IARC MONOGRAPHS

TRICHLOROETHYLENE, TETRACHLOROETHYLENE, AND SOME OTHER CHLORINATED AGENTS

VOLUME 106

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

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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, 2-9 October 2012

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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.

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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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[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. Invited Specialists do not serve as Meeting Chair or Subgroup Chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

Each participant was asked to disclose pertinent research, employment, and financial interests. Current financial interests and research and employment interests during the past 4 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US $1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

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[2] 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.

[3] Observer for the American Chemistry Council. Paul Dugard was Director (until September 2011) of Halogenated Solvents Industry Alliance (HSIA), an Employment-Trade Association representing manufacturers of trichloroethylene and tetrachloroethylene. He is sole proprietor of PHD Consulting which consults for HSIA. His mutual fund includes stock in chemical companies. He is sponsored by HSIA.

[4] Observer for the European Chlorinated Solvents Association (ECSA). Gerard Swaen is employed by and holds stock in Dow Chemical company. He is sponsored by the European Chlorinated Solvents Association.

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-sixth volume of the *IARC Monographs* evaluated the evidence for carcinogenicity of seven chlorinated solvents, including trichloroethylene, tetrachloroethylene, and their metabolites (dichloroacetic acid, trichloroacetic acid, and chloral hydrate). These solvents share metabolic pathways and have anaesthetic properties via their effects on the central nervous system. All the agents listed here were evaluated previously by the *IARC Monographs* programme (IARC, 1979, 1986, 1987, 1995, 1999a, 2004). In light of new epidemiological and mechanistic evidence published since the previous evaluation published in 1995, the Advisory Group to the *IARC Monographs* in 2008 recommended the re-evaluation of trichloroethylene and other chlorinated solvents (IARC, 2008). A summary of the findings of this Volume has appeared in *The Lancet Oncology* (Guha et al., 2012).

Trichloroethylene

Trichloroethylene is a chlorinated solvent that has been produced commercially since the 1920s. Trichloroethylene was previously used as an anaesthetic, a stain-remover in dry-cleaning, an ingredient in paints, adhesives, cleaners, and more recently, for degreasing metal parts and as a feedstock for producing chlorinated chemicals. Although occupational exposure was once widespread due to the myriad uses for trichloroethylene, exposure levels and the number of exposed workers in Europe and North America have declined by at least one order of magnitude since the 1940s. Some of the heaviest exposures have occurred in workers in industries involving the manufacture and repair of aircraft and automobiles, and in screw-cutting.

Trichloroethylene has been found in outdoor and indoor air, water, soil, food, and animal tissues, and exposure from environmental sources (including hazardous-waste sites and contaminated water) is common in the USA and elsewhere throughout the world.

Trichloroethylene was evaluated previously by the *IARC Monographs* (Volumes 20, 63, and supplement 7; IARC, 1979, 1987, 1995). In 1995, the Working Group classified trichloroethylene as *probably carcinogenic to humans (Group 2A)* based on sufficient evidence for carcinogenicity in experimental animals and limited evidence in humans. The epidemiological assessments at that time were largely driven by observations of an elevated risk of cancer of the liver and biliary tract and non-Hodgkin lymphoma (IARC, 1995).

The re-evaluation of trichloroethylene by IARC in 2012 resulted in a new classification in Group 1, *carcinogenic to humans*, based on sufficient epidemiological evidence for cancer of the kidney, with strong mechanistic support from studies in experimental animals and exposed humans. The epidemiological data also

identified limited evidence for an association with liver cancer and non-Hodgkin lymphoma. The Working Group also noted that the data for trichloroethylene are very informative with regard to demonstrating tumour-site concordance between humans and experimental animals; several rare cancers were observed in animals in the absence of common "background" tumours.

Tetrachloroethylene

Tetrachloroethylene is one of the most important chlorinated solvents worldwide. Used today in dry-cleaning and as a feedstock for the synthesis of fluorocarbons, applications in the past included degreasing metals and the production of chlorofluorocarbons. Occupational exposure has been, and continues to be, widespread. Despite considerable reduction in exposure resulting from technological advances in dry-cleaning and degreasing in the USA and in Europe, high-exposure situations continue to exist in some countries. Individuals living or working in the vicinity of dry-cleaning shops are also exposed, and exposure from environmental sources (including hazardous-waste sites and contaminated water) is common. Tetrachloroethylene has been detected in indoor and outdoor air, water, food and in animal and human tissues.

Tetrachloroethylene was evaluated previously by the *IARC Monographs* (Volumes 20, 63, and supplement 7; IARC, 1979, 1987, 1995). In 1995, the Working Group classified tetrachloroethylene as probably carcinogenic to humans (Group 2A), based on sufficient evidence in experimental animals and limited evidence in humans for cancers of the oesophagus and cervix and non-Hodgkin lymphoma (IARC, 1979, 1987, 1995). This classification was maintained in the re-evaluation of tetrachloroethylene by the Working Group in 2012 and was based on sufficient evidence in experimental animals and limited evidence in humans for an excess of cancer of the urinary bladder in dry-cleaning workers. The urinary bladder was a new tumour site identified from the epidemiological data; the Working Group noted the paucity of supporting evidence from mechanistic data. A systematic review and meta-analysis describing the evaluation of tetrachloroethylene by the Working Group, focusing on studies of dry-cleaning workers, has been prepared (Vlaanderen et al., 2014).

The Working Group decided against presenting a separate *Monograph* on dry-cleaning workers, as was done previously (Volume 63; IARC, 1995). This is consistent with the strategy of the IARC Monographs programme to evaluate specific agents, rather than mixtures or occupations, whenever possible. The primary rationale supporting the focus on specific agents in this case was the improved exposure assessment available for trichloroethylene and tetrachloroethylene in epidemiological studies addressing their carcinogenic effects. In addition, a more diverse array of dry-cleaning agents has come into use, and the frequency and intensity of exposure to trichloroethylene and tetrachloroethylene in dry-cleaning workers have decreased drastically in the last decades, in regions where epidemiological studies have been performed.

Chloral hydrate

Chloral hydrate is a metabolite of trichloroethylene and is also a by-product of chlorine-based water disinfection. It has been used as a hypnotic drug since the 1870s, and was once widely used for sedating children before dental, medical, or diagnostic procedures. Although still in use, it has largely been replaced by newer drugs with a lower potential for overdose. Chloral hydrate was previously classified in Group 3 by the Working

Group in 1995 and 2004 (IARC, 1995, 2004). In 2012, the Working Group re-classified chloral hydrate in Group 2A based on sufficient evidence of carcinogenicity in animals and strong evidence for genotoxicity (DNA damage) in mammalian and other test systems, both *in vivo* and *in vitro*. The Working Group considered noteworthy one study in infants exposed orally to chloral hydrate, which reported a significant increase in micronucleus formation in peripheral blood lymphocytes (Ikbal et al., 2004). The only available epidemiological study was uninformative as to the relationship between exposure to chloral hydrate and risk of cancer in humans. Thus the mechanistic data were crucial for characterizing the carcinogenic hazard in the absence of epidemiological data.

Other evaluations

Three of the remaining compounds – trichloroacetic acid (IARC, 1995, 2004), 1,1,1,2-tetrachlorethane and 1,1,2,2-tetrachlorethane (IARC, 1999b) – were previously classified in Group 3. In 2012, the Working Group classified these compounds as *possibly carcinogenic to humans* (Group 2B) based on *sufficient* evidence in experimental animals. The Working Group also affirmed the classification of dichloroacetic acid in Group 2B (IARC, 2004). Supporting evidence included cancer bioassays demonstrating increased incidence of hepatocellular tumours in mice.

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TRICHLOROETHYLENE

Trichloroethylene was considered by previous IARC Working Groups in 1979, 1987, and 1995 (IARC, 1979, 1987, 1995). New data have since become available, and these have been 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.: 79-01-6

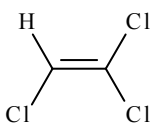
Deleted CAS Reg. No.: 52037-46-4

Chem. Abstr. Name: 1,1,2-Trichloroethene

IUPAC Systematic Name: Trichloroethylene

Synonyms: Ethinyl trichloride; ethylene trichloride; TCE; 1,1,2-trichloroethylene

1.1.2 Structural and molecular formulae and relative molecular mass



C_2HCl_3

Relative molecular mass: 131.39

1.1.3 Chemical and physical properties of the pure substance

Description: Nonflammable, mobile liquid. Characteristic odour resembling that of chloroform (O'Neil *et al.*, 2006)

Boiling-point: 86.9 °C (O'Neil *et al.*, 2006)

Melting-point: –84.8 °C (O'Neil *et al.*, 2006)

Density: 1.4642 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism, 185; grating, 62), nuclear magnetic resonance (proton, 9266; C-13, 410) and mass (583) spectral data have been reported (Sadtler Research Laboratories, 1980; Weast & Astle, 1985).

Solubility: Slightly soluble in water (1.1 g/L at 25 °C); soluble in ethanol, diethyl ether, acetone and chloroform (O'Neil *et al.*, 2006); dissolves most fixed and volatile oils (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 100 Pa at 39 °C (Haynes, 2012); relative vapour density (air = 1.0), 4.53 (O'Neil *et al.*, 2006)

Stability: Photo-oxidized in air by sunlight (half-time, 5 days) giving phosgene and

dichloroacetyl chloride (EPA, 1985), which slowly decomposes with formation of hydrogen chloride by light in the presence of moisture (O'Neil *et al.*, 2006).

Reactivity: Incompatible with strong caustics and alkalis and with chemically active metals such as barium, lithium, sodium, magnesium, titanium and beryllium (NIOSH, 1994a)

Octanol/water partition coefficient (P): log P, 2.61 (Hansch *et al.*, 1995)

Conversion factor: $mg/m^3 = 5.37 \times ppm$

Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming normal temperature (25 °C) and pressure (101 kPa).

1.1.4 Technical products and impurities

Trichloroethylene is available in the USA in vapour-degreasing, general-purpose and high-purity grades (DOW Chemical, 2012). In France, industrial-grade trichloroethylene is generally of high purity (> 99%). Depending on its intended use, it can contain one or more stabilizers, including amines, alcohols or epoxides (thymol, triethylamine, trimethyloxirane, epoxybutane, etc.) at low concentration (< 1%) (Table 1.1; Commission for Health and Safety at Work, 2012).

Possible impurities depend on the manufacturing route, the type and quality of feed stock used, the type of distillation equipment, and the technical specification being met. It is uncommon for any individual impurity to be present at a level in excess of 100 mg/kg and for the total impurities to exceed 1000 mg/kg (WHO, 1985).

Stabilizers, in the form of antioxidants or acid-receptors (such as phenolic, olephinic, pyrrolic, and/or oxiranic derivatives and aliphatic amines), are usually added in concentrations that normally range from 20 to 600 mg/kg; however, for limited quantities and special uses, concentrations as high as 5000 mg/kg may be used. The stabilizers used will depend on patent ownership and the technical specification being met (WHO, 1985).

Table 1.1 Some impurities and stabilizers found in commercial trichloroethylene

Impurities	Stabilizers
Carbon tetrachloride	Pentanol-2
Chloroform	Thymol
1,2-Dichloroethane	Triethanolamine
trans 1,2-Dichloroethylene	Triethylamine
cis 1,2-Dichloroethylene	2,2,4-Trimethylpentene
Pentachloroethane	Cyclohexene oxide
1,1,1,2-Tetrachloroethane	*n*-Propanol
1,1,2,2-Tetrachloroethane	Isobutanol
1,1,1-Trichloroethane	*N*-methylmorpholine
1,1,2-Trichloroethane	Diisopropylamine
1,1-Dichloroethylene	*N*-Methylpyrrole
Bromodichloroethylene	Methyl ethyl ketone
Tetrachloroethylene	Epichlorohydrin
Bromodichloromethane	
Benzene	

From WHO (1985)

Trade names for trichloroethylene include: Algylen, Anamenth, Benzinol, Cecolene, Chlorilen, Chlorylea, Chlorylen, CirCosolv, Crawhaspol, Densinfluat, Dukeron, Dow-Tri, Fleck-Flip, Flock Flip, Fluate, Germalgene, Lanadin, Lethurin, Narcogen, Narkosoid, Nialk, Perm-AChlor, Petzinol, Philex, Threthylen, Threthylene, Trethylene, Tri, Triad, Trial, Trichloran, Trichloren, Triclene, Trielene, Trielin, Trieline, Trilen, Trilene, Trimar, TRI-Plus M, Vestrol, Vitran, and Westrosol.

1.1.5 Analysis

Methods for the analysis of trichloroethylene have been reviewed by Delinsky *et al.* (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of trichloroethylene in various matrices are identified in Table 1.2.

Table 1.2 Methods for the analysis of trichloroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
	Air collected in specially prepared canister; desorb from cold trap to GC	GC/MS	NR	EPA (1999a)
Water	Purge with inert gas and trap; desorb to GC	GC/PID	0.02 µg/L	EPA (1988, 1995a)
		GC/HECD	0.01 µg/L	
	Purge with inert gas and trap; desorb to GC	GC/PID	0.01 µg/L	EPA (1988)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.02 µg/L	EPA (1988, 1995b)
	Extract with methyl-*t*-butyl ether or pentane;	GC/EC	0.002 µg/L	EPA (1995c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	0.02 µg/L	EPA (1996)
		GC/HECD	0.01 µg/L	
Blood	Purge with inert gas and trap on Tenax	GC/MS	0.004 ppb	Ashley *et al.* (1992)

EC, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HECD, Hall electrolytic conductivity detection; MCD, microcoulometric detection; MS, mass spectrometry; PID, photoionization detection; ppb, parts per billion; NR, not reported

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Trichloroethylene was first prepared in 1864 by Emil Fischer in experiments on the reduction of hexachloroethane with hydrogen (Hardie, 1964). Commercial production of trichloroethylene began in Germany in 1920 and in the USA in 1925 (Mertens, 1993). Originally, the acetylene-based process consisted of two steps: first, acetylene was chlorinated with a chloride catalyst to produce 1,1,2,2-tetrachloroethane, and then this product was dehydrohalogenated to trichloroethylene (Mertens, 1993).

The current method of manufacture is from ethylene. Trichloroethylene can be produced using oxichlorination or noncatalytic chlorination of ethylene dichloride or other C2-chlorinated hydrocarbons. Another method involves using catalytic hydrogenation of tetrachloroethylene (ECSA, 2012). Trichloroethylene can also be produced by direct chlorination of ethylene in the absence of oxygen, giving a mixture of tetrachloroethane and pentachloroethane. The products are thermally cracked to produce a mixture of trichloroethylene, tetrachloroethylene and hydrochloric acid. This process was developed and is used in Japan (Linak *et al.*, 1992).

(b) Production volume

In general, there has been a continuing decline in demand for trichloroethylene over the years. Concern about the environmental and health and safety implications of chlorinated solvents has resulted in regulations and controls that have had an impact on the use and production of trichloroethylene. For example, in the USA the demand for trichloroethylene dropped from 244 939 tonnes (540 million pounds) in 1971 to only 68 038 tonnes (150 million pounds) in 1990 (NICNAS, 2000). In Sweden, 4564 tonnes were used in 1993, but only 91 tonnes by 2009 (KEMI, 2012). The production volume of trichloroethylene in the European Union in 1996 was thought to be between 51 000 and 225 000 tonnes per year (SCOEL/SUM, 2009). In 1995, this declining trend was reversed when the Montreal Protocol on Substances that Deplete the Ozone Layer was enacted: trichloroethylene was required in increasing amounts as a precursor in the manufacture of chlorofluorocarbon alternatives and as

a substitute for chemicals such as 1,1,1-trichloroethane (NICNAS, 2000).

In 2007, the USA was the largest consumer of trichloroethylene, followed by western Europe, China, and Japan (Glauser & Ishikawa, 2008).

1.2.2 Use

Trichloroethylene is best known for its use as a solvent for cleaning and degreasing metal parts. However, it has had numerous other uses, including as an anaesthetic, a heat-transfer medium, an extraction agent for fats and oils, as an intermediate in producing chlorofluorocarbons and other chemicals, and as an ingredient in many products for industrial and consumer use (Doherty, 2000).

Demand for trichloroethylene was generated mainly by the development of vapour degreasing after the 1920s and by the growth of the dry-cleaning industry in the 1930s. Trichloroethylene was replaced in dry-cleaning by tetrachloroethylene in the mid-1950s. By 1989, about 85% of the trichloroethylene produced in the USA was used in metal cleaning; the remaining 15% was equally divided between exports and miscellaneous applications. The pattern in Japan was similar to that in the USA, at 83% and 17%, respectively. In western Europe, 95% was used in vapour degreasing and 5% for other uses (Mertens, 1993). Similar use patterns have been reported for Canada (Moore *et al.*, 1991), and Finland (Mroueh, 1993). The use of trichloroethylene as solvent in Europe dropped by 85% from 1984 until 2006, with a further estimated decline of 60% from 2006 until 2010 (ECSA, 2012).

Currently the main use of trichloroethylene is as a feedstock material to produce other chemicals, such as fluorinated hydrocarbons and fluorinated polymers, which are being phased out under the Montreal Protocol. About 80% of current production in the European Union is used for this purpose (ECSA, 2012).

(a) Metal degreasing

Before the 1990s, the major use of trichloroethylene was in metal degreasing. Degreasing is important in all metalworking and maintenance operations to remove oils, greases, waxes, tars and moisture before final surface treatments, such as galvanizing, electroplating, painting, anodizing and application of conversion coatings. Trichloroethylene has been used in degreasing operations in five main industrial groups: furniture and fixtures, fabricated metal products, electric and electronic equipment, transport equipment and miscellaneous manufacturing industries. It has also been used in plastics, appliances, jewellery, automobile, plumbing fixtures, textiles, paper, glass and printing (Papdullo *et al.*, 1985; Linak *et al.*, 1992).

Metal degreasing operations using trichloroethylene are of two main types: cold degreasing and vapour degreasing. In cold degreasing, trichloroethylene is applied at room temperature; in vapour degreasing, the solvent vapours are condensed on the part to be cleaned. Cold degreasing by hand will result in higher exposures than vapour degreasing.

Cold degreasing refers to the process of degreasing by dipping or soaking articles in a degreasing liquid, or spraying, brushing, or wiping the cleaner onto articles at temperatures below boiling point. The cold process is frequently used in maintenance operations and on small parts. Cold degreasing activities include immersion in tanks, drums, or other containers, and spraying, brushing and wiping.

Vapour degreasing requires a tank with heating coils on the bottom and a condensing zone near the top. The solvent is heated to boiling, and the hot vapour fills the condensing zone near the top of the tank. Soiled objects are lowered into this zone, where the vapour condenses into a pure liquid solvent on the piece and dissolves and carries off dirt as it drains back into the tank. The part dries immediately (Papdullo *et al.*, 1985;

Linak *et al.*, 1992). Vapour degreasers can incorporate spraying as part of the degreasing process. In western Europe, in 1990, 120 000 tonnes of trichloroethylene were used in vapour degreasing and only 10 000 tonnes in cold degreasing (Linak *et al.*, 1992). Similarly, vapour degreasing was more common than cold degreasing in Australia in 1995 (NICNAS, 2000).

Ultrasonic agitation can be employed in hot or cold immersion degreasing, and is sometimes incorporated into vapour-degreasing systems. In ultrasonic degreasing, a transducer mounted on the bottom or side of a solvent-containing tank creates vibrations that cause the rapid expansion and contraction of microscopic bubbles in the solvent, resulting in a scrubbing action on parts that are immersed in the tank (NICNAS, 2000).

(b) Dry-cleaning industry

Trichloroethylene was used in the dry-cleaning industry in the 1930s, but was very harsh on clothes and was replaced by tetrachloroethylene in the mid-1950s. Trichloroethylene is still used in spotting agents to remove spots before cleaning the garments in the dry-cleaning machine or after the garments have been cleaned in the machine (Wolf & Morris, 2007).

(c) Other industrial applications

(i) Chemical intermediates

Trichloroethylene is used as a molecular-weight control agent in the manufacturer of polyvinyl chloride. About 10 million pounds [4500 tonnes] of trichloroethylene are used each year in the USA in the manufacture of polyvinyl chloride (IARC, 1995). As noted above, the largest use of trichloroethylene currently is as a feedstock for chlorofluorocarbons and hydrofluorocarbons (Doherty, 2000). Under the Montreal Protocol of the 1990s, chlorofluorocarbons are being phased-out due to their contribution to ozone depletion.

(ii) Textile industry

In the textile industry, trichloroethylene has been used as a carrier solvent for spotting fluids and as a solvent in dyeing and finishing (Fishbein, 1976; Linak *et al.*, 1992; Mertens, 1993). The main use of trichloroethylene in the textile industry is to clean cotton, wool and other fabrics. It is also used as a solvent for waterless dyeing (Doherty, 2000).

(iii) Consumer products

Some consumer products that have contained trichloroethylene include automotive products, wood finishes, typewriter correction fluids, cleaners and polishes, including for electronic equipment, treatments for leather and fabric, adhesives, paint-related products and lubricants (Sack *et al.*, 1992; ATSDR, 1997).

(iv) Other uses

Trichloroethylene has been used in miscellaneous chemical synthesis and solvent applications, including: as a synthesis feedstock for products such as paints, adhesives and cleaners; as a reactant to produce pesticide intermediates; in the chemical synthesis of flame-retardant chemicals; as a solvent in pharmaceutical manufacture; and as a carrier solvent in formulated consumer products such as insecticides, fungicides, paint removers, and paint strippers (EPA, 1989; Doherty, 2000).

(d) Miscellaneous

During the 20th century, trichloroethylene was used as an anaesthetic for dental and surgical procedures and in veterinary medicine (Doherty, 2000). It was also used as an extraction solvent for natural fats and oils (such as palm, coconut and soya bean oils), and for spices, hops, and the decaffeination of coffee (Linak *et al.*, 1992). The United States Food and Drug Administration (FDA, 1977) banned these uses of trichloroethylene because of its toxicity; its use in cosmetic and drug products was also discontinued (Mertens, 1993).

Table 1.3 Mean concentrations[a] of trichloroethylene in air in the USA, by year

Year	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Concentration (μg/m³)	1.4	1.39	1.68	4.87	1.69	1.84	2.86	1.37	1.12	0.95	0.78	0.65	0.74	0.88

[a] Representing about 1200 measurements in 25 states
From Wu & Schaum (2000)

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Trichloroethylene has been reported in temperate, subtropical and tropical algae, and in one red microalga (Abrahamsson *et al.*, 1995).

1.3.2 Environmental occurrence

Trichloroethylene is widely distributed in the environment due to industrial emissions. Potential environmental exposure to trichloroethylene in the air, rainwater, surface waters, and drinking-water has been reviewed (ATSDR, 1997; Wu & Schaum, 2001). The partitioning tendency of trichloroethylene in the environment has been calculated as follows: air, 97.7%; water, 0.3%; soil, 0.004%; sediment, 0.004% (Boutonnet *et al.*, 1998).

A common method for disposal of trichloroethylene is incineration after mixing with a combustible fuel (Sittig, 1985).

(a) Air

Measurements of trichloroethylene in air in the USA, representing about 1200 measurements in 25 states (Table 1.3), suggest a general downward trend in mean concentrations of trichloroethylene in air, from about 1.5 μg/m³ in the late 1980s to 0.8 μg/m³ in the late 1990s. Concentrations in urban air were about three times higher than in rural areas, and mean concentrations of trichloroethylene were highest in commercial/industrial areas and lowest in forest areas (Wu & Schaum, 2000).

Table 1.4 presents some recent data on concentrations of trichloroethylene in air measured worldwide in remote, rural, suburban and urban sites. The data showed trends similar to those observed in the USA.

Concentrations of trichloroethylene in indoor air can increase when trichloroethylene-contaminated water is used domestically, for example, during showering (Andelman, 1985; Ömür-Özbek *et al.*, 2011).

(b) Soil

Trichloroethylene can be released into the soil through industrial discharges into surface waters and through leaching from landfill sites. It has been estimated that at least 9612 kg of trichloroethylene was released into soil from manufacturing and processing facilities in the USA in 1988 (ATSDR, 1997).

(c) Water

Owing to its widespread use, trichloroethylene occurs frequently at low concentrations in water supplies and in groundwater. Table 1.5 summarizes some recent concentrations of trichloroethylene reported in surface water, groundwater and drinking-water worldwide.

(d) Food

As a part of the Total Diet Program in the USA, 20 samples of 70 different foods purchased in supermarkets or restaurants in 1996–2000 were analysed for trichloroethylene. Trichloroethylene was found at a low frequency in 29 out of 70 food items at concentrations in the low microgram-per-kilogram range (Fleming-Jones & Smith, 2003).

In a survey of 35 whole milk samples in Las Vegas, NV, USA, trichloroethylene was found

Table 1.4 Concentrations of trichloroethylene in air

Location	Concentration (µg/m³)		Comments	Reference
	Mean	Range		
Outdoor air				
Remote				
Antarctica	NR	0.016–0.024	Five remote sites	Zoccolillo *et al.* (2009)
Atlantic Ocean	NR	[0.03–0.05]	Background tropospheric levels	Class & Ballschmiter (1986)
North America	< 0.02	NR	Remote background concentration	McCarthy *et al.* (2006)
Urban and rural				
Canada, Ottawa	0.08	0.01–1.49	Near 74 homes	Zhu *et al.* (2005)
Italy	NR	0.312–0.940	Four urban and suburban sites	Zoccolillo *et al.* (2009)
	NR	0.022–0.107	At 12 rural sites	
Japan, Shizuoka Prefecture	0.23[c]	0.11–0.80[a]	Near 25 homes	Ohura *et al.* (2006)
Japan, Hyogo Prefecture	NR	Range of medians, 0.053–0.22	At six sites over 5 years	Okada *et al.* (2012)
Spain, Tarragona	0.74	2.20[b]	Near large industrial complex	Ramírez *et al.* (2012)
USA, Georgia	[0.96]	4.59[b]	Near degreaser facility	Martin *et al.* (2005)
USA, five cities in NJ, NC, ND, CA	NR	0.083–1.78		Rappaport & Kupper (2004)
USA, Dallas, TX	8.5	1.1–327	Ambient air near gas wells	Rich (2011)
USA, Minnesota	0.43	< 0.04–25.31	At 25 sites in state (1991–1998)	Pratt *et al.* (2000)
USA, Seattle, WA	0.21	SD, 0.23	Ambient air at six sites	Wu *et al.* (2011)
Indoor air				
Canada, Ottawa, ON	0.06	0.01–0.87	75 homes	Zhu *et al.* (2005)
France	0.5[a]	< 0.4–4087	490 homes	Billionnet *et al.* (2011)
Japan, Shizuoka Prefecture	0.22[c]	0.10–0.78[a]	25 homes	Ohura *et al.* (2006)
USA, Endicott, NY	16[a]	0.18–140	70 housing blocks near contaminated site	Forand *et al.* (2012)
USA, Minneapolis, MN	0.7	0.2–1.4	284 households	Adgate *et al.* (2004)
USA, Missoula, MT	[0.02[c]][d]	[< 0.001–4.6][d]	80 homes	Ward *et al.* (2009)
USA, New Jersey	NR	< 1.1–13	100 urban and suburban homes	Weisel *et al.* (2008)

[a] 10th and 90th percentiles
[b] Maximum
[c] Median
[d] The Working Group noted that the units of concentration were not reported clearly in the original publication but assumed that these values were in µg/m³.
NR, not reported

at a mean concentration of 0.04 µg/L (range, < 0.01–0.27) (Hiatt & Pia, 2004).

The average concentration of trichloroethylene in 3 out of 17 samples of brown grease from food-preparation facility grease traps was 321.3 µg/L (range, 146–600) (Ward, 2012).

1.3.3 Occupational exposure

Degreasing is the main source of occupational exposure to trichloroethylene: cold degreasing by hand will result in higher exposures than vapour degreasing.

The United States National Institute for Occupational Safety and Health (NIOSH, 1994b)

Table 1.5 Concentrations of trichloroethylene in water

Country	Location	Concentration (µg/L)		Comments	Reference
		Mean	Range		
Ground-water					
China	Eastern China	NR	< 0.2–28	At five sites	Bi *et al.* (2012)
China, Taiwan	Country-wide	NR	1–231	Near eight contaminated sites	Fan *et al.* (2009)
China, Taiwan	Taoyuan City	253	0.1–1791	Near contaminated site	Lee *et al.* (2002)
Croatia	Sašnak	8.55	5.05–12.90	1995–1996	Vedrina-Dragojević & Dragojević (1997)
USA	Camp Lejeune, NC	NR	NR–57	Supply well near contaminated site	Sonnenfeld *et al.* (2001)
USA	Minnesota	NR	0.2–144	Near hazardous-waste disposal sites	Sabel & Clark (1984)
USA	Country-wide	NR	0.02–230	5000 samples, 1985–2002	Moran *et al.* (2007)
USA	Arizona	NR	1–239 ppb	Seven municipal wells near a contaminated site	Kioski *et al.* (1990)
Surface water					
Europe	Southern North Sea	0.049	< 0.012–0.27	10 locations, 1998–2000	Huybrechts *et al.* (2005)
Greece	Northern Greece	NR	< 0.02–40		Kostopoulou *et al.* (2000)
USA	Bush River, MD	NR	450–1600	Contaminated site near Aberdeen Proving Ground	Burton *et al.* (2002)
Drinking-water					
Malaysia	County-wide	NR	0.3–0.7		Soh & Abdullah (2007)
USA	Camp Lejeune, NC	399	1–1400		NRC (2009)
USA	Woburn, MA	267	NR		Costas *et al.* (2002)

NR, not reported

estimated that about 401 000 employees in 23 225 plants in the USA were potentially exposed to trichloroethylene. This estimate was based on a survey of products used in companies in 1981–83 and did not involve actual measurements.

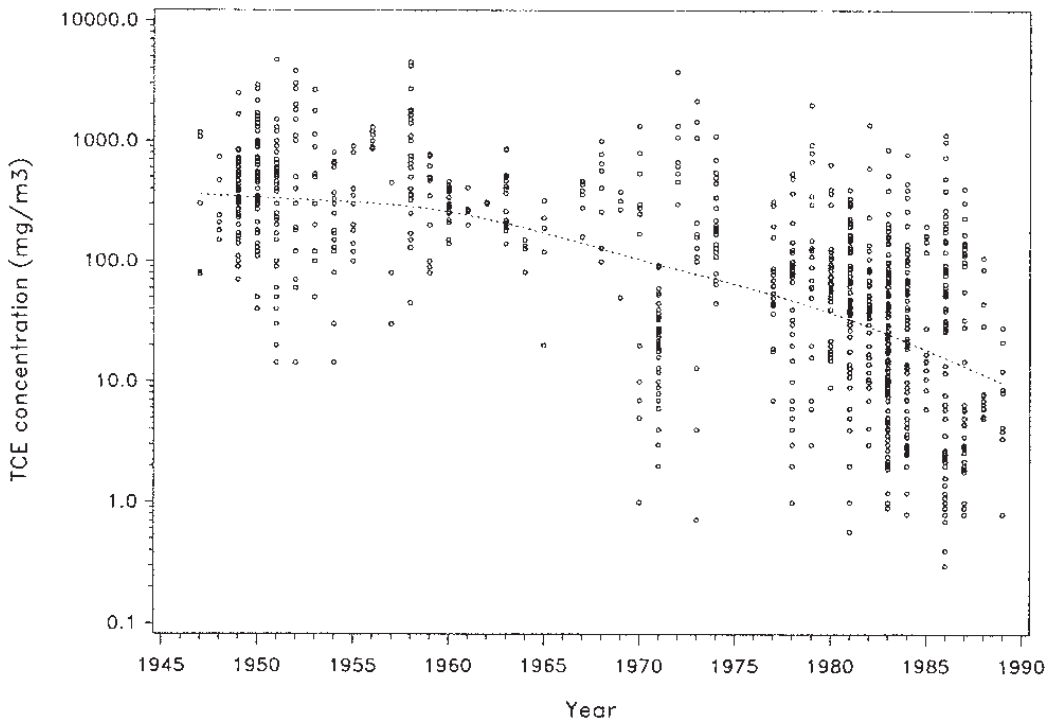
The European CAREX (CARcinogen EXposure) project estimated the number of exposed workers in 15 countries of the European Union (EU-15) to be approximately 276 000 in the early 1990s. The majority of the exposures occurred in industries producing metal products, machinery (including electrical machinery, apparatus and appliances) and in the manufacture of transport equipment. Considerable numbers of exposed workers were also found in construction, the wholesale and retail trades, restaurants and hotels, and in personal and household services, including dry-cleaning shops (Kauppinen *et al.*, 2000).

An update of CAREX for Italy by Mirabelli & Kauppinen (2005) showed a declining number of workers exposed from 42 000 to 34 500 for 1990–93 and 2001 respectively (excluding jobs considered as low-level or with low confidence in their assessment).

The total number of exposed individuals around metal vapour-degreasers in Germany was estimated at almost 11 000 in 1985, and this number decreased considerably to only about 400 in 1999 (von Grote *et al.*, 2003).

Results from CAREX Canada (2012) showed that approximately 13 000 workers were exposed

Fig. 1.1 Area and personal measurements (*n* = 1075) of trichloroethylene in occupational environments in Denmark, 1947–89



TCE, trichloroethylene
The curve shows a smoothing spline estimate of the average concentration
From Raaschou-Nielsen *et al.* (2002). Reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandf.co.uk/journals/).

to trichloroethylene, 69% of these being men. Most exposures occur in metal manufacturing, printing and related support activities, textile-furnishing mills, other textile-product mills, and plastic-product manufacturing. Most of these jobs involve metal degreasing. With trichloroethylene no longer commonly in use as a solvent in dry-cleaning, it was estimated that only 200 workers are still exposed in this industry.

A cross-industry survey (2009–10) on use of organic solvents in almost 1500 workplaces in Japan revealed that use of trichloroethylene for degreasing, cleaning and wiping had ceased, and trichloroethylene has largely been replaced by isopropyl alcohol (Nagasawa *et al.*, 2011a). In a similar study in 1909 laboratories from four large research institutes, trichloroethylene was found infrequently in the air in biology laboratories (0.6%), medical laboratories (0.2%) and technology and engineering laboratories (0.9%), but not in agricultural and science laboratories (Nagasawa *et al.*, 2011b).

Table 1.6 summarizes the results of measurements of trichloroethylene in air for occupational exposures. For biomonitoring of its metabolite, trichloroacetate, in urine, the reader is referred to the *Monograph* on Trichloroacetic Acid in this Volume.

Two studies showed clear negative temporal trends in the concentrations of trichloroethylene measured in workplaces. In the USA, estimates of decline in concentration were 6.7% per year, indicating a halving of concentrations each decade (Hein *et al.*, 2010). In Denmark (Raaschou-Nielsen *et al.*, 2002), exposure to trichloroethylene declined at an similar rate from

Table 1.6 Occupational exposure to trichloroethylene

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Australia [1995]	1	Metal industry; degreasing; after replacement of FC113 with trichloroethylene [1995]	5	[47]	NR	Neghab *et al.* (1997)
Canada	1	Textile; spot removing	12	NR	5–118	Mirza *et al.* (2000)
China 2006	6	Degreasing processes in metal manufacturing (n = 4), Optical lenses manufacturing (n = 1), circuit boards manufacturing (n = 1)	235 (P) (80 workers)	[118.22]	-	Lan *et al.* (2010)
Denmark 1947–89	Many	Variety of industries	318 (A) 74 (A) 192 (A+P) 491 (A+P)	1947–1959: 586 1960–1969: 318 1970–1979: 198 1980–1989: 75	NR	Raaschou-Nielsen *et al.* (2002)
Finland 1982–85	11	Vapour degreasing	24 (A) 13 (P) TWA	[43.0] [37.6]	[< 5.4–20.9] [< 5.4–161]	Rantala *et al.* (1992)
	1	Rubber bonding	1 (A) TWA	NR	[32.2]	
	1	Museum textile restoration	2 (P) 1 hour	NR	[3303]	
Italy	1	Printing on glass industry	49 (P)	83.3	2.7–387	Imbriani *et al.* (2001)
	1	Printing industry, degreasing process	12 (P)	31–38	NR	Iavicoli *et al.* (2005)
Republic of Korea	90	Variety of industries	196	[18.5][b]	[ND–1719]	Moon *et al.* (2001)
Netherlands 1988	9	Rubber degreasing, cementing	137 (P)	4	NR	Kromhout *et al.* (1994)
Singapore	1	Metal degreasing	12 (P)	[159] TWA	[48–704]	Goh *et al.* (1998)
Sweden	14	Degreasing	336 (A)	[328]	[0–2230]	Ahlmark *et al.* (1963)
	570	Degreasing	35 000–40 000 (A)	[86]	3% [> 161]	
	19	Degreasing	29 (P)	27	3–144	Ulander *et al.* (1992)
Switzerland	10	Degreasing	96 (P)	[304]	[5.4–1799]	Grandjean *et al.* (1955)
United Kingdom	32	Degreasing	212 (P)	91% [< 161] 97% [< 269] 99% [< 537]	NR	Shipman & Whim (1980)
USA Period NR	60	Degreasing	433 (P)	[725]	[16–4833]	Morse & Goldberg (1943)
		Condenser, nonvented	187	[515]	[27–2110]	
		Condenser, vented	149	NR	NR	
Period NR	NR	Degreasing	146 (A)[b]	86% [< 537] 96% [< 1074]	NR	Hargarten *et al.* (1961)

Table 1.6 (continued)

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Period NR	1	Degreasing	11 (P)	[302]	[199–419]	Vandervort & Polakoff (1973)
Period NR	1	Degreasing ignition coils	(P)	NR	0–[537]	Bloom *et al.* (1974)
Period NR	1	Electronic cleaning	3 (P)	[446]	[408–483]	Gilles & Philbin (1976)
Period NR	1	Semi-conductor degreasing	10 (P)	16.1	2–57	Gunter (1977)
Period NR	1	Degreasing operator	20 (P)	[736]	[140–2024]	Kominsky (1976)
		Degreasing operator	7 (P)	[88.1]	[37.6–456]	
		Degreasing operator	6 (P)	[67.7]	[37.6–199]	
		Lathe operator next to degreaser	7 (P)	[52.1]	[37.6–129]	
Period NR	1	Aircraft degreasing	4 (P)	[21.5]	[5.4–37.6]	Okawa *et al.* (1978)
Period NR	1	Tank relining	8 (P)	[1.3]	ND–[5.4]	Burroughs (1980)
Period NR	1	Degreasing sheet metal	2 (P)	11	10–12	Johnson (1980)
			2 (A)	11	4–18	
Period NR	1	Degreasing, custom finishing	23 (P)	8.3	1–38	Ruhe & Donohue (1980)
			2 (A)	6	4–8	
Period NR	1	Vapour degreasing	14 (P)	333	26.9–1670	Burgess (1981)
Period NR	1	Degreasing, bus maintenance	3 (A)	3.0	ND–8.9	Love & Kern (1981)
Period NR	1	Degreasing	24 (STEL)	742	56–2000	Ruhe *et al.* (1981)
			9 (TWA)	145	37–357	
Period NR	1	Degreasing, plastics	2 (P)	4.8	2.7–7.0	Burroughs & Moody (1982)
Period NR	1	Degreasing, electronics	79 (P)	10.2	ND–209	Lee & Parkinson (1982)
Period NR	1	Degreasing, medical	5 (P)	5.4	1–16	Ruhe (1982)
			2 (A)	6.5	4–9	
Period NR	1	Degreasing, energy conservation products	2 (P)	36.5	22–51	Almaguer *et al.* (1984)
			10 (A)	1.1	0.54–3.2	
Period NR	1	Degreasing	9 (P)	716	39–2288	Belanger & Coye (1984)
		Degreasing	2 (A)	184	0.54–367	
		Silk screening	5 (P)	23.6	1.6–81.1	
Period NR	1	Degreasing aircraft	29 (TWA, P)	30.7	ND–208	Gorman *et al.* (1984)
			11 (TWA, A)	28.5	2–121	
			22 (STEL)	320	ND–1256	

Table 1.6 (continued)

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Period NR	1	Taxidermy	2 (A) 2 (P)	8.9 8.9	1.1–16.6 1.7–16	Kronoveter & Boiano (1984)
Period NR	1	Degreasing	(TWA) (STEL)	205 1084	117–357 413–2000	Landrigan *et al.* (1987)
Period NR	1	Metal insignia; degreasing	1 (TWA) 1 (Peak)	[478] [1558]	NR	Rosa (2003)
1940–98	Many	Variety of industries	484	[37.6][c]	[0.0011–5911]	Hein *et al.* (2010)[d]
11 countries	6	Paper and pulp industry, maintenance work	10 (A)	[715]	[ND–5406]	Teschke *et al.* (1999)

[a] P, personal air samples (breathing zone); A, area samples
[b] Geometric mean
[c] Median
[d] The study by Hein *et al.* (2010) overlaps with several of the studies in the USA presented above. Most measurements were taken after observation of operating deficiencies of degreasers between 1952 and 1957.
ND, not detected; NR, not reported; STEL, short-term exposure limit; TWA, time-weighted average

Table 1.7 Exposure to trichloroethylene in the general population

Country	Subjects	No. of subjects	Age (years)	Concentration in blood (µg/L)		Concentration in air (µg/m³)	Reference
				Mean	Range		
Germany	No known occupational exposure	39	23–52	< 0.1[a]	< 0.1–1.3	NR	Hajimiragha *et al.* (1986)
USA	NHANES 1994–96	677	20–59	0.017	NR	NR	Wu & Schaum (2000)
USA	SHIELD study, 2000–01	134	6–10	0.007[b]	NR	NR	Sexton *et al.* (2005)
USA	NHANES 1999–2000	290	20–59	0.013	0.007–0.332	NR	Jia *et al.* (2012)
USA	1981–87, adults	NR	NR	NR	NR	NR	Rappaport & Kupper (2004)
	Bayonne	139	NR	NR	NR	2.2	
	Elizabeth	191	NR	NR	NR	3.5	
	Greensboro	24	NR	NR	NR	1.1	
	Devils Lake	23	NR	NR	NR	0.5	
	Los Angeles	176	NR	NR	NR	1.6	
USA	MNCPES study	72	3–12	NR	NR	0.8	Adgate *et al.* (2004)

[a] Median
[b] Geometric mean, estimated

approximately 300 mg/m³ in the early 1950s to about 10 mg/m³ in 1990 (Fig. 1.1).

Numbers of exposed workers and reported concentrations have declined considerably in Europe and North America. Recently reported concentrations from Asia appeared to be somewhat higher than current exposures in Europe and North America (Table 1.6).

Trichloroethylene has been measured in the blood of 157 metal workers in the USA, who had an average concentration of 2.5 µg/L (range, 0–22 µg/L) (Pfaffenberger *et al.*, 1984).

1.3.4 Exposure of the general population

Several studies have examined blood concentrations of trichloroethylene in the general population (Table 1.7). The number of individuals with measurable concentrations of trichloroethylene is generally low and has declined in more recent years.

In a study of 134 children in the USA who wore charcoal-based passive air samplers for 2 days before blood collection, no association was seen between personal exposure to trichloroethylene and concentration in the blood, although few subjects had measurable blood concentrations (Sexton *et al.*, 2005).

In the National Health and Nutrition Examination Survey (NHANES) 1999–2000 in the USA, blood samples were taken from 290 subjects (Jia *et al.*, 2012). The mean concentration of trichloroethylene was 0.013 µg/L and 88% of samples were below the limit of detection. Samples of exhaled air were also taken for 361 subjects; for these samples the mean concentration of trichloroethylene was 3.48 µg/m³, and 76% of samples were below the limit of detection. Concentrations in air and blood were moderately associated. In the most recent survey in 2005–06, all 3178 subjects had concentrations below the limit of detection. Exposure of the general population from air, water and food was several orders of magnitude lower than occupational exposure (see Table 1.6).

Table 1.8 Occupational exposure limits for trichloroethylene worldwide

Country or region	Concentration (mg/m³)	Interpretation
Australia	54	TWA
Austria	3.3	TWA
Belgium	55	TWA
Canada (Quebec)	269	TWA
Denmark	55	TWA
France	405	TWA
Germany, Committee on Hazardous Substances	60	–
Hungary	270	–
New Zealand	269	TWA
Singapore	269	TWA
Sweden	50	–
Switzerland	260	TWA
USA, Occupational Safety and Health Administration	537	TWA
USA, American Conference of Governmental Industrial Hygienists	269	TWA
United Kingdom	550	TWA

TWA, 8-hour time-weighted average
From GESTIS (2012)

1.4 Regulations and guidelines

Concern began to arise in the end of the 1970s about the potential environmental and health effects of trichlororoethylene (Birkenfeld *et al.*, 2005). In the USA, several regulations at the county, state and national levels were passed to limit emissions of trichloroethylene. In Europe, directives were instituted to restrict marketing and sales to end-users (76/769/EC). During the 1980s, several European countries, and the European Union, began passing regulations to protect workers from exposure to trichloroethylene.

The classification of trichloroethylene as a carcinogen carrying an R45 risk phrase ("may cause cancer") in the European Union in the 1990s resulted in replacement of trichloroethylene in many processes. The National Toxicology Program (NTP) of the USA first listed trichloroethylene in 2000 (NTP, 2000), and it is currently classified as "reasonably anticipated to be a human carcinogen" (NTP, 2011).

International time-weighted averages (TWAs) for trichloroethylene vary considerably (Table 1.8) from less than 100 mg/m³ in Australia and many European countries to more than 500 mg/m³ in the United Kingdom and the USA. Risk phrases and evaluations are presented in Table 1.9.

2. Cancer in Humans

2.1 Introduction

There is substantial epidemiological literature on cancer and trichloroethylene, consisting of both cohort and case–control studies, as well as ecological studies of environmental exposures. These study designs cover exposure by dermal and inhalation routes in a variety of settings and are complementary regarding strengths and weaknesses. The cohort design typically provides a narrower range of occupations for exposure assessment than the case–control and also allows focus on heavily exposed workers,

Table 1.9 Regulations and evaluations for trichloroethylene worldwide, as of October 2012

Country	Organization	Classification	Significance
Europe	GHS	H350	May cause cancer (1B)
	European classification	R 45	May cause cancer
Germany	TRGS 905	K3	Substances that possibly are carcinogenic for humans and thus give cause for concern
	MAK Commission	1	Substances that cause cancer and make a considerable contribution to the risk of cancer
USA	EPA	Group C	Possible human carcinogen with threshold
	ACGIH	A2	Suspected human carcinogen
	NTP		Reasonably anticipated to be carcinogen

ACGIH, American Conference of Governmental Industrial Hygienists; EPA, Environmental Protection Agency; GHS, Global Harmonization System; MAK, Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area; NTP, National Toxicology Program; TRGS, Technical Regulations on Hazardous Substances.

while the case–control design allows control for some important potential confounders that may be difficult to capture from cohort studies. Since information on smoking was typically not available for cohort studies, results were reported for cancer of the lung as an indicator for tobacco smoking in the cohort, not because this cancer was of primary interest in relation to exposure to trichloroethylene.

Many cancers have been evaluated, but there has been a focus on tumours of the kidney, liver, and non-Hodgkin lymphoma. Exposure estimates have been based on a range of techniques, including simple job-title determinations, quantitative assessments, and biological monitoring. Trichloroethylene has been used in several industries and occupations, especially where metal degreasing is performed. Cohort studies have been conducted on workers in the aerospace and aircraft-repair industries, as well as in other industries. In many workplaces where trichloroethylene is used, other chlorinated solvents can also be found. There was some overlap of exposures to tetrachloroethylene and trichloroethylene in the studies evaluated in this *Monograph*, but this was likely to be small because of the different patterns of use in different industries. Overlap in exposures complicated the interpretation of study findings, but it should be remembered that workers are exposed to multiple agents in nearly all workplaces. Trichloroethylene and tetrachloroethylene have largely been used over different periods in different industries, therefore the exposure overlap was not likely to be great. For this evaluation, the Working Group included only studies that specifically mentioned exposure to trichloroethylene. Other studies on exposure to non-specified chlorinated solvents were reviewed, but were considered uninformative with regard to trichloroethylene.

2.2 Cohort studies

See Table 2.1 for an overview, and Table 2.2 for a detailed description of the studies.

2.2.1 Workers in the dry-cleaning industry

Trichloroethylene has been used in in dry-cleaning as the main solvent (from the 1930s to the 1950s) and is still used as a spotting agent to remove stains. From the 1940s, it began to be replaced by tetrachloroethylene, which is less damaging to fabric (Ludwig, 1981; IARC, 1995; Doherty, 2000). Dry-cleaning workers active in this early period may have been exposed to trichloroethylene, as well as to carbon tetrachloride, tetrachloroethylene and

other petroleum-derived solvents. Since use of these chlorinated solvents overlapped, and since all continued to be used as spotting agents, the literature contained few reports of cohort studies of health effects and cancer outcomes among dry-cleaning workers exposed exclusively to trichloroethylene (IARC, 1995). However, the main dry-cleaning solvent used from the 1960s onwards was tetrachloroethylene. [See Section 1.2.2 of this *Monograph* for a more detailed description of exposure to trichloroethylene in the dry-cleaning industry.]

No large studies of dry-cleaning workers exposed specifically to trichloroethylene were available to the Working Group. In a small study of 65 male dry-cleaning workers exposed heavily to trichloroethylene in Prague, Czech Republic, starting in the 1950s, the foIlow-up period was 5–50 years, all had 1–35 years of exposure to trichloroethylene, and 86% (n = 57) were traced until 1979 (Malek *et al.*, 1979). Nearly 60% of those tested had a urinary concentration of trichloroacetic acid in excess of 100 mg/L, with sporadic values in the region of 1000 mg/L. In total, six men were diagnosed with cancer: three had cancer of the lung, one had cancer of the tongue, one had cancer of the rectum, and one had tumours of the bladder and rectum. None were diagnosed with cancer of the kidney or liver. [There were not enough data to estimate relative risks. The Working Group believed this study was too small to be informative for the evaluation of trichloroethylene.]

[The Working Group did not consider that any other cohort studies on dry-cleaning workers were directly relevant to the evaluation of trichloroethylene, given the limited exposure to trichloroethylene and extensive exposure to tetrachloroethylene (see the *Monograph* on Tetrachloroethylene, Section 2.1, for a further discussion of cohort studies of dry-cleaning workers).]

2.2.2 Workers in the aircraft and aerospace industries

See Table 2.2

Several cohort studies of aircraft and aerospace workers potentially exposed to trichloroethylene in the USA have been published, almost all with cancer mortality as the outcome. Only two of these studies (Garabrant *et al.*, 1988; Spirtas *et al.*, 1991) were included in the previous evaluation (IARC, 1995). For some of these cohorts, including Spirtas *et al.* (1991), multiple follow-ups were published for a single study. In such situations, the Working Group evaluated only results from the most recent study, unless otherwise described. Since information on smoking was typically not available for cohort studies, results were reported for cancer of the lung as an indicator for tobacco smoking in the cohort, but not because lung cancer was of primary interest in relation to exposure to trichloroethylene.

Garabrant *et al.* conducted a retrospective cohort study of 2169 women and 11 898 men employed for at least 4 years at an aircraft-manufacturing company in San Diego county, USA, between 1958 and 1982 (Garabrant *et al.*, 1988). The cohort was established from company records. No information on individual exposures was available. Based on a small sample of 14 deaths and 56 controls with a total of 362 jobs held within the company, the estimated prevalence of exposure to trichloroethylene among the cohort was 37%. [It was unclear whether this was an opportunistic sample.] The cause of death was retrieved from the death certificate or from the California state death tapes, and coded by a trained nosologist. The national age-, sex-, race-, calendar-year-, and cause-specific mortality rates for the USA were used to calculate expected numbers of specific causes of death. Similar rates for San Diego County were also applied. In total, 12.8% of cohort members had died by the end of follow-up, and 95.3% of death certificates were obtained. The study included

Table 2.1 Selected results (estimated relative risks) of cohort studies on the association between cancer and exposure to trichloroethylene

Reference, study location	Cancer							
	Kidney	Non-Hodgkin lymphoma	Leukaemia	Combined haematological	Liver/biliary	Lung	Bladder	Cervix
Europe								
Axelson *et al.* (1994) Sweden	1.16 (0.42–2.52)	1.56 (0.51–3.64)	NR	NR	1.41 (0.38–3.60)	0.69 (0.31–1.30)	1.02 (0.44–2.00)	NR
Anttila *et al.* (1995a) Finland	0.87 (0.32–1.89)	1.81 (0.78–3.56)	1.08 (0.35–2.53)	1.51 (0.92–2.33)	2.27 (0.74–5.29)	0.92 (0.59–1.35)	0.82 (0.27–1.90)	2.42 (1.05–4.77)
Henschler *et al.* (1995) Germany	7.97 (2.59–18.59)	NR	NR	[1.10 (0.01–77.3)]	NR	NR	NR	NR
Hansen *et al.* (2001) Denmark	0.9 (0.2–2.6)	3.5 (1.5–6.9)	1.9 (0.6–4.4)	NR	2.6 (0.8–6.0)	0.8 (0.5–1.3)	1.1 (0.5–2.0)	3.8 (1.0–9.8)
Raaschou-Nielsen *et al.* (2001) Denmark	[1.21 (0.95–1.52)]	[1.26 (1.02–1.53)]	[1.15 (0.91–1.42)]	NR	[1.35 (1.02–1.75)]	[1.43 (1.32–1.55)]	[1.06 (0.92–1.21)]	1.9 (1.42–2.37)
USA								
Garabrant *et al.* (1988) San Diego, USA	0.93 (0.48–1.64)	NR	0.82 (0.47–1.34)	0.78 (0.56–1.08)	0.94 (0.40–1.86)	0.80 (0.68–0.95)	1.26 (0.74–2.03)	NR
Greenland *et al.* (1994) Massachusetts, USA	0.99 (0.30–3.32)	0.76 (0.24–2.42)	1.10 (0.46–2.66)	NR	0.54 (0.11–2.63)	1.01 (0.69–1.47)	0.85 (0.32–2.23)	NR
Morgan *et al.* (1998) Arizona, USA	1.32 (0.57–2.60)	NR	1.05 (0.50–1.93)	0.99 (0.64–1.47)	0.98 (0.36–2.13)	1.10 (0.89–1.34)	1.36 (0.59–2.68)	0
Ritz (1999) Ohio, USA	0.65 (0.21–1.51)	NR	1.09 (0.56–1.91)	1.28 (0.90–1.77)	1.66 (0.71–3.26)	1.03 (0.85–1.24)	1.17 (0.50–2.31)	NR
Zhao *et al.* (2005)[c] California, USA	7.40 (0.47–116)[a]	0.20 (0.03–1.46)[b]	0.20 (0.03–1.46)[b]	5.15 (1.20–22.2)[a]	NR	3.10 (1.09–8.79)[a]	3.68 (0.87–15.5)[a]	NR

Table 2.1 (continued)

Reference, study location	Cancer							
	Kidney	Non-Hodgkin lymphoma	Leukaemia	Combined haematological	Liver/biliary	Lung	Bladder	Cervix
Boice *et al.* (2006)[c] California, USA	2.22 (0.89–4.57)	0.21 (0.01–1.18)	1.08 (0.35–2.53)	0.74 (0.34–1.40)	1.28 (0.35–3.27)	1.24 (0.92–1.63)	1.66 (0.54–3.87)	NR
Lipworth *et al.* (2011) California, USA	0.66 (0.38–1.07)	1.31 (0.97–1.73)	0.88 (0.61–1.23)	NR	0.89 (0.57–1.33)	0.80 (0.71–0.90)	1.03 (0.72–1.43)	0
Radican *et al.* (2008) Utah, USA	1.18 (0.47–2.94)	1.36 (0.77–2.39)	0.64 (0.35–1.18)	1.06 (0.75–1.51)	1.25 (0.31–4.97)	0.83 (0.63–1.08)	0.80 (0.41–1.58)	1.67 (0.54–5.22)
Bahr *et al.* (2011) Kentucky, USA	0 cases	1.49 (1.02–2.10)	1.40 (0.46–4.24)	NR	0.43 (0.10–1.84)	0.75 (0.72–0.79)	NR	NR

[a] High exposure; 20-year lag; cancer incidence
[b] Non-Hodgkin lymphoma plus leukaemia combined
[c] Study populations overlap
NR, not reported

Table 2.2 Cohort studies of workers exposed to trichloroethylene

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Shindell & Ulrich (1985) Illinois, USA 1957–83	2646 employees	Not specific. Workers from a factory using trichloroethylene as a degreasing agent	All cancers		21	[0.85 (0.53–1.30)]	Age and calendar time Only SMR for overall cancer
			Respiratory cancer		9	[0.75 (0.34–1.42)]	
			Non-respiratory cancer		12	[0.49 (0.26–0.86)]	
Garabrant *et al.* (1988) San Diego, USA 1958–82	14 067 (11 898 men, 2169 women)	Not specific. Follow-up of aircraft-manufacturing workers employed ≥ 4 yr.	All causes mortality	Overall	1804	0.75 (0.72–0.79)	Age, sex, race, calendar year No information on individual exposure to trichloroethylene; 37% of jobs involved exposure to trichloroethylene. Expected numbers based on USA rates. Trend towards increasing mortality from cancer of the oesophagus, pancreas, and bladder with increasing duration of employment.
			All cancer deaths	Overall	453	0.84 (0.77–0.93)	
			Oesophagus	Overall	14	1.14 (0.62–1.92)	
			Liver and biliary tract	Overall	8	0.94 (0.40–1.86)	
			Breast	Overall	16	0.91 (0.52–1.48)	
			Cervix	Overall	ND	–	
			Kidney	Overall	12	0.93 (0.48–1.64)	
			Haematological	Overall	38	0.78 (0.56–1.08)	
			NHL	Overall	ND	–	
			Leukaemia	Overall	16	0.82 (0.47–1.34)	
			Lung	Overall	138	0.80 (0.68–0.95)	
			Bladder & urinary organs	Overall	17	1.26 (0.74–2.03)	
Axelson *et al.* (1994) Sweden 1958–87	1670 (1421 men, 249 women aged ≤ 79 yr)	Individual urinary measurement of TCA (trichloroethylene metabolite)	All cancers	Men	107	0.96 (0.80–1.16)	Age, calendar time SIR
			All cancers	Women	22	1.32 (0.85–1.99)	
			Liver	Men	4	1.41 (0.38–3.60)	
			Kidney	Men	6	1.16 (0.42–2.52)	
			NHL	Men	5	1.56 (0.51–3.64)	
			Lung	Men	9	0.69 (0.31–1.30)	
			Bladder	Men	8	1.02 (0.44–2.00)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Greenland *et al.* (1994) Massachusetts, USA 1969–84	512 cases, 1202 controls	JEM	Kidney		ND	0.99 (0.30–3.32)	Nested case–control study. White men only.
			Liver/biliary tract		ND	0.54 (0.11–2.63)	
			Lymphoma		ND	0.76 (0.24–2.42)	
			Leukaemia		ND	1.10 (0.46–2.66)	
			Lung		ND	1.01 (0.69–1.47)	
			Bladder		ND	0.85 (0.32–2.23)	
Anttila *et al.* (1995a) Finland 1967–92	1698 men, 1391 women	Individual measurement of urinary concentration of TCA (trichloroethylene metabolite)	All cancers	Both sexes	208	1.05 (0.92–1.20)	Age, calendar time SIR
				Years since first measurement:			
				0–9 yr	65	1.07 (0.82–1.36)	
				10–19 yr	83	0.84 (0.67–1.04)	
				≥ 20 yr	60	1.57 (1.20–2.02)	
				< 100 µmol/L	127	1.17 (0.98–1.38)	
				≥ 100 µmol/L	63	0.97 (0.74–1.23)	
			Liver	Both sexes	5	2.27 (0.74–5.29)	
				Years since first measurement:	0		
				0–9 yr	0		
				10–19 yr	2	1.74 (0.21–6.29)	
				≥ 20 yr	3	6.07 (1.25–17.7)	
				< 100 µmol/L	2	1.64 (0.20–5.92)	
				≥ 100 µmol/L	2	2.74 (0.33–9.88)	
			Cervix	Women	8	2.42 (1.05–4.77)	
				Years since first measurement:			
				0–9 yr	6	3.39 (1.24–7.38)	
				10–19 yr	1	0.84 (0.02–4.67)	
				≥ 20 yr	1	2.89 (0.07–16.1)	
				< 100 µmol/L	3	1.86 (0.38–5.45)	
				≥ 100 µmol/L	5	4.35 (1.41–10.1)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Anttila *et al.* (1995a) Finland 1967–92 (cont.)			Kidney	Both sexes	6	0.87 (0.32–1.89)	
				Years since first measurement:			
				0–9 yr	1	0.53 (0.01–2.95)	
				10–19 yr	5	1.39 (0.45–3.24)	
				≥ 20 yr	0		
			Haematological	Both sexes	20	1.51 (0.92–2.33)	
			NHL	Both sexes	8	1.81 (0.78–3.56)	
				Years since first measurement:			
				0–9 yr	1	0.83 (0.02–4.64)	
				10–19 yr	4	1.75 (0.48–4.47)	
				≥ 20 yr	3	3.24 (0.67–9.45)	
				< 100 µmol/L	5	2.01 (0.65–4.69)	
				≥ 100 µmol/L	2	1.40 (0.17–5.04)	
			Leukaemia	Both sexes	5	1.08 (0.35–2.53)	
				Years since first measurement:			
				0–9 yr	3	1.76 (0.36–5.16)	
				10–19 yr	0		
				≥ 20 yr	2	2.72 (0.33–9.83)	
				< 100 µmol/L	1	0.39 (0.01–2.19)	
				≥ 100 µmol/L	4	2.65 (0.72–6.78)	
			Lung		25	0.92 (0.59–1.35)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Henschler *et al.*(1995) 1956–92	169	Exposure to trichloroethylene during cleaning and degreasing at a cardboard factory. Exposure in the cardboard-machine area is considered high. Exposure was lower in the locksmiths' area and in the electrical workshop. Trichloroethylene was also regularly used to clean floors, work-clothes and hands.	All cancers		15	[0.87 (0.40–1.92)]	Adjusted for age. Further, age and calendar time rates from Denmark and East Germany were applied Probably cluster data Only data for kidney and haematological cancers
			Kidney		5	7.97 (2.59–18.59)	
			Haematological		1	[1.10 (0.01–77.3)]	
Morgan *et al.* (1998) Arizona, USA 1950–93	2555 men and 2178 women exposed to trichloroethylene	Company employees with ≥ 30 yr of experience rated exposure for each job, and a company industrial hygienist compiled ratings into a four-level JEM.	All cancers	Overall	270	0.92 (0.81–1.03)	Age, sex, calendar year SMR. Expected numbers based on national rates
				Low exposure	114	1.04 (0.86–1.25)	
				High exposure	156	0.84 (0.71–0.98)	
			Liver and biliary tract	Overall	6	0.98 (0.36–2.13)	
				Low exposure	1	0.49 (0.01–2.74)	
				High exposure	5	1.38 (0.45–3.21)	
			Liver		ND		
			Biliary tract		ND		
			Breast	Overall	16	0.75 (0.43–1.22)	
				Low exposure	11	1.03 (0.51–1.84)	
				High exposure	5	0.47 (0.015–1.11)	
			Cervix		0	0	
			Kidney	Overall	8	1.32 (0.57–2.60)	
				Low exposure	1	0.47 (0.01–2.62)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Morgan *et al.* (1998) Arizona, USA 1950–93 (cont.)				High exposure	7	1.78 (0.72–3.66)	
			Haematological	Overall	25	0.99 (0.64–1.47)	
				Low exposure	10	1.07 (0.51–1.96)	
				High exposure	15	0.95 (0.53–1.57)	
			Non-Hodgkin lymphoma		ND	–	
			Leukaemia	Overall	10	1.05 (0.50–1.93)	
				Low exposure	3	0.85 (0.17–2.47)	
				High exposure	7	1.17 (0.47–2.41)	
			Lung		97	1.10 (0.89–1.34)	
			Emphysema		15	1.36 (0.76–2.23)	
Ritz (1999) 1951–89 Ohio, USA	3814 white men	Job title and plant areas based historical exposure to chemical assessed by plant experts. Workers were classified into four categories of exposure, from none to heavy.	All cancers		328	1.10 (0.99–1.23)	Age, time since first hire, pay type, radiation dose Initiated to look at radiation effects.
			Liver		8	1.66 (0.71–3.26)	
				Light, > 5 yr	3	1.90 (0.35–10.3)	
				Medium, > 5 yr	1	8.82 (0.79–98.6)	
			Kidney		5	0.65 (0.21–1.51)	
			Haematological		37	1.28 (0.90–1.77)	
				Light, > 5 yr	15	1.85 (0.87–3.95)	
			NHL		ND	–	
			Leukaemia and aleukaemia		12	1.09 (0.56–1.91)	
			Lung		112	1.03 (0.85–1.24)	
			Emphysema		3	0.21 (0.04–0.61)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Hansen *et al.* (2001) Denmark 1968–96	658 men, 145 women	Individual measurement of urinary trichloroethylene metabolite	All cancers	Men	109	1.0 (0.9–1.3)	Age and calendar time SIR. For cervical cancer, an association was seen with earlier period of first employment. Overlapped with Raaschou-Nielsen *et al.* (2001, 2002).
			Oesophagus	Men	6	4.2 (1.5–9.2)	
			Liver & biliary tract	Men	5	2.6 (0.8–6.0)	
			Breast	Women	4	0.9 (0.2–2.3)	
			Cervix	Women	4	3.8 (1.0–9.8)	
			Kidney	Both sexes	[4]	[1.1 (0.3–2.8)]	
			NHL	Men	8	3.5 (1.5–6.9)	
			Leukaemia	Men	5	1.9 (0.6–4.4)	
			Lung	Men	16	0.8 (0.5–1.3)	
Zhao *et al.* (2005) California, USA 1950–2001	5049 for cancer incidence; 6044 men for total mortality	A JEM for potentially carcinogenic exposures, including job titles and periods was constructed based on extensive industrial-hygiene reviews	All cancers		ND	–	Considerable overlap with the study by Boice et al. (2006). RR for cancer incidence reported Time since first employment, SES, age at event
			Liver		ND	–	
			Kidney	Low	6	1.0 (ref)	
				Medium	6	1.87 (0.56–6.20)	
				High	4	4.90 (1.23–19.6)	
			P for trend			0.023	
			NHL and leukaemia	Low	28	1.0 (ref)	
				Medium	16	0.88 (0.47–1.65)	
				High	1	0.20 (0.03–1.46)	
			P for trend			0.097	
			Bladder	Low	20	1.0 (ref)	
				Medium	19	1.54 (0.81–2.92)	
				High	11	1.98 (0.93–4.22)	
			P for trend			0.069	
			Lung	High (mortality)	33	1.02 (0.68–1.53)	
				High (incidence)	14	1.11 (0.60–2.06)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Boice *et al.* (2006) California, USA 1948–99	8372 workers	Individual job titles were collapsed into categories and designated as administrative/ scientific or non-administrative. Test-stand mechanics and technicians were considered as having the greatest likelihood of exposure to trichloroethylene.	All cancers	Overall	121	1.00 (0.83–1.19)	SMR. Overlapped with Zhao et al. (2005). Use of trichloroethylene was discontinued in cleaning of (i) engines (middle to late 1960s); and (ii) small metal parts (1974). SMRs for < 5 yr or > 5 yr employment were above 1, but not statistically significant for kidney cancer.
			Oesophagus	Overall	3	0.88 (0.18–2.58)	
			Liver & biliary tract	Overall	4	1.28 (0.35–3.27)	
			Kidney	Overall	7	2.22 (0.89–4.57)	
			Haematological	Overall	9	0.74 (0.34–1.40)	
			NHL	Overall	1	0.21 (0.01–1.18)	
			Leukaemia	Overall	5	1.08 (0.35–2.53)	
			Lung	Overall	51	1.24 (0.92–1.63)	
			Emphysema	Overall	5	0.90 (0.29–2.11)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Radican *et al.* (2008) Utah, USA 1952–2000	14 455	Individual exposure to 21 solvents (including trichloroethylene) and chemicals was assessed based on different exposure surrogates (historical records, walk-through surveys and interviews of long-term employees). For trichloroethylene only, frequency and exposure patterns (intermittent and continuous) were assessed based on tasks and a cumulative exposure score was calculated	All cancer deaths	Overall	854	1.03 (0.91–1.17)	Age, race, sex HR reported. No significantly increased risks. Highest category of exposure generally had the highest risk.
			Emphysema		59	0.90 (0.56–1.44)	
			Lung		166	0.83 (0.63–1.08)	
			Oesophagus	Overall	17	1.88 (0.61–5.79)	
			Liver/biliary tract	Overall	31	1.12 (0.57–2.19)	
			Liver	Overall	8	1.25 (0.31–4.97)	
				Lowest tertile, men	4	3.28 (0.37–29.45)	
				Highest tertile, men	4	4.05 (0.45–36.41)	
			Breast	Overall	26	1.23 (0.73–2.06)	
			Cervix	Overall	6	1.67 (0.54–5.22)	
			Kidney	Overall	18	1.18 (0.47–2.94)	
				Lowest tertile, men	10	1.87 (0.59–5.97)	
				Second tertile, men	1	0.31 (0.03–2.75)	
				Highest tertile, men	5	1.16 (0.31–4.32)	
			Haematological	Overall	106	1.06 (0.75–1.51)	
				Lowest tertile, men	34	1.04 (0.63–1.74)	
				Second tertile, men	21	1.06 (0.59–1.88)	
				Highest tertile, men	33	1.25 (0.75–2.09)	
			NHL	Overall	46	1.36 (0.77–2.39)	
				Lowest tertile, men	18	1.83 (0.79–4.21)	
				Second tertile, men	7	1.17 (0.42–3.24)	
				Highest tertile, men	12	1.50 (0.61–3.69)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Radican *et al.* (2008) Utah, USA 1952–2000 (cont.)			Leukaemia	Overall	27	0.64 (0.35–1.18)	
			Bladder	Overall	25	0.80 (0.41–1.58)	
Bahr *et al.* (2011) 1952–2003	6820 male workers	JEM based on discussions with current and past employees at the plant. Each job was ranked by likelihood of short-term exposure to trichloroethylene in four categories.	All cancer	Overall	146	1.08 (0.79–1.48)	Age and calendar time
			Oesophagus		ND	–	
			Liver and biliary tract	Overall	ND	0.43 (0.10–1.84)	
				Category 2	ND	0.34 (0.05–2.07)	
				Category 3	ND	0.39 (0.08–1.94)	
			Kidney		0	–	Surprisingly low number. Approximately same number as for NHL expected
			NHL	Overall	32	1.49 (1.02–2.10)	
				Category 2	ND	1.31 (0.47–3.65)	
				Category 3	ND	0.75 (0.27–2.12)	
			Leukaemia	Overall	24	1.40 (0.46–4.24)	
				Category 2	ND	0.73 (0.15–3.45)	
				Category 3	ND	1.89 (0.61–5.86)	
			Lung		146	0.75 (0.72–0.79)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Lipworth *et al.* (2011) California, USA 1960–2008	5443 workers	Job history from company records; 784 job codes were categorized into eight groups with similar factory-work activities, assessed by walk-through survey. On this basis, individuals were classified for exposure to trichloroethylene, tetrachloroethylene, mixed solvents and chromates (routine, intermittent, no likely exposure).	All cancers	Overall	986	0.92 (0.86–0.97)	Common exposure to other chemicals, including tetrachloroethylene Overlapped with Boice et al. (1999). Trend in increasing risk with increasing duration of exposure, this was statistically significant only for lung cancer ($P < 0.01$). Trichloroethylene was the primary organic solvent used for vapour degreasing until 1966, when it was replaced by tetrachloroethylene.
			Oesophagus	Overall	19	0.65 (0.39–1.01)	
				0	30	1.00 (ref)	
				< 1	7	0.53 (0.22–1.24)	
				1–4 yr	5	0.62 (0.23–1.63)	
				≥ 5 yr	7	0.77 (0.32–1.86)	
			Liver and biliary tract		24	0.89 (0.57–1.33)	
			Liver	Overall	ND	–	
				0	32	1.00 (ref)	
				< 1	10	0.67 (0.32–1.42)	
				1–4 yr	6	0.69 (0.28–1.71)	
				≥ 5 yr	8	0.83 (0.36–1.91)	
			Biliary tract	Overall	ND	–	
			Breast	Overall	12	1.03 (0.53–1.80)	
				0	61	1.00 (ref)	
				< 1	6	0.82 (0.34–1.98)	
				1–4 yr	1	0.31 (0.04–2.32)	
				≥ 5 yr	4	1.47 (0.50–4.32)	
			Cervix	Overall	0	[1.3 expected]	
			Kidney	Overall	16	0.66 (0.38–1.07)	
				0	33	1.00 (ref)	
				< 1	6	0.52 (0.21–1.30)	
				1–4 yr	3	0.42 (0.13–1.42)	
				≥ 5 yr	6	0.85 (0.33–2.19)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Lipworth *et al.* (2011) California, USA 1960–2008 (cont.)			Haematological	Overall			
			NHL	Overall	50	1.31 (0.97–1.73)	
				0	50	1.00 (ref)	
				< 1	18	0.84 (0.48–1.47)	
				1–4 yr	14	1.10 (0.59–2.04)	
				≥ 5 yr	15	1.02 (0.55–1.90)	
			Leukaemia	Overall	23	0.88 (0.61–1.23)	
			Lung		267	0.80 (0.71–0.90)	

DCA, dichloroacetic acid; HR, hazard ratio; JEM, job-exposure matrix; ND, no data; NHL, non-Hodgkin lymphoma; ref., reference; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TCA, trichloroacetate; yr, year

222 100 person-years of follow-up and 453 cancer deaths. The calculated standardized mortality ratio (SMR) for all causes and cancer overall was significantly decreased. No significant increase in standardized mortality ratio was seen for any cancer. The standardized mortality ratio for cancer of the lung was 0.80 (95% CI, 0.68–0.95) and there were significant deficits for cancers of the stomach and larynx. Deficits also appeared from diabetes mellitus, alcoholism, diseases of the nervous system, the circulatory system and the genitourinary system, as well as accidental and violent death. [The deficits indicated a strong healthy-worker effect and/or a strong selection of healthy workers for follow-up. The Working Group also noted that a relatively small proportion of workers was exposed to trichloroethylene in this study and therefore this study had a low impact on evaluate human carcinogenicity after exposure to trichloroethylene.]

Radican *et al*. extended follow-up of a cohort of civilian aircraft-maintenance workers from the Hill Air Force Base in Utah, USA, until 2000 (Spirtas *et al*., 1991; Blair *et al*., 1998; Radican *et al*., 2008). The cohort was composed of 14 444 people (10 730 men and 3725 women) employed for at least 1 year between 1952 and 1956 and identified from personnel records from the facility. Many different chemical exposures, including to other potential carcinogens, occurred at the facility. The methods for assessment of exposure within this cohort have been described previously (Stewart *et al*., 1991). Personal and area samples were available for some chemicals, including trichloroethylene (Stewart *et al*., 1991). Individual exposures for trichloroethylene, other solvents and chemicals were estimated based on job history, historical records, walk-through surveys and interviews of long-term employees and traditional monitoring results. Dichotomous exposure assessment was done for 21 solvents and chemicals. For trichloroethylene only, frequency and exposure patterns (intermittent and continuous) were assessed based on information on job tasks. Trichloroethylene levels in the degreasers was used until 1968 when trichloroethylene was replaced by 1,1,1-trichloroethane (Stewart *et al*., 1991). The National Death Index was used to assess the cause of death on the most recent follow-up. As of 31 December 2000, 8580 cohort members (68.1%) had died. The Cox model hazard ratio (HR) for all cancers was 1.03 (95% CI, 0.91–1.17; 854 deaths). No significantly increased hazard ratio appeared for any specific cancer in either men or women. Among the men potentially exposed to trichloroethylene compared with unexposed workers, the hazard ratios were 1.24 (95% CI, 0.41–3.71) for cancer of the kidney, 1.36 (95% CI, 0.59–3.11) for cancer of the biliary passage and liver, 1.12 (95% CI, 0.72–1.73) for lymphatic or haematopoietic cancer, and 1.66 (95% CI, 0.48–5.74) for cancer of the oesophagus. In women, the hazard ratios were 1.23 (95% CI, 0.73–2.06) for cancer of the breast and 1.67 (95% CI, 0.54–5.22) for cancer of the cervix. All the dose–response gradients for estimated exposure to trichloroethylene were relatively flat and based on small numbers. The hazard ratio for cancer of the lung was 0.83 (95% CI, 0.63–1.08). [The major strength of the study was the unusually long follow-up time, accounting for deaths of more than 68% of cohort members, and qualitative information on level of exposure to trichloroethylene].

Morgan *et al*. (1998) conducted a cohort study of mortality in 20 508 workers at Hughes Aircraft Manufacture, Arizona (Morgan *et al*., 1998) of whom 4733 (2555 men, 2178 women) were potentially exposed to trichloroethylene (1952–57). Study participants were identified from company records, and were eligible for inclusion in the study if they had been employed for at least 6 months between 1950 and 1985. Vital status was determined through the Social Security Administration and the National Death Index. Exposure to trichloroethylene was assessed by a four-level job-exposure matrix. In total, 4052 deaths occurred based on 105 852

person-years during 1950–93. The standardized mortality ratios for overall cancer were less than 1 for the entire cohort (SMR, 0.87; 95% CI, 0.82–0.92) and for the trichloroethylene-exposed subcohort (23% of workers) (SMR, 0.92; 95% CI, 0.81–1.03). The standardized mortality ratios were not significantly increased for any of the following cancers: kidney (SMR, 1.32; 95% CI, 0.57–2.6), lymphatic and haematological tissue (SMR, 0.99; 95% CI, 0.64–1.47), biliary passage and liver (SMR, 0.98; 95% CI, 0.36–2.13); breast (SMR, 0.75; 95% CI, 0.43–1.22), and lung (SMR, 1.10; 95% CI, 0.89–1.34). In an internal cohort analysis, the relative risk for cancer of the kidney was 1.89 (95% CI, 0.85–4.23; eight cancer deaths) for those workers with medium/high peak exposure versus those with none/low exposure. The internal comparison group minimizes concerns over the healthy-worker effect when an external comparison group is used. [The long-term follow-up was a strength of the study].

Zhao *et al.* reported a follow-up for mortality of 6044 selected male Rocketdyne workers from the aerospace division of the Santa Susana Field Laboratory in California, USA (Zhao *et al.*, 2005). The study was based on a previously established cohort in a study that focused on exposure to hydrazine and mortality (Ritz *et al.*, 1999). The source population was 55 000 Rockwell/Rocketdyne workers (now Boeing North America) at several facilities in the Los Angeles area, California, USA (Zhao *et al.*, 2005). Workers had been employed at least 2 years before 1980, and had never been monitored for exposure to radiation, indicating that they had never been employed at the nuclear facility. All employee records were matched with multiple sources to obtain information on vital status, and date of death. Underlying cause of death was coded by a licensed nosologist. Standardized mortality ratios for death during 1950–2001 were calculated. A subcohort of 5049 of these workers was followed up for cancer incidence. The cohort was matched against the Californian cancer registry and first primary incident cancer in 1988–2000 was identified. Cancer data were also retrieved from eight other state cancer registries to identify incident cancers for some of the cohort members who had left California during follow-up. A job-exposure matrix for potentially carcinogenic exposures, using job titles, was constructed based on extensive industrial hygiene reviews. Individual personnel records for all cohort members included job title and period engaged in each job. Each job was assigned to a category of cumulative exposure: none, low, medium, or high. Information on tobacco smoking was obtained from a small subset of 200 study participants. Proportional hazard modelling was used to estimate site-specific relative risks for cancer incidence and mortality. In the entire mortality cohort, 2117 (35%) had died by the end of 2001, and 600 people had died from cancer. Estimated medium or high cumulative exposure to trichloroethylene was positively associated with incidence of cancer of the kidney compared with low cumulative exposure (medium exposure: HR, 1.87; 95% CI, 0.56–6.20; high exposure: HR, 4.90; 95% CI, 1.23–19.6; *P* for trend, 0.023). Results were similar when further adjusted for all other carcinogens, including hydrazine (medium exposure: HR, 1.26; 95% CI, 0.26–1.14; high exposure: HR, 7.61; 95% CI, 0.65–9.14). For mortality from cancer of the kidney, the estimated relative risks were lower (medium exposure: HR, 1.43; 95% CI, 0.49–4.16; high exposure: HR, 2.03; 95% CI, 0.50–8.32). No significant associations were observed between estimated exposure to trichloroethylene and any other cancers. The relative risk of cancer of the lung was around 1, with the rate ratio for mortality being 1.02 (95% CI, 0.68–1.53) and for incidence, 1.11 (95% CI, 0.60–2.06). It was estimated that 40.7% of the trichloroethylene-exposed workers were smokers. [The Working Group noted that confounding by smoking was unlikely. The major strength of the study was the follow-up for mortality of more than 50 years,

and follow-up for cancer incidence since 1988. There was considerable overlap between this study and that of Boice *et al.* (2006), described below.]

The previous study by (Zhao *et al.*, 2005) overlapped considerably with a study of 8372 Rocketdyne workers (7083 men and 1289 women) employed for at least half a year in 1948–99; however, no information on cancer incidence was reported (Boice *et al.*, 2006). Study subjects were retrospectively identified from different overlapping sources, and information included name, date of birth, date of first employment, and termination, and job history. Information on death and vital status was retrieved from multiple sources. The cause of death was coded by a trained nosologist. The overall standard mortality ratio for any cancer in the trichloroethylene-exposed group was 1.00 (95% CI, 0.83–1.19). Similarily, the standard mortality ratio was 2.22 (95% CI, 0.89–4.57) for cancer of the kidney, 0.21 (95% CI, 0.01–1.18) for non-Hodgkin lymphoma, 1.28 (95% CI, 0.35–3.27) for cancer of the liver and biliary passage, and 1.24 (95% CI, 0.92–1.63) for cancer of the lung. The relative risk of kidney cancer associated with any potential exposure to trichloroethylene compared with never-exposed by duration of work as test-stand mechanic (considered to be the job with most intensive exposure) by internal comparison was 1.21 (95% CI, 0.33–4.35) for 0 test-years, 2.51 (95% CI, 0.27–23.5) for < 4 test-years and 3.13 (95% CI, 0.74–13.2) for ≥ 4 test-years.

Cancer mortality has been evaluated at the Lockeed Martin aircraft-manufacturing facility in Burbank, California, USA (Boice *et al.*, 1999; Lipworth *et al.*, 2011). In total, 77 943 workers employed on or after 1 January 1960 for at least 1 year were identified. Of these, 32 625 workers (41.9%) were employed in non-factory positions and had no chemical exposure. Each factory worker was classified as having routine, intermittent or no likely exposure to trichloroethylene, tetrachloroethylene, mixed solvents, or chromates, based on a procedure to estimate historical exposure (Marano *et al.*, 2000). Trichloroethylene was the primary organic solvent used for vapour degreasing until 1966, when it was replaced by tetrachloroethylene. [The proportion of workers involved in vapour degreasing was not available from the paper]. Deaths occurring between 1960 and 2008 (n = 34 298; 44%) were identified by several different methods, and coded by a trained nosologist. Expected numbers of deaths were based on race, age by calendar year, and sex-specific rates in the general population of California for white workers, and from the general population of the USA for non-white workers. In addition to calculation of standardized mortality ratios, relative risks of death from cancer were estimated for internal comparisons among factory workers' duration of potential exposure (0, < 1, 1–4, > 5 years) to trichloroethylene, tetrachloroethylene, mixed solvents, or chromates. The reference group for internal comparison consisted of 9520 factory workers considered to have no exposure to organic solvents or chromates. For factory workers potentially exposed to trichloroethylene, the standardized mortality ratio for overall cancer was 0.92 (95% CI, 0.86–0.97) based on 1 435 459 person-years. No significant increase was observed for any cancer. The standardized mortality ratio for cancer of bronchus, trachea and lung was significantly decreased (0.80; 95% CI, 0.71–0.90). The standardized mortality ratios for other cancers were: 0.66 (95% CI, 0.38–1.07) for kidney, 1.31 (95% CI, 0.97–1.73) for non-Hodgkin lymphoma, 0.89 (95% CI, 0.57–1.33) for biliary passage and liver, 0.65 (95% CI, 0.39–1.01) for oesophagus, 1.03 (95% CI, 0.53–1.80) for breast, and 0.00 (95% CI, 0.00–2.87) [1.3 expected] for cervix uteri,. No indication of increasing relative risk with increasing duration of potential exposure to trichloroethylene was seen in the internal cohort comparisons (P for trend, > 0.05), although the highest risk was seen in the highest exposure category for some cancer sites, including the kidney, liver, oesophagus

and prostate. [Overall, the results showed some weak but statistically significant decreases in standardized mortality ratios for cerebrovascular disease, nonmalignant respiratory disease, cirrhosis of the liver, all external causes of death, accidents, and suicides, indicating an healthy-worker effect. The indications of healthy-worker effect, and hence potential selection bias, limited the utility of this cohort. Furthermore, despite the long-term follow-up from a large cohort, the number of cases for most cancer diagnoses was relatively small. Finally, the period of exposure to trichloroethylene for vapour degreasers may have been relatively short for an unknown proportion of workers because exposure stopped in 1966].

2.2.3 Biological monitoring of trichloroacetic acid

In Sweden, Finland, and Denmark, routine urinary measurements of the main metabolite of trichloroethylene, trichloroacetic acid, have been conducted independently for several decades, and workers with such measurements have been linked to nationwide cancer registries (Axelson *et al.*, 1994; Anttila *et al.*, 1995a; Hansen *et al.*, 2001).

The study from Sweden is based on historical laboratory files from workers enrolled in a monitoring programme for trichloroacetic acid, offered to costume companies by the Swedish producer of trichloroethylene (Axelson *et al.*, 1994). The study was an update and expansion of a previous study (Axelson *et al.*, 1978). In total, 1727 exposed workers from 115 different companies, which had used the surveillance service at least once between 1955 and 1975, were identified from the laboratory files (Axelson *et al.*, 1978). Some files from the early period were not available. After exclusion of subjects who could not be unequivocally identified or who had emigrated, the final cohort included 1421 men and 249 women. The majority (81%) of the male cohort members had a urinary concentration of trichloroacetic acid of < 50 mg/L, roughly corresponding to an average concentration trichloroethylene of 20 ppm, or 110 mg/m^3. Study subjects were followed-up for mortality from 1955 until 1986, and for incident cancers from 1958 until 1987. Observed numbers of deaths and incident cancers were compared with the expected numbers, calculated from those for the standardized population. In the mortality analysis, the male cohort provided 22 446 person-years of observation with an overall standardized mortality ratio of 0.97 (95% CI, 0.86–1.10). In men, the standardized mortality ratio for overall cancer mortality was 0.65 (95% CI, 0.47–0.89) and the standardized incidence ratio (SIR) for all incident cancers was 0.96 (95% CI, 0.80–1.16; 107 observed cases; 23 516 person-years). In men, only the standardized incidence ratio for skin cancer was significantly elevated (SIR, 2.36; 95% CI, 1.02–4.65) while the SIRs for other cancers were 1.16 (95% CI, 0.42–2.52) for cancer of the kidney, 1.41 (95% CI, 0.38–3.60) for cancer of the liver, 1.56 (95% CI, 0.51–3.64) for non-Hodgkin lymphoma and 0.69 (95% CI, 0.31–1.30) for cancer of the lung. Overall mortality in women was significantly increased (SMR, 1.55; 95% CI, 1.02–2.31; n = 24). The standardized incidence ratio for overall cancer morbidity in women was 1.32 (95% CI, 0.85–1.99). Among the 22 observed cancers, 11 were cancers of the breast or the genital organs. There were no cases of cancer of the liver or lymphoma among the female members of the cohort. The standardized incidence ratio for cancer of the lung was not reported for women.

The study from Finland (Anttila *et al.*, 1995a, b), updated and expanded from a previous study (Tola *et al.*, 1980), was based on a database of workers who were monitored by the Finnish Institute of Occupational Health for urinary concentration of trichloroacetic acid after exposure to trichloroethylene between 1965 and 1982. The same database also included subjects monitored biologically for exposure to tetrachloroethylene and 1,1,1-trichloroethane, but for

more than 94% of the subjects, measurements were only recorded for one of the three organic solvents, usually trichloroethylene. The overall median concentration of urinary trichloroacetic acid was 10 mg/L for women and 8 mg/L for men, which indicated a low level of exposure, and lower than that in Sweden (Axelson *et al.*, 1994). There was a total of 11 534 measurements for all three organic solvents, and a single person could be identified for 3976 (> 93%) measurements. Of these, 3089 subjects (1698 men and 1391 women) had files with measurements of trichloroacetic acid in urine, with an average of 2.5 measurements per subject. Study subjects were followed up for mortality from 1965 until 1991 in the files of the Central Statistical Office of Finland, and for 1967 until 1992 in the files of the Finnish Cancer Registry. The standardized mortality ratios and incidence ratios were based on Finnish national rates. The overall mortality was close to the expected value. During the maximum of 26 years of follow-up for cancer among the entire cohort of workers exposed to organic solvents, a total of 31 552 person-years among men and 28 353 among women exposed to trichloroethylene contributed to the 237 observed cancers (112 men, 125 women). The standardized incidence ratio for entire cohort exposed to the three organic solvents (both sexes combined) was 1.04 (95% CI, 0.91–1.17), which was similar to that for workers exposed to only trichloroethylene (SIR, 1.05; 95% CI, 0.92–1.20). Only the standardized incidence ratio for cancer of the cervix was significantly increased (SIR, 2.42; 95% CI, 1.05–4.77). The standardized incidence ratios were 0.87 (95% CI, 0.32–1.89) for cancer of the kidney, 2.27 (95% CI, 0.74–5.29) for cancer of the liver, 1.81 (95% CI, 0.78–3.56) for non-Hodgkin lymphoma and 0.92 (95% CI, 0.59–1.35) for cancer of the lung.

The Danish cohort study (Hansen *et al.*, 2001; Raaschou-Nielsen *et al.*, 2001, 2002) was based primarily on information from files on measurements of urinary trichloroacetic acid performed by the Danish Institute of Occupational Health between 1947 and 1989 (Hansen *et al.*, 2001). A total of 2397 samples from workers at 275 companies were analysed, with an average of 2.2 measurements of urinary trichloroacetic acid available per worker. For the entire period, the mean and median values were 40 mg/L and 15 mg/L, respectively.These values were higher than the corresponding values in the study in Finland, and similar to those in the study in Sweden. Concentrations tended to decrease between 1947 and 1989. In addition, files for a total of 472 measurements of trichloroethylene in individual breathing-zone samples were available from 81 companies for 1974–89. The mean air trichloroacetic acid measurement level was 101 mg/m^3. A total of 658 men and 145 women, born between 1901 and 1979, were identified as exposed to trichloroethylene from the urinary trichloroacetic acid and the breathing-zone measurement files. Only persons alive as of 1 April 1968, when a unique personal identification number for each Danish resident was introduced, could be identified. Individuals could be identified for 64% of the trichloroacetic-acid files, and for 52% of the air-measurement files. These people were followed-up in the files of the Danish Cancer registry from 1968 until 1996, or from the first day of measurement, if later. A total of 109 men and 19 women with incident cancers were identified based on 13 796 and 2934 person-years, respectively. Standardized incidence ratios were based on reference data from the general Danish population. Standardized incidence ratios were reported and were 1.0 for all cancers combined for men and women, 1.1 (95% CI, 0.3–2.7) for cancer of the kidney for both sexes combined, 4.2 (95% CI, 1.5–9.2) for cancer of the oesophagus in men, 3.5 (95% CI, 1.5–6.9) for non-Hodgkin lymphoma, 2.6 (95% CI, 0.8–6.0) for cancer of the liver and biliary passages in men, and 3.8 (95% CI, 1.0–9.8; 4 observed cases) for cancer of the cervix. The standardized incidence ratio for cancer of lung was 0.9 (95% CI, 0.5–1.3) in men and 0.7 (95% CI, 0.01–3.8) in women.

[An overall strength of the three relatively similar Nordic cohort studies was the confirmed individual exposure to trichloroethylene as documented by measurement of a trichloroethylene metabolite, trichloroacetic acid, in the urine. The studies had a long-term follow-up for cancer incidence from reliable nationwide cancer registries established in the 1940s and 1950s. The limitations were the relatively small numbers, the lack of information on duration of exposure, and that measurement of biomarkers at a single point of time may not reflect quantitative exposure in the past or in the future. Finally, workers with high exposures may have been removed from tasks involving trichloroethylene exposure, or local levels of exposure may have been lowered, since the main aim of the monitoring programme was to avoid high exposures.]

2.2.4 Other cohorts of workers exposed to trichloroethylene

Shindell & Ulrich conducted a cohort study of 2646 employees (2216 white men and 430 women) at a manufacturing plant using trichloroethylene as a degreasing agent almost exclusively throughout the study period from 1957 to 1983 in Illinois, USA (Shindell & Ulrich, 1985). Workers who had been employed for at least 3 months were included. At the end of follow-up (1983), 618 of the workers (23.4%) were still working at the plant. National mortality rates were used to calculate the expected numbers of deaths. Nine deaths from respiratory cancer were identified versus 12 expected [SMR, 0.75; 95% CI, 0.34–1.42]. A significant decrease in mortality from non-respiratory cancer was found [SMR, 0.49; 95% CI, 0.26–0.86]. [The cohort was young, and few study participants were deceased.]

Greenland *et al.* (1994) performed a nested case–control study in workers (white men only) employed at a large transformer-manufacturing plant in Massachusetts, USA, to address earlier reports of excess mortality from cancer in this population. Only workers employed at the facility before the end of 1984, who died between 1969 and 1984, and with an available job history were included (512 cases and 1202 controls, which were primarily deaths from cardiovascular disease). Interviews with long-term management employees selected for their historical knowledge of the plant operations were used to identify chemicals used in the plant and to build a job-exposure matrix. The case–control study focused on specific exposures with potential carcinogenic effects: pyranol, benzene, trichloroethylene, other organic solvents, machine fluids, asbestos, resin systems. Trichloroethylene was used from 1930 to 1977 as a degreasing agent. No significant increase in relative risk of mortality was found for men who had any exposure to trichloroethylene. An odds ratio (OR) of 1.64 (95% CI, 0.82–3.29) was observed for cancer of the pancreas, and 1.26 (95% CI, 0.51–3.08) for the group of oral, laryngeal and pharyngeal cancers. The odds ratios for other sites were 0.99 (95% CI, 0.30–3.32) for cancer of the kidney, 0.76 (95% CI, 0.24–2.42) for lymphoma, 0.54 (95% CI, 0.11–2.63) for cancer of the liver and 1.01 (95% CI, 0.69–1.47) for cancer of the lung. [The Working Group noted that although this was a nested case–control study, results for the cohort had never been published. Furthermore, the Working Group agreed with the authors of this study that the potential for bias associated with loss to follow-up and exposure misclassification was large.]

Henschler *et al.* describe a cohort of 169 male trichloroethylene-exposed cardboard-factory workers from Germany (Henschler *et al.*, 1995). Although other organic solvents had been used at the factory, use of these solvents relative to trichloroethylene was considered small. An unexposed group of 190 male workers from the same factory, matched on age and physical job activity, was established. No information on air concentrations was available and no personal biological measurements of trichloroethylene had been conducted. Based on interviews with long-term

employees, and walk-through surveys, workers were considered to have high and long-term exposure to trichloroethylene. Of the 183 male cardboard factory workers exposed to trichloroethylene for at least 1 year between 1956 and 1975 who were identified from individual employee's records, 14 (7.6%) could not be contacted or refused to participate at closing day of the study, 31 December 1992. Information on dates of employment, work tasks and occupational exposures, intake of diuretics, and smoking habits was obtained by questionnaire. Vital status was identified by individual tracing. Physical examination included abdominal sonography. Fifty people in the trichloroethylene-exposed group and 52 people in the control group died during the study period. In cases of cancer, the date of diagnosis was the date of surgery. All renal cell tumours were verified by histopathological examination. The latency period between first exposure to trichloroethylene and date of diagnosis of cancer of the kidney was between 18 and 34 years. Five men exposed to trichloroethylene were diagnosed with cancer of the kidney (four renal cell tumours, and one urothelial cancer of the renal pelvis). An additional two men exposed to trichloroethylene were diagnosed in 1993, the year after closing the study. No cases of cancer of the kidney occurred in the group of people who were not exposed to trichloroethylene. The standardized incidence ratio for cancer of the kidney ($n = 5$), using reference rates for Denmark, was 7.97 (95% CI, 2.59–18.59). [The small size of the cohort and the use of abdominal sonography may indicate a cluster study.]

Ritz studied patterns of cancer mortality in 3814 white male uranium-processing workers employed for at least three months at the Fernald Feed Materials Production Center in Fernald, Ohio, USA (Ritz, 1999). The facility produced uranium metal products, and workers were potentially exposed to several non-radioactive chemicals, including trichloroethylene and cutting fluids. The facility operated from 1951 to 1989, which also was the period of follow-up for mortality. Workers were identified from company rosters and personal records, which included information on employment duration. Exposure to trichloroethylene and other chemicals, including crude levels (none, light, medium, high) was based on a job and plant-area exposure matrix developed by experts having been employed in the long term at the plant in the late 1970s and early 1980s. Vital status was determined from the social security system until 1979, and afterwards from the national death index. Death certificate information was available for 1045 workers. Persons not identified as deceased were assumed to be alive at the end of follow-up. Expected number of deaths was calculated from the national mortality rates for white men and with the NIOSH-CORPS cohort (Zahm, 1992). Smoking history was available for a subsample of approximately 20% of the workers. Overall mortality in the cohort was lower than among white American men (SMR, 0.84; 95% CI, 0.79–0.90), while cancer mortality was slightly increased (SMR, 1.10; 95% CI, 0.99–1.23). Non-significantly increased standardized mortality ratios for liver and biliary tract cancer were observed for workers with more than 5 years of exposure to trichloroethylene: 1.90 (95% CI, 0.35–10.3) for light exposure and 8.82 (95% CI, 0.79–98.6) for medium exposure (no workers were in the category of high exposure). The standardized mortality ratio for haematopoietic and lymphopoietic cancer and light exposure (the only category with exposed cases) was 1.85 (95% CI, 0.87–3.95), and some increases in ratios for these cancers were seen with increasing duration, and when exposure was lagged. Internal comparison showed a non-significantly elevated relative risk of haematopoietic and lymphopoietic cancers (combined) for workers with more than 10 years of exposure to trichloroethylene (RR, 2.17; 95% CI, 0.88–5.33). [Results not reported for other cancers of primary interest.] The standardized mortality ratio for lung cancer was 1.03 (95%

CI, 0.85–1.24). Based on the sample of tobacco smokers, there was no clear association between patterns of tobacco smoking and general level of exposure to chemicals. [The Working Group noticed that the study was initiated to look at the effects of radiation. Interpretation of the results was limited by the small number of cases. Tobacco smoking was not likely to be a confounder because the risk of cancer of the lung was not increased in the cohort.]

Raaschou-Nielsen *et al.* established a retrospective cohort of 40 049 "blue-collar" workers from Danish companies with documented use of trichloroethylene (Raaschou-Nielsen *et al.*, 2003). Information on 457 companies using trichloroethylene came from historical records (1947–89) of measurements of trichloroacetic acid in urine (Raaschou-Nielsen *et al.*, 2001) or air (Raaschou-Nielsen *et al.*, 2002) performed by the Danish National Institute of Occupational Health, the Danish Product Registry, the files of a dry-cleaning survey, and the archives of the company that for decades had been the main supplier of trichloroethylene to companies in Denmark. In total, 110 companies each with more than 200 employees were excluded due to the low proportion of employees expected to be exposed to trichloroethylene. Using the unique company identification number, all employees at the remaining 347 companies, including their unique national identification number, were retrieved from the records of a national pension fund with compulsory membership since its establishment in 1964. For all 152 726 employees identified, information on vital status, including date of death, emigration or disappearance, and job title was retrieved from the Central Population Registry. Based on the job title, 40 049 "blue-collar" workers who had been employed for at least 3 months at the companies using trichloroethylene were included in the study. Each worker was followed up in the Danish Cancer Registry from 1968 to 1997. National cancer rates were used to calculate standardized incidence rates. During follow-up, men contributed 588 047 person-years, while women contributed 118 270 person-years. The overall standardized incidence rate for cancer was 1.08 (95% CI, 1.04–1.12) in men and 1.23 (95% CI, 1.14–1.33) in women. The individual standardized incidence rates were calculated for the following cancers: renal cell carcinoma, 1.2 (95% CI, 0.93–1.51) for men and 1.2 (95% CI, 0.53–2.44) for women; non-Hodgkin lymphoma, 1.2 (95% CI, 0.98–1.52) for men and 1.4 (95% CI, 0.73–2.34) for women; primary cancer of the liver, 1.1 (95% CI, 0.74–1.64) for men and 2.8 (95% CI, 1.13–5.8) for women; and cancer of the gallbladder and biliary passages, 1.1 (95% CI, 0.61–1.87) for men, and 2.8 (95% CI, 1.28–5.34) for women. The standardized incidence rate for cancer of the cervix was significantly increased (SIR, 1.9; 95% CI, 1.42–2.37). For cancer of the oesophagus, the standardized incidence rate was 1.1 (95% CI, 0.81–1.53) in men, and 2.0 (95% CI, 0.54–5.16) in women. The standardized incidence ratio for oesophageal adenocarcinoma was 1.8 (95% CI, 1.2–2.7) in men, while there were no cases in women. The risk of cancer of the lung was significantly increased in both men and women, (SIR, 1.4; 95% CI, 1.28–1.51; and SIR, 1.9; 95% CI, 1.48–2.35, respectively). Standardized incidence rates for renal cell carcinoma and for non-Hodgkin lymphoma tended to increase with duration of employment and by employment before 1970 versus later. For cancer of the cervix, the highest risk was seen for women with initial potential exposure before 1970 (SIR, 2.4; 95% CI, 1.6–3.4) and with less than 1 year of potential exposure (SIR, 2.5; 95% CI, 1.7–3.5). [There may have been a bias in social selection because the risk for blue-collar workers was compared with that for the general population, which is a mixture of blue- and white-collar workers. Furthermore, uncontrolled confounding from tobacco smoking may have occurred, as indicated by the excess risk of cancer of the lung, particularly in women. This study population

partially overlapped with that of Hansen *et al.* (2001).]

Bahr *et al.* described a cohort of 6820 workers (90% white) from the Paducah gaseous-diffusion plant, Kentucky, USA, an uranium-enrichment plant that had been operating since 1952 (Bahr *et al.*, 2011). A job-exposure matrix with initially five levels of exposure to trichloroethylene was developed based on the experience of current and former workers. Each job held at the facility was applied to the matrix, including duration in that job. By the end of follow-up, 1638 workers (24.2%) had died out of the 6766 workers for whom usable data existed. The overall standardized mortality ratio for death among workers potentially exposed to trichloroethylene compared with the national population was 0.76 (95% CI, 0.72–0.79) based on 1340 deaths. Among men, the standardized mortality ratios were 0.75 (95% CI, 0.72–0.79) for cancer of the lung, trachea and bronchus, 1.49 (95% CI, 1.02–2.10) for non-Hodgkin lymphoma, and 1.15 (95% CI, 0.74–1.72) for leukaemia and aleukaemic leukaemia. The standardized mortality ratio for for cancer of the lung was 0.75 (95% CI, 0.72–0.79). Corresponding standardized rate ratios for the subcohort of white men were 0.72 (95% CI, 0.29–1.76) for cancer of the lung, 0.99 (95% CI, 0.40–2.46) for non-Hodgkin lymphoma, 1.40 (95% CI, 0.46–4.24) for leukaemia and aleukaemic leukaemia, and 0.43 (0.10–1.84) for cancer of the biliary passages and liver. No cancers of the kidney were reported. No clear dose–response patterns were seen for overall death, overall cancer, or for site-specific cancers. [Overall, this study was not well described, which limited interpretation. No numbers of incident cases, deaths, or rates were given for the SRR calculations. The number of people included in the different subcohorts was unclear. The source of information for vital status, date of death, and cause of death was not clearly described.]

2.2.5 Nonspecified chlorinated solvents

There were several studies of cohorts exposed to nonspecified chlorinated solvents (Chang *et al.*, 2003, 2005; Pukkala *et al.*, 2005; Sung *et al.*, 2007; Lindbohm *et al.*, 2009). Since exposures to trichloroethylene and tetrachloroethylene were not measured separately, and because of other confounding exposures, the Working Group considered that these studies were not informative for the evaluation of trichloroethylene.

2.3 Case–control studies

2.3.1 Cancer of the kidney

See Table 2.3

Seven case–control studies have specifically addressed the association between trichloroethylene and cancer of the kidney. Three of the studies were carried out in Germany.

In 1998, Vamvakas *et al.* published the first case–control study identifying the specific association between trichloroethylene and renal cell cancer (Vamvakas *et al.*, 1998). Cases were patients who underwent nephrectomy between 1987 and 1992 in a hospital in North Rhine-Westphalia, Germany: of the 73 patients treated during the study period, 58 patients were interviewed and enrolled. [None of the cases overlapped with those in the cohort study by Henschler *et al.* (1995) in the same area.] Controls were 84 patients from the accident wards of three other hospitals during 1993 (participation rate, 75%). Cases were histologically confirmed, and controls underwent abdominal echography to exclude cancer of the kidney. Information on occupational history, including exposure to other hazardous chemicals, and other risk factors was obtained by individual interview. The level of individual exposure to trichloroethylene was rated by applying a system that integrated total exposure time, as well as frequency and severity of acute pre-narcotic symptoms. Of the

Table 2.3 Case–control studies of renal cell cancer and exposure to trichloroethylene

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Vamvakas *et al.* (1998), Germany, 1987–93	58 84	Hospital	Interview and score including exposure time, frequency and severity of acute pre-narcotic symptoms	No exposure Ever Low Medium High	39 19 2 9 8	1 10.80 (3.36–34.75) 6.61 (0.50–87.76) 11.92 (2.55–55.60) 11.42 (1.96–66.79)	Age, sex, smoking, BMI, blood pressure, intake of diuretics Age, diastolic blood pressure Subjects may have had high exposure to trichloroethylene through cold degreasing and permanent high background exposures. Few subjects exposed to tetrachloroethylene.
Dosemeci *et al.* (1999) Minnesota, USA 1988–90	273 men and 165 women 462 men and 225 women	Cancer registry and general population	Occupational questionnaire and JEM	Trichloroethylene-exposed Men Women	55 33 22	1.30 (0.9–1.9) 1.04 (0.6–1.7) 1.96 (1.0–4.0)	Age, sex, smoking, BMI, hypertension and/or use of diuretics and/or anti-hypertension drugs Only current and usual jobs plus duration of employment in 13 specific occupations/industries and seven jobs were considered
Pesch *et al.* (2000a) Germany 1991–96	570 men and 365 women 2650 men and 1648 women	Population based, Cases from several hospitals, controls from local residency registries	Occupational questionnaire and JEM	*Men* No exposure Medium[a] High[a] Substantial[a] *Women* No exposure Medium[a] High[a] Substantial[a]	 68 59 22 11 7 5	 1 1.3 (1.0–1.8) 1.1 (0.8–1.5) 1.3 (0.8–2.1) 1 1.3 (0.7–2.6) 0.8 (0.4–1.9) 1.8 (0.6–5.0)	Smoking, age, region Not adjusted for BMI. No precision available on the types of jobs and tasks involving trichloroethylene exposure. [a] Exposure categories defined by 30th, 60th and 90th percentiles of exposure index in controls. Job-task matrix (JTEM)
Brüning *et al.* (2003) Germany 1999–2000	134 401	Hospital	Interview with patient or next-of-kin. Self-assessed exposure	No exposure < 10 yr 10- < 20 yr ≥ 20 yr	109 11 7 6	1 3.78 (1.54–9.28) 1.80 (0.67–4.79) 2.69 (0.84–8.66)	Smoking; Frequency-matched by sex and age Large difference in the results obtained from matrix assessment compared with subjects' self-assessment. No overlap with the cases in the Vamvakas *et al.* (1998) study

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Charbotel *et al.* (2006) France 1993–2003	87 316	Population	Occupational questionnaire and JTEM Cumulative dose	No exposure	49	1	Tobacco smoking, BMI; matched on sex and age
				Low	12	1.62 (0.75–3.47)	
				Medium	9	1.15 (0.47–2.77)	
				High	16	2.16 (1.02–4.60)	
				High	16	1.96 (0.71–5.37)	The study was carried out in a geographical area with high prevalence of trichloroethylene exposure among the general population
				Cumulative dose, plus peaks			
				No exposure	49	1	Trichloroethylene exposure was strongly associated with exposure to cutting fluids and petroleum oils.
				Low/medium, no peaks	18	1.35 (0.69–2.63)	
				Low/medium, plus peaks	3	1.61 (0.36–7.30)	Tobacco smoking, BMI, cutting fluids, other petroleum oils; Matched on sex and age
				High, no peaks	8	1.76 (0.65–4.73)	Tobacco smoking, BMI; matched on sex and age
				High, plus peaks	8	2.73 (1.06–7.07)	
				High, plus peaks	8	2.63 (0.79–8.83)	Tobacco smoking, BMI, cutting fluids, other petroleum oils; matched on sex and age
Charbotel *et al.* (2009) France	87 316	Population	Occupational questionnaire and JTEM	No exposure to trichloroethylene or cutting fluid	46	1	Tobacco smoking, BMI, other petroleum oils; matched on sex & age
				Exposure to cutting fluids but not trichloroethylene	3	2.39 (0.52–11.03)	Cases diagnosed between 1993 and 2003, complementary analysis to Charbotel *et al.*, (2006)
				Exposure to trichloroethylene but not to cutting fluids	15	1.62 (0.76–3.44)	
				Exposure to both and trichloroethylene < 50 ppm	12	1.14 (0.49–2.66)	
				Exposure to both and trichloroethylene ≥ 50 ppm	10	2.70 (1.02–7.17)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Moore *et al.* (2010) Central-eastern Europe 1999–2003	1097 1476	Hospital	Occupational questionnaire and JTEM	No exposure	777	1	Age, sex, centre Subjects with high confidence assessments only Increased risk in trichloroethylene-exposed subjects with at least one intact *GSTT1* allele, but not in subjects with two deleted alleles
				Any exposure	29	2.05 (1.13–3.73)	
				Below the median average intensity	31	1.73 (0.75–4.02)	
				Above the median average intensity	16	2.41 (1.05–5.56)	
				P for trend	0.02		
				Among patients with at least one intact GSTT1 allele:			
				Any exposure	32	1.88 (1.06–3.33)	
				Below the median average intensity	20	1.56 (0.79–3.10)	
				Above the median average intensity	12	2.77 (1.01–7.58)	
				Among subjects with two deleted alleles:			
				Any exposure	10	0.93 (0.35–2.44)	
				Below the median average intensity	6	0.81 (0.24–2.72)	
				Above the median average intensity	4	1.16 (0.27–5.04)	
Christensen *et al.* (2013) Montreal, Canada 1979–85	177 2532	533 population controls and 1999 cancer controls	Occupational questionnaire and expert assessment	Any level	5	0.9 (0.4–2.4)	Age, census tract median income, educational attainment (years), ethnicity (French-Canadian versus other), questionnaire respondent (self vs proxy) and smoking (cigarette-years)
				Substantial	2	0.6 (0.1–2.8)	

BMI, body-mass index; JEM, job-exposure matrix; JTEM, job-task exposure matrix; vs, versus

cases, most subjects had been engaged in cold-degreasing processes and there was an additional permanent background exposure from open tubs containing trichloroethylene, and evaporation from trichloroethylene-cleaned metal parts. Exposures to trichloroethylene and tetrachloroethylene were combined in the study, but only two controls and none of the cases had been exposed to tetrachloroethylene. The odds ratio for any exposure to trichloroethylene, adjusted for age, sex, smoking, body-mass index, blood pressure and intake of diuretics, was 10.80 (95% CI, 3.36–34.75). The analysis of intensity of exposure (including assessment from task description and pre-narcotic symptoms) compared with that of the unexposed individuals, adjusted for age and diastolic blood pressure, gave odds ratios of 6.61 (95% CI, 0.50–87.76) in the low-level category, 11.92 (95% CI, 2.55–55.60) in the medium -level and 11.42 (95% CI, 1.96–66.79) in the high-level category. [The Working Group noted that although the authors combined exposures to trichloroethylene and tetrachloroethylene "because of identical toxicological mechanisms," the reported associations for exposure to trichloroethylene were notable since no cases and only 2 out of 84 controls were exposed to tetrachloroethylene. Additionally, the authors reported permanent background exposure, and past exposure due to the use of trichloroethylene for all cleaning purposes in the plants, including cleaning floors, cloths, and also hands and arms. Narcotic symptoms, an indicator of substantially high peak exposures to solvents, were self-reported and therefore could be subject to reporting bias. There were several limitations to this study that might have biased the probability of exposure in the controls: cases and controls were not recruited in the same hospital; controls were younger than cases (mean age ± standard deviation: cases, 62 ± 9.7; and controls, 51 ± 13.0), but odds ratios were age-adjusted. Finally, the exposure assessment was self-reported, and not blinded to the outcome (case or control status). However, some of the cases were highly exposed to trichloroethylene according to their job tasks.]

Brüning *et al.* (2003) performed a case–control study in the same geographical area as Vamvakas *et al.* (1998). Histologically confirmed cases of renal cell cancer in people who had undergone a nephrectomy between June 1992 and April 2000 were included. [There was no overlap with the cases in the Vamvakas *et al.* (1998) study.] A total number of 162 incident eligible cases were identified, from which 134 cases (82.7%) were enrolled either with face-to-face interviews (n = 113) or with next-of-kin interviews (n = 21). Controls (n = 401) were recruited in the same hospital, in surgery departments or geriatric departments between 1999 and 2000 and interviewed face-to-face with the same structured questionnaire used in the previous study by Vamvakas *et al.* (1998). It included information on occupational history (job titles), tasks, and exposure to specific agents. The frequency and duration of exposure to trichloroethylene and tetrachloroethylene were self-assessed. Narcotic symptoms among subjects exposed to trichloroethylene and tetrachloroethylene were documented and considered to be indicators of substantially high peak exposures. Every job held for at least 1 year was classified according to a British job-exposure matrix (Pannett *et al.*, 1985). The job-exposure matrix provided an expert rating in terms of the probability and intensity of exposure to specified agents including organic solvents, but not specifically trichloroethylene. The products of duration, probability, and intensity of exposure were cumulated over all jobs held to obtain an estimate of lifetime exposure. The corresponding industries were classified according to the United Nations' International Standard Industrial Classification of all Economic Activities (ISIC), 1968. Data on exposure corresponding to these industries were obtained from the public database CAREX. Subjects' self-assessment of exposure to trichloroethylene and tetrachloroethylene (separately) was also used to allow a comparison

with the results of the study of Vamvakas *et al.* (1998).

Conditional logistic regression models were applied for risk estimation adjusted for sex, age and smoking. When considering employment in any industry with exposure to trichloroethylene or tetrachloroethylene using the CAREX database, an odds ratio of 1.80 (95% CI, 1.01–3.20) was observed. An odds ratio of 1.45 (95% CI, 0.59–3.58) was reported for exposure to organic solvents assessed using the British job-exposure matrix [exposure to trichloroethylene was not detailed in this specific assessment]. Finally when considering self-assessed exposure to trichloroethylene, compared with never exposed, the odds ratio was 3.78 (95% CI, 1.54–9.28) among subjects exposed for 1–10 years, 1.80 (95% CI, 0.67–4.79) exposed for 10–20 years and 2.69 (95% CI, 0.84–8.66) exposed for > 20 years. [The participation rate for controls was not provided. The use of proxy interviews to assess exposures may provide poor quality information, which would increase exposure misclassification. In this study there was a difference in the odds ratios associated with exposures obtained from matrix assessment (British job-exposure matrix) compared with subjects' self-assessment. Self-assessment of specific exposures may also lead to exposure misclassification. Other occupational exposures were found to increase the risk of renal cell cancer in this study but no model adjusted for these factors.]

Pesch *et al.* carried out a population-based case–control study in five different regions of Germany that differed from those in the studies by Vamvakas and Brüning (Pesch *et al.*, 2000a). Among the German nationals recruited, there were 935 confirmed cases of renal cell cancer (570 men and 365 women); the 4298 controls (2650 men and 1648 women) were frequency-matched to cases by region, sex and age (5-year groups). The response rate was 88% for cases and 71% for controls. Subjects were interviewed face-to-face with a structured questionnaire. The exposure assessment was based on the subject's occupational history (job titles) as well as on job-task descriptions for every job held for at least 1 year. A job-exposure matrix and a job-task exposure matrix were used to provide expert ratings in terms of the probability and the intensity of exposure to a specific agent. To obtain the subject's lifetime exposure, exposure indices were constructed using duration, probability and intensity of exposure over all job periods. For metal degreasing, the odds ratios were slightly, but insignificantly elevated for the majority of exposure categories in men and women. The odd ratios for exposure to solvents, especially to trichloroethylene, tetrachloroethylene and carbon tetrachloride, were slightly elevated in all exposure categories in males and females. Using the matrix specifically developed for the study to assess exposure to trichloroethylene (the job-task exposure matrix), the odds ratios in men adjusted for smoking, age and region were 1.3 (95% CI, 1.0–1.8), 1.1 (95% CI, 0.8–1.5) and 1.3 (95% CI, 0.8–2.1) for medium, high and substantial levels of exposure, respectively. In women, the corresponding odds ratios were 1.3 (95% CI, 0.7–2.6), 0.8 (95% CI, 0.4–1.9) and 1.8 (95% CI, 0.6–5.0), respectively. An overall estimate combining these category-specific odds ratios was provided by Pesch for a meta-analysis published in 2011 (Scott & Jinot, 2011); the odds ratio was 1.24 (95% CI, 1.03–1.49) for substantial exposure to trichloroethylene. [The Working Group noted several limitations of this study. The methods used for exposure assessment were not described in detail. The low prevalence of exposure observed for several occupations and specific exposures may reduce the power of the study. Prevalence of exposure and results differed according to which job-exposure matrix was used (job-exposure versus job-task exposure). No adjustment was performed for the other occupational exposures found to be significantly associated with risk of renal cell cancer in the study. The authors reported that no dose–response

trend was observed, but the results of statistical tests were not given. The strengths of the study were that cases and controls appeared to have been properly recruited and response rates were in accordance with those in the literature. No details were available on the types of jobs and tasks involving exposure to trichloroethylene.]

A case–control study was performed using the Minnesota cancer registry by Dosemeci *et al.* (1999). From 1988 to 1990, 796 white patients newly diagnosed with histologically confirmed renal cell cancer were identified. Population controls (n = 707) matched on race, age, and sex were recruited using random-digit dialling, or a listing of the Health Care Financing Administration for those aged > 65 years. A questionnaire, including demographic and ethnic variables, occupational and residential history, diet, smoking habits, medical history, and drug use, was administered face-to-face by trained interviewers. The participation rate for the occupational part was 64% for interviewed cases and 97% for interviewed controls. A previously developed job-exposure matrix was used to assess exposures to several chemicals, including trichloroethylene. After adjustment for age, sex, smoking, hypertension and/or use of diuretics and/or anti-hypertension drugs, and body-mass index, the risk of renal cell cancer associated with exposure to trichloroethylene was 1.30 (95% CI, 0.9–1.9) in the entire population, 1.04 (95% CI, 0.6–1.7) among men and 1.96 (95% CI, 1.0–4.0) among women. [The Working Group noted that only current and usual jobs were considered rather than a lifetime work history. Duration of employment in 13 specific occupations/industries and 7 jobs was ascertained, but no dose–response analysis was reported.]

A case–control study in Montreal, Canada, included cases of cancer in men occurring between 1979 and 1985 from 18 of the largest hospitals in the Montreal metropolitan area. Several cancer sites were considered (Christensen *et al.*, 2013). Only incident and histologically confirmed cancers were included. Of 4576 eligible patients with cancer, 3730 (participation rate, 82%) were recruited. For the population controls recruited from the general population, 533 were included out of the 740 eligible (participation rate, 72%). Cancer controls were also included in the analysis [but there was no description of which cancer sites or how many people were included in this group]. A panel of industrial hygienists reviewed each job history reported by study subjects and assessed exposure to 294 substances. Exposure assessment included degree of confidence that exposure had actually occurred, frequency of exposure during a normal working week, and concentration of the agent. Unconditional logistic regression was used to estimate odds ratios for risk of cancer at each site. An analysis was conducted including cancer controls and population controls, weighting the two groups equally. A total of 177 cases of cancer of the kidney were included. For exposure to trichloroethylene, the odds ratio was 0.9 (95% CI, 0.4–2.4) when considering any level of exposure, and 0.6 (95% CI, 0.1–2.8) for substantial exposure, after adjustment for age, income, education, ethnicity, questionnaire respondent, and smoking. [The Working Group noted the low precision and power of this study, which may not have been able to detect an effect due to the low prevalence of exposure to trichloroethylene in the controls (~3%).]

In France, Charbotel *et al.* (2006) carried out a case–control study in a region where trichloroethylene had been widely used as a degreasing agent in the screw-cutting industry. Cases were selected retrospectively from 1993, and prospectively for 1 year until the end of June 2003 from urology and oncology practices and hospitals. Deceased cases and controls were eligible, and for these, the next-of-kin were interviewed. Controls resident in the geographical study area at the time of diagnosis of the case's disease and matched on sex and year of birth were randomly selected from lists of patients at urology or

general practice clinics. Exclusion criteria for controls were: chronic kidney disease or cancer of the bladder, renal pelvis or ureter. Exposure to solvents (trichloroethylene, other chlorinated solvents) and other occupational exposures (oils, including cutting fluids and other oils; welding fumes, lead, cadmium, asbestos and ionizing radiation) was assessed by an industrial hygienist using information from the occupational questionnaires and a job task-exposure matrix (Fevotte *et al.*, 2006). The exposure assessment comprised semiquantitative estimates for trichloroethylene and qualitative estimates (low/medium/high) for the other occupational agents. The effect of cumulative and peak exposure was assessed. Conditional logistic regression analyses were performed to assess the association between trichloroethylene and risk of renal cell cancer. A total of 87 cases of renal cell cancer (participation rate, 74%) and 316 controls (participation rate, 78%) were included. Among general factors studied, only tobacco smoking and body-mass index were found to significantly increase the risk of renal cell cancer. An increased risk was identified for high cumulative exposure to trichloroethylene: the crude odds ratio was 2.23 (95% CI, 1.09–4.57) and the odds ratio adjusted for tobacco smoking and body-mass index was 2.16 (95% CI, 1.02–4.60). A dose–response relationship was identified (*P* for trend, 0.04). The odds ratios were even higher among the highest class of exposure (cumulative dose) plus peaks (crude OR, 2.70; 95% CI, 1.09–6.67; adjusted OR, 2.73; 95% CI, 1.06–7.07). When exposure to cutting fluids and to other petroleum oils were added to the conditional logistic regression model, the odds ratios for renal cell cancer were elevated, but not statistically significant for the highest class of cumulative exposure to trichloroethylene (OR, 1.96; 95% CI, 0.71–5.37) and in the high-exposure group with peaks (OR, 2.63; 95% CI, 0.79–8.83).

In a complementary analysis (Charbotel *et al.*, 2009) among the same subjects as reported in Charbotel *et al.* (2006), the odds ratios for semiquantitative estimates of 8-hour average exposure to trichloroethylene at the thresholds of 35 ppm, 50 ppm, and 75 ppm were respectively 1.62 (95% CI, 0.77–3.42), 2.80 (95% CI, 1.12–7.03), and 2.92 (95% CI, 0.85–10.09). The authors assessed the potential confounding effect of exposure to cutting fluids. In subjects exposed to trichloroethylene only, the odds ratio was 1.62 (95% CI, 0.76–3.44). In subjects exposed to cutting fluids and trichloroethylene at concentrations > 50 ppm, the odds ratio adjusted for body-mass index, tobacco smoking and exposure to other oils reached 2.70 (95% CI, 1.02–7.17). [The Working Group noted that exposure to trichloroethylene was not measured, but was estimated in ppm. This study is notable because it was carried out in a geographical area with a high prevalence of exposure among the general population. Risk factors for kidney cancer, and other occupational exposures were assessed and included in statistical models.]

A hospital-based case–control study on trichloroethylene and renal cell cancer was carried out between 1999 and 2003 in seven centres in four countries of central and eastern Europe (Moscow, the Russian Federation; Bucharest, Romania; Lodz, Poland; and Prague, Olomouc, Ceske-Budejovice and Brno, Czech Republic) (Moore *et al.*, 2010). This region is of interest for the study of occupational exposures because the prevalence and intensity of exposure have been greater than in other industrialized regions. This analysis assessed the interaction between exposure to trichloroethylene and *GSTT1* genotype because: (i) trichloroethylene-associated kidney damage occurs only after bioactivation through the reductive metabolic pathway that requires prior conjugation by hepatic and renal glutathione *S*-transferase (GSH); (ii) the GSTT1 enzyme conjugates small, halogenated compounds such as trichloroethylene; and (iii) GSTT1 is highly active in the kidney. Newly diagnosed and histologically confirmed cases

of cancer of the kidney (ICD-O2 code C.64) were included and controls were chosen among subjects admitted with non-tobacco-related conditions in the same hospital as the cases, and frequency matched with cases by sex, age, and study centre. The final study population included 1097 cases and 1476 controls. Face-to-face interviews were performed using standard questionnaires (tobacco consumption, anthropometric measures 1 year before diagnosis, personal and familial medical history). Information on each job held for at least 1 year was collected using a general questionnaire including a description of the tasks performed, machines used, working environment, location of tasks performed, and time spent on each task. In each centre, a team evaluated the frequency and intensity of exposure to various agents and groups of agents (including trichloroethylene), based on the questionnaires and their own experience and knowledge of historical working conditions at specific plants in their study area. An increased risk of renal cell cancer was observed among subjects ever exposed to trichloroethylene (OR, 1.63; 95% CI, 1.04–2.54). A trend was observed in relation to average intensity of exposure: below the median average intensity of exposure in controls (0.076 ppm), the odds ratio was 1.73 (95% CI, 0.75–4.02) while above the median the odds ratio was 2.41 (95% CI, 1.05–5.56; *P* for trend, 0.02). In subjects with at least one intact *GSTT1* allele, a significant association was found for those ever exposed to trichloroethylene (OR, 1.88; 95% CI, 1.06–3.33), and the odds ratios below and above the average exposure intensity were 1.56 (95% CI, 0.79–3.10) and 2.77 (95% CI, 1.01–7.58), respectively (*P* for trend, 0.02). In contrast, no increase in risk of renal cell cancer was observed among subjects with two deleted *GSTT1* alleles: the odds ratios were, respectively, 0.93 (95% CI, 0.35–2.44) in ever-exposed subjects, 0.81 (95% CI, 0.24–2.72) with below-average exposure, and 1.16 (95% CI, 0.27–5.04) with over-average exposure intensity. [The participation rate was stated to be high, but was not provided in the paper. Tobacco smoking, body-mass index and self-reported history of hypertension were evaluated as risk factors, but did not alter the odds ratios by > 10%, and therefore were not included in the final models. Despite being justified by the inclusion of hospital controls, the exclusion of tobacco-related conditions for control recruitment may have led to a selection effect for social class, i.e. controls may be less likely to be blue-collar workers and consequently less likely to be exposed to trichloroethylene. A selection bias due to the use of hospital controls may have occurred as suggested by a lack of association observed between tobacco smoking and risk of renal cell cancer. Studies *in vitro* and *in vivo* in humans have demonstrated that the GST pathway is active, but it is unclear which isoform is most active.]

2.3.2 Haematological malignancies

See Table 2.4

(a) Non-Hodgkin lymphoma

The association between non-Hodgkin lymphoma and exposure to trichloroethylene has been investigated in eight case–control studies in several countries.

In Sweden, a case–control study recruited all men with non-Hodgkin lymphoma from an oncology department between 1974 and 1978 (Hardell *et al.*, 1994). Controls were selected from the national population registry and matched according to sex, age, place of residence, and vital status. Deceased controls, drawn from the national registry for causes of death, were also matched for year of death. The self-administered questionnaire contained questions on job history and exposure to chemicals. The study included 105 patients with confirmed non-Hodgkin lymphoma and 335 controls. An increased risk of non-Hodgkin lymphoma was found in workers reporting exposure to trichloroethylene (crude OR, 7.2; 95% CI, 1.3–42.0). [Exposure assessment

was not described in detail in this publication. The odds ratio from this matched study did not appear to include only discordant pairs; the analysis was difficult to verify as there was no information on the non-exposed subjects.]

Data from two case–control studies with similar designs were pooled to investigate risk factors for non-Hodgkin lymphoma (Persson *et al.*, 1989, 1993; Persson & Fredrikson, 1999). In these studies, exposure assessment was based on information reported in a questionnaire posted to the subjects. A total of 199 cases and 479 controls were included. Among workers exposed to trichloroethylene, the odds ratio stratified by age and sex was 1.2 (95% CI, 0.5–2.4). [The Working Group noted that the exposure assessment was based on self-reported exposure.]

A population-based case–control study of lymphohaematopoietic tumours included all new cases of non-Hodgkin lymphoma diagnosed during 1991–93 in eight areas in Italy (Miligi *et al.*, 2006). The participation rate was 85% among cases of non-Hodgkin lymphoma (n = 1428) and 73% among controls (n = 1530). Cases were identified from hospital and pathology departments. Controls were randomly selected from residents in the general population. Face-to-face interviews were performed to collect information on occupational history, and exposure was assessed by an industrial hygienist. Odds ratios were adjusted for age, sex, education, and area. When considering all subtypes of non-Hodgkin lymphoma combined, the odds ratios associated with occupational exposure to trichloroethylene were 0.8 (95% CI, 0.5–1.3) for exposure at very low or low levels, or 1.2 (95% CI, 0.7–2.0) at medium or high levels. No association was found with duration of exposure. A non-statistically significant increased risk was observed for diffuse lymphoma (OR, 1.9; 95% CI, 0.9–3.7; 13 exposed cases), but not for other types of non-Hodgkin lymphoma. [The strengths of this study were that it included a large number of subjects and an expert assessment of exposure was performed.]

A case–control study in six regions in Germany included patients with malignant lymphoma (n = 710; participation rate, 87%) and controls (matched by sex, region, and age) recruited from population registers (n = 710; participation rate, 44%) (Seidler *et al.*, 2007). Interviewers collected a complete history of all jobs held for more than 1 year and specific job tasks. On the basis of job task-specific questionnaires, a trained occupational physician assessed exposure to chlorinated hydrocarbons, including trichloroethylene. Workers in the category of highest exposure to trichloroethylene (> 35 ppm-years) had an increased risk of malignant lymphoma (OR, 2.1; 95% CI, 1.0–4.8), but no significant dose–response relationship was observed (P for trend, 0.14). Estimated increased risks for workers in the category of highest exposure to trichloroethylene were also observed for B-cell non-Hodgkin lymphoma, T-cell non-Hodgkin lymphoma, Hodgkin lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma, but most precise for the B-NHL subtype (n = 554; OR, 2.3; 95% CI, 1.0–5.3). [The Working Group noted that the participation rate among controls was half that of cases and could represent a selection bias. In addition, the cumulative exposures appeared to have been very low.]

In Connecticut, USA, a case–control study investigated the association between non-Hodgkin lymphoma and occupational exposure to solvents among women (Wang *et al.*, 2009). Subjects were recruited between 1996 and 2000 and included 601 cases (participation rate, 72%) and 717 controls selected by random-digit dialling (participation rate, 69%) or random selection from Medicare service files. Exposure assessment was based on occupational questionnaire and expert assessment using a job-exposure matrix. An increased risk of non-Hodgkin lymphoma in workers ever exposed to trichloroethylene was found (OR, 1.2; 95% CI, 0.9–1.8), after adjustment for age, family history of

Table 2.4 Case–control studies of non-Hodgkin lymphoma, and other haematological malignancies, and exposure to trichloroethylene

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 Comments
Hardell *et al.* (1994) Sweden 1974–78	105 335	National population registry (alive) and national registry for causes of death (deceased)	Questionnaire soliciting working history and leisure-time activities	NHL	Trichloroethylene-exposed	4	7.2 (1.3–42)	Matched for sex, age, place of residence, and vital status NHL was associated with exposure to phenoxyacetic acids and chlorophenols, but trichloroethylene results were not adjusted for these exposures. Exposure assessment was not described in detail.
Persson & Fredrikson (1999) Sweden 1964–86	199 479	Population	Occupational agent exposure assessed by questionnaires mailed to participants	NHL	Any exposure to trichloroethylene	16	1.2 (0.5–2.4)	Stratified on age and sex
Miligi *et al.* (2006) Italy	1428 1530	Population	Face-to-face interviews, assessed by an industrial hygienist	All NHL subtypes	Very low/low	35	0.8 (0.5–1.3)	Sex, age, area, education
					Medium or high level	35	1.2 (0.7–2.0)	
					Test for trend		$P = 0.80$	
					> 15 year exposure duration	12	1.0 (0.5–2.6)	
				Small lymphocytic NHL	Any exposure	7	0.9 (0.4–2.1)	
				Diffuse NHL	Any exposure	13	1.9 (0.9–3.7)	
Seidler *et al.* (2007) Germany	710 710	Population	Using job-task-specific questionnaires	All malignant lymphoma	0	610	ref.	Age, sex, region, smoking, alcohol Increased risks were observed for workers in the highest category of exposure for malignant lymphoma nd several subtypes (B-cell NHL, T-cell NHL, Hodgkin lymphoma, DLBCL, FL, and marginal zone lymphoma)
					< 4.4 ppm-years	40	0.7 (0.4–1.1)	
					> 4.4–35 ppm-years	32	0.7 (0.5–1.2)	
					> 35 ppm-years	21	2.1 (1.0–4.8)	
					Test for trend		$P = 0.14$	
				B-NHL	0	47+	ref.	
					< 4.4 ppm-years	32	0.7 (0.5–1.2)	
					> 4.4–35 ppm-years	27	0.8 (0.5–1.3)	
					> 35 ppm-years	17	2.3 (1.0–5.3)	
					Test for trend		$P = 0.08$	

Table 2.4 (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 Comments
Seidler *et al.* (2007) Germany (cont.)				T-NHL	0	27	Ref.	
					< 4.4 ppm-years	2	0.7 (0.2–3.3)	
					> 4.4–35 ppm-years	2	1.1 (0.2–5.1)	
					> 35 ppm-years	2	4.7 (0.8–26.1)	
					Test for trend		*P* = 0.09	
Wang *et al.* (2009) Conneticut, USA 1996–2000	601 717	Population	Linking coded occupational data with a JEM	Histopathologically verified NHL	Ever exposed	77	1.2 (0.9–1.8)	Age, family history, alcohol, race
					Low exposure intensity	64	1.1 (0.8–1.6)	
					Medium–high exposure intensity	13	2.2 (0.9–5.4)	Increased risk with exposure to other organic, solvents, benzene, formaldehyde
					Test for trend		*P* = 0.06	Overlapped with Deng *et al.* (2012)
Cocco *et al.* (2010) multicentre Europe 1998–2004	2348 2462	Population	In-person interviews on occupational history	B-NHL	All exposed	71	0.8 (0.6–1.1)	Age, sex, education, centre
					Low	26	0.9 [no CI]	
					Medium	16	0.5 [no CI]	
					High	29	1.0 [no CI]	
					Test for trend		*P* = 0.16	
				DLBCL	All exposed	17	0.7 (0.4–1.1)	
					Low	6	0.7 [no CI]	
					Medium	4	0.4 [no CI]	
					High	7	0.9 [no CI]	
					Test for trend		*P* = 0.16	
				FL	All exposed	11	1.2 (0.6–2.3)	
					Low	7	2.4 [no CI]	
					Medium	1	0.3 [no CI]	
					High	3	1.0 [no CI]	
					Test for trend		*P* = 0.65	
				CLL	All exposed	18	0.9 [0.5–1.5)	
					Low	6	1.0 [no CI]	
					Medium	3	0.4 [no CI]	
					High	9	1.2 [no CI]	
					Test for trend		*P* = 0.94	

Table 2.4 (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 Comments
Cocco *et al.* (2010) multicentre Europe 1998–2004 (cont.)				MM	All exposed	9	0.6 [0.3–1.2]	
					Low	1	0.2 [no CI]	
					Medium	4	0.7 [no CI]	
					High	4	0.8 [no CI]	
					Test for trend		$P = 0.22$	
Purdue *et al.* (2011) USA; four SEER registries 1998–2000	1189 982	Population	Face-to-face interview for occupational exposures	NHL	Any exposure			Age, sex, study centre, race, education No assessment of exposure to other organic solvents
					Unexposed	599	Ref.	
					Possible exposure	545	1.1 (0.9–1.3)	
					Probable exposure	45	1.4 (0.8–2.4)	
					Test for trend		$P = 0.40$	
					Average weekly exposure (ppm-hours):			
					0	599	ref.	
					1–60	15	1.6 (0.7–3.8)	
					61–50	7	0.5 (0.2–1.4)	
					> 150	23	2.5 (1.1–6.1)	
					151–360	3	0.4 (0.1–1.8)	
					> 360	20	7.9 (1.8–34.3)	
					Per 90 estimated ppm-hours/week		1.11 (1.02–1.21)	
					Test for trend		$P = 0.02$	
					Years exposed:			
					0	599	Ref.	
					1–6 yr	22	2.1 (1.0–4.7)	
					7–16 yr	10	0.8 (0.3–2.1)	
					> 16 yr	13	1.3 (0.5–3.1)	
					17–24 yr	6	1.0 (0.3–3.4)	
					> 24 yr	7	1.7 (0.5–5.8)	
					Per 10 yr		1.13 (0.85–1.51)	
					Test for trend		$P = 0.40$	

Table 2.4 (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 Comments
Purdue *et al.* (2011) USA; four SEER registries 1998–2000 (cont.)					Cumulative exposure (estimated ppm-hours):			
					0	599	Ref.	
					1–46 800	14	1.4 (0.6–3.3)	
					46 801–112 320	7	0.6 (0.2–1.7)	
					> 112 320	24	2.3 (1.0–5.0)	
					112 321–234 000	8	1.4 (0.5–4.4)	
					> 234 000	16	3.3 (1.1–10.1)	
					Per 65 520 estimated ppm-hours		1.10 (0.99–1.22)	
					Test for trend		$P = 0.08$	
					Average exposure intensity (estimated ppm):			
					0	599	ref	
					1–99	23	1.5 (0.8–2.9)	
					> 99	22	1.3 (0.7–2.7)	
					Per 99 estimated ppm		1.18 (0.80–1.76)	
					Test for trend		$P = 0.41$	

Table 2.4 (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 Comments
Deng *et al.* (2012) Connecticut, USA 1996–2000	518 597	Population	Standardized questionnaire on job history	NHL	Trichloroethylene exposed and IL12A_07 genotype (rs582054)			Age, race Also, significantly increased risk with formaldehyde (NHL, DLBCL) and benzene (DLBCL); IL12A was only one of 30 SNPs selected from Th1/Th2 genes involved in immune function Overlapped with Wang *et al.* (2009)
					TT genotype	14	0.70 (0.34–1.42)	
					AT/AA genotype	51	2.09 (1.28–3.42)	
					Test for interaction		$P = 0.009$	
				DLBCL	TT genotype	4	0.59 (0.19–1.85)	
					AT/AA genotype	21	2.66 (1.42–4.96)	
					Test for interaction		$P = 0.0119$	
				FL	TT genotype	4	0.82 (0.25–2.72)	
					AT/AA genotype	10	1.71 (0.78–3.77)	
					Test for interaction		$P = 0.3498$	
Christensen *et al.* (2013) Montreal 1979–85	215 2341	Population plus controls with cancer at another site (excluding lung, and seven sites with low numbers)	Face-to-face interview on lifetime occupational history and assessment by industrial hygienists	NHL (ICD-9 200, 202)	Any trichloroethylene exposure	7	1.2 (0.5–2.9)	Age, income, education, ethnicity (French-Canadian versus other), questionnaire respondent (self versus proxy) and smoking (cigarette-years)
					Substantial exposure	3	1.0 (0.3–3.5)	

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma

haematopoietic cancers, alcohol consumption, and race. Compared with non-exposed subjects, the risk was elevated at a medium-to-high intensity of exposure (OR, 2.2; 95% CI, 0.9–5.4), but was still not statistically significant compared with that in the group with a low level of exposure (OR, 1.1; 95% CI, 0.8–1.6; *P* for trend, 0.06). [This study overlapped with that of Deng *et al.* (2012).]

Complementary analyses were carried out to determine whether the association between non-Hodgkin lymphoma and solvent exposure was modified by variation in genes of the immune system (Deng *et al.*, 2012). Samples of blood or buccal cells were collected from 518 patients and 597 controls, and the interaction between exposure to trichloroethylene and IL12A (rs582054) genotype was assessed. Among women with AT/AA genotypes who had been exposed occupationally to trichloroethylene, increased risks were observed for non-Hodgkin lymphoma overall (OR, 2.09; 95% CI, 1.28–3.42), diffuse large B-cell lymphoma (OR, 2.66; 95% CI, 1.42–4.96), and follicular lymphoma (OR, 1.71; 95% CI, 0.78–3.77). In contrast, among women who carried the IL12A (rs582054) TT genotype and who had been exposed occupationally to trichloroethylene, the risk estimates were below one and not statistically significant. [The Working Group noted that in this analysis, IL12A was only one of 30 single-nucleotide polymorphisms selected from Th1/Th2 genes involved in immune function. This study overlapped with that of Wang *et al.* (2009).]

A case–control study in six regions in Germany included patients with malignant lymphoma (*n* = 710; participation rate, 87%) and controls (matched by sex, region, and age) recruited from population registers (*n* = 710; participation rate, 44%) (Seidler *et al.*, 2007). Interviewers collected a complete history of all jobs held for more than 1 year and specific job tasks. On the basis of job task-specific questionnaires, a trained occupational physician assessed exposure to chlorinated hydrocarbons, including trichloroethylene. Workers in the category of highest exposure to trichloroethylene (> 35 ppm-years) had an increased risk of malignant lymphoma (OR, 2.1; 95% CI, 1.0–4.8), but no significant dose–response relationship was observed (*P* for trend, 0.14). Estimated increased risks for workers in the category of highest exposure to trichloroethylene were also observed for B-cell non-Hodgkin lymphoma, T-cell non-Hodgkin lymphoma, Hodgkin lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma, but most precise for the B-NHL subtype (*n* = 554; OR, 2.3; 95% CI, 1.0–5.3). [The Working Group noted that the participation rate among controls was half that of cases and could represent a selection bias. In addition, the cumulative exposures appeared to have been very low.]

A multicentric case–control study on occupational exposure to trichloroethylene and lymphoma, the Epilymph study, was conducted in the Czech Republic, France, Germany, Ireland, Italy, and Spain, from 1998 to 2004 (Cocco *et al.*, 2010). The study included 2348 cases of lymphoma and 2462 controls (hospital and population-based; matched by age, sex, residence in Germany and Italy). The overall participation rates were 88% for cases, 81% for hospital controls and 52% for population controls. Face-to-face interviews were carried out to collect data on occupational history, and exposures were assessed by an industrial hygienist. Odds ratios were adjusted for age, sex, education, and study centre. No association was found between occupational exposure to trichloroethylene and any subtype of non-Hodgkin lymphoma (B-cell non-Hodgkin lymphoma, diffuse large B-cell lymphoma, or follicular lymphoma). [This was a large study with expert assessment of exposure.]

Purdue *et al.* carried out a case–control study to analyse the association between non-Hodgkin lymphoma and exposure to trichloroethylene (Purdue *et al.*, 2011). Cases came from four

Surveillance, Epidemiology, and End Results (SEER) registry areas in the USA (Iowa, Los Angeles County, Seattle, Detroit) and were diagnosed between July 1998 and June 2000 (n = 1189; participation rate, 76%). Controls were recruited from the general population (n = 982; participation rate, 52%). Subjects were interviewed face-to-face to obtain a job history. Occupational exposure to trichloroethylene was assessed by an industrial hygienist. After adjustment for age, sex, study centre, race and education, workers who had an estimated average weekly exposure of > 150 ppm-hours had an odds ratio of 2.5 (95% CI, 1.1–6.1; P for trend, 0.02). An increased risk of non-Hodgkin lymphoma was also identified for cumulative exposure exceeding 112 320 estimated ppm-hours (OR, 2.3; 95% CI, 1.0–5.0; P for trend, 0.08). [The study included a large number of subjects and an expert assessment of exposure was performed. The Working Group noted the low participation rate among controls.]

A case–control study from Montreal (described in Section 2.3.1) included 215 men diagnosed with non-Hodgkin lymphoma and 2341 population and cancer controls (Christensen *et al.*, 2013). The criteria for inclusion of cancer controls were as follows: (i) contiguous sites were excluded as controls for the index cancer series; (ii) cancer of the lung was excluded; and (iii) subsamples were constituted such that no cancer site constituted more than 20% of any series of controls. The cancer controls were selected on the principle that for any solvent:site association, the odds ratio would not be greatly biased except in the implausible scenario that a particular solvent is a risk factor for most cancer sites, which should be detectable in the analysis using population controls. The odds ratio associated with exposure to trichloroethylene was 1.2 (95% CI, 0.5–2.9; seven exposed cases) for any exposure, and 1.0 (95% CI, 0.3–3.5; three exposed cases) for substantial exposure, after adjustment for age, income, education, ethnicity, questionnaire respondent, and smoking.

(b) Other haematological malignancies

Several studies reported associations between exposure to trichloroethylene and haematological malignancies other than non-Hodgkin lymphoma. Persson *et al.* reported a non-statistically significant increased risk of Hodgkin lymphoma (crude OR, 2.0; no P-value or confidence intervals provided) related to exposure to trichloroethylene in Sweden (Persson *et al.*, 1993). The study by Seidler *et al.* (2007) in Germany (described above) reported a non-significant increased risk of Hodgkin lymphoma (OR, 2.0; 95% CI, 0.4–10.5) in the category of highest exposure. The international Epilymph study (Cocco *et al.*, 2010) did not find any association between occupational exposure to trichloroethylene and Hodgkin lymphoma, chronic lymphocytic leukaemia, or multiple myeloma. Gold *et al.* (2011) found an association between (cumulative) occupational exposure to trichloroethylene and multiple myeloma. Seidler *et al.* (2007) found no association between occupational exposure to trichloroethylene and multiple myeloma or chronic lymphocytic leukaemia. Nordström *et al.* reported a non-significant association (OR, 1.5; 95% CI, 0.7–3.3) between exposure to trichloroethylene and hairy cell leukaemia in a study in Sweden (Nordström *et al.*, 1998).

Studies of childhood cancer, including leukaemia, have also evaluated exposure to trichloroethylene. Two of these studies identified an association between childhood leukaemia and paternal exposure to trichloroethylene (Lowengart *et al.*, 1987; McKinney *et al.*, 1991). In McKinney *et al.* (1991), the odds ratios for paternal exposure to trichloroethylene during the preconception, periconception and gestational, and postnatal periods were 2.27 (95% CI, 0.84–6.16), 4.40 (95% CI, 1.15–21.01), and 2.66 (95% CI, 0.82–9.19), respectively. In the study by Lowengart *et al.* (1987), a twofold non-significant increase in risk of leukaemia was found with paternal exposure to trichloroethylene 1 year

before pregnancy (OR, 2.0; P = 0.16), during pregnancy (OR, 2.0; P = 0.16), and after delivery (OR, 2.7; 95% CI, 0.64–15.60; P = 0.07). There were no associations with maternal exposure to trichloroethylene; few mothers were occupationally exposed to trichloroethylene.

Costas *et al.* (2002) conducted a case–control study evaluating 16 cases of childhood leukaemia occurring between 1969 and 1986, and 37 matched controls in Woborn, Massachussetts where, in 1979, two of the city's eight municipal drinking-water wells were closed when tests identified contamination with solvents including trichloroethylene. A personal interview gathered information specific to risk factors for leukaemia and use of public drinking-water in the home. An exposure value was assigned to each subject according to the addresses of residence. Non-significant increased risks were observed for different indices of exposure to contaminated municipal drinking-water wells before conception and during pregnancy (Costas *et al.*, 2002).

2.3.3 Cancer of the liver

A single case–control study investigated the association between occupational exposure to trichloroethylene and cancer of the liver (Christensen *et al.*, 2013) (described in Section 2.3.1). The analysis was based on 33 cases. Odds ratios were 1.1 (95% CI, 0.1–8.5; one exposed case) for any level of exposure and 2.1 (95% CI, 0.2–18; one exposed case) for substantial exposure.

2.3.4 Other sites

(a) Cervix

A case–control study to investigate the association between occupational exposure to trichloroethylene and cancer or dysplasia of the cervix was carried out in a region of France where trichloroethylene had been widely used (Charbotel *et al.*, 2013). Exposure assessment was the same as in the French renal cancer case–control study previously described (interview with specific questionnaire and assessment by an industrial hygienist using a job task-exposure matrix). Case and control subjects (67 subjects in each group) were recruited by gynaecologists on a voluntary basis. Interviews were performed to collect information on job history and tasks. Exposure assessment was conducted by an industrial hygienist. The crude odds ratio was 1.17 (95% CI, 0.54–2.52; 31 exposed cases). After adjustment for general risk factors that correlated significantly with cervical dysplasia or cervical cancer, the odds ratio was 1.51 (95% CI, 0.42–5.41; 17 exposed cases). [The Working Group noted that the number of subjects included was small.]

(b) Rectum and colon

Two case–control studies have reported on the association between cancers of the rectum and colon and exposure to trichloroethylene. The Montreal case–control study investigated risk for cancers of the rectum and colon (Christensen *et al.*, 2013). For rectal cancer, after adjustment for age, education, respondent status, cigarette smoking, beer consumption, and body-mass index, the odds ratio was 1.8 (95% CI, 0.9–3.6) for any exposure to trichloroethylene and 0.7 (95% CI, 0.2–2.6) for substantial exposure. For colon cancer, the odds ratios were 1.0 (95% CI, 0.5–2.0) and 1.2 (95% CI, 0.5–2.7), respectively.

A case–control study in Sweden showed a small non-significant increased risk of cancer of the colon (ICD-8 code, 153.01–153.89) in subjects who had been exposed to trichloroethylene (OR, 1.5; 95% CI, 0.4–5.7) (Fredriksson *et al.*, 1989). Among dry-cleaning workers reporting an exposure to trichloroethylene, the estimated risk of cancer of the colon increased sevenfold (OR, 7.4; 95% CI, 1.1–47.0, while the odds ratio for dry-cleaning workers was 2.0 (95% CI, 0.5–7.1; five cases and five controls). The odds ratios accounted for age, sex, physical activity. [The Working Group noted the sevenfold increased

risk in trichloroethylene-exposed dry-cleaning workers. The paper did not report when these workers were employed.]

(c) Brain

In northern New Jersey and Philadelphia, Pennsylvania, USA, a case–control study was carried out based on death certificates of 300 white men who died from astrocytic cancer of the brain between 1978 and 1980, and 320 controls who died in the same period (Heineman *et al.*, 1994). Information on occupational history was obtained from next of kin, and a job-exposure matrix was used to assess exposure to organic solvents. A statistically significant association was found between astrocytic cancer of the brain and exposure to some organic solvents, but not trichloroethylene (OR, 1.1; 95% CI, 0.8–1.6; 128 exposed cases).

A hospital-based case–control study was carried out in three hospitals in the USA to investigate the association between brain tumours and occupational exposures to several solvents (Neta *et al.*, 2012). Cases were recruited between 1994 and 1998. Controls were recruited from the same hospitals. An interview was carried out by a trained nurse, and occupational exposures were assessed by an industrial hygienist. Participation rates were near 90% for cases and controls. Proxy interviews were conducted for 16% of cases of glioma, 8% of cases of meningioma, and 3% of controls. No association was found between occupational exposure to trichloroethylene and risk of glioma or meningioma. Odds ratios were equal to or under one, and non-significant whatever the category of exposure to tricholoroethylene considered.

Ruder *et al.* (2013) evaluated risk of glioma from non-farm occupational exposure (ever/never and estimated cumulative exposure) to trichloroethylene among 798 cases and 1175 population-based controls aged 18–80 years, and non-metropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin, USA [farmers were included, but exposures were "non-farm" because all farmers were considered to be exposed to all chlorinated solvents]. The methodology for the exposure assessment was the same as that used in Neta *et al.* (2012). Unconditional logistic regression was used to calculate odds ratios adjusted for frequency-matching variables, age group and sex, and age and education. Ever-exposure to trichloroethylene was associated with reduced risk of glioma (OR, 0.7; 95% CI, 0.6–0.9; 302 cases and 515 exposed controls). Mean estimated cumulative exposure was lower for cases (85.9 ppm-years) than controls (98.9 ppm-years); the odds ratio for glioma was 1.0 (95% CI, 0.9–1.1). In analyses limited to 904 participant blood donors (excluding controls reporting a previous cancer diagnosis) genotyped for glutathione-*S*-transferases *GSTP1, GSTM3*, and *GSTT1*, solvent-exposed individuals with functional *GST* genes (that might convert chlorinated solvents crossing the blood–brain barrier into cytotoxic metabolites) were not at increased risk of glioma. [The limitations of this study included the high percentage of proxy respondents and the lack of measurements of solvent levels at the workplace or in serum.]

Another study aimed at identifying paternal occupations associated with an increased risk of childhood cancer of the brain, with a focus on specific exposures using an assessment by an industrial hygienist (De Roos *et al.*, 2001). The analysis included 405 case fathers and 302 control fathers. A statistically non-significant increased risk was found with paternal exposure to trichloroethylene when considering self-assessed exposure (OR, 1.4; 95% CI, 0.7–2.9; 22 exposed cases; OR adjusted by age, maternal race, maternal age, and maternal education), but it was not confirmed when the analysis was restricted to exposure as assessed by an industrial hygienist (adjusted OR, 0.9; 95% CI, 0.3–2.5).

(d) Bladder

From 1991 to 1995, a population based case–control study was carried out in five regions of Germany (West Berlin, Bremen, Leverkusen, Halle, Jena) to evaluate the risk of urothelial cancer associated with several occupational exposures, including solvents (Pesch *et al.*, 2000b). The study included 1035 histologically confirmed cases of urothelial cancer, including urinary bladder (80% of cases in females, 90% in males), ureter and renal pelvis, and 4298 randomly selected controls recruited from local residency registries. The exposure assessment was based on the subject's history of jobs held for at least 1 year and job-task descriptions. Two job-exposure matrices adapted to the specific context of the study provided an expert rating in terms of the probability and the intensity of exposure to a specific agent. To characterize lifetime exposure, exposure indices were constructed using duration, probability and intensity of exposure over all periods of work. Conditional logistic-regression models were applied for risk estimation while adjusting for tobacco smoking as a confounder. A significantly increased risk was observed in males reporting the longest time for metal degreasing (OR, 2.3; 95% CI, 1.4–3.8). When considering exposures based on the job-task exposure matrix, a significantly increased risk was identified in men exposed to trichloroethylene at the highest (substantial) level (OR, 1.8; 95% CI, 1.2–2.7) or to tetrachloroethylene (OR, 1.8; 95% CI, 1.1–3.1). [Patients were probably exposed to both trichloroethylene and tetrachloroethylene, since 48 cases were exposed to substantial levels of chlorinated solvents, 22 to substantial level of tetrachloroethylene, and 36 to substantial levels of trichloroethylene. No dose–response relationships were reported and not all important potential confounders were accounted for.]

In a case–control study in Montreal, no association was found between cancer of the bladder and exposure to trichloroethylene (Christensen *et al.*, 2013). Adjusted odds ratios were 0.7 (95% CI, 0.3–1.4) for any level of exposure, and 0.6 (95% CI, 0.2–1.5) for substantial exposure.

(e) Other sites

A case–control study investigated the association between mortality from cancer of the pancreas and exposure to trichloroethylene (Kernan *et al.*, 1999). It was based on death certificates from 24 states in the USA (63 097 cases who died between 1984 and 1993). For each case, four controls were frequency-matched by state, race, sex, and age group. Occupations and industries were coded and a job-exposure matrix was used to assess occupational exposures. A significantly increased risk of death from cancer of the pancreas was found in black females with a low (OR, 1.1;95% CI, 1.0–1.3) or medium (OR, 2.3; 95% CI, 1.3–4.0) level of exposure to trichloroethylene, and in white females with a low (ORs 1.0; 95% CI, 1.0–1.1) or high (OR, 1.1; 95% CI, 1.0–1.3) level of exposure, and in white males with a medium level of exposure (OR, 1.1; 95% CI, 1.1–1.5). In the other categories of exposure, odds ratios ranged from 0.8 to 1.2 and were not statistically significant. [In this study, exposure assessment was based on job and industry information available on the death certificate. Only the most recent occupation and type of industry held by the decedent may be reported on death certificates, although usual occupation and industry were requested. This may have increased the possibility of misclassification of exposure.]

In the case–control study in Montreal, Canada, by Christensen *et al.* (2013), exposure to trichloroethylene was assessed in relation to risk of pancreatic cancer (n = 116; two exposed cases; OR, 0.8; 95% CI, 0.2–3.6), stomach cancer (n = 251; OR for any exposure, 0.6; 95% CI, 0.2–1.8; four exposed cases; OR for substantial exposure, 0.5; 95% CI, 0.1–2.4; two exposed cases), oesophageal cancer (OR, 0.9; 95% CI, 0.1–6.7; n = 99; one exposed case); melanoma (n = 103; eight exposed

cases; OR for any level of exposure, 3.0; 95% CI, 1.2–7.2; OR for substantial exposure, 3.2; 95% CI, 1.0–9.9; five exposed cases), and prostate cancer (n = 449; fourteen exposed cases; OR for any level of exposure, 1.3; 95% CI, 0.7–2.6; OR for substantial exposure, 1.2; 95% CI, 0.5–3.1; seven exposed cases).

In a separate publication concerning the case–control study in Montreal, solvent exposure was assessed in relation to cancer of the lung (Vizcaya *et al.*, 2013). Cancer of the lung was not included in the study by Christensen *et al.* (2013), but cases were recruited during two different periods, 1980–86 and 1995–2001. A pooled analysis of both studies (Vizcaya *et al.*, 2013; Siemiatycki, 1991) included 1313 male cases and 1225 male controls. Odds ratios were adjusted for age, smoking habits, education, socioeconomic status, ethnicity, exposure to eight known carcinogens and study period. Odds ratios were 1.7 (95% CI, 0.9–3.4) for any level of exposure to trichloroethylene and 1.1 (95% CI, 0.5–2.7) for substantial exposure. When stratified by histological subtype, a significantly increased risk of adenocarcinoma was reported for substantial exposure to trichloroethylene (OR, 2.7; 95% CI, 1.0–7.6).

2.4 Ecological studies

Several ecological studies have evaluated the risk of cancer associated with consumption of drinking-water contaminated by trichloroethylene and tetrachloroethylene; however, since these chemicals are volatile, exposure may occur through other routes, such as inhalation. One study evaluated indoor air pollution (ATSDR, 2006), and another study did not specify exposure route (Coyle *et al.*, 2005). Despite the different methodologies and cancer sites evaluated, these studies shared several limitations in the exposure assessments carried out, which suggested that the results should be interpreted with caution. First, no estimates of personal exposure were made; although measurements in the environment were taken in some studies, all exposure estimates were based on ecological approaches based on the address of residence. Second, the measurements available were mostly taken at the same time as the diagnosis or death, contributing to exposure misclassification. Third, most of the studies were based on residence at time of diagnosis or death, assuming that study subjects had been living in the same place for the relevant time-points of exposure. Fourthly, since exposure to trichloroethylene usually occurred simultaneously with exposure to tetrachloroethylene, other solvents and volatile chemicals, it was difficult to attribute the observed effects to a single chemical. Finally, the lack of covariates and adjustment for potential confounders in ecological studies may lead to confounding in risk estimates.

An ecological study was carried out in the Endicott area (Broome County, New York, USA), which had experienced contamination of groundwater by volatile organic compounds (VOCs), including trichloroethylene and tetrachloroethylene, originating from leaks, spills and runoff from local landfills (ATSDR, 2006, 2008). In some areas, groundwater pollution contaminated the adjacent soil vapour, which migrated through the soil into structures through cracks in building foundations (soil vapour intrusion). In the eastern study area, trichloroethylene was the most commonly found vapour intrusion-related contaminant in indoor air, at concentrations ranging from 0.18 to 140 mg/m^3. In the western study area, tetrachloroethylene was the most commonly found vapour intrusion-related contaminant in indoor air, at concentrations ranging from 0.1 to 3.5 mg/m^3. The study was conducted to evaluate the incidence of cancer in 1980–2001 and determine whether rates in the Endicott area differed from those in the rest of the state for the same years. Incidence data were obtained from the New York State cancer registry, and age-adjusted standardized incidence ratios

were calculated by dividing the observed number of cancer cases by the expected number. The total number of cancers for the study period (n = 347) was similar to that expected. The incidence of cancer of the testes was significantly elevated in the western study area (where concentrations of trichloroethylene were lower), while cancer of the kidney in men was significantly elevated in the eastern study area (where concentrations of trichloroethylene were higher). For the two geographical areas combined, the incidence of cancer of the testes, and incidence of cancer of the kidney in men and women combined, was significantly elevated (ATSDR, 2006). Childhood cancer (including ages 0–19 years) was evaluated separately. No significant increase in the incidence of leukaemia among children was noted in the study areas, nor was there any significant elevation in the incidence of overall or specific cancers among children during this period. The re-evaluation conducted in 2008 (ATSDR, 2008) limited the analysis to white individuals and showed little differences in overall cancer rates or standardized incidence ratios. The only difference was that cancer of the lung was borderline statistically significantly elevated. This re-evaluation showed also evidence for increased prevalence of smoking among those with cancer of the kidney, and some indication that several individuals diagnosed with testicular or kidney cancer may have been recent arrivals to the study area.

Coyle *et al.* (2005) conducted an ecological study in Texas, USA, to evaluate the influence of releases of some industrial chemicals, including trichloroethylene and tetrachloroethylene, on the incidence of cancer of the breast in 1995–2000. Assessment of exposure from air used data from the Toxic Release Inventory (a publicly accessible database compiled by the United States Environmental Protection Agency (EPA)). Counties were classified as exposed for a specific chemical if a release was reported to the Toxic Release Inventory. A total of 54 487 cases were identified from the Texas cancer registry. Counties reporting releases of trichloroethylene showed statistically significant increases in age-adjusted incidence of cancer of the breast compared with counties without reported releases (P = 0.010). Counties reporting releases of tetrachloroethylene also showed a higher incidence of cancer of the breast (P = 0.038). Specific exposure routes and levels of exposure were not provided (Coyle *et al.*, 2005).

Morgan & Cassady (2002) evaluated incident cases of cancer diagnosed between 1 January 1988 and 21 December 1998 in San Bernardino County, covering the greater Redlands area, USA. The drinking-water supply for the city of Redlands was contaminated by trichloroethylene and tetrachloroethylene, as confirmed by monitoring of wells from 1980, which detected trichloroethylene at concentrations of > 5 parts per billion (ppb). Concentrations of tetrachloroethylene in 2001 ranged from 5 to 98 ppb, although the city of Redlands had not delivered water containing tetrachloroethylene in excess of 18 ppb since testing began. The observed number of cases of cancer divided by the expected number defined the standardized incidence ratio, which was calculated for all cancer sites and 16 site-specific cancers. All cancer sites combined accounted for 3098 cases. More cases than expected were observed for cancer of the uterus (n = 124; SIR, 1.35 [95% CI, 1.06–1.70]) and skin melanoma (n = 137; SIR, 1.42 [95% CI, 1.13–1.77]). There were no significant differences between observed and expected numbers of cases for all cancers [combined], cancer of the thyroid (n = 40), or 11 other cancers. Significantly fewer than expected cases of cancer of the lung and bronchus were observed (n = 356; SIR, 0.71 [95% CI, 0.61–0.81]), and of cancer of the colon and rectum (n = 327; SIR, 0.86 [95% CI, 0.74–0.99]) (Morgan & Cassady, 2002).

A study conducted in 1979–87 in New Jersey, USA, included 75 towns (Cohn *et al.*, 1994), of which 27 were included in a study reported by Fagliano *et al.* (1990). Concentrations of

trichloroethylene were measured in 1984–85, and an average concentration was assigned to each town. The highest concentration assigned was 67 µg/L. Co-existing chemicals included tetrachloroethylene (maximum, 14 ppb) and other volatile chemicals. The water supply of six towns contained trichloroethylene at concentrations of > 5 µg/L (average, 23.4 µg/L). The total incidence of leukaemia in women in these towns was significantly higher than in towns where the concentration of trichloroethylene in drinking-water was < 0.1 µg/L (RR, 1.4; 95% CI, 1.1–1.9); no such effect was seen for men (RR, 1.1; 95% CI, 0.84–1.4). The risk for women was particularly elevated for acute lymphocytic leukaemia, chronic lymphocytic leukaemia, and chronic myelogenous leukaemia. The risk of acute lymphocytic leukaemia in childhood was also significantly increased, in girls but not in boys. Increased risks of non-Hodgkin lymphoma were apparent in towns in the highest category of contamination with trichloroethylene (RR, 0.2; 0.94–1.5 for men; and RR, 1.4; 95% CI, 1.1–1.7 for women) and was particularly elevated for high-grade lymphoma.

Vartiainen *et al.* (1993) collected 24-hour urine samples from 95 and 21 inhabitants of two Finnish villages where the groundwater was contaminated with trichloroethylene (≤ 212 µg/L) and tetrachloroethylene (≤ 180 µg/L). The average excretion of trichloroethylene by inhabitants of the two villages was 0.55 and 0.45 µg/day, and that of two control groups was 0.36 and 0.32 µg/day; the corresponding figures for excretion of dichloroacetic acid were 0.78 and 1.3 µg/day versus 1.3 and 1.3 µg/day, and those for the excretion of trichloroacetic acid were 19 and 7.9 µg/day versus 2.0 and 4.0 µg/day. With the possible exception of non-Hodgkin lymphoma, which occurred in marginal excess in one of the villages (SIR, 1.4; 95% CI, 1.0–2.0; 31 cases), but not in the other (0.6; 95% CI, 0.3–1.1; 14 cases), neither overall cancer incidence nor the incidence of cancer of the liver or lymphohaematopoietic cancers was increased in the two villages compared with the control groups (Vartiainen *et al.*, 1993).

Studies were conducted in two counties in Arizona, USA, to address the possible association between consumption of drinking-water from trichloroethylene-contaminated wells and childhood leukaemia (Maricopa County, Flood *et al.*, 1990), or all childhood cancers and cancer of the testes (Pima County, Kioski *et al.*, 1990). In Maricopa County, two wells that were occasionally used to supplement the water supply were found to contain trichloroethylene at concentrations of 8.9 and 29.0 ppb [mg/L] in 1982; they were then taken out of service. The concentrations of trichloroethylene in contaminated municipal wells in Pima County were 1–239 ppb, with levels as high as 4600 ppb in wells at an Air Force facility in the area. No association was found between cancer at any of the sites examined and residence in the counties with contaminated wells, as opposed to residence in other areas of the county. The incidence rates in Maricopa and Pima counties were similar to those in other areas included in the United States SEER programme (Arizona Department of Health Services, 1995)

Mallin (1990) investigated incident cases of and deaths from cancer of the bladder among residents of eight north-western Illinois counties where a cluster of cases of cancer of the bladder had been observed in 1978–85. Incidence data from the Illinois State cancer registry (available from 1985) and medical records from hospitals in the study area were abstracted to identify incident cases. Expected numbers of cases were based on census data. Age-adjusted standard incidence ratios were calculated by county of residence and zip code (n = 97). Results revealed no excess risks by county, but there were two zip codes associated with significantly elevated risks, one of these had a significant excess in men (SIR, 1.5; 95% CI, 1.1–1.9) and women (SIR, 1.9; 95% CI, 1.2–2.8). This excess was primarily confined to one town in which standardized incidence ratios were

significantly elevated in men (SIR, 1.7; 95% CI, 1.1–2.6) and women (SIR, 2.6; 95% CI, 1.2–4.7). Further investigation revealed that one of four public drinking-water wells in this town had been closed due to contamination and that tests of two other wells revealed traces of trichloroethylene, tetrachloroethylene, and other solvents (Mallin, 1990).

Lagakos *et al.* (1986) studied childhood leukaemia in Woburn, a community in Massachusetts, USA, where water from two wells was contaminated with trichloroethylene and tetrachloroethylene, as well as other chemicals. Measurements made in 1979 showed that well-water contained trichloroethylene at a concentration of 267 ppb [µg/L], tetrachloroethylene at 21 ppb, and also arsenic at 2 ppb and chloroform at 11.8 ppb. [Exposure was estimated by algorithm.] Twenty cases of childhood leukaemia were diagnosed in the community in 1964–83, and these children had a significantly higher estimated cumulative exposure to water from the two contaminated wells than a random sample of children from the community (observed cumulative exposure, 21.1; expected cumulative exposure, 10.6; $P = 0.03$) (Lagakos *et al.* 1986).

Isacson *et al.* (1985) tabulated the average annual age-adjusted incidence rates for cancers of the bladder, breast, colon, lung, prostate or rectum per 100 000 population in towns in Iowa, USA, in 1969–81, by concentration of detectable VOCs in finished ground-water supplies. The concentrations of trichloroethylene were < 0.15 µg/L in one group of areas, and ≥ 0.15 µg/L in another. The concentrations of tetrachloroethylene were < 0.30 µg/L in one group of areas, and ≥ 0.30 µg/L in another. Other volatile chemicals present were 1,2-dichloroethane and 1,1,1-trichloroethane. There were virtually no differences in cancer incidence between these two groups for trichloroethylene and tetrachloroethylene (Isacson *et al.*, 1985).

2.5 Meta-analyses and pooled analyses

2.5.1 Cancer of the kidney and non-Hodgkin lymphoma

Several meta-analyses of the epidemiological literature on risk of cancer among persons exposed to trichloroethylene were available to the Working Group. The Working Group selected for discussion those meta-analyses that were recent and comprehensive, and assembled, presented, or analysed the literature in ways beyond the text of the individual publications, and that provided new information to the Working Group for their evaluation.

The EPA conducted a meta-analysis of epidemiological studies focusing on non-Hodgkin lymphoma and cancers of the kidney and liver as part of its evaluation of the carcinogenicity of trichloroethylene (Scott & Jinot, 2011). The meta-analysis followed an approach recommended by the National Research Council (2006). Criteria for inclusion in the meta-analysis included: (1) cohort or case–control design; (2) appropriate comparability of exposed and unexposed in cohort studies, and cases and controls in case–control studies; (3) potential for exposure to trichloroethylene and an actual estimate of exposure for individuals in the study; and (4) estimates of relative risk for non-Hodgkin lymphoma and cancers of the liver and kidney. Twenty-four studies met the inclusion criteria. Fixed- and random-effects models were fitted to data on overall exposure and on the highest exposure group. Sensitivity analyses examined the influence of individual studies and selection of alternative risk estimates from the publications. [It is important to point out that two studies with very high relative risks, (Henschler *et al.*, 1995, and Vamvakas *et al.*, 1998), were not included in this meta-analysis as they did not meet the inclusion criteria due to incomplete cohort identification or potential selection bias of study

controls.] Although information on smoking was not available in all the studies included, the meta-relative-risk (meta-RR) for cancer of the lung was 0.96 (95% CI, 0.76–1.21) indicating that, overall, smoking was not elevated among the individuals exposed to trichloroethylene in these studies. Fixed- and random-effects models were used in the primary analyses to calculate summary meta-relative-risk estimates for overall exposure to trichloroethylene and for the groups with highest exposure. Sensitivity analyses were conducted to evaluate the effect of including alternative risk estimates (when multiple estimates were available from a study) and to examine the impact of individual studies on the summary estimates. Heterogeneity among the studies was assessed using the Q-statistic and inconsistency between studies was assessed with the I^2 value. Publication bias was assessed in several ways, including funnel plots, the "trim and fill" procedure, forest plots of studies sorted by standard error, and cumulative meta-analyses of studies sorted by standard error. There was no major heterogeneity in overall exposure for any cancer. No single study was overly influential for any cancer. There was no evidence of publication bias for cancers of the kidney and liver, but there was a relationship between relative risk and study size for non-Hodgkin lymphoma. Overall meta-RRs for those exposed to trichloroethylene were 1.27 (95% CI, 1.13–1.43) for cancer of the kidney, 1.29 (95% CI, 0.07–1.56) for cancer of the liver and intrahepatic bile ducts, and 1.23 (95% CI, 1.07–1.42) for non-Hodgkin lymphoma. An adjustment technique to control for possible publication bias reduced the meta-RR for non-Hodgkin lymphoma to 1.15 (95% CI, 0.97–1.36). Meta-RRs for individuals in the groups with higher exposure for each study were higher than all-exposed combined. The meta-RRs in the categories of highest exposure were 1.58 (95% CI, 1.28–1.96) for cancer of the kidney, and 1.64 (95% CI, 1.31–2.04) when combining the 10 studies that reported results by exposure level), but similar for liver and intrahepatic bile ducts (1.28; 95% CI, 0.93–1.77), and 1.43 (95% CI, 1.13–1.82) for non-Hodgkin lymphoma.

Karami *et al.* (2012) conducted a meta-analysis of 15 cohort studies and 13 case–control studies evaluating the risk of cancer of the kidney associated with occupational exposure to trichloroethylene. [The Working Group noted that this meta-analysis largely overlapped with that by Scott & Jinot (2011), but included a slightly different set of studies due to updates or new publications]. Karami *et al.* (2012) included studies that specifically evaluated exposure from trichloroethylene, degreasing agents, or chlorinated solvents. Studies were classified by exposure to either trichloroethylene or chlorinated solvents. Studies of dry-cleaning workers were excluded, except for the one study that focused specifically on trichloroethylene. Also excluded were studies reporting proportionate mortality ratios only, and those not providing confidence intervals or numbers of observed and expected cases. Meta-RRs were estimated using a random-effects model. Heterogeneity across studies was evaluated by Higgin's I^2 statistic and Cochrane's Q test. No evidence for publication bias was found using Egger and Begg methods and funnel plots. The meta-RR for cancer of the kidney from cohort studies was 1.41 (95% CI, 0.98–2.05), and 1.26 (95% CI, 1.02–1.56) when the study by Henschler *et al.* (1995) was excluded. The meta-RR for case–control studies was 1.55 (95% CI, 1.18–2.05), and 1.35 (95% CI, 1.17–1.57) when the study by Vamvakas *et al.* (1998) was excluded. The combined relative risk for cohort and case–control studies was 1.41 (95% CI, 1.16–1.70). The meta-RR for exposure to chlorinated solvents was 1.13 (95% CI, 0.70–1.83). [The Working Group noted that exposure to other chlorinated solvents did not explain the association between trichloroethylene and cancer of the kidney in cohort studies, but it might in case–control studies.] The meta-RR from case–control studies was 1.96 (95% CI, 1.24–3.08)

for high-exposure groups and 1.55 (95% CI, 1.05–2.28) for low-exposure groups. Evaluation by year of publication found that meta-RRs were stronger when more recent publications were included, and it was suggested that this might reflect improved exposure assessment and less exposure misclassification. [The Working Group noted that studies with information on tobacco use were not selected for a separate analysis, although some case–control studies could adjust for smoking. The Working Group also noted that this meta-analysis included a subgroup of the exposed population from the Danish study.]

An early review by Wartenberg *et al.* (2000) found a meta-RR of 1.7 (95% CI, 1.1–2.7) for cancer of the kidney, and a meta-RR of 1.5 (95% CI, 0.9–2.3) for non-Hodgkin lymphoma. Kelsh *et al.* (2010) reviewed 23 studies on trichloroethylene and reported meta-RRs for cancer of the kidney of 1.24 (95% CI, 1.06–1.45) from all cohort studies with outlier data removed, 1.57 (95% CI, 1.06–2.30) for case–control studies, 1.34 (95% CI, 1.07–1.67) for cohort studies with higher-quality exposure data, and 0.88 (95% CI, 0.58–1.33) for cohort studies with lower-quality exposure data with outlier data removed. Mandel *et al.* (2006) in a meta-analysis of 18 studies (14 cohort and 4 case–control) of non-Hodgkin lymphoma reported meta-RRs of 2.33 (95% CI, 1.39 to 3.91) for non-Hodgkin lymphoma from studies with higher-quality exposure data, 0.84 (95% CI, 0.73–0.98) from studies with lower-quality exposure data, and 1.39 (95% CI, 0.62–3.10) from case–control studies.

2.5.2 Other cancers

In the meta-analysis by Wartenberg *et al.* (2000) (cited above), studies were classified into tier I (studies in which exposure to trichloroethylene is best characterized and exposure inferred for individual study subjects). For tier I studies, meta-RRs were 1.5 (95% CI, 0.6–3.7) for Hodgkin lymphoma, 1.0 (95% CI, 0.5–2.1) for leukaemia, 1.5 (95% CI, 0.7–3.3) for multiple myeloma, 1.9 (95% CI, 1.0–3.4) for cancer of the liver, 1.0 (95% CI, 0.6–1.6) for cancer of the bladder, 2.4 (95% CI, 1.2–4.8) for cancer of the cervix, and 0.8 (95% CI, 0.6–1.1) for cancer of the lung. For tier II studies (studies in which there is putative exposure to trichloroethylene, but individuals are not identified as uniquely exposed to trichloroethylene), meta-RRs were 1.0 (95% CI, 0.5–2.1) for cancer of the bladder, and 0.6 (95% CI, 0.3–1.3) for cancer of the lung.

Alexander *et al.* (2007) reported meta-RRs for higher quality studies of 1.30 (95% CI, 1.09–1.55) for cancers of the liver and biliary tract combined, and 1.41 (95% CI, 1.06–1.87) for primary cancer of the liver. The meta-RR for lower-quality studies was 0.87 (95% CI, 0.55–1.38) for cancers of the liver and biliary tract. Alexander *et al.* (2006) reported a meta-RR of 1.05 (95% CI, 0.80–1.38) for multiple myeloma, and 1.11 (95% CI, 0.93–1.32) for leukaemia.

[The Working Group noted that the lack of an excess for cancers of the lung and bladder indicated that smoking did not explain the excesses observed for other cancers; if individuals exposed to trichloroethylene were heavier smokers than the comparison populations, an excess of cancer of the lung would be observed.]

3. Cancer in Experimental Animals

Several recent reports have summarized the evidence for carcinogenicity associated with exposure to trichloroethylene (EPA, 2011a, b; NTP, 2011a). In most studies in rodents (i.e. rats and mice, with one study in hamsters), trichloroethylene has been administered by inhalation, or orally by gavage rather than in drinking-water, because of its volatility.

Studies of exposure to trichloroethylene in rats and mice are summarized in Table 3.1 and Table 3.2, respectively. Information is provided

on the experimental paradigm, tumour incidence, statistical significance of findings, and comments. These studies were carried out over 26 years and varied with regard to completeness of reporting, statistics performed, and nomenclature used to describe tumour pathology. In some earlier studies, the number of individual tumour diagnoses was presented for each exposure group (rather than the incidence of tumour-bearing animals). The presence of stabilizers is indicated due to concerns regarding their potential contribution to carcinogenicity.

3.1 Mouse

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given epichlorohydrin-free trichloroethylene at a dose of 0 or 1000 mg/kg bw per day by gavage in corn oil, 5 days per week, for 103 weeks (NTP, 1990). Survival was poor in males and considered to have resulted from "toxic nephropathy." [The Working Group noted the limited power of the study due to the use of a single dose and the premature mortality observed.]

Groups of male and female B6C3F$_1$ mice (age, 35 days) were given commercial-grade trichloroethylene (containing epichlorohydrin as a stabilizer) by gavage in corn oil at time-weighted average doses of 0 (n = 20), 1169 (n = 50), or 2339 (n = 50) mg/kg bw per day for males, and 0 (n = 20), 869 (n = 50), or 1739 (n = 50) mg/kg bw per day for females, 5 days per week; the mice were exposed non-continuously at variable concentrations for 78 weeks, and surviving animals were killed at 90 weeks (NCI, 1976). Survival was lower in males than in females, and to a greater extent, in treated versus vehicle controls (male mortality before termination: 12 out of 20 controls, 15 out of 50 at the lower dose, and 27 out of 50 treated with the higher dose; female mortality: 0 out of 20 controls, 8 out of 50 at the lower dose, and 8 out of 47 at the higher dose). The decreased survival in male mice treated with trichloroethylene at the higher dose was attributed to the presence of hepatocellular tumours. [The Working Group noted that "toxic nephrosis" may have also contributed to premature mortality]. An increased incidence of hepatocellular carcinoma was reported in males and females with decreased latency of tumour development also reported for males at the higher dose. Increased compound-related "toxic nephrosis" was reported in both sexes (> 90% at both doses in both sexes), but without further definition by the study authors. While control males were noted to have specific diagnoses of hydronephrosis, chronic inflammation, pyelonephritis, and amyloidosis, none were diagnosed with "toxic nephrosis." No kidney pathology was noted for female controls. A single male treated with the higher dose was diagnosed with a rare renal tubule adenoma. The incidence of malignant lymphoid tissue tumours and bronchiolo-alveolar tumours was elevated in both sexes, although not significantly. [The Working Group noted the low number of controls (n = 20), some reporting deficiencies, and early mortality in trichloroethylene-treated groups that decreased the sensitivity of the assay to detect a response. In addition, study animals were housed in rooms together with animals exposed to volatile agents, but the Working Group did not find an apparent effect on the tumour response.]

Groups of male B6C3F$_1$ mice (age, 8 weeks) were given commercial-grade trichloroethylene at doses of 0 (n = 10) or 800 (n = 75) mg/kg bw per day by gavage in corn oil, 5 days per week, for 76 weeks (Anna *et al.*, 1994). [No indication was given regarding purity or presence of stabilizers in the trichloroethylene administered, but commercial-source information was provided.] Only liver was examined for histopathology. Treatment with trichloroethylene increased the incidence of hepatocellular adenoma and hepatocellular carcinoma (P < 0.05). [The Working

Table 3.1 Studies of carcinogenicity with trichloroethylene in mice

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wk NTP (1990)	Gavage 0, 1000 mg/kg bw in corn oil 1/day, 5 days/wk, 50/group per sex	*Males*		Epichlorohydrin-free trichloroethylene (purity, 99.9%) Significantly reduced survival of treated males ($P = 0.004$) (toxic nephropathy). Decreased latency of hepatocellular tumours in exposed males; hepatocellular carcinomas observed after 57 wk in treated group and in controls after 75 wk. Statistical tests: compared with controls: [a]life table, [b]incidental tumour test, [c]Fisher exact test
		Hepatocellular adenoma: 7/48, 14/50[a,b]	[a]$P = 0.002$, [b]$P = 0.048$	
		Hepatocellular carcinoma: 8/48, 31/50[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Hepatocellular adenoma or carcinoma (combined): 14/48, 39/50[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Harderian gland adenoma: 0/48, 4/48[a]	[a]$P = 0.044$	
		Renal tubule adenoma: 1/49, 0/50	NS	
		Renal tubule carcinoma: 0/49, 1/40	NS	
		Females		
		Hepatocellular adenoma: 4/48, 16/49[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.003$	
		Hepatocellular carcinoma: 2/48, 13/49[a,b,c,]	[a]$P < 0.001$, [b]$P < 0.002$, [c]$P < 0.002$	
		Hepatocellular adenoma or carcinoma (combined): 6/48, 22/49[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Malignant lymphoma: 7/48, 13/49[a]	[a]$P = 0.047$	
		Lymphoma or leukaemia (combined): 7/48, 14/49[a]	[a]$P = 0.032$	
		Leukaemia: 0/48, 1/49	NS	
		Bronchioloalveolar adenoma: 0/48, 4/48[a]	[a]$P = 0.040$	
		Bronchioloalveolar carcinoma: 1/48, 0/48	NS	
		Harderian gland adenoma: 0/48, 3/49	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 90 wk NCI (1976)	Gavage 0, 1169, 2339 mg/kg bw in corn oil (M) 0, 869, 1739 mg/kg bw in corn oil (F) 1/d, 5 d/wk for 78 wk 20, 50, 50	*Male*		Commercial-grade trichloroethylene (purity > 99%), stabilized with 0.09% epichlorohydrin and 0.19% 1,2-epoxybutane. Initial doses of 1000 and 2000 mg/kg bw for males and 700 and 1400 mg/kg bw for females were increased after 12 wk (TWA doses, 1169 and 2339 mg/kg bw for males and 869 and 1739 mg/kg bw for females). Study animals were housed with animals exposed to volatile agents. For males, 12/20 controls, 15/50 low-dose, and 27/50 high-dose died before scheduled termination. For females, 0/20 controls, 8/50 low-dose, and 8/47 high-dose died before scheduled termination. Increased mortality in treated males was attributed to the hepatocellular tumours. In highest-dose male mice, the first hepatocellular carcinomas were seen at 27 wk; nine others were found in males dying by wk 78. Early occurrence was not observed in low-dose males or in females. Agent-related increase in the incidence of "toxic nephrosis" (> 90% at both doses in both sexes). Elevated incidences of malignant lymphoid tissue tumours and bronchioloalveolar tumours in both sexes, although not significant. Statistical tests: Trend: [d]age-adjusted tests (Tarone) for linear trend Compared with control: [e]differences between treated and matched control: modifications of Cox and Tarone tests.
		Renal tubule adenoma: 0/20, 0/50, 1/48	NS	
		Hepatocellular carcinoma: 1/20[d], 26/50[e], 31/48[e]	[d]$P < 0.001$, [e]$P = 0.04$, [e]$P = 0.001$	
		Malignant lymphoma, reticulum-cell sarcoma or lymphosarcoma [malignant lymphoid tissue tumours]: 1/20, 4/50, 2/48	NS	
		Bronchioloalveolar adenoma: 0/20, 5/50, 1/48	NS	
		Bronchioloalveolar carcinoma: 0/20, 0/50, 1/48	NS	
		Bronchioloalveolar adenoma or carcinoma (combined): 0/20, 5/50, 2/48	NS	
		Female		
		Hepatocellular carcinoma: 0/20[d], 4/50, 11/47[e]	[d]$P = 0.002$, [e]$P = 0.008$	
		Malignant lymphoma, reticulum-cell sarcoma or lymphosarcoma [malignant lymphoid tissue tumours]: 1/20, 5/50, 6/47	NS	
		Bronchioloalveolar adenoma: 1/20, 2/50, 5/47	NS	
		Bronchioloalveolar carcinoma: 0/20, 2/50, 2/47	NS	
		Bronchiolo alveolar adenoma or carcinoma (combined): 1/20, 4/50, 7/47	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M) 76–77 wk Anna *et al.* (1994)	Gavage 0, 800 mg/kg bw in corn oil, 1/d, 5 d/wk for 76 wk 10, 75	Hepatocellular adenoma: 2/10, 50/75* Hepatocellular adenoma (average number of tumours/mouse): 0.2/mouse, 1.27/mouse Hepatocellular carcinoma: 0/10, 30/75* Hepatocellular carcinoma (average number of tumours/mouse): 0/mouse, 0.57/mouse	*[$P < 0.05$] NR *[$P < 0.05$] NR	Trichloroethylene, commercial grade. No indication of stabilizer presence or purity. Anna *et al.* (1994): text reports 800 mg/kg bw as daily exposure level, but Table 1 reports 1700 mg/kg bw. Only liver tumours were examined histopathologically. All treated mice were alive at wk 76.
Mouse, B6C3F$_1$ (M) 79 wk Bull *et al.* (2002)	Gavage 0, 1000 mg/kg bw in 5% aqueous solution of Alkamuls, 1/d, 7 d/wk 15, 40	Liver, gross lesions identified as "tumours": 3/15, 33/40* Hepatocellular adenoma: 2/15, 23/36 Hepatocellular carcinoma: 1/15, 7/36	*$P < 0.05$ NR NR	Purity and source, NR. Only liver tumours were examined. Statistics: Tumour incidence compared using Fisher exact test.
Mouse, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	Drinking-water 0 (NaCl control), 22–40 mg/l [volatilization reduced concentration from 40 to 22 mg/L after 3 d] 22, 32	Hepatocellular adenoma: 2/22, 3/32 Hepatocellular carcinoma: 0/22, 3/32	NS NS	Purity, 99%. No indication of stabilizer addition. Only liver tumours were examined histopathologically.
Mouse, Swiss Ha/ICR (M, F) 89 wk Van Duuren *et al.* (1979)	Gavage 1 d/wk 0 (control), 0.5 mg/mouse in 0.1 mL trioctanoin 30/group	Forestomach papilloma: 0/30, 1/30 (M) 0/30, 2/30 (F)	 NS NS	Industrial grade. Purified, with no indication of stabilizer addition. Only lung, liver and stomach were examined histopathologically; only data for forestomach tumours were presented.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) Lifespan Maltoni *et al.* (1986, 1988) Male (Exp. BT306 bis) Female (Exp. BT306)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 78 wk 90 M and 90 F/group	Hepatoma [malignant] (M): 17/90, 20/90, 27/90, 21/90 Hepatoma [malignant] (F): 3/90, 4/90, 4/90, 9/90 Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (F): 4/90, 6/90, 7/90, 15/90* Pulmonary adenocarcinoma (M): 0/90, 1/90, 0/90, 0/90	NS NS *$P < 0.05$ (Fisher exact test) NS	Highly purified and epoxide-free trichloroethylene was used with butyl-hydroxy-toluene added as stabilizer. Experiment [using mice from NCI source] (BT306) was repeated with males [from Charles River Laboratory] (BT306 bis) due to excessive mortality in males from fighting. The incidence of hepatoma increased in males (BT306 bis) and females (BT306). Incidence of pulmonary tumours increased in females primarily from later-stage adenomas. The previous IARC Working Group (IARC, 1995) found dose-related increases in the incidence of pulmonary tumours in females (Cochran-Armitage linear trend text).
Mouse, Swiss (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT305)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 78 wk 90/group/sex	Hepatoma [malignant] (M): 4/90, 2/90, 8/90, 13/90* Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (M): 10/90, 11/90, 23/90*, 27/90** Pulmonary adenocarcinoma (M): 0/90, 0/90, 0/90, 1/90 Hepatoma [malignant] (F): 0/90, 0/90, 0/90, 1/90 Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (F): 15/90, 15/90, 13/90, 20/90	*$P < 0.05$ (Fisher exact test) *$P < 0.05$, **$P < 0.01$ (Fisher exact test) NS NS NS	Highly purified (purity, 99.9%) and epoxide-free trichloroethylene was used with butyl-hydroxy-toluene added as stabilizer. The previous IARC Working Group (IARC, 1995) found dose-related increases in the incidences of lung and liver tumours in males (Cochran-Armitage linear trend test).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, Swiss (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT303)	Inhalation 0, 100, 600 ppm, 7 h/d, 5 d/wk for 8 wk 100, 60, 72	Hepatoma [malignant] (M): 1/100, 3/60, 4/72	NS	Highly purified (purity, 99.9%) and epoxide-free trichloroethylene, with butyl-hydroxy-toluene added as stabilizer.
Mouse, Crj:CD-1 (ICR) (F) 107 wk Fukuda *et al.* (1983)	Inhalation 0, 50, 150, 450 ppm, 7 h/d, 5 d/wk, for 104 wk 50/group	Lung adenocarcinoma: 1/49, 3/50, 8/50*, 7/46*	*$P < 0.05$	Reagent-grade trichloroethylene (purity, 99.8%) containing 0.13% carbon tetrachloride, 0.02% benzene and 0.02% epichlorohydrin. Only one hepatocellular adenoma at highest dose, none in other dose groups).Treatment-related increase in lung tumours observed. The previous IARC Working Group (IARC, 1995) found a significant dose–response trend: $P = 0.034$, Cochran-Mantel-Haenszel test.

d, day; mo, month; NR, not reported; NS, not significant; wk, week; yr, year.

Table 3.2 Studies of carcinogenicity with trichloroethylene in rats

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, F344/N (M, F) 103 wk NTP (1990)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group per sex	*Males* Kidney carcinoma: 0/48[d,e,f], 0/49, 3/49[a,b] Kidney adenoma or carcinoma (combined): 0/48[d,e], 2/49, 3/49[a,b] Peritoneum, malignant mesothelioma: 1/50, 5/50[a], 0/49 Peritoneum, all mesothelioma: 1/50, 5/50[a], 1/49	[d]$P = 0.009$, [e]$P = 0.009$, [f]$P = 0.038$, [a]$P = 0.028$, [b]$P = 0.028$ [d]$P = 0.019$, [e]$P = 0.030$, [a]$P = 0.028$, [b]$P = 0.028$ [a]$P = 0.042$ [a]$P = 0.042$	Epichlorohydrin-free trichloroethylene (purity, 99.9%). Significantly reduced survival in trichloroethylene-treated groups compared with vehicle controls due to chronic "toxic nephropathy". High mortality due to gavage error: 1 male control, 3 low-dose males, 10 high-dose males, 2 female controls, 5 low-dose females, and 5 high-dose female rats were killed. Renal tubular adenocarcinoma occurred in a single female at the highest dose. Statistical tests: Compared with controls: [a]life table, [b]incidental tumour test, [c]Fisher exact test Trend tests: [d]life table, [e]incidental tumour test, [f]Cochran-Armitage trend test.
Rat, Osborne-Mendel (M, F) 110 wk NCI (1976)	Gavage 0, 549, 1097 mg/kg bw in corn oil, 1/d, 5 d/wk for 78 wk Non-continuous exposure (cycle of 1 wk untreated followed by 4 wk of treatment) 20, 50, 50	*Males* Kidney carcinoma: 0/20, 1/50, 0/50 *Females* Mammary gland fibroadenoma (multiple): 0/20, 1/45, 3/48	NS NS	Commercial-grade trichloroethylene (purity, > 99%), stabilized with 0.09% epichlorohydrin and 0.19% 1,2-epoxybutane. Doses were changed after 7 and 16 weeks of treatment based on monitoring of body-weight changes and survival. Study animals were housed with animals exposed to volatile agents. High incidence of chronic respiratory disease. Chronic "toxic nephropathy" observed in treated rats of both sexes. For males, 17/20 control, 42/50 low-dose, and 47/50 high-dose animals died before scheduled termination. For females, 12/20 control, 35/48 low-dose, and 37/50 high-dose animals died before scheduled termination. Reporting was inconsistent. [The Working Group considered that the slight increase in the incidence of multiple mammary fibroadenoma was associated with exposure.]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Osborne-Mendel (M, F) 103 wk NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males*		trichloroethylene (99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathy" observed in treated rats of both sexes, fatal gavage error increased in treated males. Statistical tests: Compared with controls: [a]Life Table, [b]incidental tumour test, [c]Fisher exact test Trend tests: [d]life table, [e]incidental tumour test, [f]Cochran-Armitage trend test.
		Kidney adenoma: 0/50, 6/50[a,b,c], 1/50	[a]$P = 0.007$, [b]$P = 0.007$, [c]$P = 0.013$	
		Kidney carcinoma: 0/50, 0/50, 1/50	NS	
		Kidney adenoma or carcinoma (combined): 0/50, 6/50[a,b,c], 2/50	[a]$P = 0.007$, [b]$P = 0.007$, [c]$P = 0.013$	
		Females		
		Kidney adenoma: 0/50, 0/50, 1/49	NS	
		Bronchioloalveolar adenoma: 0/50, 0/50, 1/50	NS	
		Bronchioloalveolar carcinoma: 0/50, 1/50, 0/50	NS	
		Adrenal cortex adenoma: 16/50[d], 13/50, 19/49[a]	[d]$P = 0.008$, [a]$P = 0.011$	
Rat, ACI (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group per sex	*Males*		Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathy" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a]life table Trend test: [d]life table, [e]incidental tumour test
		Kidney carcinoma: 0/50, 1/49, 0/49	NS	
		Bronchioloalveolar adenoma: 0/50, 2/49, 0/49	NS	
		Testis, benign interstitial cell tumours: 36/49[d,e], 23/49[a], 17/49[a]	[d]$P < 0.001$, [e]$P = 0.019$, [a]$P = 0.024$, [a]$P < 0.001$	
		Females		
		Kidney adenoma: 0/48, 2/47, 0/43	NS	
		Kidney, tubular cell adenocarcinoma [kidney carcinoma] or NOS adenocarcinoma: 0/48, 1/47 (NOS), 1/43	NS	
		Kidney, tubular cell adenocarcinoma [kidney carcinoma] or NOS adenocarcinoma, or adenoma: 0/48, 3/47[a], 1/43	[a]$P = 0.044$	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, August (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males* Kidney adenoma: 0/50, 1/50, 1/49	NS	Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathology" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a] life table, [b] incidental tumour test, Trend tests: [d] life table, [e] incidental tumour test, [f] Cochran-Armitage trend test.
		Kidney carcinoma: 0/50, 1/50, 0/49	NS	
		Subcutaneous tissue, sarcoma: 0/50[d,e], 1/50, 3/49[b]	[d]$P = 0.033$, [e]$P = 0.032$, [b]$P = 0.050$	
		Testis, benign interstitial cell tumours: 34/50, 30/50[a], 26/49	[a]$P = 0.049$	
		Females		
		Kidney adenoma: 1/49, 2/48, 0/50	NS	
		Kidney carcinoma: 0/49, 2/48, 0/50	NS	
		Kidney adenoma or carcinoma (combined): 1/49, 4/48, 0/50	NS	
		Leukaemia (monocytic type or NOS): 1/50[d,e,f], 0/50, 5/50	[d]$P = 0.027$, [e]$P = 0.020$, [f]$P = 0.037$	
		Thyroid, C-cell adenoma or carcinoma (combined): 0/49, 4/49, 1/50	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Marshall (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males* Kidney adenoma: 0/49, 1/50, 0/47 Kidney carcinoma: 0/49, 0/50, 1/47 Testis, benign or malignant interstitial cell tumours: 17/46[d,e,f], 21/48[a,b], 32/48[a,b,c] In one high-dose treated rat, a malignant interstitial cell tumour was diagnosed. All sites, mesothelioma: 2/50, 2/50, 3/50 *Females* Kidney adenoma: 1/50, 1/48, 0/44 Kidney carcinoma: 0/50, 1/48, 1/44 Kidney adenoma or carcinoma (combined): 1/50, 2/48, 1/44	NS NS [d]$P < 0.001$, [e]$P < 0.001$, [f]$P = 0.003$, [a]$P < 0.001$, [b]$P < 0.001$, [a]$P < 0.001$, [b]$P < 0.001$, [c]$P = 0.004$ NS NS NS NS	Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine was used. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathology" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a] life table, [b] incidental tumour test, [c] Fisher exact test Trend tests: [d] life table, [e] incidental tumour test, [f] Cochran-Armitage trend test
Rat, Sprague-Dawley (M, F) Lifespan Maltoni *et al.* (1986) (Exp. BT301)	Gavage 0, 50, 250 mg/kg bw in olive oil, 1/d, 4–5 d/wk for 52 wk 30/group/sex	*Males* Leukaemia (malignant neoplasms of the hemolymphoreticular system): 0/30, 2/30, 3/30	NS	Trichloroethylene was highly purified (purity, 99.9%) and epoxide-free with butyl-hydroxy-toluene (20 ppm) added as stabilizer.

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Sprague-Dawley (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT304 and Exp. BT304 bis)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 2 yr 135, 130, 130, 130 (M) 145, 130, 130, 130 (F)	*Male*		Trichloroethylene was highly purified (purity, 99.9%) and epoxide-free with butyl-hydroxy-toluene (20 ppm) added as stabilizer. Adjusted incidences (EPA, 2011a) reflect the number of rats alive at 47 wk. Rare kidney carcinomas reported for males (4/130) and females (1/130) at the highest dose (600 ppm). One testicular tumour was malignant at the highest dose. Slight dose-related increase in incidences of pituitary adenoma in female rats in BT 304 but not BT304 bis (data not shown). The previous IARC Working Group (IARC, 1995) reported significant, dose-related increase in the incidence of Leydig cell interstitial tumours of the testis ($P < 0.001$; Cochran-Mantel-Haenszel test)
		Kidney carcinoma – overall (corrected): 0/135 (0/122), 0/130 (0/121), 0/130 (0/116), 4/130 (4/124)	[NS]	
		Leukaemia (malignant neoplasms of the haemolymphoreticular system): 9/135, 13/130, 14/130, 15/130	NS	
		Immunoblastic lymphosarcoma [lymphoma]: 1/135, 5/130, 4/130, 2/130	[NS]	
		Testis, interstitial cell tumours (mainly benign): 6/135, 16/130*, 30/130**, 31/130**	*$P < 0.05$, **$P < 0.01$	
		Female		
		Kidney carcinoma – overall (corrected): 0/145 (0/141), 0/130 (0/128), 0/130 (0/127), 1/130 (1/127)	[NS]	
		Leukaemia (malignant neoplasms of the haemolymphoreticular system): 7/145, 9/130, 2/130, 11/130	NS	
		Immunoblastic lymphosarcoma [lymphoma]: 0/145, 4/130*, 1/130, 1/130	*$P < 0.05$	

d, day; mo, month; NOS, not otherwise specified; NS, not significant; wk, week; yr, year

Group noted the limitations in reporting and design of the study.]

Groups of male B6C3F$_1$ mice (age, 6 weeks) were given trichloroethylene (in a 5% aqueous solution of Alkamuls) by gavage at doses of 0 (n = 15), or 1000 (n = 40) mg/kg bw for 79 weeks (Bull *et al.*, 2002). Trichloroethylene purity, source, and presence of stabilizers were not reported. Only liver was examined for treatment-related induction of tumours. The incidence of gross lesions (also identified as liver "tumours") was higher in treated mice ($P < 0.05$) than in controls. Not all liver "tumours" (nodules, hepatocellular adenoma, or hepatocellular carcinoma) were confirmed by histopathological examination of the 40 trichloroethylene-treated mice. However, 1 out of 15 controls and 23 out of 36 treated mice developed hepatocellular adenoma, and 1 out of 15 controls and 7 out of 36 treated mice developed hepatocellular carcinoma. [The Working Group noted the limitations in reporting and design of the study (e.g. differences in the number of animals in control versus treatment groups, low numbers of animals studied, and limited focus on liver lesions).]

Groups of male B6C3F$_1$ mice (age, 4 weeks) were given drinking-water containing sodium chloride at a concentration of 2 g/L (n = 22), or trichloroethylene (purity, 99%) at a concentration of 40 mg/L (n = 32) for 61 weeks (Herren-Freund *et al.*, 1987). Because of volatilization, the concentration of trichloroethylene was reduced to 22 mg/L after 3 days (drinking-water was changed twice per week). Only liver was examined for treatment-related induction of tumours. A few hepatocellular carcinomas were observed in three treated mice, but not in control mice. [The Working Group noted the low number of animals, limited pathology examination, shortened exposure period, and variable concentrations of trichloroethylene, which limited the power of the study to detect a carcinogenic response.]

Groups of 50 male and 50 female Swiss (ICR/HA) mice (age, 5 weeks) were exposed to trichloroethylene in corn oil by daily gavage five times per week for 18 months, and were observed for an additional 6 months (Henschler *et al.*, 1984). Four groups were exposed to purified trichloroethylene (purity, > 99.9%) alone, or with either epichlorohydrin, 1,2-epoxybutane, or a combination of both epichlorohydrin and 1,2-epoxybutane as stabilizers. A fifth group was exposed to industrial-grade trichloroethylene (purity, 99.4% with 0.11% epichlorohydrin, 0.20% 1,2-epoxybutane, 0.05% carbon tetrachloride, and 0.01% chloroform). A control group was exposed to corn oil only. Exposure doses were 2.4 g/kg bw for males and 1.8 g/kg bw for females, but exposure was stopped for all groups during weeks 35–40, 65, and 69–78, and all doses were reduced by a factor of two from the fortieth week onwards because of toxicity attributable to gavage. Survival for females was higher than for males in all groups. The number of tumours diagnosed in all mice for each exposure group was reported, rather than incidence of tumours.

No significant differences between treatment groups were reported, except for increases in the incidence of forestomach tumours in several groups exposed to trichloroethylene and stabilizers. A single renal cystadenoma was diagnosed in an untreated male and none in untreated females, while there was one renal cystadenoma in males and four in females treated with purified trichloroethylene only. There was a slight, not statistically significant, increase in the incidence of lung bronchioloalveolar adenoma in the four groups of females treated with trichloroethylene versus the control groups. The previous Working Group (IARC, 1995) had noted that the incidence of hepatocellular tumours (adenoma and carcinoma combined) in male mice was: 3 out of 50 controls; 6 out of 50 mice treated with purified trichloroethylene; and 9 out of 50 mice treated with industrial-grade trichloroethylene, and that no survival-adjusted analysis of tumour

incidence was performed. The Working Group noted the reporting deficiencies in this study, the non-continuous exposure, uncertainty in diagnostic terminology for kidney tumours, and the attribution of carcinogenic responses to trichloroethylene rather than stabilizers.]

Groups of 30 male and 30 female Swiss (ICR/HA) mice (age, 6–8 weeks) were given purified trichloroethylene at a dose of 0 or 0.5 mg by gavage in 0.1 mL of trioctanoin as a vehicle, once per week, for 79 weeks [no indication of stabilizers, purity not reported] (Van Duuren *et al.*, 1979). Only sections of lung, liver, and stomach were taken for histopathological examination. No excess incidence of tumours was reported. [The Working Group noted that the dose was ~400 times lower than in other gavage studies. The Working Group also noted limitations in the design (e.g. short exposure duration, few animals, and histopathology of only lung, liver, and stomach), and reporting of the study.]

3.1.2 Inhalation

Groups of 90 male and 90 female B6C3F$_1$ mice (age, 12 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0, 100, 300, or 600 ppm for 7 hours per day, 5 days per week, for 78 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Due to excessive fighting and mortality, experiments in males were repeated using a different animal source. "Hepatoma" described all malignant tumours of hepatic cells of different histological subtypes, and of various degrees of malignancy. Diagnoses of pulmonary tumour included adenomatous hyperplasia–early adenoma, adenoma, and adenocarcinoma. Statistically significant increases in the incidence of pulmonary tumours were observed in treated females at the highest dose; increases were reported to be primarily in later-stage adenoma. An increased incidence of hepatoma was observed in males and females, with statistical significance achieved as a combination of tumours between both sexes. [The previous IARC Working Group (IARC, 1995) had found a dose-related increase in the incidence of pulmonary tumours in female B6C3F$_1$ mice (Cochran-Armitage linear trend test). The Working Group noted the poor presentation of data, and that combining descriptors of pulmonary hyperplasia with early adenoma decreased the ability to discern emergence of less common but more malignant neoplastic states.]

In a second experiment in the same report, groups of 90 male and 90 female Swiss mice of unspecified strain (age, 11 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0, 100, 300, or 600 ppm for 7 hours per day, 5 days per week, for 78 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Using the same pathological descriptions as above, an increased incidence of pulmonary tumours was reported in males (statistically significant at the two higher doses; primarily early-stage tumours). Increased incidence of hepatoma was noted for males at the two higher concentrations (statistically significant only at the highest concentration). Overall, a lower background rate and increase in incidence of hepatoma by trichloroethylene was seen in male Swiss mice than in male B6C3F$_1$ mice using the same experimental paradigm. [The previous Working Group (IARC, 1995) had found dose-related increases in the incidence of pulmonary tumours and hepatoma in male Swiss mice (Cochran–Armitage linear trend test). The Working Group noted similar issues concerning data presentation and pathological descriptors as for the studies in B6C3F$_1$ mice discussed above.] In a third experiment in the same report, groups of male and female Han:NMRI Swiss mice (age, 11 weeks) were similarly exposed to epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0 (n = 100), 100 (n = 60) or

600 (n = 72) ppm for a shorter time (8 weeks). A non-significant higher incidence of hepatoma was observed in males, but not females.

Groups of 30 male and 30 female Han:NMRI mice [age not reported] were exposed by inhalation to highly purified trichloroethylene (containing triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and observed for 12 additional months (Henschler *et al.*, 1980). The number of tumours diagnosed in all mice for each exposure group, rather than the tumour incidence, was reported. A separate diagnosis was used for renal cystadenoma and renal adenoma. A single renal adenoma was reported in males at the lower dose, and a single renal adenocarcinoma [renal tubule carcinoma] was diagnosed in the control group. [The Working Group noted that the low frequency of renal tumours in this strain during this time period (one renal adenocarcinoma and one anaplastic carcinoma out of 614 males, with no kidney tumours observed in 591 females) (Bomhard & Mohr, 1989).] Statistically significant increases in age-adjusted incidence ($P < 0.01$) and trend in the incidence ($P < 0.05$; EPA, 2011a) of malignant lymphoma (9 out of 29, 17 out of 30, and 18 out of 28 for groups at 0, 100, or 500 ppm, respectively) were reported in treated females. [The Working Group noted the low number of animals studied and limited reporting.]

Groups of 50 female Crj:CD-1(ICR) mice (age, 7 weeks) were exposed by inhalation to reagent-grade trichloroethylene (containing epichlorohydrin as a stabilizer) at a concentration of 0, 50, 150, or 450 ppm for 7 hours per day, 5 days per week, for 104 weeks (Fukuda *et al.*, 1983). The experiment was terminated after 107 weeks. Only malignant tumours were counted when both malignant and benign tumours were observed in the same organ. The number of tumours diagnosed in all animals for each exposure group was reported, rather than tumour incidence, with the exception of lung adenocarcinoma. A low number of liver tumours was reported (only one hepatocellular adenoma in the group at the highest dose, but none in other groups). [The Working Group noted that the background rate for hepatocellular adenoma was low for this strain and sex in contemporary studies (Maita *et al.*, 1988).] The increased incidence of lung adenocarcinoma was statistically significant [$P < 0.05$] in the groups at the two higher doses compared with controls. [The Working Group noted the limited reporting of this study.]

3.1.3 Skin application

In a study of two-stage carcinogenesis in mouse skin, groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with single doses of 1.0 mg of trichloroethylene [no indication of stabilizers, purity not reported] in 0.1 mL of acetone by topical application to the shaved dorsal skin; after 14 days, 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 2.5 µg in 0.1 mL of acetone) was applied topically, three times per week for at least 49 weeks (Van Duuren *et al.*, 1979). A total of 9 skin papillomas were found in 4 out of 30 trichloroethylene-treated mice, and 10 papillomas were found in 9 out of 120 TPA-treated controls. Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were also treated with trichloroethylene by repeated topical application, at a dose of 1.0 mg per mouse, three times per week, for 83 weeks. No tumours were observed at the site of application. [The Working Group noted the small number of animals studied and the low dose used. The volatility of the compound was also noted as a limitation to this study protocol.]

3.1.4 Subcutaneous injection

Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with purified trichloroethylene [no indication of stabilizers, purity not reported] by subcutaneous injection at a dose of 0 or 0.5 mg in 0.05 mL of trioctanoin, once per

week, for at least 74 weeks (Van Duuren *et al.*, 1979). No tumours were observed at the injection site in either group. [The Working Group noted the limited number of animals studied and the low dose used.]

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female F344/N rats (age, 8 weeks) were treated with epichlorohydrin-free trichloroethylene by gavage in corn oil at a dose of 0, 500, or 1000 mg/kg bw per day for 103 weeks (NTP, 1990). Significantly reduced survival in trichloroethylene-treated groups compared with vehicle controls was due to gavage error and chronic "toxic nephropathy." Rarely seen in controls (background incidence in male rats, 0.4%), toxic nephropathy was distinct from the spontaneous chronic progressive nephropathy commonly observed in aged rats. Cytomegaly and karyomegaly of tubular epithelial cells of the inner renal cortex were observed in > 98% of all trichloroethylene-treated rats. Although decreased survival was noted in trichloroethylene-treated groups, an increased incidence, and positive trend in the incidence of rare kidney adenoma or carcinoma (combined), and of kidney carcinoma, were observed in males at the higher dose (carcinomas in 3 out of 49 rats at the higher dose versus 0 out of 48 controls). One case of kidney carcinoma was also reported in females at the higher dose, but no statistically significant increases in tumour incidence were reported. [The Working Group noted the rarity of kidney adenoma and carcinoma in this rat strain, with the background incidence in males reported as 0.7% (10 out of 1352) for adenoma and 0.2% (3 out of 1352) for carcinoma; in females, the background incidence has been reported as 0% (0 out of 1348) for adenoma and 0.1% (1 out of 1348) for carcinoma (Haseman *et al.*, 1998). A low background incidence of kidney adenoma or carcinoma was also reported in F344/N rats treated with trichloroethylene in corn oil by gavage, in studies conducted by the NTP. The EPA (2011a) cited the incidence of kidney carcinoma in unexposed rats fed an NIH-07 diet (conditions used before 1995, when the NTP studies on trichloroethylene were conducted), and treated with corn oil by gavage, as 0.5% (2 out of 400) for males and 0% (0 out of 400) for females. For F344/N rats fed an NIH-07 diet, the historical control rate for kidney adenoma or carcinoma (combined) was 1.3% (5 out of 400) for males and 0.3% (1 out of 400) for females.] A statistically significant increase in the incidence of peritoneal malignant mesothelioma was also observed in males at the lower dose (5 out of 50 treated rats versus 1 out of 50 controls). [The Working Group noted that mesothelioma (at all sites) is an uncommon tumour in untreated male F344/N rats, with the background incidence reported as 3.0% (40 out of 1354 rats in feeding studies and 28 out of 905 rats in inhalation studies) (Haseman *et al.*, 1998).]

Groups of male and female Osborne-Mendel rats (age, about 45 days) were treated with commercial-grade trichloroethylene (containing epichlorohydrin as stabilizer) at time-weighted average doses of 0 (n = 20), 549 (n = 50), or 1097 (n = 50) mg/kg bw in corn oil by gavage for 78 weeks (non-continuous exposure at concentrations that were varied due to toxicity and mortality) and killed after 110 weeks (NCI, 1976). Premature mortality was high (84–94% in males, and 50–74% in females). A high incidence of chronic respiratory disease was noted among the rats without differences in type, severity, or morbidity by sex or group, and chronic "toxic nephropathy" was observed only in treated rats of both sexes. A few malignant mixed tumours and hamartomas of the kidney were observed in controls, with one observed in a male at the lower dose, which were considered not related to chemical treatment. A single trichloroethylene-treated male had a rare kidney carcinoma.

No statistically significant increases in tumour incidence were reported in either sex. [The Working Group considered that a slight increase in the incidence of multiple mammary fibroadenoma was associated with exposure to trichloroethylene in females (3 out of 48 at the highest dose versus 0 out of 20 untreated); the incidence of single fibroadenoma was not increased with treatment (3 out of 20, 5 out of 45, and 7 out of 48 for controls, and rats at the lower and higher doses, respectively). The Working Group noted the high rate of early mortality in control and treated rats, limited duration of treatment, and lower numbers of animals studied in control groups. In addition, study animals were housed in the same rooms as animals exposed to volatile agents, but the Working Group did not observe an effect on the tumour response.]

Groups of 50 male and 50 female Osborne-Mendel rats (age, 8 weeks) were treated with trichloroethylene (containing diisopropylamine as a stabilizer) at a dose of 0, 500, or 1000 mg/kg bw in corn oil by gavage for 103 weeks (NTP, 1988). More trichloroethylene-treated male rats than controls were killed by gavage error (controls, 1 out of 50; lower dose, 6 out of 50; and higher dose, 7 out of 50), while similar rates of accidental death occurred in all groups of females (six to eight per group). One bronchioloalveolar adenoma and one bronchioloalveolar carcinoma were diagnosed in two trichloroethylene-treated females, but no such tumours were observed in those receiving the vehicle only. After adjustment for survival, a positive trend and an increase (for the group at the higher dose) in the incidence of adrenal cortex adenoma, was observed in females. Treatment with trichloroethylene also caused cytomegaly and karyomegaly of proximal tubule cells, and chronic "toxic nephropathy" [see description above in NTP (1990)], but not in controls. Tubular hyperplasia [distinct from chronic progressive nephropathy] was noted to occur at low rates in trichloroethylene-treated rats, and less frequently in controls. All animals exposed to trichloroethylene for up to 2 years had renal tubular cell cytomegaly, and most rats (80%) had chronic "toxic nephropathy." Incidences of kidney adenoma, and kidney adenoma or adenocarcinoma (combined) were increased in trichloroethylene-treated males (0 out of 50 controls, 6 out of 50 at the lower dose, and 2 out of 50 at the higher dose), and were statistically significant in animals at the lower dose. A rare kidney adenoma was diagnosed in a single trichloroethylene-treated female. [The Working Group noted that the background incidence in this strain for kidney adenoma and carcinoma was reported to be 0% (0 out of 100 in males and females; 50 untreated and 50 with corn oil by gavage) (Solleveld & Boorman, 1986). After inclusion of the data for 70 control rats from the NCI (1976) and NTP (1988) studies, the average background rate was 0% (0 out of 170) for male and female rats of this strain.] [Chemical-induced toxicity, reduced survival, and incomplete documentation of experimental data were noted by the Working Group.]

In the same publication, groups of male and female ACI rats (age, 6.5 weeks) were treated with trichloroethylene using the same experimental paradigm (NTP, 1988). Higher numbers of trichloroethylene-treated male and female rats were killed by gavage error, than controls (males: 0 out of 50 controls, 11 out of 49 at the lower dose, and 18 out of 49 at the higher dose; females: 2 out of 48 controls, 14 out of 47 at the lower dose, and 12 out of 43 at the higher dose). Renal tubular cell cytomegaly was noted in 80–98% of treated males and 90% of treated females exposed for up to 2 years, while approximately 40% of treated males and females had chronic "toxic nephropathy;" these pathologies were not seen in controls. A single male treated with trichloroethylene at the lower dosewas diagnosed with a kidney carcinoma. While no kidney neoplasms were reported in females in the control group, females treated with trichloroethylene at the lower dose had an incidence of 2 out of 47 for kidney

adenoma, and 3 out of 47 for tubular cell adenocarcinoma [kidney carcinoma] or adenocarcinoma, NOS (not otherwise specified) or kidney adenoma (statistically significant); the group treated with the higher dose had an incidence of one out of 43 for tubular cell adenocarcinoma [kidney carcinoma] or adenocarcinoma, NOS. [The Working Group noted that these tumours are rare in this strain of rat (0 out of 98 males and 0 out of 97 females for kidney adenoma or carcinoma) (Solleveld & Boorman, 1986). After inclusion of the control data from this study, the average number of control rats with kidney tumours was 0% (0 out of 148 for males and 0 out of 147 for females).] An increased incidence of testicular interstitial cell adenoma was diagnosed in surviving trichloroethylene-treated males (a positive trend and a higher incidence in both groups treated with trichloroethylene). Although the incidence of interstitial cell tumours was not significantly higher than that in vehicle controls (using the incidental tumour test), there was a positive trend when the incidence of interstitial cell tumours was based on survival of animals until the week in which the first tumour appeared (i.e. at week 75, incidence in males was 36 out of 43, 23 out of 26, and 17 out of 17 for rats in the control group, at the lower dose and higher dose, respectively). Bronchioloalveolar adenoma was diagnosed in one trichloroethylene-treated female, but not in females receiving vehicle only.

The same publication also provided results for male and female August rats (age, 8 weeks) using the same experimental paradigm (NTP, 1988). Higher numbers of trichloroethylene-treated male and female rats were killed by gavage error than controls (males: 6 out of 50 controls, 12 out of 50 at the lower dose, and 11 out of 49 at the higher dose; females: 1 out of 49 controls, 6 out of 48 at the lower dose, and 13 out of 50 at the higher dose). More than 90% of treated males and females exposed for up to 2 years had renal tubular cell cytomegaly, and about 20–60% of males and females had chronic "toxic nephropathy." Tubular cell hyperplasia, distinguishable from that observed for chronic progressive nephropathy, was also noted in a few individuals in trichloroethylene-treated groups only. In males, the incidence of kidney adenoma was zero out of 50 controls, one out of 50 at the lower dose, and one out of 49 at the higher dose, with one male treated with trichloroethylene at the lower dose also diagnosed with kidney carcinoma. In females, the incidence of kidney adenoma or adenocarcinoma (combined) was one out of 49 controls, four out of 48 at the lower dose, and zero out of 50 at the higher dose, with two females treated with trichloroethylene at the lower dose diagnosed with rare kidney carcinoma. [The Working Group noted that, although not statistically significant, these kidney tumours are rare in this strain (0% incidence of kidney adenoma in males (0 out of 100 rats) and 1% incidence in females (1 out of 99 rats), and 0% incidence of kidney carcinoma in either sex) (Solleveld & Boorman, 1986). With the addition of the controls from this study, the number of unexposed rats with kidney tumours was 0 out of 150 (0%) for each sex of this strain.] There was a statistically significant trend in increasing incidence of subcutaneous tissue sarcoma (the incidence was significantly increased for rats at the higher dose) in trichloroethylene-treated males. After adjustment for survival, there was also a small increase in the incidence of testicular interstitial cell tumours at the lower dose. A positive trend in the incidence of leukaemia was observed in females. After taking survival into account, the incidence of leukaemia was slightly increased in the group at the higher dose (1 out of 50 controls versus 5 out of 50 females at the higher dose). The incidence of thyroid C-cell adenoma or carcinoma (combined) was slightly increased in females at the lower dose (0 out of 49 controls versus 4 out of 49 females at the lower dose).

In a fourth study reported in the same publication (NTP, 1988), groups of 50 male and 50

female Marshall rats (age, 7 weeks) were exposed to trichloroethylene using the same experimental paradigm. Higher numbers of trichloroethylene-treated rats were killed by gavage error than controls (males: 2 out of 49 controls, 12 out of 50 at the lower dose, and 25 out of 47 at the higher dose; females: 3 out of 50 controls, 14 out of 48 at the lower dose, and 18 out of 44 at the higher dose). More than 90% of treated males and females, exposed for up to 2 years, had renal tubular cell cytomegaly, and 36–49% of males and 63% of females had chronic "toxic nephropathy." Tubular cell hyperplasia, distinguishable from that observed for chronic progressive nephropathy, was also noted in a few individuals in trichloroethylene-treated groups only. With regard to kidney tumours, results were similar to the other strains tested by this paradigm, with a few treated male and female rats exhibiting rare kidney tumours. In males, the incidence of kidney carcinoma was 1 out of 47 rats at the higher dose compared with 0 out of 49 controls. In females, the incidence of kidney adenoma or adenocarcinoma (combined) was 1 out of 50 controls, 2 out of 48 rats at the lower dose, and 1 out of 44 rats at the higher dose, with an incidence of kidney carcinoma of 0 out of 50 controls, 1 out of 48 rats at the lower dose, and 1 out of 44 rats at the higher dose. For males, a much higher trichloroethylene treatment-related increase in the incidence of testicular interstitial cell tumours was observed compared with the other strains. The increased incidence of interstitial cell tumours had statistically significant trends, with and without survival adjustment; the increased incidence was also statistically significant compared with controls in both treatment groups after survival adjustment, and in the group treated with the higher dose, without survival adjustment. One trichloroethylene-treated male at the higher dose was diagnosed with a rare malignant interstitial cell tumour. In addition, there was a slight increase in the total number of mesotheliomas (peritoneal and tunica vaginalis) in males at the lower dose, after adjustment for survival.

[The Working Group noted the early mortality described in the four studies encompassed in the NTP (1988) publication, as well as the observation of rare tumours and increased incidence of more common tumours after treatment with trichloroethylene. Comparison of tumour incidence with historical background rates is difficult for the strains employed in these studies, especially for sites other than kidney. The Working Group also noted that the early mortality reduced the statistical power of the bioassays.]

Groups of 30 male and 30 female Sprague-Dawley rats (age, 13 weeks) were exposed to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as stabilizer) at a dose of 0, 50, or 250 mg/kg bw per day in olive oil by gavage, 4–5 days per week, for 52 weeks, and observed for their lifespans (Maltoni *et al.*, 1986). As in F344/N rats (NTP, 1990), an increased incidence of renal tubule meganucleocytosis (cytokaryomegaly) was observed in males at the higher dose. Slight increases in the incidence, or trend in the incidence of leukaemia were observed in trichloroethylene-treated males. Leukaemia was reported to be primarily immunoblastic lymphosarcoma [lymphoma]. [The Working Group noted that although deaths attributable to gavage error were not reported, the power of the study was limited by the small number of animals in each group and the short duration of exposure in older animals (see additional comments from the Working Group below regarding the diagnosis of lymphoma in rats from this laboratory).]

3.2.2 Inhalation

Groups of 130–145 male and female Sprague-Dawley rats (age, 12 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as stabilizer) at a concentration of 0, 100,

300, or 600 ppm, 7 hours per day, 5 days per week, for 104 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Tumour results were reported separately, and as a combination of two experiments that were conducted at the same time; both experiments used the same protocol and "were divided by litter distribution within the two experiments." The EPA (2011a) adjusted the incidence of kidney tumours to reflect the number of animals alive at 47 weeks (i.e. the date at which the first diagnosis of renal tubular meganucleocytosis was made). There was an increase in the incidence of testicular interstitial cell tumours (mainly benign) in all groups of trichloroethylene-exposed males. As with Marshall rats exposed by gavage (see Section 3.2.1; NTP, 1988), one male rat at the higher dose was diagnosed with a rare malignant testicular cell tumour. [The Working Group noted that the previous IARC Working Group (IARC, 1995) had reported a significant, dose-related increase in the incidence of Leydig cell interstitial tumour of the testis ($P < 0.001$; Cochran–Mantel–Haenszel test).] A slight dose-related increase in the incidence of pituitary adenoma in female rats in one of the two studies was noted, but the data were not shown. There was also an increase in the incidence of immunoblastic lymphosarcoma [lymphoma] in females at the lower dose. [The Working Group noted the increased power of this study to detect a response from the large number of animals in the combined exposure groups, and the use of four, rather than three, exposure groups. The Working Group also noted the limited reporting of findings for individual animals, and that the diagnoses of rat lymphoma in this laboratory (especially those in the lung) have been questioned in other studies; however, other diagnoses of solid tumour, such as testicular interstitial cell adenoma, were confirmed in the reviews carried out by those other studies (NTP, 2011b, c).] As for observations in studies in other rat strains (NTP, 1988, 1990), and the gavage study from the same laboratory (see Section 3.2.1; Maltoni *et al.*, 1986), an increased incidence of renal tubule meganucleocytosis (cytokaryomegaly) was reported in male rats exposed to trichloroethylene by inhalation at the two higher doses. Rare kidney carcinomas, originating from tubular cells, were reported in 4 out of 130 males at the highest dose versus 0 out of 135 controls; one kidney carcinoma, identified as cortical, was reported in a female at the highest dose. No kidney carcinomas "of the same pattern [originating from tubular cells] has ever been observed in nearly 50 000 Sprague-Dawley rats (untreated, vehicle treated, or treated with different chemicals) examined" in their laboratory. [The Working Group noted that variations of this strain have a low background incidence of kidney tumours (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010); for kidney carcinoma, background incidence has been reported as 0% (0 out of 350 males and 0 out of 350 females from various dietary groups) (Keenan *et al.*, 1995), 0.51% (3 out of 585 males) and 0% (0 out of 584 females) (McMartin *et al.*, 1992), 0.21% (1 out of 472 females) (Dinse *et al.*, 2010) and 0.27% (1 out of 370 females) (Brix *et al.*, 2005). Overall, the average background incidence of kidney carcinoma was 0.11% in females and 0.32% in males.]

Groups of 30 male and 30 female Han:WIST rats [age not reported] were exposed by inhalation to purified trichloroethylene (containing triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and killed after 36 months (Henschler *et al.*, 1980). The number of tumours diagnosed in all animals for each exposure group, but not tumour incidence, was reported. Survival did not differ among groups, and no statistically significant treatment-related increase in the number of tumours was seen. [For this study as a whole, the Working Group noted the small number of animals studied in each group, and the inability to compare tumour findings from this study with those of other

studies and historical databases, given that only the number of tumours per exposure group was reported and not tumour incidence. The Working Group noted that use of the term "cystadenoma" has only been used by these authors among the studies included in this review and does not appear in other historical databases (Solleveld & Boorman, 1986; Haseman *et al.*, 1998; Harlan, 2011).]

Groups of 50 female CrJ:CD(SD) rats (age, 7 weeks) were exposed by inhalation to reagent-grade trichloroethylene (containing epichlorohydrin as a stabilizer) at a concentration of 0, 50, 150, or 450 ppm for 7 hours per day, 5 days per week, for 107 weeks (Fukuda *et al.*, 1983). Increased mortality was reported in the control group compared with treated groups (i.e. about 75% of the rats in the three treated groups and 50% of controls were alive at 100 weeks). Only malignant tumours were counted when both malignant and benign tumours were observed in the same organ. The number of tumours per total number of animals in each group was reported, but not the tumour incidence. No statistically significant increases in number of tumours per exposure group were reported, but rare tumours were reported in trichloroethylene-treated groups.

A renal clear cell carcinoma [kidney carcinoma] was reported in the group treated with the highest dose. A single bronchioloalveolar adenoma was reported in each of the groups at the intermediate and highest doses, but not in controls. [The Working Group noted that bronchioloalveolar adenoma is rare in Harlan Sprague-Dawley female rats (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010).] A single mammary gland carcinosarcoma was diagnosed in in each of the groups at the intermediate and highest doses. A rare hepatocellular carcinoma was observed in the group at the highest dose, and a cystic cholangioma observed in the group at the lowest dose, but not in controls. [The Working Group noted that hepatocellular carcinomas and bile duct tumours are rare in Harlan Sprague-Dawley female rats (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010.] [The Working Group noted the increased mortality in the control group, the limited reporting, and the rarity of some tumour findings in trichloroethylene-treated groups. The Working Group also noted the inability to compare tumour incidence with other studies, given that only the number of tumours per exposure group was reported.]

3.3 Hamster

Groups of 30 male and 30 female Syrian hamsters [age unspecified] were treated by inhalation with trichloroethylene (purity, > 99.9%; containing 0.0015% triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and observed for 30 months (Henschler *et al.*, 1980). The number of tumours diagnosed in all hamsters for each exposure group was reported, but not the incidence. Survival was reported to be similar in treated and control groups. No significant increase in number of tumours was reported. [The Working Group noted that the low number of animals studied in each group and the abbreviated exposure period limited the power of the study to detect a response.]

3.4 Administration with known carcinogens or other modifying factors

Seven groups of 23–33 male B6C3F$_1$ mice (age, 15 days) were given a single intraperitoneal injection of *N*-ethyl-*N*-nitrosourea (ENU) in 0.1 mol/L sodium acetate at a dose of 0, 2.5, or 10 mg/kg bw; from age 4 weeks, the mice were given drinking-water containing trichloroethylene at a dose of 0, 3, or 40 mg/L for 61 weeks (Herren-Freund *et al.*, 1987). [Trichloroethylene was reported to

have a purity of more than 99% and the presence of stabilizers was not addressed.] Because of volatilization, the concentration of the 40 mg/L dose was reduced to 22 mg/L after 3 days. [The previous Working Group (IARC, 1995) noted that the highest concentration of trichloroethylene given was equivalent to a daily dose of 6 mg/kg bw, which was low.] Only livers were examined for treatment-related induction of tumours. The incidence of hepatocellular adenoma or hepatocellular carcinoma was not increased in mice that received trichloroethylene only, in comparison with vehicle controls, and trichloroethylene did not promote liver tumours in mice initiated with ENU. [The Working Group noted the low levels of exposure to trichloroethylene, the small number of animals examined, and the abbreviated exposure duration, all of which limited the interpretation of results.]

3.5 Effects of stabilizers

Although some studies used trichloroethylene containing two carcinogenic compounds as stabilizers (epichlorohydrin [IARC Group 2A] and 1,2-epoxybutane [IARC Group 2B]), consistent patterns of tumour induction were observed in studies using trichloroethylene with or without small amounts (< 1%) of these carcinogenic stabilizers.

The study in mice by Henschler *et al.* (1984) was designed to specifically analyse the effect of stabilizers on tumour induction by trichloroethylene and reported no significant differences in systemic tumorigenesis between groups treated with pure trichloroethylene, industrial trichloroethylene, or trichloroethylene containing very small amounts of stabilizers. The relatively low susceptibility to liver tumours in the strain tested (Swiss HA:ICR) decreased the ability of the study to detect differences in liver tumorigenesis, but also decreased the probability of premature mortality before termination of the study. The NTP (1990) study was a better example of the lack of a confounding effect of stabilizers on the results of trichloroethylene carcinogenicity studies; rats and mice exhibited patterns of tumour induction without stabilizers that were similar to those reported for similar paradigms using stabilizer-containing trichloroethylene). Thus, the evidence suggested that concentrations of these stabilizers were too low to be the cause of tumours.

3.6 Carcinogenicity of metabolites

See also individual *Monographs* on Dichloroacetic acid, Trichloroacetic acid, and Chloral Hydrate in this Volume.

Groups of female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with trichloroethylene oxide in 0.1 mL of acetone by skin application at a dose of 0 (n = 30) or 2.5 mg (n = 100) three times per week, for their lifespans. In a separate experiment, groups of female ICR/Ha Swiss mice (age, 6–8 weeks) were treated by injection in the flank with trichloroethylene oxide (purity, 90%, with 10% dichloroacetyl chloride) in 0.05 mL of trioctanoin at a dose of 0 (n = 30) or 500 µg (n = 100) once per week (Van Duuren *et al.*, 1983). No skin papillomas were reported in the skin-application experiment and only one fibrosarcoma was reported in the subcutaneous-injection experiment. Liver, stomach, and kidney tissue were routinely sectioned, but results were not reported. Trichloroethylene epoxide had a rapid rate of hydrolytic decomposition (half-life,1.5 minute). [The Working Group noted the low number of animals tested and raised questions regarding the stability of the compound tested.]

In a study of two-stage carcinogenesis in mouse skin, groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with purified trichloroethylene oxide at a dose of 1.0 mg in 0.1 mL of acetone by application to the shaved dorsal skin; after 14 days, 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 2.5 µg in 0.1 mL of acetone)

was applied topically, three times per week for at least 49 weeks (Van Duuren *et al.*, 1979). A total of three skin papillomas were found in 3 out of 30 trichloroethylene oxide-treated mice, and 10 papillomas were found in 9 out of 120 TPA-treated controls. [The Working Group noted the low number of animals tested and raised questions regarding the stability of the compound tested.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Trichloroethylene is a lipophilic solvent of low relative molecular mass, and can readily cross biological membranes. It is taken up in the lungs, with pulmonary absorption approaching steady-state within a few hours after the start of exposure (Monster *et al.*, 1976; Vesterberg & Astrand, 1976; Vesterberg *et al.*, 1976; Fernández *et al.*, 1977). Measured values of pulmonary retention range from 35% to 70% in individuals, with generally greater values at rest and lower values associated with physical activity (Soucek & Vlachova, 1960; Bartonicek, 1962; Astrand & Ovrum, 1976; Monster *et al.*, 1976; Jakubowski & Wieczorek, 1988). One factor in pulmonary absorption is the equilibrium ratio between blood and air concentrations of trichloroethylene (blood:air partition coefficient), which has been measured *in vitro* using vial equilibrium methods. Mean values reported for human blood range from 8 to 12 (Sato *et al.*, 1977; Sato & Nakajima, 1979; Fiserova-Bergerova *et al.*, 1984; Gargas *et al.*, 1989; Koizumi, 1989; Fisher *et al.*, 1998).

Data on oral absorption were derived from case reports of accidental occupational or intentional (suicidal) ingestion, and suggested that trichloroethylene is also readily absorbed by this route. Trichloroethylene and its metabolites were reported in blood and/or urine at the first available sampling times after exposure, the earliest of which was 13 hours, with peak amounts in blood within the first 24 hours (Brüning *et al.*, 1998; Perbellini *et al.*, 1991; Yoshida *et al.*, 1996). However, quantitative estimates of oral bioavailability in humans were not available because the ingested amounts were not known precisely, and because all cases underwent gastric intubation and/or lavage.

Dermal absorption of vapour and liquid in humans has been shown to be rapid on contact, with peak values of trichloroethylene in exhaled breath reported within 5–30 minutes after exposure (Sato & Nakajima, 1978; Kezic *et al.*, 2000, 2001). In addition, Kezic *et al.* (2000) reported high inter-individual variability in dermal flux, as well as varying degrees of skin irritation that may have increased absorption (Kezic *et al.*, 2001).

(b) Experimental systems

Dallas *et al.* (1991), using a nose-only face-mask exposure system in rats, reported retention of about 70% during the second hour of a 2-hour exposure by inhalation. Data on exposure by whole-body inhalation in rats and mice were also consistent with considerable pulmonary absorption (Fisher *et al.*, 1991; Greenberg *et al.*, 1999; Simmons *et al.*, 2002).

Mean values for the blood:air partition coefficient in rats and mice, measured *in vitro* using vial equilibrium methods, ranged from 13 to 26 (Sato *et al.*, 1977; Gargas *et al.*, 1989; Koizumi, 1989; Fisher *et al.*, 1991; Barton *et al.*, 1995; Abbas & Fisher, 1997; Simmons *et al.*, 2002; Mahle *et al.*, 2007). [The Working Group noted that these values were higher than those measured in humans, but the higher partition coefficient in rodents only partially explained their greater retention by inhalation, as pulmonary absorption

also depends on other factors such as alveolar ventilation, hepatic blood flow, and metabolism.]

Most of the data from experimental systems *in vivo* involved oral exposures, and were consistent with rapid and extensive absorption after ingestion. Radiolabelling studies have reported that up to 98% of the administered dose was excreted in the urine and about 1–4% was eliminated unchanged in expired air, consistent with virtually complete absorption (Prout *et al.*, 1985; Dekant *et al.*, 1986a). The rate of absorption is affected by stomach contents and vehicle. Fasted animals exhibited greater bioavailability, higher peak blood levels, and shorter terminal half-lives compared with non-fasted animals (D'Souza *et al.*, 1985). Withey *et al.* (1983) and Staats *et al.* (1991) examined the effect of vehicle on gastrointestinal absorption of several compounds in rats, including trichloroethylene. Withey *et al.* (1983) noted faster and more extensive uptake with an aqueous vehicle than with oil, with significant differences in peak concentration and time to reach peak concentration. Staats *et al.* (1991) showed similar differences between aqueous and oil vehicles in rats, and also estimated gastrointestinal absorption and transfer coefficients by fitting their data to a physiologically based pharmacokinetic model. Stomach absorption coefficients were estimated to be three to four times greater for aqueous vehicle than for oil, but estimated coefficients for stomach–duodenal transfer and duodenal absorption were not affected by the vehicle.

4.1.2 Distribution and body burden

(a) Humans

Once absorbed, trichloroethylene enters the blood circulation and undergoes rapid systemic distribution to tissues. Data in humans on tissue distribution *in vivo* were limited to tissues taken from autopsies after accidental poisonings, or from surgical patients exposed environmentally, so the level of exposure was typically unknown. Tissue levels reported in autopsies after accidental poisonings showed wide systemic distribution across all tested tissues, including the brain, muscle, heart, kidney, lung, and liver (Ford *et al.*, 1995; De Baere *et al.*, 1997; Dehon *et al.*, 2000; Coopman *et al.*, 2003). Human populations exposed environmentally show detectable levels of trichloroethylene in various tissues, including the liver, brain, kidney, and adipose, and also in maternal milk (McConnell *et al.*, 1975; Pellizzari *et al.*, 1982; Kroneld, 1989). In addition, trichloroethylene has been shown to cross the human placenta during childbirth (Laham, 1970).

Because of its lipophilicity, trichloroethylene preferentially partitions to tissues with a high lipid content, such as fat. The equilibrium ratio of tissue:blood concentrations (the partition coefficient) has been measured *in vitro* using vial equilibrium methods in human fat, brain, kidney, liver, lung, and muscle (Sato *et al.*, 1977; Fiserova-Bergerova *et al.*, 1984; Fisher *et al.*, 1998). The reported human tissue: blood partition coefficients were highest for fat (52–64), with those for the remaining tissues ranging from 0.5 to 6.0.

(b) Experimental systems

Reports of detailed tissue-distribution experiments in rats and mice exposed to trichloroethylene orally or by inhalation (Savolainen *et al.*, 1977; Pfaffenberger *et al.*, 1980; Abbas & Fisher, 1997; Greenberg *et al.*, 1999; Simmons *et al.*, 2002; Keys *et al.*, 2003) suggested that trichloroethylene is distributed to all tissues examined, including the kidney, liver, lung, brain, fat, muscle, gastrointestinal tract, heart, and spleen, with the highest concentrations measured in fat.

Partition coefficients have been measured *in vitro* for a large array of tissues in rats and mice, including the brain, fat, heart, kidney, liver, lung, muscle, spleen and testis (Sato *et al.*, 1977; Fisher *et al.*, 1989, 1991; Gargas *et al.*, 1989; Koizumi, 1989; Barton *et al.*, 1995; Abbas & Fisher, 1997;

Simmons *et al.*, 2002; Rodriguez *et al.*, 2007). The reported rodent tissue: blood partition coefficients were highest for fat (23–36), with those for the remaining tissues ranging from 0.5 to 3.

4.1.3 Metabolism

(a) Overview

Metabolism is critical to the various adverse effects of trichloroethylene in biological systems. Moreover, with the exception of solvent effects that occur at extremely high exposures to trichloroethylene, all the adverse effects can be attributed to specific metabolites of trichloroethylene. The basic metabolic pathways for trichloroethylene have been deciphered over many years and have been summarized in several relatively recent reviews and published documents (e.g. IARC, 1995; Lash *et al.*, 2000a; Chiu *et al.*, 2006; EPA, 2011a). This section summarizes the two major pathways by which trichloroethylene is metabolized in humans and experimental animals: the cytochrome P450 (CYP)-dependent oxidative pathway and the glutathione (GSH)-conjugation pathway. Key urinary metabolites are identified that are often used to estimate exposure in environmental or occupational settings.

Quantitatively, flux through the CYP-dependent oxidative pathway far exceeds that through the GSH-conjugation pathway in all species studied, including humans. The metabolites generated by the CYP-dependent oxidative pathway are mostly chemically stable, while several of those generated by the GSH-conjugation pathway are highly reactive. [The Working Group noted that the high flux and chemical stability of most of the oxidative metabolites do not infer that these metabolites are not linked to adverse effects. Also, some highly reactive metabolites generated by the GSH-conjugation pathway may be difficult to detect, thus suggesting that interpretations regarding toxicological importance that are based on quantitative differences in estimated flux must be made with caution.]

(i) CYP-dependent oxidation

The metabolism of trichloroethylene by the oxidative pathway is initiated by the action of several CYP enzymes. While this step occurs predominantly in the liver, it can also occur in a large number of other tissues, including the kidney (Cummings *et al.*, 2000a, 2001), lungs (Odum *et al.*, 1992; Green *et al.*, 1997a; Forkert *et al.*, 2005, 2006), and male reproductive tissues (Forkert *et al.*, 2002, 2003). The overall scheme of oxidative metabolism of trichloroethylene is shown in Fig. 4.1. Several of the metabolites are chemically stable and have been detected in urine; these are highlighted in the scheme.

The initial step in the metabolism of trichloroethylene is catalysed by one of several CYP enzymes and results in formation of an enzyme-bound intermediate (trichloroethylene-epoxide-CYP), which is chemically unstable and, therefore, shown in parentheses. This trichloroethylene-epoxide-CYP can have one of three fates: Conversion to (i) trichloroethylene epoxide; (ii) *N*-hydroxy-acetyl-aminoethanol; or (iii) chloral, which is in equilibrium with chloral hydrate. The majority of the flux is towards chloral hydate. In fact, chloral hydrate is typically the first stable metabolite recovered in incubations of tissues, cells, or microsomes with trichloroethylene.

Trichloroethylene epoxide spontaneously generates either dichloroacetyl chloride (another chemically unstable and reactive species), or oxalic acid, which is readily excreted in the urine. Dichloroacetyl chloride undergoes spontaneous dechlorination to produce dichloroacetate.

Chloral hydrate/chloral undergoes either reduction by alcohol dehydrogenase or CYP to generate trichloroethanol, or oxidation by aldehyde dehydrogenase to form trichloroacetate. Trichloroacetate is typically the major urinary metabolite of trichloroethylene that is recovered, although trichloroethanol is also a significant urinary metabolite. Trichloroethanol can be oxidized by CYPs to yield trichloroacetate,

or can undergo glucuronidation by uridine diphosphate (UDP)-glucuronosyltransferases (UGTs) to produce trichloroethanol glucuronide. Both trichloroethanol and its glucuronide are recovered in the urine, although it is typically trichloroethanol that is mostly recovered due to hydrolytic removal of the glucuronide moiety during sample preparation. Although trichloroacetate is generally poorly metabolized and readily excreted in urine, it may undergo dechlorination to yield dichloroacetate (see *Monograph* on Trichloroacetic Acid in this Volume). Hence, there are two sources of dichloroacetate, dichloroacetyl chloride derived from trichloroethylene epoxide, and trichloroacetate derived from chloral/chloral hydrate.

Although dichloroacetate is a urinary metabolite of trichloroethylene, it can also undergo further metabolism: dichloroacetate can be dechlorinated to form monochloroacetate, which is excreted in the urine; or dichloroacetate is metabolized by a fairly unique GST isoform GST-zeta to yield glyoxylic acid, which is eventually broken down to carbon dioxide (see *Monograph* on Dichloroacetic Acid in this Volume).

Thus, the major CYP-derived, oxidative metabolites of trichloroethylene that are found in urine of exposed animals or humans include dichloroacetate, trichloroacetate, trichloroethanol and its glucuronide, monochloroacetate, and oxalic acid.

A summary of the major oxidative metabolites formed from trichloroethylene, site of formation, the data source (animals, humans, or both), and whether the metabolite is systemically available, is presented in Table 4.1. Systemic availability is based on the chemical stability of the metabolite; those that are relatively stable may be transported from their site of formation into the blood stream and be delivered to other potential target organs, while those that are chemically unstable and reactive tend to remain near their site of formation and react with cellular molecules, including DNA, proteins, and lipids.

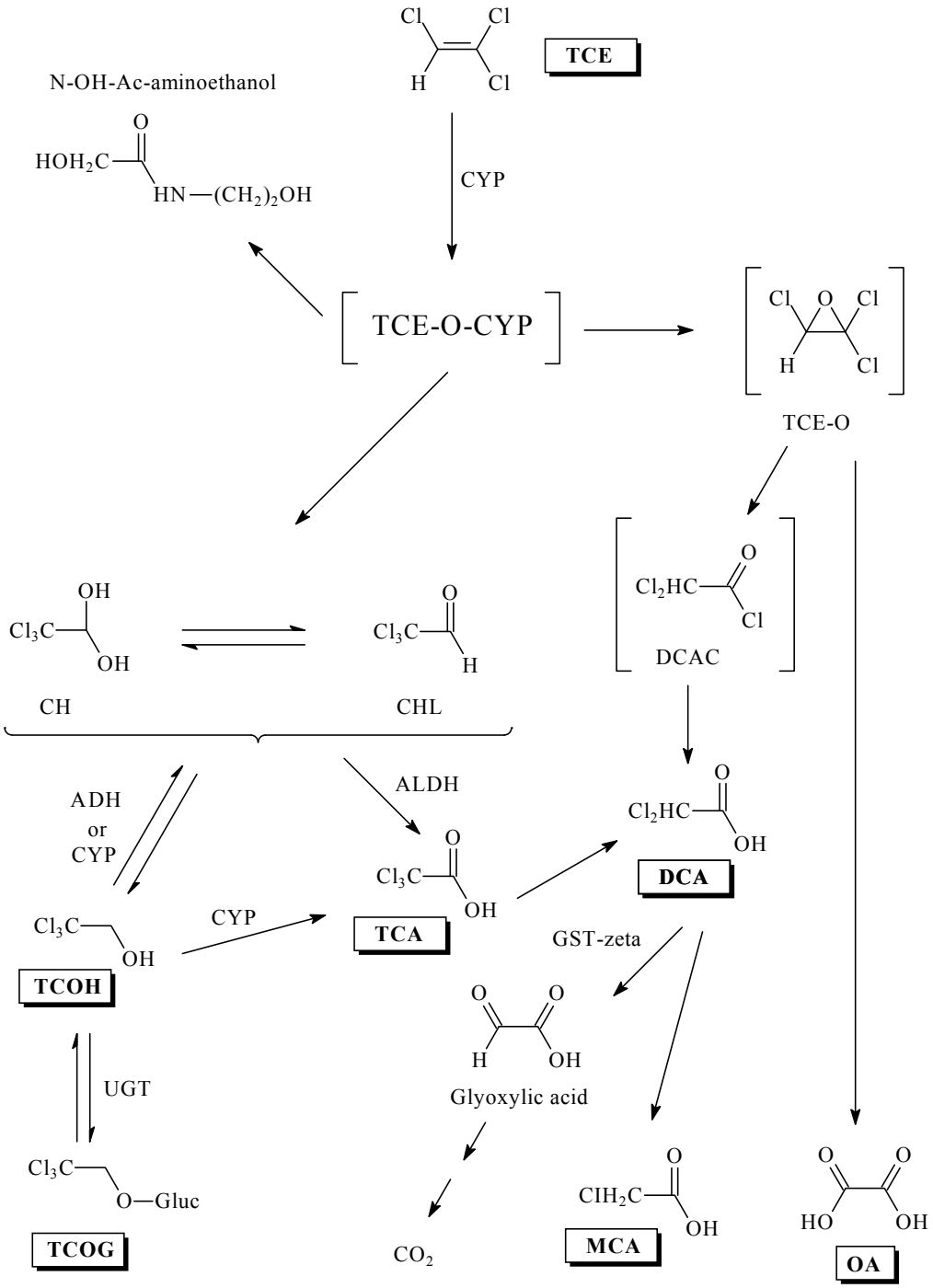
(ii) GSH conjugation

As shown in Fig. 4.2, trichloroethylene undergoes a substitution nucleophilic S_N2 displacement reaction with GSH, releasing Cl^- ion and *S*-(1,2-dichlorovinyl)glutathione (DCVG) as products. Although this initial GSH-conjugation step can occur in many tissues, it occurs primarily in the liver owing to first-pass metabolism and the high content of GSTs in the liver (the various GST isoforms can account for as much as 5% of total cytosolic protein in rat or human liver).

DCVG, whether it is formed within the kidneys or in the liver, is then processed predominantly in the kidneys by a sequence of two hydrolytic enzymes on the proximal tubular brush-border membrane, γ-glutamyltransferase (GGT) and cysteinylglycine dipeptidase, to yield the corresponding cysteine conjugate, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC).

DCVC can be viewed as a major branching point in this metabolic pathway, as it can have three potential fates. First, DCVC can be *N*-acetylated by the microsomal cysteine conjugate *N*-acetyltransferase to form the mercapturate *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC). Second, DCVC can be a substrate for cysteine-conjugate *β*-lyase (CCBL) activities to generate the reactive thiolate *S*-(1,2-dichlorovinyl)-thiol (DCVT). DCVT spontaneously rearranges to form either chlorothioketene or chlorothionoacetyl chloride (Dekant *et al.*, 1988; Völkel & Dekant, 1998), both of which are chemically unstable and reactive and are believed to be the molecular species responsible for formation of covalent adducts derived from DCVC with nucleic acids (Müller *et al.*, 1998a, b), proteins (Hayden *et al.*, 1991), and phospholipids (Hayden *et al.*, 1992). Third, DCVC can be a substrate for the flavin-containing monooxygenase (FMO), yielding a reactive sulfoxide, *S*-(1,2-dichlorovinyl)-L-cysteine

Fig. 4.1 Scheme for oxidative metabolism of trichloroethylene



Trichloroethylene undergoes cytochrome P450 (CYP)-dependent oxidation to form either a trichloroethylene–CYP intermediate or an epoxide intermediate. Further processing through either non-enzymatic rearrangements or the actions of aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), CYPs, or glutathione-*S*-transferase zeta (GSTZ) yield a variety of metabolites, including chloral (CHL) and chloral hydrate (CH), dichloroacetate (DCA), trichloroacetate (TCA), trichloroethanol (TCOH) and its glucuronide (TCOG), monochloroacetate (MCA), and oxalate (OA). Names of metabolites that are recovered in urine are shown in boxes and those that are chemically unstable or reactive are shown in brackets. Other abbreviations: DCAC, dichloroacetyl chloride; GSH, glutathione; N-OH-Ac-aminoethanol, *N*-hydroxyacetyl aminoethanol; trichloroethylene-O, trichloroethylene epoxide; UGT, UDP-glucuronosyltransferase.

Table 4.1 Formation and systemic availability of trichloroethylene and its metabolites

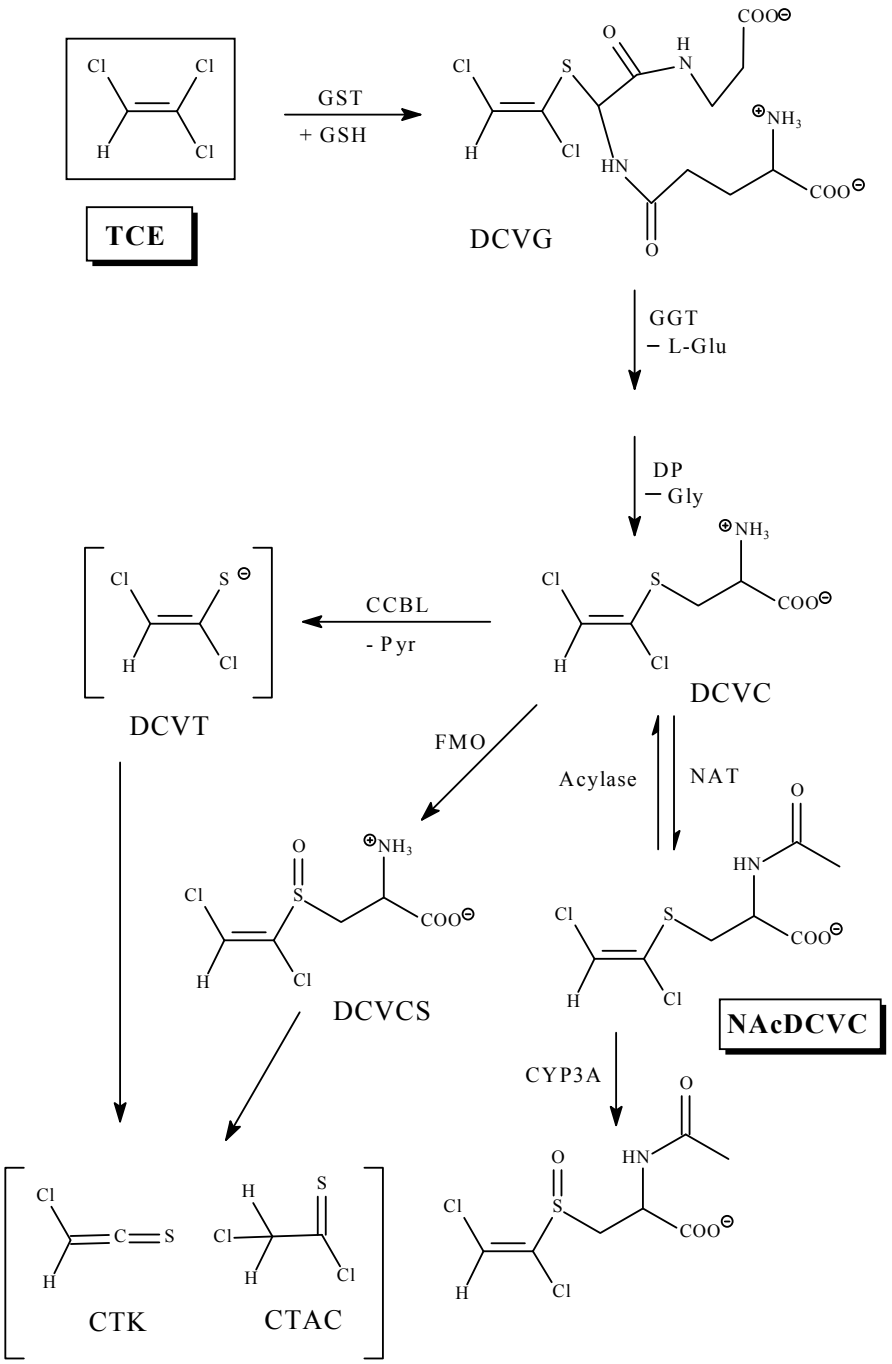
Compound or metabolite	Portal of entry or tissues where formed (A/H)	Systemic availability (A/H)
Trichloroethylene	Lung Gastrointestinal tract Skin	Yes (A,H)
Oxidative metabolites (CYP pathway)		
Trichloroethylene epoxide Dichloroacetyl chloride	Liver (A, H) Lung (A, H) Testes (A, H)	No
Chloral hydrate/chloral	Liver (A, H) Lung (A, H) Testes (A, H)	Yes
Trichloroethanol	Liver (A, H) Lung (A) GI (A, H) Testes (A,H)	Yes
Trichloroacetate	Liver (A, H) Lung (A,H) Testes (H)	Yes
Trichloroethanol glucuronide	Liver (A,H)	Yes
Dichloroacetate	Liver (A) Lung (A) Testes (H)	Yes (low amount)
GSH-conjugation metabolites		
DCVG	Liver (A, H) Kidney (A, H)	Yes
DCVC	Liver (A, H) Kidney (A, H)	Yes
DCVT DCVCS CTK/CTAC	Kidney (A, H) Haematopoietic (A)	No
NAcDCVC NAcDCVCS	Liver (A, H) Kidney (A, H)	Yes

A, animal; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene; DCA, dichloroacetate; DCAC, dichloroacetyl chloride; DCVC, *S*-(1,2-dichlorovinyl)-*L*-cysteine; DCVCS, DCVC sulfoxide; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVT, *S*-(1,2-dichlorovinyl)-thiol; GSH, glutathione; GST, glutathione-*S*-transferase; H, human; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; NAcDCVCS, NAcDCVC sulfoxide

sulfoxide (DCVCS). Because of the reactive nature of the various intermediates from this pathway, only NAcDCVC has been recovered in urine of experimental animals (Dekant *et al.*, 1986b; Bernauer *et al.*, 1996) and humans (Birner *et al.*, 1993; Bernauer *et al.*, 1996) exposed to trichloroethylene or DCVC.

The mercapturate NAcDCVC can have three potential fates. Apart from being excreted into the urine, NAcDCVC can be deacetylated within the renal proximal tubular cell by aminoacylase III to reform DCVC (Uttamsingh & Anders, 1999; Uttamsingh *et al.*, 2000; Newman *et al.*, 2007). Additionally, mercapturates of several nephrotoxic haloalkenes, including NAcDCVC, are substrates for CYP3A enzymes, yielding sulfoxides (Werner *et al.*, 1995a, b, 1996). Thus, although NAcDCVC is considered a stable end product of the metabolism of trichloroethylene and is recovered in urine, it can undergo additional metabolic transformations that serve to reactivate it. [The Working Group noted that

Fig. 4.2 Scheme for glutathione-dependent metabolism of trichloroethylene



Trichloroethylene (trichloroethylene) undergoes conjugation with glutathione (GSH) to yield the GSH S-conjugate DCVG. After processing to yield the cysteine S-conjugate DCVC, three potential fates are detoxication to yield the mercapturate NAcDCVC or bioactivation by either the cysteine conjugate β-lyase to yield dichlorovinylthiol, which rearranges to yield thioacylating species, or the flavin-containing monooxygenase to yield DCVC sulfoxide. The mercapturate can also be deacetylated to regenerate DCVC or it can undergo CYP3A-dependent sulfoxidation. Names of metabolites than are recovered in urine are shown in boxes and those that are chemical unstable or reactive are shown in brackets. Abbreviations: CCBL, cysteine conjugate β-lyase; CYP3A, cytochrome P-450 3A; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVCS, DCVC sulfoxide; DCVT, 1,2-dichlorovinylthiol; DP, dipeptidase; FMO, flavin-containing monooxygenase; GGT, γ-glutamyltransferase; GLy, glycine; GSH, glutathione; GST, GSH S-transferase; L-Glu, L-glutamic acid; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine; NAcDCVCS, NAcDCVC sulfoxide; NAT, *N*-acetyltransferase.

these additional fates of the putative end product of the GSH-conjugation pathway highlight both the complexity of the metabolism of trichloroethylene by this pathway and the potential difficulties in equating overall flux through the GSH-conjugation pathway when using urinary NAcDCVC as a surrogate measurement.]

A summary of the site of formation and systemic availability of the major metabolites from the GSH-conjugation pathway is presented in Table 4.1.

Of the two potential bioactivation pathways for DCVC, the CCBL and FMO reactions, the former has received the most study and is thought to account for most of the bioactivation activity for DCVC (Lash *et al.*, 2000a). CCBL activity is actually a catalytic function of a diverse array of enzymes (Cooper & Pinto, 2006; Lash, 2007). While CCBL activity has also been detected in liver and other tissues, only renal CCBL activity is toxicologically important for renal toxicity, because of the tissue localization of plasma-membrane transporters and several of the enzymes of the GSH-conjugation pathway that determine the distribution of trichloroethylene metabolites.

Studies in the mid-1960s first identified a thiol metabolite of a sulfonamide that was formed by a C–S lyase activity (Colucci & Buyske, 1965). Schultze and coworkers (Anderson & Schultze, 1965; Bhattacharya & Schultze, 1967) subsequently identified hepatic and renal enzymes that catalysed this reaction, and Tateishi *et al.* (1978) were the first to use the term "cysteine conjugate β-lyase" to describe this activity in rat liver. All the known CCBL enzymes contain pyridoxal 5′-phosphate as coenzyme. Although the overall β-lyase reaction mechanism is cleavage of a C–S bond to yield a reactive, thioacylating species, subsequent studies (Stevens *et al.*, 1986; Elfarra *et al.*, 1987) showed that the reaction mechanism can occur by either a direct β-elimination reaction or a transamination reaction with a suitable α-keto acid cosubstrate to yield either the thiolate or a propionic acid derivative, respectively; the latter is chemically unstable and rearranges to release the thiolate.

According to Cooper & Pinto (2006), there are 11 distinct mammalian enzymes capable of catalysing the CCBL reaction (Table 4.2). Some of the CCBL enzymes catalyse both β-elimination and transamination reactions, while others can only catalyse the former. The relative importance of each of these activities in the bioactivation of DCVC, however, is not presently known.

The FMOs, like the CYPs, represent a multigene family of enzymes. Both enzyme systems also share several other characteristics, including localization in the endoplasmic reticulum, requirement for NADPH as a reductant, and overall catalysis of a mixed-function oxidation reaction. Differences do exist, however, that make some of the functions of the FMOs rather distinctive. For example, although there are more than 50 individual functional CYP enzymes from more than 40 gene families in humans, there are only five FMO genes in mammals. FMOs catalyse the oxidation of chemicals containing sulfur, selenium, and nitrogen (Ziegler, 1993; Cashman & Zhang, 2006). Although FMOs and some CYPs share substrates and catalyse the same overall reactions, FMOs have some distinctive substrates, including cysteine *S*-conjugates of various haloalkenes and haloalkanes.

4.1.4 Excretion

(a) Humans

The main routes for the excretion of trichloroethylene are exhalation of the parent compound at high doses, and urinary excretion of metabolites. After exposure by inhalation, pulmonary excretion during and up to 5 days after exposure has been estimated to account for 19–35% of intake, with trichloroethanol and trichloroacetic acid in urine accounting for 24–39% of intake, and the balance still retained in the body at the end of the reporting period (Monster *et al.*, 1976; Opdam, 1989; Chiu *et al.*, 2007). The terminal

half-life of trichloroethylene in alveolar air has been estimated to be about 6–44 hours (Sato *et al.*, 1977; Opdam, 1989; Chiu *et al.*, 2007), reflecting release from storage in fat. The half-lives of trichloroethanol and trichloroacetate in urine have been estimated to be in the range of 15–50 and 36–73 hours, respectively (Bartonicek, 1962; Stewart *et al.*, 1970; Ikeda *et al.*, 1971; Nomiyama & Nomiyama, 1971; Ogata *et al.*, 1971; Ikeda & Imanura, 1973).

Limited information was available on faecal elimination of trichloroethylene and its metabolites. On the third day after exposure by inhalation, Bartonicek (1962) reported detecting trichloroacetate and trichloroethanol in the faeces at concentrations similar to those in urine. However, by the seventh day after exposure, neither substance was detected. Since daily faecal production is 10 times less than daily urine production, this implies that excretion in the urine is about 10-fold that in faeces.

(b) Experimental systems

Exhalation of trichloroethylene has been measured in rodents given trichloroethylene via inhalation, oral, and dermal exposure (Dekant *et al.*, 1984; Green & Prout, 1985; Prout *et al.*, 1985; Dekant *et al.*, 1986a; Dallas *et al.*, 1991; Poet *et al.*, 2000). Radiolabelling studies have also reported exhaled carbon dioxide as an excretion product in addition to unchanged parent (Dekant *et al.*, 1984, 1986a; Green & Prout, 1985; Prout *et al.*, 1985). In rats and mice, there is a general trend towards increasing exhalation of unchanged trichloroethylene with increasing dose from 2 to 2000 mg/kg bw, suggesting saturation of a metabolic pathway. In mice, the percentage recovered in air increased from 1–6% to 10–18% in this dose range, while in rats, the increase was from 1–3% to 43–78%. This was consistent with the greater overall metabolic capacity in mice than in rats. At exposures below saturation, most of the trichloroethylene administered is excreted in urine as metabolites. In addition, urinary excretion is relatively rapid in rodents, with reported half-lives of 14–17 hours (Ikeda & Imanura, 1973), and complete elimination within 1 or 2 days after exposure (Dekant *et al.*, 1984; Green & Prout, 1985; Prout *et al.*, 1985). [The Working Group noted that excretion rates in rodents are faster than those inferred from studies in humans.]

With respect to faecal elimination, Dekant *et al.* (1984) and Kim & Ghanayem (2006) reported that total radioactivity recovered in mouse and rat faeces after oral exposures accounted for about 1–5% of total radiolabel administered. However, Green & Prout (1985) and Prout *et al.* (1985) reported higher amounts of faecal excretion, about 8–24%, in various strains of mice.

4.2 Genotoxicity and related effects

4.2.1 Humans

(a) Chromosomal aberration, sister-chromatid exchange and related genotoxic effects

The limited number of studies on the genotoxicity of trichloroethylene have focused on the association between exposure to trichloroethylene and the development of chromosomal aberrations in exposed individuals. Chromosomal aberrations are disruptions in the normal content of the chromosomes and can include both numerical and structural changes, such as translocations and deletions (Natarajan *et al.*, 1996). There have been no additional studies of genotoxicity with trichloroethylene since the previous IARC evaluation in 1995 (IARC, 1995; Table 4.3; reviewed in Tabrez & Ahmad, 2009). The evidence for genotoxic effects of trichloroethylene in humans was limited and based on a small number of studies (IARC, 1995; Table 4.3). Exposure to trichloroethylene did not result in a significantly increased frequency of sister-chromatid exchange (SCE) in lymphocytes in one occupational study comparing trichloroethylene-exposed workers and controls, while in

Table 4.2 Mammalian enzymes capable of catalysing cysteine-conjugate β-lyase activity

Enzyme	Tissue (A/H)	Competing transamination	Subunit molecular weight (kDa)	Reference(s)
Cytoplasmic enzymes				
GTK/KAT (EC 2.6.1.64/EC 2.6.1.7)	Kidney (A/H)	Yes	45 (homodimer)	Stevens *et al.* (1986), Lash *et al.* (1990), Perry *et al.* (1993, 1995), Yamauchi *et al.* (1993)
GTK/KAT	Choroid plexus (A)	Yes	45 (homodimer)	Cooper *et al.* (1993), Alberati-Giani *et al.* (1995)
Kynureninase (EC 3.7.1.3)	Liver (A/H)	ND	55 (homodimer)	Tateishi *et al.* (1978), Stevens & Jakoby (1983), Stevens (1985), Tomisawa *et al.* (1986)
cytASAT (EC 2.6.1.1)	Heart (H)	No	45 (monomer)	Gaskin *et al.* (1995)
ALAT (EC 2.6.1.2)	Heart (H)	No		Gaskin *et al.* (1995)
$BCAT_c$ (EC 2.6.1.42)	Brain (H)	No	44 (homodimer)	Cooper *et al.* (2003)
High-molecular-weight CC β-lyase	Liver, kidney (A)	Yes	330	Abraham *et al.* (1995a)
Mitochondrial enzymes				
mitASAT (EC 2.6.1.1)	Liver (A)	Yes	45 (monomer)	Cooper *et al.* (2002)
$BCAT_m$ (EC 2.6.1.42)	Brain (H)	No	44 (homodimer)	Cooper *et al.* (2003)
GTK/KAT (EC 2.6.1.64/EC 2.6.1.7)	Brain (A)	Yes	45 (homodimer)	Malherbe *et al.* (1995)
High-molecular-weight CC β-lyase	Liver, kidney (A)	Yes	330	Abraham *et al.* (1995b), Cooper *et al.* (2001)

A, studies in experimental animals; ALAT, alanine aminotransferase; $BCAT_{c/m}$, branched-chain aminotransferase (cytoplasm/mitochondria); CC β-lyase, cysteine-conjugate β-lyase; cyt/mitASAT, cytosolic and mitochondrial aspartate aminotransferase; GTK, glutamine transaminase K; H, studies in human tissue; KAT, kynurenine aminotransferase; ND, not determined
Modified from Lash (2007).

another study it was reported that the frequency of structural aberrations and hyperdiploid cells in cultured lymphocytes was significantly increased in metal workers exposed to trichloroethylene compared with unexposed people (Tabrez & Ahmad, 2009). In another study, while quantitative estimates of exposure to trichloroethylene were not reported for degreasing workers, only workers with the highest levels of exposure (i.e. exposures > 20 hours per week) were included in the analysis (Rasmussen *et al.*, 1988). The reference group (not exposed) for this analysis was drawn from a population-based study and parents of offspring with stable chromosome abnormalities, and therefore may be less comparable to the exposed workers than in other studies. There is some evidence that the frequency of SCE may be significantly elevated in lymphocytes in male smokers exposed to trichloroethylene in an occupational setting, compared with male smokers in the control group, but these findings were based on only 15 subjects and similar associations were not observed for women or for nonsmokers (Seiji *et al.*, 1990). In this study, non-exposed workers were matched to exposed workers by age, sex, smoking habits, and working location in the factory. One study in India, including 97 trichloroethylene-exposed individuals and 220 individuals without exposure, evaluated genotoxicity in lymphocyte cultures *in vitro* in relation to polymorphisms in detoxifying genes (*CYP1A1, GSTM1, GSTT1, GSTP1;* Kumar *et al.*, 2009). Peripheral lymphocytes were treated *in vitro* with trichloroethylene at concentrations of 2, 4, and 6 mM and were examined for chromosomal aberration and micronucleus formation. [The Working Group noted that these high, non-physiological concentrations

of solvent typically cause non-specific effects.] Genotype frequencies for the detoxifying genes examined were similar in the solvent-exposed and control groups, and increased frequencies of chromosomal aberrations and micronucleus induction were generally not observed, except in rare cases at the higher levels of exposure to trichloroethylene, although no differences based on genotype were reported. Therefore while the available data from studies in humans provided inconclusive evidence of a genotoxic effect of trichloroethylene, the findings of an elevated frequency of structural chromosomal aberration and SCE in a subset of exposed individuals, combined with the weak mutagenic activity of trichloroethylene observed in some experimental studies, suggested that such effects cannot be ruled out (Tabrez & Ahmad, 2009).

(b) Mutations in the VHL gene

Several studies have evaluated the association between exposure to trichloroethylene and mutation of the von Hippel-Lindau (*VHL*) tumour suppressor gene, which has been reported in a relatively large percentage of cases of renal cell carcinoma (Table 4.3). *VHL* is thought to be a target for environmental carcinogens. The results regarding the association between renal cell carcinoma and mutations in the *VHL* gene were inconclusive.

The first study was of 23 patients in Germany with histologically confirmed renal cell carcinoma who had previously been exposed to trichloroethylene for an average of about 22 years, and who were estimated to have exposure levels exceeding 50 ppm based on reported symptoms (Brüning *et al.*, 1997a). Tumour tissue and normal tissue from each patient was isolated and individual *VHL* exons were amplified and subsequently analysed for single-strand conformation polymorphism (SSCP). All 23 patients had abnormal SSCP patterns in one of the exons considered when compared with wild-type *VHL* sequences, with most differences being in exon 2 (44% of patients), followed by exon 1 (30% of patients), and exon 3 (26% of patients). Further sequencing analysis of a subset of the aberrations confirmed these as *VHL* mutations. This frequency of mutation was higher than found in other studies reviewed below, and differed with that found in a later study in Germany in terms of the specific *VHL* exon that showed the highest frequency of mutation (Brauch *et al.*, 1999).

A case–control study in Germany enrolled 44 patients with renal cell carcinoma (a subset of whom were previously enrolled in the study by Brüning *et al.* 1997a) who had previously worked in metal-processing factories and had high cumulative exposures to trichloroethylene, and 107 control patients without reported exposure to trichloroethylene (Brauch *et al.*, 1999). [The Working Group noted that it did not see these two studies as independent.] The patients with exposure to trichloroethylene had a mean age of 24 years when they first began working with trichloroethylene, and were estimated to have exposure levels > 50 ppm based on reported clinical symptoms such as headache and nausea. Exposure intensity for the workers was scored on the basis of criteria that included the duration and frequency of working with trichloroethylene, associated clinical symptoms, and how the liquid trichloroethylene was regularly handled (Brauch *et al.*, 1999). *VHL* mutations were assessed by SSCP analysis, and microsatellite analysis was used to assess loss of heterozygosity (LOH). Of the 44 patients with renal cell carcinoma who had been exposed to trichloroethylene, *VHL* mutations were identified in 33 patients, and 14 of these had multiple, mainly missense (54%), mutations. The majority of mutations were found at exon 1 (52%), followed by exon 3 (28%), and exon 2 (20%) (Brauch *et al.*, 1999). Moreover, a mutational hot spot at nucleotide 454 of the *VHL* gene, which resulted in a C→T base change, was found in 39% of patients with a *VHL* mutation and 18 out of 18 renal cell carcinomas with intragenic somatic *VHL* mutation were reported to have LOH at the

Table 4.3 Genetic effects of trichloroethylene in humans

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
Chromosomal aberrations in lymphocytes; sperm count and morphology, % two fluorescent bodies, structural aberrations, hyperdiploid cells	15	14 for semen examination and 669 for lymphocyte analysis	Population-based (all degreasing workers identified)	Trichloroethylene exposures > 20 hr per week in 15 workers included in genotoxic analyses; exposure level, NR	Exposed workers had significantly increased occurrence of structural aberrations and hyperdiploid cells compared with controls	No personal exposure monitoring conducted; exposure data collected via occupational medical interview; reference group for lymphocyte analysis was drawn from a population study and parents of offspring with stable chromosome abnormalities.	Rasmussen *et al.* (1988) Denmark
SCE in human lymphocytes *in vivo*	6	9	Workers exposed to trichloroethylene	Exposed level, NR	Exposed workers had significant increase in SCE	Personal exposures were assessed by the measure of trichloroethylene metabolites in the blood (trichloroethanol and trichloroacetate).	Gu *et al.* (1981)
SCE	22	22	Cross-sectional	Exposed: U-TTC, 183.6 mg/L	No differences between exposed and controls, including in smokers	Mean employment duration of workers was 9.7 years; U-TTC used as index for degree of exposure, no monitoring conducted; unexposed controls matched to exposed workers by sex, age, and smoking habits	Nagaya *et al.* (1989) Japan

Table 4.3 (continued)

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
SCEs in peripheral blood lymphocytes.	38	51	Cross-sectional	Exposed: 7 ppm	No overall difference in SCEs in exposed workers and controls, but significant increase in male exposed smokers compared with male smoking controls.	Diffuse sampling techniques used to monitor workers in breathing zone as TWA for 8 h working day; concurrent controls matched to exposed workers by age, sex, smoking, location of factory	Seiji *et al.* (1990) Japan
VHL mutation	23	NA	Patients from two case–control studies	Semi-quantitative exposure estimates; average exposure time, 21.8 years	All 23 patients had aberrations of the *VHL* gene (exon 1, 30%; exon 2, 44%; exon 3, 26%)	Higher than expected mutation frequency; mutation prevalence highest in exon 2 whereas prevalence was highest in exon 1 in subsequent study (Brauch *et al.*, 1999)	Brüning *et al.* (1997a) Germany
VHL mutation	44	107	Case–control	Exposure determined by occupational hygenists and ranked as one of three levels. Scoring system took into account total exposure time, frequency, and duration of acute adverse effects.	75% mutation prevalence in trichloroethylene-exposed samples (multiple mutations, $n = 14$); 54% of mutations were missense; mutational hotspot at nucleotide 454 in 39% of patients and only in trichloroethylene-exposed.	Majority of mutations at exon 1 (52%); exon 1 is very difficult to sequence in FFPE tissue; mutations at nucleotide 454 were also observed in clear cell and papillary subtypes.	Brauch *et al.* (1999) Germany

Table 4.3 (continued)

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
VHL mutation	17 (RCC cases exposed to trichloroethylene)	21 (RCC cases not exposed to trichloroethylene)	Case–control	Used same exposure scoring system as in previous study by Brauch *et al.* (1999).	Exposed cases had a higher frequency of mutations (82% versus 10% unexposed), multiple mutations (50% versus 0% unexposed), and 454 C→T mutation (38% versus 0% unexposed).	Reported methodological difficulties including insufficient DNA yield; 75% of *VHL* coding gene amplified; mutation rate only 10% in the unexposed cases.	Brauch *et al.* (2004) Germany
VHL mutation	69 cases of RCC	NA	Patients from a case–control study	Expert evaluated occupational questionnaires and used a task-exposure matrix; exposures assigned as low/medium/high level.	No significant differences in frequency of mutations between RCC cases according to trichloroethylene exposure status; 4 mutations detected in 48 cases of RCC.	Potential trichloroethylene exposure misclassification, OR for trichloroethylene and RCC from case–control study was higher after restricting to higher confidence trichloroethylene exposures.	Charbotel *et al.* (2007) France
VHL mutation	470 sporadic clear cell RCC cases	NA	Case series	Exposure assessment based on expert review; estimated frequency, intensity, and cumulative exposure.	*VHL* inactivation reported in 86.6% of clear cell RCC cases, but differences in alteration prevalence according to trichloroethylene exposure status.	Only one unexposed case had a *VHL* mutation at nt454 and the *VHL* mutation prevalence was similar to trichloroethylene-exposed and unexposed cases.	Moore *et al.* (2011) Europe

FFPE, formalin-fixed, paraffin-embedded; NA, not applicable: normal tissue used as control or known control with additional band as positive control; nt, nucleotide; OR, odds ratio; RCC, renal cell carcinoma; SCE, sister-chromatid exchange; TWA, time-weighted average; U-TTC, urinary total trichloro compounds; ND, not determined.

3p allele. Notably, only patients with high and medium exposure to trichloroethylene, but not low exposure, had *VHL* mutations, and there was a significant correlation between severity of exposure and presence of multiple mutations.

A follow-up of the previous study compared the characteristics of *VHL* mutations in cases of renal cell carcinoma in people exposed to trichloroethylene (n = 17) and cases in people not exposed to trichloroethylene (n = 21) (Brauch *et al.*, 2004). Samples of tissues from tumour and non-tumour areas of the kidney were collected from the 38 cases, microdissected, and amplification and sequencing of the individual *VHL* exons was conducted using polymerase chain reaction (PCR). Cases of renal cell carcinoma associated with occupational exposure to trichloroethylene were reported to be diagnosed at a younger age (median, 57.5 years) compared with cases with no exposure to trichloroethylene (median, 67 years). In addition, mutation characteristics of the *VHL* gene differed according to trichloroethylene-exposure status, as exposed cases had a higher frequency of somatic mutations (82% in exposed versus 10% in unexposed), multiple mutations (50% in exposed versus 0% in unexposed), and frequency of the nucleotide 454 C→T hot spot mutation previously identified (38% in exposed versus 0% in unexposed). Methodological difficulties were reported relating to insufficient DNA yield, which may have explained the lower than expected frequency of *VHL* mutation in the unexposed cases (10%), compared with the 44% expected from published case series (Brauch *et al.*, 2004). Specifically, failure of the PCR amplification for the GC-rich *VHL* exon 1 occurred in 25% of cases, while the failure rate in cases was 76% for exon 3. This, combined with mutation-detection issues resulting from template disequilibrium, could have resulted in the observed frequency (Brauch *et al.*, 2004).

A study in France analysed somatic mutations in the *VHL* gene in 69 cases of renal cell carcinoma from a case–control study, of which 48 were clear cell renal cell carcinomas (Charbotel *et al.*, 2007). Formalin and Bouin's fixed paraffin-embedded tissue samples (n = 64) and frozen samples (n = 5) were obtained from patients, and screening for *VHL* mutation was conducted using PCR. The *VHL* gene was amplified and sequenced for all of the frozen samples, and for 71% and 38% of the formalin-fixed and Bouin's fixed samples, respectively, resulting in a complete sequence, with 100% of the coding region analysed, for 26 tumours (54%). There was no statistically significant difference between the trichloroethylene-exposed and not-exposed cases (P = 0.40) with respect to the frequency of mutations in codon 81, or the frequency of mutation overall. For the cases of clear cell renal cell carcinoma for which the *VHL* gene was fully sequenced (trichloroethylene-exposed, 15; not exposed, 11) and those for which the *VHL* gene was not entirely sequenced (trichloroethylene-exposed, 25; not exposed, 23), there were two mutations in each group. The low prevalence of alterations in *VHL* could be attributed to the method of DNA extraction, as formalin-fixing methods generally yield DNA of lower quality than that that obtained after freezing (Moore *et al.*, 2011). Another limitation was potential exposure misclassification, since sensitivity analyses from the case–control study that evaluated the association between exposure to trichloroethylene and renal cell carcinoma reported that the odds ratio increased when the analysis was restricted to cases for which exposure information was judged to be of good quality (Charbotel *et al.*, 2006).

A large case-series of clear cell renal cell carcinomas (n = 470) enrolled from a hospital-based case–control study in Europe evaluated *VHL* inactivation in relation to risk of sporadic clear cell renal cell carcinoma (Moore *et al.*, 2011). All cases were newly diagnosed and histologically confirmed. Exposure to chlorinated solvents was assessed using job-specific questionnaires, and expert review (blinded to disease status) was

conducted to estimate cumulative exposure, and frequency and intensity of exposure. In 86.6% of cases of sporadic clear cell renal cell carcinoma, *VHL* was inactivated through genetic (sequence alterations) or epigenetic (promoter methylation) mechanisms. The most common alterations observed were deletions (37.8%) and missense mutations (27.9%), and most alterations were found in exon 1 (36.7%). Consideration of exposure to trichloroethylene and the prevalence of *VHL* mutation did not reveal a difference between exposed and non-exposed cases with respect to overall frequency of alteration, or frequency of multiple mutation, and no specific hotspot mutation at nucleotide 454 (codon 81) was identified among cases exposed to trichloroethylene (Moore *et al.*, 2011).

The methodological issues relating to the interpretation of the results from these studies have been recently reviewed (EPA, 2011a). These issues include the method of tissue preparation (i.e. frozen, formalin-fixed), potential contamination of the tumour tissue with neighbouring healthy tissue resulting in a lower mutation frequency, and biases involving subject selection and misclassification. Furthermore, discrepancies between studies may be attributed to differences in levels of exposure to trichloroethylene (Moore *et al.*, 2011).

In summary, epidemiological studies of somatic mutations in the *VHL* gene have been inconclusive, as three studies conducted in Germany have reported alterations in the *VHL* gene associated with exposure to trichloroethylene, but have differed with respect to which exon was most commonly affected. These findings were not replicated in a study in France, or in a large case-series of clear cell renal cell carcinomas in Europe. Reported methodological difficulties and concern for bias in some studies prevented firm conclusions from being drawn.

4.2.2 Experimental systems

The genetic effects of trichloroethylene in experimental systems, as measured in DNA-binding studies; in bacterial systems; in fungal and yeast systems; and in mammalian systems have been studied. For information on the genotoxicity of some oxidative metabolites of trichloroethylene, see the *Monographs* on Chloral and Chloral Hydrate, Dichloroacetic Acid, and Trichloroacetic Acid in this Volume. The genetic toxicology of trichloroethylene has been reviewed (Baden & Simmon, 1980; Fabricant & Chalmers, 1980; Vainio *et al.*, 1985; Crebelli & Carere, 1989; Candura & Faustman, 1991; Jackson *et al.*, 1993; ECETOC, 1994; EPA, 2011a). The possible mechanisms for genotoxicity of trichloroethylene were discussed by Henschler (1987).

In considering the genotoxicity of trichloroethylene, it should be recognized that stabilizers, such as epichlorohydrin and 1,2-epoxybutane, are often used in commercial preparations of trichloroethylene. These stabilizers are mutagenic, rendering problematic the interpretation of positive results in assays for mutagenicity with trichloroethylene (McGregor *et al.*, 1989). Humans are exposed mostly, if not exclusively, to preparations of trichloroethylene containing stabilizers. Table 4.4 lists the known tests for genotoxicity that have been conducted on samples of pure trichloroethylene (without stabilizers), and Table 4.5 lists results of tests for genotoxicity with samples for which the content of stabilizers was unclear.

(a) Binding to DNA and protein

Evidence suggests that exposure to trichloroethylene can lead to binding to DNA and proteins, probably due to the conversion of trichloroethylene to reactive metabolites. In studies *in vitro*, ^{14}C-labelled trichloroethylene was found to bind to salmon sperm DNA and calf thymus DNA under conditions that favoured the presence of CYP enzymes and inhibited the activity

of epoxide hydrolase (Banerjee & Van Duuren, 1978; DiRenzo *et al.*, 1982). The work of Miller & Guengerich (1983) and Cai & Guengerich (2001a) indicated that liver microsomes or whole hepatocytes from phenobarbital-induced rats enhanced binding of trichloroethylene metabolites to DNA and to protein. The levels of DNA adducts (binding to calf thymus DNA) and protein adducts formed in the presence of microsomal preparations of human liver were approximately the same as those observed in liver microsomes from untreated rats. Trichloroethylene epoxide was found to be responsible for the binding to protein, and also to DNA to a lesser extent.

In studies in rodents *in vivo*, radiolabelling of protein was greater than of DNA (Stott *et al.*, 1982; Mazzullo *et al.*, 1992).

Covalent binding of radiolabel to hepatic and renal proteins in male F344 rats and $B6C3F_1$ mice after exposure to [^{14}C]trichloroethylene was studied by Eyre *et al.* (1995a). Association of radiolabel from [^{14}C]trichloroethylene was observed in liver (peak, 2–4 hours) and kidney (peak, 8 hours) proteins in rats and mice. The formation of acid-labile protein adducts from [^{14}C]trichloroethylene was approximately two times greater in mice than in rats. In the same study, there was no evidence for formation of these adducts in rats and mice treated with [^{14}C]-labelled trichloroacetic acid or [^{14}C]-labelled dichloroacetic acid, while treatment with [^{14}C]-labelled DCVC resulted in the formation of renal acid-labile protein adducts at a level that was greater by approximately 12-fold in male $B6C3F_1$ mice than in male F344 rats.

A low level of covalent interaction was reported with DNA from rat and mouse liver, kidney, lung, and stomach (estimated at 0.15 adducts per million nucleotides (Mazzullo *et al.*, 1992); and from mouse liver (maximum, 0.62 alkylations per million nucleotides, Stott *et al.*, 1982). Binding of [^{14}C]trichloroethylene to DNA *in vitro* was enhanced by the addition of GSH and was reduced by the addition of SKF-525-A, a CYP inhibitor (Mazzullo *et al.*, 1992). High-performance liquid chromatography indicated the possible presence of a DNA adduct, which was not identified. In other studies, DNA binding could not be demonstrated *in vivo* in several tissues of mice (Bergman, 1983), or in the liver of rats (Parchman & Magee, 1982); however, Bergman (1983) noted incorporation of radiolabel into nucleosides.

(b) *Mutation*

(i) *Bacterial systems*

See Table 4.4 and Table 4.5

The Ames assay for gene mutation has been conducted with trichloroethylene (pure, or of unspecified purity) in various strains of *Salmonella typhimurium*, with and without metabolic activation. Both positive and negative results have been reported, but several studies showed positive results with metabolic activation in strain TA100. The pure samples of trichloroethylene (non-technical grade) did not induce mutation in other strains.

Trichloroethylene (pure, or of unspecified purity) gave negative results in the SOS chromotest in *Escherichia coli* with or without metabolic activation, and in the Mutatox assay in the absence of metabolic activation. In the presence of metabolic activation, analytical-grade trichloroethylene induced *arg*$^+$reverse mutations, but not forward mutations or *gal*$^+$or *nad*$^+$ reversions, in *E. coli*.

(ii) *Fungi and yeast systems*

See Table 4.4 and Table 4.5

The ability of trichloroethylene to induce gene mutation, conversion, and recombination has been studied in fungi and yeast systems. Trichloroethylene (pure, or of unspecified purity) induced gene conversion in *Saccharomyces cerevisiae* in two out of three studies, and induced reverse mutation in the presence of metabolic activation in all four studies available. In a single study, pure trichloroethylene or trichloroethylene

Table 4.4 Genetic and related effects of trichloroethylene without mutagenic stabilizers in experimental systems

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SOS chromotest, *Escherichia coli* PQ37	–	–	7325[c]	Mersch-Sundermann *et al.* (1989)
Salmonella typhimurium BAL13, forward mutation (ara test)	–	–	190	Roldán-Arjona *et al.* (1991)
Salmonella typhimurium TA100, reverse mutation	–	+	160 vapour[c]	Simmon *et al.* (1977)
Salmonella typhimurium TA100, reverse mutation	–	+	160 vapour[c]	Baden *et al.* (1979)
Salmonella typhimurium TA100, reverse mutation	–	–	420 (8% vapour) 16 h	Bartsch *et al.* (1979)
Salmonella typhimurium TA100, reverse mutation	–	+	18 vapour	Crebelli *et al.* (1982)
Salmonella typhimurium TA100, reverse mutation	–	–	260 vapour[d]	Shimada *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1535, reverse mutation	–	–	2800 vapour[c]	Simmon *et al.* (1977)
Salmonella typhimurium TA1535, reverse mutation	–	–	526 vapour[c]	Baden *et al.* (1979)
Salmonella typhimurium TA1535, reverse mutation	(+)	0	50[c]	Kringstad *et al.* (1981)
Salmonella typhimurium TA1535, reverse mutation	(+)	–	50 vapour[d]	Shimada *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA1537, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA98, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA98, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
Salmonella typhimurium YG7108 pin 3ERb5, reverse mutation	0	–	3000 µg/plate	Emmert *et al.* (2006)
Saccharomyces cerevisiae D7, gene conversion	–	+	2600	Bronzetti *et al.* (1978)
Saccharomyces cerevisiae D7, reverse mutation	–	+	1300	Bronzetti *et al.* (1978)
Aspergillus nidulans, diploid yA2/+ strain 35×17, quiescent conidia, mitotic crossing-over	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35×17, growth-mediated assay, mitotic crossing-over	–	0	90 vapour	Crebelli *et al.* (1985)
Schizosaccharomyces pombe P1, stationary phase, forward mutation	–	–	3280	Rossi *et al.* (1983)
Schizosaccharomyces pombe P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Aspergillus nidulans, haploid strain 35, quiescent conidia, forward mutation (methionine suppressor)	–	0	100 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, haploid strain 35, 'growth-mediated assay', forward mutation (methionine suppressor)	+	0	13 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, quiescent conidia, nondisjunctional diploids	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, quiescent conidia, haploids	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, 'growth-mediated assay', nondisjunctional diploids	+	0	40 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, 'growth-mediated assay', haploids	+	0	90 vapour	Crebelli *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		2500[c] injection	Foureman *et al.* (1994)
Drosophila melanogaster, sex-linked recessive lethal mutation	?		5000 feeding[c]	Foureman *et al.* (1994)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour[d]	Shimada *et al.* (1985)
Unscheduled DNA synthesis, human lymphocytes *in vitro*	(+)	0	[7], 2.5–10µg/mL	Perocco & Prodi (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	+	146[c]	Caspary *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	(+)	(+)	401[c]	Galloway *et al.* (1987)
Micronucleus induction, Chinese hamster ovary (CHO-K_1) cells *in vitro*	+	0	150 [0.8–1.4 ppm]	Wang *et al.* (2001)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–[e]	–[e]	14 900[c]	Galloway *et al.* (1987)
Cell transformation, RLV/Fischer rat F1706 embryo cells *in vitro*	+	0	144	Price *et al.* (1978)
Micronucleus induction, human hepatoma Hep G2 cells	+	0	0.5 mM [65.7 µg/mL]	Hu *et al.* (2008)
Gene mutation, human lymphoblastoid TK6 cells *in vitro*	–	–	600	Caspary *et al.* (1988)
Inhibition of intercellular communication, B6C3F_1 mouse hepatocytes *in vitro*	+	0	1.3	Klaunig *et al.* (1989)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of intercellular communication, F344 rat hepatocytes *in vitro*	–	0	13	Klaunig *et al.* (1989)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D4 recovered from CD-1 mouse liver, lungs and kidneys	+		400 po × 1[f]	Bronzetti *et al.* (1978)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D7 recovered from CD-1 mouse liver and kidneys	+		400 po × 1	Bronzetti *et al.* (1978)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D7 recovered from CD-1 mouse lungs	–		400 po × 1	Bronzetti *et al.* (1978)
HMM, Host-mediated assay, reverse mutation in *Saccharomyces cerevisiae* D7 from CD-1 mouse liver, lungs and kidneys	+		400 po × 1	Bronzetti *et al.* (1978)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1, CD-1 × C57Bl hybrid mouse	–		2000 iv or ip × 1	Rossi *et al.* (1983)
Gene mutation, *Lac Z* transgenic mice, base change or small-deletion mutation in kidney, spleen, liver, lung, testis *in vivo*	–		3144 mg/kg bw, inhalation,[c] 6 h/day × 6 days	Douglas *et al.* (1999)
DNA single-strand breaks, mouse liver *in vivo*	–		2000 ip × 1	Parchman & Magee (1982)
DNA single-strand breaks (alkaline unwinding) in liver and kidney of male NMRI mice *in vivo*	+[g]		790 ip × 1	Walles (1986)
DNA single-strand breaks (alkaline unwinding), mouse liver *in vivo*	+		1500 po × 1[c]	Nelson & Bull (1988)
DNA single-strand breaks (alkaline unwinding), rat liver *in vivo*	+		3000 po × 1[c]	Nelson & Bull (1988)
Mouse spot test (DNA-alterations) *in vivo*	–		350 ip × 1	Fahrig (1977)
Unscheduled DNA synthesis, CD-1 mouse primary hepatocytes *in vivo*	–		1000 po × 1	Doolittle *et al.* (1987)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		750 po × 2	Duprat & Gradiski (1980)
Micronucleus induction, B6C3F_1 mouse bone-marrow erythrocytes *in vivo*	–		2500 ip × 3[c]	Shelby *et al.* (1993)
Micronucleus induction, mouse spermatocytes *in vivo* (spermatids examined) *in vivo*	–		565 inhalation 6 h/d × 5	Allen *et al.* (1994)
Micronucleus induction, mouse splenocytes *in vivo*	–		9800 inhalation 6 h	Kligerman *et al.* (1994)
Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	+		5 inhalation 6 h	Kligerman *et al.* (1994)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		8800 inhalation 6 h	Kligerman *et al.* (1994)
Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Micronucleus induction, rat kidney cells *in vivo*	+		3591 po × 1	Robbiano *et al.* (2004)
Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		8800 inhalation 6 h	Kligerman *et al.* (1994)
Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Sister chromatid exchange, mouse splenocytes *in vivo*	–		9800 inhalation 6 h	Kligerman *et al.* (1994)
Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	– [e]		8800 inhalation 6 h	Kligerman *et al.* (1994)
Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	– [e]		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Chromosomal aberrations, mouse splenocytes *in vivo*	– [e]		9800 inhalation 6 h	Kligerman *et al.* (1994)
Dominant lethal mutation, male NMRI-Han/BGA mice *in vivo*	–		3400 inhalation 24 h[c]	Slacik-Erben *et al.* (1980)
Binding (covalent) to salmon sperm DNA *in vitro*	–	+	270	Banerjee & Van Duuren (1978)
Binding (covalent) to calf thymus DNA *in vitro*	–	+	340[c]	Bergman (1983)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	13	Miller & Guengerich (1983)
Binding (covalent) to DNA of isolated rat hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
Binding (covalent) to DNA of isolated mouse hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	131	DiRenzo *et al.* (1982)
Binding (covalent) to RNA of NMRI mouse spleen, lung, liver, kidney, pancreas, testis and brain *in vivo*	–[h]		67 ip × 5[c]	Bergman (1983)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to DNA of NMRI mouse spleen, pancreas, lung, testis, kidney and brain *in vivo*	–[h]		67 ip × 5	Bergman (1983)
Binding (covalent) to DNA of NMRI mouse liver *in vivo*	?		67 ip × 5	Bergman (1983)
Binding (covalent) to DNA of B6C3F_1 mouse liver *in vivo*	?		1200 po × 1	Stott *et al.* (1982)
Binding (covalent) to DNA of B6C3F_1 mouse liver *in vivo*	?		250 ip × 1	Parchman & Magee (1982)
Binding (covalent) to DNA of Sprague-Dawley rat liver *in vivo*	?		1000 ip × 1	Parchman & Magee (1982)
Dichloroacetyl chloride				
λ Prophage induction, *Escherichia coli* WP2	–	–	10 000	DeMarini *et al.* (1994)
Salmonella typhimurium TA100, reverse mutation	+	–	3	DeMarini *et al.* (1994)
Trichloroethanol				
Salmonella typhimurium TA98, TA100, reverse mutation	–	–	7500	Waskell (1978)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	8 µg/plate	Bignami *et al.* (1980)
Salmonella typhimurium TA100	–	–	0.5 µg/cm^3 vapour	DeMarini *et al.* (1994)
Salmonella typhimurium TA104, reverse mutation	–	+	2500 µg/plate	Beland (1999)
Sister-chromatid exchanges, human lymphocytes *in vitro*	+	0	178	Gu *et al.* (1981)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested.

[b] LED, lowest effective dose; HID, highest effective dose. In-vitro tests, µg/mL; in-vivo tests, mg/kg bw; ip, intraperitoneally; po, orally.

[c] Purity, 99% or greater.

[d] Stabilizers, 0.001%

[e] It should be noted that results of most assays for chromosomal aberration report the combined incidence of multiple effects, including chromatid breaks, isochromatid or chromosome breaks, chromatid exchanges, dicentric chromosomes, ring chromosomes, and other aberrations.

[f] Also positive by gavage at 150 mg/kg bw for 5 days per week, 22 times, with 400 mg/kg bw on the last day.

[g] No DNA strand breaks in lungs of mice treated at 1300 mg/kg bw ip × 1

[h] Metabolic incorporation of ^{14}C into nucleotides was observed.

containing stabilizers did not induce forward mutation in *Schizosaccharomyces pombe* (Rossi *et al.*, 1983). Pure trichloroethylene induced forward mutation in one study of growing cultures of *Aspergillus nidulans*, which are capable of some metabolic-activation reactions, but this effect was not seen in quiescent conidia. Trichloroethylene (of unspecified purity) induced aneuploidy in *S. cerevisiae* in the presence of growth-mediated metabolic activation, and the pure compound induced aneuploidy in *A. nidulans*. In a single study, trichloroethylene (of unspecified purity) induced gene mutation in *Tradescantia* (Schairer & Sautkulis, 1982). Pure trichloroethylene did not cause recessive lethal mutations in *Drosophila melanogaster* after injection, and equivocal results were obtained after feeding (Foureman *et al.*, 1994).

As with bacterial systems, the parent compound trichloroethylene was not itself genotoxic, but the metabolites of trichloroethylene appeared to be responsible for positive effects in the various assays. Yeast strains with a high CYP content showed an increase in frequencies of mitotic gene conversion and recombination after exposure to trichloroethylene, while a strain with a much lower CYP content did not (Callen *et al.*, 1980). However, Rossi *et al.* (1983) saw no change in mutation frequency in yeast exposed to trichloroethylene in the presence or absence of supernatant S9. Likewise, Koch *et al.* (1988) did not observe any change in mitotic gene conversion, or reverse mutation with or without S9 in yeast exposed to trichloroethylene.

(iii) Mammalian systems

See Table 4.4 and Table 4.5

There were several studies of mutation induced by trichloroethylene in mammalian systems. In a host-mediated assay, gene conversion and reverse mutation were induced in *S. cerevisiae* recovered from the liver, lungs and kidneys of mice treated orally with pure trichloroethylene. Forward mutation was weakly induced by trichloroethylene of unspecified purity in *Schizosaccharomyces pombe* cells injected into the peritoneum of mice in one of two studies; no effect was seen in the only study available in rats. *S. pombe* cells recovered from mice after intravenous injection showed no forward mutation in one study; a positive result was seen in another study in mouse liver, but not in kidneys or lungs, after treatment with trichloroethylene of unspecified purity. In a more direct test for mutagenicity in mammals (Douglas *et al.*, 1999), male and female transgenic *lac*Z mice were exposed by inhalation to trichloroethylene at varying levels and mutation frequencies in multiple tissues were determined after 60 days. No positive findings were observed. In a single study, pure trichloroethylene did not induce dominant-lethal mutation in mice (Slacik-Erben *et al.*, 1980).

(c) Cytogenetic effects

There were some reports of studies on trichloroethylene-induced chromosomal aberrations. No chromosomal aberrations were observed by Galloway *et al.* (1987) in trichloroethylene-exposed Chinese hamster ovary cells, with or without S9. Kligerman *et al.* (1994) exposed rats and mice to trichloroethylene at varying levels by inhalation and examined peripheral blood lymphocytes for chromosomal aberrations, SCE, and micronucleus formation. No chromosomal aberrations were observed.

Studies both *in vitro* and *in vivo* indicated that exposure to trichloroethylene can induce micronucleus formation. Wang *et al.* (2001) found a dose-dependent increase in micronucleus formation in trichloroethylene-exposed Chinese hamster ovary cells, while studies by Robbiano *et al.* (2004) observed increases in micronucleus formation in exposed, cultured rat kidney cells. Hu *et al.* (2008), using exposure concentrations similar to those used by Robbiano *et al.* (2004), found a trichloroethylene-induced increase in micronucleus frequency in cultured human hepatoma cells. In the inhalation studies

Table 4.5 Genetic and related effects of trichloroethylene containing mutagenic stabilizers or of unknown purity in experimental systems

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli PQ37, SOS chromotest	–	–	13 140	von der Hude *et al.* (1988)
Photobacterium phosphorium, mutatox assay, derepression of luminescence operon,	–	0	NR	Elmore & Fitzgerald (1990)
Salmonella typhimurium TA100, reverse mutation	–	–	14 650 plate incorporation assay	Henschler *et al.* (1977)
Salmonella typhimurium TA100 and TA100 reverse mutation	–	–	525 vapour	Waskell (1978)
Salmonella typhimurium TA100, reverse mutation	+	+	260 vapour[c]	Shimada *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	+	+	NR	Milman *et al.* (1988)
Salmonella typhimurium TA100, reverse mutation	(+)	(+)	130 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1535, reverse mutation	+	+	50 vapour[c]	Shimada *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	+	+	NR	Milman *et al.* (1988)
Salmonella typhimurium TA1535, reverse mutation	+	+	33 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1537, reverse mutation	–	–	NR	Milman *et al.* (1988)
Salmonella typhimurium TA98, reverse mutation	–	–	525 vapour	Waskell (1978)
Salmonella typhimurium TA98, reverse mutation	–	–	0.00	Milman *et al.* (1988)
Salmonella typhimurium TA98, reverse mutation	–	–	65 vapour	McGregor *et al.* (1989)
Escherichia coli K12, forward mutation	–	–	434	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (*arg*$^+$)	–	+	434	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (*gal*$^+$) or (*nad*$^+$)	–	–	434	Greim *et al.* (1975)
Saccharomyces cerevisiae D7, log-phase cultures, gene conversion	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D4, log-phase cultures, gene conversion	0	–	2900[e]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, gene conversion	–	–	2900[e]	Koch *et al.* (1988)
Saccharomyces cerevisiae XV185–^{14}C, reverse mutation (*lys*1–1, *his*1–7, *hom*3–10)	0	+	1460	Shahin & Von Borstel (1977)
Saccharomyces cerevisiae D7, log-phase cultures, reverse mutation	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase cultures, mitotic recombinants or otherwise genetically altered colonies (*ade*2)	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, reverse mutation	–	(+)	2900[e]	Koch *et al.* (1988)
Schizosaccharomyces pombe P1, stationary phase, forward mutation	–	–	3285	Rossi *et al.* (1983)
Schizosaccharomyces pombe P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)

Table 4.5 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae D61.M, growing cells, aneuploidy	+	+	725	Koch *et al.* (1988)
Tradescantia species, mutation	+	0	0.0003	Schairer & Sautkulis (1982)
DNA strand break, comet assay, rat kidney cells *in vitro*	+	0	130	Robbiano *et al.* (2004)
DNA strand break, comet assay, human kidney cells *in vitro*	+	0	130	Robbiano *et al.* (2004)
Unscheduled DNA synthesis, phenobarbital-induced rat hepatocytes *in vitro*	+	0	368	Costa & Ivanetich (1984)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour	Shimada *et al.* (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	NR	Milman *et al.* (1988)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	1445	Williams *et al.* (1989)
Unscheduled DNA synthesis, B6C3F_1 mouse primary hepatocytes *in vitro*	+	0	NR	Milman *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	0	–	9	White *et al.* (1979)
Chromosomal aberrations, Chinese hamster lung (CHL) cells *in vitro*	–	–	1000	Sofuni *et al.* (1985)
Micronucleus induction, rat kidney cells *in vitro*	+	0	16.5	Robbiano *et al.* (2004)
Cell transformation BALB/c–3T3 mouse cells, *in vitro*	(+)	0	250	Tu *et al.* (1985)
Morphological transformation Syrian hamster embryo cells, *in vitro*	(+)	0	25	Amacher & Zelljadt (1983)
Micronucleus induction, human kidney cells *in vitro*	+	0	16.5	Robbiano *et al.* (2004)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse kidneys and lungs	–	0	2000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse liver	(+)	0	2000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in CD-1 mouse peritoneum	(+)	0	1000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in Spraque-Dawley rat peritoneum	–	0	1000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1CD-1 × 7BL hybrid mouse	–	0	2000 iv or ip × 1	Rossi *et al.* (1983)
DNA strand break, comet assay, Sprague-Dawley rat kidney *in vivo*	–		2000 ppm	Clay (2008)
Unscheduled DNA synthesis, Fischer 344 male rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)

Table 4.5 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, male and female B6C3F$_1$ mouse hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
Chromosomal aberrations, CD-1 mouse bone-marrow cells *in vivo*	–		1000 po × 1	Loprieno & Abbondandolo (1980)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		1200 po × 1	Sbrana *et al.* (1985) (abstract)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		795 inhalation 7 h × 5 d/wk, 10 wk	Sbrana *et al.* (1985) (abstract)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		1200 po × 1	Sbrana *et al.* (1985) (abstract)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		460 ip × 1[f]	Hrelia *et al.* (1994)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	3.2	Mazzullo *et al.* (1992)
Binding (covalent to DNA of BALB/c mouse liver, kidney, lung and stomach *in vivo*	(+)		0.76 ip × 1	Mazzullo *et al.* (1992)
Binding (covalent) to DNA of Wistar rat liver, kidney, lung and stomach *in vivo*	(+)		0.76 ip × 1	Mazzullo *et al.* (1992)
Enzyme-altered foci in male Osborne-Mendel rat liver *in vivo*, promotion protocol, with and without NDEA as an initiator	–		1300 mg/kg bw, 5 d/wk, 7 wk	Milman *et al.* (1988)
Enzyme-altered foci in male Osborne-Mendel rat *liver in vivo*, initiation protocol, phenobarbital as promoter	–		1300 mg/kg bw	Milman *et al.* (1988)
S-phase induction, male and female B6C3F$_1$ mouse hepatocytes *in vivo*	+		200 mg/kg	Mirsalis *et al.* (1989)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested

[b] LED, lowest effective dose; HID, highest in effective dose. In-vitro tests, µg/mL; in-vivo tests, mg/kg bw; NR, dose not reported; ip, intraperitoneally; po, orally

[c] Purity, ≥ 99%

[d] CYP content fivefold greater in D7 strain

[e] High toxicity at 22 mM [2900 µg/mL]

[f] Correlate with trichloroethanol in urine

NDEA; *N*-nitrosodiethylamine

of Kligerman *et al.* (1994) in rats and mice, micronucleus formation was increased in the bone-marrow cells of rats, but not mice. A high oral dose of trichloroethylene in rats (Robbiano *et al.*, 2004) resulted in micronucleus formation in kidney cells. A high intraperitoneal dose (457 mg/kg bw) in mice resulted in an increase in micronucleus formation in bone-marrow cells, and the micronucleus frequency correlated with measures of urinary oxidative metabolites of trichloroethylene (Hrelia *et al.*, 1994).

Studies both *in vivo* and *in vitro* have reported that trichloroethylene induces SCE. In studies *in vitro* using Chinese hamster ovary cells, White *et al.* (1979) found no induction of SCE in response to trichloroethylene, while Galloway *et al.* (1987) saw a response in the same cell line both with and without metabolic activation. Gu *et al.* (1981) observed a positive response in cultured human lymphocytes. In studies conducted *in vivo*, no increase in the frequency of SCE was observed in peripheral lymphocytes of rats, or in splenocytes of mice exposed to trichloroethylene by inhalation (Kligerman *et al.* (1994). Analysis of peripheral blood lymphocytes in humans occupationally exposed to trichloroethylene showed no increase in the frequency of SCE (Nagaya *et al.*, 1989).

(d) Other types of DNA damage and related effects

Unscheduled DNA synthesis (UDS), DNA strand breaks and cell transformation have all been studied in relation to exposure to trichloroethylene. Primary cultures of hepatocytes from rats did not show any induction of UDS, even at relatively high exposures (Shimada *et al.*, 1985). In hepatocytes from rats treated with phenobarbital, exposure to trichloroethylene was found to induce UDS (Costa & Ivanetich, 1984). Mirsalis *et al.* (1989) found no induction of UDS in rats and mice exposed *in vivo*. UDS was studied in human lymphocytes cultured *in vitro* with and without metabolic activation from S9; an increase in UDS was observed only in the presence of metabolic activation (Perocco & Prodi, 1981).

In the studies by Robbiano *et al.* (2004), DNA strand breaks were observed in cultured rat and human kidney cells exposed to trichloroethylene, and in kidney cells of a rat exposed to trichloroethylene at a high dose. In contrast, a study by Clay (2008) in rats exposed by inhalation to trichloroethylene found no DNA strand breaks with the comet assay in rat kidney cells.

Assays for cell transformation with trichloroethylene showed weakly positive and negative responses. In three different assays, trichloroethylene (of unspecified purity, except in one study) weakly induced cell transformation in mouse, Syrian hamster and rat cells (pure trichloroethylene) *in vitro*, without exogenous metabolic activation. Pure trichloroethylene inhibited intercellular communication in mouse hepatocytes, but not in rat hepatocytes *in vitro*.

(e) Genotoxicity of metabolites of trichloroethylene

Several experimental studies of the genotoxicity of trichloroethylene metabolites were performed, and are reviewed here. For discussion of the genotoxicity of the trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, and chloral and chloral hydrate, see the corresponding *Monographs* in this Volume.

(i) Trichloroethanol

A limited number of studies on the genotoxicity of trichloroethanol were available (see Table 4.4). Trichloroethanol gave negative results in *S. typhimurium* strain TA100 (Waskell, 1978; Bignami *et al.*, 1980; DeMarini *et al.*, 1994). A study by Beland (1999) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation; however, it did increase mutant frequency after preincubation of trichloroethanol at a dose of ≥ 2500 μg/plate with an exogenous metabolic activation system for 30 minutes before addition

of the tester strains. Trichloroethanol was not evaluated in the other recommended screening assays.

(ii) DCVG

See Table 4.6

Vamvakas *et al.* (1988a) investigated the mutagenicity of DCVG in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA (a pyridoxal phosphate-dependent β-lyase inhibitor) to inhibit genotoxicity. DCVG exhibited direct-acting mutagenicity, with kidney mitochondria, cytosol, or microsomes enhancing the effects and AOAA diminishing, but not abolishing, the effects. Importantly, addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with metabolism in situ (via GGT and dipeptidase) playing a significant role in the genotoxicity of the resulting cysteine conjugates in the kidney (Vamvakas *et al.*, 1988a).

(iii) DCVC

See Table 4.6

Dekant *et al.* (1986c) demonstrated mutagenicity of DCVC in S. *typhimurium* strains (TA100, TA2638, and TA98) using the Ames assay in the absence of S9. The effects were decreased with the addition of AOAA, a β-lyase inhibitor, suggesting that bioactivation by this enzyme plays a role in genotoxicity. Furthermore, Vamvakas *et al.* (1988a), in another experiment, investigated the mutagenicity of DCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA to inhibit genotoxicity. DCVC exhibited direct-acting mutagenicity, with kidney mitochondria or cytosol enhancing the effects and AOAA diminishing, but not abolishing, the effects.

DCVC has shown predominantly positive results in other available assays for genotoxicity *in vitro* and *in vivo*. Jaffe *et al.* (1985) reported DNA strand breaks after administration of DCVC *in vivo*, in isolated perfused kidneys (*ex vivo*), and in isolated proximal tubules of albino male rabbits (*ex vivo*). Vamvakas *et al.* (1989) reported dose-dependent increases in UDS in porcine kidney tubular epithelial LLC-PK1 cells at noncytotoxic concentrations. In addition, Vamvakas *et al.* (1996) reported that exposure of LLC-PK1 cells to DCVC at noncytotoxic concentrations for 7 weeks induced morphological and biochemical dedifferentiation of single cells (clones), which persisted for at least 30 passages after removal of the compound. This study also reported increased expression of the proto-oncogene c-*Fos* in the DCVC-derived clones. In Syrian hamster embryo fibroblasts, DCVC induced UDS, but not micronucleus formation (Vamvakas *et al.*, 1988b).

Two more recent studies are discussed in more detail. Mally *et al.* (2006) isolated primary rat kidney epithelial cells from *Tsc-2*$^{Ek/+}$ (Eker) rats, and reported increased transformation when the cells were exposed to DCVC at a concentration of 10 µM [2 µg/mL], like the genotoxic renal carcinogen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Horesovsky *et al.*, 1994). The frequency of transformation was variable, but consistently higher than background. No LOH of the tuberous sclerosis complex 2 tumour suppressor *Tsc-2* gene was reported either in these DCVC transformants or in renal tumours (which were not increased in incidence) in Eker rats treated with trichloroethylene. Mally *et al.* (2006) suggested that these data support a nongenotoxic mechanism, because a substantial fraction of spontaneous renal tumours in Eker rats showed LOH at this locus (Kubo *et al.*, 1994; Yeung *et al.*, 1995), and because LOH was demonstrated both *in vitro* and *in vivo* after treatment with 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone in Eker rats (Yoon *et al.*, 2001). However, 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone is not genotoxic in standard assays for mutagenicity (Yoon *et al.*, 2001), and Kubo *et al.* (1994) also reported that none of the renal tumours induced by the genotoxic carcinogen,

Table 4.6 Genetic and related effect of glutathione-derived metabolites of trichloroethylene in experimental systems

Test system/end-point	Result		Dose (LED or HID)	Comments	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation			
S-(1,2-dichlorovinyl)glutathione (DCVG)					
S. typhimurium, TA2638 gene mutations	+	+	50–300 nmol 20–120 µg/plate	Kidney subcellular fractions cytosol, mitochondria or microsomes used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects. Liver subcellular fractions did not enhance mutagenicity.	Vamvakas *et al.* (1988a)
S-(1,2-dichlorovinyl)-*L*-cysteine (DCVC					
S. typhimurium, TA100, TA2638, TA98, gene mutations	+	ND	0.1–0.5 nmol 0.02–0.11 µg/plate	DCVC was mutagenic in all three strains of *S. typhimurium* without the addition of mammalian subcellular fractions. Decreased response when AOAA (β-lyase inhibitor) added.	Dekant *et al.*, (1986c)
S. typhimurium, TA2638, gene mutations	+	+	50–300 nmol 11–65 µg/plate	Kidney subcellular fractions mitochondria or cytosol used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects.	Vamvakas *et al.* (1988a)
Gene mutation, LOH in *Tsc* gene, rat kidney epithelial cells *in vitro*	–	NA	10 µM [2 µg/mL]	Only 1/9 transformed cell lines showed LOH of the *Tsc-2* gene.	Mally *et al.* (2006)
Gene mutation, *Vhl* gene (exons 1–3), rat kidney epithelial cells *in vitro*	–	NA	10 µM [2 µg/mL]	No mutations in *Vhl* gene. Note: *Vhl* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally *et al.* (2006)
Unscheduled DNA synthesis, porcine kidney tubular epithelial cell line (LLC-PK1) *in vitro*	+	NA	2.5 µM [0.55 µg/mL], 5, 10, 15, 24 h; 2.5–100 µM [0.55–21.5 µg/mL]	Dose-dependent in UDS up to 24 h tested at 2.5 µM. Also, there was a dose-dependent increase at lower concentrations. Higher concentrations were cytotoxic as determined by cellular release of lactate dehydrogenase release.	Vamvakas *et al.* (1989)
Unscheduled DNA synthesis, Syrian hamster embryo fibroblasts *in vitro*	+	NA	2.5–10 µM [0.5–2 µg/mL]	Increase in UDS in treatment groups.	Vamvakas *et al.* (1988b)
DNA single-strand breaks, male rabbit renal tissue (perfused kidneys and proximal tubules)	+ +	ND	10–20 mg/kg, iv; 50–100 mg/kg bw, ip	Dose-dependent increase in DNA single-strand breaks after iv or ip injections (iv injections only at 10 and 20 mg/kg bw)	Jaffe *et al.* (1985)

Table 4.6 (continued)

Test system/end-point	Result		Dose (LED or HID)	Comments	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation			
DNA single-strand breaks, isolated perfused kidneys *in vitro*	ND	+	10–1000 µM	Perfusion of rabbit kidney (45-min exposure) and proximal tubules (30-min exposure) resulted in a dose-dependent difference in the amount of DNA single-strand breaks.	Jaffe *et al.* (1985)
DNA strand breaks, proximal tubules *in vitro*	ND	+	10–1000 µM [2–200 µg/mL]		
DNA strand breaks, comet assay, male Sprague-Dawley rats *in vivo*	NA	+/–	1 or 10 mg/kg bw, single oral dose, evaluated at 1, 2, and 16 h	Positive at 10 mg/kg bw, 2 h after treatment. No significant increase at other doses/time-points.	Clay (2008)
Micronucleus formation, Syrian hamster embryo fibroblasts *in vitro*	–	NA	10 µM [2 µg/mL]	No micronucleus formation	Vamvakas *et al.* (1988b)
Cell transformation, kidney tubular epithelial cell line (LLC-PK1)	+	NA	1 or 5 µM; 7 wk [0.2 or 1 µg/mL]	Induced morphological cell transformation at both concentrations tested. Furthermore, in clone cells biochemical and morphological alterations remained stable for 30 passages.	Vamvakas *et al.* (1996)
Cell transformation, rat kidney epithelial cells *in vitro*	+	NA	10 µM [2 µg/mL]; 24 h exposure, 7 wk post-incubation	Cell transformation was higher than in controls; however, cell survival ranged from 39% to 64%, indicating cytotoxicity.	Mally *et al.* (2006)
Gene expression, kidney tubular epithelial cell line (LLC-PK1)	+	NA	1 or 5 µM	Increased c-*Fos* expression in 1 and 5 µM DCVC-derived clones after 30, 60 or 90 min incubation without the compound.	Vamvakas *et al.* (1996)
N-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC)					
S. typhimurium, TA2638, gene mutation	+	+	5–100 nmol [1.3–26 µg/plate]; LED 25 nmol [6.5 µg/plate]	Kidney cytosolic fractions used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects.	Vamvakas *et al.* (1987)

AOAA, aminooxyacetic acid; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; HID, highest ineffective dose; LED, lowest effective dose; LOH, loss of heterozygosity; *N*-AcDCVC, *N*-acetylDCVC; NA, not applicable; ND, not determined.

N-ethyl-*N*-nitrosourea, showed LOH. Therefore, the lack of LOH at the *Tsc-2* locus after treatment with DCVC *in vitro*, or trichloroethylene *in vivo*, reported by Mally *et al.* (2006), was actually more similar to the response to the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea than to the nongenotoxic carcinogen 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone. Therefore, these data did not substantially contradict the body of evidence on the genotoxicity of DCVC.

Finally, Clay (2008) evaluated the genotoxicity of DCVC *in vivo* using the comet assay to assess DNA stand breakage in the proximal tubules of rat kidneys. Rats were given a single oral dose of DCVC (1 or 10 mg/kg bw) and killed 2 or 16 hours after dosing. There was no significant damage to DNA from rat kidney proximal tubules after treatment with DCVC at either dose after 16 hours, or with DCVC at a dose of 1 mg/kg bw after 2 hours. While Clay (2008) concluded that these data were insufficient to indicate a positive response in this assay, the study did report a statistically significant increase in percentage tail DNA 2 hours after treatment with DCVC at 10 mg/kg bw, despite the small number of rats at each dose (n = 5) and sampling time. Therefore, these data did not substantially contradict the body of evidence on the genotoxicity of DCVC.

Overall, DCVC has shown genotoxicity based on consistent results in several available studies. While some recent studies (Mally *et al.*, 2006; Clay, 2008) have reported a lack of positive responses in some measures of genotoxicity *in vivo*, these studies did not, due to the limitations discussed above, substantially contradict the body of evidence on the genotoxicity of DCVC. These metabolites are formed *in vivo* after exposure to trichloroethylene, specifically in the kidney, so they have the potential to contribute to the genotoxicity of trichloroethylene, especially in that tissue. Moreover, genotoxic responses associated with exposure to DCVC were enhanced when metabolic activation with kidney subcellular fractions was used (Vamvakas *et al.*, 1988a). Finally, the lack of similar responses in assays for genotoxicity with trichloroethylene *in vitro*, even with metabolic activation, was likely to be the result of the small yield (if any) of DCVC under conditions *in vitro*, since GGT and other bioactivating enzymes that form DCVC are present *in vivo* in higher concentrations in the kidney than in the liver, from which S9 fractions are typically derived.

(iv) NAcDCVC

See Table 4.6

Vamvakas *et al.* (1987) investigated the mutagenicity of NAcDCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA to inhibit genotoxicity. NAcDCVC exhibited direct-acting mutagenicity in the absence of exogenous metabolic activation, with kidney cytosol enhancing the effects and AOAA diminishing, but not abolishing, the effects.

4.3 Non-genotoxic mechanisms of carcinogenesis

The sections below describe the available data on non-genotoxic mechanisms of carcinogenesis for cancers of the kidney, liver, lung, immune system, and testes induced by trichloroethylene (see also Section 3).

4.3.1 Kidney

The available studies in humans and experimental animals have addressed multiple hypotheses for non-genotoxic mechanisms of carcinogenesis in the kidney associated with exposure to trichloroethylene. These include accumulation of α2u-globulin, activation of peroxisome proliferator-activated receptor α (PPARα), and sustained chronic nephropathy and regeneration (independent of α2u-globulin). The sections that follow address these three mechanisms in detail.

(a) Accumulation of α2u-globulin

Accumulation of α2u-globulin is a histopathological phenomenon elicited by long-term chemical exposure in the male rat kidney. The hypothesized sequence of key events comprises:

- Excessive accumulation of hyaline droplets containing α2u-globulin in renal proximal tubules;
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium;
- Sustained regenerative tubule-cell proliferation
- Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and linear papillary mineralization;
- Foci of tubule hyperplasia in the convoluted proximal tubules;
- Tumours of the renal tubule.

Seven criteria have been specified for demonstrating that α2u-globulin is the sole mechanism for carcinogenesis in the male rat kidney (Swenberg & Lehman-McKeeman, 1999):

- Characteristic histopathology;
- Toxicity in the kidney is specific for male rats;
- Accumulation of α2u-globulin;
- Reversible binding to α2u-globulin;
- Increased, sustained cell proliferation;
- Similarities in dose–response relationships for histopathology and tumour outcome;
- Lack of genotoxicity.

This mechanism is posited to occur only in rodents; accordingly, only studies in experimental animals were identified, and are discussed below.

Experimental animals

Few studies of trichloroethylene in experimental animals were available, and the evidence did not meet the criteria for proof of this mechanism. Induction of α2u-globulin nephropathy has been investigated by Goldsworthy *et al.* (1988), who reported that trichloroethylene (1000 mg/kg bw for 10 days by gavage) did not induce increases in this urinary protein, nor did it stimulate tubular cellular proliferation in rats. Thus, direct evidence for several of the required criteria, including that trichloroethylene induces the characteristic histopathology, accumulation of α2u-globulin or reversible binding to α2u-globulin in a male rat-specific manner, was lacking. However, indirect evidence supporting an increase in α2u-globulin and associated histopathological changes was provided by a separate study in which groups of 60 male Fischer rats were exposed to the trichloroethylene metabolite trichloroethanol in drinking-water at concentrations of 50 and 1000 mg/L for 52 weeks (Green *et al.*, 2003). A dose-related increase in the incidence and severity of hyaline-droplet accumulation was observed. Increases in α2u-globulin were observed and were not considered sufficient to account for the renal pathology.

Concerning the remaining criteria, no studies were identified that would establish similarities in the dose–response relationships for histopathology and tumour outcome. Additionally, the evidence presented in Section 4.2 does not clearly rule out the potential role of genotoxicity, particularly in the kidney.

(b) Cytotoxicity/sustained chronic nephrotoxicity in the absence of α2u-globulin nephropathy

This hypothetical mechanism involves renal cytotoxicity and subsequent cellular proliferation in the absence of α2u-globulin accumulation. Evidence supporting this mechanism from studies in humans and experimental animals is summarized in the following sections.

(i) Humans

Epidemiological studies have demonstrated increased excretion of markers of nephrotoxicity (*N*-acetylglucosaminidase, NAG; protein,

albumin) at occupational (Green *et al.*, 2004) and higher (Brüning *et al.*, 1999a, b; Bolt *et al.*, 2004) levels of exposure to trichloroethylene. A more recent study that used the sensitive marker of kidney toxicity, kidney injury molecule-1 (Kim-1), demonstrated toxicity at low concentrations of trichloroethylene (22.2 ± 35.9 ppm; mean ± standard deviation), with exposures < 12 ppm (the median concentration of trichloroethylene) exhibiting a statistically significant elevation in urinary concentrations of Kim-1 (Vermeulen *et al.*, 2012).

Other evidence for renal toxicity associated with exposure to trichloroethylene came from studies of acute toxicity with GSH-derived metabolites in human cells *in vitro* (Chen *et al.*, 1990; Cummings & Lash, 2000; Cummings *et al.*, 2000b; Lash *et al.*, 2001a, 2003; Krause *et al.*, 2003; McGoldrick *et al.*, 2003). Additionally, changes in gene expression and mitochondrial disfunction induced by DCVC have been observed in primary human proximal tubular cells (Lash *et al.*, 2005; Lock *et al.*, 2006; Xu *et al.*, 2008).

(ii) Experimental animals

There was substantial evidence that trichloroethylene is nephrotoxic in experimental animals. Long-term bioassays have reported very high (nearly 100%) incidences of "nephrotoxicity" of the proximal tubule in rats (NTP, 1988, 1990) and mice (NCI, 1976; NTP, 1990) at the highest doses tested. Overt signs of subchronic nephropathy have been reported in multiple studies (see Section 3 for details) (Green *et al.*, 1997b, 1998). Maltoni *et al.* (1986) also reported cytomegaly and karyomegaly in rats exposed to trichloroethylene by inhalation. Studies i*n vivo* examining the effect of exposure to trichloroethylene on nephrotoxicity showed increased proximal tubule damage after intraperitoneal injection and inhalation of trichloroethylene in rats (Chakrabarti & Tuchweber, 1988) and intraperitoneal injection in mice (Cojocel *et al.*, 1989).

The available evidence also supported the nephrotoxicity of the trichloroethylene metabolite DCVC in rats (Terracini & Parker, 1965; Elfarra *et al.*, 1986) and mice (Jaffe *et al.*, 1984; Darnerud *et al.*, 1989). Studies *in vitro* showed that DCVC was more a more potent cytotoxic agent in the kidney than trichloroethylene (Stevens, 1985; Lash *et al.*, 1986; Stevens *et al.*, 1986). These studies also confirmed the greater susceptibility of male rats and mice to cytotoxicity induced by DCVC. Additionally, histological changes caused by DCVC in the kidney were similar to those induced by trichloroethylene, including cytokaryomegaly. Increased formation and urinary excretion of formic acid mediated by the oxidative metabolites trichloroacetic acid or trichloroethanol (Green *et al.*, 1998; Dow & Green, 2000; Green *et al.*, 2003) have also been posited to contribute to the observed nephrotoxicity of trichloroethylene. However, the contribution of these oxidative metabolites does not appear to be sufficient to explain the range of renal effects observed after exposure to trichloroethylene, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. Multiple mechanisms of cytotoxicity, including alteration of calcium-ion homeostasis and mitochondrial dysfunction have been identified in kidney cells *in vitro* (Lash & Anders, 1986, 1987; Vamvakas *et al.*, 1990, 1992; van de Water *et al.*, 1993, 1994; Yu *et al.*, 1994) (see Section 4.5.1a).

The primary limitation to the evidence supporting this mechanism was that nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but tumours of the kidney are only observed at low incidence in rats at the highest doses tested (NCI, 1976; NTP, 1990). In rats carrying the Eker mutation (*Tsc-2*$^{EK/+}$), Mally *et al.* (2006) reported increased DNA synthesis as measured by bromodeoxyuridine (BrdU) incorporation in rats receiving the highest dose of trichloroethylene (1000 mg/kg bw per day) for 13 weeks, but there was no evidence of

clonal expansion or tumorigenesis in the form of increased preneoplastic or neoplastic lesions when compared with controls.

Therefore in studies of trichloroethylene exposure in rodents and humans, data demonstrating a causal link between compensatory proliferation and the induction of tumours in the kidney were lacking.

(c) Activation of PPARα

(i) Humans

No studies were identified addressing activation of PPARα in the human kidney. However, studies of transactivation *in vitro* have shown that human PPARα is activated by trichloroacetic acid and dichloroacetic acid, while trichloroethylene itself is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). A limited number of studies have examined PPARα activation by the trichloroethylene metabolites dichloroacetic acid and trichloroacetic acid in cultured human liver cells (Walgren *et al.*, 2000). However, direct evidence of these effects from studies of human kidney *in vivo* or *in vitro* was lacking.

(ii) Experimental animals

No evidence was available concerning activation of PPARα in kidney. Peroxisome proliferation in the kidney has been evaluated in a single study of trichloroethylene (Goldsworthy & Popp, 1987), using increases in cyanide-insensitive palmitoyl-coenzyme A (palmitoyl-coA) oxidation activity as a marker in male rats and mice. Increases in renal palmitoyl-coA oxidation activity were observed in rats (3.0-fold) and mice (3.6-fold) treated with trichloroethylene by gavage at a dose of 1000 mg/kg bw per day for 10 days, with smaller increases in both species after treatment with trichloroacetic acid at 500 mg/kg bw per day for 10 days. No significant increases in kidney weight:body weight ratios were observed in either species.

(d) Conclusion

The kidney is a target organ in mammalian species for trichloroethylene and other related chlorinated ethanes and ethylenes, and trichloroethylene causes cancer of the kidney in male rats. There was inadequate evidence to support any of the three non-genotoxic mechanisms, that is, α2u-globulin nephropathy, cytotoxicity not associated with α2u-globulin accumulation, and PPARα activation. Very little evidence was available to support the hypothesized α2u-globulin nephropathy mechanism, and the identified evidence is insufficient to satisfy the criteria specified for establishing that this mechanism is operative. Concerning a mechanism dependent on sustained chronic nephrotoxicity, chronic exposure to trichloroethylene is indeed associated with damage to the kidney in humans as well as rats and mice. Nonetheless, no studies that have experimentally tested the hypothesis of ensuing cellular proliferation, or of mechanisms that would otherwise provide support for toxicity as a key step in carcinogenesis, were identified. One *in vivo* experimental study provides support for the view that short-term trichloroethylene exposure induces peroxisome proliferation in the kidney of exposed rodents. However, the effects occurred in both rats of mice whereas tumours occur only in male rats, suggesting a lack of species specificity of the mechanism. Additionally, direct evidence in the kidney is lacking from experimental animals (or humans) for activation of the PPAR receptor. Moreover, no studies have experimentally tested the link between peroxisome proliferation to kidney tumorigenesis for trichloroethylene or other compounds.

In conclusion, the overall evidence base supporting the three hypothesized mechanisms is inadequate in each case.

4.3.2 Liver

The available evidence for the non-genotoxic mechanisms for the induction of rodent (mouse) liver tumours by trichloroethylene comprised the following: (1) epigenetic effects (especially DNA hypomethylation); (2) cytotoxicity and oxidative stress; (3) alteration of proliferation and apoptosis, and clonal expansion; (4) PPARα activation; and (5) disruption of gap-junctional communication.

(a) Epigenetic effects

Experimental evidence for hypomethylation of DNA was limited to studies of trichloroacetic acid and dichloroacetic acid in mice (Tao *et al.*, 1998; 2004; Ge *et al.*, 2001). Since changes in methylation represent common early molecular events in most tumours (Zingg & Jones, 1997; Baylin *et al.*, 1998), these data supported the hypothesis that dysregulation of gene methylation plays a role in trichloroethylene-induced tumorigenesis. However, no data from studies in humans or experimental animals were available that specifically tested this hypothesis for trichloroethylene.

(b) Cytotoxicity and secondary oxidative stress

(i) Humans

Few studies on liver toxicity in humans and exposure to trichloroethylene were identified. Of these, three studies reported significant changes in serum liver-function tests (widely used in clinical settings partly to identify patients with liver disease) in metal degreasers whose exposure to trichloroethylene was assessed using urinary trichloro-compounds as biomarkers (Nagaya *et al.*, 1993; Rasmussen *et al.*, 1993a; Xu *et al.*, 2009). Two additional studies reported changes in plasma or serum bile acids (Driscoll *et al.*, 1992; Neghab *et al.*, 1997). The results of one study including subjects from a subregistry of the Agency for Toxic Substances and Disease Registry (ATSDR) were suggestive of liver disorders associated with exposure to trichloroethylene, but it was not possible to determine whether trichloroethylene caused these conditions given the limitations of this study (Davis *et al.*, 2005). Furthermore, several case reports existed of liver toxicity, including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens–Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity syndrome (Kamijima *et al.*, 2007), in addition to jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in trichloroethylene-exposed workers (Thiele *et al.*, 1982). Cohort studies have examined mortality attributable to cirrhosis and exposure to either trichloroethylene (Garabrant *et al.*, 1988; Blair *et al.*, 1989; 1998; Morgan *et al.*, 1998; Boice *et al.*, 1999, 2006; Ritz, 1999; ATSDR, 2004; Radican *et al.*, 2008) or solvents (Leigh & Jiang, 1993), but were greatly limited by their use of death certificates for which there is a high degree (up to 50%) of under-reporting (Blake *et al.*, 1988), so these null findings did not rule out an effect of trichloroethylene on cirrhosis.

Overall, while some evidence exists for liver toxicity as assessed by tests for liver function, the data were inadequate for making conclusions regarding causality. Additionally, no data on secondary oxidative stress were identified.

(ii) Experimental animals

Numerous studies in experimental animals have demonstrated that trichloroethylene is hepatotoxic. In experimental animals, exposure to trichloroethylene is associated with a wide array of hepatotoxicity end-points. Like humans, experimental animals exposed to trichloroethylene have been observed to have increased levels of serum bile acids (Wang & Stacey, 1990; Bai & Stacey, 1993; Hamdan & Stacey, 1993), although the toxicological importance of this effect is unclear (see Section 4.5.2b). Increases in liver weight proportional to trichloroethylene dose have been consistently reported

in numerous studies and appear to be accompanied by periportal hepatocellular hypertrophy (Kjellstrand *et al.*, 1981, 1983a, b; Tucker *et al.*, 1982; Buben & O'Flaherty, 1985; Elcombe *et al.*, 1985; Goldsworthy & Popp, 1987; Melnick *et al.*, 1987; Merrick *et al.*, 1989; Goel *et al.*, 1992; Dees & Travis, 1993; Berman *et al.*, 1995; Nakajima *et al.*, 2000; Tao *et al.*, 2000; Nunes *et al.*, 2001; Laughter *et al.*, 2004).

Several studies have attempted to study oxidative stress and DNA damage resulting from exposure to trichloroethylene. Toraason *et al.* (1999) measured 8-hydrodeoxyguanosine adducts (8-OHdG) and a marker of oxidative damage to cell membranes, 8-epi-prostaglandin $F_{2\alpha}$ (8-epiPGF), excretion in the urine and thiobarbituric acid reactive substances (TBARS) (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats given single intraperitoneal injections of trichloroethylene. TBARs and the 8-OHdG/dG ratio were significantly elevated in the liver after treatment with trichloroethylene at 500 mg/kg bw, but there was significant toxicity and it was suggested that the animals would not have survived another 24 hours.

With regard to dichloroacetate and trichloroacetate, Larson & Bull (1992b) exposed male $B6C3F_1$ mice or F344 rats to single doses of trichloroacetate or dichloroacetate in distilled water by gavage (n = 4). In the first experiment, TBARS was measured from liver homogenates and assumed to be malondialdehyde equivalents. A preliminary experiment had shown that the maximum concentration of TBARS was increased 6 hours after dosing with dichloroacetate and 9 hours after dosing with trichloroacetate in mice, and that by 24 hours, TBARS concentrations had declined to control levels. Time-course information in rats was not presented. At a dose of 100 mg/kg bw, dichloroacetate (rats or mice) and trichloroacetate (mice) did not elevate concentrations of TBARS over those in liver of controls. However, trichloroacetate was not examined at this dose in rats. For trichloroacetate, there was a slight dose-related increase in concentration of TBARS over control values starting at 300 mg/kg bw in mice, with the increase in concentration of TBARS increasing at a rate that was lower than the magnitude of increase in dose. The induction of TBARS in mice was transient and subsided within 24 hours of a single dose of dichloroacetate or trichloroacetate. The response in mice appeared slightly greater with dichloroacetate than trichloroacetate at similar doses; for dichloroacetate, there was similar TBARS induction between rats and mice at similar dose levels.

Austin *et al.* (1996) is a follow-up publication of the preliminary experiment cited in Larson & Bull (1992b). Male $B6C3F_1$ mice were treated with single doses (300 mg/kg bw) of dichloroacetate or trichloroacetate via gavage and nuclear DNA from liver was examined for 8-OHdG as an indicator of oxidative stress. To reduce the number of animals used, controls were not employed at each time-point. A statistically significant increase in 8-OHdG for dichloroacetate in mice was seen at 4 and 6 hours (~1.4- and 1.5-fold levels for controls, respectively), but not 8 hours. For trichloroacetate, there was a statistically significant increase in 8-OHdG at 8 and 10 hours (~1.4- and 1.3-fold levels for controls, respectively).

Consistent results as to low, transient increases in markers of oxidative stress were also reported by Parrish *et al.* (1996), who in addition to examining oxidative stress alone, attempted to examine its possible relationship to palmitoyl-coA oxidation and liver weight in male $B6C3F_1$ mice exposed to trichloroacetate or dichloroacetate for 3 or 10 weeks (n = 6). While there was a dose-related increase in palmitoyl-coA oxidation activity at 21 days with trichloroacetate, there was only a statistically significant increase in palmitoyl-coA oxidation activity (~1.8-fold levels for controls) at 21 days with dichloroacetate at a concentration of 2.0 g/L. After 71 days of treatment, trichloroacetate induced dose-related increases in palmitoyl-coA oxidation activities

that were approximately twice those reported at 21 days. Treatment with dichloroacetate at 0.1 or 0.5 g/L produced statistically significant increases in palmitoyl-coA oxidation activity of ~1.5- and 2.5-fold levels for controls, respectively. The positive control, drinking-water containing clofibric acid at 1.25 g/L, produced increases of six- to sevenfold in palmitoyl-coA oxidation activity relative to controls after 21 and 71 days of exposure. Parrish *et al.* (1996) reported that the activity of laurate hydroxylase was elevated significantly only by trichloroacetate at 21 days, and to approximately the same extent (~1.4–1.6-fold levels for controls) at all doses tested. At 71 days, there was a statistically significant increase in the activity of laurate hydroxylase (i.e. 1.6- and 2.5-fold levels for controls, respectively) with trichloroacetate at concentrations of 0.5 and 2.0 g/L. No change in the activity of laurate hydroxylase was reported after exposure to dichloroacetate (activity was within the ranage for control values, varying 1.7-fold between days 21 and 71). Levels of 8-OHdG in isolated liver nuclei were not altered after exposure to trichloroacetate or dichloroacetate at concentrations of 0.1, 0.5, or 2.0 g/L for 21 days, and results remained negative even when treatment was extended to 71 days. The level of 8-OHdG increased in control mice with age (i.e. levels increased by about twofold between day 21 and day 71 in control mice).

Thus the increases in palmitoyl-coA oxidation activity reported after exposure to dichloroacetate or trichloroacetate were not associated with 8-OHdG levels (which were unchanged), nor with the observed changes in laurate hydrolase activity.

(c) Cell proliferation, apoptosis, and clonal expansion

(i) Humans

No studies were identified providing evidence of alteration of cell proliferation and apoptosis, or clonal expansion of initiated cells, in humans after exposure to trichloroethylene.

(ii) Experimental animals

Mirsalis *et al.* (1989) studied S-phase DNA synthesis in primary hepatocytes from male F344 rats and male and female B6C3F_1 mice given single doses (up to 1000 mg/kg bw) of trichloroethylene by gavage in corn oil. They reported that the only positive result was considered to be for a dose of 1000 mg/kg bw in male mice at 48 hours (~2.2% of hepatocytes), but no statistical analysis was performed. Dees & Travis (1993) reported elevated incorporation of tritiated thymidine in DNA from mouse liver after exposure to trichloroethylene at a dose of 250–1000 mg/kg bw. This study also reported that mitotic figures were more frequently observed after exposure to trichloroethylene. Channel *et al.* (1998) assessed liver-cell proliferation in B6C3F_1 mice given trichloroethylene orally at a dose of 0, 400, 800, or 1200 mg/kg bw per day in corn oil, 5 days per week, for 8 weeks. The number of proliferating cell nuclear antigen (PCNA)-positive cells, a measure of the number of cells that have undergone DNA synthesis, was elevated only on day 10 and only in the group at 1200 mg/kg per day. Laughter *et al.* (2004) found an increase in the level of DNA synthesis (BrdU labelling index) in mice given trichloroethylene by gavage at a dose of 500 and 1000 mg/kg bw for 3 weeks. Sano *et al.* (2009) examined cell proliferation in the liver using the K_i-67 antigen in male Sprague-Dawley rats and B6C3F_1 mice exposed by gavage to trichloroethylene at a dose of 1500 mg/kg bw per day for 14 days. A small number of K_i-67-positive hepatocytes and mitotic figures were

found in trichloroethylene-treated mice, but not in rats, but no quantitative analysis was reported.

With regard to changes in apoptosis, the study by Dees & Travis (1993) was the only one to report a qualitative increase in liver apoptotic bodies in $B6C3F_1$ mice exposed to trichloroethylene at a dose of 1000 mg/kg bw per day for 10 days. Channel *et al.* (1998), and Sano *et al.* (2009) observed no differences in liver apoptosis between control or trichloroethylene-treated animals.

Several studies have examined cell proliferation in *Pparα*-null mice. BrdU incorporation, a measure of DNA synthesis that may reflect cell division, polyploidization, or DNA repair, was diminished in null mice compared with wild-type mice given trichloroethylene at a dose of 500 or 1000 mg/kg bw per day (Laughter *et al.*, 2004). However, BrdU incorporation in *Pparα*-null mice was still higher by about threefold in than controls, although it was not statistically significantly different due to the small number of mice, high variability, and the baseline levels of BrdU incorporation in control *Pparα*-null mice that were two- to threefold those in control wild-type mice.

Other measures of proliferation, including liver-weight changes, have been examined. Trichloroethylene-induced increases in liver weight have been reported in male and female mice lacking a functional *Pparα* receptor (Nakajima *et al.*, 2000). On the other hand, Laughter *et al.* (2004) found no significant difference in liver weight in *Pparα*-null mice before and after exposure to trichloroethylene.

(d) Activation of PPARα

The sections below review the evidence that trichloroethylene or its metabolites induces PPARα activation or its markers.

(i) Humans

No studies were identified addressing peroxisome proliferation or the key events in the PPARα-activation mechanism in human liver. However, studies of transactivation *in vitro* have shown that human (as well as murine) versions of PPARα are activated by trichloroacetate and dichloroacetate, while trichloroethylene itself is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Limited studies have examined PPARα activation by the trichloroethylene metabolites dichloroacetate and trichloroacetate in cultured human cells of the liver (e.g. Walgren *et al.*, 2000).

(ii) Experimental animals

Numerous studies have reported that the administration by gavage of trichloroethylene to mice and rats leads to proliferation of peroxisomes in hepatocytes. Some studies have used changes in the volume and number of peroxisomes as measures of proliferation, while others have measured peroxisomal-enzyme activity, such as catalase and cyanide-insensitive palmitoyl-coA oxidation.

Elcombe *et al.* (1985) reported increases in the percentage of cytoplasm occupied by peroxisomes in $B6C3F_1$ and Alderley Park mice treated with trichloroethylene at 500–1500 mg/kg bw per day for 10 days. Although the increase compared with controls appeared greater in the $B6C3F_1$ strain, this was largely due to lower levels for controls in that strain. Nakajima *et al.* (2000) treated male wild type Sv/129 mice with trichloroethylene by gavage at 750 mg/kg bw per day for 14 days, and found even higher baseline values for the percentage peroxisomal volume, but with an absolute level after treatment similar to that reported by Channel *et al.* (1998) in $B6C3F_1$ mice treated with trichloroethylene at 1200 mg/kg bw per day for 14 days. Nakajima *et al.* (2000) also noted that the treatment-related increases were smaller for female wild-type mice, and that there were no increases in peroxisomal volume in male

or female *PPARα*-null mice, although levels for vehicle controls were slightly elevated (not statistically significant). Only Elcombe *et al.* (1985) examined peroxisomal volume in rats, and reported smaller treatment-related increases in two strains Osborne-Mendel and Alderley-Park, but higher and variable baseline levels.

In various strains of mice ($B6C3F_1$, Alderley-Park, Swiss albino, Sv/129 wild type) exposed to trichloroethylene at doses of 500–2000 mg/kg bw per day for 10–28 days, increases in catalase activity tended to be more modest (1.3–1.6-fold levels for controls) than increases in palmitoyl-coA oxidation activity (1.4–7.9-fold levels for controls) (Elcombe *et al.*, 1985; Goldsworthy & Popp, 1987; Goel *et al.*, 1992; Nakajima *et al.*, 2000; Watanabe & Fukui, 2000; Laughter *et al.*, 2004). In rats, Elcombe *et al.* (1985) reported no increases in catalase activity or palmitoyl-coA oxidation in Alderley-Park rats given trichloroethylene at 1000 mg/kg bw per day for 10 days. In F344 rats treated with trichloroethylene at 600–4800 mg/kg bw per day for 10–14 days, Goldsworthy & Popp (1987) and Melnick *et al.* (1987) reported increases of up to twofold in catalase activity, and 4.1-fold in palmitoyl-coA oxidation activity, relative to controls. The changes in catalase activity in rats were similar to those in mice at similar doses, with increases of 1.1–1.5-fold at doses of 1000–1300 mg/kg bw per day compared with controls (Elcombe *et al.*, 1985; Melnick *et al.*, 1987). However, the changes in palmitoyl-coA oxidation activity at these doses compared with controls were smaller, with increases of 1.1–1.8-fold in rats compared with 6.3–7.9-fold in mice (Goldsworthy & Popp, 1987; Melnick *et al.*, 1987).

Nakajima *et al.* (2000) and Laughter *et al.* (2004) investigated the dependence of these changes on PPARα using *Pparα*-null Sv/129 mice. Nakajima *et al.* (2000) reported that neither male nor female wild-type or null mice showed statistically significant increases in catalase activity after 14 days of treatment with trichloroethylene at 750 mg/kg bw per day. However, given the small number of mice (four per group) and the relatively small changes in catalase activity observed in other (wild-type) mouse strains, this study had limited power to detect such changes. Several other markers of peroxisome proliferation, including acyl-CoA oxidase and CYP4A1, were induced by trichloroethylene in male wild-type mice, but not in male *Pparα*-null mice or female mice of either type. Unfortunately, none of these markers have been investigated in female mice of any other strain, so it is unclear whether the lack of response to trichloroethylene is characteristic of female mice in general, or just in this strain. Interestingly, as noted above, increases in liver weight:body weight ratio were observed in male and female *Pparα*-null mice in this study. Laughter *et al.* (2004) only quantified activity of the peroxisome proliferation marker, palmitoyl-coA oxidation, in their study, and found a slight decrease (0.8 times lower than controls) in *Pparα*-null mice given trichloroethylene at 500 mg/kg bw per day, and an increase (1.5-fold levels for controls) at 1500 mg/kg bw per day after 3 weeks of treatment, with neither result being statistically significant (four to five mice per group). However, baseline activity of palmitoyl-coA oxidation was almost twofold higher in the null mice, and the treated wild-type and null mice differed in palmitoyl-coA oxidation activity by only about 1.5-fold.

In summary, oral administration of trichloroethylene for up to 28 days causes proliferation of peroxisomes in hepatocytes and associated increases in peroxisomal-enzyme activity in mice and rats. Male mice tend to be more sensitive to such effects: at similar doses, rats and female mice tend to exhibit weaker responses. For example, changes in peroxisomal volume and palmitoyl-coA oxidation were three to six times lower in rats than in mice, but changes in catalase activity were in F344 rats and mice. No longer-term studies or studies of inhalation were located, and only one study examined these

changes at more than one time-point. Therefore, little is known about the route-dependence, time course, and persistence of these changes. Finally, two studies (Nakajima *et al.*, 2000; Laughter *et al.*, 2004) found diminished responses in terms of increase in peroxisomal volume and peroxisomal enzyme activity in *Pparα*-null mice compared with wild-type mice, although there was some confounding due to baseline differences between null and wild-type mice in several measures.

The hypothesis that trichloroethylene-induced PPARα activation, along with its sequelae, is a key or causative event in trichloroethylene-induced hepatocarcinogenesis has not been directly tested (e.g. in bioassays with knockout mice or involving the blocking of hypothesized key events). Support for this hypothesis is based mainly on the observations that trichloroacetate induces tumours through PPARα activation, and that trichloroacetate is formed after exposure to trichloroethylene *in vivo*. To summarize, trichloroacetate activates PPARα, and induces proliferation of peroxisomes and hepatocytes. However, several inconsistencies and gaps in data reduce confidence in the conclusion that trichloroacetate induces hepatocarcinogenesis solely through a mechanism involving PPARα activation. First, while trichloroacetate induces peroxisome proliferation (a marker for PPARα agonism) in rats and in mice, it has been shown to be tumorigenic in B6C3F_1 mice, but not in a limited study in F344 rats (DeAngelo *et al.*, 1997) (the only strains tested for carcinogenicity). Second, the pattern of H-*ras* mutation in murine liver tumours induced by trichloroacetate differs from that in tumours induced by dichloroacetate and other peroxisome proliferators (Fox *et al.*, 1990; Hegi *et al.*, 1993; Stanley *et al.*, 1994; Bull *et al.*, 2002). Other effects of trichloroacetate, including increased expression of *c-myc* and hypomethylation of DNA (Tao *et al.*, 2000), are not specific to the PPARα-activation mechanism, and other data also contribute uncertainty as to whether PPARα-independent mechanisms may be involved in the induction of tumours by trichloroacetate in mice.

To conclude, trichloroethylene clearly activates PPARα. However, based on data from trichloroethylene and its metabolites alone, there is only limited evidence that activation of PPARα and its sequelae are key events in trichloroethylene-induced hepatocarcinogenesis. PPARα agonism may play a significant role in the induction of tumours in mouse liver by some compounds, such as Wy-14 643. However, recent studies suggest that di(2-ethylhexyl) phthalate (DEHP) can induce tumours in a PPARα-independent manner without any loss of potency (Ito *et al.*, 2007), and that PPARα agonism alone is not a sufficient cause for tumorigenesis in hepatocytes (Yang *et al.*, 2007). For trichloroethylene and most PPARα agonists, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient.

4.3.3 Immune system

Tumours most strongly associated with exposure to trichloroethylene in humans include non-Hodgkin lymphoma. In experimental animals, an increased incidence of lymphoma was observed in female B6C3F_1 mice given trichloroethylene in corn oil by gavage (NTP, 1990), and the incidence of leukaemia was increased in male Sprague-Dawley rats and female August rats (Maltoni *et al.*, 1986, 1988; NTP, 1988). Evidence supporting hypothesized non-genotoxic mechanisms for these tumour types was limited to studies of immunological and haematological toxicity, as described in the sections that follow.

(a) Humans

Studies in humans provide evidence of associations between exposure to trichloroethylene and several immunotoxicological end-points. The relationship between occupational exposure to trichloroethylene and systemic autoimmune diseases, such as scleroderma, has been reported in several recent studies. A meta-analysis of

studies of scleroderma (Nietert *et al.*, 1998; Diot *et al.*, 2002; Garabrant *et al.*, 2003) conducted by the United States EPA resulted in a statistically significant combined odds ratio for any exposure in men (OR, 2.5; 95% CI, 1.1–5.4), with a lower odds ratio in women (OR, 1.2, 95% CI, 0.58–2.6) (Cooper *et al.*, 2009). The incidence of systemic sclerosis among men is very low (approximately 1 per 100 000 person-years), and is approximately 10 times lower than that in women (Cooper & Stroehla, 2003). Thus the available data for humans did not allow determination of whether the difference in effect estimates between men and women reflected the relatively low background risk of scleroderma in men, sex-related differences in exposure prevalence, or the reliability of exposure assessment (Messing *et al.*, 2003), a sex-related difference in susceptibility to the effects of trichloroethylene, or chance. Changes in levels of inflammatory cytokines were reported in a study of degreasers exposed occupationally to trichloroethylene (Iavicoli *et al.*, 2005), and in a study of infants exposed to trichloroethylene via indoor air (Lehmann *et al.*, 2001, 2002).

There were a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to trichloroethylene, with prevalences as high as 13% among workers in the same location (Kamijima *et al.*, 2007, 2008; see Section 4.5.3b).

Several molecular epidemiology studies in humans have evaluated the effect of exposure to trichloroethylene on immune markers and potential for immunosuppressive effects. Most studies have been of workers exposed occupationally to trichloroethylene and unexposed control workers, and were conducted using a cross-sectional design with an exposure-monitoring period preceding collection of blood or other specimens (Table 4.7; Lan *et al.*, 2010; Hosgood *et al.*, 2011; Bassig *et al.*, 2013; Zhang *et al.*, 2013; see Section 4.5.3a).

(b) Experimental animals

Experimental studies provide additional support for the immunotoxicity of trichloroethylene. Numerous studies have demonstrated accelerated autoimmune responses in mice that are prone to autoimmune disease (Griffin *et al.*, 2000a, b; Blossom *et al.*, 2004, 2007, 2008; Cai *et al.*, 2008). With shorter exposure periods, effects include changes in cytokine concentrations similar to those reported in studies in humans. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and these effects differed somewhat from "normal" expression in these mice. Immunotoxic effects (including increases in anti-double-stranded DNA antibodies in adult animals), decreased thymus weights, and decreased plaque-forming cell (PFC) response with prenatal and neonatal exposure, have been also reported in B6C3F_1 mice which are not known to be particularly susceptible to autoimmune disease (Peden-Adams *et al.*, 2006; Keil *et al.*, 2009). Recent mechanistic studies focused on the roles of various measures of oxidative stress in the induction of these effects by trichloroethylene (Wang *et al.*, 2007a, 2008).

Evidence of a treatment-related increase in delayed type hypersensitivity response accompanied by hepatic damage has been observed in guinea-pigs treated by intradermal injection (Tang *et al.*, 2002, 2008). Increase in delayed type and hypersensitivity response was also seen in mice offspring exposed to trichloroethylene in drinking-water between day 0 of gestation until age 8 weeks (Peden-Adams *et al.*, 2006).

Evidence for localized immunosuppression, as measured by pulmonary response to bacterial challenge (streptococcus aerosol infection), was seen in two studies of acute exposure in CD-1 mice (Aranyi *et al.*, 1986; Selgrade & Gilmour, 2010).

Table 4.7 Studies of the effects of exposure to trichloroethylene on the immune system, and autoimmune disease

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Lan *et al.* (2010) China	80	96	Cross-sectional	Exposed: 22.19 ppm; < 12 ppm: mean, 5.19 ppm; ≥ 12 ppm: mean, 38.6 ppm	WBC, granulocytes, monocytes, lymphocytes, T-cells: $CD4^+$, $CD8^+$, B-cells, NK-cells, sCD27, sCD30	Exposed workers had reduced WBC (≥ 12 ppm only), lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, T cells, sCD27, and sCD30 compared to controls.	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Iavicoli *et al.* (2005) Italy	35	70	Cross-sectional	Exposed: 5mg/m³;13.3mg/g creatinine urinary trichloroacetic acid	IL-4, IL-2, IFN-γ	Exposed workers had significant increase in IL-2 and IFN-γ; significant decrease in IL-4 levels compared to both control groups.	Urinary trichloroacetic acid levels measured in exposed and internal-control workers; Personal air sampling for 40% of exposed workers during three consecutive working shifts were collected using sorbent tubes; Analyses used both internal and external control group, but personal monitoring conducted on a subset of subjects and limited control for confounding.
Hosgood *et al.* (2011) China	80	96	Cross-sectional	Exposed: 22.19 ppm; < 12 ppm: 5.19 ppm; ≥ 12 ppm: 38.6 ppm	$CD4^+$ naïve, $CD4^+$ central and effector memory T cells, regulatory T cells subsets, $CD8^+$ naïve, $CD8^+$ central and effector memory cells	Exposed workers had reduced $CD4^+$ naïve (≥ 12 ppm) and $CD8^+$ naïve T cells, and $CD4^+$ effector memory T cells, compared with controls.	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Bassig *et al.* (2013) China	71	78	Cross-sectional	Exposed: 23.4 ppm; < 12 ppm: 5.1 ppm; ≥ 12 ppm: 41.2 ppm	IL-10, IL-6, TNF-α	Exposed workers had significantly reduced levels of serum IL-10, but not IL-6 or TNF-α, compared with controls	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI, lymphocyte counts) and characteristics were similar in exposed and non-exposed workers.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Zhang *et al.* (2013) China	80	45	Cross-sectional	Exposed: 22.2 ppm < 12 ppm: 5.2 ppm ≥ 12 ppm: 38.4 ppm	IgM, IgG, IgE	Exposed workers had reduced levels of serum IgM and IgG, but not IgE	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Lehmann *et al.* (2001) Germany	121	NA	Cohort	Median, 0.42 µg/m³	IgE antibodies, IL-4 + $CD3^+$ T cells, IFN-γ + $CD8^+$ T cells	Trichloroethylene not significantly associated with any end-point evaluated	Passive monitoring was conducted for VOCs for 4 weeks using 3M badges in the child's bedroom (children aged 36 months); Information on VOC exposure and IgE available for only 60.5% of children; VOCs highly correlated; population studied was children at risk for atopy, so external generalizability may be an issue.
Lehmann *et al.* (2002) Germany	85	NA	Cohort	Median, 0.6 µg/m³	IL-4, IL-2, TNF-α, and IFN-γ in cytokine-producing T cells.	Trichloroethylene associated with elevated IFN-γ OR, 3.6 (95% CI, 0.9–14.9) and reduced IL-4 OR, 4.4 (95% CI, 1.1–17.8) in multivariate analyses; reduced IL-2 in univariate analysis.	Passive monitoring was conducted for VOCs for 4 weeks using 3M badges in the child's bedroom (newborns).
Arif & Shah (2007) USA	550	NA	Cross-sectional	Geometric mean, 0.03 µg/m³	Physician diagnosed asthma/wheezing in previous 12 months	Physician-diagnosed asthma: OR, 0.94 (95% CI, 0.77–1.14); 1–2 wheezing attacks: OR, 1.29 (95% CI, 0.98–1.68); ≥ 3 wheezing attacks: OR, 0.21 (95% CI, 0.04–1.05).	Trichloroethylene and other VOCs measured in subjects using 3M personal monitoring for 48–72 h; limited ability to evaluate temporal relationship given cross-sectional design; no objective assessment of asthma available.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Nietert *et al.* (1998) USA	178	200	Case–control	NR	Scleroderma	Max. intensity, men: OR, 3.3 (95% CI, 1.0–10.3) Cumul. intensity, men: OR, 2.0 (95% CI, 0.7–5.3) Max. probability, men: OR, 5.1 (CI not calculated) Max. intensity, women: OR, 0.9 (95% CI, 0.3–2.3) Cumul. intensity, women: OR, 1.2 (95% CI, 0.5–2.6) Max. probability, women: OR, 0.7 (95% CI, 0.2–2.2)	Exposure assessment based on personal interviews and JEM, which was used to create semi-quantitative exposure scores based on intensity, probability of exposure, and cumulative intensity exposure score; used clinic-based control group but geographical location disregarded (> 97% controls lived in South Carolina versus 65% of cases).
Diot *et al.* (2002) France	80	160	Case–control	NR	Systemic sclerosis	Ever exposure: OR, 2.39 (95% CI, 1.04–5.22); high cumulative exposure: OR, 7.58 (95% CI, 1.54–37.36); ever exposure, women: OR, 2.10 (95% CI, 0.65–6.75); ever exposure, men: OR, 4.67 (95% CI, 0.99–21.89)	Expert review-assessed exposures and developed semi-quantitative exposure scores based on probability, frequency, intensity, and duration of exposure; the controls were matched to cases by age, sex, and smoking habits, and were hospitalized during the same time and in the same department as the cases.
Garabrant *et al.* (2003) USA	660	2227	Case–control	NR	Scleroderma	Exposed, self-report: OR, 2.0 (95% CI, 0.8–4.8) Exposed, expert review: OR, 1.9 (95% CI, 0.6–6.6)	Exposure assessment based on personal interviews and expert review which either confirmed or did not confirm reported exposures; controls frequency matched to cases by race, age, and residence and recruited using random-digit dialling.
Beaudreuil *et al.* (2005) France	60	120	Case–control	NR	ANCA (small vessel vasculitis)	No association: OR, 1.1 (95% CI, 0.5–2.4)	Exposure assessment based on self-reported exposures from personal interview and expert review developed exposure score (product of probability × intensity ×frequency × duration); In patient controls enrolled from same hospital as cases and matched by age and sex.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m^3 or ppm)	End-points	Notable effects	Comments
Lacey *et al.* (1999) USA	205	2095	Case–control	NR	Undifferentiated connective tissue disease	Self-reported exposure: OR, 0.88 (95% CI, 0.11–6.95) Expert-review exposure: OR, 1.67 (95% CI, 0.19–14.90) Dry-cleaning: OR, 1.38 (95% CI, 0.68–2.78).	OR for self-report and expert review based on only 1 exposed case; exposure assessment based on personal interviews and expert review which either confirmed or did not confirm reported exposures; controls enrolled via random-digit dialling and matched to cases by age, race/ethnic group, and geographical region.

ANCA, antineutrophil cytoplasmic antibodies; BMI, body-mass index; CD, cluster of differentiation; CI, confidence interval; cumul., cumulative; IL, interleukin; IFN-γ, interferon-gamma; JEM, job-exposure matrix; max., maximum; NA, not available; NK, natural killer cells; NR, not reported; OR, odds ratio; sCD, soluble CD receptor, TNF-α, tumour necrosis factor-alpha; VOCs, volatile organic compounds, WBC, white blood cells

4.3.4 Lung

The hypothesized non-genotoxic mechanisms for induction of tumours of the lung by trichloroethylene include cytotoxicity. Evidence for this mechanism is limited to the demonstration of acute cytotoxicity in bronchiolar Clara cells and transient cell proliferation in mice exposed to trichloroethylene (Forkert *et al.*, 1985; Yost *et al.*, 1989; Forkert & Forkert, 1994; Henschler *et al.*, 1980; Fukuda *et al.*, 1983; Maltoni *et al.*, 1988). Because the cell type (or types) of origin for the observed tumours of the lung in mice has not been determined, the contribution to carcinogenicity of toxicity in Clara cells and subsequent regenerative cell division is largely unknown. Similarly, induction of dichloroacetyl-lysine protein adducts has only been studied over short duration and after intraperitoneal exposure (Forkert *et al.*, 2006), and the contribution of these adducts to tumorigenesis has not been investigated. Chloral hydrate, a genotoxic metabolite of trichloroethylene, is probably formed in the mouse lung and may therefore contribute to carcinogenicity (see the *Monograph* on Chloral Hydrate in this Volume). However, data were insufficient to determine whether chloral hydrate is formed in the human lung. Overall, the evidence for lung as a target tissue for trichloroethylene is moderate and there is only weak mechanistic support for trichloroethylene-induced carcinogenesis in the lung.

4.3.5 Testes

The data for trichloroethylene do not include an extensive characterization of the mechanisms for testicular tumorigenesis in the rat. Relevant evidence concerned a potential hormonal-disruption mechanism, for which key events leading to trichloroethylene-induced formation of testicular tumours were not specified. Two studies suggested that trichloroethylene causes hormonal disruption in male rats (Kumar *et al.*, 2000, 2001). Two studies in humans examined endocrine function in men (Chia *et al.*, 1997; Goh *et al.*, 1998), both studies followed up the cohort reported in Chia *et al.* (1996); increases in levels of the sulfated analogue of dehydroepiandrosterone, and decreases in levels of follicle-stimulating hormone, sex-hormone-binding globulin, and testosterone were seen with increased years of exposure to trichloroethylene. Other observed adverse effects on the male reproductive system included altered sperm morphology, hyperzoospermia, decreased sexual drive and function, and altered fertility (Bardodej & Vyskocil, 1956; El Ghawabi *et al.*, 1973; Saihan *et al.*, 1978).

4.4 Susceptibility data

4.4.1 Inter-individual variability

(a) Humans

The oxidative metabolism of trichloroethylene is largely dependent on CYP2E1, and clinically relevant genetic polymorphisms are known to exist in the gene encoding this enzyme (Catanzaro *et al.*, 2012; Daly, 2012). Neafsey *et al.* (2009) reviewed the literature and found that while a variety of *CYP2E1* variant alleles have been found, the functional significance of these variants is still unclear. Some, but not all, studies suggested that several upstream 5′ flanking mutations affect gene expression and response to inducers such as ethanol or obesity. None of the coding-region variants consistently affects enzyme function. The only indirect evidence for the potential role of *CYP2E1* polymorphisms in toxicity associated eith trichloroethylene has been reported in a study by Povey *et al.* (2001), who compared *CYP2E1* alleles in 7 patients who had developed scleroderma after exposure to solvents versus 71 patients with scleroderma without solvent exposure (“sporadic” disease) and 106 population controls. The *2E1*3* allele was found in two of the seven patients who had been exposed to organic solvents, with a greater

frequency than in either the disease controls or the population controls (OR, 9.1; [95% CI, 1.5–59.1]; and OR, 10.2 [95% CI, 1.8–62.2], respectively). [The Working Group commented that while this study provided some information on trichloroethylene exposure, *CYP2E1* polymorphisms and an adverse health effect (i.e. scleroderma), it did not evaluate the potential relationship to any cancer outcomes.]

GSH conjugation is a trichloroethylene-metabolism pathway that results in formation of several toxicologically relevant metabolites. Several transporter proteins in the kidney (e.g. organic anion transporters 1 and 3, OAT1 and OAT3) that are likely to be responsible for the uptake and cellular accumulation of the GSH metabolites DCVG and DCVC into the renal proximal tubular cells, are known to be polymorphic in humans (Erdman *et al.*, 2006; Lash *et al.*, 2006b; Urban *et al.*, 2006). Thus different subpopulations of humans may have a markedly different capacity to accumulate DCVG or DCVC, which may affect their susceptibility to nephrotoxicity.

While it has not been firmly established which GST enzymes are responsible for the metabolism of trichloroethylene, allelic polymorphisms of these enzymes in humans have been associated with variations in enzyme activity (Katoh *et al.*, 2008); such polymorphisms may thus affect the concentration of trichloroethylene metabolites in the body (Caldwell & Keshava, 2006).

Brüning *et al.* (1997b) investigated polymorphisms of *GSTM1* and *GSTT1* and risk of renal cell cancer in workers with long-term high occupational exposure to trichloroethylene [identified as "trichloroethene"]. The study comprised 45 cases with histologically verified renal cell cancer and a history of long-term occupational exposure to high concentrations of trichloroethylene. A reference group consisted of 48 cancer-free workers from the same geographical region with similar histories of occupational exposure to trichloroethylene. Among patients with renal cell carcinoma, 27 carried at least one functional *GSTM1* gene (*GSTM1*+) and 18 carried at least one functional *GSTT1* gene (*GSTT1*+). Among the 48 reference workers, 17 were *GSTM1*+ and 31 were *GSTT1*+. The odds ratios for renal cell cancer 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 study were re-evaluated by Wiesenhütter *et al.* (2007) who used data from additional control subjects to increase the size of the study population. No genetic influence on the development of renal cell cancer due to trichloroethylene (e.g. deletion polymorphisms of *GSTT1* and *GSTM1*, or *NAT2* rapid/slow acetylator) was found. There was a somewhat higher proportion of the homozygous *GSTP1* 313A wild type (*GSTP1*A*) among cases of renal cell cancer, although this was not statistically significant.

Moore *et al.* (2010) conducted a case–control study in central Europe (1097 cases and 1476 controls) to assess risk of renal carcinoma associated with occupational exposure to trichloroethylene (assessed from the work history). Increased risk was observed among subjects ever exposed to trichloroethylene. A significant association was found among trichloroethylene-exposed subjects with at least one intact *GSTT1* allele (active genotype), but not among subjects with two deleted alleles (null genotype). Similar associations for all exposure metrics including average intensity were observed among *GSTT1*-active subjects, but not among *GSTT1* nulls.

Bronley-DeLancey *et al.* (2006) used cryogenically preserved human hepatocytes to simultaneously evaluate the kinetics of chloral hydrate metabolism and aldehyde dehydrogenase (*ALDH*) or alcohol dehydrogenase (*ADH*) genotype. Thirteen samples of human hepatocytes were examined and large inter-individual variation in the V_{max} values for formation of trichloroethanol and trichloroacetate were reported; no correlation with *ADH*/*ALDH* genotype was apparent, although the sample size was limited.

Furthermore, despite the large variation in V_{max} values among individuals, disposition of chloral hydrate into downstream metabolites was found to be relatively constant. Therefore, cellular factors other than genotype may contribute to the observed variability in metabolism of chloral hydrate in human liver.

Among many inter-individual differences in lifestyle and nutrition, alcohol intake is the most prominent factor affecting susceptibility to trichloroethylene. Trichloroethylene is metabolized to chloral hydrate, and then to trichloroacetate by aldehyde dehydrogenase, and to trichloroethanol by alcohol dehydrogenase. The effects of trichloroethylene (probably through chloral hydrate) on alcohol and acetaldehyde metabolism have been suggested as a mechanism for the dramatic effects of coexposure to chlorinated solvents and alcohol. Such coexposures lead to more than additive sedative effects in humans (Sellers *et al.*, 1972). Adverse health effects indicative of elevated blood levels of acetaldehyde have been described as "degreaser's flush" (Stewart *et al.*, 1974). Additionally, aldehyde and alcohol dehydrogenases are polymorphic in humans, and these polymorphisms have a major impact on cancer susceptibility in humans who consume alcoholic beverages, especially in Asian countries (IARC, 2010, 2012). It has been suggested that polymorphisms in these metabolic pathways may yield subpopulations with greater than expected formation of trichloroacetate and enhanced risk of adverse health effects after exposure to chloral hydrate or other chlorinated solvents.

(b) Experimental animals

Bradford *et al.* (2011) studied the metabolic and genetic basis for differences in trichloroethylene toxicity using a panel of genetically diverse inbred mouse strains. Trichloroethylene (2100 mg/kg bw) or corn oil vehicle was given by gavage to male mice of 15 strains (age, 6–8 weeks). Serum and liver were collected 2, 8, and 24 hours after dosing and were analysed for trichloroethylene metabolites, hepatocellular injury, and hepatic gene expression. Metabolism of trichloroethylene through oxidative and conjugative pathways varied considerably between strains. Trichloroethylene-specific effects on hepatic gene expression were strongly dependent on genetic background. Conversely, effects on cell death, liver necrosis, and immune-mediated response pathways, which are altered in the liver by treatment with trichloroethylene, were largely independent of genetic background.

Chloral hydrate has been shown to be an inhibitor of aldehyde dehydrogenase (Wang *et al.*, 1999), thus suggesting that production of trichloroacetic acid from chloral hydrate may not increase in linear fashion with dose. An inhibitory effect of chloral hydrate on the activity of alcohol dehydrogenase in liver was also reported in studies in mice (Sharkawi *et al.*, 1983). In a short-term study in rats, Poon *et al.* (2002) showed that exposure to drinking-water containing chloral hydrate led to a significant reduction in the activity of liver aldehyde dehydrogenase, while the activity of liver aniline hydroxylase (associated with CYP2E1) was significantly elevated in males and females receiving chloral hydrate at a concentration of 200 ppm. This study confirmed previous findings (Wang *et al.*, 1999) that chloral hydrate was a potent inhibitor of liver aldehyde dehydrogenase *in vitro*, with a 50% inhibition concentration (IC_{50}) of 8 μM, while trichloroacetic acid was weakly inhibitory and trichloroethanol was without effect.

Coexposure to chlorinated solvents and alcohol lead to more than additive sedative effects in rodents (Sharkawi *et al.*, 1983), as also noted above in humans (Sellers *et al.*, 1972). Because alcohol consumption leads to increased activity of CYP2E1 in the liver (Bradford *et al.*, 2005), the metabolism of trichloroethylene via the oxidative pathway may be increased. Indeed, Nakajima *et al.* (1992a) observed an increase in metabolism of trichloroethylene in rat liver microsomes from rats pre-treated with alcohol.

4.4.2 Life-stage susceptibility

(a) Early life stages

Many studies have considered differences in exposure as an important element of early life-stage susceptibility to adverse health outcomes associated with trichloroethylene. It has been shown that trichloroethylene can be transferred to the fetus by the placenta in all mammalian species studied, including humans. Infants that are breastfed by mothers exposed to trichloroethylene by various routes (including inhalation) have been shown to receive appreciable amounts of trichloroethylene (Fisher *et al.*, 1990, 1997; Abbas & Fisher, 1997). Differences in diet between infants and adults may also be a factor in differences in exposure, since dairy products have been found to contain trichloroethylene and children eat a generally higher proportion of these foodstuffs than adults (Wu & Schaum, 2000). Exposure dermally and by inhalation was also estimated to be higher in children than in adults when bathing in trichloroethylene-containing water (Fan, 1988).

Since children have a higher ventilation rate than adults, and a relatively higher alveolar surface area for the first 2 years of life, absorption of trichloroethylene is expected to be greater in early life stages, although no data were available to prove this. As trichloroethylene is a lipophilic compound, the percentage of adipose tissue in the body will have an impact on distribution and retention of the absorbed dose; children, who have a lower percentage of body fat per unit body weight, may thus have higher concentrations of trichloroethylene in the body fat, although no data to corroborate this were available. While trichloroethylene has been found in the blood and tissues of fetuses born to exposed mothers, there is disagreement on whether such concentrations are higher or lower than those found in maternal tissues (Laham, 1970; Withey & Karpinski, 1985). More recent studies have demonstrated that the blood: air partition coefficient of trichloroethylene varies with age. Rodriguez *et al.* (2007) have demonstrated that in rats exposed to trichloroethylene via inhalation, blood concentrations of trichloroethylene were higher on postnatal day 10 than in adults, which may be attributed to the lower metabolic capacity of the pre-weaning rats. In addition, Mahle *et al.* (2007) reported that the tissue: air partition coefficient for trichloroethylene increases with age in rats, but decreases in humans.

It is well established that hepatic expression of most CYP and also GST enzymes is dramatically different in the fetus than in adults (Ring *et al.*, 1999). Within months after birth, the metabolic capacity of the human liver changes and becomes more similar to that in adults. Expression of CYP2E1 and GSTs in the developing fetus is detectable, albeit not in all samples, and is dependent on the stage of pregnancy (Carpenter *et al.*, 1996; McCarver & Hines, 2002; Johnsrud *et al.*, 2003). Thus differences in the metabolism of trichloroethylene between early life stages and adults represent a potential susceptibility factor.

Several early life stage-specific adverse health outcomes attributable to exposure to trichloroethylene during pregnancy or neonatal development have been reported in humans and other species. These include cardiac birth defects, neural tube defects, oral clefts, and choanal atresia (Bove *et al.*, 2002). It should be noted that a large number of epidemiological and experimental studies observed no association between trichloroethylene and these developmental abnormalities (Watson *et al.*, 2006). Other non-cancer adverse health effects in children or pre-weaning animals have been evaluated. Overall, the evidence base of available information was largely inconsistent with regard to whether early life stage is a susceptibility factor for adverse health outcomes in the nervous and immune systems, kidney, liver or lung.

Several studies have evaluated the potential for susceptibility to cancer outcomes in early life stages. Total incidence of childhood cancer, and

incidence of childhood leukaemia and tumours of the central nervous system have been considered. Most studies have found no evidence that children may be more susceptible than adults; however, these studies included small numbers of cases and poor characterization of exposure (see Section 2.2.2b, Section 2.2.3c; McKinney *et al.*, 1991; Shu *et al.*, 1999; De Roos *et al.*, 2001).

(b) Advanced life stages

Limited evidence existed to suggest that exposure to trichloroethylene in adults of advanced age (> 65 years for humans) may lead to greater adverse health effects than in younger people. Some studies have suggested that toxicokinetic parameters in later life stages are different from those in young adults (Benedetti *et al.*, 2007); however, there was little evidence to suggest that expression of CYP2E1 or GSTs differs with age in adults. While several studies have documented significant age-related declines in the amount, specific activity and rate of induction of liver microsomal mono-oxygenases in inbred male rats, on the basis of a variety of clinical tests, most liver functions in humans appear to be well preserved with age [reviewed in Schmucker (2001)]. There was some evidence suggesting a reduction in CYP2E1 activity in the elderly (O'Shea *et al.*, 1994; George *et al.*, 1995); however, studies *in vitro* that used non-human primate or human liver tissues, or isolated cells, did not detect age-related deficiencies in the activity of CYP-dependent microsomal mono-oxygenases [reviewed in Schmucker (2001)].

Mahle *et al.* (2007) observed that blood:air partition coefficients for trichloroethylene in the rat increased with age (age 60 days versus age 2 years). Another study used a modelling approach to predict age-appropriate pharmacokinetics of trichloroethylene in the rat (Rodriguez *et al.*, 2007). These authors predicted that the steady-state concentration of trichloroethylene in the blood would be reached more slowly and be higher at age 2 years than in young adults. In addition, they predicted that concentrations of trichloroethylene in the brain would increase with age. No experimental confirmation of this pharmacokinetic model was available.

4.4.3 Sex-specific differences

Several studies examined sex-specific differences in the toxicokinetic parameters of trichloroethylene using physiologically-based pharmacokinetic modelling. Sato *et al.* (1991) evaluated the influence of body size, body-fat content, and sex on the pharmacokinetic behaviour of trichloroethylene. Absorption, distribution, metabolism, and excretion of trichloroethylene were found to vary according to the different anatomical features of men and women. Body build (body weight and body-fat content) also affected the pharmacokinetic behaviour of trichloroethylene. In a follow-up study, Sato (1993) concluded that there was a sex difference in the pharmacokinetic profiles of trichloroethylene, and although retention of trichloroethylene in the body was greater in men than in women, the blood concentration of trichloroethylene in women was higher than in men 16 hours after exposure. Fisher *et al.* (1998) evaluated sex-specific differences in the uptake and metabolism of trichloroethylene using data on human exposure, and concluded that only minor differences existed in the toxicokinetics of trichloroethylene.

Lash *et al.* (2006a) evaluated the metabolism and tissue distribution of trichloroethylene in male and female Fischer 344 rats given doses of 2, 5, or 15 mmol/kg bw in corn oil by gavage, and monitored for trichloroethylene and its metabolites in the liver, kidneys, blood, and urine for up to 48 hours. Higher concentrations of trichloroethylene were generally observed in tissues of males at lower doses. Higher concentrations of oxidative metabolites were observed in the livers of males than in females, with the opposite pattern being observed in the kidneys.

DCVG was recovered in the liver and kidneys of female rats only, and in blood of males and females, with amounts being generally higher in females. DCVC, the nephrotoxic metabolite, was recovered in liver of males and females, kidneys of females, blood of males, and in the urine of males and females.

While most of the experimental systems used to study the mechanisms of DCVC-related nephrotoxicity have been derived from the male rat, studies have also been conducted in tissue from female rats and in mice. Darnerud *et al.* (1989) characterized the relationship between developmental stage, activities of CCBL and OAT, and nephrotoxicity. Female mice were found to be the most susceptible to DCVC-induced nephrotoxicity at low doses due to higher CCBL activity, while male mice were more susceptible at higher doses.

Nakajima *et al.* (1992b) used liver microsomes from male and female rats aged 3 and 18 weeks to study the metabolism of trichloroethylene (by measuring formation of chloral hydrate) *in vitro*. No differences between males and females were seen with age or concentration of trichloroethylene administered.

Expression and function of OAT1 and OAT3 and other organic anion transporters (such as OAT polypeptide 1) have been shown to exhibit sex-specific differences in humans and experimental animals (Gotoh *et al.*, 2001; Kato *et al.*, 2002; Kobayashi *et al.*, 2002; Buist *et al.*, 2003; Buist & Klaassen, 2004; Ljubojevic *et al.*, 2004), suggesting that transport differences may be another contributing factor to sex-specific differences in susceptibility to trichloroethylene metabolites.

No sex-specific susceptibility to toxicity in the liver or respiratory tract associated with exposure to trichloroethylene has been reported. Two studies evaluating kidney effects in humans have concluded that females may be more susceptible to kidney disease and diabetes associated with exposure to trichloroethylene (Davis *et al.*, 2005). However, males were reported to be more sensitive to renal toxicity in studies in rats (Lash *et al.*, 1998; Lash *et al.*, 2001b). With regard to data on immunotoxicity in humans, a meta-analysis of three case–control studies of scleroderma with a measure of occupational trichloroethylene exposure found that the combined odds ratio was 2.5 (95% CI, 1.1–5.4) in men and 1.2 (95% CI, 0.58–2.6) in women (Cooper *et al.*, 2009).

Sex-specific differences in susceptibility to cancer associated with exposure to trichloroethylene are well established. In rats, exposure to trichloroethylene by inhalation or gavage caused cancer of the kidney (tubular adenocarcinoma) only in males. Leukaemia was also observed in a single study in male rats, although low survival was noted as a challenge in interpreting this study (Maltoni *et al.*, 1988). Testicular tumours have also been observed in studies in rats. In studies in mice, no sex-specific differences in the incidence of cancer of the liver or lung were observed (Maltoni *et al.*, 1988). Lymphomas were reported in female mice (Henschler *et al.*, 1980).

Raaschou-Nielsen *et al.* (2003) evaluated risk of cancer among workers at Danish companies using trichloroethylene in a cohort study (see Section 2). No significant sex-specific differences in the incidence of tissue-specific cancer were observed in this study. Most other epidemiological studies of cancer and exposure to trichloroethylene did not report sex-specific differences.

4.5 Other adverse effects

4.5.1 Kidney: chronic nephropathy

(a) Humans

Brüning *et al.* (1996) detected substantially more tubular damage in patients with renal cell carcinoma who were also exposed to trichloroethylene than in those who were not exposed. In a second study, Brüning *et al.* (1999b) performed a retrospective analysis of 39 workers exposed to high levels of trichloroethylene for 19 years.

While the standard biomarkers of tubular function that indicate significant renal damage (i.e. total urinary protein, blood urea nitrogen, and urinary and serum creatinine) were not elevated, elevated urinary excretion of α1-microglobulin and GSTA were significantly elevated.

Vermeulen *et al.* (2012) studied a small group of 80 factory workers exposed to trichloroethylene and 45 non-exposed workers. Precise exposure information was obtained (mean ± standard deviation, 22.2 ± 35.9 ppm). All workers exposed to trichloroethylene were stratified according to their exposure level, with 12 ppm as the threshold. Significant elevation of urinary Kim-1 (a very sensitive and selective indicator of renal damage in humans and rodents) was observed (Hoffmann *et al.*, 2010; Harpur *et al.*, 2011). Even workers with exposures of < 12 ppm exhibited a statistically significant elevation in urinary Kim-1.

Green *et al.* (2004) investigated the nephrotoxic potential of trichloroethylene in a cross-sectional study of 70 workers currently exposed to trichloroethylene. Data from age- and sex-matched control populations were also available. The mean exposure to trichloroethylene, estimated from urinary concentrations of trichloroacetate, was 32 ppm (range, 0.5–252 ppm) with an average duration of exposure of 4.1 years (range, 1–20 years). Significant differences between the exposed and control populations were found for nephrotoxicity markers NAG, albumin, and formic acid. Neither NAG nor albumin showed any significant correlation with either the magnitude or duration of exposure to trichloroethylene. However, there was a significant correlation between urinary concentrations of formic acid and trichloroacetate. Within the exposed population there were dose-dependent increases in urinary concentrations of methylmalonic acid and urinary GSTA activity. Although not outside the range for controls, these changes were clearly dose-dependent.

(b) Experimental systems

Exposure of Eker rats to trichloroethylene in corn oil by gavage for 13 weeks (0, 100, 25, 500, and 1000 mg/kg bw) led to an increased incidence of nephrotoxicity, but no significant increases in the incidence of preneoplastic or neoplastic lesions when compared with controls (Mally *et al.*, 2006). Chronic nephropathy was also observed in male and female Osborne-Mendel rats exposed to trichloroethylene (549 and 1097 mg/kg bw per day) by gavage for 78 weeks (NCI, 1976).

Overt signs of short-term nephrotoxicity, such as changes in blood or urinary biomarkers, are primarily seen at higher doses, although histopathological changes are evident at lower doses. In Fischer 344 rats given trichloroethylene by gavage in corn oil at a dose of 2000 mg/kg bw per day for 42 days, there were increases of about twofold in urinary markers of nephrotoxicity such as urine volume and protein (both 1.8 times), NAG (1.6 times), glucose (2.2 times) and alkaline phosphatase (2.0 times) compared with controls (Green *et al.*, 1997b). No morphological changes were observed in the kidney of treated rats or controls (Green *et al.*, 1997b). At lower doses, Green *et al.* (1998) reported that plasma and urinary markers of nephrotoxicity were unchanged. In particular, after 28 days of exposure to trichloroethylene at a concentration of 250 or 500 ppm for 6 hours per day, there were no statistically significant differences in plasma concentrations of blood urea nitrogen, or in urinary concentrations of creatinine, protein, alkaline phosphatase, NAG, or GGT. However, increased urinary excretion of formic acid, accompanied by changes in urinary pH and increased ammonia, was found at these exposures. Interestingly, at the same exposure level of 500 ppm (6 hours per day, 5 days per week, for 6 months) in Long-Evans rats, Mensing *et al.* (2002) reported elevated excretion of low-molecular-weight proteins and NAG, biomarkers

of nephrotoxicity, but after the longer exposure duration of 6 months.

Numerous studies have reported histological changes after short- and long-term exposure trichloroethylene (Maltoni *et al.*, 1986; NTP, 1988, 1990; Mensing *et al.*, 2002).

After 1–2 years of exposure to trichloroethylene by gavage (NCI, 1976; NTP, 1988, 1990) or inhalation (Maltoni *et al.*, 1986, 1988), mice and rats exhibited lesions in the tubular epithelial cells of the inner renal cortex that are characterized by cytomegaly, karyomegaly, and "toxic nephrosis" (see Section 3 for details). These long-term studies reported cytomegaly and karyomegaly of tubular cells. NTP (1990) specified the area of damage as the pars recta, located in the corticomedullary region. These effects are distinct from the chronic nephropathy and inflammation observed in control mice and rats (NCI, 1976; Maltoni *et al.*, 1986, 1988; Lash *et al.*, 2000b).

These effects of trichloroethylene on the kidney appear to be progressive. Maltoni *et al.* (1986, 1988) and NTP (1988, 1990) noted that the incidence and degree of renal toxicity increased with exposure duration and with time from the start of treatment.

Wallin *et al.* (1992) characterized the renal cellular response to DCVC in male Sprague-Dawley rats. Rats given ^{35}S-labelled DCVC at a dose of 30 mg/kg bw exhibited a doubling of blood urea nitrogen levels within 2 days. Formation of covalent adducts was seen within 3 hours and peaked at 6 hours, but was still detectable after 120 hours. BrdU staining indicative of cell proliferation increased within 24 hours, and the increase of vimentin-positive cells indicative of loss of differentiation was evident.

Eyre *et al.* (1995b) demonstrated that administration of DCVC (1, 5, or 25 mg/kg bw) or trichloroethylene (1000 mg/kg bw) to male F344 rats or male B6C3F$_1$ mice resulted in increased cell proliferation, as indicated by BrdU staining. Moreover, they observed greater formation in mice than in rats of acid-labile adducts from both DCVC and trichloroethylene that correlated with a higher proliferative response. These results were consistent with the documented greater rates of GSH-dependent metabolism and CCBL-dependent bioactivation in mice than in rats.

Cummings *et al.* (2000c) studied cytotoxicity of trichloroethylene and DCVC in primary cultures of rat proximal tubular cells and found that exposure of cells for 72 hours to trichloroethylene (10 mM) or DCVC (10 µM) resulted in the appearance of vimentin-positive cells, indicating loss of differentiation.

Mally *et al.* (2006) assessed renal cell proliferation and transformation *in vivo* and *in vitro* in Eker rats (a strain that carries the Eker mutation, *Tsc-2*$^{Ek/+}$, and is thus extremely susceptible to renal carcinogens). *In vivo*, exposure to trichloroethylene (0, 100, 250 500, and 1000 mg/kg bw) by gavage, 5 days per week, for 13 weeks significantly increased cell proliferation, but did not enhance formation of preneoplastic lesions or renal tumours. *In vitro,* exposure of primary cultures of kidney epithelial cells to DCVC (10 µM) reduced cell viability to ~50% after a 24-hour incubation, and caused transformation. These effects were not associated with known, carcinogen-specific mutations in either the *VHL* or *Tsc-2* tumour suppressor genes.

Mehendale and colleagues studied tissue repair and survival in male Swiss-Webster mice exposed to DCVC in a series of studies (Korrapati *et al.*, 2005, 2006, 2007; Dnyanmote *et al.*, 2006). These studies showed that intraperitoneal injection of a sublethal dose of DCVC could stimulate renal repair processes and protect mice from subsequent exposure to normally lethal doses of either DCVC or other nephrotoxicants, such as mercuric chloride. These studies also identified potential mechanisms for this stimulation of renal repair, including changes in the expression of certain cyclins and cyclin-dependent kinases.

Counts *et al.* (1995) demonstrated that incubation of rabbit proximal tubules with a relatively high concentration of DCVC inhibited the ability of the renal cells to undergo repair and regeneration.

Nowak and colleagues (Nowak *et al.*, 1999; Nowak, 2003; Shaik *et al.*, 2008) provided insight into how the compensatory repair and proliferation response might occur in rabbit proximal tubules, by showing that activation of certain regulatory enzymes, such as protein kinase C and protein kinase B (Akt), or epidermal growth factor promoted recovery of renal mitochondrial function and promoted repair after DCVC-induced injury.

4.5.2 Liver

(a) Cytotoxic injury and hepatitis

(i) Humans

In a cross-sectional epidemiological study of the early hepatic effects of long-term exposure to low levels of trichloroethylene, Nagaya *et al.* (1993) evaluated serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and GGT (called GGtranspeptidase in this report) in 148 workers occupationally exposed to trichloroethylene by inhalation. Of the main cohort, 13 workers were followed for 2 years. Exposure to trichloroethylene was ascertained from the urinary concentrations of total trichloro-compounds, and subjects were divided into groups with low, moderate, and high exposure. Slight, but not statistically significant, increases in total and HDL cholesterol were observed with increasing exposure levels. No effect was found on the activities of the serum enzymes. In the follow-up study, concurrent fluctuations in urinary concentrations of total trichloro-compounds and subclinical (e.g. not exceeding the normal range in this study) changes in HDL cholesterol, AST, and GGT were observed. While there was some evidence for liver toxicity of trichloroethylene in occupationally exposed individuals, these effects were subclinical and reversible.

Rasmussen *et al.* (1993a) examined dose–response relationships between exposure to degreasing solvents (mainly trichloroethylene) and liver function. The study included 99 metal degreasers using trichloroethylene (range of full-time degreasing, between 1 month and 36 years); no control group was used. Based on the estimated total number of hours of exposure, subjects were divided into four groups. Present or recent exposure to trichloroethylene was quantified by blood and urine analyses of the metabolites trichloroacetate and trichloroethanol. Of several serum markers of liver injury, a dose–response relationship was found to be statistically significant only for GGT. However, when age and alcohol abuse were included as covariates in the multiple-regression analysis, this association was no longer significant.

Xu *et al.* (2009) evaluated liver-injury markers in 21 subjects with severe hypersensitivity dermatitis who were employed as metal degreasers. Exposure was evaluated from workplace air measurements and further documented by urinary concentrations of trichloroacetate. In most subjects, exposure was classified as exceeding recommended occupational levels. In 76–90% of subjects, an increase in serum activities of liver enzymes (ALT, AST) and total bilirubin was detected.

Kamijima *et al.* (2007) performed a comprehensive literature analysis to evaluate the possible relationship between idiosyncratic generalized skin disorders and accompanying hepatitis, and occupational exposure to trichloroethylene. They reported, based on primary evidence presented in Xia *et al.* (2004), that hepatitis was diagnosed in 46–94% of trichloroethylene-exposed subjects who also had various types of dermatitis ranging from hypersensitivity syndrome to erythema

multiforme, Stevens–Johnson syndrome, or toxic epidermal necrolysis.

(ii) Experimental systems

Acute exposure to trichloroethylene, even at high doses, is not known to cause significant hepatocellular necrosis. Okino *et al.* (1991) exposed male Wistar rats to trichloroethylene via inhalation for 2 hours (up to 8000 ppm) or 8 hours (up to 2000 ppm). Mild hepatocellular necrosis, evaluated by histopathology, and small increases in serum enzyme markers of liver injury were observed only at the highest concentrations. In a study of oral exposure (up to 5000 mg/kg bw by gavage), Berman *et al.* (1995) reported no elevation in serum enzyme activities, but histopathological evidence of mild hepatocellular necrosis in female F344 rats. Sano *et al.* (2009) observed no liver injury in either male Sprague-Dawley rats or $B6C3F_1$ mice given trichloroethylene at 1500 mg/kg bw by gavage.

Several, although not all, studies reported an increased incidence of liver necrosis after short-term exposure to trichloroethylene. Buben & O'Flaherty (1985) observed central lobular necrosis in male Swiss-Cox mice exposed to trichloroethylene at a dose of 1600 mg/kg bw per day for 6 weeks. Melnick *et al.* (1987) reported single-cell necrosis in male F344 rats fed or gavaged with trichloroethylene at doses of 2.2 g/kg bw per day or higher for 14 days. Merrick *et al.* (1989) found that the use of corn oil as vehicle for trichloroethylene (4 weeks at 600, 1200 and 2400 mg/kg bw per day for males and 450, 900 and 1800 mg/kg bw per day for females) promotes liver necrosis in male, but not female $B6C3F_1$ mice. When aqueous vehicle was used, no liver necrosis was found. In addition, mild increases in serum enzymes were found in male mice treated with trichloroethylene diluted in corn oil. Dees & Travis (1993) observed slight to mild liver necrosis in $B6C3F_1$ mice treated with trichloroethylene at doses of up to 1000 mg/kg bw per day for 10 days. Berman *et al.* (1995) reported histopathological evidence for hepatocellular necrosis in female F344 rats exposed to trichloroethylene at a dose of 1500 mg/kg bw per day or higher for 14 days. Ramdhan *et al.* (2008, 2010) found an increase in serum enzyme activities (ALT and AST) in Sv/129 mice exposed to trichloroethylene at concentrations exceeding 1000 ppm via inhalation for 7 days.

Other investigators reported no evidence of liver necrosis after short-term exposure to trichloroethylene. These included a study in $B6C3F_1$ mice given doses of up to 1200 mg/kg bw per day in corn oil for up to 8 weeks (Channel *et al.*, 1998); a study in rats given a dose of 2000 mg/kg bw per day in corn oil for 7 days (Nunes *et al.*, 2001); a study in Sv/129 mice given doses of up to 1500 mg/kg bw per day for 3 weeks (Laughter *et al.*, 2004); and a study in male Sprague-Dawley rats and $B6C3F_1$ mice given doses of up to 1500 mg/kg bw per day in corn oil for 14 days (Sano *et al.*, 2009). In a 2-year cancer bioassay (NTP, 1990), no evidence of increased focal necrosis in the liver was found in $B6C3F_1$ male and female mice, although the incidence of neoplasms of the liver was increased.

Two studies examined end-points for liver injury in MRL mice, which are prone to autoimmune disease. Kaneko *et al.* (2000) observed dose-dependent mild liver inflammation after exposure to trichloroethylene (up to 2000 ppm) via inhalation for up to 8 weeks (4 hours per day, 6 days per week). Gilbert *et al.* (2009) evaluated the effects of long-term (32-week) low-level exposure to trichloroethylene (0.5 mg/mL in drinking-water) in female MRL mice; trichloroethylene-induced autoimmune hepatitis could be detected in as little as 26 weeks. Exposure to trichloroethylene also generated a time-dependent increase in the number of antibodies specific for liver proteins, and altered the hepatic expression of selected genes associated with immunity and inflammation.

Several studies reported liver inflammation and/or evidence for inflammatory cell infiltrates.

Kjellstrand *et al.* (1983b) exposed male and female NRMI mice to trichloroethylene at 150 ppm, and found inflammatory cell infiltration and an increase in the number and size of Kupffer cells in mice exposed for 120 days. Elcombe *et al.* (1985) observed isolated inflammatory foci in livers of two strains of rats exposed to trichloroethylene at 1500 mg/kg bw per day for 10 days. Goel *et al.* (1992) found "proliferation of hepatic sinusoidal endothelial cells" in male Swiss mice exposed to trichloroethylene at 1000–2000 mg/kg bw per day for 28 days.

(b) Cholestatic injury

(i) Humans

Driscoll *et al.* (1992) evaluated concentrations of individual serum or plasma bile acids in subjects exposed occupationally to trichloroethylene. The study cohort of 22 volunteers (21 men) was divided into two groups: exposed (n = 16) and unexposed (n = 6) to trichloroethylene on the basis of their job tasks. Blood was collected at the beginning of a shift after an overnight fast. Highly significant increases in the exposed group, after controlling for age and alcohol intake, were seen for plasma concentrations of chenodeoxycholic acid, and its glycine and taurine conjugate, and for total glycine, taurine conjugates, and total cholate, and total chenodeoxycholate (free plus conjugates), and total bile acids.

Neghab *et al.* (1997) examined hepatobiliary function in subjects exposed occupationally to trichloroethylene. This study included 10 healthy workers (5 unexposed controls and 5 exposed) and evaluated end-points before (first day of the working week) and after exposure (2 days into the working week). Statistically significant elevations in concentrations of total serum bile acids, particularly deoxycholic acid and the subtotal of free bile acids, among "exposed" subjects were reported before exposure compared with after exposure. Furthermore, serum bile acid concentrations correlated with level of exposure to trichloroethylene (r = 0.94).

(ii) Experimental systems

Several studies of exposure to trichloroethylene by intraperitoneal injection from one laboratory reported increases in serum bile-acid concentrations in male rats exposed to trichloroethylene (Wang & Stacey, 1990; Bai & Stacey, 1993; Hamdan & Stacey, 1993). Serum increases in cholic, chenodeoxycholic, deoxycholic, taurocholic, and tauroursodeoxycholic acids in serum were dose-related in male Sprague-Dawley rats treated with high doses of trichloroethylene via intraperitoneal injection (up to 1300 mg/kg bw per day for 3 days) (Wang & Stacey, 1990). Studies *in vitro* supported a dose-related suppression of initial rates of uptake of cholic and taurocholic acids, with no significant effect on enzyme leakage or intracellular potassium-ion content (Bai & Stacey, 1993). The inhibition of uptake of cholic and taurocholic acids was confirmed and shown to be reversible in accompanying studies *in vivo*.

(c) Cirrhosis

(i) Humans

There is mixed evidence with regard to a possible association between exposure to trichloroethylene and cirrhosis of the liver. A cohort study on deaths from cirrhosis in California between 1979 and 1981 (Leigh & Jiang, 1993), which examined various occupational risk factors from job titles, reported elevated risks of cirrhosis among white males with occupational titles of sheet metal workers and metalworkers. Ala *et al.* (2006) found that the prevalence ratio for primary biliary cirrhosis, an uncommon liver disease of unknown etiology that has been linked to environmental factors, was significantly higher in zip codes containing or adjacent to Superfund sites (a discarded site where hazardous waste is located). However, no specific link to trichloroethylene was made in this study.

Contrary to the findings listed above, a deficit in mortality from cirrhosis was observed in three epidemiological studies that evaluated potential occupational exposure to trichloroethylene and cirrhosis (Garabrant *et al.*, 1988; Morgan *et al.*, 1998; Boice *et al.*, 1999, 2006), while several other studies found either null or non-significantly elevated associations (Blair *et al.*, 1989, 1998; Ritz, 1999).

(ii) Experimental systems

No data were available to the Working Group.

(d) Hepatomegaly

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Increases in liver weight after single, short-term and long-term exposure to trichloroethylene have been observed in numerous studies in mice, rats and other species. However, single-exposure studies with trichloroethylene, even at doses exceeding 1500 mg/kg bw, in male Sprague-Dawley rats and $B6C3F_1$ mice (Sano *et al.*, 2009), or in a panel of inbred mouse strains (Bradford *et al.*, 2011) found no elevation in relative liver weight. Nonetheless, trichloroethylene-induced increases in liver weight have been reported to occur as early as after 2 days of exposure by inhalation in NMRI mice (Kjellstrand *et al.*, 1981). Laughter *et al.* (2004) found increased liver weight in Sv/129 mice after 3 days of treatment by gavage, and Tao *et al.* (2000) reported an increase in liver weight in female $B6C3F_1$ mice after 5 days. Elcombe *et al.* (1985) and Dees & Travis (1993) reported that mice and rats given trichloroethylene by gavage for 10 days showed significant increases in relative liver weight. Tucker *et al.* (1982) observed that male CD-1 mice given trichloroethylene by gavage at 24 or 240 mg/kg bw for 14 days showed a dose-dependent increase in liver weight. Sano *et al.* (2009) found an increase in relative liver weight in male Sprague-Dawley rats and $B6C3F_1$ mice given trichloroethylene at 1500 mg/kg bw per day by gavage for 14 days, but they reported greater increases in mice than in rats.

Studies have provided little evidence for a major role of PPARα in hepatomegaly associated with treatment with trichloroethylene. Nakajima *et al.* (2000) treated groups of Sv/129 wild-type and *Pparα*-null male and female mice (n = 6) with trichloroethylene at a dose of 750 mg/kg bw by gavage for 2 weeks. Relative liver weight increased by 1.5-fold in wild-type males, and by 1.3-fold in *Pparα*-null males. In female mice, the increase was about 1.25-fold in both strains. Laughter *et al.* (2004) treated Sv/129 wild-type and *Pparα*-null male mice with three daily doses of trichloroethylene at in 0.1% methyl cellulose for either 3 days (trichloroethylene, 1500 mg/kg bw) or 3 weeks (trichloroethylene, 0, 10, 50, 125, 500, 1000, or 1500 mg/kg bw, 5 days per week). After 3 days, the percentage liver weight:body weight ratio was 1.4-fold control levels in the wild-type mice and 1.07-fold control levels in the null mice. After 3 weeks of exposure to trichloroethylene at varying concentrations, wild-type mice were reported to have percentage liver weight:body weight ratios that were close to control values, with the exception of the groups at 1000 and 1500 mg/kg bw (increased by 1.18- and 1.30-fold, respectively, compared with controls). For the *Pparα*-null mice, the variability in percentage liver weight:body weight ratios was reported to be greater than that of the wild-type mice in most of the groups receiving trichloroethylene, and the baseline levels of percentage liver weight:body weight ratio for control mice were 1.16-fold that of wild-type mice. Of note was the higher toxicity of trichloroethylene in *Pparα*-null mice; some mice at 1500 mg/kg bw per day died during the study. Ramdhan *et al.* (2010) exposed male wild-type, *Pparα*-null, and humanized *PPARα* mice on an Sv/129 genetic background to trichloroethylene at 0, 1000, and 2000 ppm by inhalation, 8 hours per day, for 7 days. Hepatomegaly was induced

in all strains to a similar extent after exposure to trichloroethylene.

Several studies showed that hepatomegaly in mice is reversible after cessation of treatment with trichloroethylene for up to 30 days (Kjellstrand *et al.*, 1981, 1983b; Elcombe *et al.*, 1985).

4.5.3 Immune system

(a) Immune markers and immunosuppression

(i) Humans

As mentioned in Section 4.3.3a, several molecular epidemiology studies have evaluated the effect of exposure to trichloroethylene on concentrations of immune markers in humans and the potential for immunosuppressive effects. Most studies have been of workers exposed occupationally to trichloroethylene, with the controls being unexposed workers, and have been of a cross-sectional design with an exposure-monitoring period preceding collection of blood or other specimens (Table 4.7; Lan *et al.*, 2010; Selgrade & Gilmour, 2010; Hosgood *et al.*, 2011; Bassig *et al.*, 2013; Zhang *et al.*, 2013).

A cross-sectional study in China evaluated peripheral blood-cell counts, total lymphocyte counts and subsets, and specific markers of B-cell activation in relation to exposure to trichloroethylene among 80 healthy exposed factory workers and 96 unexposed controls in separate factories in the same geographical area in Guangdong (Lan *et al.*, 2010). Two to three full-shift air-exposure measurements for all exposed subjects and a subset of controls were collected over 3 weeks using 3M organic-vapour monitoring badges, and additional monitoring for other organic solvents was completed to rule out potential confounding exposures. The mean exposure level in the exposed workers was 22.19 ppm (± 35.94). Specific markers evaluated included peripheral blood cell counts, lymphocyte subsets, and soluble levels of CD27 (sCD27) and CD30 (sCD30), which regulate immune-cell activation (Table 4.7). Compared with unexposed factory workers, total lymphocytes (15% reduction), specific lymphocyte subsets including $CD4^+$ T-cells (8%), $CD8^+$ T-cells (14%), B-cells (24%), natural killer (NK) cells (30%), and plasma levels of sCD27 (62%) and sCD30 (34%) were significantly decreased in a dose-dependent manner after adjustment for age, sex, and other demographic variables, including smoking.

Subsequent analyses using data from this cross-sectional study examined levels of $CD4^+$ and $CD8^+$ T-cell subsets (Hosgood *et al.*, 2011), levels of key cytokines with a role in immune regulation and suspected to be relevant to lymphomagenesis (Bassig *et al.*, 2013), and serum levels of immunoglobulins (Zhang *et al.*, 2013) in the exposed workers and controls. Significant decreases in $CD4^+$ naïve, $CD8^+$ naïve, and in $CD4^+$ effector memory T-cell counts were reported in exposed workers compared with controls, and significantly decreased trends were demonstrated for each of these end-points. Conversely, levels of $CD8^+$ memory T-cells, $CD4^+$ central memory T-cells, or T-cell regulation subsets were not significantly different in exposed and unexposed workers. This study provided evidence that exposure to trichloroethylene may result in immunosuppressive effects, and potentially a reduced capacity to respond to antigen-related inflammation (Hosgood *et al.*, 2011) and immune dysregulation.

Serum concentrations of the cytokines IL-6, IL-10, and TNF-α were examined in a subset of exposed and unexposed workers in the cross-sectional study, including a total of 71 exposed and 78 unexposed workers (Bassig *et al.*, 2013). Compared with the unexposed workers, the concentration of IL-10 in exposed workers was significantly reduced by about 70%, but there were no significant differences in IL-6 or TNF-α. The decrease in IL-10 concentration was similar in workers exposed to trichloroethylene at < 12 ppm or ≥ 12 ppm compared with unexposed workers, suggesting that these effects may occur

at relatively low exposures. IL-10 is an anti-inflammatory cytokine that is involved in the regulation of T-cell mediated immune inflammation, and previous studies have indicated that altered levels of this marker are associated with risk of lymphoma. A subsequent analysis of the same study population (Zhang *et al.*, 2013) found that workers exposed to trichloroethylene had significant declines in serum concentrations of IgG (18% reduction) and IgM (38% reduction), but not in levels of IgE compared with unexposed workers, after adjustment for demographic variables. These reductions were apparent in workers exposed to trichloroethylene at < 12 ppm or ≥ 12 ppm. For each of these analyses in the cross-sectional study, potential confounding by age, sex, smoking, alcohol consumption, recent infections, and body-mass index was evaluated, and personal exposure monitoring was available for each of the enrolled subjects. Therefore, confounding with respect to these variables was unlikely.

A cross-sectional study in Italy (Iavicoli *et al.*, 2005) evaluated serum concentrations of the cytokines IL-2, IL-4, and INF-γ in 35 healthy male workers employed in the printing sector who were exposed to trichloroethylene in the degreasing process (exposed group) and in two groups of control workers. The first group included 30 healthy male workers in the same factory, but not involved in degreasing or any work involving exposure to trichloroethylene (internal controls), while the second group included 40 healthy male office workers in the same factory (external controls). Personal air monitoring of trichloroethylene was conducted for four exposed workers and four workers in the internal control group for three consecutive working shifts, with a total of 24 samples. Urinary concentrations of trichloroacetate were higher in exposed workers (13.3 ± 5.9 mg/g creatinine) than in the internal control group (0.02 ± 0.02 mg/g creatinine). Compared with workers in the internal or external control groups, exposed workers had significantly increased levels of type-1 cytokines IL-2 (11.5% increase compared with the internal control group, 8.5% increase compared with the external control group) and INF-γ (38% increase compared with internal or external control groups), and a significantly decreased concentration of the type-2 cytokine IL-4 (52% decrease compared with internal or external control groups). There were no significant differences in cytokine concentrations between the two control groups.

Two studies of children in Germany evaluated levels of IgE antibodies to food and allergens and of cytokines in relation to exposure to trichloroethylene and other volatile organic compounds (Lehmann *et al.*, 2001, 2002). In the first study, blood samples were taken during a medical examination; serum concentrations of IgE were measured in 121 children, and cytokine measurements (IFN-γ, IL-4) were available for 28 children (Lehmann *et al.*, 2001). The subjects were drawn from those enrolled in the birth cohort study at the age of 36 months, and subjects were at high risk of atopy based on a family history of atopy and cord-blood levels of IgE of > 0.9 kU/L. Trichloroethylene and other exposures were passively monitored using 3M badges for 4 weeks in each child's bedroom, and the median level of exposure to trichloroethylene was reported to be 0.42 μg/m^3. Indoor exposure to trichloroethylene in these children was not significantly associated with allergic sensitization to indoor or outdoor allergens, or with cytokine-producing T-cells (Lehmann *et al.*, 2001).

In a subsequent study, exposure to volatile organic compounds and levels of cytokines in cord blood were evaluated for 85 neonates randomly selected from 976 children enrolled in a prospective cohort study in Germany (Lehmann *et al.*, 2002). Intracellular-cytokine staining was used to assess cord blood T-cell function, and levels of trichloroethylene and other volatile organic compounds were monitored passively using 3M badges for 4 weeks in each child's bedroom.

Median concentrations of trichloroethylene were reported to be 0.6 μg/m³. Exposure to trichloroethylene was associated with increased levels of IFN-γ (OR for > 75th percentile, 3.6; 95% CI, 0.9–14.9) and reduced levels of IL-4 (OR for < 25th percentile, 4.4; 95% CI, 1.1–17.8) after the multivariate adjustment. Moreover, a reduction in levels of IL-2 was reported for higher degrees ofexposure to trichloroethylene in the univariate analysis, but there was no significant association after adjustment for family history of atopy, sex, and maternal smoking during pregnancy (Lehmann *et al.*, 2002). However, the findings of this study of children known to be at high risk of atopy may not be generalizable to children not at high risk (Lehmann *et al.*, 2001). There was no association between exposure to trichloroethylene and levels of cytokines in these children at high risk for atopy.

Apart from the studies evaluated above, there were limited data available concerning the immunosuppressive effects of trichloroethylene in humans. Some studies of residents of Woburn, MA, USA, which was the site of several industrial facilities, examined immunosuppressive effects associated with ingestion of drinking-water contaminated with chlorinated solvents (Lagakos *et al.*, 1986; Byers *et al.*, 1988). Environmental testing of municipal wells in the town in 1979 suggested levels of exposure to trichloroethylene of 267 ppb, although tetrachloroethylene and other chemicals were also detected (Byers *et al.*, 1988). These studies provided some evidence for a positive association between higher levels of cumulative exposure and a history of infections involving the kidney, urinary tract, and respiratory system, including asthma, chronic bronchitis, and pneumonia (Lagakos *et al.*, 1986). A study of 23 family members of patients with leukaemia in Woburn, and 70 control subjects living in Boston, also suggested that levels of T-cells, CD4, and CD8 T-cells were elevated and the CD4:CD8 ratio was reduced in the family members compared with the controls (Byers *et al.*, 1988). However, cell counts in the family members declined and were no longer statistically different from those in control subjects on reassessment 18 months later. These findings suggested that there may be alterations in lymphocyte subpopulations and altered susceptibility to infection in subjects exposed to chlorinated solvents.

In summary, these findings provided evidence that exposure to trichloroethylene at concentrations of < 12 ppm can result in an altered immune response, as indicated by a decrease in cell count for lymphocytes and specifically $CD4^+$ T-cells, and immune dysregulation via altered levels of cytokines that mediate the Th1/Th2 immune response in otherwise healthy individuals. There is a well established connection between immune status and carcinogenesis, particularly for lymphoma, as an increased risk of cancer has been associated with a history of use of immunosuppressive medication, with certain chronic infections such as HIV, and with certain autoimmune diseases and lifestyle factors that result in immune alterations and abnormalities (Whiteside, 2006). Furthermore, more subtle changes in immune function, including imbalances in Th1/Th2 responses resulting from cytokine alterations, have been implicated in the oncogenic process via regulation of transcriptional factors and of tumour growth, angiogenesis, and cell differentiation and survival (Tan & Coussens, 2007).

(ii) Experimental systems

Several studies investigated the effect of exposure to trichloroethylene by inhalation on pulmonary defences as measured by the degree of susceptibility to respiratory bacterial infections. Aranyi *et al.* (1986) exposed female CD-1 mice to trichloroethylene at various concentrations (up to 50 ppm) as a single dose (3 hours) or as repeated doses (5 days, 3 hours per day) by inhalation. Susceptibility to *Streptococcus zooepidemicus* aerosol infection and pulmonary

bactericidal activity to inhaled *Klebsiella pneumoniae* were evaluated. Single 3-hour exposures to trichloroethylene at 10 ppm or more resulted in significant increases in mortality after infection. Pulmonary bactericidal activity was significantly decreased after a single exposure at 10 ppm. A similar experimental design was used by Selgrade & Gilmour (2010). Female CD-1 mice were exposed by inhalation to filtered air (control) or trichloroethylene (5 to 200 ppm) for 3 hours. Immediately after exposure to trichloroethylene, mice were challenged with an aerosol of *Streptococcus zooepidemicus* and monitored for clearance of bacteria from the lung and for mortality. In separate experiments, exposed mice were injected intratracheally with viable bacteria and phagocytic function was evaluated in macrophages obtained from lung washes 30 minutes later. Mortality due to infection was significantly increased with exposure to trichloroethylene at concentrations of 50 ppm and higher. Significant differences in clearance of bacteria from the lung were noted in mice exposed to trichloroethylene at concentrations greater than 50 ppm, and differences in alveolar macrophage phagocytic index were noted at concentrations greater than 100 ppm.

Humoral immunity end-points were assessed in studies in animals exposed to trichloroethylene in drinking-water. Groups of female and male mice were exposed to trichloroethylene at a concentration of 0.1, 1.0, 2,5, and 5 mg/mL for up to 6 months. The immunological parameters assessed were humoral immunity, cell-mediated immunity, lymphocyte responsiveness, bone-marrow function, and macrophage function. Females were more affected than males by exposure to trichloroethylene, particularly after 4 months. In females, cell-mediated immunity and bone-marrow stem-cell colonization were inhibited by trichloroethylene at all concentrations, while humoral immunity was inhibited only at the highest concentrations. The males were relatively unaffected after exposure for 4 or 6 months.

In a study by Peden-Adams *et al.* (2006), $B6C3F_1$ mice were given drinking-water containing trichloroethylene (0, 1400, 14 000 ppb) from day 0 of gestation until age 8 weeks. Decreased sheep-erythrocyte-specific production of IgM (plaque-forming cell response) was noted in male offspring at age 3 or 8 weeks and at both concentrations. Plaque-forming cell responses in female offspring were suppressed by treatment with trichloroethylene at 14 000 ppb at both ages assessed and at 1400 ppb at 8 weeks. Splenic numbers of B220 cells were only decreased in pups aged 3 weeks exposed to trichloroethylene at 14 000 ppb. The most pronounced alteration in T-cell subpopulations was increases in all thymic T-cell types ($CD4^+$, $CD8^+$, $CD4^+/CD8^+$, and $CD4^-/CD8^-$) in mice aged 8 weeks. Delayed-type hypersensitivity (DTH) was increased in females at both concentrations of trichloroethylene and in males at the higher dose only.

Blossom & Doss (2007) studied central and peripheral immune function in autoimmune disease-prone $MRL^{+/+}$ mice given occupationally relevant doses of trichloroethylene (0, 0.5, or 2.5 mg/mL in drinking-water) throughout development until adulthood (i.e. age 7–8 weeks). Decreased spleen cellularity and reduced numbers of $CD4^+$, $CD8^+$, and $B220^+$ lymphocyte subpopulations were observed in the post-weaning offspring. Thymocyte development was altered by exposure to trichloroethylene, as shown by significant alterations in the proportions of double-negative ($CD4^-/CD8^-$) subpopulations and inhibition of apoptosis in immature thymocytes *in vitro*. Trichloroethylene was also shown to induce a dose-dependent increase in IFN-γ production by $CD4^+$ and $CD8^+$ T-lymphocytes in peripheral blood by age 4–5 weeks, although these effects were no longer observed at age 7–8 weeks. Serum anti-histone autoantibodies and total IgG_{2a} were significantly increased in

trichloroethylene-exposed offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys.

In a follow-up study, Blossom *et al.* (2008) exposed MRL$^{+/+}$ mice to trichloroethylene (0.1 mg/mL in drinking-water) from mating until postnatal day 42. Offspring were evaluated at various time-points. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at postnatal day 20 (sexes combined). Treatment with trichloroethylene also appeared to promote T-cell differentiation and maturation at postnatal day 42, and evaluation of cultured thymocytes *ex vivo* indicated increased generation of reactive oxygen species at postnatal day 20. Evaluation of peripheral blood indicated that splenic CD4$^+$ T-cells from trichloroethylene-exposed mice at postnatal day 42 produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes.

Peden-Adams *et al.* (2008) investigated lifetime exposure to trichloroethylene (up to 14 000 ppb in drinking-water) in MRL$^{+/+}$ mice from day 0 of gestation until age 12 months. Splenic CD4$^-$/CD8$^-$ cells were altered in female mice (but not males) at 1400 ppb only. Splenic T-cell populations, numbers of B220$^+$ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8$^+$, CD4$^-$/CD8$^-$, and CD4$^+$) were significantly decreased in male but not female mice after exposure to trichloroethylene at 14 000 ppb, and CD4$^+$/CD8$^+$ cells were significantly reduced in males by treatment with trichloroethylene. Autoantibody levels (anti-dsDNA and anti-glomerular antigen) were not increased in the offspring over the course of the study, indicating that trichloroethylene did not contribute to the development of autoimmune disease markers after developmental exposures that continued into adult life.

(b) Autoimmune disease and allergy

(i) Humans

Several epidemiological studies have evaluated the association between exposure to trichloroethylene and autoimmune disease (Nietert *et al.*, 1998; Lacey *et al.*, 1999; Diot *et al.*, 2002; Garabrant *et al.*, 2003; Beaudreuil *et al.*, 2005; Table 4.7). Exposure to trichloroethylene has been associated with generalized skin disorders with accompanying hepatitis that resemble drug hypersensitivities, as previously reviewed in Section 4.3.3 (Kamijima *et al.*, 2007). The clinical manifestations of this disorder generally involve the presence of rash on the extremities, neck, trunk, or face and potentially fever that occurs within 2 weeks to 2 months after first occupational exposure (Kamijima *et al.*, 2007). The disorders have been classified into four groups, including exfoliative dermatitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis, depending on the specific skin manifestation. While the incidence of these disorders has been estimated to be between 1–13% in trichloroethylene-exposed workers, being most common in Asia, between 9–13% of cases have been reported to be fatal in a review of case series (Kamijima *et al.*, 2007). A cross-sectional study in China included 19 hospitalized patients with these disorders who worked with trichloroethylene in factories, three to six healthy workers from each factory who performed job duties similar to those of the hospitalized patients, and two control workers from each factory not exposed to trichloroethylene (Kamijima *et al.*, 2008). Levels of trichloroethylene metabolites were measured in the patients, and exposure assessments at the factories included both personal and area monitoring for trichloroethylene and other volatile organic compounds (VOCs). This study provided evidence that the skin hypersensitivity disorders were attributable to trichloroethylene, given the absence of common impurities in the factories, and suggested based on the exposure assessments

that urinary trichloroacetate concentrations of less than 50 mg/L may be needed to reduce the risk of these disorders (Kamijima *et al.*, 2008).

A case–control study conducted in France evaluated the risk of systemic sclerosis in 80 case patients and 160 controls matched by age, sex, and smoking habits (Diot *et al.*, 2002). The study assessed exposure to organic solvents and other occupational exposures, and an expert review panel was used to semiquantitatively assess exposures in cases and controls. This assessment involved the use of an exposure score that considered the probability, intensity, frequency, and duration of exposure. A significantly elevated risk of systemic sclerosis was observed for ever exposure to trichloroethylene as well as for high cumulative exposure, and stratified analyses by sex suggested a higher risk in males than females (Diot *et al.*, 2002; Table 4.7).

A higher risk of scleroderma in men was similarly reported in a case–control study of 178 case patients and 200 controls conducted in the USA, while there was no elevated risk in women (Nietert *et al.*, 1998). The exposure assessment for trichloroethylene and other organic solvents was conducted using a job exposure matrix and semiquantitative exposure scores were assigned based on the probability and intensity of exposure. The exposure intensity and probability were each defined as none, low, medium, or high. Risk of systemic sclerosis associated with exposure to trichloroethylene was evaluated according to the cumulative and maximum exposure intensities and the maximum probability of exposure, which was the highest probability score of exposure for all of a given subject's jobs (Nietert *et al.*, 1998). An increased risk of systemic sclerosis was reported for each of these trichloroethylene exposure metrics (Table 4.7; Nietert *et al.*, 1998). Sensitivity analyses taking into account geographic residence suggested similar results (Nietert *et al.*, 1998).

A case–control study in the USA evaluated the risk of systemic sclerosis in relation to trichloroethylene exposure only in women (Garabrant *et al.*, 2003). The study included 660 cases of scleroderma (8 self-reported exposed trichloroethylene cases) and 2227 frequency matched controls. Self-reported exposures that were not considered plausible or were judged to be "trivial" with respect to frequency, intensity, or duration were not classified as exposures by the expert review (Garabrant *et al.*, 2003). Risk estimates for scleroderma were similar for self-reported and expert confirmed trichloroethylene exposure, and were non-significantly elevated for both exposure metrics (Table 4.7).

An evaluation of community exposure to chlorinated solvents was conducted among residents of Tucson, Arizona, where ground water contamination of the Santa Cruz River aquifer resulted from percolation of metal-cleaning products (Kilburn & Warshaw, 1992). This study included 345 residents of Tucson who agreed to provide blood samples and a regional comparison group consisting of 158 residents of Phoenix. Levels of antinuclear antibodies measured by fluorescence (FANA) and the prevalence of symptoms related to systemic lupus erythematosus were compared between the Tucson residents and reference subjects in Phoenix. Residents of Tucson reported higher frequencies of all examined symptoms, with significant differences reported for arthralgia, Raynards's phenomenon, malar rash, skin lesions, seizure or convulsion (Kilburn & Warshaw, 1992). Furthermore, exposed subjects had increased frequencies of having three and four or more symptoms and an increased prevalence of FANA titre levels consistent with an autoimmune response. However, the Tucson residents were exposed to multiple chemicals and therefore the effects of trichloroethylene specifically could not be evaluated.

There were few data concerning the risk of other autoimmune conditions associated with exposure to trichloroethylene, other than a small case–control study in France, which found

no association between exposure to trichloroethylene and antineutrophil cytoplasmic autoantibodies (ANCA) (Beaudreuil *et al.*, 2005). Cases included hospital in-patients admitted between 1990 and 2000 who were ANCA-positive, and in-patient controls admitted between 2000 and 2001 were matched to cases by age (± 5 years) and sex. The exposure assessment included both qualitative and semiquantitative methods and exposures were assessed by a panel of experts blinded to the disease status of the patients. The associated odds ratio for ANCA-positivity was based on 11 cases exposed to trichloroethylene and was null (Table 4.7). One additional study has evaluated the risk of undifferentiated connective tissue disease (UCTD) in women in relation to self-reported solvent exposure, including trichloroethylene (Lacey *et al.*, 1999). All self-reported exposures were subsequently examined and confirmed by expert review. While exposure to trichloroethylene was not significantly associated with UCTD for either self-reported or expert confirmed trichloroethylene exposures (Table 4.7), these results were based on only one exposed case. Similar null findings were observed for dry-cleaning workers, including nine cases exposed to trichloroethylene.

One study using data from the National Health and Nutrition Examination Survey (NHANES) has evaluated the association between exposure to trichloroethylene and physician-diagnosed asthma and wheezing episodes during the previous 12 months (Arif & Shah, 2007). A subsample of the subjects participating in the NHANES survey were selected to undergo personal-exposure monitoring and subjects with non-missing data for VOCs were included in the study. The sample included a total of 550 subjects each of whom were monitored for 48–72 hours using 3M organic-vapour monitors for 10 VOCs, including trichloroethylene and tetrachloroethylene. The geometric mean exposure to trichloroethylene in the population was 0.03 μg/m^3 (95% CI, 0.02–0.04). Exposure to trichloroethylene was not significantly associated with physician-diagnosed asthma or with episodes of wheezing in the previous 12 months (Table 4.7). The cross-sectional design made it difficult to evaluate the temporal relationship between asthma and exposure to VOCs in this study, and furthermore the self-reported diagnosis of asthma could be subject to some misclassification in the absence of objective assessments of lung function (Arif & Shah, 2007).

Overall, while case–control studies of autoimmune disease have included a larger number of subjects compared with the considered cross-sectional studies of immune parameters, a relatively small number of trichloroethylene-exposed cases has been included and therefore the available evidence was based on small samples. Furthermore, the implications of the stronger trichloroethylene-related effect for autoimmune disease in men, particularly for scleroderma, are unclear, but may be attributed to the lower background risk of this condition in men, differences in prevalence of exposure to trichloroethylene, or genuine differences in susceptibility for trichloroethylene-induced toxicity (Cooper *et al.*, 2009) (see Section 4.3.3 and Section 4.4.3 in this *Monograph*). Moreover, differences in the magnitude of risk between men and women may partially be attributed to exposure misclassification, particularly in studies without quantitative exposure assessments and personal monitoring. While personal-exposure monitoring was generally not available in the studies of autoimmune disease, the exposure assessment was improved through the use of expert review. Nevertheless, these studies have provided some evidence for an elevated risk of autoimmunity associated with exposure to trichloroethylene, particularly for scleroderma.

(ii) Experimental systems

Skin hypersensitivity and immune-mediated hepatitis attributable to exposure to trichloroethylene were studied in guinea-pigs. Tang

et al. (2002) evaluated the contact allergenicity potential of trichloroethylene in FMMU strain. Oedema and erythema indicative of skin sensitization (and confirmed by histopathology) were observed. Sensitization rates were reported to be 71.4% for trichloroethylene, when compared with a reference positive-control response rate (i.e. 100% for 2,4-dinitrochlorobenzene). Trichloroethylene was judged to be a strong allergen.

In a second study, female FMMU guinea-pigs were given trichloroethylene as an intradermal injection at 0, 167, 500, 1500, or 4500 mg/kg bw, or as a dermal patch at 0 or 900 mg/kg bw, and killed 48 hours after treatment Tang *et al.* (2008). With regard to dermal hypersensitivity, treatment with trichloroethylene resulted in dermal erythema and oedema, and the sensitization rate was 66% (i.e. classified as a strong sensitizer). In addition, liver end-points associated with hypersensitive skin reaction were evaluated. At the intradermal dose of 1500 mg/kg bw, a significant increase ($P < 0.05$) in serum AST levels was observed. At 4500 mg/kg bw, significantly ($P < 0.01$) increased ALT and AST levels were reported, and total protein and globulin decreased significantly ($P < 0.05$). Histopathological examination of the liver revealed fatty degeneration, hepatic sinusoid dilation, and inflammatory cell infiltration. No changes were observed at the intradermal doses of ≤ 500 mg/kg bw, or the dermal patch dose of 900 mg/kg bw.

Peden-Adams *et al.* (2006) exposed $B6C3F_1$ mice to drinking-water containing trichloroethylene (0, 1400, 14 000 ppb) from day 0 of gestation to up to age 8 weeks and reported an increased delayed hypersensitivity response in female offspring aged 8 weeks at both concentrations and in males at the higher concentration of 14 000 ppb.

Seo *et al.* (2008) investigated trichloroethylene exposure-associated antigen-induced histamine release and inflammatory mediator production *in vivo* and *in vitro*. Male Wistar rats were injected with trichloroethylene (0.1, 1 and 10 mg/kg bw) intraperitoneally, together with 0.1 mL of anti-dinitrophenol (DNP) monoclonal IgE antibody (anti-DNP IgE) diluted to 1:1000 or 1:4000, injected subcutaneously into both abdominal sides. After 48 hours, 1 mL of antigen (DNP-conjugated bovine serum albumin B5A)-Evans blue solution was injected into the tail vein. The rats were killed after 30 minutes, and passive cutaneous anaphylaxis was evaluated. A significant dose-dependent increase in cutaneous anaphylaxis was seen after exposure to trichloroethylene. In addition, a part of this study *in vitro* used non-purified rat peritoneal mast cells (NPMC) and rat basophilic leukaemia (RBL-2H3) cells that were sensitized with anti-DNP IgE antibody and then stimulated with DNP-BSA plus trichloroethylene. Trichloroethylene enhanced antigen-induced histamine release from NPMC and RBL-2H3 cells in a dose-related manner, and increased IL-4 and TNF-α production from the RBL-2H3 cells.

A series of studies evaluated the ability of trichloroethylene to promote autoimmune reactions in mouse strains that are prone to autoimmune disease. $MRL^{+/+}$ mice treated with trichloroethylene either by intraperitoneal injection (Khan *et al.*, 1995; Wang *et al.*, 2007b, 2008), or via drinking-water (Gilbert *et al.*, 1999; Griffin *et al.*, 2000a, b; Cai *et al.*, 2008). Both short-term and long-term exposure scenarios were evaluated and a range of trichloroethylene doses were used. All the studies have observed that exposure to trichloroethylene was associated with increases in antinuclear-, anti-ssDNA-, and anti-dsDNA antibodies. Increased activation of splenic $CD4^+$ cells, increased weights of spleen and increases in IgG levels were also commonly reported. Interestingly, Keil *et al.* (2009) showed trichloroethylene autoimmune-mediating effects in $B6C3F_1$ mice, which are not prone to spontaneous autoimmune disorders. Mice were exposed to trichloroethylene (0, 1, 1400 or 14 000 ppb) via drinking-water for 30 weeks.

During the exposure period, serum levels of total IgG, and autoantibodies (anti-ssDNA, -dsDNA, and -glomerular antigen) were monitored. Trichloroethylene did not alter NK cell activity, or T- and B-cell proliferation. Numbers of activated T-cells ($CD4^+/CD44^+$) were increased in the $B6C3F_1$ mice. Serum levels of autoantibodies to dsDNA and ssDNA were also increased.

5. Summary of Data Reported

5.1 Exposure data

Trichloroethylene, a chlorinated solvent, has been produced commercially since the 1920s by chlorination of ethylene or acetylene. In the 1930s, it was introduced in the dry-cleaning industry, but was replaced by tetrachloroethylene in the 1950s. Trichloroethylene has had numerous other uses, including as an anaesthetic and in veterinary medicine. Its use as a degreasing agent began in the 1930s, reached its peak in 1990s, and declined thereafter. Currently, trichloroethylene is still used as a spot remover in dry cleaning, but the main use of trichloroethylene is as feedstock to produce chlorinated chemicals. Trichloroethylene has been found in both outdoor and indoor air, water, soil, food, and animal tissues; exposure from environmental sources (including hazardous waste sites and contaminated water) is common throughout the United States and elsewhere in the world. Exposure in humans occurs mainly by inhalation. The most heavily occupationally exposed people are those involved in degreasing of metals and other materials. In Europe and North America, exposure levels have declined by at least one order of magnitude since the 1940s, and the number of exposed workers has also declined.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological data, the Working Group recognized positive associations between trichloroethylene and cancer of the kidney, non-Hodgkin lymphoma, and cancer of the liver. The Working Group concluded that the evidence for cancer of the kidney was stronger than that for non-Hodgkin lymphoma or cancer of the liver.

5.2.1 Cancer of the kidney

The largest body of evidence regarding the carcinogenicity of trichloroethylene comes from studies of cancer of the kidney. In the previous evaluation by IARC (Volume 63), information regarding carcinogenicity in the kidney came from a small cohort of workers in a cardboard-manufacturing factory in Germany and two Nordic cohorts monitored for exposure to trichloroethylene using biological measurements in urine, which gave conflicting results. Since that time, studies of several cohorts of aircraft and aerospace workers in the USA and seven case–control studies in several countries have reported data relevant to the association of cancer of the kidney and trichloroethylene. In general, the case–control studies adjusted for smoking and other potential confounders, but such adjustments seldom had a notable impact on the risk estimates. Although these adjustments were typically not possible in cohort studies, confounding by smoking was unlikely as relative risks for cancer of the lung were not elevated in most cohorts or in a meta-analysis.

A significant amount of new evidence had become available from cohort studies of aircraft and aerospace workers in the USA and a study of workers employed in industries using trichloroethylene in Denmark. Some of these cohort studies reported modestly elevated relative risks for cancer of the kidney, with an indication of an exposure–response relationship in one study.

The other important group of cohort studies comprised biologically monitored workers from three Nordic countries. These studies showed little evidence of increased risk of cancer of the kidney. However, the Working Group noted that the cohorts included workers recruited at different points in time from diverse industries with a broad range of exposures and only a small number of measurements per worker, on average. The studies were also relatively small and may have had limited ability to detect a modest increase in risk. A small elevation in the risk of cancer of the lung was also noted in the Danish study.

Case–control studies provide stronger and more consistent evidence than cohort studies of a positive association between kidney cancer and exposure to trichloroethylene. The most detailed exposure assessments were carried out for two studies in France and eastern Europe. Both studies evaluated confounding for several risk factors for cancer of the kidney, and both provided evidence for an exposure–response relationship. The study in France was conducted in an area with a high prevalence of occupational exposure to trichloroethylene and assessed the potentially confounding effects of exposure to cutting oils. These analyses found a non-statistically significant odds ratio of 1.62 (95% CI, 0.76–3.44) for the association with trichloroethylene in workers not exposed to cutting oils, and a statistically significant odds ratio of 2.70 (95% CI, 1.02–7.17) for workers in the highest category of exposure to trichloroethylene who were also exposed to cutting oils. The study in eastern Europe was larger than the study in France, but the prevalence of exposure to trichloroethylene was lower. The odds ratio for any exposure to trichloroethylene was 1.63 (95% CI, 1.04–2.54), and in the highest category of exposure intensity it was 2.34 (95% CI, 1.05–2.51).

Two recent, independently conducted meta-analyses based on a largely similar set of case–control and cohort studies of cancer of the kidney reported statistically significant meta-relative risks (meta-RR) for cancer of the kidney and exposure to trichloroethylene of 1.3 and 1.4. One meta-analysis reported a higher meta-RR of 1.6 (95% CI, 1.3–2.0) for groups with a higher exposure. Neither analysis found significant heterogeneity between studies.

5.2.2 Non-Hodgkin lymphoma

Information on non-Hodgkin lymphoma was available from eight independent cohort studies and eight case–control studies. Cohort studies of aircraft and aerospace workers in the USA reported modestly elevated relative risks for non-Hodgkin lymphoma. The cohort studies of biologically monitored workers in the Nordic countries show evidence of modestly increased risk for non-Hodgkin lymphoma, in contrast to the findings for cancer of the kidney.

Interpretation of the results from case–control studies on non-Hodgkin lymphoma was complicated by the variety of systems used to classify the lymphomas. Modest positive associations of non-Hodgkin lymphoma with exposure to trichloroethylene were observed in several case–control studies, but the results were inconsistent overall. There were some indications of an exposure–response relationship, but these were also inconsistent and generally not statistically significant.

A meta-analysis of cohort and case–control studies of non-Hodgkin lymphoma reported statistically significant meta-RRs of 1.2 (95% CI, 1.1–1.4) for non-Hodgkin lymphoma and any exposure to trichloroethylene and 1.4 (95% CI, 1.1–1.8) for higher exposure. The meta-RR was 1.33 (95% CI, 1.13–1.58) for cohort studies and 1.11 (95% CI, 0.89–1.38) for case–control studies and heterogeneity between studies was found. There was also some indication of publication bias. [The Working Group emphasized the results of the meta-analysis in its evaluation and noted

that the evidence from case–control studies was weaker than that from cohort studies.]

5.2.3 Cancer of the liver

Information about the association of cancer of the liver with exposure to trichloroethylene was available from one case–control study and nine cohort studies. Although some positive associations were observed in cohort studies, the results were somewhat inconsistent. The cohort studies were unable to adjust for other risk factors, such as consumption of alcoholic beverages. The only case–control study available had only one exposed case and was considered uninformative. A meta-analysis reported similar meta-RRs for overall exposure (meta-RR, 1.29; 95% CI, 1.07–1.56) and for higher exposure (meta-RR, 1.28; 95% CI, 0.93–1.77).

5.2.4 Other sites

Statistically significant excess risks of cancers of the lung, cervix and oesophagus were also observed in isolated studies, but these observations were considered insufficient to make an evaluation.

5.3 Animal carcinogenicity data

In four studies in $B6C3F_1$ mice, treatment with trichloroethylene by gavage increased the incidence of hepatocellular adenoma and hepatocellular carcinoma in males and/or females. In one of these studies, there was also an increase in the incidence of Harderian gland adenoma in males. In one study in Swiss mice treated by inhalation, there was an increase in the incidence of malignant hepatoma in males.

In one study in female mice treated by inhalation, there was an increase in the incidence of lung adenocarcinoma. In two other studies of exposure by inhalation, there was an increase in the incidence of pulmonary tumours (mainly adenomas) in male Swiss mice and female $B6C3F_1$ mice. In one study in $B6C3F_1$ mice treated by gavage, there was also an increase in the incidence of bronchioloalveolar adenoma in females. Increased incidences of malignant lymphoma were reported in two strains of mice in one study of exposure by gavage and one study of exposure by inhalation.

In three gavage studies and one inhalation study in rats, increases in the incidence of benign interstitial cell tumours of the testis were reported in trichloroethylene-treated rats of four different strains, along with the occurrence of a few rare malignant interstitial cell tumours in two of these studies.

In female rats, an increasing trend in the incidence of leukaemia (of the monocytic type or not otherwise specified) has been reported in one study in rats treated by gavage, and an increased incidence of lymphoma was observed in one study in rats treated by inhalation. The incidence of subcutaneous tissue sarcoma was also increased in male rats treated with trichloroethylene by gavage.

In three studies in three different strains of rats, treatment with trichloroethylene by gavage increased the incidences of adenoma or carcinoma (combined) of the kidney in males or females, while incidence of kidney carcinoma also increased in one of these studies. Overall, nine studies of exposure by gavage or inhalation in several different strains reported rare kidney adenoma or carcinoma in at least one or more rats treated with trichloroethylene per study.

After adjustment for survival, an increase in the incidence of malignant peritoneal mesothelioma was observed in male rats in one gavage study, and an increase in the incidence of adrenal cortex adenoma was observed in females in another gavage study. Although not statistically significant, rare liver tumours (i.e. haemangiosarcoma, cholangioma, cholangiocarcinoma, and hepatocellular carcinoma) were reported in several trichloroethylene-treated groups of three

different strains of rats, and some rare bronchioloalveolar tumours were also reported in five groups of treated rats of three different strains.

5.4 Mechanistic and other relevant data

A comprehensive body of literature exists to characterize the absorption, distribution, metabolism and excretion of trichloroethylene in humans and in experimental animals; it is clear that qualitative similarities are evident between humans and rodents. Two major metabolic pathways of trichloroethylene have been characterized in humans and laboratory animals. The major pathway is cytochrome P450 (CYP)-mediated oxidation, resulting in formation of a variety of short- and long-lived metabolites. Subsequent processing of oxidative metabolites involves alcohol and aldehyde dehydrogenases and glucuronidation. In all species, trichloroacetic acid and trichloroethanol/trichloroethanol glucuronide are measured in vastly larger amounts than other oxidative metabolites. Evidence exists supporting quantitative differences between species in the extent of oxidative metabolism of trichloroethylene at higher exposures, but at lower exposures oxidation is limited by hepatic blood flow. Glutathione (GSH) conjugation is another important metabolic pathway resulting in formation of short-lived and reactive metabolites. The initial conjugation reactions primarily occur in the liver to form *S*-(1,2-dichlorovinyl) glutathione (DCVG). Subsequent processing of DCVG occurs primarily in the kidney. In humans and rodents, urinary excretion of stable end products is orders of magnitude greater for the oxidative pathway than the GSH-conjugation pathway. However, this is not an accurate indication of the overall flux through each pathway, because it does not account for the formation of reactive and chemically unstable metabolites by the GSH-conjugation pathway.

Data from studies in humans have suggested possible mutagenicity of trichloroethylene leading to *VHL* gene mutations in renal cell carcinoma, but only a limited number of studies have reported an association. Carefully controlled studies evaluating trichloroethylene alone found it to be incapable of inducing gene mutations in most standard assays for bacterial mutagenesis. Therefore, it appears unlikely that trichloroethylene is a direct-acting mutagen, although trichloroethylene has shown the potential to affect DNA and chromosomal structure. There is strong evidence that the GSH-conjugation metabolites of trichloroethylene, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), and to a lesser degree, DCVG and *N*-acetyl-*S*-(1,2-dichloroWvinyl)-L-cysteine (NAcDCVC), are genotoxic on the basis of consistent results in several studies. For oxidative metabolites of trichloroethylene, the Working Group concluded that weak to moderate evidence was available to suggest that dichloroacetic acid may cause genotoxicity (see *Monograph* on Dichloroacetic Acid in this Volume for details); that evidence suggested that trichloroacetic acid is not genotoxic (see *Monograph* on Trichloroacetic Acid in this Volume for details); and that strong evidence was available to suggest that chloral hydrate may cause genotoxicity (see *Monograph* on Chloral and Chloral Hydrate in this Volume for details). The data on genotoxicity of trichloroethanol were limited. Overall, there was strong evidence to conclude that, after metabolism, trichloroethylene can be genotoxic, particularly in the kidney where metabolism in situ occurs.

Trichloroethylene has been shown to be associated with adverse health outcomes in the kidney, liver, and lung, and in the immune, central nervous, and reproductive systems. The evidence for kidney as a target tissue for trichloroethylene, from cancer and toxicity findings in humans and experimental animals was strong. The data supporting the non-genotoxic mechanisms of kidney carcinogenesis were limited.

However, strong evidence of genotoxicity of DCVC, the metabolite of trichloroethylene in the kidney, supported an overall conclusion that the evidence of mechanisms of carcinogenesis in kidney is strong. There was strong evidence for liver as a target tissue for trichloroethylene from cancer and toxicity findings in experimental animals. The evidence for non-genotoxic and/or genotoxic mechanisms of liver carcinogenesis was moderate. The available data suggested that multiple non-genotoxic mechanisms of carcinogenesis exist, and that there is the potential for genotoxic mechanisms from trichloroethylene metabolites dichloroacetic acid and chloral hydrate. The evidence for the immune system as a target tissue for trichloroethylene from findings of a generalized hypersensitivity syndrome and of alterations of immune response in humans and experimental animals was strong. Evidence from studies in humans and experimental animals identifying active metabolites or the mechanisms for cancers of the immune system was weak, being limited to studies of immunological and haematological toxicity in humans and experimental animals. The evidence for the lung as a target tissue for trichloroethylene, from cancer and toxicity findings in experimental animals, was moderate. The data supporting the mechanisms of carcinogenesis in the lung were weak. The evidence for the nervous system as a target tissue for trichloroethylene on the basis of a variety of neurobehavioural effects in studies in humans and experimental animals was strong. The relevance of these effects to the potential cancer hazard of trichloroethylene in the nervous system is unknown. The data regarding the mechanism of carcinogenesis of trichloroethylene in the central nervous system were inconclusive. Trichloroethylene has been shown to adversely affect the male and female reproductive systems. The evidence for the male reproductive system as a target tissue for trichloroethylene was strong, on the basis of studies of toxicity in humans and experimental animals and studies of cancer in rats. The overall data supporting the mechanisms of carcinogenesis of trichloroethylene in the testes were weak, with limited data from humans and experimental animals available to support a mechanism involving hormonal disruption for trichloroethylene-induced testicular tumours. The overall support for an association between exposure to trichloroethylene and reproductive toxicity in females was weak.

The carcinogenicity and toxicity of trichloroethylene, particularly in the liver and kidney, are associated with its metabolism. Inter-individual differences in the formation of trichloroethylene metabolites that are thought to be responsible for toxic and carcinogenic effects of trichloroethylene in the kidney and liver exist in humans and rodents. Susceptibility to the adverse health effects of trichloroethylene may be influenced by genetics, sex, life stage and other conditions that influence the extent and nature of its metabolism. Polymorphisms in genes involved in oxidative metabolism (e.g. *CYP2E1*, *ADH*, *ALDH*) and glutathione conjugation (e.g. GSTs) have been studied in connection with susceptibility to toxicity and carcinogenicity caused by trichloroethylene. Polymorphisms in genes for plasma-membrane transporters (e.g. *OAT1* and *OAT3*) may also influence rates or extent of cellular accumulation of key metabolites. With respect to life-stage susceptibility, data were available to support conclusions concerning differences in exposure (e.g. transplacental transfer or exposure through breast milk in early life stages) or life-stage-specific differences in toxicokinetics. Lifestyle factors (e.g. consumption of alcoholic beverages) may also affect the metabolism of trichloroethylene, while nutrition or obesity may affect internal concentrations of trichloroethylene and its metabolites.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of trichloroethylene. Trichloroethylene causes cancer of the kidney. A positive association has been observed between exposure to trichloroethylene and non-Hodgkin lymphoma and liver cancer.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Trichloroethylene is *carcinogenic to humans (Group 1).*

6.4 Rationale

The Working Group was unanimous in its conclusion that trichloroethylene is a Group 1 carcinogen.

The majority of the Working Group concluded that the epidemiological data were sufficient; however, a minority had concerns because most of the positive evidence came from case–control studies, while the data from cohort studies were weaker.

In reaching unanimous agreement, the Working Group took into consideration the following supporting evidence:

- The absorption, distribution, metabolism and excretion of trichloroethylene are well characterized in experimental animals and humans.
- In experimental animals and humans, oxidative metabolism of trichloroethylene is catalysed by cytochrome P450 enzymes and GSH conjugation of trichloroethylene is catalysed by GST enzymes.
- The formation of reactive metabolites of trichloroethylene in the kidney from processing of GSH-conjugation metabolites in situ has been observed in experimental animals and in human kidney cells.
- The reactive GSH-conjugation metabolites of trichloroethylene are genotoxic on the basis of consistent results in several available test systems.

Consistent with the importance of the GSH-conjugation metabolic pathway for kidney carcinogenesis, one study demonstrated a statistically significant association among trichloroethylene-exposed people with at least one intact *GSTT1* allele, but not among those with two deleted alleles.

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TETRACHLOROETHYLENE

Tetrachloroethylene was considered by previous IARC Working Groups in 1979, 1987, and 1995 (IARC, 1979, 1987, 1995). New data have since become available and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

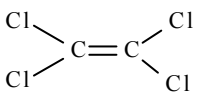
Chem. Abstr. Serv. Reg. No.: 127-18-4

Chem. Abstr. Name: Tetrachloroethene

IUPAC Systematic Name: Tetrachloroethylene

Synonyms: Ethylene tetrachloride; PCE; 'per'; PER; PERC; perchlorethylene; perchloroethene; perchloroethylene; tetrachlorethylene; 1,1,2,2-tetrachloroethene; 1,1,2,2-tetrachloroethylene

1.1.2 Structural and molecular formulae and relative molecular mass



C_2Cl_4

Relative molecular mass: 165.83

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless, nonflammable liquid; ethereal odour (O'Neil *et al.*, 2006)

Boiling-point: 121 °C (O'Neil *et al.*, 2006)

Melting-point: –22 °C (O'Neil *et al.*, 2006)

Density: 1.6230 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism [5422]; grating [469]), ultraviolet [1485] and mass [1053] spectral data have been reported (Sadtler Research Laboratories, 1980; Weast & Astle, 1985).

Solubility: Slightly soluble (0.206 g/L at 25 °C; HSDB, 2012); miscible with alcohol, ether, chloroform, benzene (O'Neil *et al.*, 2006); soluble in ethanol, diethyl ether and benzene (Haynes, 2012)

Volatility: Vapour pressure, 1 kPa at 10 °C (Haynes, 2012)

Stability: Photo-oxidized in air with sunlight (half-time, about 12 hours), giving phosgene and trichloroacetyl chloride (EPA, 1982; Hickman, 1993)

Reactivity: Incompatible with chemically active metals such as barium, lithium and beryllium, and with sodium hydroxide, potash and strong oxidizers (NIOSH, 1994)

Octanol/water partition coefficient (P): log P, 3.40 (Hansch *et al.*, 1995)

Conversion factor: mg/m^3 = 6.78 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa).

1.1.4 Technical products and impurities

Tetrachloroethylene is available commercially in several grades, including a vapour degreasing grade, a dry-cleaning grade, an industrial grade for use in formulations, a high-purity low-residue grade, a spectrophotometric grade, and a grade specifically formulated for use as a transformer fluid (IARC, 1995; ATSDR, 1997a). It typically has a purity of 95% or more for dry-cleaning and industrial grades, 99% or more for more refined grades, and 99.995% for isomerization and fluorocarbon grades. The various grades differ in the amount and type of stabilizers added to prevent decomposition (ATSDR, 1997a).

Trade names for tetrachloroethylene include Ankilostin, Antisol 1, Didakene, Dow-per, Dilatin PT, Fedal-Un, Nema, Perawin, Perchlor, Perclene, Percosolv, Perklone, PerSec, Tetlen, Tetracap, Tetraleno, Tetralex, Tetravec, Tetroguer and Tetropil (IARC, 1995; Doherty, 2000a; EPA, 2013).

1.1.5 Analysis

Several methods for the analysis of tetrachloroethylene in air, solids, liquids, water, and food were reviewed by WHO (1984), EPA (1985), Greenberg *et al.* (1992) and Demeestere *et al.* (2007). Selected methods for the analysis of tetrachloroethylene in various matrices are presented in Table 1.1.

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Tetrachloroethylene was first prepared in 1821 by Michael Faraday using thermal decomposition of hexachloroethane (Doherty, 2000a). For many years, the most important process for producing tetrachloroethylene was from acetylene via trichloroethylene, but because of the increasing price of acetylene feedstock in the 1970s, newer processes involving direct chlorination or oxychlorination of other hydrocarbons were introduced (ATSDR, 1997a).

(b) Production volume

Production of tetrachloroethylene in Japan, western Europe and the USA reached its peak in the 1980s (Linak *et al.*, 1992). In 1992, annual capacity was 10 000 tonnes in Austria, 30 000 tonnes in Belgium, 62 000 tonnes in France, 100 000 tonnes each in Germany and in Italy, 21 000 tonnes in Spain, 130 000 tonnes in the United Kingdom of Great Britain and Northern Ireland, 96 000 tonnes in Japan and 223 000 tonnes in the USA (Linak *et al.*, 1992). In 2007, the USA was the largest consumer of tetrachloroethylene (43% of demand), followed by western Europe (19%), China (10%) and Japan (9%) (Glauser & Ishikawa, 2008).

1.2.2 Use

In the 1950s, about 80% of tetrachloroethylene was used in dry-cleaning, and 15% in metal-cleaning and vapour degreasing (Doherty, 2000a). By the 1980s, the pattern was changing as a result of the establishment of environmental regulations and improved technology, and about 50% of tetrachloroethylene was used for dry-cleaning, 28% for chemical intermediates (mostly for production of fluorocarbons), and 10–15% for metal cleaning and degreasing

Table 1.1 Methods for the analysis of tetrachloroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Air collected in specially-prepared canister; desorb	GC/MS	NR	EPA (1999a)
		GC/ECD	NR	
		GC/FID	NR	
		GC/PID	NR	
	Analyte collected on sorbent tube; thermally desorb to GC from cold trap	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	0.05 µg/L	EPA (1988, 1995a)
		GC/HECD	0.04 µg/L	
	Purge with inert gas and trap; desorb to GC	GC/PID	0.01 µg/L	EPA (1988)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.05 µg/L	EPA (1988, 1995b)
	Extract with methyl-*t*-butyl ether or pentane	GC/ECD	0.002 µg/L	EPA (1995c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	0.05 µg/L	EPA (1996a)
		GC/HECD	0.04 µg/L	
	Purge with inert gas and trap; and various other methods	GC/MS	5 µg/kg (soil/ sediment)	EPA (1996b)
			500 µg/kg (wastes)	
			5 µg/L (groundwater)	
Blood	Purge with inert gas and trap on Tenax	GC/MS	0.022 ppb	Ashley *et al.* (1992)

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

(Linak *et al.*, 1992; Doherty, 2000a). The relative proportions used for dry-cleaning and chemical production have continued to shift, with more than 50% being used as intermediates and 15% for dry-cleaning in the 1990s (ATSDR, 1997a). Currently the most common use of tetrachloroethylene is as a feedstock for producing fluorocarbons (Glauser & Ishikawa, 2008).

(a) Dry-cleaning industry

Since the 1950s, tetrachloroethylene has been the most commonly used dry-cleaning solvent (Doherty, 2000a). Tetrachloroethylene is ideally suited for dry-cleaning as it is nonflammable and a good degreaser that does not saturate the fabric fibres, thus avoiding swelling and shrinking of the fabric (Linak *et al.*, 1992; NICNAS, 2001).

By the 1960s, almost all dry-cleaning facilities in the USA used tetrachloroethylene, and this continued until the 1990s (Doherty, 2000a). In 2007, tetrachloroethylene was used by about 70% of dry-cleaners in the USA (State Coalition for Remediation of Drycleaners, 2009). It is currently used by two thirds of dry-cleaners in Denmark, and 90% of dry-cleaners in France (ECSA, 2012).

Technological changes in dry-cleaning machines are well documented for the USA (Earnest, 2002), Europe (von Grote *et al.*, 2003; Johansen *et al.*, 2005), and Australia (NICNAS, 2001). Before the 1960s, most machines were transfer types for which the clothes (which had been immersed in tetrachloroethylene) were moved manually from the washer to the dryer.

In the 1960s, dry-to-dry machines were invented that did not require manual transfer. In addition, the newer machines were equipped with carbon absorbers and did not vent into the atmosphere. Improvements in recycling dry-cleaning solvents in closed machines have meant that more than 95% of tetrachloroethylene is recovered in these modern machines. However, in practice, the replacement of the transfer machines took about two decades. In a study in New Jersey communities in 1984, dry-to-dry machines were present in 75% of the facilities included (Garetano & Gochfeld, 2000).

Alternatives for tetrachloroethylene have been developed, including 1-bromopropane, carbon dioxide, hydrocarbon solvents, and propylene glycol ether in response to restrictions on the use of tetrachloroethylene in the dry-cleaning industry (California EPA, 2008).

(b) Metal-degreasing in the automotive and metal industries

Tetrachloroethylene is used as a degreasing agent in vapour and liquid forms. It dissolves many organic compounds (including pitches and waxes) and inorganic compounds, and can be used to clean metal parts. Tetrachloroethylene is used as a solvent in aerosol products for cleaning tyres, brakes, engines, carburettors and wire; in 2004, such uses accounted for 12% of the total use of tetrachloroethylene in the USA (TURI, 2006). These aerosol automotive products may be used by the public, as well as by professionals.

Tetrachloroethylene has been used in cleaning products for electrical equipment; these products can be applied by spraying, brushing or dipping (NICNAS, 2001).

(c) Other industrial applications

(i) Chemical intermediates

Currently the most common use of tetrachloroethylene is as a feedstock for the production of chlorofluorocarbons and hydrofluorocarbons. Under the Montreal Protocol on Substances that Deplete the Ozone Layer of the 1990s, production of chlorofluorocarbons is being phased out by 2030 due to their contribution to ozone depletion (Doherty, 2000a).

(ii) Textile industry

In textile processing, tetrachloroethylene is used as a solvent to remove oils from fabrics after knitting and weaving operations, and as a carrier solvent for scouring, sizing and desizing, and for fabric finishes and water repellents. Tetrachloroethylene is able to dissolve fats, greases, waxes, and oils without harming natural or synthetic fibres (IARC, 1995).

(iii) Printing industry

Flexographic printing is a method that is similar to letterpress printing, but uses flexible plates. Tetrachloroethylene is used to clean unpolymerized coatings from the flexible film. The cleaning is performed in automated enclosed machinery (NICNAS, 2001). Tetrachloroethylene has also been used in printing inks (EPA, 2013).

(iv) Miscellaneous

There are several other uses of tetrachloroethylene (NICNAS, 2001), including for testing in the coal industry; as a source of chlorine in the catalytic reforming process in petroleum refineries; to clean prints and negatives of cinema film; in typewriter correction fluid; in carpet stain-removal products; and as an antihelminthic agent (O'Neil *et al.*, 2006).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Tetrachloroethylene has been reported in temperate, subtropical and tropical algae, and in one red microalga (Abrahamsson *et al.*, 1995).

1.3.2 Environmental occurrence

Tetrachloroethylene is a volatile organic compound that is widely distributed in the environment due to industrial emissions. Potential environmental exposure to tetrachloroethylene in air, rainwater, surface water, and drinking-water has been reviewed (ATSDR, 1997a). The partitioning tendency of tetrachloroethylene in the environment has been calculated as follows: air, 99.7%; water, 0.3%; soil, < 0.01%; sediment, < 0.01% (Boutonnet *et al.*, 1998).

(a) Air

Measurement data from remote North American sites indicate that background concentrations of tetrachloroethylene have decreased since 1995 by more than 5% per year (McCarthy *et al.*, 2006).

Table 1.2 presents some recent data on concentrations of tetrachloroethylene in air, measured worldwide in remote, rural, suburban, and urban sites, and in indoor air near dry-cleaning shops.

(b) Water

Tetrachloroethylene occurs at low concentrations in drinking-water supplies and frequently occurs as a contaminant of groundwater, owing to its widespread use and physical characteristics. Table 1.3 presents recent measurements of concentrations of tetrachloroethylene in surface waters, groundwater and drinking-water.

(c) Food

As a part of the Total Diet Study in the USA, 20 samples of 70 different foods purchased in supermarkets or restaurants were analysed for tetrachloroethylene during 1996–2000. Tetrachloroethylene was found at low concentrations (up to 102 µg/kg) in 29 out of 70 food items (Fleming-Jones & Smith, 2003).

In a survey of 35 samples of whole milk from Las Vegas, NV, USA, tetrachloroethylene was found at a mean concentration of 0.09 µg/L (range, < 0.01–0.39) (Hiatt & Pia, 2004).

The average concentration of tetrachloroethylene in 4 out of 17 samples of brown grease from food-preparation facility grease traps was 2771.1 µg/L (range, 31.5–8510 µg/L) (Ward, 2012).

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 by the United States National Institute for Occupational Safety and Health (NIOSH, 2013) estimated that about 688 000 workers in a wide range of industries, notably dry-cleaning, textiles, metals, automotive, printing and cleaning, were potentially exposed to tetrachloroethylene in the USA at that time. This estimate was based on a survey of products used in companies in 1981–1983 and did not involve actual measurements.

The European CAREX project estimated the number of exposed workers in 15 countries of the European Union (EU-15) to be approximately 820 000 in the early 1990s. Most exposures occurred in dry-cleaning shops (Kauppinen *et al.*, 2000). An update of CAREX for Italy by Mirabelli & Kauppinen (2005) showed a negligible increase in the number of workers exposed, from 102 500 to 106 300, for 1990–1993 and 2001, respectively (excluding jobs flagged as low-level or low confidence).

The number of exposed individuals in Germany in the dry-cleaning industry only was estimated to total almost 25 700 in 1975, decreasing considerably to about 5900 in 2001. This was mainly due to a reduction in the number of garments being dry-cleaned and, to a lesser extent, the replacement of tetrachloroethylene by nonchlorinated solvents. Merging of smaller shops into larger facilities also decreased the number of dry-cleaning machines by 77% (von Grote *et al.*, 2006).

Table 1.4 presents exposure to tetrachloroethylene in the dry-cleaning industry by country and

Table 1.2 Concentrations of tetrachloroethylene in air

Location	Concentration (μg/m³)		Comments	Reference
	Mean	Range		
Outdoor air				
Remote				
Antarctica	NR	0.032–0.075	Five remote sites	Zoccolillo *et al.* (2009)
Atlantic Ocean	NR	NR–30 ppt	Background tropospheric levels	Class & Ballschmiter (1986)
North America	0.022	0.015–0.029	Remote background concentration	McCarthy *et al.* (2006)
Urban and rural				
Canada	0.19	0.015–2.44	Near 74 homes	Zhu *et al.* (2005)
North Rhine – Westphalia, Germany	NR	0.03–7.33	Over four seasons at two sites	Begerow *et al.* (1996)
Italy	NR	0.109–0.719	Twelve rural sites	Zoccolillo *et al.* (2009)
	NR	0.461–4.314	Four urban & suburban sites	
Leipzig, Germany	0.01	NR	At seven sites	Gokhale *et al.* (2008)
Shizuoka Prefecture, Japan	0.11[a]	0.05–0.32	Near 25 homes	Ohura *et al.* (2006)
Hyogo Prefecture, Japan	NR	0.07–0.28	At six sites	Okada *et al.* (2012)
Tarragona, Spain	0.67	NR–1.0	Near large industrial complex	Ramírez *et al.* (2012)
Endicott, NY, USA	NR	0.1–24	Above contaminated soil	Forand *et al.* (2012)
Minneapolis, MN, USA	0.8	0.2–1.7	Near 284 households	Adgate *et al.* (2004)
Five cities, USA	NR	0.858–4.34	24-hour air concentration	Rappaport & Kupper (2004)
Dallas, TX, USA	[2.2]	[1.4–13]	Ambient air near gas wells	Rich (2011)
Minnesota, USA	0.44	< 0.21–25.08	25 sites in state (1991–1998)	Pratt *et al.* (2000)
Seattle, WA, USA	0.21	NR	Ambient air at six sites	Wu *et al.* (2011)
Indoor air				
Ottawa, ON, Canada	1.15	0.015–9.23	In 75 homes	Zhu *et al.* (2005)
France	1.3[b]	0.4–72	In 490 homes	Billionnet *et al.* (2011)
North Rhine-Westphalia, Germany	NR	0.10–50.04	Over four seasons at two sites	Begerow *et al.* (1996)
Leipzig, Germany	0.33	NR	In homes	Gokhale *et al.* (2008)
Shizuoka Prefecture, Japan	0.16[a]	0.06–0.34	In 25 homes	Ohura *et al.* (2006)
Minneapolis, MN, USA	3.5	0.5–5.2	In 284 homes	Adgate *et al.* (2004)
New Jersey, USA	NR	< 1.4–540	Urban and suburban homes	Weisel *et al.* (2008)
Near dry-cleaning shops				
France	NR	20–2900	Indoor air	Chiappini *et al.* (2009)
Mulheim, Germany	1360[b]	< 10–> 10 000[c]	Indoor air	Altmann *et al.* (1995)
New York, NY, USA	35	3–5000	Indoor air	McDermott *et al.* (2005)
New York, NY, USA	2150	1800–2400	Indoor air	Schreiber *et al.* (2002)
New York, NY, USA	340[a]	127–700	Indoor air	New York State Department of Health (2010)

[a] Geometric mean
[b] Median
[c] Read from graph
ppt, parts per trillion

Table 1.3 Concentrations of tetrachloroethylene in water

Country	Location	Concentration µg/L		Comments	Reference
		Mean	Range		
Ground and surface water					
China	Eastern China	NR	NR–35.6	Groundwater at five sites	Bi *et al.* (2012)
Croatia	Sašnak	16.28	9.08–21.35	Groundwater samples, 1995–1996	Vedrina-Dragojević & Dragojević (1997)
Europe	Southern North Sea	0.023	NR–0.280[a]	Ten locations, 1998–2000	Huybrechts *et al.* (2005)
Greece	Northern Greece	NR	< 0.02–0.19	Surface water	Kostopoulou *et al.* (2000)
Taiwan, China	Country-wide	NR	2–21	Groundwater near eight contaminated sites	Fan *et al.* (2009)
	Taoyuan City	520	0.1–5228	Groundwater near contaminated site	Lee *et al.* (2002)
USA	Bush River, MD	NR	30–90	Contaminated surface water near Aberdeen Proving Grounds	Burton *et al.* (2002)
	Minnesota	NR	1–120	Groundwater near hazardous waste disposal sites	Sabel & Clark (1984)
	Cape Cod, MA	NR	1.5–7750	Leaching into drinking-water from pipes	Janulewicz *et al.* (2008)
	Camp Lejune, NC	NR	NR–1580 ppb	Contaminated groundwater	Sonnenfeld *et al.* (2001)
	Country-wide	NR	0.02–4800	5000 groundwater samples, 1985–2002	Moran *et al.* (2007)
Drinking-water					
USA	Camp Lejune, NC	153	2–400	*Drinking-water*	NRC (2009)

NR, not reported; ppb, parts per billion

time period. Gold *et al.* (2008) reported average personal exposures of 59 ppm for dry-cleaning workers in the USA in 1936–2001. Lynge *et al.* (2011) provided insight into temporal trends in exposure to tetrachloroethylene in Nordic countries over a 60-year period. In the mid-1970s, personal exposures were reported as a median concentration of about 20 ppm, and this decreased to about 3 ppm in 2000. Stationary measurements indicated concentrations up to 100 ppm in the 1950s and 1960s (see Fig. 1.1). Recent studies in Egypt and the Islamic Republic of Iran showed that high exposures in dry-cleaning still occur, with concentrations of 100 ppm in air reported in both countries (Azimi Pirsaraei *et al.*, 2009; Emara *et al.*, 2010; Rastkari *et al.*, 2011).

Exposure to tetrachloroethylene in other industries and occupations (mainly degreasing, particularly in the metal and plastics industries) is summarized in Table 1.5, presented by industry and country. Gold *et al.* (2008) reported average concentrations for personally measured exposure among workers degreasing metal and plastics in the USA to be 95 ppm during 1944–2001. In these industries, exposure levels have decreased by two orders of magnitude over a 60-year period.

Concentrations of tetrachloroethylene in blood among workers in dry-cleaning and other industries are summarized in Table 1.6. For studies on biological monitoring of urine for trichloroacetic acid, a metabolite of tetrachloroethylene,

Table 1.4 Exposures to tetrachloroethylene in dry-cleaning shops

Country (year)	No. of plants	Job/task/industry	No. of samples	Air concentration				Reference
				Mean		Range		
				ppm	mg/m³	ppm	mg/m³	
Europe								
Belgium	6	Dry-cleaning	26 subjects (P) TWA	20.8	141	8.9–37.5	60–254	Lauwerys *et al.* (1983)
Finland (1982–85)	6	Dry-cleaning	10 (S) TWA	13	88	3–29	20–197	Rantala *et al.* (1992)
Finland	6 shops	Dry-cleaning operators shops		4.1	28	NR	NR	Räisänen *et al.* (2001)
	3 industrial	Operators, industrial		4.6	32	NR	NR	
		Pressers, shops		1.1	7.7	NR	NR	
		Presser, industrial		0.5	3.4	NR	NR	
		Customer-service shops	6 (P) TWA	0.1	0.8	NR	NR	
Europe; Nordic countries (1947–2001)		Dry-cleaning (total)	609 (S)	11.92		NR		Lynge *et al.* (2011)
			687 (P)	7.27		NR		
		Dry-cleaner	461 (P)	7.50		NR		
		Shop assistant	104 (P)	6.25		NR		
France	26	Dry-cleaning	> 100 per shop	NR	NR	0–100	0–678	Davezies *et al.* (1983)
France (1989–1990)	36	Dry-cleaning operator	5–10 per shop[a]	17.3	117.3	NR–100	NR–678	Anon (1991)
Germany		Dry-cleaning	19 workers		62 (end of wk)		16–672	Pannier *et al.* (1986)
			55 (P)		43 (following Monday)		NR	
Germany		Dry-cleaners	101 workers (P) TWA		205		NR	Seeber (1989)
Germany (1987, 1989)	15	Dry-cleaning	75 (S)		45% > 50		3.1–331	Gulyas & Hemmerling (1990)
					33% > 100		NR	
					9% > 200		NR	
Germany (1993–1994)	21	Dry-cleaning operator	100		7.4		< 0.02–27	Klein & Kurz (1994)
Italy	47	Dry-cleaning	143 workers	11.3	77	1–80.8	7–548	Missere *et al.* (1988)
Italy (1992–93)	28	Dry-cleaning	60 workers[b]					Aggazzotti *et al.* (1994)
			(P) TWA		NR		2.6–221.5	
			(S)		36		0.19–308	

Table 1.4 (continued)

Country (year)	No. of plants	Job/task/industry	No. of samples	Air concentration				Reference
				Mean		Range		
				ppm	mg/m³	ppm	mg/m³	
Italy	12	Dry-cleaning	(P) TWA					Gobba *et al.* (1997)
			Group A (19 workers)	4.35		0.21–23.4		
			Group B (14 workers)	0.66		0.01–6.2		
Italy	7	Dry-cleaning shops	26 workers		44.2		5.6–224.6	Gobba *et al.* (2003)
Netherlands (1976)	1	Dry-cleaning	48 (5 workers)	6.7	45	3.7–25.9	25–176	van der Tuin & Hoevers (1977a)
Netherlands (1977)	1	Dry-cleaning	86 (10 workers)	41.3	280	11–101	75–685	van der Tuin & Hoevers (1977b)
Netherlands (1978)	1	Dry-cleaning	80 (9 workers)	59.7	405	10.0–250	68–1695	van der Tuin (1979)
Netherlands	4	Dry-cleaning, industrial	82 workers		7.9 (extrapolated from biomonitoring values)		1–221	Verplanke *et al.* (1999)
Norway	1	Dry-cleaning shop	13 (P) long-term		6.7		4.2–11	Steinsvåg *et al.* (2007)
		Offshore	4 (P) short-term		69		28–177	
Switzerland	10	Dry-cleaning	49 workers (P) 1 wk	18.5	125			Boillat *et al.* (1986)
United Kingdom	90	Dry-cleaning shops	333 (P)	74% < 30	203			Shipman & Whim (1980)
				88% < 50	339			
				97% < 100	678			
	41	Dry-cleaning factories	160 (P)	53% < 30	203			
				76% < 50	339			
				93% < 100	678			
United Kingdom (1990–91)	81	Dry-cleaning operator	405 (P) TWA	22.5	153	0–360	0–2441	Edmondson & Palin (1993)

Table 1.4 (continued)

Country (year)	No. of plants	Job/task/industry	No. of samples	Air concentration				Reference
				Mean		Range		
				ppm	mg/m³	ppm	mg/m³	
Middle East and Asia								
Egypt		Dry-cleaning shops	40 workers (S)	< 140		NR		Emara *et al.* (2010)
Islamic Republic of Iran	69	Dry-cleaning shops	179 workers					Azimi Pirsaraei *et al.* (2009)
		Machine operator	71 workers (P)	11.5		0.6 –81		
		Presser	63 workers (P)	9.6		0.6 –132.3		
		Clerk	45 workers (P)	7.2		0.6–51		
Islamic Republic of Iran		Dry-cleaning shops	30 workers					Rastkari *et al.* (2011)
			10 (8 kg machines) (P)		31.04		NR	
			10 (12 kg machines) (P)		50.87		NR	
			10 (18 kg machines) (P)		120.99		NR	
Japan	3	Dry-cleaning	56 workers TWA	20	136	3.8–94.4	26–640	Cai *et al.* (1991)
Republic of Korea	8	Dry-cleaning shops	8 (S)		4.2		NR	Jo &Kim. (2001)
North America								
USA		Dry-cleaners, commercial	19 (S) (12 workers)	91.5	621	31–270	210–1831	Kerr (1972)
		Dry-cleaners, coin-operated	11 (S) (4 workers)	125	848	87–264	590–1790	
USA		Dry-cleaning area	3 (S)	33	222	21–48	142–325	Eddleston & Polakoff (1974)
		Spotter and dry cleaners	4 (P)	62	420	10–171	68–1160	
USA		Dry-cleaners	96 (P)	41	278	5–125	34–848	Center for Chemical Hazard Assessment (1985)
USA (1975)		5 machine operators	(S)	37.2	252	12.2–62.2	83–422	Tuttle *et al.* (1977)
		2 pressers		11.4	77	4.6–18.3	31–124	
		5 counters		1.3	9	0.4–2.3	3–16	
		7 miscellaneous		3	20	0.9–5.1	6–35	
		5 machine operators	(P)	20.5	139	2.3–38.7	16–262	
		2 pressers		4.48	30	4.1–4.9	28–33	
		5 counters		0.95	6.4	0.3–1.6	2–11	
		7 miscellaneous		2	14	0–4.3	0–29	

Table 1.4 (continued)

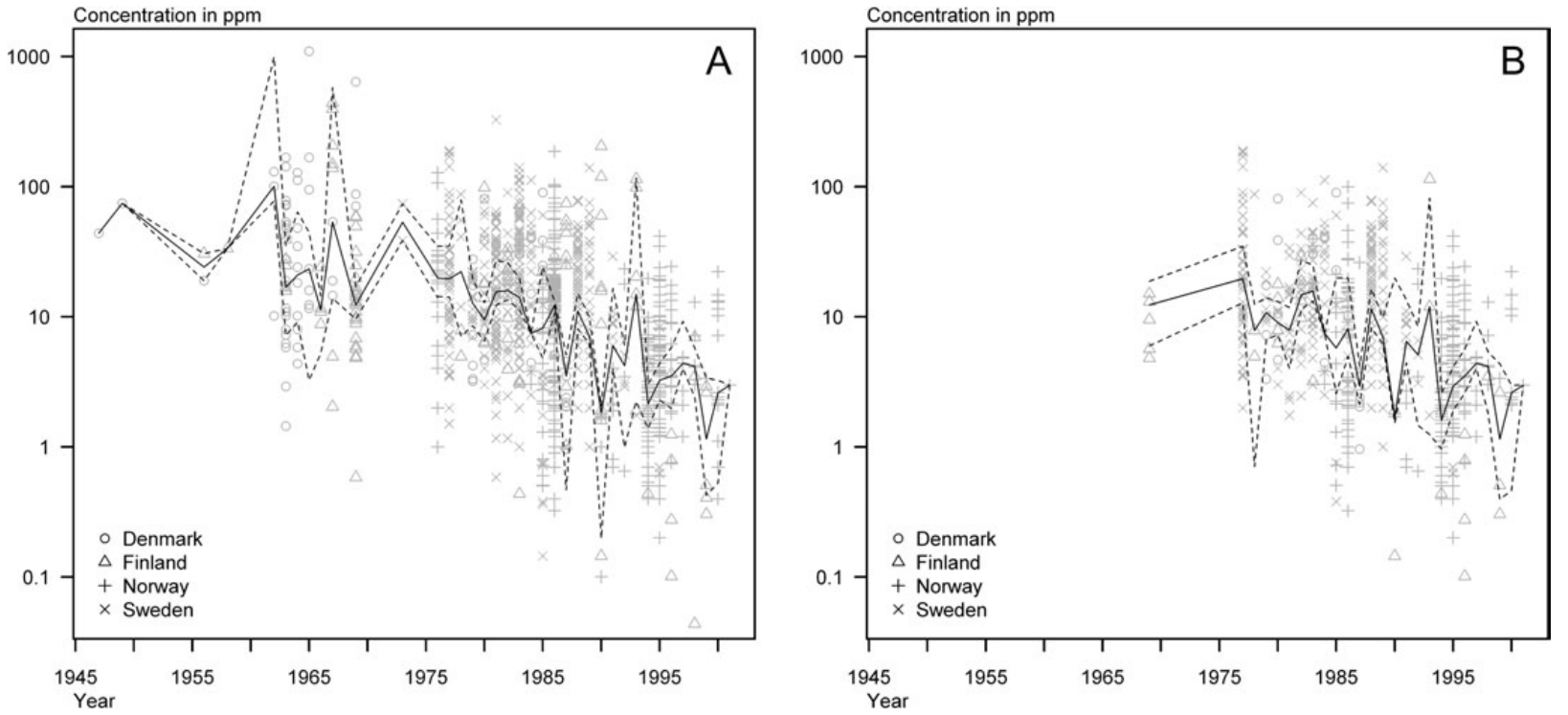
Country (year)	No. of plants	Job/task/industry	No. of samples	Air concentration				Reference
				Mean		Range		
				ppm	mg/m³	ppm	mg/m³	
USA (1977–79)	44	Machine operator	45 (P) TWA	31[c] 22[d]	210[c] 149[d]	4.0–149	27–1010	Ludwig (1981), Ludwig *et al.* (1983)
	35	Presser	52 (P) TWA	5.7[c] 3.3[d]	39[c] 22[d]	0.1–37	0.7–251	
	12	Seamstress	12 (P) TWA	6.6[c] 3[d]	45[c] 20[d]	0.6–29	4–197	
	31	Counter area	31 (P) TWA	5.9[c] 3.1[d]	40[c] 21[d]	0.3–26	2–176	
	39	Machine operator during transfer	134 (P) 5-min peak	76[c] 44[d]	515[c] 298[d]	3.3–366	22–2482	
	30	Machine operator during transfer	49 (P) 15-min peak	55[c] 33[d]	373[c] 224[d]	1–269	7–1824	
USA	44	Washer to dryer transfer	175 (S)	95.8	650	1.0–775	7–5255	NIOSH (1981)
		Counter area	39 (S)	4.8	33	0.3–26.4	2–179	
		Dry-cleaning area	36 (S)	32.8	222	0.5–177	3.4–1200	
		Washer area	25 (S)	22	169	2.0–91	14–617	
		Pressing area	26 (S)	6	41	0.2–40	1.4–271	
		Spotting area	14 (S)	12.3	83.4	0.9–35	6.1–237	
USA (1982)	17	Transfer	(P) TWA	86.6	587	28.5–302.7	193–2053	Materna (1985)
			(P) 5-min peak	135.9	921	11.3–533.8	77–3620	
	3	Dry-to-dry	(P) TWA	28.2	191	3.0–75.9	20–515	
USA (1984)	1	Dry-cleaning (transfer process)	2 (P) TWA	54.5	370	45–64	305–434	Pryor (1985)
			10 (P) 15-min ceiling	306	2075	68–597	461–4049	
USA (1985)	1	Dry-cleaning (dry-to-dry process)	4 (S) TWA		119		79–135	Burr & Todd (1986)

Table 1.4 (continued)

Country (year)	No. of plants	Job/task/industry	No. of samples	Air concentration				Reference
				Mean		Range		
				ppm	mg/m³	ppm	mg/m³	
USA (1980s)		Transfer				NR	NR	International Fabricare Institute (1987)
	471	Machine operator	TWA	49.8	338			
	43	Counter person, etc.		16.7	113			
	57	Spotter, finisher		18.1	123			
		Dry-to-dry						
	157	Machine operator		23.2	157			
	16	Counter person, etc.		10.8	73			
	24	Spotter, finisher		12.1	82			
USA		Dry-cleaning	34 workers (P)		7.9		0.002–55	Eskenazi *et al.* (1991)
USA	10	Operator/presser in dry-cleaning (transfer and dry-to-dry)	60 (P) (13 workers)	10	68	0.17–58.24	1.2–395	Petreas *et al.* (1992)
USA	4		35 (P) (18 workers)	3.15		NR		McKernan *et al.* (2008)
USA (1936–2001)		Dry-cleaning, all job titles	1395 (P)	59		0–4636		Gold *et al.* (2008)
			481 (S)	54		0–1648		
		Operator transfer	441 (P)	150		0–1000		
		Operator (dry-to-dry)	149 (P)	19		0.3–257		
		Spotter	72 (P)	6.6		0.01–39		
		Presser/seamstress	179 (P)	5.6		0.1–52		
		Counter clerk	92 (P)	3.4		0–15		
USA	7		18 workers	3.8		NR		Tucker *et al.* (2011)

[a] Worst-case sampling (highest exposed worker)
[b] Six to eight spot samples taken at each plant
[c] Arithmetic mean
[d] Geometric mean
min, minute; NR, not reported; P, personal air sample; S, stationary air sample; TWA, time-weighted average

Fig. 1.1 Air concentrations of tetrachloroethylene in dry-cleaning shops in the Nordic countries



A All measurements, 1947–2001
B Personal measurements, 1969–2001
The straight and dotted lines represent the median and 95% confidence intervals, respectively.
From Lynge *et al.* (2011), by permission of Oxford University Press

Table 1.5 Exposures to tetrachloroethylene in industries and occupations other than those associated with dry-cleaning

Country, time Period	No. of plants	Job, task or industry	No. of samples	Air concentration [mg/m³]		Reference
				Mean	Range	
Poland	1	Repair rubber belts in brown coal mining	13 (P)	0.6	0.1–5.5	Gromiec *et al.* (2002)
Republic of Korea	90	Solvent workshops in 90 factories	173 (P)	[27.2] (GM)	[2.2]–[5629]	Moon *et al.* (2001)
USA	60	Degreasing (11 non-condensing machines)	68	[1498]	[163–5966]	Morse & Goldberg (1943)
	8	Degreasing	14	[1220] (work in)	[170–2102]	Crowley *et al.* (1945)
			24	[3282] (work out)	[542–12 204]	
	1	Degreasing, auto industry	Short-term	NR	[1573–2610]	Coler & Rossmiller (1953)
	1	Degreasing, printing plates	4 (S)	78	25–99.3	Pryor (1977)
			2 (P)	57	28–86.4	
	1	Degreasing, medical equipment	3 (S)	[106]	[48–197]	Tharr & Donahue (1980)
	1	Degreasing	6 (P)	[271]	[34–1180]	Burgess (1981)
	1	Degreasing, foundry	1 (P)	86.1	NR	Hartle & Aw (1984)
			3 (S)	130.3	40.9–250	
	1	Cutlery manufacture, blade degreasing	2 (P)	[115]	[104–126]	Center for Chemical Hazard Assessment (1985)
	1	Electroplating	5 (P)	[753]	[557–1253]	Daniels & Kramkowski (1986)
	1	Plating, degreasing	1 (P)	11	NR	Abundo *et al.* (1994)
			1 (S)	2	NR	
	NR	Degreasing metal and plastics	206 (P)	95 ppm	0–1800	Gold *et al.* (2008)
			49 (S)	2.3 ppm	0.1–37	
	1	Polyether urethane foam, car industry	3 (S)	2.1	0.4–4.2	White & Wegman (1978)
			9 (P)	4.2	< 0.05–8.0	
	1	Urethane foam	3 (S)	0.6	0.344–0.714	Costello (1979)
	1	Protective coatings	11	[2.7]	[ND–27]	Burroughs (1980)
	1	Filling aerosol cans with carburettor cleaner	30 (S)	[311]	[31–1248]	Hervin *et al.* (1972)
			30 (P)	[403]	[104–1010]	
	1	Coal-testing laboratory	1 (P)	[1010]	NR	Jankovic (1980)
			Several (S)	NR	[746–1315]	
	1	Automotive brake manufacture	11 (P)	103	10–350	Hervin & Lucas (1973)
			11 (S)	145	10–350	

Table 1.5 (continued)

Country, time Period	No. of plants	Job, task or industry	No. of samples	Air concentration [mg/m³]		Reference
				Mean	Range	
USA (cont.)	1	Specialty packaging	4 (P)	[4]	[1.4–5.4]	Hanley (1993)
			5 (S)	[15]	[1.8–41]	
	1	Rubber moulding	15 (P)	[17.6]	[ND–36]	Cook & Parker (1994)
			1 (S)	[8]	NR	
	14	Motion-picture film processing	119 (P)	189	2.7–1606	Mosely (1980)
			51 (S)	111	2.2–965	
	1	Spray painting	9 (P)	21.4	4.4–50	Hartle & Aw (1983)
	1	Automotive parts, fasteners	2 (S)	1.3	1.1–1.5	Ahrenholz & Anderson (1982)
	1	Motion-picture film processing	4 (S)	9.5	6.5–11.3	Okawa & Coye (1982)
			7 (P)	16.4	7.8–54.5	
	1	Graphic arts	4 (P)	13	0.01–30	Love (1982)
	1	Painters, power plant	6 (P)	0.13	< 0.01–0.46	Chrostek & Levine (1981)
			2 (S)	0.29	< 0.01–0.88	
	1	Taxidermy	9 (P)	403	< 0.01–1546	Gunter & Lybarger (1979)

NR, not reported; ND, not detected; P, personal air sample (breathing zone); S, stationary air sample

Table 1.6 Biomonitoring of occupational exposure to tetrachloroethylene

Country Year of study	Job/Task	Number of subjects	Air concentrations Mean (range)	Blood concentrations Mean (range)	Reference
Finland 1974–83	Various	3976	NA	Men, 0.12 mg/L Women, 0.07 mg/L	Anttila *et al.* (1995a)
Germany 1992	Dry-cleaners	12	NR	NR (0.20–3.10) mg/L	Popp *et al.* (1992)
Italy	Dry-cleaners	26	44.2 (5.6–224.6) mg/m^3	725.6 (96.8–3303) µg/L	Gobba *et al.* (2003)
Republic of Korea 1993	Metal degreasers	13	22.4 (0–61) ppm	0.85 (0.2–2.5) mg/L	Jang *et al.* (1993)
USA 2008	Dry-cleaners (4 facilities)	18 women	3.15 ppm	0.07 mg/L pre-shift	McKernan *et al.* (2008)

NA, not applicable; NR, not reported

the reader is referred to the *Monograph* on trichloroacetic acid in this Volume.

1.3.4 Exposure of the general population

Exposure to tetrachloroethylene has been measured in several populations not exposed occupationally, primarily in Germany and the USA (Table 1.7). While concentrations are generally low, living near a dry-cleaning facility considerably increases the level of exposure (Altmann *et al.*, 1995; Schreiber *et al.*, 2002; Storm *et al.*, 2011).

In a study of breast milk in the USA, tetrachloroethylene was detected in seven out of eight samples analysed (Pellizzari *et al.*, 1982).

1.4 Regulations and guidelines

Tetrachloroethylene has been registered on the Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) of the European Commission (ECHA, 2013). Allowable time-weighted averages range from 70 mg/m^3 in Denmark and Sweden, to 345 mg/m^3 in some other European countries (Table 1.8). The European Union recommendation for an occupational exposure limit (OEL) according to the Scientific Committee on Occupational Exposure Limits (SCOEL) is 20 ppm for workers (European Commission, 2009). The American Conference of Governmental Industrial Hygienists (ACGIH, 2002) has established a threshold limit value (TLV) of 25 ppm and biological exposure indices (BEIs) of 5 ppm in end-exhaled air collected before the last shift of the working week, 0.5 mg/L in blood collected before the last shift of the working week, and 3.5 mg/L as trichloroacetic acid in urine collected at the end of the working week.

The state of California, USA, has started to phase out the use of tetrachloroethylene for dry-cleaning, and any new uses have been banned as from 2008, while use in pre-existing machines is to be discontinued by 2023 (California EPA, 2008). No information on bans in any other countries was available to the Working Group.

2. Cancer in Humans

In the previous evaluation by the *IARC Monographs* IARC (1995), the Working Group concluded that tetrachloroethylene was *probably carcinogenic to humans* (Group 2A) based on *limited* evidence in humans and *sufficient*

Table 1.7 Population exposures to tetrachlorethylene

Country Year of study	Subjects	No. of subjects	Age (years)	Blood concentrations			Personal air sample	Reference
				Mean	Range	Measurable %		
Croatia	General population	38	26–62	0.07 µg/L	0–2.54 µg/L	82%	–	Skender *et al.* (1993)
Germany	No known occupational exposure	39	23–52	0.4 µg/L median	< 0.1–3.7µg/L	95%	–	Hajimiragha *et al.* (1986)
Germany	Living above dry-cleaners	29	6–76	106 µg/L	< 6.5–1330 µg/L	–	–	Popp *et al.* (1992)
Germany	Living above dry-cleaners	19	NR	17.8 µg/L	NR	NR	NR	Altmann *et al.* (1995)
Germany		191	Children (5–7 yr)	0.02 µg/L	< 0.004–0.131	NR	NR	Begerow *et al.* (1996)
		223	Adult women	0.05 µg/L	0.01–6.34			
	Not near dry-cleaners	86 urban		0.05 µg/L	0.01–0.80			
		127 rural		0.04 µg/L	0.01–0.05			
	Near dry-cleaners	5 urban		0.33 µg/L	0.09–6.34			
		4 rural		0.45 µg/L	0.15–2.41			
USA	NHANES III	590	20–59	0.19 ppb	ND–0.62 ppb	–	–	Ashley *et al.* (1994)
USA	NHEXAS	147	Adult	0.21 µg/L	NR	39%	–	Clayton *et al.* (1999)
USA	Living near dry-cleaners	10	NR	6.43 µg/L (afternoon)	1.1–18	NR	948 µg/m^3 (daytime)	Schreiber *et al.* (2002)
			NR	4.87 µg/L (morning)	1.1–7	NR	420 µg/m^3 (overnight)	
USA		73	Children	NR	NR	NR	3.7 µg/m^3	Adgate *et al.* (2004)
USA, 1981–87	Bayonne	139	Adults	NR	NR	NR	7.8	Rappaport & Kupper (2004)
	Elizabeth	191					8.9	
	Greensboro	24					3.1	
	Devils Lake	23					5.2	
	Los Angeles	179					7.1	

Table 1.7 (continued)

Country Year of study	Subjects	No. of subjects	Age (years)	Blood concentrations			Personal air sample	Reference
				Mean	Range	Measurable %		
USA	SHIELD	113	6–10	0.03 µg/L	NR	37–62%	–	Sexton *et al.* (2005)
USA	Live near dry-cleaners; indoor air	11	Adult	1.28 µg/L	–	–	–	Storm *et al.* (2011)
		7	Child	0.51 µg/L				
	> 100 µg/m³	39	Adult	0.13 µg/L				
	Live near dry-cleaners; indoor air	28	Child	0.11 µg/L				
	< 100 µg/m³	39	Adult	0.05 µg/L				
	Not living near dry-cleaners	32	Child	0.03 µg/L				
USA	NHANES	2940	12–59	NR	LOD–0.17 µg/L	–	–	CDC (2013)

LOD, limit of detection; NR, not reported

Table 1.8 Regulations and guidelines for tetrachlorethylene

Country or region	TWA concentration (mg/m³)	Carcinogenicity[a]
Australia	340	–
Austria	345	–
Belgium	172	–
Canada, Quebec	170	–
Denmark	70	–
Europe GHS	–	H351
European classification	–	R40
France	138	–
Germany AGS	138	–
Germany TRGS	–	K3
Germany MAK	–	3B
New Zealand	335	–
Singapore	170	–
Spain	172	–
Sweden	70	–
Switzerland	345	–
USA OSHA	170	A3
ACGIH	25	–
NTP	–	Reasonably anticipated
United Kingdom	345	–

[a] Carcinogenicity: H351, suspected of causing cancer; R 40, limited evidence of a carcinogenic effect; K3, substances which possibly are carcinogenic for humans and thus give cause for concern; 3B, substances which are proved/possibly carcinogenic and therefore give reason for concern; Group C, possible human carcinogen; A3, confirmed animal carcinogen with unknown relevance to humans
ACGIH, American Conference of Governmental Industrial Hygienists; EPA, Environmental Protection Agency; GHS, Global Harmonization System; MAK, maximum occupational concentrations; NTP, National Toxicology Program; OSHA, Occupational Safety and Health Administration; TRGS, Technical rules for hazardous substances; TWA, 8-hour time-weighted average
From GESTIS (2012)

evidence in experimental animals. In studies in humans, associations occurred with cancers of the oesophagus, cervix, and with non-Hodgkin lymphoma, but confounding could not be entirely excluded. No consistent pattern of elevated risk was observed for cancer of the kidney.

A substantial body of literature on the epidemiology of cancer and exposure to tetrachloroethylene was available to the Working Group and included both cohort and case–control studies. The two designs complement each other in that cohort designs typically provide a narrower range of occupations for exposure assessment than do case–control designs, while case–control studies are able to control for some important potential confounders. While many cancers have been evaluated, there has been a focus on lymphatic and haematopoietic cancers, and tumours of the urinary tract. Exposure assessments range from simple job-title determinations to quantitative assessments. While tetrachloroethylene has been used in several occupations, including vehicle repair, other mechanics, printers, and electricians, it is particularly associated with dry-cleaning (see Section 1). In many workplaces where tetrachloroethylene is used, other chlorinated solvents can also be found. There is some overlap between exposures to tetrachloroethylene and trichloroethylene in studies evaluated for this *Monograph*; this complicated the interpretation of study findings, but it should be remembered that nearly all workplaces have multiple exposures.

The Working Group did not consider in its evaluation several studies that reported results only for "laundry and dry-cleaning workers" combined (Malker & Weiner, 1984; McLaughlin *et al.*, 1987; Lynge & Thygesen, 1990; Minder & Beer-Porizek, 1992; Lynge *et al.*, 1995; Andersen *et al.*, 1999; Travier *et al.*, 2002; Pukkala *et al.*, 2009). Similarly, studies based on occupations coded on death certificates that combined laundry and dry-cleaning workers (Katz & Jowett, 1981; Duh & Asal, 1984; Nakamura, 1985; Walker *et al.*, 1997) were also considered to be uninformative with regard to tetrachloroethylene exposure. Launderers typically handle soap and other chemical cleaning agents, while persons engaged in dry-cleaning work have used different types of chlorinated solvents, mainly tetrachloroethylene, supplemented with trichloroethylene and fluorocarbons (Andersen *et al.*, 1999). Since the exposure assessment was not specific to tetrachloroethylene, this would consequently result in a dilution of the magnitude of any observed effect.

2.1 Cohort studies

Most of the studies of cohorts exposed to tetrachloroethylene focused on dry-cleaning and related occupations. However, a few cohorts of non-dry-cleaning workers are discussed in Section 2.1.2. In addition to studies that characterized exposure by employment in occupation or industry categories combining "laundry and dry-cleaning," the Working Group also excluded studies of exposure to mixed solvents without further distinction.

2.1.1 Dry-cleaning workers

Tetrachloroethylene became the most commonly used dry-cleaning solvent in the 1950s, replacing carbon tetrachloride, which was considered to be more toxic, and trichloroethylene, which was harsher on fabrics (Ludwig, 1981; IARC, 1995; Doherty, 2000a, b). Large studies of cohorts of dry-cleaning workers have been conducted in Europe and the USA. Because of the large number of small shops with a few employees each and the high turnover in this industry, the United States National Cancer Institute (NCI) cohort was assembled through union records (Blair *et al.*, 1979; Blair *et al.*, 1990, 2003), as was the NIOSH cohort (Brown & Kaplan, 1987; Ruder *et al.*, 1994, 2001; Calvert *et al.*, 2011), while most of the European studies, with the exception of Anttila *et al.* (1995a, b), Lynge *et al.* (2006) and Seldén & Ahlborg (2011), used census records linked to cancer-registry or mortality data. None of the cohort studies of dry-cleaning workers assessed exposure to tetrachloroethylene directly. Table 2.1 presents the most recent results of cohort studies on eight cancer sites of interest: cancers of the lung, kidney, bladder, liver, breast, cervix, oesophagus, and lympohaematopoietic system (Hodgkin disease, non-Hodgkin lymphoma, multiple myeloma, and leukaemia).

The NCI cohort study enrolled members of a union of dry-cleaning workers in Missouri, USA, which had 11 062 members between 1945 and 1978, of whom 5790 had held membership for 1 year or more. After exclusion of 425 members for whom information on race, sex, or date of birth was unavailable, the analysis was restricted to 5365 members who were followed from entry to the union or on 1 January 1948 (whichever came later) until 1 January 1979: follow-up was extended to 31 December 1993 for the most recent update and included 5369 members (Blair *et al.*, 2003). The cohort provided 146 082 years of follow-up until 1993 and expected numbers of deaths were calculated from national rates. The mortality rate was as expected for all causes combined (standardized mortality rate [SMR], 1.0; 95% CI, 1.0–1.1; 2351 deaths), but slightly higher than expected for cancer (SMR, 1.2; 95% CI, 1.1–1.3; 590 deaths). Excesses were found for cancers of the oesophagus (SMR, 2.2; 95%

Table 2.1 Cohort studies of occupational exposure to tetrachloroethylene

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Bond *et al.* (1990), Michigan, USA 1940–82	44 (liver cancer deaths)	Employees from chemical plant; company work records	Liver, gallbladder or bile ducts (ICDA-8 155–156 and 197.8)	Exposed to tetrachloroethylene	6	1.8 (0.8–4.3)	Age
Anttila *et al.* (1995a) Finland 1967–92	849	All workers biologically monitored for tetrachloroethylene in blood. Median: women, 0.4 µmol/L; men, 0.7 µmol/L	Kidney	Exposed to tetrachloroethylene	2	1.82 (0.22–6.56)	SIR. Occupation not specified "tetrachloroethylene was used in dry-cleaning, and to a small extent also in degreasing and in the graphic industry"
			Lymphohaematopoietic tissues (200–204)		3	1.38 (0.28–4.02)	
			Non-Hodgkin lymphoma (200, 202)		3	3.76 (0.77–11.0)	
			Multiple myeloma (203)		0	–	
			Cervix uteri (171)		2	3.20 (0.39–11.6)	
			Lung, bronchus (162.0–162.1)		5	1.92 (0.62–4.48)	
Boice *et al.* (1999), California, USA 1960–66	2631	Aircraft-manufacturing workers. JEM without quantitative estimate of intensity, 1987–1988, 8-h TWA, tetrachlorethylene concentration (atmospheric monitoring): 3 ppm [mean] and 9.5 ppm [median]	Oesophagus (150)	Routine exposure to tetrachlorethylene	6	1.47 (0.54–3.21)	SMR. Employed ≥ 1 year from 1960 onwards; 30% exposed to tetrachlorethylene and probably other solvents also
			Cervix uteri (180)		0	0.47 expected cases	
			Kidney (189.0–189.2)		2	0.69 (0.08–2.47)	
			Bladder and other urinary tract (188, 189.3–189.9)		2	0.70 (0.09–2.53)	
			NHL (200, 202)		8	1.70 (0.73–3.34)	
			Hodgkin disease (201)		0	0.63 expected cases	
			Bronchus, trachea, and lung (162)		46	1.08 (0.79–1.44)	

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
Blair *et al.* (2003) Missouri, USA 1948–93	5369	Dry-cleaning workers: occupational history from union records	Kidney (189)	Overall	8	1.0 (0.4–2.0)	SMR adjusted for age at death, year of death, race, and sex. Reference was population of USA.
				Little/no exposure	1	0.3 (< 0.1–1.6)	
				Medium/high exposure	7	1.5 (0.6–3.1)	
			Bladder (188)	Overall	12	1.3 (0.7–2.4)	
				Little/no exposure	5	1.4 (0.4–3.2)	
				Medium/high exposure	7	1.5 (0.6–3.1)	
			Liver (155)	Overall	10	0.8 (0.4–1.5)	
			Breast (174)	Overall	68	1.0 (0.8–1.3)	
				Little/no exposure	30	0.8 (0.6–1.2)	
				Medium/high exposure	29	1.2 (0.8–1.7)	
			Lymphatic, haematopoietic	Overall	39	1.0 (0.7–1.3)	
				Little/no exposure	18	1.0 (0.6–1.5)	
				Medium/high exposure	17	0.9 (0.5–1.4)	
			NHL (200, 202)	Overall	12	0.9 (0.5–1.6)	
			Hodgkin disease (201)	Overall	5	2.0 (0.6–4.6)	
			Multiple myeloma (203)	Overall	7	0.8 (0.3–1.6)	
			Leukaemia (204–207)	Overall	12	0.8 (0.4–1.4)	
			Oesophagus (150)	Overall	26	2.2 (1.5–3.3)	
				Little/no exposure	7	2.1 (0.9–4.4)	
				Medium/high exposure	16	2.2 (1.2–3.5)	
			Cervix (180)	Overall	27	1.6 (1.0–2.3)	
				Little/no exposure	12	1.5 (0.8–2.7	
				Medium/high exposure	11	1.4 (0.7–1.7)	
		Overall	Lung		125	1.4 (1.1–1.6)	

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
Lynge *et al.* (2006) Denmark, Finland, Norway, Sweden 1970–2001	46 768 (695 dry-cleaner and other exposed; 183 other in dry-cleaning; 2420 unexposed)	Laundry and dry-cleaning workers identified from the 1970 censuses in Denmark, Norway, Sweden, and Finland; duration of employment assessed through pension scheme data (Denmark, Finland) and biography of dry-cleaning shop owners & yellow pages of telephone books (Denmark)	Bladder cancer (excluding *in situ*)	Dry-cleaning workers	93	1.44 (1.07–1.93)	RR adjusted for matching criteria and, where relevant, for smoking and alcohol use. Case–control study nested in Nordic cohort; three controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time.
Radican *et al.* (2008) USA 1973–2000	14 455 (851 ever exposed to tetrachloroethylene)	Aircraft-maintenance workers from Hill Air Force Base, Utah; employed > 1 yr; 1952–1956, JEM (intensity), internal referent (workers with no chemical exposures)	Lymphatic & haematopoietic (men)	Any exposure to TETRA	14	1.92 (1.00–3.69)	Age, race HR for mortality. Lung cancer mortality not reported.
			NHL (men)		5	2.32 (0.75–7.15)	
			NHL (women)		2	2.35 (0.52–10.71)	
			Multiple myeloma (men)		3	1.71 (0.42–6.91)	
			Multiple myeloma (women)		2	7.84 (1.43–43.06)	
			Lung		NR	–	
			Non-malignant respiratory diseases (men)		46	1.83 (1.28–2.60)	

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
Calvert *et al.* (2011) California, Illinois, Michigan, New York, USA 1940–2004	1704	Dry-cleaners: occupational history from union records	Kidney (189)	Overall	5	1.1 (0.4–2.7)	SMR. Reference was population of USA. All employed in plant using tetrachloroethylene at least 1 year by 1960
				TETRA only	2	1.4 (0.2–4.9)	618 workers exposed to tetrachloroethylene only
			Bladder (188)	Overall	10	1.8 (0.9–3.3)	
				TETRA only	0		
				Employed < 5 years; < 20 years since first employed	0		*P* by employment duration, 0.12
				Employed ≥ 5 years; < 20 years since first employed	0	–	
				Employed < 5 years; ≥ 20 years since first employed	1	0.5 (0.03–2.5)	
				Employed ≥ 5 years; ≥ 20 years since first employed	9	4.1 (2.1–7.1)	
			Liver, biliary tract (155, 156)	Overall	1	0.1 (0.0–0.7)	
				TETRA only	0	–	
			Breast (174)	Overall	28	1.1 (0.7–1.5)	
				TETRA only	10	1.1 (0.5–1.9)	
			Lymphatic, haematopoietic	Overall	19	0.9 (0.5–1.4)	
				TETRA only	11	1.5 (0.8–2.7)	
			NHL (200, 202)	Overall	11	1.6 (0.8–2.8)	
				TETRA only	6	2.5 (0.9–5.4)	
			Oesophagus (150)	Overall	16	2.4 (1.4–4.0)	
				TETRA only	6	2.7 (0.98–5.8)	

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
Calvert et al. (2011) California, Illinois, Michigan, New York, USA 1940–2004 (cont.)				Employed < 5 years; < 20 years since 1st employed	0	–	*P* by employment duration, 0.09
				Employed ≥ 5 years; < 20 years since first employed	0	–	
				Employed < 5 years; ≥ 20 years since first employed	5	2.2 (0.9–4.5)	
				Employed ≥ 5 years; ≥ 20 years since first employed	11	4.8 (2.7–7.9)	
			Cervix (180)	Overall	13	1.8 (0.98–3.1)	
				TETRA only	5	2.1 (0.7–4.9)	
				Employed < 5 years; < 20 years since first employed	2	0.8 (0.2–2.7)	*P* by employment duration, 0.66
				Employed ≥ 5 years; < 20 years since first employed	4	2.6 (0.9–6.0)	
				Employed < 5 years; ≥ 20 years since first employed	4	2.8 (0.9–6.3)	
				Employed ≥ 5 years; ≥ 20 years since first employed	3	2.1 (0.6–5.4)	
		Overall	Lung		77	1.3 (1.0–1.6)	

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
Seldén & Ahlborg (2011) Sweden 1985–2006	9440	Questionnaire mailed to all "washing establishments" on company workers for past 11 years, production volumes, washing techniques, chemicals used (1973–1983): TETRA group = subgroup of dry-cleaners and laundries with a proportion of dry-cleaning with tetrachloroethylene only	Liver, gallbladder (155)	TETRA group (men)	8	2.14 (0.92–4.21)	SIR; cancer incidence study; no information on exposure at the company or individual level was available, but estimates of the proportion of tetrachloroethylene and other detergents employed (as reported by the companies over the period of interest) were used as proxy; response rate, 38%; no data on workers from nonresponding companies.
				Employed < 1 year	0	0	
				1–4 years	3	3.19 (0.66–9.31)	
				5–11 years	5	2.06 (0.67–4.80)	
			Breast (174)	TETRA group (women)	140	0.85 (0.72–1.00)	
			Hodgkin disease (201)	TETRA group (men)	3	3.22 (0.66–9.40)	
			Non-Hodgkin lymphoma (200, 202)	TETRA group [men & women]	[33]	[1.42 (0.98–2.0)]	
				employed < 1 year	[10]	[3.28 (1.57–6.03)]	
				1–4 years	[7]	[1.03 (0.41–2.12)]	
				5–11 years	[16]	[1.20 (0.69–1.95)]	
			Oesophagus (150)	TETRA group (women)	3	1.25 (0.26–3.65)	
			Cervix (171)	TETRA group (women)	16	1.19 (0.64–1.93)	
				Employed < 1 year	1	0.32 (0.01–1.78)	
				1–4 years	8	1.72 (0.74–3.40)	
				5–11 years	7	1.24 (0.50–2.56)	

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
Seldén & Ahlborg (2011) Sweden 1985–2006 (cont.)			Lung (162)	TETRA group (men)	23	1.30 (0.82–1.94)	
				TETRA group (women)	35	1.09 (0.76–1.51)	
			Liver	Dry cleaner	11	0.76 (0.38–1.52)	
			Cervix	Dry cleaner	36	0.98 (0.65–1.47)	
			Kidney	Dry cleaner	29	0.67 (0.43–1.05)	

HR, hazard ratio; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NR, not reported; TETRA, tetrachloroethylene; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TCE, trichloroethylene; TWA, time-weighted average

CI, 1.5–3.3; 26 deaths), lung (SMR, 1.4; 95% CI, 1.1–1.6; 125 deaths), cervix uteri (SMR, 1.6; 95% CI, 1.0–2.3; 27 deaths) and bladder (SMR, 1.3; 95% CI, 0.7–2.4; 12 deaths), and for Hodgkin disease (SMR, 2.0; 95% CI, 0.6–4.6; five deaths). There was no excess of cancer of the liver or of the kidney (see Table 2.1). The risk of cancer of the oesophagus was highest for African-American men (SMR, 3.1; 95% CI, 1.9–5.0; 18 deaths). Exposure assessment was based on the published literature, applied to the job titles in the union records. The relative risk for cancer of the oesophagus for all cohort members was not related to the estimated exposure level; for those with little or no exposure it was 2.1; for those with medium or high exposure it was 2.2. There were 18 deaths from lymphatic and haematopoietic malignancies among those with little or no exposure (SMR, 1.0) and 17 (SMR, 0.9) among those with medium or high exposure. When mortality was compared by year of joining the union (before or after 1960, when tetrachloroethylene became the predominant solvent used), there was no difference at most sites except bladder, for which the SMR for those joining the union before 1960 was 1.1 (95% CI, 0.5–2.0, nine deaths), and for those joining after 1960 the SMR was 2.9 (95% CI, 0.6–9.5, three deaths). [The size of the cohort and the extended follow-up made this a valuable study. There was little evidence of an exposure–response effect, but the Working Group noted that the higher mortality for cancer of the bladder after the introduction of tetrachloroethylene supported the involvement of an occupational rather than a lifestyle risk factor. The Working Group also noted, however, that mortality from cancer of the oesophagus was in excess, possibly supporting an effect of smoking.]

The NIOSH cohort included 1704 unionized dry-cleaning workers from four cities in California, Illinois, Michigan and New York, USA. The inclusion criteria were: employment for at least 1 year before 1960 in a shop where tetrachloroethylene was the primary solvent used, and no known exposure to carbon tetrachloride. A survey in 1977–79 (Brown & Kaplan, 1987) showed geometric mean, time-weighted average concentrations of tetrachloroethylene in the range of 3–22 ppm (20.3–149 mg/m^3); other solvents used for spot cleaning were not detected in the samples. Visits were made to many of the facilities that were still operating, and data on solvent use and solvent levels were collected (Ludwig, 1981). These monitoring data, and the job-title information available for about one third of the NIOSH cohort, were not used to create a job-exposure matrix for the NIOSH study, but were used to verify exposure to tetrachloroethylene and other solvents used in dry-cleaning, and to exclude workers who had been exposed to carbon tetrachloride or trichloroethylene.

In the analyses, two subcohorts were defined: people employed only in shops where tetrachloroethylene was the primary solvent used, and people whose work also involved exposure to other solvents. The most recent update added mortality follow-up until 31 December 2004, accounted for 94% of the women and 97% of the men, and generated 63 426 person-years at risk, but did not extend employment follow-up (Calvert *et al.*, 2011). The mean duration of employment in dry-cleaning shops until 31 December 1990 was 6.4 years for those exposed only to tetrachloroethylene and 11.4 years for those also exposed to other solvents; the latter group had a mean duration of 6.0 years of exposure to tetrachloroethylene. Expected numbers of deaths were calculated from the national death rates. There were 1255 deaths (SMR, 1.0; 95% CI, 1.0–1.1) and 322 cancer deaths (SMR, 1.2; 95% CI, 1.1–1.4). The SMRs were significantly increased for cancers of the oesophagus (SMR, 2.4; 95% CI, 1.4–4.0; 16 deaths), tongue (SMR, 4.5; 95% CI, 1.5–10; five deaths), and trachea, bronchus and lung (SMR, 1.3; 95% CI, 1.0–1.6; 77 deaths). Mortality from cancer of the pancreas was elevated (SMR, 1.5; 95% CI, 1.0–2.3; 22 deaths). When the analysis was restricted to workers with a 20-year latency

since first employment and with a duration of employment > 5 years, the SMRs were increased for cancers at all sites combined (SMR, 1.3; 95% CI, 1.2–1.5; 130 deaths) and, notably, for cancers of the oesophagus (SMR, 4.8; 95% CI, 2.7–8; 11 deaths) and urinary bladder (SMR, 4.1; 95% CI, 2.1–7; nine deaths). There were also three cancers of the tongue (SMR, 3.5; 95% CI, 0.73–10). Only one death from cancer of the liver and biliary tract was found (with 7.7 expected). The SMR for chronic obstructive pulmonary disease was 1.2 (95% CI, 0.8–1.6; 33 deaths). [In this study, exposure to tetrachloroethylene and other solvents was verified (until the end of work-history collection in 1978). The elevated SMRs for all cancers and oesophageal and bladder cancers for those who had worked 5 years or more support an exposure–response effect. However, because two thirds of the workers were exposed to solvents other than tetrachloroethylene, and there were no deaths from cancer of the bladder among workers exposed to tetrachloroethylene only, the possible contribution of exposure to other solvents must be considered. In addition, the cancer sites with excess mortality are all known to be associated with consumption of tobacco, and the elevated SMR for chronic obstructive pulmonary disease indicated that consumption of tobacco could play a role in the excess mortality at these sites. The excesses for cancers of the bladder and oesophagus, however, appear to be accounted for entirely by consumption of tobacco, given the relatively small increase in mortality from cancer of the lung.]

In a case–control study nested within the Nordic population (Lynge *et al.*, 2006), the authors evaluated incident cases of selected cancers (bladder, oesophagus, gastric cardia, pancreas, cervix, kidney, liver, and non-Hodgkin lymphoma) reported in the respective national registers from 1997 to 2001. Three controls for each case (six for esophageal cancer) were randomly selected from the cohort and were frequency-matched by country, sex, 5-year age group, and date of case diagnosis by 5-year calendar period. The cohort focused on 46 768 laundry and dry-cleaning workers registered in the 1970 census in Denmark, Finland, Norway, and Sweden, because tetrachloroethylene was by far the most commonly used dry-cleaning solvent in these countries before and during the study period. Dry-cleaning workers were defined as "persons stated to be dry-cleaners, owners of dry-cleaning shops, and other persons employed in dry-cleaning shops with < 10 workers." The last category was included because of the shared work tasks and physical proximity to equipment in small shops. Census and registry data were supplemented with implied exposure status (working as a dry-cleaner or in a dry-cleaning shop), based on original texts from the census forms (Denmark and Norway), interviews (Norway and Sweden), and pension-scheme data (Denmark and Finland) for cases and controls.

There was a statistically significant excess incidence of cancer of the bladder among dry-cleaners (relative risk [RR], 1.4; 95% CI, 1.1–1.9; 93 cases) and the risk of pancreatic cancer was also elevated (RR, 1.27; 95% CI, 0.90–1.80; 57 cases). There were no statistically significant excesses of cancer at any of the other sites. For those working in dry-cleaning for 10 years or more, there were elevated risks of cancers of the bladder (RR, 1.6; 95% CI, 1.1–2.3; 53 cases), pancreas (RR, 1.2; 95% CI, 0.7–2.0; 23 cases) and cervix (RR, 1.2; 95% CI, 0.6–2.2; 16 cases) and no increases in risk of cancer of the oesophagus, gastric cardia, kidney, liver, or of non-Hodgkin lymphoma (Lynge *et al.*, 2006). [Tests for trend by increasing duration of employment were not presented.] [Table 2.1 presents results for those definitely known to be dry-cleaners only. The unexposed comparison group comprised laundry workers, rather than the general population used for comparison in cohorts from studies in the USA, to indirectly adjust for tobacco smoking habits.]

Seldén & Ahlborg (2011) assembled a cohort of 10 389 dry-cleaning and laundry workers in

Sweden in 1984, based on a questionnaire mailed to all "washing establishments" (response rate, 38%) about workers, production volume, and chemicals used. Data on cancer incidence were obtained by matching to the national cancer register for 1985–2006. Use of tetrachloroethylene in dry-cleaning was reported by 61% of the cohort members (6356 out of 10 389). Among those who were exposed to tetrachloroethylene through dry-cleaning work, there was an increase in incidence of cancer among men only (standardized incidence ratio [SIR], 1.11; 95% CI, 0.97–1.26), with excesses of non-Hodgkin lymphoma (SIR, 2.0; 95% CI, 1.1–3.3; 15 cases) and cancers of the liver (SIR, 2.1; 95% CI, 0.9–4.2; eight cases) and lung (SIR, 1.3; 95% CI, 0.8–1.9; 23 cases). There was no significant excess of cancer of the bladder. [The study population may overlap with that of Lynge *et al.* (2006). Despite the large number of study participants and the identification of those exposed to tetrachloroethylene, the lack of quantitative data on exposure and the low response rate for the questionnaire were limitations of this study.]

A cohort of 3974 workers in Finland who were biomonitored for occupational exposure to halogenated hydrocarbons, including tetrachloroethylene, during 1974–1983 was followed for cancer incidence from 1967 to 1992 (Anttila *et al.*, 1995a, b). These workers were exposed to tetrachloroethylene in dry-cleaning, and to a small extent also in degreasing and in the graphics industry. Among workers exposed to tetrachloroethylene, there was no overall increased risk of cancer (SIR, 0.9; 95% CI, 0.6–1.3; 31 cases), but there were indications of an increased risk of non-Hodgkin lymphoma (SIR, 3.8; 95% CI, 0.8–11.0; three cases) and cancers of the pancreas (SIR, 3.1; 95% CI, 0.6–9.0; three cases), cervix (SIR, 3.2; 95% CI, 0.4–12.0; two cases), and lung (SIR, 1.92; 95% CI, 0.62–4.48; five cases) [based on a small number of cases].

2.1.2 Workers in other industries

Bond *et al.* (1990) conducted a case–control study that was nested within a cohort of workers in a chemical plant in Michigan, USA (see Section 2.2.7). The risk estimate for cancer of the liver and biliary tracts associated with exposure to tetrachloroethylene, assessed via company work records, was 1.8 (95% CI, 0.8–4.3).

Boice *et al.* (1999) studied aircraft-manufacturing workers exposed to tetrachloroethylene and trichloroethylene in or after 1960 and followed for vital status until 1996. Exposure was assessed by industrial hygiene walk-through inspections of factories to become familiar with the manufacturing processes and patterns of use of chemicals; interviews with over 50 long-term employees (retired and active); review of existing industrial hygiene files, job descriptions going back to the 1940s, and other historical documents; and detailed job histories from the work history cards of factory workers. In 2681 workers exposed to tetrachloroethylene, overall cancer mortality was not elevated (SMR, 0.90; 95% CI, 0.82–0.98; 476 exposed cases), and for those workers with > 5 years of exposure, the relative risk was 0.74 (95% CI, 0.6–0.9; 123 deaths; *P* for trend, 0.01) compared with no exposure. For non-Hodgkin lymphoma, exposure was associated with increased risk without exposure–response relationship; for workers with > 5 years exposure, the relative risk was 1.4 (95% CI, 0.7–3.0; 10 deaths; *P* for trend, > 0.20) compared with no exposure. [Workers were exposed to multiple solvents so any increased risks may not have been attributable to tetrachloroethylene.]

A study of mortality in aircraft-maintenance workers (Radican *et al.*, 2008) is discussed in more detail in the *Monograph* on trichloroethylene in this volume, because tetrachloroethylene was not the major or only solvent exposure. The following cancer outcomes were assessed in relation to exposure to tetrachloroethylene: cancer of the breast (one exposed case), non-Hodgkin

lymphoma (hazard ratio [HR] in men, 2.3, 95% CI, 0.8–7.2, five exposed cases; HR in women, 2.4; 95% CI, 0.5–10.7; two exposed cases), and multiple myeloma (HR in men, 1.7, 95% CI, 0.4–6.9; three exposed cases; HR in women, 7.8; 95% CI, 1.4–43.1; two exposed cases), accounting for age, race, and sex.

2.2 Case–control studies

The association between occupational exposure to tetrachloroethylene and various cancers has been evaluated in numerous case–control studies. While a few studies assessed tetrachloroethylene specifically, the majority of studies assessed occupations and industries with potential exposure to tetrachloroethylene, such as dry-cleaning and aircraft workers. As discussed in Section 2.1, studies that assessed exposure to mixed solvents without distinguishing further, or that assessed the combined occupational category of "laundry and dry-cleaning workers" were excluded from this review (i.e. United States National Bladder Cancer Study by Silverman *et al.*, 1989, 1990). No study further subdivided exposures by job tasks that would likely incur higher exposure (i.e. among dry-cleaning workers, no distinction was made between machine operators, who handle tetrachloroethylene-soaked garments, and other workers). One research group assessed the risk for several cancers associated with environmental exposure to tetrachloroethylene (Aschengrau *et al.*, 1993, 1998, 2003; Vieira *et al.*, 2005; Gallagher *et al.*, 2011; Paulu *et al.*, 1999).

2.2.1 Cancer of the bladder

The results of case–control studies of cancer of the bladder are presented in Table 2.2. All these studies adjusted for smoking.

The only study to report risk of cancer associated with residential exposure to tetrachloroethylene in drinking-water was carried out in Massachusetts, USA (Aschengrau *et al.*, 1993). Living and deceased cases diagnosed in 1983–1986 were identified from the state cancer registry. Population controls from the same geographical area and matched by vital status and age group were selected by random-digit dialling, death registrations, or health insurance rolls, if aged > 65 years. Information about occupational history, water consumption, bathing habits, exposure to specific chemicals, and potential confounding factors was obtained by telephone or in-person interviews with subjects or their next of kin. Semiquantitative estimates of exposures to tetrachloroethylene were developed using an algorithm based on residence and water-system design. Multivariable logistic regression models were adjusted for sex, age at diagnosis or index year, vital status at interview, educational level, and occupational exposure to benzene and other solvents. The analysis included 61 cases of cancer of the bladder and 852 controls. The adjusted odds ratio was 1.39 (95% CI, 0.67–2.91) for any exposure to tetrachloroethylene, and 4.03 (95% CI, 0.65–25.10) for exposure above the 90th percentile of the semiquantitative estimates. The number of exposed cases was too small to allow analysis to account for latency. [This study included estimates of specific exposure to tetracholorethylene, and adjustment for an array of risk factors. However, interpretation was hampered by the small number of exposed cases.]

The NCI conducted the National Bladder Cancer Study, a case–control study of 2982 incident cases and 5782 controls carried out over 18 months, starting in 1977, in 10 areas of the USA (Hartge *et al.*, 1984). As part of this study, Schoenberg *et al.* (1984) studied risk of cancer of the bladder according to occupation in white men in New Jersey, USA. Information on incident cases and age-stratified population controls selected by random-digit dialling was collected by interviewing in person to collect information about smoking, occupational

Table 2.2 Case-control studies of cancer of the bladder and exposure to tetrachloroethylene

Reference, study location and period	Total cases Total controls	Control source (Hospital, Population	Exposure Assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Aschengrau *et al.* (1993) USA, Massachusetts: 1983–89	61 852	Population	Telephone/in-person interviews with subjects or next of kin on occupational history, water consumption, bathing habits, exposure to specific chemicals and potentially confounding factors; tetrachloroethylene-contaminated water estimated from exposure model	Any exposure to tetrachloroethylene	13	1.39 (0.67–2.91)	Sex, age at diagnosis, vital status at interview, education, cigarette smoking, urinary-tract infection and past occupational exposure Tetrachloroethylene is not the only compound to which the population was residentially exposed
				Took mostly baths		1.99 (0.40–10.01)	
				High exposure to tetrachloroethylene	4	4.03 (0.65–25.10)	RDD > 90th percentile
Schoenberg *et al.* (1984) USA, New Jersey: 1978–79	658 1258	Age-stratified population controls	Caucasian men, age 21–84 yr, in-person interview with questionnaire, industry and job title surrogate exposure metric	White men, ever dry-cleaning workers	7	1.3 (0.5–3.6)	Age, cigarette smoking Study population overlaps with the US National Bladder Cancer Study and had the same method of exposure assessment. Launderers may be included in the same exposure category as dry-cleaners.
Steineck *et al.* (1990) Sweden, Stockholm: 1985–87	254 287	Population	Men, birth years, 1911–1945, living in County of Stockholm 1985–1987, mailed questionnaire, occupational title as surrogate,	Male dry-cleaning workers	2	1.2 (0.2–9.2)	OR adjusted for birth year and smoking, exposure after 1981 excluded
Burns & Swanson (1991) USA, Michigan	2160 3979	Hospital	Men and women, age 40–84 yr, telephone interview, longest period (usual) employed in occupation or industry	Usual occupation as dry-cleaning workers	8	1.9 (0.7–4.9)	OR adjusted for cigarette smoking, race, sex, and age at diagnosis Rectal or colon cancer controls; possible overlap with Swanson & Burns (1995)

Table 2.2 (continued)

Reference, study location and period	Total cases Total controls	Control source (Hospital, Population	Exposure Assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Swanson & Burns (1995) USA, Michigan	627 1972 (all sites)	Population	Age 40–84 yr; phone interview (occupation, smoking)	Women whose usual occupation is dry-cleaning worker	6	2.0 (0.7–6.2)	Adjusted for age at interview, race, and cigarette smoking Possible overlap with Burns &Swanson (1991)
Pesch *et al.* (2000a) Germany, 5 regions: 1991–95	704 2650	Population controls (case age ± 5 yr, same sex)	Hospital record study, in-person interview (case in hospital, control at home), tetrachloethylene JEM	JEM, men, medium exposure	162	1.1 (0.9–1.3)	Age, smoking, study centre
				JEM, women, medium exposure	21	1.8 (1.0–3.0)	Age, smoking, study centre
				JEM, men, high exposure	172	1.2 (1.0–1.5)	Age, smoking, study centre
				JEM, women, high exposure	15	1.0 (0.6–1.9)	Age, smoking, study centre
				JEM, men, substantial exposure	71	1.4 (1.0–1.9)	Age, smoking, study centre
				JEM, women, substantial exposure	3	0.7 (0.2–2.5)	Age, smoking, study centre
				JTEM, men, medium exposure	37	1.0 (0.7–1.5)	Age, smoking, study centre No JTEM data for women
				JTEM, men, high exposure	47	1.2 (0.8–1.7)	Age, smoking, study centre
				JTEM men, substantial exposure	22	1.8 (1.1–3.1)	Age, smoking, study centre
Gaertner *et al.* (2004) Canada, 7 Provinces: 1994–97	535 men 1430 men	Population	Occupational title reported on mailed questionnaire	Male dry-cleaners	4	1.24 (0.23–6.64)	Age, province, race, smoking status, consumption of fruit, fried food, coffee, past occupational exposure
Colt *et al.* (2011) USA, Maine, New Hampshire, Vermont: 2001–04	263 371	Population	Aged 30–79 yr, occupational histories through interview coded by occupation (SOC 7658) and industry (SIC 721)	Women in dry-cleaning plants, except rug	6	2.2 (0.4–11.9)	Age, race, Hispanic ethnicity, state, smoking status, and employment in a high-risk occupation Controls frequency-matched by age (within 5 yr), state, and sex

Table 2.2 (continued)

Reference, study location and period	Total cases Total controls	Control source (Hospital, Population	Exposure Assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Colt et al. (2011) USA, Maine, New Hampshire, Vermont: 2001–04 (cont.)	895 1031	Population	Aged 30–79 yr, occupational histories through interview coded by occupation (SOC 7658) and industry (SIC 721)	Men in dry-cleaning plants, except rug	4	0.9 (0.2–3.8)	Age, race, Hispanic ethnicity, state, smoking status, and employment in a high-risk occupation
Christensen *et al.* (2013) Montreal, Canada	484 533	Population	Occupational exposures derived using subject-reported job history and expert assessment	Men–any exposure to tetrachloroethylene Men–substantial tetrachloroethylene exposure	2 2	0.5 (0.1–3.0) 0.9 (0.1–7.3)	Age, census tract median income, educational attainment (years), ethnicity (French Canadian vs others), questionnaire respondent (self vs proxy), smoking (cigarettes-years), coffee intake, aromatic amines An update of Siemiatycki *et al.* (1994)

JEM, job-exposure matrix; JTEM, job-task exposure matrix; OR, odds ratio; RDD, relative delivered dose; vs, versus

history and exposures. The analysis included 658 cases and 1258 controls. The adjusted odds ratio for dry-cleaning workers was 1.33 (95% CI, 0.50–3.58; seven cases). [It is likely that all the analyses of the National Bladder Cancer Study used the same strategies for exposure assessment, and therefore the "dry-cleaning workers" group may have included launderers.].

In a study of men with urothelial cancer diagnosed in Stockholm, Sweden, in 1985–7, which included 254 cases and 287 referents, there was no increase in risk of urothelial cancer among men who reported working in dry-cleaning (RR, 1.2; 95% CI, 0.2–9.2; two cases) (Steineck *et al.*, 1990).

A case-referent study of cancer of the bladder in Detroit, Michigan, USA, included 2160 cases of cancer of the bladder that were compared with 3979 cases of colorectal cancer identified from a surveillance programme (Burns & Swanson, 1991). The odds ratio for dry-cleaning workers, adjusted for smoking, race, sex, and age, was 1.9 (95% CI, 0.7–4.9; eight cases). Using the same source of data, cases of cancer of the bladder in women diagnosed in 1984–91 were compared with 1972 population controls for "usual occupation" and "ever employed" (Swanson & Burns, 1995). Having a "usual occupation" of dry-cleaning worker was associated with an adjusted (for age, race, and smoking) odds ratio of 2.0 (95% CI, 0.7–6.2; six cases). [The Working Group noted that there may have been some overlap between the cases included in these two studies].

Pesch and colleagues (Pesch *et al.*, 2000a) carried out a study of urothelial carcinoma (bladder, ureter, and renal pelvis) in which exposure to tetrachloroethylene was evaluated using exposure matrices for job title or job task. Analyses with a job-exposure matrix reported relative risks for medium (men: 1.1, 95% CI, 0.9–1.3; women: 1.8, 95% CI, 1.0–3.0), high (men: 1.2, 95% CI, 1.0–1.5; women: 1.0, 95% CI, 0.6–1.9), and substantial (men: 1.4, 95% CI, 1.0–1.9; women: 0.7, 95% CI, 0.2–2.5) exposure. Analyses with a job-task exposure matrix reported relative risks for medium (1.0, 95% CI, 0.7–1.5), high (1.2, 95% CI, 0.8–1.7), and substantial exposure (1.8, 95% CI, 1.1–3.1), for men only.

Risk factors for cancer of the bladder in seven provinces of Canada were examined for 887 cases (535 men) diagnosed in 1994–97 compared with 2847 frequency-matched controls (1430 men) surveyed in 1996 (Gaertner *et al.*, 2004). Among men, the odds ratio for employment as a dry-cleaning worker was 1.24 (95% CI, 0.23–6.64; four cases), adjusted for age, province, race, smoking, and nutritional factors. Data were not reported for women.

A study of cancer of the bladder in Maine, New Hampshire, and Vermont, USA, compared the occupations of 1158 people with cancer of the bladder newly diagnosed in 2001–4 and 1402 population controls. The odds ratio for workers in dry-cleaning plants was 0.86 (95% CI, 0.19–3.81; four cases) for men and 2.19 (95% CI, 0.41–11.85; six cases) for women after adjusting for several covariates, including smoking and occupation with a high risk of bladder cancer (Colt *et al.*, 2011).

Christensen *et al.* (2013) conducted a study of occupational risk factors for selected cancers in men aged 35–70 years in metropolitan Montreal, Canada. In an analysis of 484 cases of cancer of the bladder and 533 population controls, odds ratios were 0.5 (95% CI, 0.1–3.0; two cases) for any exposure to tetrachloroethylene, and 0.9 (95% CI, 0.1–7.3; two cases) for substantial exposure to tetrachloroethylene, after adjusting for smoking and other covariates (Christensen *et al.*, 2013).

2.2.2 Cancer of the upper aerodigestive tract

See Table 2.3.

Two case–control studies evaluated cancer of the upper aerodigestive tract and exposure to tetrachloroethylene, or employment as a dry-cleaner, through personal interviews.

Vaughan *et al.* (1997) reported odds ratios > 1 (not statistically significant) and dose–response or duration–response patterns for laryngeal cancer, although there were few exposed cases. Christensen *et al.* (2013) classified study subjects by degree of occupational exposure to tetrachloroethylene; none of the oesophageal cancer cases were exposed to tetrachloroethylene. [The study by Christensen *et al.* (2013) is an update of the study by Siemiatycki (1991).]

2.2.3 Lymphatic and haematopoietic cancers

See Table 2.4.

Associations between haematopoietic cancers and occupational exposure to tetrachloroethylene have been evaluated in several case–control studies.

In a study in residents exposed to drinking-water contaminated by tetrachloroethylene in Cape Cod, Massachusetts, USA [described in Section 2.2.1] (Aschengrau *et al.*, 1993), the odds ratio for leukaemia was 8.33 (95% CI, 1.53–45.29; two exposed cases) for people with estimated exposure above the 90th percentile. [There were few exposed cases and exposure to tetrachloroethylene was derived from a model.]

Occupational exposure to dry-cleaning fluids was assessed in a case–control study in the state of New York, USA (Kato *et al.*, 2005). The study included incident cases of non-Hodgkin lymphoma in women aged 20–79 years diagnosed between October 1995 and September 1998; 722 were eligible and 376 were included. Population controls (aged < 65 years, from driving-licence records; aged > 65 years, from healthcare records) were selected (1498 eligible, 463 included). Exposure data were collected via telephone interview. For the seven cases in people exposed to dry-cleaning fluids (not further specified), the odds ratio was 1.59 (95% CI, 0.49–5.13), after adjusting for several covariates.

In a case–control study of several haematological malignancies in 12 areas of Italy, incident cases in people aged 20–74 years diagnosed in 1991–1993 were recruited (Miligi *et al.*, 2006; Costantini *et al.*, 2008). The cases included diagnoses of non-Hodgkin lymphoma, including chronic lymphocytic leukaemia (821 men, 607 women); and Hodgkin lymphoma (159 men, 145 women), leukaemia (345 men, 241 women), and multiple myeloma (129 men, 134 women). Controls were randomly selected from the general population. Data on occupational history were collected by person-to-person interviews, and exposures assessed by experts. Age-adjusted Mantel–Haenszel odds ratios were calculated, or odds ratios were calculated with multiple logistic regression taking potential confounders into account. Fourteen cases of non-Hodgkin lymphoma had medium/high exposure to tetrachloroethylene (OR, 1.2; 95% CI, 0.6–2.5) (Miligi *et al.*, 2006). For leukaemia, seven cases had medium/high exposure to tetrachloroethylene (OR, 1.0; 95% CI, 0.4–2.7) (Costantini *et al.*, 2008).

A case–control study in six regions in Germany included 710 incident cases of malignant lymphoma in people aged 18–80 years and an equal number of population controls. Controls were identified from the population register, and recruitment continued until one participating control had been identified for each participating case. Face-to-face interviews were conducted. An industrial physician estimated cumulative exposure to tetrachloroethylene (intensity × frequency × years). The odds ratios associated with levels of exposure to tetrachloroethylene were 1.1 (95% CI, 0.5–2.3; 16 cases) for low exposure; 1.0 (95% CI, 0.5–2.2; 14 cases) for medium exposure; and 3.4 (95% CI, 0.7–17.3; six cases) for high exposure (Seidler *et al.*, 2007).

Gold *et al.* (2011) conducted a case–control study in the Seattle-Puget Sound region of Washington and the Detroit metropolitan area of Michigan, USA, between 1 January 2000 and 31 March 2002. The analysis included 181 incident cases of multiple myeloma in people aged

Table 2.3 Case–control studies of cancers of the upper aerodigestive tract and exposure to tetrachloroethylene

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 Comments
Vaughan *et al.* (1997), Washington (USA), 1983–87 and 1987–90	491 oral cavity and pharynx, 235 larynx, 404 oesophagus and gastric cardia 724 controls	Population	Personal interview		Duration of employment in dry-cleaning industry			Age, sex, education, study period, alcohol consumption, cigarette smoking
				Oral cavity	Never (< 6 mo)	484	1.0 (ref)	
					Ever (≥ 6 mo)	7	1.2 (0.3–4.6)	
					1–9 yr	6	1.4 (0.3–5.7)	
					≥ 10 yr	1	0.4 (0.0–31.6)	
					Exposure to tetrachloroethylene			
					Possible	7	1.2 (0.3–4.6)	
					Probable	4	1.5 (0.2–9.5)	
					1–29 ppm-yr	3	1.0 (0.1–7.0)	
					≥ 30 ppm-yr	4	1.4 (0.2–8.7)	
					Duration of employment in dry-cleaning industry			
				Larynx	Never (< 6 mo)	230	1.0 (ref)	
					Ever (≥ 6 mo)	5	2.7 (0.6–10.9)	
					1–9 yr	3	1.9 (0.3–10.8)	
					≥ 10 yr	2	5.5 (0.4–75.0)	
					Exposure to tetrachloroethylene			
					Possible	4	2.3 (0.5–10.2)	
					Probable	1	0.9 (0.1–12.9)	
					1–29 ppm-yr	2	2.0 (0.2–17.9)	
					≥ 30 ppm-yr	2	2.5 (0.3–19.1)	
					Duration of employment in dry-cleaning industry			
				Oesophagus (SCC)	Never (< 6 mo)	107	1.0 (ref)	
					Ever (≥ 6 m)	2	3.6 (0.5–27.0)	
					1–9 yr	2	4.6 (0.5–39.4)	

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 Comments
Vaughan et al. (1997), Washington (USA), 1983–87 and 1987–90 (cont.)					≥ 10 yr	0	–	
					Exposure to tetrachloroethylene			
					Possible	2	3.6 (0.5–27.0)	
					Probable	2	6.4 (0.6–68.9)	
					1–29 ppm-yr	2	11.9 (1.1–124)	
					≥ 30 ppm-yr	0	–	
					Duration of employment in dry-cleaning industry			
				Oesophagus (ADC)	Never (< 6 mo)	293	1.0 (ref)	
					Ever (≥ 6 mo)	2	1.1 (0.2–5.7)	
					1–9 yr	1	0.8 (0.1–7.7)	
					≥ 10 yr	1	1.7 (0.1–26.5)	
					Exposure to tetrachloroethylene			
					Possible	2	1.1 (0.2–5.7)	
					Probable	1	0.9 (0.1–10.0)	
					1–29 ppm-yr	1	2.0 (0.2–21.7)	
					≥ 30 ppm-yr	1	0.7 (0.1–6.8)	
Christensen *et al.* (2013), Montreal (Canada) 1979–85	99 533	Population	Personal interview + expert assessment	Oesophagus (ICD-9 150)	Tetrachloroethylene exposure	0	–	

ADC, adenocarcinoma; mo, month; SCC, squamous cell carcinoma; yr, year

Table 2.4 Case–control studies of haematopoietic cancers and exposure to tetrachloroethylene

Reference, study location, period	Total cases Total controls	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Convariates Comments
Kato *et al.* (2005), New York State, USA, 1995–98	376 female NHL patients 463 selected from driver licence/health care records	Telephone interview	Exposed to dry-cleaning fluid	7	1.59 (0.49–5.13)	Age at index date, family history of hematologic cancer, college education, surrogate status, year of interview, body mass index 10 years before interview, average frequency of use of pain-relieving drugs, total number of episodes of systemic antibiotic use, total number of uses of household pesticide products, duration of work involving pesticide exposures
Miligi *et al.* (2006); Costantini *et al.* (2008)	1135 NHL+chronic lymphocytic leukaemia patients 1246 population controls	Interview	Expert-assessed exposure to TETRA:			Sex, age, education, area Costantini *et al.* (2001), Miligi *et al.* (1999, 2006) derive from the same data set
			Very low/low	18	0.6 (0.3–1.2)	
			Medium/high	14	1.2 (0.6–2.5)	
	586 with leukaemia 1278 population controls	Interview	Expert assessed exposure to TETRA:	6	0.6 (0.2–1.6)	Sex, age, education, area
			Very low/low			
			Medium/high exposure	7	1.0 (0.4–2.7)	
Seidler *et al.* (2007), Germany	710 lymphoma patients 710 population controls	Interview	Expert-assessed TETRA exposure			Smoking and alcohol; Controls were matched on region, sex and age
			Low (> 0 to ≤ 9.1)	16	1.1 (0.5–2.3)	
			Medium (> 9.1 to ≤ 78.8)	14	1.0 (0.5–2.2)	
			High (> 78.8)	6	3.4 (0.7–17.3)	
Gold *et al.* (2011), Seattle-Puget Sound/Detroit, USA, 2000–02	181 with multiple myeloma 481 controls identified by random-digit dialling/Medicare records	Interview	Job-exposure-matrix for TETRA	29	1.4 (0.9–2.4)	Age, race, study site, gender and years of education
			Highest category of cumulative exposure	14	2.5 (1.1–5.4)	

Table 2.4 (continued)

Reference, study location, period	Total cases Total controls	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Convariates Comments
Christensen *et al.* (2013) Montreal, Canada, 1979–85	215 hospitalized NHL patients	Personal interview + expert assessment				Age, census tract median income, educational attainment (years), ethnicity (French Canadian vs others), questionnaire respondent (self vs proxy), smoking
	533 population controls		Any TETRA exposure	3	2.2 (0.5–10)	Update of Siemiatycki (1991)
			Substantial TETRA exposure	2	2.6 (0.4–19)	
	2341 cancer controls		Any exposure	3	1.7 (0.5–6.2)	
			Substantial exposure	2	1.7 (0.3–8.5)	

NHL, non-Hodgkin lymphoma; TETRA, tetrachloroethylene

35–74 years and 481 population controls selected by random-digit dialling (aged < 65 years) and from Medicare files (aged ≥ 65 years). In-person interviews were conducted with recording of occupational history and a job-exposure matrix for solvents was applied. Twenty-nine cases had ever been exposed to tetrachloroethylene (OR, 1.4; 95% CI, 0.9–2.4). A significant trend (P = 0.04) was observed with cumulative exposure level (intensity × frequency × years summed over all exposed jobs): in the highest exposure category, the odds ratio was 2.5 (95% CI, 1.1–5.4). In a further analysis excluding jobs for which the exposure assessor had low confidence the odds ratio was 1.5 (95% CI, 0.8–2.9) for ever exposure and 3.3 (95% CI, 1.2–9.5) in the highest category (Gold *et al.*, 2011). [The Working Group noted that only half of the identified cases participated in the study.]

Cases in men aged 35–70 years diagnosed in Montreal, Canada, between September 1979 to June 1985 were included in a case–control study by Christensen *et al.* (2013) based on the population studied previously by Siemiatycki (1991). In total, 215 cases of non-Hodgkin lymphoma were recruited and 2341 cases of other cancers, and a population sample served as controls. Personal interviews were conducted and exposure was assessed by expert evaluation. Exposure to tetrachloroethylene was associated with an odds ratio of 1.7 (95% CI, 0.5–6.2; three exposed cases) when combining cancer and population controls, using proportional weighting.

2.2.4 Cancer of the kidney

See Table 2.5.

Seven case–control studies have examined risk of cancer of the kidney associated with occupations involving exposure to tetrachloroethylene, particularly dry-cleaning. All studies except one conducted personal interviews with study subjects.

The study by Delahunt *et al.* (1995) was based on cancer-registry data and exposure assessment relied on occupational data recorded in the cancer registry. Two studies applied job-exposure matrices and classified subjects by degree of exposure to tetrachloroethylene (Dosemeci *et al.*, 1999; Pesch *et al.*, 2000b). In one study, a team of chemists and industrial hygienists translated job titles into potential exposures (Christensen *et al.*, 2013). In one study, subjects were classified according to occupational exposure to dry-cleaning solvents (Mandel *et al.*, 1995). In the remaining studies, subjects were classified by job title (Asal *et al.*, 1988; Delahunt *et al.*, 1995; Karami *et al.*, 2012). All studies reported positive associations for men, or for women, or both, although statistical significance was only reached in three studies (Delahunt *et al.*, 1995; Mandel *et al.*, 1995; Pesch *et al.*, 2000b), and one of them reported only crude risk estimates (Delahunt *et al.*, 1995). Among the three studies that evaluated dose–response or duration–response trends (Mandel *et al.*, 1995; Pesch *et al.*, 2000b; Karami *et al.*, 2012), only Karami *et al.* (2012) reported monotonic positive trends. Two studies reported protective (non-significant) associations among women (Dosemeci *et al.*, 1999; Pesch *et al.*, 2000b) and one study among men (Asal *et al.*, 1988). [The study by Christensen *et al.* (2013) was based in the same population included in the study by Siemiatycki (1991), with refinements in exposure assessment and case classification.]

Environmental exposure to tetrachloroethylene has been evaluated in a case–control study (Aschengrau *et al.*, 1993, described in Section 2.2.1) which included cancers of the kidney and other sites. None of the 35 kidney cancer cases were classified as exposed to tetrachloroethylene.

2.2.5 Cancer of the breast

Several analyses have been undertaken to evaluate the risk of cancer of the breast among women exposed to drinking-water contaminated with

Table 2.5 Case–control studies of kidney cancer and occupational exposure to tetrachloroethylene

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 Comments
Asal *et al.* (1988), Oklahoma (USA), 1981–84	315 313 (hospital), 336 (population)	Hospital and population	Personal interview	Renal cell carcinoma (ICD not reported)	Dry-cleaning workers, men Dry-cleaning workers, women	3 8	0.7 (0.2–2.3) 2.8 (0.8–9.8)	Age, smoking, weight Response rates not reported; [In Oklahoma petroleum solvents are more often used in dry-cleaning than tetrachloroethylene]
Delahunt *et al.* (1995), New Zealand, 1978–86	710 (men), from the cancer registry 12 756 (men)	Cancer registry, primary tumours from sites other than the urinary tract	Occupational data recorded in the cancer registry. Occupation classified using the New Zealand Standard Classification of Occupations, a modification of the ISCO	Malignant neoplasms of the kidney, excluding the renal pelvis (ICD 189.0)	Dry-cleaning workers	Not reported	1.92 (0.27–13.89)	Unadjusted estimates Occupational code was available for 98.9% cases and for all controls
Mandel *et al.* (1995), Australia, Denmark, Germany, Sweden, USA	1732 2309	Population	Personal interviews	Renal cell adenocarcinoma (ICD-9 189.0)	Dry-cleaning solvents (men) Duration of occupational exposure 1–7 years 8–25 years 26–60 years Dry-cleaning solvents (women)	245 70 98 75 57	1.4 (1.1–1.7) 1.2 (0.9–1.8) 1.7 (1.2–2.4) 1.2 (0.9–1.8) 1.6 (1.0–2.7)	Age, smoking status, body mass index, education and study centre Response rates: 72.3% (cases), 74.7% (controls) Overlaps with Mellemgaard *et al.* (1994) and McCredie & Stewart (1993) The only statistically significant association among women

Table 2.5 (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 Comments
Dosemeci *et al.* (1999) Minnesota (USA), 1988–90	438 687	Population	Personal interview and job-exposure matrix (JEM)	Renal cell carcinoma (ICD not reported)	Tetrachloroethylene exposure, All	50	1.07 (0.7–1.6)	Age, gender (for total), smoking, hypertension and/or use of diuretics and/or anti-hypertension drugs and body mass index Response rates: 87% (cases), 86% (controls)
					Tetrachloroethylene exposure, Men	42	1.12 (0.7–1.7)	
					Tetrachloroethylene exposure, Women	8	0.82 (0.3–2.1)	
Pesch *et al.* (2000b) Germany, 1991–95	935 4298	Population	Interviewer-administered standardized questionnaire, plus job-exposure (JEM) and job task-exposure matrices (JTEM)	Renal cell carcinoma (ICD not reported)	German JEM, Men			Age, study centre, smoking Response rates: 88% (cases), 71% (controls)
					Unexposed and < 30th percentile	not reported	1.0 (ref)	
					Medium (> 30th percentile)	154	1.4 (1.1–1.7)	
					High (> 60–90th percentile)	119	1.1 (0.9–1.4)	
					Substantial (> 90th percentile)	50	1.4 (1.0–2.0)	
					German JEM, Women			
					Unexposed and < 30th percentile	not reported	1.0 (ref)	
					Medium (> 30th percentile)	12	0.7 (0.4–1.3)	
					High (> 60–90th percentile)	19	1.1 (0.7–1.9)	
					Substantial (> 90th percentile)	4	0.7 (0.3–2.2)	
					JTEM, Men			
					Unexposed and < 30th percentile	not reported	1.0 (ref)	
					Medium (> 30th percentile)	44	1.2 (0.9–1.7)	
					High (> 60–90th percentile)	39	1.1 (0.7–1.5)	

Table 2.5 (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 Comments
Pesch et al. (2000b) Germany, 1991–95 (cont.)					Substantial (> 90th percentile) JTEM, Women	15	1.3 (0.7–2.3)	
					Unexposed and < 30th percentile	not reported	1.0 (ref)	
					Medium (> 30th percentile)	8	2.2 (0.9–5.2)	
					High (> 60–90th percentile)	6	1.5 (0.6–3.8)	
					Substantial (> 90th percentile)	3	2.0 (0.5–7.8)	
Karami *et al.* (2012), Detroit and Chicago (USA), 2003–07	1217 1235	Population	Personal interviews	Kidney (ICD-O C64)	Industry: dry-cleaning plants, except rug			Sex, age at reference date, race, study centre, education level, history of hypertension, smoking status, BMI (5 years before interview), family history of cancer Response rates: 77.5% (cases), 54.4% (controls); controls frequency matched by age group, race, sex, study centre
					never	1169	1.0 (ref)	
					ever	15	2.0 (0.9–4.4)	
					< 5 years	11	1.8 (0.6–5.4)	
					≥ 5 years	4	2.5 (0.4–14.4)	
					P for trend		0.093	

Table 2.5 (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 Comments
Christensen *et al.* (2013), Montreal 1979–85	177 533	Population	Personal interviews	Kidney (ICD-9 189)	Occupational exposure to tetrachloroethylene			Age, census track median income, educational attainment (years), ethnicity (French Canadian versus other), questionnaire respondent (self versus proxy), smoking (cigarette-years), coffee, beer, wine, and spirit intake Only men. Response rates: 82% (all cancer patients, unspecific for kidney), 72% (controls)
					Any exposure	2	1.6 (0.3–9.4)	
					Substantial exposure	2	3.1 (0.4–24)	

tetrachloroethylene in Cape Cod, Massachusetts, USA (Aschengrau *et al.*, 1998, 2003; Vieira *et al.*, 2005; Gallagher *et al.*, 2011). The Working Group focused on the results of the latest re-analysis of these data (Gallagher *et al.*, 2011). Study participants were permanent residents of eight towns in the Cape Cod region. Incident cancers of the breast diagnosed in 1983–1993 were identified from the Massachusetts cancer registry. Controls were demographically similar women living in Cape Cod in 1983–1993, identified by random-digit dialling (age < 65 years), from Medicare files (age ≥ 65 years), or death certificates. Telephone or personal interviews were used to collect residential history and other data. Exposure to tetrachloroethylene was estimated using modelling techniques (manual and automated algorithms). [The automated model redefined many unexposed subjects from the manual model as having low exposure.] The analysis included 930 cases and 1302 controls for the manual assessment, and 920 cases and 1293 controls for the automated assessment. Adjusted for age at diagnosis, vital status at interview, family history of breast cancer, personal history of breast cancer (before current diagnosis or index year), age at first live birth/stillbirth, occupational exposure, and study of origin, the odds ratio estimates based on the manual exposure assessment was 1.0 (95% CI, 0.8–1.2). Estimated exposure at > 75th and > 90th percentiles gave adjusted odds ratios of 1.6 (95% CI, 1.1–2.4) and 1.3 (95% CI, 0.7–2.6), respectively. For the automated assessment, the odds ratio was 1.3 (95% CI, 0.9–1.9). [There may be selection bias as only a small proportion of the selected controls could be reached.]

2.2.6 Cancer of the lung

See Table 2.6.

Two case–control studies of cancer of the lung investigated occupational exposure to tetrachloroethylene; both reported increased risks of lung cancer among those exposed to tetrachloroethylene after adjusting for smoking (Brownson *et al.*, 1993; Vizcaya *et al.*, 2013). One case–control study of cancer of the lung assessed residential exposure to tetrachloroethylene through contaminated drinking-water (Paulu *et al.*, 1999). Results showed increased risks for those in the highest categories of exposure.

One of the studies of occupational exposure included cases in nonsmokers (Brownson *et al.*, 1993). Both studies of occupational exposure conducted face-to-face personal interviews. Risks were reported by job title (Brownson *et al.*, 1993) or by degree of exposure to tetrachloroethylene Vizcaya *et al.* (2013). Increased risks of cancer of the lung in female dry-cleaning workers were reported by Brownson *et al.* (1993) (OR, 1.8; 95% CI, 1.1–3.0), adjusted for age, smoking and previous lung disease. For lifetime nonsmokers, the odds ratio was 2.1 (95% CI, 1.2–3.7), adjusted for age and previous lung disease. Increased risk of cancer of the lung associated with exposure to tetrachloroethylene was reported by Vizcaya *et al.* (2013) (OR, 2.54; 95% CI, 1.25–5.26, for any exposure; and OR, 2.4; 95% CI, 0.8–7.7, for substantial exposure). [The study by Vizcaya *et al.* (2013) was a re-evaluation of the Siemiatycki (1991) study, including improved exposure assessment in the same study population.]

Paulu *et al.* (1999) conducted a population-based case–control study to evaluate the risk of cancers of the lung, colon and rectum, brain and pancreas associated with residential exposure to tetrachloroethylene. Cases were incident cancers diagnosed between 1983 and 1986, and resident in the upper Cape towns. Among the selected study subjects, 79.2% of cases of lung cancer, 83.2% of Medicare controls, and 81.1% of next-of-kin for deceased controls were contacted and interviewed. Among controls identified by random-dialling, 73.9% of the eligible and contacted subjects were interviewed. The relative delivered dose of tetrachloroethylene was estimated using a model that took into account residential location, duration of residence, water flow,

Table 2.6 Case–control studies of lung cancer and occupational exposure to tetrachloroethylene

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 Comments
Brownson *et al.* (1993), Missouri (USA), 1986–91	429 (non-smoking women, from the cancer registry) 1021 (non-smoking women)	Population (driver's license and Medicare files)	Interview, occupational and medical history	Lung cancer (ICD not reported)	Dry-cleaning workers (all subjects)	30	1.8 (1.1–3.0)	Age, history of previous lung disease, and active smoking
					Dry-cleaning workers (lifetime nonsmokers)	23	2.1 (1.2–3.7)	Age and history of previous lung disease Response rates: 69% (cases), 73% (controls)
Vizcaya *et al.* (2013), Montreal (Canada). 1980–86 (study 1), 1995–2001 (study 2)	2016 [study 1: 851 (all men), study 2: 430 (women), 735 (men)] 2001 [study 1: 533 (all men), study 2: 570 (women), 898 (men)]	Population	Personal interview	Lung cancer (ICD not reported)	Tetrachloroethylene exposure			Age, smoking habit, educational attainment, socioeconomic status, ethnicity and exposure to eight known carcinogens Response rates: Study 1: 79% (cases), 70% (controls). Study 2: 86% (cases), 70% (controls). Odds ratios are only reported for men
					Any, pooled	23	2.5 (1.2–5.6)	
					Substantial, pooled	10	2.4 (0.8–7.7)	

and pipe characteristics. Adjusted odds ratios for cancer of the lung were moderately elevated among subjects whose exposure level was above the 90th percentile, whether or not a latent period was assumed [ORs and 95% CIs, 3.7 (1.0–11.7), 3.3 (0.6–13.4), 6.2 (1.1–31.6), and 19.3 (2.5–141.7) for 0, 5, 7, and 9 years of latency, respectively]. Results for other cancer sites considered in this study are reported in a subsequent section.

2.2.7 Cancer of the liver

See Table 2.7.

The risk of cancer of the liver associated with occupational exposure to tetrachloroethylene was evaluated in three case–control studies. Two studies included deceased subjects only, while the study by Christensen *et al.* (2013) included both living and deceased subjects. Exposure assessment was based on personal interviews with living study subjects (Christensen *et al.*, 2013), company work history records (Bond *et al.*, 1990) or occupation and kind of business or industry as recorded on the death certificate (Suarez *et al.*, 1989). Subjects were classified by exposure to tetrachloroethylene (Bond *et al.*, 1990; Christensen *et al.*, 2013), or by job title (Suarez *et al.*, 1989). Increased risk of cancer of the liver associated with exposure to tetrachloroethylene was reported by two studies (Bond *et al.*, 1990; Christensen *et al.*, 2013).

2.2.8 Cancer of the brain

See Table 2.8.

Three case–control studies of cancer of the brain evaluated occupational exposure to tetrachloroethylene. None of the studies reported statistically significant increased risks.

Heineman *et al.* (1994) studied white men with astrocytic tumours in the USA. A total of 111 cases had job titles that were compatible with exposure to tetrachloroethylene (OR, 1.2; 95% CI, 0.8–1.6). None of the risk estimates for subgroups of increasing probability, duration or intensity of exposure reached statistical significance. Neta *et al.* (2012) evaluated glioma and meningioma in Arizona, Massachusetts and Pennsylvania in relation to occupational exposure to tetrachloroethylene. Exposure was evaluated through personal interviews, and odds ratios tended to be around 1 and non-statistically significant. These studies are described in more detail in the *Monograph* on trichloroethylene.

Ruder *et al.* (2013) evaluated glioma risk from non-farm occupational exposure (ever/never and estimated cumulative exposure) to tetrachloroethylene among 798 cases and 1175 population-based controls, aged 18–80 years and non-metropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin, United States. Solvent use was estimated based on occupation, industry, and era, using a bibliographic database of published exposure levels and exposure determinants. Unconditional logistic regression was used to calculate odds ratios adjusted for frequency matching variables age group and sex, and age and education. Ever exposure to tetrachloroethylene was associated with reduced risk of glioma (OR, 0.8; 95% CI, 0.6–0.9, 299 cases and 500 controls exposed). Mean estimated cumulative exposure was similar for cases (3.5 ppm-years) and controls (3.1 ppm-years), with OR of glioma of 1.0 (0.9–1.1) for a 1-unit increase in natural-log transformed exposures in ppm–years. In analyses limited to 904 participant blood donors (excluding controls reporting a previous cancer diagnosis) genotyped for glutathione-*S*-transferases *GSTP1, GSTM3*, and *GSTT1*, solvent-exposed individuals with functional *GST* genes that might convert chlorinated solvents crossing the blood-brain barrier into cytotoxic metabolites were not at increased risk of glioma. [Study limitations include the high percentage of proxy case responses and the lack of workplace or serum measurements of solvent levels.]

Brain cancer and non-occupational tetrachloroethylene exposure was evaluated by Paulu

Table 2.7 Case–control studies of liver cancer and occupational exposure to tetrachloroethylene

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 Comments
Suarez *et al.* (1989), Texas (USA) 1969–80,	1742 (cancer deaths), only males 1742	Death certificate records	Occupation and kind of business or industry as recorded on the death certificate	Primary cancer of the liver (ICD 155.0)	Dry-cleaning services	11	0.98 (0.44–2.20)	Age, race, ethnicity [Dry-cleaning solvent in Texas more likely to be Stoddard solvent than PCE];, Controls excluded all neoplasms (ICD 140–239), diseases of the liver and gallbladder (ICD 570–576), infectious hepatitis (ICD 070) and alcoholism (ICD 303)
					Dry-cleaning operators	4	0.55 (0.17–1.75)	
Bond *et al.* (1990), Michigan, USA 1940–82	44 1888 controls		Company work records	Liver, gallbladder or bile ducts (ICDA-8 155–156 and 197.8)	Exposed to tetrachloroethylene	6	1.8 (0.8–4.3)	Age
Christensen *et al.* (2013), Montreal (Canada) 1979–85	48 533	Population	Personal interviews	kidney (ICD-9 189)	Occupational exposure to tetrachloroethylene			Age, census track median income, educational attainment (years), ethnicity (French Canadian versus Other), questionnaire respondent (self versus Proxy), smoking (cigarette-years), beer, wine, and spirit intake Only males. Response rates: 82% (all cancer patients, unspecific for kidney), 72% (controls)
					Any exposure	1	3.3 (0.2–60)	
					Substantial exposure	1	4.4 (0.2–103)	

Table 2.8 Case–control studies of cancer of the brain and exposure to tetrachloroethylene

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 Comments
Heineman *et al.* (1994), Lousiana (USA) 1978–80 and New Jersey and Philadelphia (USA) 1979–81	654 (from death certificates) 612	Death certificates	Next-of-kin personal interview	Brain or other central nervous system tumour (ICD-9 191, 192, 225, 239.7)	Occupational tetrachloroethylene exposure			Age and study area Response rates: 88% (cases), 83% (controls)
					Ever			
					Any	111	1.2 (0.8–1.6)	
					Low probability, ever	72	1.3 (0.8–1.9)	
					Medium probability, ever	30	0.9 (0.5–1.6)	
					High probability, ever	9	1.2 (0.4–3.5)	
					Exposed 2–20 years			
					Any	71	1.1 (0.7–1.6)	
					Low probability	50	1.1 (0.7–1.8)	
					Medium probability	15	0.9 (0.4–1.9)	
					High probability	6	1.0 (0.3–3.7)	
					Low-medium average intensity	64	1.0 (0.7–1.6)	
					High intensity	7	1.2 (0.4–4.4)	
					Exposed ≥21 years			
					Any	28	1.4 (0.7–2.7)	
					Low probability	14	1.6 (0.6–4.0)	
					Medium probability	11	1.0 (0.4–2.6)	
					High probability	3	∞	
					Low-medium average intensity	25	1.3 (0.7–2.4)	
					High intensity	3	∞	
					Low cumulative exposure			
					Any	33	0.8 (0.5–1.4)	
					Low probability	25	0.8 (0.4–1.5)	
					Medium probability	7	1.0 (0.3–3.1)	
					High probability	1	0.5 (0.0–7.4)	

Table 2.8 (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 Comments
Heineman et al. (1994), Lousiana (USA) 1978–80 and New Jersey and Philadelphia (USA) 1979–81 (cont.)					Medium cumulative exposure			
					Any	45	1.3 (0.8–2.2)	
					Low probability	27	1.6 (0.8–3.1)	
					Medium probability	13	1.0 (0.4–2.4)	
					High probability	5	1.2 (0.3–5.4)	
					High cumulative exposure			
					Any	21	1.5 (0.7–3.2)	
					Low probability	12	1.8 (0.7–5.1)	
					Medium probability	6	0.8 (0.2–2.6)	
					High probability	3	∞	
					Summary measures			
					Low-medium average intensity, total	89	1.1 (0.8–1.6)	
					High intensity, total	10	1.8 (0.6–5.9)	
					Low probability	Not reported	1.0 (0.5–1.8)	Age, study area employment in electronics occupations/industries and exposure to other chlorinated aliphatic hydrocarbons
					Medium probability	Not reported	0.5 (0.2–1.3)	
					High probability	Not reported	1.2 (0.4–3.9)	
Paulu et al. (1999), 5 upper Cape towns (USA) 1983–86	37 703	Population	Personal interview	Not specified	Drinking-water exposure			Crude Response rates: 86% (cases), 74% (random-digit-dial controls), 76% (Health Care Financing Administration controls), 79% (next-of-kin for deceased controls)
					0 yrs latent period	3	0.6 (0.1–1.7)	
					5 yrs latent period	3	1.0 (0.2–2.9)	
					7 yrs latent period	2	0.9 (0.1–3.0)	
					9 yrs latent period	1	0.7 (0.0–3.4)	

Table 2.8 (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 Comments
Neta *et al.* (2012), Arizona, Massachusetts and Pennsylvania (USA), 1994–98	489 glioma, 197 meningioma 799	Hospital	Personal interviews	Glioma or other neuroepitheliomatous neoplasm (ICD-O-2 9380–9473 and 9490–9506), meningioma (ICD-O-2 9530–9538) or acoustic neuroma (ICD-O-2 9560)	Occupational tetrachloroethylene exposure			Age group, race, sex, hospital site and proximity of residence to hospital Response rates: 92% (glioma cases), 94% (meningioma cases), 86% (controls)
					Possible, men	102	0.7 (0.5–1.0)	
					Probable, men	6	1.2 (0.4–3.8)	
					Possible, women	34	0.7 (0.5–1.1)	
					Probable, women	3	0.5 (0.1–1.7)	
					Possible, all	136	0.7 (0.5–0.9)	
					Probable, all	9	0.7 (0.3–1.6)	
Ruder *et al.* (2013) Iowa, Michigan, Minnesota, Wisconsin (USA) 1995–97	798 1175	Population	Personal interviews plus industrial hygienist evaluation	Glioma ICD-O-9380–948	Any tetrachloroethylene	299	0.75 (0.62–0.91)	Age, education, sex
					Men	216	0.81 (0.64–1.04)	
					Women	83	0.66 (0.48–0.91)	

et al. (1999) among 37 cases, in a study setting where exposure occurred through contaminated drinking-water. [Low numbers of brain tumours hampered the estimation of risks.] Only crude associations were reported, showing non-significant protective associations with wide confidence intervals.

2.2.9 Other cancers

Christensen *et al.* (2013) evaluated cancers of the prostate, colon, stomach and rectum, and melanoma associated with occupational tetrachloroethylene exposure. Substantial exposure was associated with a significant increased risk of prostate cancer (9 exposed cases; OR, 6.0; 95% CI, 1.2–30), and non-statistically significant increased risk of colon cancer (3 exposed cases; OR, 1.8; 95% CI, 0.3–11), stomach cancer (2 exposed cases; OR, 2.1; 95% CI, 0.3–17), rectal cancer (1 exposed case; OR, 1.1; 95% CI, 0.1–13) and melanoma (1 exposed case; OR, 2.6; 95% CI, 0.2–33). Pancreatic cancer was evaluated but no cases were exposed to tetrachloroethylene.

Environmental tetrachloroethylene exposure has been investigated by Paulu *et al.* (1999) (see Section 2.2.8) in relation to colorectal cancer (326 cases) and pancreatic cancer (37 cases). The adjusted ORs for colorectal cancer were modestly elevated among ever-exposed subjects, and did vary substantially as more years of latency were assumed. Adjusted ORs for rectal cancer among ever-exposed subjects were more elevated than the corresponding estimates for colon cancer. Low numbers for pancreatic cancer hampered the estimation of risks. Only crude associations were reported, showing non-significant protective associations with wide confidence intervals.

2.3 Ecological studies

Exposure to tetrachloroethylene rarely occurs in an isolated manner. Episodes of water pollution usually occur from industrial sources and have involved different solvents including tetrachloroethylene and trichloroethylene. Most of the ecological studies reviewed in the *Monograph* on trichloroethylene also consider tetrachloroethylene, and results cannot disentangle the effects of the two chemicals. Specific methods, results and limitations concerning these studies are detailed in the *Monograph* on trichloroethylene in this volume. Some of these studies reported increased incidence rates of cancers of the testis and kidney (ATSDR, 2006, 2008), breast (Coyle *et al.*, 2005), bladder (Mallin, 1990) and uterus, and skin melanoma (Morgan & Cassady, 2002), while another study (Isacson *et al.*, 1985) that evaluated cancers of the bladder, breast, colon, lung, prostate, and rectum did not observe associations with exposure.

Haematopoietic cancers have been evaluated in ecological studies, with mixed results. Cohn *et al.* (1994) found increased rates of leukaemia and non-Hodgkin lymphoma among women, and increased rates of childhood acute lymphocytic leukaemia among girls. Vartiainen *et al.* (1993) observed a marginally increased risk of non-Hodgkin lymphoma, while ATSDR (2006) did not find evidence for an association with childhood leukaemia. One study was conducted in Finland (Vartiainen *et al.*, 1993), while the others were conducted in the USA.

Exposure to tetrachloroethylene may also occur through inhalation. Ma *et al.* (2009) conducted an ecological study in New York City, USA, where there were about 900 small dry-cleaning facilities using tetrachloroethylene. The risk of cancer of the kidney was evaluated in association with living near a dry-cleaning facility using tetrachloroethylene. The unit of analysis was the population with a particular postal code. The outcome variable was the number of hospital discharges for each postal code with a diagnosis of cancer of the kidney. The density of dry-cleaning facilities using tetrachloroethylene in each postal code was a surrogate measure of exposure. Higher densities of dry-cleaning facilities using

tetrachloroethylene were associated with higher rate ratios of kidney cancer, with a rate ratio of 1.15 (95% CI, 1.01–1.30) among those living in areas with the highest densities compared with those in the lowest. A non-monotonic increasing dose–response pattern was observed. [*P*-values for trend were not presented.]

2.4 Meta-analyses

In a meta-analysis on cancer of the pancreas and exposure to solvents, Ojajärvi *et al.* (2001) calculated a meta-relative risk for pancreatic cancer among dry-cleaning workers of 1.4 (95% CI, 1.1–2.4; based on eight populations). [Exposures to other solvents also occurred in these populations.]

3. Cancer in Experimental Animals

The carcinogenicity of tetrachloroethylene in experimental animals was last reviewed by an IARC Working Group in 1995 (IARC, 1995), and more recently by the United States Environmental Protection Agency (EPA, 2012).

3.1 Mouse

See Table 3.1

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age 5–7 weeks) were given tetrachloroethylene (purity, 99%) by gavage in corn oil on 5 days per week for 78 weeks (NCI, 1977; Weisburger, 1977). Groups of 20 male and 20 female mice were given vehicle only and served as controls. Dosage adjustments were made during the exposure period: male mice were given tetrachloroethylene at a dose of 450 or 900 mg/kg body weight (bw) for 11 weeks, and then at 550 or 1100 mg/kg bw for 67 weeks; female mice were given tetrachloroethylene at a dose of 300 or 600 mg/kg bw for 11 weeks, and then at 400 or 800 mg/kg bw for 67 weeks. Time-weighted average doses of tetrachloroethylene were 536 and 1072 mg/kg bw per day, respectively, for males, and 386 and 772 mg/kg bw per day, respectively, for females. The treatment period was followed by a 12-week observation period. Mortality was significantly increased in treated mice compared with controls. Significant dose-related positive trends and increased incidences of hepatocellular carcinoma in all treatment groups were observed in males and females. In male mice, the incidences were 2 out of 20 (vehicle controls), 32 out of 49 (lower dose), and 27 out of 48 (higher dose); the corresponding incidences in females were 0 out of 20, 19 out of 48, and 19 out of 48. Exposure to tetrachloroethylene caused toxic nephropathy (characterized in this study as degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla, with cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium) in male mice (0/20, 40/49, 45/48) and female mice (0/20, 46/48, 48/48). A rare renal tubular cell carcinoma was observed in a male at the lower dose. [The Working Group noted that the study animals were housed in the same rooms as animals exposed to volatile agents, the group size for vehicle controls was small, and decreased survival and the 78-week exposure period reduced the power of this study to detect the full carcinogenic potential of the test agent.]

3.1.2 Inhalation

Groups of 49–50 male and 49–50 female B6C3F$_1$ mice (age, 8–9 weeks) were exposed to air containing tetrachloroethylene (purity, 99.9%) at concentrations of 0, 100, or 200 ppm (0, 680, or 1360 mg/m^3) for 6 hours per day on 5 days per week for up to 103 weeks (Mennear *et al.*, 1986; NTP, 1986). Survival was significantly reduced for males at both doses, and for females at the

Table 3.1 Studies of carcinogenicity in experimental animals exposed to tetrachloroethylene

Species, strain (sex) Duration Reference	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 90 wk NCI (1977); Weisburger, (1977)	Gavage in corn oil 0 (vehicle control), 536, 1072 mg/kg bw per day, 5 days/wk for 78 wk, 20, 50, 50/group	Hepatocellular carcinoma: 2/20, 32/49, 27/48	Cochran-Armitage test, Fisher exact test $P < 0.01$ (trend), $P < 0.001$ (lower dose, higher dose)	Purity, 99%, survival (reduction): 50%, 38%, 20%
Mouse, B6C3F$_1$ (F) 90 wk NCI (1977); Weisburger (1977)	Gavage in corn oil 0 (vehicle control), 386, 772 mg/kg bw per day, 5 days/wk for 78 wk, 20, 50, 50/group	Hepatocellular carcinoma: 0/20, 19/48, 19/48	Cochran-Armitage test, Fisher exact test $P < 0.01$ (trend), $P < 0.001$ (lower dose, higher dose)	Purity, 99%, survival (reduction): 90%, 22%, 14%
Mouse, B6C3F$_1$ (M) 24 mo NTP (1986)	Inhalation 0, 100, 200 ppm, 6 h/day, 5 days/wk, 50, 50, 50/group	Hepatocellular adenoma: 12/49, 8/49, 19/50 Hepatocellular carcinoma: 7/49, 25/49, 26/50 Hepatocellular adenoma or carcinoma (combined): 17/49, 31/49, 41/50	Incidental tumour test $P < 0.01$ (trend), $P < 0.05$ (higher dose) $P < 0.01$ (trend), $P < 0.05$ (lower dose, higher dose) $P < 0.001$ (trend), $P < 0.05$ (lower dose, higher dose)	Purity, 99.9%; survival (reduction): 94%, 50%, 64%
Mouse, B6C3F$_1$ (F) 24 mo NTP (1986)	Inhalation 0, 100, 200 ppm, 6 h/day, 5 days/wk, 49, 50, 50/group	Hepatocellular carcinoma: 1/48, 13/50, 36/50 Hepatocellular adenoma or carcinoma (combined): 4/48, 17/50, 38/50	Incidental tumour test $P < 0.001$ (trend), $P < 0.001$ (low dose, high dose) $P < 0.001$ (trend), $P < 0.001$ (low dose, high dose)	Purity, 99.9%; survival (reduction): 73%, 62%, 38%

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF1 (M) 24 mo JISA (1993); EPA (2012)	Inhalation 0, 10, 50, 250 ppm, 6 h/day, 5 day/wk, 50/group	Hepatocellular adenoma: 7/50, 13/50, 8/50, 26/50 Hepatocellular carcinoma: 7/50, 8/50, 12/50, 25/50 Hepatocellular adenoma or carcinoma (combined): 13/50, 21/50, 19/50, 40/50 All organs, haemangioma or haemangiosarcoma (combined): 2/50, 1/50, 6/50, 8/50 Spleen, haemangiosarcoma: 1/50, 1/50, 3/50, 5/50 Liver, haemangiosarcoma: 1/50, 1/50, 5/50, 5/50 Harderian gland adenoma: 2/50, 2/50, 2/50, 8/50	Peto test, Fisher exact test $P < 0.001$ (trend), $P < 0.01$ (highest dose) $P < 0.001$ (trend), $P < 0.01$ (highest dose) $P < 0.001$ (trend), $P < 0.01$ (highest dose) $P < 0.05$ (trend) $P < 0.05$ (trend) $P < 0.05$ (trend) $P < 0.01$ (trend)	Purity, 99%, survival (reduction): 62%, 70%, 56%, 44%. The US EPA re-analysed the data on individual animals from this study and reported the overall incidence of haemangioma or haemangiosarcoma (combined) for all organs (primarily liver or spleen) to be 4/50, 2/50, 7/50, 11/50 (EPA, 2012)
Mouse, Crj:BDF1 (F) 24 mo JISA (1993); EPA (2012)	Inhalation 0, 10, 50, 250 ppm, 6 h/day, 5 day/wk, 50/group	Hepatocellular adenoma: 3/50, 3/47, 7/49, 26/49 Hepatocellular carcinoma: 0/50, 0/47, 0/49, 14/49 Hepatocellular adenoma or carcinoma (combined): 3/50, 3/47, 7/49, 33/49 All organs, haemangioma or haemangiosarcoma (combined): 1/50, 0/47, 2/49, 3/49	Peto test, Fisher exact test $P < 0.001$ (trend), $P < 0.001$ (highest dose) $P < 0.001$ (trend), $P < 0.001$ (highest dose) $P < 0.001$ (trend), $P < 0.001$ (highest dose) $P < 0.05$ (trend)	Purity, 99%, survival (reduction): 64%, 57%, 45%, 34%
Rat, Osborne-Mendel (M) 110 wk NCI (1977); Weisburger (1977)	Gavage in corn oil 0 (vehicle control), 471, 941 mg/kg bw per day, 5 days/wk for 78 wk, 20, 50, 50/group	No significant differences in tumour incidence between control and treated animals	NS	Purity, 99%; survival (reduction): 10%, 12%, 4%

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Osborne-Mendel (F) 110 wk NCI (1977); Weisburger (1977)	Gavage in corn oil 0 (vehicle control), 474, 949 mg/kg/d bw, 5 d/wk for 78 wk, 20, 50, 50/group	No significant differences in tumour incidence between control and treated animals	NS	Purity, 99%; survival (reduction): 40%, 34%, 28%
Rat, F344 (M) 24 mo NTP (1986)	Inhalation 0, 200, 400 ppm, 6 h/day, 5 day/wk, 50/group	Mononuclear cell leukaemia (all three stages): 28/50, 37/50, 37/50	Life-table test $P < 0.01$ (trend), $P < 0.05$ (lower dose, higher dose)	Purity, 99.9%; survival (reduction): 46%, 40%, 24%
		Mononuclear cell leukaemia (stage 3 only): 20/50, 24/50, 27/50	$P < 0.05$ (trend), $P < 0.05$ (higher dose)	
		Kidney, tubular cell adenoma or carcinoma (combined): 1/49, 3/49, 4/50	NS	Historical control incidence in NTP studies: 4/1968 (0.2 ± 0.6%, no adenocarcinoma)
		Kidney, tubular cell carcinoma: 0/49, 0/49, 2/50	NS	
		Glioma: 1/50, 0/50, 4/50	Life-table test $P < 0.05$ (trend)	Historical control incidence in NTP studies: 16/1971 (0.8%)
		Testis, interstitial cell tumour: 35/50, 39/49, 41/50	Incidental tumour test $P < 0.05$ (trend), $P < 0.05$ (lower dose, higher dose)	Historical control incidence in NTP studies: 740/1055 (70.1%)
Rat, F344 (F) 24 mo NTP (1986)	Inhalation 0, 200, 400 ppm, 6 h/day, 5 days/wk, 50/group	Mononuclear cell leukaemia (all three stages): 18/50, 30/50, 29/50	Life table test $P = 0.053$ (trend), $P < 0.05$ (lower dose)	Purity, 99.9%
		Mononuclear cell leukaemia (stage 3 only): 10/50, 18/50, 21/50	$P < 0.05$ (trend), $P < 0.05$ (higher dose)	
Rat, F344/DuCrj (M) 24 mo JISA (1993); EPA (2012)	Inhalation 0, 50, 200, 600 ppm, 6 h/day, 5 days/wk, 50/group	Mononuclear cell leukaemia: 11/50, 14/50, 22/50, 27/50	Peto test, Fisher exact test $P < 0.001$ (trend), $P < 0.05$ (highest dose)	Purity, 99%; survival (reduction): 74%, 68%, 60%, 56%
Rat, F344/DuCrj (F) 24 mo JISA (1993); EPA (2012)	Inhalation 0, 50, 200, 600 ppm, 6 h/day, 5 days/wk, 50/group	Mononuclear cell leukaemia: 10/50, 17/50, 16/50, 19/50	Peto test, Fisher exact test $P < 0.001$ (trend), $P < 0.05$ (lowest dose)	Purity, 99%; survival (reduction): 84%, 68%, 68%, 68%
		Mammary gland fibroadenoma: 3/50, 13/50, 1/50, 0/50		

EPA, Environmental Protection Agency; F, female; h, hour; mo, month; M, male; NS, not significant; NTP, National Toxicology Program; wk, week

higher dose, compared with controls. Significant positive trends were observed in males for the incidence of hepatocellular adenoma and in both sexes for hepatocellular carcinoma. The incidence of hepatocellular carcinoma were also significantly increased at both doses in males (7/49, 25/49, 26/50) and females (1/48, 13/50, 36/50); and the incidence of hepatocellular adenoma or carcinoma (combined) was also significantly increased at both doses in males (17/49, 31/49, 41/50) and females (4/48, 17/50, 38/50). The incidence of hepatic degeneration was increased in male mice at both doses (2/49, 8/49, 14/50) and in female mice at the higher dose (1/49, 2/50, 13/50). The incidence of karyomegaly of renal tubular cells was also increased in exposed mice (males: 4/49, 17/49, 46/50; females: 0/48, 16/49, 38/50). [The Working Group noted that decreased survival reduced the power of this study to detect the full carcinogenic potential of the test agent.]

Groups of 50 male and 50 female Crj:BDF1 mice (age, 5 weeks) were exposed to air containing tetrachloroethylene (purity, 99%) at concentrations of 0, 10, 50, or 250 ppm [0, 68, 340, or 1695 mg/m^3] for 6 hours per day on 5 days per week for up to 103 weeks (JISA, 1993; EPA, 2012). Survival decreased with increasing concentration among males and females; however, no statistical analysis of the survival data was provided. In males and females, significant positive trends in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were observed, and the incidences of these neoplasms were significantly increased in the group at the highest dose, compared with controls. The incidences of hepatocellular carcinoma were 7 out of 50 (control), 8 out of 50 (lowest dose), 12 out of 50 (intermediate dose), 25 out of 50 (highest dose) in male mice, and 0 out of 50, 0 out of 47, 0 out of 49, and 14 out of 49, respectively, in female mice. The incidences of hepatocellular adenoma or carcinoma (combined) were 13 out of 50, 21 out of 50, 19 out of 50, and 40 out of 50, respectively, in male mice, and 3 out of 50, 3 out of 47, 7 out of 49, and 33 out of 49 in female mice. The incidence of hepatic degeneration was also increased at the highest dose in males (1/50, 1/50, 4/50, and 37/50) and in females (0/50, 1/50, 2/50, and 30/50). In addition, there was a positive trend in the incidence of haemangiosarcoma of the liver and of the spleen in males, of haemangioma or haemangiosarcoma (combined) for all organs in males and females (primarily of the liver or spleen in males), and of Harderian gland adenoma in males (2/50, 2/50, 2/50, and 8/50). The incidence of karyomegaly of renal tubular cells was also increased in the highest dose compared with controls (males: 0/50, 0/50, 6/50, 38/50; females: 0/50, 0/50, 1/50, 49/50).

3.1.3 Skin application

Two groups of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were given tetrachloroethylene [purity not reported] at a dose of 18 or 54 mg per application in 0.2 mL of acetone by repeated topical application (three times per week) for at least 63 weeks. A control group of 30 mice was given an application of 0.1 mL acetone only (Van Duuren *et al.*, 1979). The effects of exposure on survival were not reported. One skin papilloma occurred in a mouse at the lower dose; no skin tumours were observed among controls or in mice at the higher dose. [Interpretation of these findings was limited by the small number of animals tested, the short exposure duration, the incomplete reporting of results, and because the study did not address volatile loss of the test compound.]

3.1.4 Intraperitoneal injection

In a screening assay for induction and increased multiplicity of lung tumours, three groups of 20 male mice of strain A/St (age, 6–8 weeks) were given tetrachloroethylene (purity unspecified, but ≥ 95%) by intraperitoneal

injection in tricaprylin, at doses of 80 mg/kg bw (14 injections), 200 or 400 mg/kg bw (24 injections), three times per week (Theiss *et al.*, 1977). A group of 50 controls was given 24 injections of tricaprylin only. Twenty-four weeks after the first injection, the mice were killed, their lungs were examined under a dissecting microscope, and the number of surface adenomas was counted. Tetrachloroethylene did not increase the number of pulmonary adenomas per mouse in treated animals compared with controls. [The small number of animals studied and the short duration of exposure limited the interpretation of these findings. In addition, the lung was the only organ examined.]

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female Osborne-Mendel rats (age, 7 weeks) were given tetrachloroethylene (purity, 99%) by gavage in corn oil on 5 days per week for 78 weeks (NCI, 1977; Weisburger, 1977). Groups of 20 male and 20 female rats (age, approximately 11 weeks) were treated with vehicle only and served as controls. Dosage adjustments were made during the exposure period: male rats were given tetrachloroethylene at a dose of 500 or 1000 mg/kg bw for 19 weeks, then 700 or 1400 mg/kg bw for 6 weeks, and 500 or 1000 mg/kg bw for 46 weeks, followed by 26 weeks of cyclic dosing comprising one treatment-free week and 4 weeks at 500 or 1000 mg/kg bw; female rats were given tetrachloroethylene at a dose of 500 or 1000 mg/kg bw for 16 weeks, then 600 or 1200 mg/kg bw for 3 weeks, 700 or 1400 mg/kg bw for 6 weeks, and 500 or 1000 mg/kg bw for 20 weeks, followed by 26 weeks of cyclic dosing with one treatment-free week and 4 weeks at 500 or 1000 mg/kg bw. Time-weighted average doses of tetrachloroethylene were 471 and 941 mg/kg bw per day for males, and 474 and 949 mg/kg bw per day for females. The treatment period was followed by a 32-week observation period. Mortality was significantly increased in treated rats compared with controls. Toxic nephropathy was observed at the lower and higher doses in 88% and 94% of males, and 50% and 80% of females, respectively, and not in controls. Toxic nephropathy in rats was morphologically similar to that described in Section 3.1.1 for $B6C3F_1$ mice treated with tetrachloroethylene by gavage (NCI, 1977; Weisburger, 1977). There were no significant differences in tumour incidence between the control and treated rats of either sex. [The Working Group noted that high mortality and the 78-week exposure period precluded reliable evaluation of carcinogenicity. In addition, study animals were housed in the same rooms as animals exposed to volatile agents, and the small group size for vehicle controls limited the power of this study.]

3.2.2 Inhalation

In an abstract, Rampy *et al.* (1977) reported no increase in tumour incidence in groups of male or female Sprague-Dawley rats (n = 96 for the treated groups and n = 192 for the control groups) exposed to vapours containing tetrachloroethylene at a concentration of 300 or 600 ppm, for 6 hours per day on 5 days per week for 12 months, and then observed for an additional 18 months. [The Working Group noted that the duration of exposure was too short to adequately evaluate the carcinogenic potential of tetrachloroethylene, and details of experimental methods and results were lacking.]

Groups of 50 male and 50 female Fischer 344/N rats (age, 8–9 weeks) were exposed to air containing tetrachloroethylene (purity, 99.9%) at concentrations of 0, 200, or 400 ppm (0, 1360, or 2720 mg/m^3) for 6 hours per day on 5 days per week for up to 103 weeks (Mennear *et al.*, 1986; NTP, 1986). Survival of male rats in the group at the higher dose was significantly lower than that of controls. Positive trends and increased incidence

of mononuclear cell leukaemia were observed in both sexes. The incidences of mononuclear cell leukaemia in males were: 28 out of 50 (controls), 37 out of 50 (lower dose), and 37 out of 50 (higher dose); and in females, the incidences were: 18 out of 50, 30 out of 50, and 29 out of 50. The historical incidence of mononuclear cell leukaemia in 2-year studies conducted in F344 rats at the study laboratory was 47 ± 15% in males and 29 ± 6% in females. The incidence of advanced (stage 3) mononuclear cell leukaemia (characterized by involvement of multiple organs) was increased in exposed males (20/50, 24/50, 27/50) and females (10/50, 18/50, 21/50). A non-significant increase in uncommonly occurring adenoma or carcinoma (combined) of the kidney tubule was observed in male rats (1/49, 3/49, 4/50); the historical incidence of these neoplasms in control male rats in inhalation studies conducted by the National Toxicology Program (NTP) at that time was 4 out of 1968 (0.2 ± 0.6%). Among the eight male rats that developed tumours of the kidney tubule in the study, carcinomas were observed in two males at the higher dose; malignant tubular cell tumours had not been observed previously in control male F344 rats in NTP inhalation studies. In addition, the incidence of renal tubular cell hyperplasia was increased in exposed male rats (0/49, 3/49, 5/50) and the incidence of karyomegaly (nuclear enlargement) in renal tubular epithelial cells was increased in males (1/49, 37/49, 47/50), and females (0/50, 8/49, 20/50). In male rats, significant positive trends in the incidence of glioma (1/50, 0/50, 4/50) and interstitial cell tumours of the testis (35/50, 39/49, 41/50) were observed. Gliomas are uncommon in male F344 rats; the historical incidence of these neoplasms in all NTP studies conducted at that time was 16 out of 1971 (0.8%). Gliomas were also observed in one female in the control group and in two females at the higher dose. Although the incidence of interstitial cell tumours of the testis was high in male F344 rats in 2-year studies, the mean incidence in historical controls in male rats in inhalation studies conducted by the NTP using NIH-07 diet was 70.1% (740/1055; NTP, 2012), similar to the incidence for controls in this study. Thus, the Working Group considered that in addition to mononuclear cell leukaemia, the increased incidences of renal tubular cell tumours, glioma, and interstitial tumours of the testis in male rats were also associated with exposure to tetrachloroethylene.

Groups of 50 male and 50 female F344/DuCrj rats (age, 5 weeks) were exposed to air containing tetrachloroethylene (purity, 99%) at concentrations of 0, 50, 200, or 600 ppm [0, 340, 1360, or 4080 mg/m^3] for 6 hours per day on 5 days per week for up to 103 weeks (JISA, 1993; EPA, 2012). Survival was lower in exposed rats than in controls, although no statistical analysis of the survival data was provided. Increased incidences of mononuclear cell leukaemia were observed in male rats (control, 11/50; lowest dose, 14/50; intermediate dose, 22/50; highest dose, 27/50) and female rats (10/50, 17/50, 16/50, 19/50); significant positive trends were observed for both sexes, and the incidence in the group at the highest dose was significantly greater than that in controls. The historical incidence of mononuclear cell leukaemia in 2-year studies in F344/DuCrj rats conducted at the study laboratory was 13% (range, 6–22%) in males and 14% (range, 2–26%) in females. Similarly to the NTP (1986) study, exposure to tetrachloroethylene induced an increase in the incidence of karyomegaly in renal tubular epithelial cells in males (0/50, 0/50, 23/50, 48/50), and females (0/50, 0/50, 1/50, 16/50). The incidences of renal tubular cell adenoma in male rats were 1 out of 50, 2 out of 50, 1 out of 50, and 2 out of 50. In female rats, a renal tubular cell adenoma was observed in the control group, and a rare renal tubular cell carcinoma was observed in the group at the highest dose. A significant increase in the incidence of mammary gland fibroadenoma was observed in females at the lowest dose (3/50, 13/50, 1/50, 0/50).

3.3 Studies with mixtures of solvents

Mouse

Groups of 33–43 male and 36–41 female ICR mice were given drinking-water containing a mixture of six chlorinated alkanes and alkenes (including tetrachloroethylene) at three different concentrations for 16 months (males) or 18 months (females) (Wang *et al.*, 2002). The concentrations of tetrachloroethylene in these mixtures were 36.0, 90.3, or 606.5 μg/mL, respectively, accounting for 38–52% of each formulation. Among mice surviving to the end of the study, there was a non-statistically significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) in males at the lowest dose (3/18) and intermediate dose (4/15) compared with controls (1/23), and a significant increase ($P < 0.05$) in the incidence of adenocarcinoma of the mammary gland in females at the highest dose (5/26) compared with controls (0/24). [The Working Group noted that because this study was designed to attain the approximate concentrations of these solvents in groundwater near an electronic appliances factory in Taiwan, China, the doses of tetrachloroethylene were much lower than those used in the study of oral carcinogenicity in mice described in Section 3.1.1 and would not be adequate to evaluate the carcinogenic potential of this chlorinated solvent. Furthermore, the influence of other agents in the mixture has not been studied. The mixture containing tetrachloroethylene at 606 μg/mL appeared to have exceeded the solubility of tetrachloroethylene in water (about 150 μg/mL), but groundwater can contain higher concentrations of this chemical.]

3.4 Initiation–promotion studies

Mouse

Tetrachloroethylene [purity unspecified] in 0.2 mL of acetone was applied as a single dose at 163 mg/mouse to the dorsal skin of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) (Van Duuren *et al.*, 1979). Topical applications (three times per week) of the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (5 μg in 0.2 mL of acetone) began 14 days later and were continued for at least 61 weeks. A control group of 90 mice received 12-*O*-tetradecanoylphorbol 13-acetate only. Seven skin papillomas were found in 4 out of 30 treated mice, and seven skin papillomas were found in 6 out of 90 controls. This difference was not statistically significant. [Interpretation of these findings was limited by the incomplete reporting of results (e.g. purity of the compound), and because the study did not address loss of the test compound due to volatility.]

3.5 Carcinogenicity of metabolites

Studies of carcinogenicity with dichloroacetic acid and trichloroacetic acid are summarized in the respective *Monographs* in this volume.

Mouse

Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) received tetrachloroethylene oxide, a metabolite of tetrachloroethylene, by skin application (5 μL [7.5 mg]/mouse followed immediately by 0.1 mL acetone) three times per week for 65 weeks, or by subcutaneous injection (500 μg/mouse in 0.05 mL of trioctanoin) once per week for up to 80 weeks (Van Duuren *et al.*, 1983). Controls received a skin application of 0.1 mL of acetone only. In mice receiving tetrachloroethylene oxide by skin application, a significant increase ($P = 0.014$) in the incidence of skin tumours at the site of application (4/30;

one keratoacanthoma, two squamous cell papillomas, and one squamous cell carcinoma) was observed compared with controls (0/30). The results of the subcutaneous-injection experiment were negative. [The Working Group noted that the half-life of tetrachloroethylene oxide is only 11.5 minutes in aqueous media.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Tetrachloroethylene is a lipophilic solvent of low relative molecular mass that readily crosses biological membranes. Pulmonary uptake is rapid, approaching steady-state within a few hours after the start of exposure.

In a study with six male volunteers, pulmonary uptake decreased during the course of the experiment to 60% of the initial value (Monster, 1979). Measured alveolar retention was 65%, averaged over six or seven volunteers (Monster *et al.*, 1979; Chiu *et al.*, 2007). Increased physical activity increases uptake, but lowers the alveolar partial pressure, thus removing more tetrachloroethylene from the alveoli, resulting in a longer time to reach tissue equilibrium (Pezzagno *et al.*, 1988). The blood–air partition coefficient represents 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* by means of vial-equilibrium methods (Gargas *et al.*, 1989), and mean values ranged from 10 to 17 for humans (Sato & Nakajima, 1979; Koizumi 1989; Gearhart *et al.*, 1993; Fisher *et al.*, 1997; Mahle *et al.*, 2007).

Data on oral absorption are limited. One case report of accidental ingestion suggested that tetrachloroethylene is also readily absorbed by this route of exposure (Köppel *et al.*, 1985). Quantitative estimates of the bioavailability of tetrachloroethylene after oral intake in humans are not available, because the ingested amounts are not precisely known, and because the exposed subjects underwent hyperventilation therapy.

Dermal absorption of tetrachloroethylene vapours by humans has been reported to be relatively insignificant (only 1%) when compared with absorption via inhalation (Riihimäki & Pfäffli, 1978; Nakai *et al.*, 1999). After liquid contact, skin absorption can be more significant: the amount of chemical absorbed during the immersion of one thumb in liquid tetrachloroethylene is equivalent to the uptake during inhalation of two to five times this amount during the same period (Stewart & Dodd, 1964). The permeability of tetrachloroethylene through skin was tested in a penetration model *in vitro* with human skin and skin of hairless guinea-pigs. The results for these two skin types were similar (Frasch & Barbero, 2009). Dermal absorption of tetrachloroethylene from contaminated soil has also been measured (Poet *et al.*, 2002).

(b) Experimental systems

Inhalation studies in experimental animals have been conducted predominantly in adult males. Tetrachloroethylene is readily absorbed via the lungs into the systemic circulation (Pegg *et al.*, 1979; Dallas *et al.*, 1994a, b, 1995). Blood–air partition coefficients for tetrachloroethylene have been measured *in vitro* by means of vial-equilibrium methods (Gargas *et al.*, 1989) and ranged from 13 to 21 for rodents (Koizumi 1989; Gearhart *et al.*, 1993; Reitz *et al.*, 1996; Mahle *et al.*, 2007).

Oral doses of tetrachloroethylene – given by gavage or in drinking-water – are almost completely absorbed from the gut, as reported in several studies in mice, rats, and dogs (Pegg *et al.*, 1979; Schumann *et al.*, 1980; Frantz & Watanabe, 1983; Dallas *et al.*, 1995).

Dermal uptake is minimal compared with pulmonary uptake during exposure to tetrachloroethylene vapour (Tsuruta, 1989), but is greater after direct application to the skin (Jakobson *et al.*, 1982; Bogen *et al.*, 1992). Permeability coefficients have been measured *in vitro* in several studies. Nakai *et al.* (1999) reported lower permeability into human skin for liquid tetrachloroethylene than for trichloroethylene or chloroform. Absorption of tetrachloroethylene from a contaminated soil matrix was higher in rats than in humans (Poet *et al.* 2002).

4.1.2 Distribution and body burden

(a) Humans

Once absorbed, tetrachloroethylene enters the blood circulation and undergoes rapid systemic distribution to tissues. The highest concentrations are expected to occur in adipose and other fatty tissue, due to the lipophilicity of the compound. Data on distribution of tetrachloroethylene in humans *in vivo* come from analyses of tissues taken from autopsies after fatal accidents. The available data show wide systemic distribution in blood and across all tissues tested, including the lung, liver, heart, kidney, and brain (Lukaszewski 1979; Levine *et al.*, 1981; Garnier *et al.*, 1996). Tetrachloroethylene has also been measured in human breast milk (Schreiber 1993, 1997; Schreiber *et al.*, 2002).

Repeated daily exposure of human volunteers to tetrachloroethylene by inhalation resulted in accumulation of the compound in the body, with blood concentrations increasing over several days. Exhalation of the compound continued over several days due to its slow release from adipose tissue (Skender *et al.*, 1991). For a given concentration in blood or air, the half time [the time required to equilibrate the adipose tissue to 50% of its final concentration] is about 25 hours (Monster, 1979). For persons exposed to tetrachloroethylene in a work schedule of 5 days per week, an equilibrium is established over 3–4 weeks.

The of tissue–blood partition coefficient has been measured *in vitro* by use of vial-equilibrium methods in human fat, kidney, muscle, and liver. The highest reported values are for fat (125), as expected due to the lipophilicity of tetrachloroethylene, with values for the remaining tissues ranging from 5 to 6 (Gearhart *et al.*, 1993).

(b) Experimental systems

Studies in experimental animals have been conducted predominantly in adult males. These experiments provide clear evidence that tetrachloroethylene is distributed widely to all tissues of the body. In rats exposed *in vivo*, tetrachloroethylene has been detected and measured in blood, fat, brain, lungs, liver, kidneys, heart, and skeletal muscle. The highest tissue concentrations were found in adipose tissue (≥ 60 times that in blood) and in brain and liver (four and five times higher than in blood, respectively), as was calculated from rat tissue-distribution data (Savolainen *et al.*, 1977; Dallas *et al.*, 1994a, b). The concentration of tetrachloroethylene in fat was 9–18 times higher than the concentrations found in other tissues. Skeletal muscle contained the lowest concentration (Dallas *et al.* (1994b). Tetrachloroethylene readily crosses the blood–brain barrier (Savolainen *et al.*, 1977; Schumann *et al.*, 1980) and the placenta (Ghantous *et al.*, 1986).

Partition coefficients have also been measured *in vitro* for a wide variety of tissues in rats and mice, including fat, liver, muscle, skin, kidney, and brain (Gargas *et al.*, 1989; Koizumi 1989; Gearhart *et al.*, 1993; Mattie *et al.*, 1994; Mahle *et al.*, 2007). The highest reported values were those for fat (90–110), whereas partition coefficients for the remaining tissues were in the range 1–4.

4.1.3 Metabolism

(a) Overview

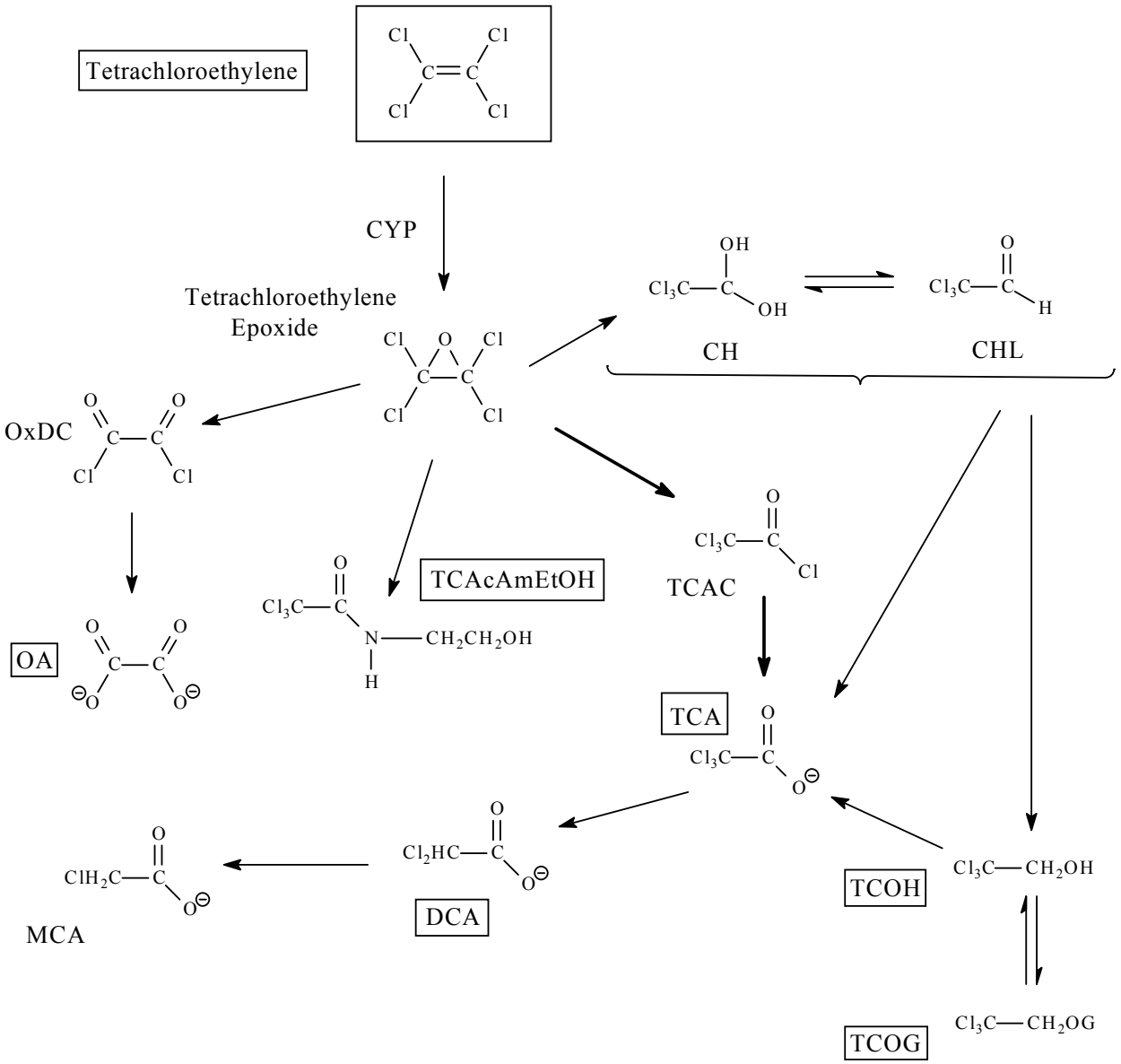
Metabolism is critical to the various adverse effects of tetrachloroethylene in biological systems: with the exception of solvent effects that occur at extremely high exposures to tetrachloroethylene, all the adverse effects of tetrachloroethylene can be attributed to specific metabolites. The basic metabolic pathways for tetrachloroethylene have for the most part been deciphered over many years and have been summarized in various publications as well as a recent review (Green, 1990; IARC, 1995; Lash & Parker, 2001; EPA, 2012). This section will describe the major pathways in the metabolism of tetrachloroethylene, i.e. the cytochrome P450 (CYP)-dependent oxidative pathway and the glutathione (GSH)-conjugation pathway, in humans and experimental animals. Key urinary metabolites that are often used to estimate exposure in environmental or occupational settings have been identified. While the basic outline of the pathways has been known for many years, this section will focus on some of the more recently identified metabolites, particularly those described during the past decade. This section comprises four subsections: (i) an overview of the major pathways of tetrachloroethylene metabolism and the enzymes involved; (ii) a review of each step of the CYP-dependent pathway, providing evidence from humans or human tissues, and from experimental systems; (iii) a review of each step of the GSH-conjugation pathway, likewise providing data from humans or human tissues and from experimental systems; where appropriate, information on membrane transport of metabolites will be discussed; and (iv) a brief discussion of comparisons between the metabolism of tetrachloroethylene and trichloroethylene (see *Monograph* in this volume). Although tetrachloroethylene and trichloroethylene share several metabolic intermediates, have very similar metabolites, or are metabolized by similar enzymes, there are several important differences that need to be carefully considered.

Of the two major pathways, the flux through the oxidative pathway far exceeds that through the GSH-conjugation pathway. Whereas the latter generates several highly reactive metabolites, those generated by the oxidative pathway are in most cases chemically stable, although there are exceptions. [The Working Group noted that some of the chemically stable oxidative metabolites may be linked to adverse effects, but the different chemical reactivities of metabolites generated by the two pathways may render some metabolites in the GSH-conjugation pathway difficult to detect. Interpretations regarding the toxicological importance of such metabolites – based on quantitative differences in estimated flux – must, therefore, be made with caution.]

(i) CYP-dependent oxidation

The overall scheme of the metabolism of tetrachloroethylene via the CYP-dependent oxidative pathway is shown in Fig. 4.1. The initial step is catalysed by CYP and is believed to lead to the formation of tetrachloroethylene epoxide, although a transient intermediate of tetrachloroethylene with CYP may also occur (Yoshioka *et al.*, 2002). Tetrachloroethylene epoxide may follow several reaction pathways (the quantitatively predominant route is indicated by the thicker arrow in Fig. 4.1): the epoxide may be de-chlorinated to oxalate dichloride and oxalate, which is excreted in the urine. Alternatively, the epoxide may be converted to trichloroacetyl aminoethanol (a urinary metabolite), which is a relatively minor pathway. The major metabolic route is conversion to trichloroacetyl chloride, which is subsequently converted to trichloroacetate, the major metabolite recovered in urine of tetrachloroethylene-exposed humans and animals. Finally, some of the trichloroacetate may be converted to dichloroacetate, which has been detected in urine, typically in very low amounts.

Fig. 4.1 Scheme for oxidative metabolism of tetrachloroethylene



Tetrachloroethylene undergoes cytochrome P450 (CYP)-dependent oxidation to primarily form an epoxide intermediate. Further processing yields a variety of metabolites, including chloral (CHL) and chloral hydrate (CH), trichloroacetyl chloride (TCAC), trichloroacetyl aminoethanol (TCAcAmEtOH), or oxalate dichloride (OxDC). Trichloroacetate (TCA) can be generated from either CHL/CH, TCAC, or trichloroethanol (TCOH) and its glucuronide (TCOG). Dichloroacetate (DCA) and monochloroacetate (MCA) are considered minor metabolites. OxDC is dechlorinated to yield oxalate (OA), which is a significant urinary metabolite. Names of metabolites that are recovered in urine are shown in boxes. The thicker arrows indicate the major pathway of metabolite flux.

The major oxidative metabolites formed from tetrachloroethylene, their site of formation or portal of entry, and the source of the information (animals and/or humans), is presented in Table 4.1. Systemic availability is dependent on the chemical stability of the metabolites: those that are relatively stable may be transferred from their site of formation into the blood stream and be delivered to other potential target organs, whereas those that are chemically unstable and reactive (tetrachloroethylene-oxide) tend to remain near their site of formation and react with cellular molecules, including DNA, proteins, and lipids.

(ii) GSH-conjugation

As shown in Fig. 4.2, tetrachloroethylene undergoes an S_N2 nucleophilic displacement reaction with GSH, releasing a chloride ion under formation of *S*-(1,2,2-trichlorovinyl)glutathione (TCVG). Although this initial GSH-conjugation step can take place in many tissues, it occurs primarily in the liver as a result of first-pass metabolism and because of the high content of glutathione-*S*-transferases (GSTs): the various GST isoforms can account for as much as 5% of total cytosolic protein in rat or human liver. This initial step in the metabolism of tetrachloroethylene leads to the formation of reactive metabolites associated with toxic effects in the kidneys, or to a non-toxic mercapturate that is readily excreted in the urine.

After formation of TCVG, which occurs predominantly in the liver but also in the kidneys (Lash *et al.*, 1998), this metabolite is processed by γ-glutamyltransferase (GGT) and dipeptidase on the brush-border plasma membrane of the renal proximal tubular cell, to form the corresponding cysteine conjugate *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC). Although GGT and dipeptidase activities are present in other tissues, the inter-organ pathways by which GSH and GSH-conjugates are processed effectively direct the GSH-conjugate to the kidneys.

The formation of TCVC represents a critical branch point in the GSH-dependent metabolism of tetrachloroethylene. This cysteine conjugate serves as a substrate for several enzymes that catalyse reactions towards its bioactivation to chemically reactive metabolites, or to its detoxification. The detoxification route involves *N*-acetylation by the microsomal enzyme *N*-acetyltransferase (NAT) to form *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (NAcTCVC). Although the latter product has been recovered in the urine of both rats and humans exposed to tetrachloroethylene (Bartels, 1994; Birner *et al.*, 1996; Völkel *et al.*, 1999), it can be metabolized via two alternative routes. First, as a substrate for acylase I, NAcTCVC can be deacetylated to regenerate TCVC (Uttamsingh & Anders, 1999). And second, it can undergo sulfoxidation as catalysed by CYP3A enzymes (CYP3A1/2 in rats and CYP3A4 in humans) (Werner *et al.*, 1996). The resulting TCVC sulfoxide (TCVCS) is highly reactive and cytotoxic.

Apart from the metabolic changes in the mercapturate/mercapturate sulfoxide reaction pathway, TCVC is metabolized to reactive species by either cysteine conjugate β-lyase (CCBL) (Dekant *et al.*, 1986a), which produces 1,2,2-trichlorovinylthiol (TCVT), or by a cysteine conjugate *S*-oxidase activity, identified as a catalytic function of flavin-containing monooxygenase 3 (FMO3) (Ripp *et al.*, 1997), which forms TCVCS. Both TCVC and TCVCS can rearrange spontaneously to form a thioketene, which is the ultimate reactive and toxic acylating agent. These additional metabolic routes leading to the formation of the putative end product of the GSH-conjugation pathway highlight both the complexity of the metabolism of tetrachloroethylene along this pathway, and the potential difficulties in assessing the overall flux through the GSH-conjugation pathway by use of urinary NAcTCVC as a surrogate marker.

The site of formation and systemic availability for the major metabolites from the

Table 4.1 Formation and systemic availablility of tetrachloroethylene metabolites

Compound or metabolite	Portal of entry, or tissue where formed in animals (A) or humans (H)
Tetrachloroethylene	Lung (A, H) Gastrointestinal tract (A, H) Skin (A, H)
Oxidative metabolites	
Tetrachloroethylene epoxide [a]	Liver (A)
Trichloroacetyl chloride	Lung (A)
Trichloroacetate	Liver (A, H) Lung (A)
Dichloroacetate	Kidney (A)
GSH-conjugation metabolites	
TCVG	Liver (A) Kidney (A)
TCVC	Liver (A) Kidney (A)
TCVT [a]	Kidney (A)
TCVCS	Kidney (A)
TCVK	Kidney (A)
NAcTCVC	Liver (A, H) Kidney (A, H)
NAcTCVCS	Liver (A) Kidney (A)

[a] Due to their reactivity, these two metabolites are not systemically available
NAcTCVC, *N*-acetyl-*S*-(1,1,2-trichlorovinyl)-L-cysteine; NAcTCVCS, NAcTCVC sulfoxide; TCVC, *S*-(1,2,2-trichlorovinyl)-L-cysteine; TCVCS, *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide; TCVG, *S*-(1,2,2-trichlorovinyl)glutathione; TCVK, trichlorovinyl thioketene; TCVT, *S*-(1,2,2-trichlorovinyl)thiol

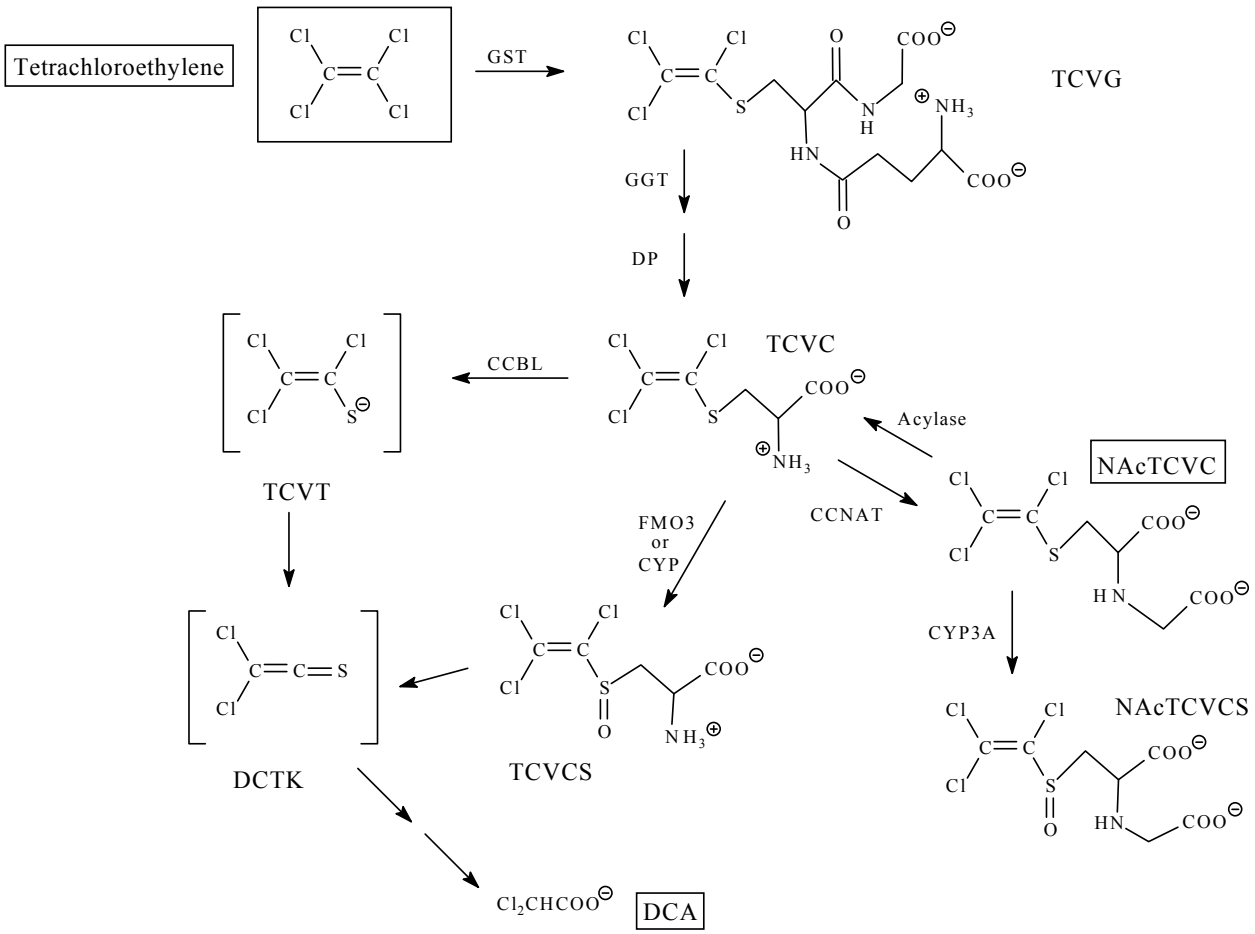
GSH-conjugation pathway is also presented in Table 4.1.

(iii) Enzymes involved in TCVC bioactivation

Of the two major bioactivation pathways for TCVC, i.e. with the enzymes CCBL or FMO3, the former has received most attention, but unlike for DCVC (Lash *et al.*, 2000) it is unclear which of the two pathways predominates. The activity of CCBL is actually a catalytic function of a diverse array of enzymes (Cooper & Pinto, 2006; Lash, 2007) that has been detected not only in the kidneys, but also in liver and other tissues.

Studies in the mid-1960s first identified a thiol metabolite of a sulfonamide that was formed by a C–S lyase activity (Colucci & Buyske, 1965). Others subsequently identified hepatic and renal enzymes from cow and turkey that catalysed this reaction (Anderson & Schultze, 1965; Bhattacharya & Schultze, 1967). More than a decade later the term 'cysteine conjugate β-lyase' was first used to describe this activity in rat liver (Tateishi *et al.*, 1978). All known CCBL enzymes contain pyridoxal-5-phosphate. Although the overall CCBL-catalysed reaction is the cleavage of a C–S-bond to yield a reactive thioacylating species, subsequent studies with the cysteine conjugate of trichloroethylene (i.e. DCVC) showed that the reaction mechanism proceeds via either a direct β-elimination or a transamination reaction with a suitable α-keto acid as co-substrate, to yield either the thiolate or a propionic-acid derivative, respectively; the latter is chemically unstable and rearranges to release the thiolate (Stevens *et al.*, 1986; Elfarra *et al.*, 1987).

Fig. 4.2 Scheme for glutathione-dependent metabolism of tetrachloroethylene



Tetrachloroethylene undergoes conjugation with glutathione (GSH) to yield the GSH S-conjugate TCVG. After processing to yield the cysteine S-conjugate TCVC, three potential fates are detoxication to yield the mercapturate NAcTCVC or bioactivation by either the cysteine conjugate β-lyase to yield trichlorovinylthiol, which rearranges to yield thioacylating species, or the flavin-containing monooxygenase to yield TCVC sulfoxide. The mercapturate can also be deacetylated to regenerate TCVC or it can undergo CYP3A-dependent sulfoxidation. Names of metabolites than are recovered in urine are shown in boxes and those that are chemically unstable or reactive are shown in brackets. Abbreviations: CCNAT, cysteine conjugate *N*-acetyltransferase; CYP3A, cytochrome P-450 3A; DCA, dichloroacetate; DCTK, 1,1-dichlorothioketene; DP, dipeptidase; FMO, flavin-containing monooxygenase; GGT, γ-glutamyltransferase; GSH, glutathione; GST, GSH *S*-transferase; TCVC, *S*-(1,2,2-trichlorovinyl)-L-cysteine; TCVG, *S*-(1,2,2-trichlorovinyl)glutathione; TCVCS, TCVC sulfoxide; NAcTCVC, N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine; NAcTCVCS, NAcTCVC sulfoxide.

Eleven mammalian enzymes are currently known to catalyse the CCBL reaction. Some of these enzymes catalyse both the β-elimination and the transamination reactions, whereas others only catalyse the former. The importance of each of these activities in TCVC bioactivation has been discussed (Cooper & Pinto, 2006; Cooper *et al.*, 2011).

The flavin-containing monooxygenases (FMOs), like the CYP enzymes, represent a multigene family of enzymes. However, although there are more than 50 individual functional CYP enzymes from more than 40 gene families known in humans, only five FMO genes have been identified in mammals. Both systems have several characteristics in common, including localization in the endoplasmic reticulum, requirement for NADPH as a proton donor, and overall catalytic activity in a mixed-function oxidation reaction. Some of the functions of the FMOs are different, however. FMOs catalyse the oxidation of sulfur-, selenium-, and nitrogen-containing chemicals (Ziegler, 1993, 2002; Cashman & Zhang, 2006). Although some FMO and CYP450 enzymes share substrates and catalyse the same overall reactions, FMOs have some distinctive substrates, including cysteine *S*-conjugates of various halo-alkenes and halo-alkanes.

(b) Humans

(i) CYP-dependent oxidation

The predominant route in the metabolism of tetrachloroethylene in all species studied, including humans, is the CYP-dependent oxidation pathway (Reitz *et al.*, 1996). This is particularly true at typical environmental exposures, because the various CYP enzymes that may act on tetrachloroethylene have higher affinities for tetrachloroethylene than do GSTs. Studies of overall metabolism in humans, however, suggest that the capacity of the CYP pathway to metabolize tetrachloroethylene readily saturates and is considered limited (Ohtsuki *et al.*, 1983) and markedly lower than metabolic rates in experimental animals such as mice (Reitz *et al.*, 1996). This conclusion was based on analyses of urine from workers exposed by inhalation to concentrations of tetrachloroethylene of up to 100 ppm for up to 8 hours. During the 8-hour exposure, 38% of the absorbed tetrachloroethylene is excreted unchanged through the lungs and < 2% of the absorbed dose is metabolized, as quantified by measurement of urinary metabolites (primarily trichloroacetate). Consistent with the conclusion that the total capacity for humans to metabolize tetrachloroethylene is limited, the reported half-life of tetrachloroethylene is 144 hours (Ikeda, 1977; Ikeda & Imanura, 1973).

The metabolism of tetrachloroethylene in humans has been modelled on the basis of literature data. The extent of metabolism was found to be quite variable and dependent on dose. Thus, at relatively high exposure levels of up to 144 ppm, as used in the study by Monster *et al.* (1979), < 2% of the absorbed dose was estimated to be metabolized. However, at ambient exposure levels (i.e. around 0.001 ppm, but as high as 1 ppm), the fraction of absorbed tetrachloroethylene estimated to be metabolized was > 36% (Bois *et al.*, 1996).

The toxicokinetics of tetrachloroethylene in humans were also examined at an exposure level of 1 ppm by inhalation (Chiu *et al.*, 2007). During and after a 6-hour exposure period, samples were taken from blood, urine, and alveolar breath at various time-points until up to 6 days after the exposure. The results were generally similar to those of earlier studies, but the variability and the substantial uncertainty in the model were emphasized. Major contributing factors were concentration-dependent differences in toxicokinetics, as previously concluded by Bois *et al.* (1996), and inter-individual differences in metabolism (Chiu *et al.*, 2007).

(ii) GSH-conjugation pathway

Only a few studies are available on the metabolism of tetrachloroethylene via the GSH-conjugation pathway in humans or in human-derived tissues. However, the available data unambiguously confirm the existence of this pathway *in vivo*.

The urinary excretion of tetrachloroethylene metabolites was studied in six workers occupationally exposed in a dry-cleaning store, with ambient air concentrations of tetrachloroethylene of 50 ± 4 ppm. Four subjects were exposed for 8 hours per day, and two subjects for 4 hours per day. The major metabolites detected were trichloroacetate and trichloroethanol; the excretion pattern correlated closely with the duration of exposure of the individuals, with total amounts of 2,2,2-trichloro-compounds ranging from 13.5 to 65.0 nmol/mg creatinine. NAcTCVC was detected at concentrations approximately 5000 times lower (2.2–14.6 pmol/mg creatinine) (Birner *et al.*, 1996).

In another study, three female and three male volunteers were exposed to tetrachloroethylene at 10, 20, and 40 ppm by inhalation for 6 hours. Over the 78 hours after the start of the exposure to the highest dose, a total of 20.4 ± 7.8 µmol of trichloroacetate and 0.21 ± 0.05 µmol of NAcTCVC were excreted. Again, while this study unequivocally demonstrated the presence of an active GSH-conjugation pathway in humans, it suggests that CYP-dependent oxidation greatly predominates. However, the relative flux through the two main pathways should not be estimated solely on the basis of urinary metabolites recovered. If that were done, the conclusion would be that no more than 1% of tetrachloroethylene is metabolized by GSH conjugation. Because NAcTCVC represents only one potential end product of the GSH-conjugation pathway, and since most of the end products are chemically reactive, these metabolites are likely to react with cellular nucleophiles, resulting in covalent adducts. DNA and protein adducts have indeed been found in incubations with tetrachloroethylene (Völkel *et al.*, 1998, 1999).

(c) Experimental systems

(i) CYP-dependent oxidation

The interaction of tetrachloroethylene with hepatic microsomal CYP enzymes and the influence of various CYP inhibitors and inducers were studied in male Long-Evans rats. Trichloroacetate was the major metabolite and neither chloral hydrate nor trichloroethanol were detectable. Induction of CYP3A1 with pregnenolone-16α-carbonitrile, or CYP2B1/2 with phenobarbital both enhanced the metabolism of tetrachloroethylene, suggesting the involvement of these CYP enzymes (Costa & Ivanetich, 1980). In a later study, the absence of trichloroethanol in the urine of chronically dosed mice was confirmed; trichloroacetate was the only metabolite reported (Buben & O'Flaherty, 1985).

Evidence for a role of CYP2B enzymes in the metabolism of tetrachloroethylene was shown in studies with rat liver and enzyme-specific inducers and substrates. Tetrachloroethylene was shown to induce the CYP2B subfamily CYP. Unlike in the studies mentioned above (Costa & Ivanetich, 1980), there was no evidence for an involvement of CYP3A1 (Hanioka *et al.*, 1995a, b). [The Working Group noted that the role of CYP2E1 in the metabolism of tetrachloroethylene has not been demonstrated directly in experimental animals, but is presumably based on the activity of this CYP with the congener trichloroethylene and with the fairly broad range of halogenated organic substrates of CYP2E1].

(ii) GSH-conjugation pathway

The presence of an active GSH-conjugation pathway for tetrachloroethylene has been demonstrated *in vivo* in experimental animals (rats and mice) through detection of NAcTCVC as a urinary metabolite (Bartels 1994; Dekant *et al.*, 1986a). The initial step in the pathway,

conjugation with GSH to form TCVG, has been shown to occur in rat liver (Dekant *et al.*, 1987, 1998), which is presumably the primary site for most of this reaction in the body.

Because GSTs are found in most tissues, it was expected that TCVG formation could also occur in the kidneys. Accordingly, incubations of cytoplasm and microsomes from kidneys of F344 rats (both sexes) and B6C3F1 mice (both sexes) with 2 mM tetrachloroethylene and 5 mM GSH demonstrated readily measurable rates of TCVG formation (Lash *et al.*, 1998). Metabolism in cytoplasm and microsomes from liver of both sexes of both species were also measured for comparison. Rates of GSH conjugation of TCVG were consistently higher in the corresponding fractions and tissues of males than in females in both species. In the kidneys, this difference was 2–3-fold for rats and 1.5–2-fold for mice. As expected, rates of TCVG formation in subcellular fractions of rat liver were 8–30 times higher than those in the corresponding kidney fraction from the same sex. While rates of TCVG formation were significantly higher in mice than in rats, the difference in rates between kidney and liver was much smaller in mice.

[The Working Group noted that although the higher rates of TCVG formation in male rats than in female rats correlates with the higher sensitivity of male rats to renal tumour formation from exposure to tetrachloroethylene, the markedly higher rates in mice of either sex does not correlate with the lack of renal tumours in this species (NTP, 1986).]

In contrast to the results described above, other investigators found no TCVG formation or much lower rates of GSH-conjugation of tetrachloroethylene. One study reported TCVG formation in rodent liver but not in human liver, and another showed TCVG formation in rat kidney at the limit of detection for the assay (i.e. 0.01 nmol/min per mg protein) (Dekant *et al.*, 1987, 1998; Green *et al.*, 1990). The latter value is similar to that reported by Lash *et al.* (1998).

The balance in the metabolism of tetrachloroethylene between CYP-dependent oxidation and GST-conjugation has been directly assessed in two studies.

Incubation of rat hepatocytes with either tetrachloroethylene or the prototypical GST-substrate 1-chloro-2,4-dinitrobenzene and NADPH resulted in a 70–85% reduction in the CYP-dependent oxidation pathway, suggesting that CYP-dependent oxidation can effectively compete with GST-conjugation in metabolizing tetrachloroethylene (Dekant *et al.*, 1987).

The second study addressed the effect of modulating the two metabolic processes in isolated rat hepatocytes and kidney cells. Treatment with non-selective CYP450 inhibitors (e.g. SKF-525A, metyrapone) or with CYP2E1-selective inhibitors (e.g. chlorzoxazone, diethyldithiocarbamate) significantly stimulated TCVG formation in both cell types. Inhibition of the CYP-dependent metabolism also resulted in increased cytotoxicity of tetrachloroethylene, but only in isolated kidney cells (Lash *et al.*, 2007). This finding highlights the importance of the GST-conjugation pathway for tetrachloroethylene-induced nephrotoxicity, but not hepatotoxicity (see Sections 4.5.1a and 4.5.2a for additional discussion of tetrachloroethylene-induced nephrotoxicity). Induction of CYP expression with either clofibrate or pyridine – compounds that enhance renal and hepatic expression of CYP2B1/2 and CYP2E1, respectively (Cummings *et al.*, 1999, 2001) – resulted in increased hepatic CYP-dependent metabolism of tetrachloroethylene in both cases, and increased renal CYP-dependent metabolism of tetrachloroethylene after treatment with clofibrate, but no effect on renal CYP-dependent metabolism of tetrachloroethylene after treatment with pyridine. These results suggest that patterns of CYP-dependent metabolism of tetrachloroethylene differ in rat liver and kidney (Lash *et al.*, 2007).

The differential roles of the CYP-dependent oxidation and GST-conjugation pathways in the bioactivation of tetrachloroethylene in rat liver and kidney were also demonstrated by modulation of the cellular GSH status. Treatment of freshly isolated rat hepatocytes with 5 mM methionine resulted in a ~50% increase in cellular GSH concentration and a significant reduction in tetrachloroethylene-induced cytotoxicity. In contrast, treatment of freshly isolated rat kidney cells with GSH at 5 mM resulted in a doubling of the cellular GSH concentration, but an increase in tetrachloroethylene-induced cytotoxicity. Conversely, depletion of tissue GSH with either L-buthionine-*S*,*R*-sulfoximine (BSO) or diethyl maleate resulted in increased cytotoxicity of tetrachloroethylene in isolated hepatocytes, but had no significant effect on cytotoxicity of tetrachloroethylene in isolated kidney cells. These results also demonstrate the distinct roles of the GSH-dependent metabolism of tetrachloroethylene in liver and kidney. In the liver, therefore, GSH plays its more traditional role with respect to tetrachloroethylene as an antioxidant and cytoprotective agent whereas in the kidneys, GSH functions primarily in the bioactivation and subsequent cytotoxicity of tetrachloroethylene (Lash *et al.*, 2007).

Two studies provide information on the metabolism of TCVC by enzymes with cysteine conjugate β-lyase activity, leading to formation of reactive thioacylating intermediates. In the first study, a bacterial lyase and *N*-dodecylpyridoxal bromide were used as catalysts to convert TCVC into dichloroacetic acid (Dekant *et al.*, 1988). In the second study, incubation of rat-kidney subcellular fractions with either tetrachloroethylene or TCVC produced *N*ε-(dichloroacetyl)-L-lysyl residues in proteins, primarily in mitochondria and cytoplasm. Formation of these adducts was inhibited by pre-incubation with the CCBL inhibitor amino-oxyacetic acid (AOAA), providing further evidence of the function of these enzymes (Birner *et al.*, 1994).

Formation of a TCVC-sulfoxide metabolite was reported to result from incubations of TCVC with rat or rabbit kidney or liver microsomes that exhibited all the properties of an FMO isoenzyme. Of the five FMO isoenzymes, TCVC is a substrate only for FMO3 (Elfarra & Krause, 2007; Ripp *et al.*, 1997; Novick & Elfarra, 2008).

Some of the cytotoxicity of TCVC that was independent of CCBL and FMO3 enzymes was shown to be actually due to formation of a mercapturic acid sulfoxide. By use of various selective inhibitors, including the CYP3A inhibitor troleanodomycin, it was shown that CYP3A enzymes were responsible for the sulfoxidation reaction (Werner *et al.*, 1996). [The Working Group noted that rates of this reaction were within a factor of 2 to 3 of those for CCBL or FMO3, suggesting that this additional pathway may play a role in tetrachloroethylene bioactivation and nephrotoxicity.]

The kinetics of the *N*-acetylation of TCVC in liver and kidney of male and female Wistar rats were determined *in vitro* by incubation of microsomes isolated from these organs with TCVC. Rates of NAcTCVC formation were substantially higher in kidney than in liver, particularly in females. Upon administration of 40 µmol TCVC/kg bw to rats, 40% of the compound was excreted as the mercapturate during 48 hours, and the rest as unmodified cysteine conjugate. This excretion of mercapturate was markedly less than that observed with DCVC (see next section). The authors hypothesized that the more lipophilic conjugates were more readily excreted in an unmodified form (Birner *et al.*, 1997).

(d) Comparison of the metabolisms of tetrachloroethylene and trichloroethylene

An understanding of the differences and similarities between the metabolism and pharmacokinetics of tetrachloroethylene and trichloroethylene is critical because much less direct information is available for tetrachloroethylene and extrapolations are often made from

data with trichloroethylene. Despite the fact that trichloroacetate is a major oxidative metabolite of both chemicals, the rates of formation from tetrachloroethylene and trichloroethylene differ substantially. Trichloroethanol and its glucuronide are oxidative metabolites of trichloroethylene that have not been consistently detected after exposure to tetrachloroethylene, and results may have been confounded in some studies due to co-exposure with trichloroethylene. The available data suggest that tetrachloroethylene is less extensively metabolized than trichloroethylene in both humans and experimental animals because it is a much poorer substrate than trichloroethylene for the CYP-dependent oxidation pathway (Ohtsuki *et al.*, 1983; Völkel *et al.*, 1998; Lash & Parker, 2001). For example, maximal rates of tetrachloroethylene metabolism by CYP-dependent oxidation in rat-liver microsomes have been estimated to be 30 times lower than those for trichloroethylene (Costa & Ivanetich, 1980). Besides its lower affinity for CYPs, tetrachloroethylene is also more lipophilic than trichloroethylene and exhibits greater sequestration in fat than trichloroethylene. This fact alone would slow the distribution of tetrachloroethylene to sites of metabolism in comparison with trichloroethylene.

A major difference between the GSH-dependent metabolism of trichloroethylene and that of tetrachloroethylene is that amounts of TCVG formed in kidney cells of male rats were approximately 5-fold those of *S*-(1,2,-dichlorovinyl)glutathione (DCVG) (Lash *et al.*, 1998, 2007). The fraction of total GSH-conjugation of trichloroethylene attributable to renal metabolism can be estimated to be 1.8%, and that of tetrachloroethylene 8.6%, i.e. 4.8-higher. In addition, oxidative metabolism of tetrachloroethylene in the liver is significantly slower than that of trichloroethylene. These data suggest that not only does renal GSH-conjugation make a relatively larger contribution towards overall GSH-conjugation for tetrachloroethylene than for trichloroethylene, but that GSH-conjugation contributes more towards the overall metabolism of tetrachloroethylene. The TCVC metabolite generated by CCBL is more chemically reactive than the metabolite generated by the action of CCBL on DCVC (Lash & Parker, 2001).

Besides differences related to the CCBL reaction route, differences also exist between the metabolic conversions of tetrachloroethylene and trichloroethylene in the FMO pathway. Studies with rabbit-liver microsomes showed that TCVC is overall a better substrate than DCVC for FMO3. Although the V_{max} for DCVC as substrate was sevenfold that for TCVC, the affinity of TCVC for the enzyme was nearly 20-fold (i.e. the K_m of FMO3 for TCVC was 20-fold lower than that for DCVC). Therefore, the catalytic efficiency of FMO3 towards TCVC is twice that towards DCVC. However, in a comparison of the kinetics of *S*-oxidation of TCVC and DCVC in bacterial membranes containing rabbit cDNA-expressed FMO3, the K_m for TCVC was about seven times lower than that for DCVC, while the V_{max} for DCVC was 50-fold that for TCVC. In this case, the catalytic efficiency with DCVC is sixfold that for TCVC (Ripp *et al.*, 1997).

The kinetics of *N*-acetylation of DCVC and TCVC in hepatic and renal microsomes from male and female Wistar rats were investigated. The rates of *N*-acetylation for both cysteine conjugates were consistently higher in kidney than in liver. In addition, in both males and females and in both organs the V_{max} values for *N*-acetylation with TCVC as substrate were generally higher than those for DCVC (Birner *et al.*, 1997).

[Considering the data for the metabolism of TCVC and DCVC by CCBL, FMO3, and the cysteine conjugate *N*-acetyltransferase (CCNAT), along with the marked difference in chemical reactivity of the thioketenes and sulfoxides generated from the two conjugates, the Working Group noted that one might expect renal proximal tubular cells to experience greater exposure to more reactive metabolites from

tetrachloroethylene as compared with trichloroethylene. However, no data making this comparison directly are available.]

4.1.4 Excretion

(a) Humans

In humans, the main excretion route of tetrachloroethylene is by exhalation of the unchanged parent compound, with smaller amounts of metabolites excreted in the urine (Ikeda *et al.*, 1972; Köppel *et al.*, 1985; Monster, 1979). Based on measured concentrations in exhaled breath, pulmonary excretion has been estimated to account for 80–100% of the intake (Monster *et al.*, 1979; Chiu *et al.*, 2007). After cessation of exposure, pulmonary excretion occurs in multiple first-order phases due to release from different tissues, with initial half-lives in the range of 5 to 20 minutes, several intermediate phases, and terminal half-lives of around 50–65 hours (Guberan & Fernandez, 1974; Monster *et al.*, 1979; Chien 1997). Urinary excretion of trichloro-metabolites (predominantly trichloroacetic acid) accounts for around 1–3% of intake (Stewart *et al.*, 1970; Essing *et al.*, 1973; Fernandez *et al.*, 1976; Monster *et al.*, 1979; Chiu *et al.*, 2007), with urinary excretion of several GSH-derived metabolic products representing an even smaller fraction (Völkel *et al.*, 1998). However, in these studies the urinary excretion was not followed for more than 3–7 days, and it is possible that a larger percentage of the tetrachloroethylene dose was eventually excreted in the urine. In studies that also measured pulmonary excretion, the entire dose was not always accounted for in the sum of exhaled tetrachloroethylene and urinary excretion of trichloroacetate (Monster *et al.*, 1979; Chiu *et al.*, 2007). Part of the administered dose may become metabolized to biotransformation products that were not measured, including oxidative products such as carbon monoxide, carbon dioxide, or oxalic acid, and additional GSH-conjugation products such as sulfoxides and reactive thiols. Physiologically based pharmacokinetic (PBPK) model-based estimates of the amount excreted via exhalation have ranged widely, with the most recent PBPK model predicting ranges of 90–99% via inhalation and 81–99% via ingestion (Chiu & Ginsberg, 2011).

(b) Experimental systems

In experimental rats and mice, excretion half-lives are in the order of hours, with pulmonary excretion virtually complete (> 99%) within 24 hours (Pegg *et al.*, 1979; Schumann *et al.*, 1980). This indicates that elimination is much more rapid in rodents than in humans. The extent of pulmonary excretion of unchanged tetrachloroethylene is dependent on species and dose. As exposure levels increase, the percentage of the compound excreted unchanged increases, which reflects saturation of metabolism (Pegg *et al.*, 1979; Schumann *et al.*, 1980). Pulmonary excretion appears greater in rats than in mice (Filser & Bolt 1979; Buben & O'Flaherty, 1985). The recently proposed PBPK model predicts the percentage tetrachloroethylene exhaled unchanged at exposures below saturation to be around 90–95% in rats and about 40–80% in mice, depending on the route of administration (Chiu & Ginsberg, 2011).

4.2 Genotoxicity and related effects

4.2.1 Humans

Several small cross-sectional studies have evaluated genotoxic and cytogenetic effects associated with exposure to tetrachloroethylene (Table 4.2). These studies have included assessments of the frequency of chromosomal aberrations and sister-chromatid exchange (SCE), the frequency of acentric fragments, and the presence of markers of oxidative stress.

(a) Chromosomal aberrations and sister-chromatid exchange

A cross-sectional study among 27 dry-cleaning workers and 26 controls in Japan reported no significant increase in the frequency of SCE in association with exposure to tetrachloroethylene. SCE was significantly increased in tetrachloroethylene-exposed male smokers compared with nonsmoking controls (Seiji *et al.*, 1990).

A study of 10 tetrachloroethylene-exposed degreasing workers and 11 non-exposed controls found no significant increase in numerical or structural chromosomal aberrations or in frequency of SCE (Ikeda *et al.*, 1980).

The frequencies of acentric fragments and chromosomal translocations – considered to be suitable indicators of chronic genotoxicity – were assessed in a cross-sectional study in the USA including 18 female tetrachloroethylene-exposed dry-cleaning workers, and 18 laundry workers not exposed to tetrachloroethylene. The average employment duration for the exposed dry-cleaners was 8 years. Air samples in the personal breathing-zone were collected from the dry-cleaning workers and from a subset of the laundry workers. A time-weighted average (TWA) exposure level of 3.8 ppm was measured in the exposed workers, while exposure levels were below the limit of detection in the controls. Chromosome painting was used to evaluate the frequency of cells with chromosomal translocations, insertions, dicentric and acentric fragments, and colour junctions. While the frequencies of each of these end-points, including translocations, were elevated in the exposed dry-cleaning workers compared with the controls, none of the increases were statistically significant (Tucker *et al.*, 2011).

(b) Oxidative stress

An earlier cross-sectional study in 18 dry-cleaning workers and 20 laundry workers (all women) showed a significantly reduced level of 8-OH-dG in leukocytes of the dry-cleaning workers, but this could not be clearly attributed to exposure to tetrachloroethylene. There were no increases in other oxidative stress biomarkers in relation to exposure to tetrachloroethylene (Toraason *et al.*, 2003).

4.2.2 Experimental systems

The genetic toxicology of tetrachloroethylene has been reviewed (Fabricant & Chalmers, 1980; Reichert, 1983; WHO, 1984; Vainio *et al.*, 1985; Illing *et al.*, 1987; European Centre for Ecotoxicology and Toxicology of Chemicals, 1990; Jackson *et al.*, 1993; IARC, 1995; ATSDR, 1997b; EPA, 2012). The mechanisms by which tetrachloroethylene causes genotoxicity have been discussed (Henschler, 1987). Table 4.3 presents the studies of genotoxicity published to date. Highlights from these studies are given below.

(a) DNA-and protein-binding

In a study *in vivo*, mice were exposed orally or by inhalation to tetrachloro[^{14}C]ethylene. Labelling was observed in protein and RNA, but not in DNA of the liver (Schumann *et al.*, 1980). In a more sensitive assay and after intraperitoneal injection of the radiolabelled compound, DNA-labelling was detected in mouse liver and kidney, and in rat and mouse stomach. Binding was highest in mouse liver and stomach. Metabolic activation was found to enhance binding of tetrachloroethylene to calf-thymus DNA *in vitro* (Mazzullo *et al.*, 1987).

(b) Mutations

Mutations were not generally observed after exposure of *Escherichia coli* or *Salmonella typhimurium* cells to tetrachloroethylene, with or

Table 4.2 Molecular cross-sectional studies of genotoxicity and exposure to tetrachloroethylene

Reference, study location	Total No. exposed	Total No. controls (unexposed)	Mean exposure levels	End-points evaluated	Notable effects	Comments
Ikeda *et al.* (1980) Japan	10	11	92 ppm (degreasing workshops; $n = 6$) and 10–40 ppm (support department; $n = 4$)	Erythrocytes, leukocytes, haemoglobin, haematocrit, structural and numerical chromosomal aberrations, SCE, mitotic index, proportion of $M_2 + M_3$ metaphases	No significant differences for SCE, chromosomal aberrations, mitotic index between exposed workers and controls	Workers divided into exposure groups based on inferred exposure according to work environment (degreasing shop *vs* support department) and work duration; urine analysis for total trichlorinated compounds and ambient air samples taken, but no personal monitoring; limited information on comparability of exposed and non-exposed groups; no evaluation of smoking
Seiji *et al.* (1990) Japan	27	26	10 ppm	SCE	Significantly elevated SCE frequency in exposed smoking men compared with nonsmoking controls, but not for other groups	Diffuse sampling techniques used to monitor workers in breathing zone as TWA for 8-hour work; concurrent controls matched to exposed workers by age, sex, smoking, location of factory
Toraason *et al.* (2003) USA	18	20	Average 3.1 ppm, for dry-cleaning workers; < 0.02 ppm, for laundry workers (below LOD)	Leukocyte and urine 8-OHdG, urinary 8-epi-PGF	Reduced leukocyte 8-OHdG in dry cleaners compared to launderers (control), but no differences for other markers of oxidative stress	Personal breathing-zone samples collected for all women over 2 days (8-hour TWA); laundry workers matched to dry-cleaning workers by race, age, and smoking status; similar distribution of BMI between groups
Tucker *et al.* (2011) USA	18	18	TWA (8-hour) exposure, 3.8 ppm for exposed group (dry cleaners); below LOD for controls (laundry workers)	Translocations, insertions, acentric fragments, and dicentrics	Significant correlation between exposure levels and percentage of cells with acentric fragments; no differences in translocation frequencies between exposed and controls	Personal breathing zone samples collected from exposed dry-cleaning workers mid-working week; non-exposed laundry workers matched to exposed dry-cleaning workers by age, race, and smoking habits

8-epi-PGF, 8-epi-prostaglandin $F_{2\alpha}$; 8-OHdG, 8-hydroxy-deoxyguanosine; BMI, body-mass index; LOD, limit of detection; SCE, sister-chromatid exchange; TWA, time-weighted average

Table 4.3 Genetic and related effects of tetrachloroethylene and trichloroacetyl chloride

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Tetrachloroethylene				
Binding (covalent) to calf thymus DNA *in vitro*	0	+	9	Mazzullo *et al.* (1987)
SOS chromotest, *Escherichia coli* PQ37	–	–	8150	Mersch-Sundermann *et al.* (1989)
Lambda-prophage induction, *Escherichia coli* WP2	–	–	10 000	DeMarini *et al.* (1994)
Salmonella typhimurium BAL13, forward mutation (*ara* test)	–	–	76	Roldán-Arjona *et al.* (1991)
Salmonella typhimurium TA100, reverse mutation	–	–	660	Bartsch *et al.* (1979)
Salmonella typhimurium TA100, reverse mutation	–	–	167	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	–	–	1000	Connor *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	–[c]	–	166 (vapour)	Shimada *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation (liver- and kidney-derived microsomes used for metabolic activation)	–	+/(+)[d]	332	Vamvakas *et al.* (1989a)
Salmonella typhimurium TA100, reverse mutation	–	–	1.3 (vapour)	DeMarini *et al.* (1994)
Salmonella typhimurium TA1535, reverse mutation	–	NT	50	Kringstad *et al.* (1981)
Salmonella typhimurium TA1535, reverse mutation	–	–	167	Haworth *et al.* (1983)
Salmonella typhimurium TA1535, reverse mutation	–[c]	(+)	66 (vapour)	Shimada *et al.* (1985)
Salmonella typhimurium TA1537, reverse mutation	–	–	167	Haworth *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	–	–	167	Haworth *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	–	–	1000	Connor *et al.* (1985)
Salmonella typhimurium UTH8413, reverse mutation	–	–	1000	Connor *et al.* (1985)
Salmonella typhimurium UTH8414, reverse mutation	–	–	1000	Connor *et al.* (1985)
Escherichia coli K12, forward mutation	–	–	150	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (arg^+)	–	–	150	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (gal^+)	–	–	150	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (nad^+)	–	–	150	Greim *et al.* (1975)
Saccharomyces cerevisiae D7, log-phase cultures, gene conversion	+	NT	1100	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, gene conversion	–	–	9960	Bronzetti *et al.* (1983)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, gene conversion	–	–	2440	Koch *et al.* (1988)

Table 4.3 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Saccharomyces cerevisiae D7, log-phase cultures, mitotic recombination or other genetic alterations (*ade2*)	+	NT	1100	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, mitotic recombination	–	–	9960	Bronzetti *et al.* (1983)
Saccharomyces cerevisiae D7, log-phase cultures, reverse mutation	(+)	NT	810	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, reverse mutation	–	–	9960	Bronzetti *et al.* (1983)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, reverse mutation	–	–	2440	Koch *et al.* (1988)
Saccharomyces cerevisiae D61.M, growing cells, aneuploidy	(+)	(+)	810	Koch *et al.* (1988)
Tradescantia species, mutation	+	NT	7 (vapour)	Schairer & Sautkulis (1982)
Tradescantia species, micronucleus induction	–	NT	600	Sandhu *et al.* (1989)
Tradescantia species, micronucleus induction	(+)	NT	2 (vapour)	Sandhu *et al.* (1989)
Drosophila melanogaster, sex-linked recessive mutation	–		1000 (injection)	Valencia *et al.* (1985)
Drosophila melanogaster, sex-linked recessive mutation	–		4000 (feeding)	Valencia *et al.* (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–[c]	NT	166 (vapour)	Shimada *et al.* (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	–	–	245	NTP (1986)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	–	164	Galloway *et al.* (1987)
Micronucleus induction, Chinese hamster ovary (CHO-K1) cells *in vitro*	+	NT	~1.85 ppm (vapour dissolved in medium)	Wang *et al.* (2001)
Micronucleus induction, Chinese hamster lung-cell line (CHL/IU) *in vitro*	–	NT	250 µg/mL	Matsushima *et al.* (1999)
Chromosomal aberrations, Chinese hamster lung (CHL) cells *in vitro*	–	–	500	Sofuni *et al.* (1985)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–	–	136	Galloway *et al.* (1987)
Cell transformation, RLV/Fischer rat embryo F1706 cells *in vitro*	+	NT	16	Price *et al.* (1978)
Cell transformation, BALB/c-3T3 mouse cells *in vitro*	–	NT	250	Tu *et al.* (1985)
Single-cell gel electrophoresis assay (comet), human leukocytes *in vitro*	–	–	5 mM	Hartmann and Speit (1995)
Unscheduled DNA synthesis, human lymphocytes *in vitro*	+	–	10 mM	Perocco *et al.* (1983)

Table 4.3 (continued)

Test system	Result[a]			Dose[b] (LED/HID)	Reference
	Without exogenous metabolic activation		With exogenous metabolic activation		
Sister-chromatid exchange, human lymphocytes *in vitro*	–		–	2 mM	Hartmann and Speit (1995)
Micronucleus induction, human lymphoblastoid cell lines with enhanced metabolic activity *in vitro*	AHH-1 (expresses CYP1A1)	+	NT	5 mM	Doherty *et al.* (1996)
	h2E1 (expresses CYP2E1)	+	NT	1 mM	
	MCL-5 (expresses CYP1A2, 2A6, 3A4, 2E1)	+	NT	1 mM	
DNA single-strand breaks (alkaline unwinding), liver/kidney of male NMRI mice *in vivo*	+[e]			660 ip × 1	Walles (1986)
Single-cell gel electrophoresis assay (comet), CD1 mouse hepatocytes *in vivo*	+/–		NT	1000 mg/kg per day[f]	Cederberg *et al.* (2010)
	+/–		NT	2000 mg/kg per day[f]	
Single-cell gel electrophoresis assay (comet), CD1 mouse kidney cells *in vivo*	–		NT	2000 mg/kg per day[f]	
Binding (covalent) to DNA in male B6C3F$_1$ mouse liver *in vivo*	–			1400 in 6 h	Schumann *et al.* (1980)
Binding (covalent) to DNA in male B6C3F$_1$ mouse liver *in vivo*	–			500 po × 1	Schumann *et al.* (1980)
Binding (covalent) to DNA in male BALB/c mouse and Wistar rat liver, kidney, lung and stomach *in vivo*	+			1.3 ip × 1	Mazzullo *et al.* (1987)
Binding (covalent) to RNA and protein in male BALB/c mouse and Wistar rat liver, kidney, lung and stomach *in vivo*	+			1.3 ip × 1	Mazzullo *et al.* (1987)
Gene conversion and reverse mutation, *Saccharomyces cerevisiae* D7 recovered from liver, lungs and kidneys of CD-1 mice *in vivo*	–		NT	11 000 po × 1	Bronzetti *et al.* (1983)
Gene conversion and reverse mutation, *Saccharomyces cerevisiae* D7 recovered from liver, lungs and kidneys of CD-1 mice *in vivo*	0		–	2000 po × 12	Bronzetti *et al.* (1983)
Micronucleus induction, mouse reticulocytes, *in vivo*	–		NT	1 × 2000	Murakami and Horikawa (1995)
Micronucleus induction, mouse hepatocytes, *in vivo*	+		NT	1 × 1000 mg/kg bw[g]	

Table 4.3 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Micronucleus induction, mouse hepatocytes, *in vivo (cont.)*	–	NT	1 × 2000 mg/kg bw[h]	
Oxidative DNA damage (8-OHdG), Fischer rats *in vivo* (urine, lymphocytes, liver)	–[i]	NT	1 × 100–1000	Toraason *et al.* (1999)
DNA single-strand breaks, male F344 rats *in vivo*	–	NT	1 × 1000 (p.o.)	Potter *et al.* (1996)
Enzyme-altered foci in male Osborne Mendel rat liver *in vivo* (promotion protocol, with or without NDEA as an initiator)	+		1000 (p.o.), 5 days/wk, 7 wks	Milman *et al.* (1988)
Enzyme-altered foci in male Osborne Mendel rat liver *in vivo* (initiation protocol with phenobarbital as a promoter)	–		1000 (p.o.)	Milman *et al.* (1988)
Sister chromatid exchange, human lymphocytes *in vivo*	–		88 inh	Ikeda *et al.* (1980)
Sister-chromatid exchange, human lymphocytes *in vivo*	–	NT	10 ppm (geometric mean)	Seiji *et al.* (1990)
Chromosomal aberrations, human lymphocytes *in vivo*	–		88 inh	Ikeda *et al.* (1980)
Chromosomal aberrations, human lymphocytes *in vivo*	+	NT	144 mg/m^3 (contaminated with trichloroethylene)	Fender (1993)
Trichloroacetyl chloride				
Lambda-prophage induction, *Escherichia coli* WP2	–	–	10 000	DeMarini *et al.* (1994)
Salmonella typhimurium TA100, reverse mutation	+	+	2.6	DeMarini *et al.* (1994)

[a] +, positive; (+), weakly positive, inadequate study; –, negative; ?, inconclusive (variable responses in several experiments, inadequate study)
[b] In-vitro tests, µg/ml; in-vivo tests, mg/kg bw
[c] Tetrachloroethylene with stabilizers was positive with and without metabolic activation
[d] Equivocal increase in activity with S9 from rat liver, unequivocal increase with kidney microsomes and glutathione (GSH); strong (fourfold) increase with rat-kidney microsomes, GSH and GSH *S*-transferase; the effect was diminished upon addition of serine borate or AOAA.
[e] Negative in lung
[f] Number of days not given
g After partial hepatectomy
[h] Without hepatectomy
[I] Substantial morbidity at the high dose limits interpretation of this result
LED, lowest effective dose; HID, highest ineffective dose; inh, inhalation ; i.p., intraperitoneal NDEA, *N*-nitrosodiethylamine; NT, not tested; p.o., oral

without the standard metabolic activation by S9. In the diploid D7 strain of the yeast *Saccharomyces cerevisiae*, tetrachloroethylene caused mitotic gene conversion and recombination in one study (Callen *et al.*, 1980), but failed to do so in two other studies (Bronzetti *et al.*, 1983; Koch *et al.*, 1988).

Tetrachloroethylene was not active in the SOS chromotest with *E. coli* and was not mutagenic to bacteria in the absence of metabolic activation. Purified tetrachloroethylene was not mutagenic to *S. typhimurium* or *E. coli* when tested in the presence of a metabolic-activation system prepared from rat-liver microsomes; however, a doubling of revertant frequencies was seen in *S. typhimurium* TA1535 in one study at both doses tested. In another study, purified tetrachloroethylene clearly increased the number of revertants in *S. typhimurium* TA100 in the presence of rat-liver GST, glutathione and kidney microsomes. This study was intended to simulate the multistep bio-activation pathway by GSH-conjugation (Vamvakas *et al.*, 1989a). The mutagenicity was accompanied by time-dependent formation of *S*-(1,2,2-trichlorovinyl) glutathione, a pro-mutagen activated by kidney microsomes, and did not occur in the absence of GSH, GST, or kidney microsomes. Bile collected from an isolated rat-liver system perfused with tetrachloroethylene was clearly mutagenic in the presence of rat-kidney particulate fractions. In both assay systems, the mutagenicity was reduced, but not abolished, by the presence of the γ-glutamyl transpeptidase inhibitor, serine borate, and the β-lyase inhibitor AOAA, suggesting that the important steps in the metabolic activation of tetrachloroethylene in renal fractions are GST-mediated GSH-conjugation, followed by γ-glutamyl-transpeptidase-mediated formation of an *S*-cysteine conjugate, and bio-activation of the *S*-cysteine conjugate by β-lyase.

Tetrachloroethylene did not induce gene conversion, mitotic recombination or reverse mutations in yeast in stationary cultures. Both negative and positive findings were reported from studies of cultures in logarithmic growth phase, which stimulates xenobiotic metabolism; and positive results were obtained with tetrachloroethylene containing 0.01% thymol as a stabilizer. In a single study in yeast, tetrachloroethylene (analytical grade) weakly induced aneuploidy in growing cells capable of xenobiotic metabolism.

In single studies with *Tradescantia*, tetrachloroethylene [purity not given] induced mutations, and a compound of 99% purity induced a slight increase in the frequency of micronucleus formation.

Studies in *Drosophila melanogaster* were negative for sex-linked recessive lethal mutations (Beliles et al., 1980; Valencia et al., 1985; NTP, 1986).

(c) Micronucleus formation, SCE, and chromosomal aberrations

Tetrachloroethylene increased the frequency of micronucleus formation in hepatocytes, but not in peripheral blood reticulocytes of ddY mice given single intraperitoneal injections after, but not before, partial hepatectomy (Murakami & Horikawa, 1995). Micronucleus induction was not observed in a Chinese hamster lung cell line (CHL/IU) exposed to tetrachloroethylene (Matsushima *et al.*, 1999). When Chinese hamster ovary (CHO K1) cells were exposed to tetrachloroethylene in a closed vapour-exposure system, a significant, dose-dependent increase in the frequency of micronuclei was found (Wang *et al.*, 2001) Micronucleus induction was studied in AHH-1 human lymphoblastoid cells and in two daughter cell lines (h2E1 and MCL5) stably expressing various human metabolic enzymes. The parental AHH-1 cells possess native CYP1A1 activity and considerable GST activity; the h2E1 cells express human CYP2E1; and MCL-5 cells express human CYP1A2, 2A6, 3A4, 2E1, and microsomal epoxide hydrolase. Tetrachloroethylene induced an increase in micronuclei formation by threefold in AHH-1

cells and ninefold in h2E1 and MCL-5 cells (Doherty *et al.*, 1996). Similar results were reported with the MCL-5 cell line (White *et al.*, 2001).

Tetrachloroethylene-induced DNA damage was not observed in the SCE assay in cultured human blood cells at a dose that reduced viability by 40% due to cytotoxicity (Hartmann & Speit, 1995). Neither chromosomal aberrations nor SCE were induced in cultured Chinese hamster ovary cells after exposure to tetrachloroethylene (Sofuni *et al.* 1985; Galloway *et al.*, 1987). Chinese hamster ovary cells exposed to tetrachloroethylene in the presence or absence of S9 fraction (derived from livers of Sprague Dawley rats) showed no increase in SCE frequency (NTP, 1986). Beliles *et al.* (1980) assessed chromosomal aberrations and aneuploidy in bone marrow of male and female Sprague-Dawley rats after single and short-term exposures to tetrachloroethylene by inhalation. The only effect reported with a single exposure was a slight increase in the percentage of cells with aberrations and aneuploidy in male, but not female rats. No significant effects were observed in any of the groups exposed for short periods. No chromosomal aberrations were found in Chinese hamster ovary cells exposed to tetrachloroethylene with or without metabolic activation (NTP, 1986).

Studies in *Drosophila melanogaster* were negative for chromosomal aberations (Beliles *et al.*, 1980; Valencia *et al.*, 1985; NTP, 1986).

(d) Unscheduled DNA synthesis and DNA strand breaks

Human fibroblasts (WI 38 cells) were tested for the induction of unscheduled DNA synthesis after exposure to tetrachloroethylene; the results were equivocal (Beliles *et al.*, 1980). However, the positive controls were only weakly positive in this study. In other studies, no evidence of unscheduled DNA synthesis was observed in human lymphocytes, human fibroblasts, or rat and mouse hepatocytes (Perocco *et al.*, 1983; Costa & Ivanetich, 1984; Shimada *et al.*, 1985; Milman *et al.*, 1988).

The results of the few assays for DNA strand break after exposure to tetrachloroethylene *in vivo* were equivocal. DNA single-strand breaks were measured in the liver and kidney of male mice exposed by intraperitoneal injection, but this effect was reversible within 24 hours (Walles, 1986). A second study examined DNA strand breaks after oral exposure of male F-344 rats to tetrachloroethylene for 1 week, and demonstrated no DNA breakage (Potter *et al.*, 1996). A recently published report on DNA strand breaks in hepatocytes showed a marginal increase in tail intensity in the comet assay after oral exposure of mice to tetrachloroethylene (Cederberg *et al.*, 2010); the statistical and biological significance of this result has been disputed (Lillford *et al.*, 2010; Struwe *et al.*, 2011).

(e) Cell transformation

Treatment with tetrachloroethylene for 3 days without metabolic activation did not induce cell transformation in BALB/c 3T3 cells after a 30-day post-treatment incubation period (Tu *et al.*, 1985). However, cells from Fischer rat embryos were transformed upon treatment with tetrachloroethylene in the absence of metabolic activation (Price *et al.*, 1978).

(f) Genotoxicity of metabolites of tetrachloroethylene

A limited number of experimental studies on the genotoxicity of tetrachloroethylene metabolites have been performed (see Table 4.4).

Trichloroacetyl chloride results from oxidative metabolism of tetrachloroethylene, and has been tested for mutagenicity in *S. typhimurium*, with inconsistent results. An early study found no mutagenicity after incubation in liquid suspension with *S. typhimurium* TA98 and TA100 strains, with or without S9 activation (Reichert *et al.*, 1983). In a second study, trichloroacetyl chloride as a vapour was tested for mutagenicity

in *S. typhimurium* TA100 and found to give positive results in the presence or absence of metabolic activation; the chemical induced mostly GC > TA transversions (the predominant background mutation). Trichloroacetyl chloride gave negative results for prophage induction in *E. coli* in the same study (DeMarini *et al.*, 1994).

Tetrachloroethylene epoxide, an intermediate in the CYP450-mediated oxidative metabolism of tetrachloroethylene (Henschler, 1977; Henschler & Bonse, 1977), has been tested for mutagenicity in a single study. It was mutagenic in *S. typhimurium* TA1535, but not in *E. coli* WP2*uvr*A (Kline *et al.*, 1982). Mutagenicity was observed at the lower doses in *S. typhimurium*, but not at higher doses, most likely due to cytotoxicity.

Only a small number of studies were available that assessed the genotoxicity of metabolites in the GSH-conjugation pathway of the metabolism of tetrachloroethylene.

TCVG formed from tetrachloroethylene in isolated perfused rat liver and excreted into bile was mutagenic in *S. typhimurium* in the presence of a rat-kidney homogenate, which contained high concentrations of GGT (Vamvakas *et al.*, 1989a). In this study, the mutagenicity assay was conducted with *Salmonella* strains TA100, TA98, and TA2638, and with tetrachloroethylene, TCVG, and bile from liver perfusates of tetrachloroethylene-exposed rats. The results show that the GST metabolites, or tetrachloroethylene in the presence of bile containing GST, led to gene mutations in *S. typhimurium* TA100. In a more recent study, TCVG showed an unequivocal dose-dependent mutagenic response in *Salmonella* TA100 in the presence of S9 protein fraction from rat kidney; TCVC was mutagenic without metabolic activation in this strain (Dreessen *et al.*, 2003). TCVC (1–10 nmol/plate) was also mutagenic in *Salmonella* strains TA98 and TA100, but not in TA2638, and β-lyase activity was blocked by the addition of AOAA (Dekant *et al.*, 1986b). A further study from the same group indicated that *Salmonella* bacteria were capable of deacetylating the urinary metabolite NAcTCVC (50–100 nmol/plate) when TA100 showed a clearly positive response without exogenous activation (Vamvakas *et al.*, 1987). Addition of cytosolic protein increased the mutagenic response, while addition of the β-lyase inhibitor AOAA reduced it.

A concentration-related increase in unscheduled DNA synthesis was found in LLC PK1 cells (derived from porcine kidney) exposed to TCVC; this effect was abolished by a β-lyase inhibitor. To determine cytotoxicity, release of lactate dehydrogenase was measured, but no increase was found (Vamvakas *et al.*, 1989c).

4.3 Non-genotoxic effects and organ toxicity

4.3.1 Kidney

The available studies in humans and experimental animals have addressed multiple non-genotoxic mechanisms for kidney carcinogenicity associated with exposure to tetrachloroethylene. These include accumulation of α2u-globulin, cytotoxicity unrelated to α2u-globulin, and peroxisome proliferator-activated receptor-α (PPARα) activation.

(a) Accumulation of α2u-globulin

Accumulation of α2u-globulin is a histopathological phenomenon elicited in the kidney of the male rat by long-term exposure to chemicals. The hypothesized sequence of key events comprises:

- Excessive accumulation of hyaline droplets containing α2u-globulin in renal proximal tubules;
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium;
- Sustained regenerative tubule cell proliferation;

Table 4.4 Studies of genotoxicity with metabolites of tetrachloroethylene

Metabolite	Test system/end-point	Results[b]		Dose[a] (LED or HID)	Reference
		Without exogenous metabolic activation	With exogenous metabolic activation		
Tetrachloroethylene epoxide	*S. typhimurium* TA1535, reverse mutation	+	NT	2.5 mM	Kline *et al.* (1982)
	E. coli WP2 *uvr*A, reverse mutation	–	NT	25 mM	Kline *et al.* (1982)
Trichloroacetyl chloride (TCAC)	Lambda-prophage induction, *E. coli* WP2	–	–	10 000	DeMarini *et al.* (1994)
	S. typhimurium TA100, reverse mutation	+	+	300/200 ppm[c]	DeMarini *et al.* (1994)
	S. typhimurium TA100, reverse mutation	–	–	5 µL/2 mL	Reichert *et al.* (1983)
Trichlorovinyl-glutathione (TCVG)	*S. typhimurium* TA100, reverse mutation	–	+	50 nmol/plate	Dreessen *et al.* (2003)
	S. typhimurium TA100, reverse mutation	–	+	25 nmol/plate	Vamvakas *et al.* (1989a)
	Unscheduled DNA synthesis, cultured porcine LLC-PK1 (kidney) cells, *in vitro*	+	NT	7.5×10^{-6} M	Vamvakas *et al.* (1989b)
Trichlorovinyl-cysteine (TCVC)	*S. typhimurium* TA100, reverse mutation	+	NT	50 nmol/plate	Dreessen *et al.* (2003)
	Unscheduled DNA synthesis, cultured porcine LLC-PK1 (kidney) cells, *in vitro*	+	NT	5×10^{-6} M	Vamvakas *et al.* (1989c)
NAcTCVC	*S. typhimurium* TA100, increased mutation frequency	+	+	< 50 nmol [d]	Vamvakas *et al.* (1987)

[a] LED, lowest effective dose; HID, highest ineffective dose; NA, not available
[b] Results: +, positive; (+), weakly positive; –, negative; NT, not tested
[c] Tested with the vaporization technique; doses in ppm given without/with activation
[d] Lower concentrations that indicate mutagenicity not specified in the article

- Development of intraluminal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization;
- Foci of tubule hyperplasia in the convoluted proximal tubules;
- Tumours of renal tubules.

Seven criteria are specified for demonstrating the α2u-globulin mechanism for carcinogenesis in male rat kidney (IARC, 1999), namely:

- Characteristic histopathology
- Specificity to male rats
- Accumulation of α2u-globulin
- Reversible binding to α2u-globulin
- Increased cell proliferation
- Similarities in dose–response relationships for histopathology and tumour outcome
- Lack of genotoxicity

This mechanism is posited to occur only in rodents; accordingly, only studies in experimental animals were identified, and are discussed below.

Three studies provide evidence satisfying four of the seven above-mentioned criteria (characteristic histopathology, specificity for the male rat, accumulation of α2u-globulin, and increased cell proliferation) for supporting the α2u-globulin mechanism, at doses of tetrachloroethylene in excess of those observed to induce tumorigenesis. These studies demonstrate that tetrachloroethylene induces accumulation of α2u-globulin and hyaline-droplet nephropathy in male rats (see Table 4.5).

Increased α2u-hyaline droplet formation was seen in male, but not female, F344 rats treated by gavage for 10 days with tetrachloroethylene at 1000 mg/kg bw per day. This finding was correlated with both protein-droplet nephropathy (crystalloid accumulation) and enhanced cellular proliferation. Cell replication was increased in the male rats specifically in damaged P_2 segments, suggesting a link between the α2u-globulin accumulation and the ensuing proliferative response (Goldsworthy *et al.*, 1988).

In short-term, high-dose, studies, oral administration of tetrachloroethylene at 1500 mg/kg bw per day for up to 42 days caused accumulation of α2u-globulin in the proximal renal tubules of male rats (Green *et al.*, 1990). [The Working Group noted the lack of a parallel experiment in female rats in this study].

Accumulation of α2u-globulin was also demonstrated to occur in P_2 segments of proximal tubule cells after oral exposure of male, but not female, rats to tetrachloroethylene at 500 mg/kg bw per day in corn oil 4 weeks (Bergamaschi *et al.*, 1992).

The primary limitation to the mechanistic support for the α2u-globulin mechanism concerns the criterion of similarities in dose–response relationships for histopathology and tumour outcome. The NTP cancer-bioassay (NTP, 1986) did not provide evidence of hyaline droplets in rats exposed to carcinogenic doses of tetrachloroethylene for up to 2 years. However, the NTP protocol at that time was not designed specifically to detect hyaline droplets or accumulation of α2u-globulin in the kidney (NTP, 1990). In addition, the nephropathy observed at the end of a 2-year bioassay would be difficult to distinguish from the nephropathy associated with advanced age in these rats. Other relevant findings concerning the correspondence in dose–response relationships for histopathology and tumour outcome are those reported by Green *et al.* (1990), who found no evidence of hyaline-droplet formation in tests with lower doses of inhaled tetrachloroethylene of up to 400 ppm for 6 hours per day, for 28 days. Because animals were killed up to 18 hours after termination of the final exposure, recovery may have been possible. The authors raised the possibility that a longer exposure to tetrachloroethylene at 400 ppm would be required for the hyaline-droplet accumulation in the rat kidney. However, accumulation has been demonstrated after

Table 4.5 Formation of renal α2u-globulin in rodents exposed to tetrachloroethylene

Test system (species, strain, sex, number)	Dose	Effects	Reference
Mouse, B6C3F$_1$, (M and F, groups of 49 or 50 mice of each sex, total of ~300 mice)	100, 200 ppm, 6 h/day, 5 days/wk for 103 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed mice; nephrosis in exposed females, casts increased in all exposed males and in females at higher dose	NTP (1986)
Rat, F344, (M and F, groups of 50 mice of each sex, total of ~300 mice)	200, 400 ppm, 6 h/day, 5 days/wk for 103 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed rats	NTP (1986)
Rat, F344 (M and F, 3 per group)	1000 mg/kg bw per day for 10 days, corn oil, gavage	Increases in α2u-hyaline droplets in exposed male but not female rats, correlated with increased cell proliferation and protein-droplet nephropathy	Goldsworthy *et al.* (1988)
Rat, F344 (M and F, 12 per group)	500 mg/kg bw per day for 4 wk, corn oil, gavage	Increases in α2u-hyaline accumulation in proximal tubule cells, correlated with albuminuria	Bergamaschi *et al.* (1992)
Rat, F344 (M and F) and mouse, B6C3F$_1$ (M and F) (10 per group for oral studies, 5 per group for inhalation studies)	1000 or 1500 mg/kg bw per day for 42 days, corn oil, gavage; 1000 ppm, 6 h/day for 10 days, by inhalation	After oral gavage, accumulation of α2u-globulin in proximal tubules of male rats; nephrotoxicity also observed in male rats (formation of granular tubular casts and evidence of tubular cell regeneration). Inhalation exposure induced formation of hyaline droplets in kidneys of male rats	Green *et al.* (1990)

F, female; h, hour; M, male; wk, week

short-term exposure (even after a single dose) to several agents, such as d-limonene, decalin, unleaded gasoline, and trimethylpentane (Charbonneau *et al.*, 1987; NTP, 1990). The renal pathology reported in the NTP bioassay is also not entirely consistent with the general findings for other chemicals that induce accumulation of α2u-globulin (NTP, 1986). For example, mineralization in the inner medulla and papilla of the kidney was not seen with tetrachloroethylene, but is a frequent finding in bioassays with chemicals that induce accumulation of α2u-globulin (e.g. for pentachloroethane, the incidence of renal papillar mineralization was 8% in controls, 59% in the group at the lowest dose, and 58% in the group at the highest dose) (NTP, 1983).

Concerning the remaining two criteria, no direct evidence demonstrating binding to α2u-globulin was identified. The evidence presented in Section 4.2 does not clearly rule out the potential role of genotoxicity, particularly in the kidney. Indeed, metabolites of tetrachloroethylene known to be produced in the kidney (e.g. TCVG and NAcTCVC) have been demonstrated to be genotoxic *in vitro*. In one study in mammalian cells, unscheduled DNA synthesis in porcine kidney cells was observed to increase in a dose-dependent manner after exposure to TCVC (Vamvakas *et al.*, 1989c). Bacterial assays found TCVG (Vamvakas *et al.*, 1989a; Dreessen *et al.*, 2003) and NAcTCVC (Vamvakas *et al.*, 1987) to be mutagenic in the presence of metabolic activation, while TCVC was mutagenic in the absence of activation (Dreessen *et al.*, 2003).

(b) *Cytotoxicity and sustained chronic nephrotoxicity, not associated with α2u-globulin*

This hypothesized mechanism for development of renal neoplasms involves renal cytotoxicity and subsequent cellular proliferation without regard to accumulation of α2u-globulin. Experimental evidence in humans and animals supporting this mechanism is summarized below.

(i) *Humans*

The renal toxicity of tetrachloroethylene has been demonstrated in studies with patients receiving high intentional exposures, and in occupational settings. High concentrations of inhaled tetrachloroethylene given as an anaesthetic are associated with symptoms of renal dysfunction, including proteinuria and haematuria (Hake & Stewart, 1977; ATSDR, 1997a). The study by Calvert *et al.* (2011) supports an association between inhalation exposure to tetrachloroethylene and end-stage renal disease, particularly hypertensive end-stage renal disease. There was an increase of more than 2.5-fold in incidence (SIR, 2.66; 95% CI, 1.15–5.23; 15 cases) among subjects who worked in a shop where tetrachloroethylene was the primary cleaning solvent compared with the expected incidence based on rates in the USA population. An exposure–response pattern was further suggested because the risk for hypertensive end-stage renal disease was highest among those employed for 5 years (SIR, 3.39; 95% CI, 1.10–7.92; five cases).

Other studies of the chronic effects of inhaled tetrachloroethylene on the kidney used measurements of urinary renal proteins as an indicator of kidney function. No effect on several urine parameters or blood urea nitrogen (a measure of kidney function) was reported with controlled inhalation exposure to tetrachloroethylene (25 or 100 ppm for 11 weeks, 12 healthy individuals) [Stewart *et al.* (1977), as reported in ATSDR (1997a)]. However, Mutti *et al.* (1992) observed an elevated prevalence of abnormal values for brush-border antigens, a higher geometric mean concentration of brush-border antigens in urine, and a higher concentration of tissue non-specific alkaline phosphatase in urine among 50 exposed dry-cleaning workers compared with 50 blood donors matched by sex and age with the exposed group. The markers of renal damage were highly

predictive of exposure status in discriminant analyses. The amount of β_2-microglobulin was not elevated among exposed subjects as compared with controls in this and two other studies that examined this protein (Lauwerys *et al.*, 1983; Vyskocil *et al.*, 1990). In two studies that measured β-glucuronidase or lysozyme, respectively, a statistically significant increase was reported in mean urinary concentration of these proteins among dry-cleaning workers compared with controls (Franchini *et al.*, 1983; Vyskocil *et al.*, 1990).

Several studies examined urinary indicators of renal tubule function – retinol-binding protein, *N*-acetyl-β-D-glucosaminidase, or alanine aminopeptidase – in workers in dry-cleaning exposed to tetrachloroethylene. One study reported a statistically significantly increased prevalence of abnormal values of retinol-binding protein, but no difference in geometric mean concentration between exposed and controls (Mutti *et al.*, 1992). Another study did find a higher geometric mean concentration of retinol-binding protein for exposed workers compared with controls (Verplanke *et al.*, 1999). However, no effect of tetrachloroethylene was seen in four studies that measured urinary excretion of *N*-acetyl-β-D-glucosaminidase (Mutti *et al.*, 1992; Verplanke *et al.*, 1999; Trevisan *et al.*, 2000) or in one that measured alanine aminopeptidase (Verplanke *et al.*, 1999).

Primary cultures of proximal tubular cells from both rat and human kidney were used to study the role of CCBL in the acute cytotoxicity caused by DCVC, TCVC, and two fluorinated cysteine conjugates of halogenated solvents. Incubation in the presence of the CCBL inhibitor AOAA resulted in partial protection only. Nonetheless, the study demonstrated a requirement for CCBL-dependent metabolism for DCVC to exert a toxic effect on human kidney cells. TCVC was less cytotoxic than DCVC. CCBL activity in proximal tubular cells from the rat kidney was threefold that in the human cells (McGoldrick *et al.*, 2003).

(ii) Experimental animals

Adverse effects on the kidney have been observed in studies of rodents exposed by inhalation (JISA, 1993; NTP, 1986) and oral gavage (NCI, 1977; Goldsworthy & Popp, 1987; Goldsworthy *et al.*, 1988; Green *et al.*, 1990; Ebrahim *et al.*, 1996, 2001; Jonker *et al.*, 1996; Philip *et al.*, 2007).

While neoplasia in the renal tubules is observed to occur only in male rats, tetrachloroethylene has been reported to produce nephrotoxicity across species, and in both sexes. Signs of tetrachloroethylene-induced kidney damage appeared in both rats and mice during the early phases of the NTP cancer bioassay (inhalation study), for example, indicating that animals of both species surviving to the scheduled termination of the study had long-standing nephrotoxicity. Although the female rats did not develop any tumours in the renal tubules, the incidence of karyomegaly was significantly elevated in females as well as in males, and one of the 50 female rats exposed at the highest dose developed tubul cell hyperplasia (NTP, 1986).

In the NTP inhalation study with mice, 'nephrosis' – generally defined as non-inflammatory degenerative kidney disease – was noted to occur at increased incidences in dosed females, casts (cylindrical structures formed from cells and proteins released from the kidney) were observed at increased frequency in exposed males and in females at the highest dose, and karyomegaly of the tubule cells was seen at increased incidences in both sexes of exposed mice (NTP, 1986). The severity of the renal lesions was dose-related, and one male at the lowest dose had a renal tubule cell adenocarcinoma. In the NCI cancer bioassay (gavage study) in B6C3F_1 mice and Osborne-Mendel rats treated with tetrachloroethylene, toxic nephropathy was not detected in control animals, but did occur in both male and female rats as well as in mice (NCI, 1977).

Nephrotoxicity after exposure to tetrachloroethylene administered by inhalation was observed in a 2-year cancer bioassay in groups of 50 male and female Fischer rats (0, 50, 200, or 600 ppm) and Crj:BDF1 mice (0, 10, 50, or 250 ppm) exposed for 6 hours per day, 5 days per week, for 104 weeks. Compared with controls, survival was decreased in all groups at the highest dose; this decrease was considered to be treatment-related. Relative kidney weight was increased in male and female rats (exposed at 200 or 600 ppm) and in male and female mice (exposed at 250 ppm). Karyomegaly in the proximal tubules of the kidneys was observed among male and female rats and mice. An increase in atypical tubular dilation of the proximal tubules was noted in male and female rats at the highest dose, and exacerbation of chronic renal disease was observed in male rats at the highest dose only (JISA, 1993).

Hayes *et al.* (1986) reported renal effects in rats exposed to tetrachloroethylene in drinking-water. The rats were given nominal amounts of 14, 400, and 1400 mg/kg bw per day for 90 consecutive days. There were no treatment-related deaths. Increased kidney-to-body weight ratios were observed.

Nephrotoxicity and increased relative kidney weights were observed in female Wistar rats treated with tetrachloroethylene at 600 or 2400 mg/kg bw per day in corn oil by gavage for 32 days. One rat at the higher dose died as a result of the treatment. Nephrotoxic effects were noted at the higher dose, with significant changes in all clinical chemistry markers related to kidney function (urea, total protein, albumin, *N*-acetyl-β-D-glucosaminidase) measured in the urine at the end of week 1 or week 4, except for urinary density, glucose, and creatinine. Karyomegaly was also observed at the higher dose in four out of five animals exposed (Jonker *et al.*, 1996).

Oral administration of tetrachloroethylene in sesame oil (3000 mg/kg bw per day for 15 days) to male and female albino Swiss mice caused a significant increase in kidney weight, an increase in glomerular nephrosis, and a decrease in blood glucose concentrations compared with controls (Ebrahim *et al.*, 1996). Concurrent administration of 2-deoxy-D-glucose (500 mg/kg bw per day, intraperitoneal injection) or vitamin E (400 mg/kg bw per day, gavage) prevented tetrachloroethylene-induced biochemical and pathological alterations. Exposure to tetrachloroethylene alone led to a decrease in blood glucose concentrations, which returned to near-normal levels with concomitant exposure to 2-deoxy-D-glucose and vitamin E. Elevated levels of glycolytic and gluconeogenic enzymes after exposure to tetrachloroethylene were also observed to decrease to near-normal levels after exposure to these two agents. Histopathology of the kidney showed hypercellular glomeruli after exposure to tetrachloroethylene, but this was not observed in mice treated with tetrachloroethylene and 2-deoxy-D-glucose, or tetrachloroethylene and vitamin E (Ebrahim *et al.*, 1996).

Mechanistic information on the nephrotoxicity of tetrachloroethylene was relatively sparse. Most studies have concentrated on the metabolites in the GSH pathway rather than on the parent compound, because much of the available information for both tetrachloroethylene and trichloroethylene suggests that this pathway generates the reactive chemical species responsible for nephrotoxicity.

The role of the GSH metabolites of tetrachloroethylene, particularly TCVC and TCVCS, in kidney toxicity was examined in a study in two groups of four male Sprague-Dawley rats exposed to TCVC or TCVCS (115 or 230 µmol/kg bw in saline) given by single intraperitoneal injections. The rats were killed 24 hours after dosing. Serum was analysed for blood urea nitrogen, and urine samples were analysed for γ-glutamyl transpeptidase activity as markers of nephrotoxicity. Rats exposed to the higher dose of TCVCS showed visible signs of kidney necrosis, while none of the other treated groups did. Histologically,

kidneys from rats exposed to TCVC or TCVCS at the lower dose showed slight-to-mild acute tubular necrosis. Analysis of kidneys at 24 hours after exposure showed mild-to-moderate acute tubular necrosis in rats exposed to TCVC at the higher dose, and severe tubular necrosis in those exposed to TCVCS at the higher dose. A significant fourfold increase in blood urea nitrogen was observed in rats exposed to TCVCS at the higher dose compared with controls, but no significant increases were noted after exposure to TCVC. Variable increases in urinary glucose concentrations and γ-glutamyl transpeptidase activity were seen after exposure to TCVC or TCVCS. A second part of this experiment demonstrated enhanced toxicity by pre-treatment with the β-lyase inhibitor AOAA (500 μmol/kg bw), given by intraperitoneal injection 30 minutes before administration of TCVC at the higher dose. In a third experiment, groups of four rats were exposed to TCVC or TCVCS (higher dose) and killed after 2 hours to investigate non-protein thiol status in the kidney. TCVCS significantly decreased non-protein thiols, but did not affect non-protein disulfides, whereas TCVC was without effect. Histological examination of the kidneys showed scattered foci of mild acute tubular necrosis (after TCVC) or widespread acute tubular necrosis, intratubular casts, and interstitial congestion and haemorrhage (after TCVCS). These results show that TCVCS has greater nephrotoxicity than TCVC (Elfarra & Krause, 2007)

The tetrachloroethylene-*S*-conjugate metabolites TCVG and TCVC caused dose-related cytotoxicity in renal cell preparations, which was prevented by a β-lyase enzyme inhibitor. Renal β-lyases are known to cleave TCVC to yield an unstable thiol, 1,2,2-trichlorovinylthiol, which may give rise to formation of a highly reactive thioketene, a chemical species that can form covalent adducts with cellular nucleophiles (Vamvakas *et al.*, 1989d). In addition, sulfoxidation of both TCVC and its *N*-acetylated product resulted in formation of toxic metabolites (Werner *et al.*, 1996; Ripp *et al.*, 1997). A study by Lash *et al.* (2002) characterized the cytotoxicity of tetrachloroethylene and TCVG in suspensions of isolated kidney cells from male and female F344 rats and the mitochondrial toxicity of tetrachloroethylene and TCVG in suspensions of renal cortical mitochondria from the kidneys of male and female F344 rats and $B6C3F_1$ mice. In both cases, responses to tetrachloroethylene and TCVG were compared with those to trichloroethylene and DCVG, respectively, from a parallel study (Lash *et al.*, 2001). Although the parent compounds are not particularly potent as acute cytotoxic agents, both tetrachloroethylene and trichloroethylene at concentrations of 1 mM each produced a modest degree of cytotoxicity in 3-hour incubations of isolated kidney cells from male F344 rats, as assessed by release of lactate dehydrogenase; tetrachloroethylene and trichloroethylene caused 38% and 24% release, respectively. In contrast, neither parent compound produced significant release of lactate dehydrogenase in isolated kidney cells from female rats. A larger difference was observed when the cytotoxicity of the GSH conjugates was compared. In 3-hour incubations with either TCVG or DCVG at 1 mM, isolated kidney cells from male rats exhibited 40% and 26% release of lactate dehydrogenase, respectively, while isolated kidney cells from female rats exhibited 18% release with both conjugates. Thus, tetrachloroethylene and its GSH-conjugate TCVG were clearly more acutely cytotoxic to isolated renal cells than trichloroethylene and DCVG, and kidney cells from male rats are significantly more sensitive than kidney cells from female rats to these compounds. Tetrachloroethylene and TCVG also induced toxic effects in mitochondria (i.e. mitochondrial dysfunction, such as a reduced respiratory control ratio and inhibition of state-3 respiration by specific inhibition of several sulfhydryl-containing enzymes) in male rats, and in

male and female mice (Lash & Parker, 2001; Lash *et al.*, 2002).

(c) PPARα activation

(i) Humans

No studies were identified on PPARα activation or peroxisome proliferation in human kidney after exposure to tetrachloroethylene. However, in transactivation studies *in vitro*, human PPARα was activated by the metabolites dichloroacetate and trichloroacetate, while tetrachloroethylene itself was relatively inactive (Maloney & Waxman, 1999). A few studies have examined PPARα activation by dichloroacetate and trichloroacetate in cultured human liver cells (e.g. Walgren *et al.*, 2000), but the evidence of these effects from studies of human kidney *in vivo* or *in vitro* is weak.

(ii) Experimental animals

Two studies addressed peroxisome proliferation in rodent kidney after short-term exposure to tetrachloroethylene (see Table 4.6), but there were no data available concerning activation of the receptor in kidney tissue.

Five male F344 rats and five male B6C3F$_1$ mice were given tetrachloroethylene at a dose of 1000 mg/kg bw per day by gavage in corn oil for 10 days. In exposed rats, cyanide-insensitive palmitoyl-coenzyme A (palmitoyl-coA) oxidation activity was modestly—although not significantly—elevated in the kidney (1.7-fold increase). In mice, the treatment enhanced palmitoyl-coA oxidation activity by 2.3-fold in the kidney. Relative kidney weight was unaffected. A comparison of corn oil with methylcellulose revealed no effect of the gavage vehicle on tetrachloroethylene-induced palmitoyl-coA oxidation. A less-than-additive effect on palmitoyl-coA oxidation was seen when trichloroethylene (1000 mg/kg bw) was administered together with tetrachloroethylene (Goldsworthy & Popp, 1987).

In the second study, male and female F344 rats and B6C3F$_1$ mice were exposed to tetrachloroethylene by inhalation (400 ppm, 6 hours per day for 14, 21, or 28 days; 200 ppm, 6 hours per day for 28 days), and palmitoyl-coA oxidation activity was measured in liver and kidney. Due to insufficient material, the analysis of the mouse kidney tissues was done on pooled samples. Slight increases in palmitoyl-coA oxidation were observed in the kidney of male mice (maximum increase of 1.6-fold at 21 days, 400 ppm), but no change in female mice. Modest increases were noted in the kidney of male rats at 200 ppm at 28 days (1.3-fold) but not at 400 ppm at 14, 21, or 28 days. In female rat kidney, palmitoyl-coA oxidation activity was elevated (approximately 1.6-fold) at all doses and times. However, peroxisome proliferation was not observed in rat or mouse kidney when analysed by microscopy (Odum *et al.*, 1988).

4.3.2 Liver

(a) Epigenetic effects

(i) Humans

No data were available from studies in humans regarding a role for epigenetic effects in tetrachloroethylene-induced tumorigenesis.

(ii) Experimental animals

No data were available from studies in experimental animals regarding a role for alteration in DNA methylation in tetrachloroethylene-induced tumorigenesis. However, experimental support for hypomethylation of DNA is available from studies in mice exposed to the metabolites trichloroacetate and dichloroacetate.

When female B6C3F$_1$ mice received an intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) as an initiator followed by exposure to trichloroacetate or dichloroacetate in drinking-water, DNA methylation in the resulting hepatocellular adenomas and carcinomas was about half that observed in non-tumour tissue

Table 4.6 Renal peroxisome proliferation in rodents exposed to tetrachloroethylene

Test system (species, strain, sex, number)	Dose	Effect	Reference
Rat, F344 (male only, 5/group) and mouse, B6C3F$_1$ (male only, 7/group)	1000 mg/kg bw per day for 10 days, corn oil, gavage	Rats: no increase in PCO activity, but increased kidney weight Mice: increased PCO activity in all exposed mice	Goldsworthy & Popp (1987)
Rat, F344; and mouse, B6C3F$_1$ (males and females, 5/group)	200 ppm, 6 h/day, 28 days; and 400 ppm, 6 h/day, 14, 21, 28 days, by inhalation	Rats of both sexes: modest increases in PCO observed in male rat kidneys only at 200 ppm for 28 d, and in female rat kidneys at all doses and times Mice: analysis limited to pooled tissue showed slight increases in β-oxidation in male mouse kidney	Odum *et al.* (1988)

d, day; h, hour; PCO, cyanide-insensitive palmitoyl coenzyme A oxidase activity

from the same animal or from animals given MNU alone (Tao *et al.*, 1998). Exposure of female B6C3F$_1$ mice to drinking-water containing trichloroacetate or dichloroacetate for 11 days reduced total liver-DNA methylation by 60% (Tao *et al.*, 1998). In a further study, changes in methylation of the c-*jun* and c-*myc* genes, and alterations in gene expression were measured in liver tumours initiated by MNU and promoted by dichloroacetate and trichloroacetate in female B6C3F$_1$ mice. Hypomethylation and overexpression of these genes were found in liver tumours promoted by dichloroacetate and trichloroacetate (Tao *et al.*, 2000). Subsequently, these investigators reported hypomethylation of a region of the *IGF-II* gene in liver and tumours from mice initiated with MNU and then exposed to trichloroacetate or dichloroacetate (Tao *et al.*, 2004).

(b) Cytotoxicity and secondary oxidative stress

(i) Humans

A group of 49 workers in the metal-processing industry was exposed to trichloroethylene and tetrachloroethylene at different concentrations. The workers were divided in three groups based on job descriptions, but no exposure assessment was performed. Several markers to assess liver functions were investigated: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, copper, and zinc. The bromphthaleine test was also performed. Statistically, there were no differences between the values found for exposed and controls, the outcome of the tests all being in the normal range (Szadkowski & Körber, 1969).

A health-surveillance study was conducted among 22 workers exposed to tetrachloroethylene (time-weighted average, 21 ppm; range, 9–38 ppm) in six dry-cleaning shops. Results of behavioural, renal, hepatic, and pulmonary tests on these workers were compared with those obtained for 33 subjects not exposed to organic solvents. No differences were observed in mean serum hepatic enzyme concentrations (Lauwerys *et al.*, 1983) [The Working Group noted the lack of proper statistical analysis].

The hepatic health status of 133 dry-cleaning plant workers (women and men) exposed to tetrachloroethylene and 107 controls was assessed by measuring ALT, AST, fatty acids, prothrombin index, bilirubin and iron in serum, by recording a proteinogram, and by conducting the thymol turbidity test. Among women, no statistically significant differences were found between exposed and controls. Among men, statistically significant differences in values (mean and standard deviation) for aminotransferases were observed. Increased activities of serum enzymes were seen in workers exposed to high concentrations of tetrachloroethylene (Gluszczowa, 1988).

Results were presented of a five-year follow-up of 130 workers exposed to tetrachloroethylene in dry-cleaning shops. The study involved measurement of exposure conditions (personal dosimetry, exposure tests) and clinical investigations (ALT, AST, alkaline phosphatase, GGT, alanine aminopeptidase, cholinesterase) and plasma concentrations of bilirubin, cholesterol and triglycerides. No signs of hepatotoxicity due to exposure to tetrachloroethylene were observed (Müller *et al.*, 1989).

Subjective symptoms and clinical indicators of liver injury were evaluated among 56 dry-cleaning workers (29 men, 27 women) who had been exposed to tetrachloroethylene vapour (20 ppm, 8-hour TWA) for several years. A control group (32 men, 37 women) of similar age recruited from the same factories was not exposed to solvents. There was an exposure-related increase in the prevalence of symptoms such as dizziness, floating sensation, and headache among the exposed workers. In haematological assays and serum biochemistry tests, no significant changes were seen in AST or ALT, alkaline phosphatase or leukocyte alkaline phosphatase, GGT or total bilirubin (Cai *et al.* (1991).

The isoenzyme pattern of serum GGT was studied in 141 male and female workers in dry-cleaning shops exposed to tetrachloroethylene (8-hour TWA, 11.3 ppm) and in 130 control subjects. None of the workers showed clinical symptoms of liver disease and their enzymatic profiles, including AST, ALT, 5′nucleotidase, alkaline phosphatase, and GGT, were within the normal range. A statistically significant increase in total GGT in serum was found in the exposed subjects, which was due to an increase in one of the two fractions normally present in healthy individuals (GGT-2), and to the appearance and progressive increase in the level of a fraction (GGT-4) considered to be indicative of hepato-biliary impairment. [It should be noted that individuals who had liver enzyme activity above the normal range, and those who had past or current liver disease were not included in this study (Gennari *et al.*, 1992).]

A study was conducted to determine whether subclinical hepatotoxicity is associated with exposure to tetrachloroethylene at concentrations commonly experienced in the workplace, and whether surveillance with serum hepatic transaminase activity underestimates such effects. Included in the study were 29 dry-cleaning operators (8-hour TWA, 16 ppm) and a control group of 29 non-exposed laundry workers. Hepatic parenchymal tissue was scanned by means of ultrasonography; hepatic transaminase activity in serum was also measured. ALT activities of up to 1.5 times the normal limits were found in five out of 27 (19%) dry-cleaning workers compared with one out of 26 (4%) laundry workers. In contrast, ultrasonography revealed a twofold increase in hepatic parenchymal changes in 18 out of 27 dry-cleaning workers (67%), compared with 10 of 26 (39%) laundry workers (Brodkin *et al.*, 1995).

(ii) Experimental animals

Numerous studies in experimental animals, including long-term bioassays, have demonstrated that tetrachloroethylene is hepatotoxic, with the mice being more sensitive than rats (NCI, 1977; Schumann *et al.*, 1980; NTP, 1986; JISA, 1993). Male and female NMRI mice were exposed by whole-body inhalation to various concentrations of tetrachloroethylene (9–3600 ppm) in different experimental designs (continuous or intermittent exposure, from 4 to 120 days). In mice continuously exposed to tetrachloroethylene for 30 days, the increase in plasma butyrylcholinesterase activity became significant at exposure levels > 37 ppm. Compared with controls, the increase was 1.7-fold in female mice and 2.5-fold in male mice after exposure for 120 days to 150 ppm. Under these conditions, the increase in liver weight was 2.3-fold in female mice and 1.9-fold in male mice. The activity of butyrylcholinesterase returned to normal within

30 days after cessation of exposure, but a 10% increase in liver weight remained. No signs of cholestasis were found upon histopathological examination of the liver (Kjellstrand *et al.*, 1984).

Hepatic toxicity was observed in rodents during short- and long-term (lifetime) bioassays with tetrachloroethylene administered by inhalation (NTP, 1986). In a 13-week study, male and female rats and mice were exposed to tetrachloroethylene (0, 100, 200, 400, 800, or 1600 ppm) by inhalation (6 hours per day, 5 days per week). In the group at the highest dose, some rats (males, 4 out of 10; females, 7 out of 10) and some mice (males, 2 out of 10; females, 4 out 10) died before the end of the study. Exposure to tetrachloroethylene (200 ppm and above) increased the incidence of hepatic congestion in male and female rats. In mice of both sexes, liver inflammatory lesions (leukocytic infiltration, centrilobular necrosis, and bile stasis) were observed at 400, 800, or 1600 ppm. Tetrachloroethylene was also administered by inhalation to F344 rats (0, 200, or 400 ppm) or $B6C3F_1$ mice (0, 100, or 200 ppm) for 103 weeks (6 hours per day, 5 days per week). In male and female mice, in addition to liver tumours (see Section 3), liver degeneration was reported in 2 out of 49, 8 out of 49, and 14 out of 50 males, and in 1 out of 49, 2 out of 50, and 13 out of 50 females in the three groups. Degeneration was characterized by cytoplasmic vacuolization, hepatocellular necrosis, inflammatory cell infiltrates, pigmented cells, oval cell hyperplasia, and regenerative foci. Liver necrosis was observed at increased incidence in males (1 out of 49, 6 out of 49, and 15 out of 50) and in females at 400 ppm (3 out of 48, 5 out of 50, and 9 out of 50). Nuclear inclusions increased in male mice (2 out of 49, 5 out of 49, and 9 out of 50). No dose-related effects on the liver were reported in the rats.

Male Swiss-Cox mice were exposed to tetrachloroethylene by oral gavage (0, 20, 100, 200, 500, 1000, 1500, or 2000 mg/kg bw per day) on 5 days per week for 6 weeks. Liver necrosis, polyploidy in the centrilobular region, and lipid accumulation were evident upon histopathological examination of mice at 200 or 1000 mg/kg bw. Liver:body weight ratios and liver triglycerides were significantly increased at doses ≥ 100 mg/kg bw per day. Enlarged hepatocytes, polyploidy in the centrilobular region, and lipid accumulation were evident upon histopathological examination of mice exposed to 200 or 1000 mg/kg bw. The percentage increase in the liver:body weight ratio was highly correlated with the amount of tetrachloroethylene metabolized (urinary levels of metabolites). Other indices of tetrachloroethylene hepatotoxicity (e.g. increased serum ALT activity) were significantly increased at doses ≥ 500 mg/kg bw (Buben & O'Flaherty, 1985).

An inhalation study by the Japanese Industrial Safety Association (JISA) used male and female Crj/BDF1 mice (exposed at 0, 10, 50, and 250 ppm tetrachloroethylene) and F344/DuCrj rats (exposed at 0, 50, 200, and 600 ppm). Exposure was for 104 weeks and the mice were killed at 110 weeks. In mice, in addition to hepatocellular carcinomas and adenomas (see Section 3), focal liver necrosis was observed in males at doses of 50 ppm and higher. Liver degeneration was noted at 250 ppm in males and females. In male but not female rats, an excess incidence of spongiosis hepatitis was reported at 200 ppm and 600 ppm (JISA, 1993).

Female F344 rats were dosed by oral gavage either once (0, 150, 500, 1500, and 5000 mg/kg bw), or daily for 14 consecutive days (0, 50, 150, 500, 1500 mg/kg bw per day). An increase in the concentration of serum ALT was seen in rats at the highest dose in the 14-day exposure experiment (Berman *et al.*, 1995).

Tetrachloroethylene-associated hepatotoxicity was evaluated after exposure of female Wistar rats at 600 or 2400 mg/kg bw per day via oral gavage in corn oil for 32 days. At the higher dose, the activities of ALT and AST in serum were significantly increased. There was also a significant increase in relative liver weight (Jonker *et al.*, 1996).

The hepatotoxic effect of repeated exposure was investigated in male Swiss Webster mice that were given tetrachloroethylene as three doses (150, 500, or 1000 mg/kg bw) via aqueous gavage, daily for up to 30 days. Tissue injury was monitored regularly during the dosing regimen on days 1, 7, 14, and 30, and over a time course of 24–96 hours after the last dose. Significant increases in serum ALT were observed after a single exposure at all three doses. Higher levels of ALT were also found in the group receiving the highest dose after 7 days, and in all three groups after 14 days of continuous exposure, but no longer after 30 days of exposure, nor in the follow-up samples. Mild to moderate centrilobular degeneration was observed in the liver of mice in the groups receiving the intermediate and highest dose after 1 day of exposure. By 30 days, both groups showed a reduction of tissue damage compared with day 1, as well as evidence of tissue repair, primarily visible as an increase in hepatocellular mitotic figures (Philip *et al.*, 2007).

A limited number of studies focused on tetrachloroethylene-induced hepatic oxidative stress. When tetrachloroethylene was given orally in sesame oil to male and female Swiss mice at 3000 mg/kg bw per day for 15 days, a significant increase in liver weight was seen, as well as degeneration and necrosis of hepatocytes. These changes occurred simultaneously with a decrease in blood glucose, enhanced activity of the enzymes hexokinase, aldolase, and phosphoglucoisomerase, and reduced activities of enzymes involved in gluconeogenesis. Most of these effects were mitigated by concomitant exposure to 2-deoxy-D-glucose or vitamin E (Ebrahim *et al.*, 1996).

In a further study, the potential protective properties of 2-deoxy-D-glucose, vitamin E, and taurine against membrane damage were investigated with a similar treatment protocol. Male albino Swiss mice were exposed to the same doses of tetrachloroethylene as used in the previous study, with the addition of a taurine-exposed group. Membrane-bound Na^+K^+-ATPase and Mg^{2+}-ATPase activity were significantly decreased ($P < 0.001$), while Ca^{2+}-ATPase activity was increased ($P < 0.001$) after exposure to tetrachloroethylene alone. These levels remained close to normal in mice exposed to tetrachloroethylene together with 2-deoxy-D-glucose, vitamin E, or taurine. This return to normal levels after exposure to vitamin E and taurine may be due to their activity as an antioxidant, and the ensuing reduction in oxidative stress in exposed cells (Ebrahim *et al.*, 2001).

(c) Cell proliferation

Increased cell proliferation in mice has been reported after exposure to tetrachloroethylene. To study the extent to which tetrachloroethylene alters cell proliferation and apoptosis, several studies have measured hepatocyte proliferation in mice in response to treatment with the metabolite trichloroacetate (Sanchez & Bull, 1990; Dees & Travis, 1994; Pereira, 1996; Stauber & Bull, 1997; DeAngelo *et al.*, 2008). For instance, Dees & Travis (1994) observed relatively small (two- to threefold) but statistically significant increases in [^{3}H]thymidine incorporation in hepatic DNA in mice exposed by gavage for 11 days to trichloroacetate at doses (100–1000 mg/kg bw) that increased the relative liver weight. Increased hepatic DNA labelling was seen at doses lower than those associated with evidence of necrosis, suggesting that trichloroacetate-induced cell proliferation is not due to regenerative hyperplasia.

In the study by Philip *et al.* (2007) above, a dose-dependent increase in [^{3}H]thymidine incorporation was observed in all dose groups on day 7 of treatment, which was sustained until 14 days in the groups at the intermediated dose and highest dose. A lower level of cell proliferation was evident after 30 days than after 14 days exposure to all three doses. PCNA immunohistochemistry was performed to confirm the findings of S-phase stimulation determined by the

[^{3}H]thymidine pulse-labelling study. The immunochemistry results and the pulse-labelling data were consistent (Philip *et al.*, 2007).

(d) PPARα activation

Tetrachloroethylene or its metabolites have been shown to induce activation of PPARα or markers of peroxisome proliferation in the liver.

(i) Humans

No studies were available on peroxisome proliferation or the key events in PPARα activation in human liver. However, transactivation studies *in vitro* have shown that in humans PPARα is activated by trichloroacetate and dichloroacetate, while tetrachloroethylene itself is relatively inactive (Zhou & Waxman, 1998). A limited number of studies focused on PPARα activation by the tetrachloroethylene metabolites dichloroacetate and trichloroacetate in cultured human liver cells. In one of these studies, trichloroacetate and dichloroacetate did not increase palmitoyl-CoA oxidation and caused a decrease in DNA synthesis in human hepatocyte cultures, in contrast to the response seen in rodents (Walgren *et al.*, 2000).

(ii) Experimental animals

Several studies in experimental animals *in vivo* examined the effect of tetrachloroethylene on peroxisome proliferation or its markers in the liver (Table 4.7).

Groups of five male and five female F344 rats and B6C3F$_1$ mice were exposed by inhalation for 6 hours per day to tetrachloroethylene at 200 ppm for 28 days, or at 400 ppm for 14, 21, or 28 days. In both sexes and in both species, hepatic palmitoyl-coA oxidation activity was increased (mice, up to 3.6-fold; rats, up to 1.3-fold). Hepatic peroxisome proliferation was noted by electron microscopy in all exposed mice, and the proportion of the cytoplasm occupied by peroxisomes was increased. In rats, variable increases in peroxisome volume were noted at 200 ppm, but results lacked statistical significance. Catalase, another peroxisomal enzyme, was unaffected by tetrachloroethylene: male mice exposed at 400 ppm showed only a moderate increase (1.4-fold). Mitochondrial proliferation was observed at 28 days in male mice in the group at 400 ppm. In addition, a time-dependent proliferation of smooth endoplasmic reticulum in the liver of males and females correlated well with centrilobular hypertrophy. Tetrachloroethylene caused centrilobular lipid accumulation in male and female mice. Relative liver weight was increased in male and female mice (Odum *et al.*, 1988).

Five male F344 rats and five male B6C3F$_1$ mice were given tetrachloroethylene (1000 mg/kg bw) in corn oil by gavage, daily for 10 days. In the rats, palmitoyl-coA oxidation was modestly, although not statistically significantly, elevated in the liver (1.4-fold). In exposed mice, palmitoyl-coA oxidation was increased 4.3-fold in. Relative liver weight was increased in rats and mice. A comparison between corn oil and methylcellulose showed no effect of the gavage vehicle on tetrachloroethylene-induced palmitoyl-coA oxidation. Administration of trichloroethylene (1000 mg/kg bw) together with tetrachloroethylene had a less-than-additive effect on induction of palmitoyl-coA oxidation (Goldsworthy & Popp, 1987).

Kyrklund *et al.* (1990) exposed male Sprague-Dawley rats to tetrachloroethylene at 320 ppm continuously for 90 days, followed by a 30-day recovery period. Relative liver weight was significantly increased in rats examined at the end of the exposure period. A slight increase in relative liver weight was also observed in the recovered, solvent-treated group.

SV129 PPARα-deficient mice exposed to trichloroacetate at doses up to 2 g/L in drinking-water for 7 days did not show the characteristic induction of acyl-coenzyme A oxidase, palmitoyl-coA oxidase, and CYP4A associated with PPARα activation and peroxisome proliferation in wild-type animals. In addition, the livers

Table 4.7 Studies of induction of hepatic peroxisome proliferation or its markers in rodents exposed to tetrachloroethylene

Test system (species, strain, sex, number)	Dose	Effect	Reference
Rat, F344 (male only, 5/group) and mouse, $B6C3F_1$ (male only, 7/group)	1000 mg/kg bw per day for 10 days, corn oil, gavage	Mice: increased relative liver weight; 4.3-fold PCO increase Rats: increased relative liver weight; modest (1.4-fold) PCO increase, not significant	Goldsworthy & Popp (1987)
Rat, F344; and mouse, $B6C3F_1$; males and females (5/group)	200 ppm, 6 h/day, 28 days; and 400 ppm, 6 h/day, 14, 21, 28 days, by inhalation	Male and female mice: increased relative liver weight, centrilobular lipid accumulation and peroxisome proliferation; increased PCO (up to 3.7-fold)	Odum *et al.* (1988)
	400 ppm, inhalation; 28 days	Male mice: mitochondrial proliferation	
	200, and 400 ppm, inhalation; 14, 21, 28 days	Male and female rats: increased PCO (up to 1.3-fold)	
Mouse, Swiss-Webster, male (4/group)	150, 500, and 1000 mg/kg bw per day, aqueous gavage; 24 h to 14 days after initial exposure	Increased plasma ALT	Philip *et al.* (2007)
	500 and 1000 mg/kg bw per day, aqueous gavage; 24 h to 30 days after initial exposure	Mild to moderate fatty degeneration and necrosis, with focal inflammatory cell infiltration	
	150, 500, and 1000 mg/kg bw per day, aqueous gavage; peaked on day 7, sustained at 14–30 days	Increased mitotic figures and DNA synthesis	
	1000 mg/kg bw per day, aqueous gavage; 7 days, but not 14 days	Increased expression of CYP4A	

d, day; h, hour; ALT, alanine aminotransferase; PCO, cyanide-insensitive palmitoyl coenzyme A oxidase activity

from wild-type, but not PPARα-deficient, mice exposed to trichloroacetate at 2 g/L developed centrilobular hepatocyte hypertrophy, although no significant increase in relative liver weight was seen (Laughter *et al.*, 2004).

CYP4A, a marker for PPARα-activation, was transiently increased (only on day 7 of a 30-day treatment) in Swiss Webster mice, and only at the highest of three oral doses (150, 500, 1000 mg/kg bw per day) (Philip *et al.*, 2007). [The Working Group noted that in the NCI cancer bioassay, liver tumours had appeared at doses around 500 mg/kg bw per day (NCI, 1977), at which no increased CYP4A activity was reported. The sensitivity of the Swiss Webster mouse strain relative to that of the $B6C3F_1$ mice used in the cancer bioassay is unknown.]

(e) Disruption of gap-junctional intercellular communication

The lucifer-yellow scrape-load dye-transfer technique was used to examine the effect of tetrachloroethylene, dichloroacetate, and trichloroacetate on cultures of clone-9 normal rat-liver cells after exposure during 1, 4, 6, 24, 48, and 168 hours. Tetrachloroethylene caused a significant effect at 0.01 mM after 48 hours. Over a 24-hour treatment period the relative efficiencies, expressed as the concentration needed to produce a 50% reduction in intercellular communication, were 0.3 mM for tetrachloroethylene, 3.8 mM for trichloroacetate, and 41 mM for dichloroacetate. The time course of the effect was similar for the three compounds (Benane *et al.*, 1996).

4.3.3 Immune system

(a) Humans

Several recent studies have evaluated the immunotoxicity of exposure to tetrachloroethylene. A small cross-sectional study conducted in the Czech Republic included 21 exposed workers in the dry-cleaning industry and 16 controls from the same plant. Several immunological markers were measured, including α-macroglobulin, phagocyte activity, T lymphocytes, concentrations of the complement proteins C3 and C4, and immunoglobulins. These parameters were compared with laboratory reference values from blood donors and healthy individuals in the same region. Biological monitoring of the workers over an 8-hour working shift showed tetrachloroethylene in exhaled air in the range of 9 to 344 mg/m^3 at the end of the shift. Compared with controls, the exposed workers had significantly elevated serum concentrations of the proteins C3 and C4 and higher salivary concentrations of IgA. Compared with reference values from 41 healthy blood donors, the serum concentration of C3 of the exposed workers was higher, while the number of T lymphocytes was reduced (Andrýs *et al.*, 1997).

In terms of sample size and the use of an appropriately matched control group, the strongest study examining immunological and haematological effects of exposure to tetrachloroethylene was that among 40 male dry-cleaning workers who had mean exposure levels up to 140 ppm, and a mean exposure duration of 7 years. Statistically significant decreases in erythrocyte count and haemoglobin levels, and increases in total leukocyte counts and lymphocyte counts were observed in the exposed workers compared with age- and smoking-matched controls. In addition, increases in several other immunological parameters, including T-lymphocyte and natural killer-cell subpopulations, IgE, and interleukin-4 levels were reported. These immunological effects suggest an augmentation of Th2 responsiveness (Emara *et al.*, 2010).

Case–control studies that evaluated the risk of autoimmune disease in relation to exposure to tetrachloroethylene have not suggested that there is a significant association.

The association with exposure to tetrachloroethylene was evaluated in 60 patients who were positive for ANCA (anti-neutrophil cytoplasmic antibody) and 120 age- and sex-matched controls in France (Beaudreuil *et al.*, 2005). Cases included hospital in-patients (admitted in 1990–2000) who were ANCA-positive, and in-patient controls (admitted in 2000–2001) matched to cases by age (± 5 years) and sex. An interviewer-administered questionnaire was used to evaluate occupational exposure to several solvents and other chemicals. The exposure assessment included both qualitative and semiquantitative methods and was assessed by a panel of experts blinded to the disease status of the patients. A total of five ANCA-positive patients were reported to be exposed to tetrachloroethylene; leading to a twofold non-significantly increased risk of ANCA-positivity associated with exposure to tetrachloroethylene.

A case–control study of 205 female patients with undifferentiated connective tissue disease (UCTD) and 2095 population-based controls in the USA evaluated the association between UCTD and exposure to petroleum distillate solvents, including tetrachloroethylene. Exposures to solvents were self-reported in a personal interview, which included an assessment of the number of years each woman had worked with the solvent, whether protective equipment was used, and job activities. The only available risk estimate was for nine exposed cases in dry-cleaning workers; the risk of UCTD in these workers was marginally elevated, but not statistically significant (Lacey *et al.*, 1999).

Two studies of children in Germany have evaluated formation of IgE antibodies to food and allergens and measured the number of

cytokine-producing peripheral T-cells in relation to exposure to tetrachloroethylene and other volatile organic compounds. In the first study, serum IgE concentrations were measured in 121 children, while cytokine measurements were available for 28 children. Exposures to tetrachloroethylene and other volatiles were passively monitored in the bedroom of each child for 4 weeks; the median exposure level was reported for tetrachloroethylene to be 2.54 μg/m^3. This indoor exposure was not significantly associated with allergic sensitization to egg white or milk, or with cytokine-producing T-cells (Lehmann *et al.*, 2001).

In a study of 22 Hispanic children with asthma living in Los Angeles, USA, symptom diaries were completed for 3 months, while outdoor 24-hour air samples were collected from a central site to assess airborne concentrations of tetrachloroethylene, other volatile organic compounds, and reference air-pollutants. The mean level of exposure to tetrachloroethylene was reported to be 0.51 ppb. More severe asthma symptoms were significantly associated with exposure to tetrachloroethylene, but this association was attenuated and no longer statistically significant after further adjustment for levels of sulfur dioxide and nitrogen dioxide (Delfino *et al.*, 2003a).

(b) Experimental animals

No studies were available on the toxicity of tetrachloroethylene in putative target tissues in the immune system in F344 rats, i.e. bone marrow, spleen, or target cells of mononuclear cell leukeamia. However, in mice the haematopoietic toxicity of tetrachloroethylene has been demonstrated in several studies.

In albino Swiss mice, administration of tetrachloroethylene in sesame oil (3000 mg/kg bw per day, for 15 days) by oral intubation resulted in a significant decrease in haemoglobin, haematocrit (erythrocyte volume fraction), and erythrocyte and platelet counts, and a significant increase in leukocyte counts (Ebrahim *et al.*, 2001). These findings are similar to those observed in studies in humans exposed to tetrachloroethylene (Emara *et al.*, 2010).

Female NMRI mice were given tetrachloroethylene in drinking-water at nominal doses of 0.05 or 0.1 mg/kg bw per day for 7 weeks. The treatment resulted in reversible haemolytic anaemia, and there was evidence from microscopical analyses of splenic involvement. Tetrachloroethylene accumulated in the spleen to a significantly greater extent than in liver, brain, or kidney; levels of tetrachloroethylene in the spleen were 20-fold those in the liver after 7 weeks (Marth, 1987). Tetrachloroethylene was also found in the spleen and fatty tissue of treated mice up to 2 months after the end of the 7 weeks of exposure (Marth *et al.*, 1989).

Female hybrid mice (C57/BL/6 × DBA/2) were exposed to tetrachloroethylene at a concentration of 270 ppm for 6 hours per day, 5 days per week for 11.5 weeks, and at 135 ppm for 6 hours per day for 7.5 weeks, followed by a 3-week exposure-free period. There was a reduction in erythrocyte count, supported by decreases in colony-forming units (CFU) and burst-forming units (BFU) of erythroid cells, and evidence of reticulocytosis. Reversible reductions in the numbers of lymphocytes/monocytes and neutrophils were also observed. The slight depression in number of granulocyte progenitor cells (CFU-C), which persisted after the exposure, could indicate the beginning of disturbance at all levels of progenitor cell (Seidel *et al.*, 1992).

Several leukaemogens (e.g. benzene) inhibit the production of both erythrocytes and various types of leukocyte in blood. A decrease in CFU-Ss has commonly been reported, but this effect is not observed after exposure to tetrachloroethylene. Leukaemogens also cause a decrease in number of several myeloid progenitors in the bone marrow; CFU-E/BFU-E was also reduced by tetrachloroethylene (Seidel *et al.*, 1989a, b). Thus, there is indirect evidence that

tetrachloroethylene induces effects associated with leukaemogenesis (NRC, 2010).

Some studies focused on the immunotoxicity of tetrachloroethylene, but their number was too small to establish whether tetrachloroethylene affects immune parameters in a way that would confirm its leukaemia-inducing potential.

Immunosuppression was observed in female $B6C3F_1$ mice given tetrachloroethylene in drinking-water (maximum concentration, 6.8 ppm) in a mixture of 24 contaminants of groundwater occurring frequently near Superfund sites in the USA (i.e. an uncontrolled or abandoned place where hazardous waste is located; Germolec *et al.*, 1989). Mice exposed to the highest dose of this mixture for 14 or 90 days showed suppression of haematopoietic stem cells and of antigen-induced antibody-forming cells. There were no effects on T-cell function or in numbers of T and B-cells in any exposed group. No changes were evident upon challenge with *Listeria monocytogenes* or PYB6 tumour cells.

Aranyi *et al.* (1986) exposed female CD-1 mice to tetrachloroethylene at a concentration of 25 or 50 ppm by inhalation as a single (3 hours) or repeated dose (5 days per week, 3 hours per day). Susceptibility to *Streptococcus zooepidemicus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella pneumoniae* were evaluated. A single 3-hour exposure to tetrachloroethylene at 50 ppm significantly increased susceptibility to respiratory infection and mortality after exposure to *S. zooepidemicus*. In addition, the 3-hour exposure to tetrachloroethylene at 50 ppm was associated with a statistically significant decrease in pulmonary bactericidal activity. No significant differences were observed in mice exposed to tetrachloroethylene at 25 ppm.

4.3.4 Central nervous system

No data were available directly concerning either the metabolites or the mechanisms that may contribute to the induction of rare tumours of the brain (glioma) occurring in rats exposed to tetrachloroethylene (see Section 3). Studies in humans and experimental animals indicate an association of neurobehavioural defects with exposure to tetrachloroethylene (see Bale *et al.* 2011 for a recent review). The primary neurobehavioural changes observed after exposure to tetrachloroethylene are changes in vision, cognitive deficits, and increased reaction time. The acute effects of tetrachloroethylene appear to have much in common with those of other chlorinated solvents such as trichloroethylene and dichloromethane, as well as toluene, volatile anaesthetics, and alcohols. It is not known how the different neurological effects are induced, but there is information available to help elucidate which areas in the brain and which specific molecular targets may be involved in the ensuing neurotoxicological outcome.

(a) Humans

Data on neurotoxicity in humans were available from controlled experimental chamber studies (Altmann *et al.*, 1990) and epidemiological investigations that used standardized neurobehavioural batteries (Altmann *et al.*, 1995; Echeverria *et al.*, 1995; Spinatonda *et al.*, 1997) or assessment of visual function (Schreiber *et al.*, 2002; Storm *et al.*, 2011), a neurological outcome known to be sensitive to volatile organic compounds. Seven epidemiological studies examined occupational exposure to tetrachloroethylene (Seeber, 1989; Ferroni *et al.*, 1992; Cavalleri *et al.*, 1994; Echeverria *et al.*, 1995; Spinatonda *et al.*, 1997; Gobba *et al.*, 1998; Schreiber *et al.*, 2002), three epidemiological studies examined residential exposure to tetrachloroethylene (Altmann *et al.*, 1995; Schreiber *et al.*, 2002; Storm *et al.*, 2011), and two were

experimental chamber studies of acute effects (Hake & Stewart, 1977; Altmann *et al.*, 1990).

Results from the studies of both occupational and residential exposure support an inference of visual deficits after long-term exposure to tetrachloroethylene. Notably, decrements in colour confusion were reported among all workers exposed to a mean TWA of 6 ppm for an average of 8.8 years (Cavalleri *et al.*, 1994).

Studies of acute effects in humans reported increased latencies of up to 3.0 ms in visual evoked potentials (Altmann *et al.*, 1990) and changes in electroencephalograms [magnitude of effect not specified] (Stewart *et al.*, 1970; Hake & Stewart, 1977) at higher exposures ranging from 340 to 680 mg/m^3.

Effects on visiospatial memory (increased response times or cognition errors) in humans were also reported in each of the studies that examined this parameter (Seeber, 1989; Echeverria *et al.*, 1994, 1995; Altmann *et al.*, 1995), in occupational and residential settings.

An occupational exposure study (n = 60) (Ferroni *et al.*, 1992) and a residential exposure study (n = 14) (Altmann *et al.*, 1995), with mean exposure levels of 15 and 0.7 ppm, respectively, reported significant increases in simple reaction time of 24 ms (11% increase) and 40 and 51.1 ms (15 and 20% increases, respectively, for two separate measurements) for the exposed subjects. A third study reported better performance on simple reaction time in 21 exposed workers (mean TWA, 21 ppm) compared with controls when measured before a work shift, but not when measured after work (Lauwerys *et al.*, 1983).

(b) Experimental animals

Mechanistic neuropathological studies have been conducted in animal models (rats, mice, gerbils) to determine how tetrachloroethylene may give rise to the neurological effects observed. Changes in fatty-acid composition of the brain after exposure to tetrachloroethylene at 320 ppm for 30 or 90 days have been reported, and some of these changes persisted for up to 30 days after cessation of exposure (Kyrklund *et al.*, 1988, 1990). Studies that examined the entire brain of animals reported reductions in astroglial proteins (GFAP and S-100), decreased brain RNA content, and decreased levels of glutamine, threonine, and serine (Savolainen *et al.*, 1977; Honma *et al.*, 1980a, b; Kyrklund *et al.*, 1984, 1987, 1988, 1990; Rosengren *et al.*, 1986; Wang *et al.*, 1993). Brain regions examined after exposure to tetrachloroethylene included the frontal cortex, the hippocampus, the striatum, and the cerebellum. Notable changes included decreased DNA content in the frontal cortex after continuous exposure at 600 ppm for 4 weeks in rats (Wang *et al.*, 1993), or exposure at 60 ppm for 3 months in Mongolian gerbils (Karlsson *et al.*, 1987). Decreased levels of taurine were noted in the cerebellum and hippocampus after exposure to tetrachloroethylene at 120 ppm for 12 months in Mongolian gerbils, but there were no changes in levels or uptake of γ-aminobutyric acid (GABA) (Briving *et al.*, 1986).

Reduced concentrations of acetylcholine were noted in the striatum of male rats exposed to tetrachloroethylene at 800 ppm for 1 month (Honma *et al.*, 1980a, b).

Voltage- and ligand-gated ion channels have been implicated in many neurological functions and have been studied as potential neurological targets for tetrachloroethylene and structurally related chlorinated solvents (e.g. trichloroethylene, 1,1,1-trichloroethane, dichloromethane). Tetrachloroethylene has been shown to perturb voltage-sensitive calcium-channel function in nerve growth factor-differentiated pheochromocytoma cells (Shafer *et al.*, 2005) and to block various acetylcholine-induced currents in human neuronal nicotinic acetylcholine receptors by 40–60% (Bale *et al.*, 2005). On the basis of the structural similarity of tetrachloroethylene and other chlorinated solvents, as well as similar neurobehavioural and mechanistic findings, it is likely that tetrachloroethylene also interacts with

the other molecular targets. This solvent class, in particular trichloroethylene, has been shown to interact with ion channels such as the $GABA_A$ and glycine receptors (Beckstead *et al.*, 2000; Krasowski & Harrison, 2000; Lopreato *et al.*, 2003). In addition, this class of solvents block the sodium-channel (Shrivastav *et al.*, 1976) and the voltage-sensitive calcium-channel function (Shafer *et al.*, 2005) when the membrane is held at or near the resting membrane potential. Overall, these solvents appear to potentiate the function of inhibitory receptors and inhibit the function of excitatory receptors (see Bowen *et al.* (2006) and Bushnell *et al.* (2007) for reviews).

Evidence from the available studies in humans and experimental animals indicated that long-term exposure to tetrachloroethylene can result in decrements in colour vision, visuospatial memory, and possibly other aspects of cognition and neuropsychological function, including reaction time.

4.4 Susceptibility

4.4.1 Genetic polymorphisms

Genetic variation is likely to play a role in the response to exposure to tetrachloroethylene in humans. Individual uptake of tetrachloroethylene was estimated from the concentrations of the parent compound and its metabolites in blood, urine, and exhaled air after a single exposure at 70 or 140 ppm for 4 hours (Monster & Houtkooper, 1979). The concentrations of tetrachloroethylene in blood at 2 hours or 20 hours after exposure, and in exhaled air at 2 hours after exposure provided a coefficient of inter-individual variation of 20–25%.

The metabolism of tetrachloroethylene differed by about fivefold among seven samples of liver from adults (Reitz *et al.*, 1996), and a twofold difference in the concentration of tetrachloroethylene in blood was reported among nine adults (Opdam, 1989); the latter difference may be attributable to differing amounts of body fat of the subjects.

The oxidative metabolism of tetrachloroethylene is mediated by several cytochrome P450 enzymes, but it is not clear which role is played by specific isoforms. CYP2E1 facilitates the metabolism of tetrachloroethylene to genotoxic intermediates (Doherty *et al.*, 1996). However, induction of CYP2E1 with pyridine had little effect on tetrachloroethylene-induced cytotoxicity (Lash *et al.*, 2007). Human lymphoblastoid cell lines expressing individual human CYP450 isoforms were used to identify the enzymes responsible for the formation of immunoreactive protein by microsomal fractions upon incubation with tetrachloroethylene. CYP1A2, CYP2B6 and CYP2C8 appeared to be responsible for the immunoreactivity, but no activation of tetrachloroethylene by CYP2E1 could be detected (White *et al.*, 2001). While CYP2E1 is a key enzyme in the metabolic activation of a variety of toxicants including nitrosamines, benzene, vinyl chloride, and halogenated solvents such as trichloroethylene (reviewed in Neafsey *et al.*, 2009), there is little evidence for a potential role of CYP2E1 polymorphisms in differences in the metabolism and toxicity of tetrachloroethylene.

Formation of reactive thiols after metabolism of tetrachloroethylene is considered a critical pathway leading to toxicity and cancer in the kidney. GSTs, β-lyase, FMOs and CYP3A enzymes have been implicated in this mechanism. Genetic variants in CYP3A enzymes are well established in humans as one of the major pharmacogenomic factors for a variety of drugs and environmental chemicals (McGraw & Waller, 2012). While it has not been firmly established which GSTs are important in the metabolism of tetrachloroethylene, allelic polymorphisms of these enzymes in humans have been reported (Rodilla *et al.*, 1998; Cummings *et al.*, 2000; Tzeng *et al.*, 2000). It is not clear, however, how the possible inter-individual differences in these isoenzymes are related to differences in the toxic

effects of tetrachloroethylene, because the specificities and reaction rates of these enzymes are not well understood.

Several transporter molecules that mediate the entry of organic ions into the renal proximal tubular cells (e.g. the organic-anion transporters OAT1 and OAT3) are known to be polymorphic in humans (Erdman *et al.*, 2006; Lash *et al.*, 2006; Urban *et al.*, 2006). These transporters are likely to be responsible for the uptake and cellular accumulation of TCVG and TCVC, two nephrotoxic metabolites of tetrachloroethylene. Thus, dependent on this transporter polymorphism, human populations may have markedly different capacities to accumulate TCVG or TCVC, which may affect their susceptibility to nephrotoxicity.

4.4.2 Life-stage susceptibility and vulnerability

Differences in the effects of exposure to tetrachloroethylene at different stages of life have been reported. Tetrachloroethylene and its metabolite trichloroacetic acid were found in the fetus and in amniotic fluid after exposure of pregnant rats by inhalation (Ghantous *et al.*, 1986). Maternal and fetal/neonatal blood and tissue dose-metrics during pregnancy and lactation were evaluated by use of a physiologically-based pharmacokinetic model for tetrachloroethylene (Gentry *et al.*, 2003). Blood concentrations of tetrachloroethylene in the fetus during gestation were found to be about 600-fold those in the neonate during the lactation period.

During perinatal development, several additional factors may contribute to a higher exposure of children to tetrachloroethylene compared with adults. Because children spend more time indoors and have a greater ventilation rate, i.e. they breathe more rapidly, they may be more vulnerable. Indoor environments in households where dry-cleaning workers live have been found to contain concentrations of tetrachloroethylene that are up to 100-fold those in control homes (Aggazzotti *et al.*, 1994; Storm *et al.*, 2011). Breast milk is an additional and unique exposure source in early life stages (Bagnell & Ellenberger, 1977; Schreiber *et al.*, 2002). Differences in diet between children and adults may lead to higher ingestion of tetrachloroethylene by children (per body weight compared with adults). Collectively, these factors may play a role in greater absorption of tetrachloroethylene in children, but this has not been evaluated quantitatively.

With regards to distribution, one study based on physiologically based pharmacokinetic modelling estimated that blood concentrations of tetrachloroethylene will be lower in children than in adults (Clewell *et al.*, 2004). Another model showed that for a given set of exposures, the younger a person is, the greater the estimated concentration of tetrachloroethylene in the brain (Rao & Brown, 1993).

It is well established that expression of most CYP450 enzymes and GSTs in fetal liver is very different from that in the adult liver. Expression of several CYP450 enzymes and GSTs has been detected in some samples of the developing fetus and is dependent on stage of pregnancy (Carpenter *et al.*, 1996; Tateishi *et al.*, 1997; McCarver and Hines, 2002; Johnsrud *et al.*, 2003). CYP3A7 accounts for up to 50% of total fetal hepatic CYP450 content, but expression of this enzyme decreases rapidly after birth. CYP1A1 and CYP2D6 have also been detected in human fetal liver, but expression of CYP2E1 remains controversial (Ring *et al.*, 1999). Within months after birth, the metabolic capacity of the human liver changes and becomes more similar to that in the adult tissue. Thus, the differences in tetrachloroethylene metabolism between early stages of life and adulthood may represent a potential determinant of susceptibility, even though there is no direct evidence to demonstrate this apart from the information collected from physiologically based pharmacokinetic modelling (Gentry *et al.*, 2003; Clewell *et al.*, 2004).

Some studies have suggested greater susceptibility to tetrachloroethylene-associated impairments in visual contrast sensitivity in children than in adults (Laslo-Baker *et al.*, 2004; Storm *et al.*, 2011). Studies that focused on possible life stage-specific susceptibility to immune system-related adverse health outcomes and hepatotoxicity after exposure to tetrachloroethylene gave inconsistent results, or were not conducted in a way that allows proper comparison between age groups.

Overall, from the available epidemiological studies and studies in experimental animals, there was little evidence of increased susceptibility to cancer from exposure to tetrachloroethylene during early life-stages.

Only few studies examined the exposure to tetrachloroethylene in the elderly (age > 65 years), and in none of these studies was a direct comparison made with another age group.

4.4.3 Sex differences

One study examined sex differences in toxicokinetic parameters of tetrachloroethylene by means of physiologically based pharmacokinetic modelling. Sex-specific differences in the metabolism of tetrachloroethylene were small but significant (Clewell *et al.*, 2004).

Expression and function of organic anion transporters have been shown to exhibit sex-dependent differences in humans and experimental animals (Gotoh *et al.*, 2001; Kobayashi *et al.*, 2002; Buist & Klaassen, 2004; Ljubojevic *et al.*, 2004; Sekine *et al.*, 2006; Sabolić *et al.*, 2007), suggesting that differences in transport into the renal tubular cells may be another factor involved in sex differences in susceptibility to the effects of tetrachloroethylene and its metabolites.

Suspensions of rat kidney cells and renal mitochondria from rats or mice were used to assess the sex- and species-dependence of acute toxicity attributable to tetrachloroethylene and its glutathione conjugate TCVG. A marked sex difference in the acute cytotoxicity – release of lactate dehydrogenase – was observed for both compounds, with a greater effect in males. In suspensions of isolated mitochondria from kidneys of male and female rats, a generally similar pattern of sensitivity was observed. Respiratory function in mitochondria from male and female mice was also significantly inhibited by tetrachloroethylene and TCVG, but there was little sex dependence in the degree of inhibition. Renal toxicity was higher in male than in female rats in a long-term bioassay with exposure to tetrachloroethylene by inhalation (NTP, 1986; see Section 3).

4.5 Mechanistic considerations

Two important metabolic pathways of tetrachloroethylene have been characterized in humans and in experimental animals. The major pathway is CYP450-mediated oxidation, resulting in formation of a variety of short- and long-lived metabolites. In all species, the oxidative metabolite trichloroacetic acid is formed in much larger amounts than other oxidative metabolites, such as dichloroacetic acid. A list of the major oxidative metabolites of tetrachloroethylene, the site of their formation, and the species in which they were detected is given in Table 4.1. There are quantitative differences in the extent of oxidative metabolism of tetrachloroethylene among species, with greater oxidative metabolism in rodents than in humans.

GSH conjugation is another important metabolic pathway for tetrachloroethylene, resulting in the formation of short-lived, reactive metabolites. The initial conjugation reaction to TCVG occurs primarily in the liver, with subsequent processing in the kidney by GGT, dipeptidase, β-lyase, *N*-acetyltransferase, FMO, and via CYP-mediated sulfoxidation. A list of the major metabolites of tetrachloroethylene formed after GSH-conjugation, the site of their formation, and

the species in which they were detected is given in Table 4.1.

Data were lacking to characterize the extent of GSH-conjugation in all species. It should be noted that dichloroacetic acid, a minor product of oxidative metabolism, is also formed through GSH conjugation.

The parent compound tetrachloroethylene and several of its metabolites have been evaluated for genotoxic potential. Tetrachloroethylene itself has been tested in a large number of assays for genotoxicity *in vitro*. The results did not clearly indicate that it is directly mutagenic in the absence or presence of metabolic activation from the standard S9 microsomal preparations from rat liver. However, when tetrachloroethylene was pre-incubated with rat liver GST, GSH, and a microsomal fraction from rat kidney, a clear dose–response relationship was obtained. These findings support a role of metabolic activation via the GSH-conjugation pathway for tetrachloroethylene in genotoxicity *in vitro*. Among the GSH-conjugation metabolites that have been tested in mutagenicity assays, TCVG and NAcTCVC were reported to be mutagenic in the presence of activation, while TCVC was mutagenic without activation. The Working Group concluded that these metabolites of tetrachloroethylene are genotoxic, particularly in the kidney, where metabolism in situ occurs. The genotoxicity of dichloroacetic and trichloroacetic acid is discussed in the respective *Monographs* in this Volume. There are few data available on the genotoxicity of other oxidative metabolites of tetrachloroethylene, i.e. trichloroacetyl chloride and tetrachloroethylene-epoxide.

Tetrachloroethylene has been associated with cancer of the kidney and liver, and of the immune and central nervous systems. For each of these, some mechanistic considerations are given below.

4.5.1 Kidney

In mammalian species, the kidney is a target organ for toxicity of tetrachloroethylene and other related chlorinated ethanes and ethylenes. There is some evidence that tetrachloroethylene is carcinogenic in the kidney in male rats (see Section 3). Besides genotoxicity, the following mechanisms of carcinogenesis have been considered: α2u-globulin-associated nephropathy, cytotoxicity not associated with accumulation of α2u-globulin, and peroxisome proliferation mediated by the receptor PPARα. Regarding the first mechanism, several experimental studies have shown an increase in hyaline droplets in the proximal tubule cells of treated male rats. However, the overall data indicated that α2u-globulin nephropathy is not the sole mechanism for nephrotoxicity or carcinogenesis. Long-term exposure to tetrachloroethylene is indeed associated with kidney damage in humans and in male and female rats and mice. Two studies *in vivo* have shown that short-term exposure to tetrachloroethylene induces peroxisome proliferation in the rodent kidney, but the effects were weak. There is no direct evidence of activation of the PPARα receptor in the kidney, and whether peroxisome proliferation is causally linked to kidney cancer has not been experimentally demonstrated for tetrachloroethylene or other compounds. With regard to cytotoxicity and peroxisome proliferation, these effects occurred in male and female rats and mice, suggesting a lack of sex- and species-specificity in these mechanisms. Overall, available data supports the notion that the likely mechanism of carcinogenisis in the kidney involves the genotoxicity of kidney-derived metabolites of this compound.

4.5.2 Liver

Tetrachloroethylene and its oxidative metabolites induce several effects that may contribute to development of hepatocellular tumours. They

include DNA hypomethylation, cytotoxicity and oxidative stress, alterations in cell proliferation and apoptosis, clonal expansion, PPARα activation, and disruption of gap-junctional intercellular communication. The epigenetic effects of trichloroacetic and dichloroacetic acid, together with the fact that changes in methylation represent common early molecular events in most tumours, support the plausibility that dysregulation of gene methylation may play a role in tetrachloroethylene-induced tumorigenesis. However, there was no specific information available to test this hypothesis. Numerous studies in humans and experimental animals, including long-term bioassays in rodents, have demonstrated that tetrachloroethylene is hepatotoxic, even though the metabolites trichloroacetic and dichloroacetic acid are not. In experimental animals treated with tetrachloroethylene, the characteristics of the hepatic injury include increased liver weight, changes in fatty acids, necrosis, inflammatory cell infiltration, increased levels of triglycerides, and regenerative cell proliferation. Reactive oxygen species can also play a role in mediating hepatotoxicity of tetrachloroethylene. Few studies were available on tetrachloroethylene-induced hepatic oxidative stress. Increased transient liver-cell proliferation in mice has been reported after exposure to tetrachloroethylene. In addition, several studies in mice have shown transient hepatocyte proliferation in response to exposure to trichloroacatic acid. Based on data from tetrachloroethylene alone, there was only limited evidence for the mechanism via PPARα activation, and a concordance with the tumour response in the liver was lacking. It is worth noting that the metabolites trichloroacetic and dichloroacetic acid do activate both human and mouse PPARα, but at concentrations that are an order of magnitude higher than for other peroxisome proliferators. Overall, there was strong evidence that the liver is a target for tetrachloroethylene, and multiple mechanisms of liver carcinogenesis are likely operational.

4.5.3 Immune system

In humans, tumours of the immune system associated with exposure to tetrachloroethylene include non-Hodgkin lymphoma and multiple myeloma. In experimental animals, cancer findings of primary concern are the increased incidence of mononuclear cell leukeamia in both sexes in the NTP (1986) and JISA (1993) inhalation bioassays (see Section 3).

Studies in humans and experimental animals in support of a mechanism for cancers of the immune system were limited to studies that focused on immunological and haematological toxicity. The mechanism by which tetrachloroethylene induces immunotoxicity, or by which the toxicity may ultimately lead to carcinogenesis, could not be identified. A limited number of studies have evaluated exposure of workers to tetrachloroethylene in association with alterations in immune-system parameters. There was some evidence that exposure to tetrachloroethylene is associated with altered blood-cell counts and immune markers indicative of immune activation and dysregulation. However, the findings were inconsistent and the sample sizes were small for nearly all studies. Although there is a well established connection between immune status and carcinogenesis in general, overall the evidence was not sufficiently strong to support a conclusion about a mechanism for tetrachloroethylene-induced carcinogenesis in cells of the immune system.

The limited available data from studies in children (Lehmann *et al.*, 2002; Delfino *et al.*, 2003a, b) do not provide substantial evidence of an effect of exposure of tetrachloroethylene during childhood on allergic sensitization or exacerbation of asthma symptomology. The observed association between exposure to tetrachloroethylene measured in the home, and reduced numbers of interferon-gamma-producing type-1 T-cells in blood samples from the umbilical cord may reflect a sensitive stage of development, and points to the

limited understanding of the potential immunotoxic effects of prenatal exposures. The available data (e.g. Emara *et al.*, 2010) pertaining to risk of autoimmune disease associated with exposure to tetrachloroethylene are limited by problems regarding ascertainment of disease incidence and by difficulties with exposure-assessment in population-based studies.

4.5.4 Other target organs

The central nervous system is clearly a target tissue for tetrachloroethylene induced toxicity. Studies in humans and experimental animals provide evidence of the association of neurobehavioural deficits, including visual changes, cognitive deficits, increased reaction time, and exposure to tetrachloroethylene. No data were available to identify the mechanism(s) that may contribute to the induction of the rare brain gliomas occurring in exposed rats.

On the basis of the number of molecular targets reported in the studies, it is likely that several mechanisms are responsible for the neurotoxicological effects of tetrachloroethylene.

4.5.5 Susceptible populations

The bladder and oesophagus may be target tissues for tetrachloroethylene-induced carcinogenesis in humans; however, there were no studies to suggest mechanisms underlying these effects.

The carcinogenicity of tetrachloroethylene is associated with its metabolism and, therefore, the susceptibility to this agent may be influenced by genetic factors, sex, life-stage and other conditions. Polymorphisms in genes involved in the oxidative metabolic pathways (e.g. CYP2E1, CYP3A) and in GSH conjugation (e.g. GSTs) are commonly found in human populations, but it is not clear what role, if any, they play in the carcinogenicity of tetrachloroethylene. With respect to life-stage susceptibility, data were available to suggest that individuals at early stages of life, especially at the prenatal and neonatal phase, may be more vulnerable to exposure to tetrachloroethylene. Most of such evidence is based on either differences in exposure routes (e.g. transplacental transfer and ingestion via breast milk in early life), or in life stage-specific differences in toxicokinetics. Sex differences have been noted in adverse health outcomes associated with exposure to tetrachloroethylene, namely a greater susceptibility of males to kidney toxicity, which could result from differences in physiological factors (e.g. hormonal status), and toxicokinetics.

5. Summary of Data Reported

5.1 Exposure data

Tetrachloroethylene is one of the most important chlorinated solvents worldwide; it has been produced commercially since the early 1900s. Between the 1950s and 1980s, the most important use of tetrachloroethylene was in dry-cleaning. Smaller amounts were used for degreasing metals and in the production of chlorofluorocarbons. Since the 1990s, the largest use has been as a feedstock for the synthesis of fluorocarbons.

Tetrachloroethylene is detected in indoor and outdoor air, water, food and in animal and human tissues.

Exposure to tetrachloroethylene occurs primarily by inhalation. Occupational exposure has been and continues to be widespread. Technological advances in dry-cleaning and degreasing have resulted in a considerable reduction in exposure, and concentrations to which workers are exposed have decreased by two orders of magnitude since the 1940s in the USA and Europe, although high-exposure situations continue to exist in some countries. Individuals living or working in the vicinity of

dry-cleaning shops are also exposed, although at lower concentrations.

5.2 Human carcinogenicity data

The Working Group focused its evaluation of the epidemiological data on studies that assessed exposure to tetrachloroethylene specifically, or that focused on employment in dry-cleaning. Excluded were studies that characterized exposure by employment in occupational or industrial categories combining 'laundry and dry-cleaning' and studies of mixed solvent exposure without further distinction. The largest cohort studies were those of dry-cleaning workers in the USA and in four Nordic countries. The studies in the USA compared cancer mortality among exposed workers and in the general population, while the Nordic study compared cancer incidence in dry-cleaning workers with that in unexposed laundry workers. None of the cohort studies specifically controlled for tobacco smoking or alcohol consumption. However, the internal comparison in the Nordic study controlled indirectly for social class, which is an effective proxy for tobacco smoking in those countries.

5.2.1 Cancer of the bladder

Information about bladder cancer was available from the cohort studies of dry-cleaning workers in the USA and Europe and from 11 case–control studies that reported data on exposure to tetrachloroethylene, or work in dry-cleaning shops in several countries. All three dry-cleaning cohorts showed an increased risk of bladder cancer, which was statistically significant in the Nordic study. This study was based on cancer incidence, which is a better outcome measure than mortality, given the low case-fatality of bladder cancer. Two of the cohort studies found no evidence of an exposure–response relationship, but one study reported a statistically significant increase in risk among workers employed for more than 5 years, with 20 years of latency. All case–control studies included adjustments for tobacco smoking and other potential confounders. Three case–control studies specifically assessed occupational or environmental exposure to tetrachloroethylene. One found a positive association for men, but did not report data for women, while another study, based on small numbers, showed an excess risk in the group with the highest exposure. The third study found a negative association, also based on small numbers. All eight studies that evaluated employment in dry-cleaning showed positive associations with bladder cancer. Three of these were from the United States National Bladder Cancer Study, and they all showed positive associations, although statistically significant only in one; furthermore two of the studies included dry-cleaners, ironers and pressers. Three other case–control studies were small, with inconsistent results. The last case–control study was based on a surveillance system and showed a statistically non-significant excess risk. While positive associations with bladder cancer were observed in several cohort and case–control studies, and smoking was adequately controlled for in the majority, employment in dry-cleaning was in most cases the only indicator of exposure to tetrachloroethylene, the number of exposed cases was small, and support for an exposure–response relationship was lacking.

5.2.2 Other cancer sites

Several studies evaluated exposure to tetrachloroethylene and the risk of cancers at other sites, including oesophagus, kidney, cervix, and non-Hodgkin lymphoma. No consistent patterns were seen across studies. Statistically significant increases in mortality from cancer of the oesophagus were observed in two studies of dry-cleaning workers in the USA, with a larger increase among the longest-employed workers; increased mortality from lung cancer in these studies points

to potential confounding by tobacco smoking among exposed workers. No increase in incidence of cancer of the oesophagus was found in the Nordic dry-cleaners study, which controlled for social class as a proxy indicator of tobacco and alcohol consumption. Two studies of aerospace workers in the USA who were also exposed to trichloroethylene reported a non-significant increase in mortality from oesophageal cancer. Two small case–control studies provided information on the association of oesophageal cancer with potential exposure to tetrachloroethylene: one evaluated employment in dry-cleaning and reported a non-significant positive association, while the other assessed exposure to tetrachloroethylene, but had no exposed cases.

For cancer of the kidney, some case–control studies were suggestive of a positive association for occupations involving exposure to tetrachloroethylene. However, the cohort studies generally did not find an association between tetrachloroethylene exposure and cancer of the kidney, and most studies did not evaluate or failed to show positive exposure– or duration–response relationships. The studies also did not account for coexposure to trichloroethylene, which has been associated with cancer of the kidney in many other studies. Increased risk of cervical cancer was seen in the two dry-cleaner cohorts in the USA and in a study of Swedish dry-cleaners, but not in the Nordic dry-cleaner study. For non-Hodgkin lymphoma, three cohort studies showed an increased risk based on small numbers, and the largest study with the best control of potential confounders did not. Case–control studies on non-Hodgkin lymphoma did not find significant associations.

5.3 Animal carcinogenicity data

Tetrachloroethylene was evaluated for carcinogenicity in male and female mice and rats exposed by gavage or by inhalation.

In mice given tetrachloroethylene in corn oil by gavage, there were increases in the trend and in the incidence of hepatocellular carcinoma in males and females. In two separate studies in mice of two different strains exposed by inhalation, significant increases in the incidence of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) were observed in males and females. In one of the studies in mice exposed by inhalation, there was a positive trend in the incidence of haemangioma or haemangiosarcoma (combined) in males and females, a positive trend in the incidence of haemangiosarcoma of the liver and of the spleen in males, and a positive trend in the incidence of adenoma of the Harderian gland in males.

In two studies in rats, exposure to tetrachloroethylene by inhalation for 2 years caused an increase in the incidence of mononuclear cell leukaemia in males and females. In one of these studies, an increased incidence of interstitial cell tumours of the testis and a positive trend in the incidence of brain glioma were observed in males. The incidences of brain glioma, and kidney adenoma or carcinoma (combined) were not statistically significantly increased compared with concurrent controls, but were markedly higher than incidences for historical controls. In the other study in rats exposed by inhalation there was an increase in the incidence of fibroadenoma of the mammary gland in the group at the lowest dose. The study in rats exposed by gavage gave negative results.

In a study in mice, skin application of tetrachloroethylene oxide, a metabolite of tetrachloroethylene, induced a statistically significant increase in the incidence of benign or malignant skin tumours (combined) at the site of application.

5.4 Mechanistic and other relevant data

A comprehensive body of literature exists to characterize the absorption, distribution, metabolism and excretion of tetrachloroethylene in humans and in experimental animals. Tetrachloroethylene is readily absorbed via all exposure routes (oral, dermal, by inhalation) in all species studied, including humans. Rapid systemic distribution has been observed in humans and experimental animals. Because of its high lipophilicity, tetrachloroethylene is distributed widely to all tissues, especially those with a high lipid content. As it is poorly metabolized in humans, a large fraction of tetrachloroethylene is expired unchanged. In rats and mice, due to more extensive metabolism, the proportion of tetrachloroethylene that is excreted unchanged is smaller, although it rises with increasing exposure. The major urinary excretion product is trichloroacetic acid.

There are qualitative similarities between humans and rodents with respect to the two important metabolic pathways of tetrachloroethylene. The major pathway is cytochrome P450-mediated oxidation, resulting in formation of a variety of short- and long-lived metabolites. In all species, the oxidative metabolite trichloroacetic acid is formed in much larger amounts than other oxidative metabolites (e.g. dichloroacetic acid). There are quantitative differences among species in the extent of oxidative metabolism of tetrachloroethylene, with rodents showing more extensive oxidative metabolism than humans. Glutathione conjugation is another important metabolic pathway for tetrachloroethylene, resulting in formation of short-lived, reactive metabolites. The initial conjugation reactions occur in the liver (formation of trichlorovinyl-glutathione), while subsequent processing primarily takes place in the kidney. Dichloroacetic acid is also formed through conjugation with glutathione. Only few data exist to further characterize the glutathione-conjugation pathway.

Tetrachloroethylene and several of its metabolites have been evaluated for genotoxic potential. Metabolic activation via the glutathione-conjugation pathway leads to the formation of genotoxic metabolites. Among the glutathione-conjugation metabolites that have been tested, trichlorovinyl-glutathione and *N*-acetyl-*S*-(1,1,2-trichlorovinyl)-L-cysteine are genotoxic. There is some evidence that dichloroacetic acid may cause genotoxicity and that trichloroacetic acid is not genotoxic (see the *Monographs* on dichloroacetic acid and trichloroacetic acid in this Volume). For other oxidative metabolites of tetrachloroethylene, i.e. trichloroacetyl chloride and tetrachloroethylene epoxide, there is weak evidence for their genotoxicity. Thus tetrachloroethylene can be converted to genotoxic metabolites, particularly in the kidney, where metabolism in situ occurs.

Tetrachloroethylene has been associated with adverse health outcomes in the kidney, liver, and the immune and central nervous systems. There is evidence from toxicological studies in experimental animals that the kidney is a target organ for tetrachloroethylene and that genotoxic metabolites are formed in that organ. The data in support of a non-genotoxic mechanism of kidney carcinogenesis are less convincing. There is also strong evidence from toxicological studies in experimental animals that the liver is a target organ for tetrachloroethylene; with respect to liver carcinogenesis, it is likely that multiple mechanisms are operational in view of the evidence supporting genotoxic and non-genotoxic mechanisms. The information that pertains to a mechanism of carcinogenesis related to the immune system is limited to studies of immunological and haematological toxicity. Multiple observations of neurobehavioural effects in exposed humans and experimental animals provide evidence that the central nervous system is a target for tetrachloroethylene. However, the relevance of

these findings to the potential cancer hazard of this compound to the central nervous system is not known. The bladder has been identified as a target organ for tetrachloroethylene-induced carcinogenesis in humans; however, there are no mechanistic studies available to support this.

Adverse health effects of tetrachloroethylene, particularly in the liver and kidney, are associated with its metabolism. Therefore, the susceptibility to tetrachloroethylene may be influenced by genetic factors, sex, life-stage and other conditions that may have an impact on the extent and nature of the metabolism. Polymorphisms in genes involved both in oxidative metabolism (e.g. CYP2E1, CYP3A) and in glutathione-conjugation pathways (e.g. glutathione-*S*-transferases) are common in the human population. However, it is not clear what role, if any, these polymorphisms play in affecting the toxicity of tetrachloroethylene. With respect to life-stage susceptibility, differences in exposure route (e.g. transplacental transfer and ingestion via breast milk in early stages of life), or on life stage-specific differences in toxicokinetics have been observed.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of tetrachloroethylene. Positive associations have been observed for cancer of the bladder.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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DICHLOROACETIC ACID

This substance was considered by previous Working Groups in February 1995 and October 2004 (IARC, 1995, 2004). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

(a) Dichloroacetic acid

Chem. Abstr. Serv. Reg. No.: 79-43-6

Deleted Chem. Abstr. Serv. Reg. No.: 42428-47-7

Chem. Abstr. Serv. Name: Dichloroacetic acid

IUPAC Systematic Name: Dichloroacetic acid

Synonyms: DCA; DCA (acid); dichloracetic acid; dichlorethanoic acid; dichloroethanoic acid; bichloracetic acid

(b) Sodium dichloroacetate

Chem. Abstr. Serv. Reg. No.: 2156-56-1

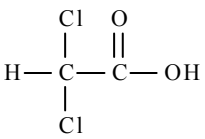
Chem. Abstr. Serv. Name: Sodium dichloroacetate

IUPAC Systematic Name: Sodium 2,2-dichloroacetate

Synonyms: Dichloroacetate, sodium salt; dichloroacetic acid sodium salt; sodium 2,2-dichloroacetate

1.1.2 Structural and molecular formulae and relative molecular mass

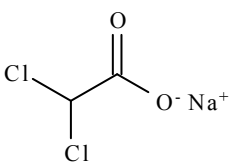
(a) Dichloroacetic acid



$C_2H_2Cl_2O_2$

Relative molecular mass: 128.94

(b) Sodium dichloroacetate



$C_2HCl_2NaO_2$

Relative molecular mass: 150.92

1.1.3 Chemical and physical properties of the pure substance

(a) Dichloroacetic acid

Description: Corrosive liquid; pungent odour (O'Neil *et al.*, 2006)

Table 1.1 Methods for the analysis of dichloroacetic acid in water

Sample preparation	Assay procedure	Limit of detection	Reference
Extract methyl-*t*-butyl ether; derivatize to methyl ester; acidify; extract with methanol	GC/ECD	0.24 µg/L	EPA (2003)
Add ammonium chloride and ^{13}C-labelled internal standards; direct injection	IC-ESI-MS/MS	0.055 µg/L	EPA (2009)

ECD, electron capture detection; ESI-MS/MS, electrospray ionization tandem mass spectrometry; GC, gas chromatography; IC, ion chromatography

Boiling-point: 193–194 °C (O'Neil *et al.*, 2006)

Melting-point: 9.7 °C and −4 °C; apparently occurs in two crystalline forms (O'Neil *et al.*, 2006)

Density: 1.563 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism [2806]), nuclear magnetic resonance [166] and mass spectral data have been reported (Weast & Astle, 1985)

Solubility: Slightly soluble in water; miscible with ethanol, ethyl ether (O'Neil *et al.*, 2006) soluble in acetone; slightly soluble in carbon tetrachloride (Haynes, 2012)

Volatility: Vapour pressure, 0.023 kPa at 25 °C (Haynes, 2012)

Stability: Dissociation constant (K_a), 5.14×10^{-2} (Morris & Bost, 1991)

Octanol/water partition coefficient (P): Log P, 0.92 (Hansch *et al.*, 1995)

Conversion factor: $mg/m^3 = 5.27 \times ppm$ (calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg)

(b) Sodium dichloroacetate

Description: White powder (Haynes, 2012)

Melting-point: 198 °C (decomposes) (Haynes, 2012)

Solubility: Soluble in cold water (Haynes, 2012)

1.1.4 Technical products and impurities

Dichloroacetic acid is commercially available as a technical-grade liquid with the following typical specifications: purity, 98.0% minimum; monochloroacetic acid, 0.2% maximum; trichloroacetic acid, 1.0% maximum; and water, 0.3% maximum (Clarian GmbH, 2002). Sodium dichloroacetate is available as a powder with a purity of 98%, containing < 2% ethyl alcohol (Sigma-Aldrich, 2012).

Trade names for dichloroacetic acid include Urner's liquid.

1.1.5 Analysis

Methods for the analysis of dichloroacetic acid have been reviewed by Delinsky *et al.* (2005). Selected methods for the analysis of dichloroacetic acid in water, exhaled air, blood and urine are identified in Table 1.1. A headspace gas chromatography–mass spectrometry method has been developed for measuring trichloroacetic acid in urine (Cardador & Gallego, 2010).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Dichloroacetic acid was reported to be first synthesized in 1864 by the further chlorination of monochloroacetic acid with chlorine (Beilstein Online, 2002).

The most common production method for dichloroacetic acid is the hydrolysis of dichloroacetyl chloride, which is produced by the oxidation of trichloroethylene. It can also be obtained by hydrolysis of pentachloroethane with 88–99% sulfuric acid or by oxidation of 1,1-dichloroacetone with nitric acid and air. In addition, dichloroacetic acid can be produced by catalytic dechlorination of trichloroacetic acid or ethyl trichloroacetate with hydrogen over a palladium catalyst (Koenig *et al.*, 1986; Morris & Bost, 1991).

Sodium dichloroacetate is readily formed when dichloroacetic acid is dissolved in an aqueous solution. In addition, haloacetic acids may form de novo as disinfection by-products in chlorinated drinking-water (Nissinen *et al.*, 2002).

(b) Production

Dichloroacetic acid was produced by two companies in the USA and one company each in China, Japan and Mexico (Chemical Information Services, 2002; IARC, 2004). It was formulated into pharmaceutical products by one company each in New Zealand and Turkey (Chemical Information Services, 2002).

1.2.2 Use

Dichloroacetic acid and its esters are intermediates in organic synthesis, used in the production of glyoxylic acid, dialkoxy and diaroxy acids, and sulfonamides and in the preparation of iron chelates in the agricultural sector (Koenig *et al.*, 2011). It can also be used as an analytical reagent in fibre manufacture (polyethylene terephthalate).

Dichloroacetic acid is used in medical practice as a cauterizing agent. It rapidly penetrates and cauterizes the skin and keratins. Its cauterizing ability compares with that of electrocautery or freezing. It is used on calluses, hard and soft corns, xanthoma palpebrarum, seborrhoeic keratoses, in-grown nails, cysts and benign erosion of the cervix (Gennaro, 2000). It can also be used as a medicinal disinfectant as a substitute for formalin. Dichloroacetic acid has also been proposed for use in targeted therapy against cancer (Tennant *et al.*, 2010).

Dichloroacetic acid and its salts have been used therapeutically to treat the rare condition of congenital lactic acidosis (Stacpoole *et al.*, 2006, 2008). They have also been tested for effects on diabetes and on tumour growth (Michelakis *et al.*, 2010; Stacpoole & Greene, 1992). Due to side-effects, these substances are not in common use as therapeutic agents.

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Dichloroacetic acid is not known to occur as a natural product.

1.3.2 Environmental occurrence

(a) Air

No data were available to the Working Group.

(b) Water

Dichloroacetic acid is produced as a by-product during the chlorination of water containing humic substances and may occur in drinking-water or swimming pools after chlorine-based disinfection of raw waters that contain natural organic substances (IARC, 2004).

Table 1.2 Concentrations of dichloroacetic acid in water

Country	Location	Concentration (µg/L)		Reference
		Mean	Range	
Drinking-water				
Australia	Seven cities	NR	1–46	Simpson & Hayes, (1998)
China	Eight water supplies	NR	0.3–10.9	Liu *et al.* (2011)
	Beijing	11.1	9.6–12.9	Wang & Wong, (2005)
	Beijing	2.69	13.02[b]	Wei *et al.* (2010)
Greece	Athens	NR	2.3–24.5	Golfinopoulos & Nikolaou (2005)
	Mytilene	NR	2.6–3.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	1.8[a]	0.7–18.0	Villanueva *et al.* (2012)
United Kingdom	England	6.8	3.12–15.0	Zhang *et al.* (2010a)
	Three large regions	9.1	23[b]	Malliarou *et al.* (2005)
		39.9	116[b]	
		23.7	58[b]	
Raw and surface water				
China	Eight water supplies	NR	29.3–155.7	Liu *et al.* (2011)
Republic of Korea	Four regions	50.4	44.2–58.1	Kim (2009)
Swimming-pool water				
Republic of Korea	*Seoul*			Lee *et al.* (2010)
	Pools treated with Chlorine	68.3	14.1–246	
	Pools treated with ozone + chlorine	12.0	ND–31.9	
	Pools treated with EGMO	33.7	1.5–98.5	

[a] Median
[b] Maximum
EGMO, electrically generated mixed oxidants; ND, not detected; NR, not reported
Note: Data from earlier periods can be found in the previous *IARC Monograph* (IARC, 2004)

Table 1.2 summarizes some recent levels of dichloroacetic acid found in surface waters, groundwater and drinking-water worldwide.

(c) Food

No data were available to the Working Group.

(d) Other

No data were available to the Working Group.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 1592 employees in 39 facilities in the USA were potentially exposed to dichloroacetic acid (NIOSH, 1994). The estimate was based on a survey of companies and did not involve measurements of actual exposure.

Recently, occupational exposure of swimming-pool attendants to dichloroacetic acid in indoor and outdoor pools was evaluated by analysis of urine samples. After an exposure of 2 hours, the urine of 24 exposed indoor-pool attendants contained dichloroacetic acid at a concentration of ~300 ng/L (range, 230–448 ng/L). Exposure levels in outdoor pools were much lower at ~50 ng/L (range, < 30–60 ng/L), despite higher concentrations of the chemical in the water of the outdoor pools than in the indoor pools (Cardador & Gallego, 2011). The concentrations in urine of indoor-pool

workers increased by 40% after the length of the shift doubled (4 hours). [The Working Group noted that it was unclear by what route the pool attendants had been exposed.]

1.3.4 Exposure of the general population

Kim & Weisel (1998) measured the amount of dichloroacetic acid excreted by people who had swum in a chlorinated pool; values ranged from 25 to 960 ng per urine void. Mean concentrations at the time of visit were 1.4 ng/mg creatinine in those having swum in water with low concentrations of chlorination-disinfection by-products, and 1.82 ng/mg creatinine in those having swum in water with high concentrations of these by-products (Weisel *et al.*, 1999).

In a study of swimmers who attended swimming pools for two sessions (duration, 1 hour) per week, the average concentrations of dichloroacetic acid in urine were 2294 ng/L in 13 adults and 3102 ng/L in 6 children in an indoor pool and 4979 ng/L in 8 swimmers in an outdoor pool (Cardador & Gallego, 2011).

1.4 Regulations and guidelines

The maximum concentration of haloacetic acids (five) (HAA5) allowable as contaminants in drinking-water is 0.060 mg/L. HAA5 is the sum of the concentrations in milligrams per litre of the haloacetic acid compounds (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid), rounded to two significant figures after addition (EPA, 2008).

Dichloroacetic acid is not listed as a carcinogen by the United States Environmental Protection Agency (EPA), the National Toxicology Program (NTP), or the European Union. Dichloroacetic acid was classified as a carcinogen in 1996 in California by the Safe Drinking-water and Toxic Enforcement Act. The only countries with established limits for occupational exposure are Belgium and the Republic of Korea, with a limit value of 0.5 ppm [2.5 mg/m^3] at 8 hours (GESTIS, 2013).

2. Cancer in Humans

Dichloroacetic acid is a chemical that occurs in drinking-water and swimming pools as part of a mixture of disinfection by-products. The chemicals in water-disinfection by-products do not occur in an isolated manner and there is no epidemiological evidence on risk of cancer associated specifically with them. A detailed description of water-disinfection by-products and cancer risk is given in *IARC Monograph* Volume 101 (IARC, 2012).

3. Cancer in Experimental Animals

Because of the potential role of dichloroacetic acid in carcinogenicity as a metabolite of trichloroethylene, studies with dichloroacetic acid have focused almost exclusively on the liver.

Therefore assessment of cancer at other sites has been very limited (see Table 3.1 and Table 3.2).

3.1 Mouse

See Table 3.1

3.1.1 Oral administration

As part of an initiation–promotion study, Herren-Freund *et al.* (1987) examined induction of liver cancer in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 5 g/L for 61 weeks. Control animals were given drinking-water containing sodium chloride (NaCl) at a concentration of 2 g/L to control for the sodium hydroxide (NaOH) used to neutralize dichloroacetic acid.

Table 3.1 Studies of carcinogenicity in mice exposed to dichloroacetic acid by oral administration or skin application

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	NaCl, 2 g/L (control), DCA, 5 g/L in drinking-water 27, 26/group	At terminal kill (five mice unaccounted for in the control group): Liver [hepatocellular] adenomas: 2/22, 25/26* Hepatocellular carcinoma: 0/22, 21/26*	*$P < 0.01$, Fisher exact test	Purity, > 99% Small numbers of mice. Short duration of exposure. Based on data from other studies, it is probable that drinking-water consumption was significantly depressed in the treated group, Pathological examination limited to the liver.
Mice, B6C3F$_1$ (M) 52 wk Bull *et al.* (1990)	0 (control), 1, 2 g/L in drinking-water 35, 11, 24/group	Total gross liver lesions: 2/35, 2/11, 23/24* Six hepatocellular carcinomas confirmed in five mice in the group at 2 g/L	Statistical analysis, NR *[$P < 0.0001$]	Analytical grade; purity, NR. Small numbers of mice/group, short duration, and only 45/120 gross liver lesions were examined and characterized. Only the liver was examined for gross pathology or histopathology. Ten females survived to 52 wk with no lesions noted.
Mice, B6C3F$_1$ (M) 60–75 wk DeAngelo *et al.* (1991)	*Experiment 1A (60 wk):* NaCl, 2 g/L (control); DCA, 0.05, 0.5, 5 g/L in drinking-water 9, 9, 9, 30/group *Experiment 1B (75 wk):* NaCl, 2 g/L (control); DCA, 0.05, 0.5 g/L 19, 20, 18/group *Experiment 2 (60 wk):* acetic acid, 2 g/L (control); DCA, 3.5 g/L 10, 12/group	Data from all experiments were combined for reporting prevalence at terminal kill *DCA, 5 g/L* Hepatocellular adenoma: 24/30* Hepatocellular carcinoma: 25/30* *Control, and DCA, 0.05, 0.5, 3.5 g/L* Hepatocellular adenoma: 0/28, 2/29, 1/27, 12/12* Hepatocellular carcinoma: 2/28, 5/29, 2/27, 8/12*	*$P < 0.001$	Purity, > 99% Drinking-water consumption significantly decreased at 5 g/L; only histopathological results from the liver were reported; limited reporting of the study.
Mice, B6C3F$_1$ (M) 104 wk Daniel *et al.* (1992)	0, 0.5 g/L in drinking-water Experiment 1: 10, 16/group Experiment 2: 10, 8/group	Data from both experiments were combined for reporting prevalence at terminal kill Liver adenoma: 1/20, 10/24* Hepatocellular carcinoma: 2/20, 15/24* Hepatic adenoma or carcinoma (combined): 3/20, 18/24*	Fisher exact test; *$P \leq 0.01$	Purity, > 95% Small number of mice/group and single dose limit statistical power. Histopathology nor reported for mice dying during experiment.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) 76 wk Anna *et al.* (1994)	0, 5 g/L drinking-water Start: 24, 110/group	Prevalence at terminal kill: Hepatocellular adenoma: 2/24, 83/89* Hepatocellular carcinoma: 2/24, 66/89*	Fisher exact test *[$P < 0.0001$]	Purity, NR Only the liver was examined grossly or microscopically for pathology. Consumption of drinking-water at this high dose was not discussed by the authors.
Mice, B6C3F$_1$ (F) Up to 586 days Pereira (1996)	NaCl, 1.15 g/L; DCA, 0.259, 0.86, 2.59 g/L in drinking-water, or repeated treatment cycle of 24 days with DCA at 2.59 g/L followed by 48 days without DCA 134, 90, 50, 40, 34/group	Prevalence: *360 days:* Hepatocellular adenoma: 1/40, 0/40, 3/20, 7/20, 0/15 Hepatocellular carcinoma: 0/40, 0/40, 0/20, 1/20, 0/15 *576 days:* Hepatocellular adenoma: 2/90, 3/50, 7/28, 16/19, 3/34 Hepatocellular carcinoma: 2/90, 0/50, 1/28, 7/19, 1/34	Kruskal–Wallis test $P < 0.01$; adenoma at high dose, 360 and 576 days $P < 0.05$; carcinoma at high dose, 576 days	Purity, NR Only cancer bioassay (except initiation-promotion studies) in the female mouse. Drinking-water consumption reduced at the high dose for the first week, but not beyond. The only organ examined was the liver.
Mice, B6C3F$_1$ (F) 104 wk Schroeder *et al.* (1997)	0, 0.5, 3.5 g/L in drinking-water 39, 25, 25/group	Hepatocellular carcinoma: 1/39, 1/25, 23/25*	*[$P < 0.001$]	Purity, NR Study was primarily intended to characterize *ras* mutation spectra in liver tumours. The only organ examined was the liver and reporting of histopathology was limited.
Mice, B6C3F$_1$ (M) Up to 100 wk DeAngelo *et al.* (1999)	0, 0.5, 1, 2, 3.5 g/L in drinking-water 88, 55, 71, 55, 46/group	*Hepatocellular adenoma* At 78 wk ($n = 10$): 10%, 10%, 20%, 50%, 50% At 79–100 wk: 10% ($n = 50$), 20% ($n = 24$), 51.4%* ($n = 32$), 42.9%* ($n = 14$), 45%*($n = 8$) *Hepatocellular carcinoma* At 78 wk ($n = 10$): 10%, 0%, 20%, 50%, 70%* At 79–100 wk: 26% ($n = 50$), 48% ($n = 24$), 71%* ($n = 32$), 95%* ($n = 14$), 100%* ($n = 8$)	Trend (Fisher-Irwin test) *$P < 0.05$	Purity, > 99%; no contaminants detected Early sacrifice of groups of 10–15 mice at wk 26, 52 and 78. Number of mice at terminal kill varied as indicated. Not always apparent what the effective number of mice was at terminal kill. Inconsistent reporting. Limited pathology examination of tumours sites other than the liver.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) Up to 87 wk Bull *et al.* (2002)	52 wk: 0, 0.1, 0.5, 2 g/L in drinking-water; 87 wk: 0, 0.5, 2 g/L Number of mice at start unclear	Combined incidence of liver hyperplastic nodules or hepatocellular adenoma or carcinoma: 52 wk: 1/20, 2/20, 5/20, 12/19* 87 wk: 4/7, 17/19*, 5/5	Fisher exact test *$P < 0.05$	Purity, NR Primarily an interaction study between DCA and TCA as they contribute to carcinogenicity of trichloroethylene. Limited statistical power of overall study, but particularly because of small numbers of mice available at 87 wk. Only liver was examined as a target organ. Limited histopathological diagnosis of lesions. Lesions include grossly observable nodules, hepatocellular adenoma or carcinoma.
Mice, Tg.AC hemizygous (M) 41 wk NTP (2007), Kissling *et al.* (2009)	0, 0.5, 1, 2 g/L in drinking-water 10, 10, 10, 10/group	Bronchioloalveolar adenoma: 1/10, 2/10, 7/10*, 3/10	Fisher exact test *$P < 0.01$	Purity, > 99% Liver tumours were not observed. Small numbers of mice. Short duration of treatment.
Mice, Tg.AC hemizygous (M) 39 wk NTP (2007), Kissling *et al.* (2009)	0, 31.25, 125, 500 mg/kg bw, skin application 10, 10, 10, 10/group	Skin papilloma: 0/10, 0/10, 2/10, 8/10*	Fisher exact test *$P < 0.01$	Small number of mice. Short duration of treatment.
Mice, Tg.AC hemizygous (F) 39 wk NTP (2007), Kissling *et al.* (2009)	0, 31.25, 125, 500 mg/kg bw, skin application 10, 10, 10, 10/group	Skin papilloma: 0/10, 0/10, 0/10, 6/10*	Fisher exact test *$P < 0.01$	Small number of mice. Short duration of treatment.

bw, body weight; DCA, dichloroacetic acid; F, female; M, male; mo, month; NR, not reported; NS; not significant; TCA, trichloroacetic acid; wk, week

Table 3.2 Integration of the studies of carcinogenicity in F344 rats given drinking-water containing dichloroacetic acid reported in Richmond *et al.* (1995) and DeAngelo *et al.* (1996)

Parameter	Study 1[a]				Study 2[b]	
	DCA (g/L)				DCA (g/L)	
	0 (NaCl, 2.0 g/L)	0.05	0.5	2.4[d]	0 (Water)[c]	1.6 [c,e]
Mean daily dose (mg/kg bw per day)	-	3.6	40.2	NR	-	139.1
No. of rats at start	50	60	60	60	78	78
No. of unscheduled deaths	6	12	10	NR	17	23
No. of rats killed at interim (45 and 60 wk)	21	27	27	NR	28	27
No. of rats killed at termination (100–104 wk)	23	21	23	NA	33	28
No. of rats surviving > 78 wk	[23]	26	29	NA	33	28
Prevalence of HN/HA/HC[f] (No.)	4.4%, 4.4%, 0% (23)	0%, 0%, 0%	10.3%, 17.2%, 10.3% (29)	NR	3%, 0%, 3%	3.6%, 10.7%, 21.4%*
Incidence of HN/HA/HC[c,f]	0/7, 0/7, 0/7	0/7, 0/7, 0/7	0/7, 1/7, 0/7	19/27,* 7/27, 1/27		
Prevalence of mononuclear cell leukaemia[b]	24%	20%	43%	NR	9%	11%

* $P < 0.05$

[a] Richmond *et al.* (1995) study, but some data for the control group, and groups receiving DCA at 0.05, 0.5 and 2.4 g/L were taken from DeAngelo *et al.* (1996)

[b] DeAngelo *et al.* (1996) study

[c] Termination for this group was at 60 wk. Data were taken from Richmond *et al.* (1995)

[d] The starting concentration for this group identified in the Richmond *et al.* (1995) study was 2.4 g/L and was maintained throughout the study; the DeAngelo *et al.* (1996) study identifies the starting concentration as 5 g/L in the abstract, and that this was lowered in stages to 1 g/L

[e] This is a time-weighted average dose: 2.5 g/L for 8 wk, 1.5 g/L from 8 to 26 wk, and 1.0 g/L from 26 wk to study termination. There was inconsistency in describing the starting concentration (5.0 g/L is mentioned in the abstract, but 2.5 g/L in the methods section).

[f] Hepatic nodules are lesions distinct from altered foci and that express similar phenotypes as hepatocellular adenoma and hepatocellular carcinoma.

bw, body weight; DCA, dichloroacetic acid; HA, hepatocellular adenoma; HC, hepatocellular carcinoma; HN, hepatic nodules; NA, not applicable; NR, not reported; wk, week

In mice receiving dichloroacetic acid for 61 weeks, 25 out of 26 ($P < 0.01$) had multiple liver [hepatocellular] adenomas (average, 4.58 ± 0.51 per mouse) and 21 out of 26 ($P < 0.01$) had multiple hepatocellular carcinomas (average, 1.69 ± 0.29 per mouse). Incidences of these lesions in mice in the control group were 2 out of 22, and 0 out 22, respectively. [The Working Group noted that this study was limited by examination of the liver only, the short duration of exposure, no reporting of consumption of drinking-water (but that this was likely to be depressed at 5 g/L based upon results of other studies), and the small numbers of mice examined.]

Male B6C3F$_1$ mice were given dichloroacetic acid (neutralized with NaOH) at a concentration of 1 g/L or 2 g/L for 37 or 52 weeks (Bull *et al.*, 1990). Controls received distilled water. There was a clear dose-related increase in the incidence of gross lesions in the liver. Some of these lesions were identified after histopathological examination as hyperplastic nodules, [hepatocellular] adenoma or hepatocellular carcinoma. The incidence of gross lesions after 52 weeks of exposure was 2 out of 35 in the control group, 2 out of 11 in the group receiving dichloroacetic acid at 1 g/L, and 23 out of 24 [$P < 0.0001$] in the group at 2 g/L. Only 45 of a total of 120 gross lesions found in the liver of mice receiving dichloroacetic acid and mice in the control group were examined histologically. In the control group and the group at 1 g/L, a single hyperplastic nodule was confirmed in each group after 52 weeks of treatment. In the group at 2 g/L, 15 hyperplastic nodules were confirmed in 9 mice, 2 liver adenomas in 2 mice, and 6 hepatocellular carcinomas in 5 mice. [The Working Group noted that observations were restricted to the liver, histopathological examination was only carried out on a fraction of the gross lesions observed, and statistical analyses were limited.]

DeAngelo *et al.* (1991) conducted two experiments in male B6C3F$_1$ mice. In the first experiment, mice were given drinking-water containing dichloroacetic acid at a concentration of 0 (control), 0.05, 0.5, or 5 g/L of for 60–75 weeks. The controls received NaCl at 2 g/L. In the second experiment, mice were given drinking-water containing acetic acid at 1.5 g/L (control) or dichloroacetic acid at 3.5 g/L. Dichloroacetic acid in the drinking-water was neutralized with NaOH.

In both experiments, mice treated with dichloroacetic acid at 3.5 or 5 g/L were killed after 60 weeks of treatment; mice treated with lower doses in the first experiment were killed after 60 weeks (nine mice per group) with the remaining mice killed after 75 weeks. The data from all experiments were combined for reporting. Statistically significant increases ($P < 0.001$) in the prevalence and multiplicity of hepatocellular adenoma and carcinoma of the liver were observed in the group at 5 g/L. A statistically significant increase ($P < 0.001$) in prevalence and multiplicity was also observed in the group at 3.5 g/L group. [The Working Group noted that liver, kidney, testes and spleen were examined for gross lesions and histopathology, but results were presented only for the liver. The treatments were of short duration, which may have prevented the expression of carcinogenesis at the lower doses. The Working Group also noted the limited reporting of the study.]

Daniel *et al.* (1992) presented the results of two experiments in which male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at a concentration of 0.5 g/L, with mice in the control group being given distilled water. Dichloroacetic acid in drinking-water was neutralized with NaOH. In the first experiment, the initial number of mice in the control group and in the treated group was 23. In the control group, five mice were killed after 30 weeks and 60 weeks, and three died prematurely. Five mice were killed after 5 weeks and two died in the treated group, leaving ten survivors in the control group and sixteen in the treated group at termination of the study (104 weeks). In the second

experiment, there were 10 mice in the control group and in the treatment group. There were no interim kills, and while there were no premature deaths in the control group, there were two in the treated group. The data were combined for reporting. After 104 weeks, the prevalence of hepatocellular adenoma in surviving mice was 1 out of 20 in the control group, and 10 out of 24 ($P \leq 0.01$) in the group receiving dichloroacetic acid. Hepatocellular carcinoma was found in 2 out of 20 mice in the control group, and in 15 out of 24 ($P \leq 0.01$) in the group receiving dichloroacetic acid. [The Working Group noted that complete histopathological examinations were not performed, and that although selected organs (kidney, liver, testes and spleen) of survivors were examined, no data other than for liver were shown or discussed. The Working Group also noted the limited reporting, that no histopathology was reported for mice dying prematurely, and that the study was limited by the single dose and small number of mice.]

A group of 110 male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at 5 g/L for 76 weeks, while a control group of 50 male mice were given distilled water (Anna *et al.*, 1994). Dichloroacetic acid in the drinking-water was neutralized with NaOH. In the control group, 24 mice were killed after 76 weeks, while the remaining mice were killed after 96, 103, or 134 weeks. [Only the 24 controls that were killed at the same time as the treated mice were considered by the Working Group.] Only the liver was examined grossly and microscopically for pathology.

Hepatocellular adenoma was detected in 2 out of 24 mice in the control group, and 2 out of these 24 mice were found to have hepatocellular carcinoma. Of the mice receiving dichloroacetic acid, 83 out of 89 [$P < 0.0001$] had hepatocellular adenoma and 66 out of 89 [$P < 0.0001$] had hepatocellular carcinoma. [The Working Group noted that the study was limited to a single high dose in a large group of mice, but only a limited number of mice in the control group were killed at the same time as the treated mice. Consumption of drinking-water at this high dose was not discussed by the authors. Liver was the only tissue for which lesions were characterized histopathologically.]

Groups of female B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at 0 (control group, $n = 134$), 2.0 mM [0.259 g/L] ($n = 90$), 6.67 mM [0.86 g/L] ($n = 50$), or 20.0 mM [2.59 g/L] ($n = 40$) (Pereira, 1996). Mice were killed after 360 or 576 days of treatment. The drinking-water of mice in the control group was supplemented with NaCl at 20.0 mM [1.15 g/L] to control for the amount of NaOH that was required to neutralize dichloroacetic acid in the drinking-water of treated mice. An additional group of 34 mice underwent repeated dosing with dichloroacetic acid at 2.59 g/L for 24 days, followed by 48 days without treatment [intermittent treatment]. The authors stated that this schedule was designed to provide the same total dose as the group receiving continuous treatment with dichloroacetic acid at 0.86 g/L. At day 360, 40 mice in the control group, 40 mice at 0.259g/L, 20 mice from each of the groups at 0.86 g/L and 2.59 g/L, and 15 mice from the intermittent-treatment group were killed. The remaining mice were killed at day 576 (90 in the control group, 50 in the group at 0.259 g/L, 28 in the group at 0.86 g/L, 19 in the group at 2.59 g/L, and 34 in the intermittent-treatment group). The incidences of hepatocellular adenoma and carcinoma in the treatment groups and by duration of treatment is shown in Table 3.1. Statistically significant increases in the incidence of hepatocellular adenoma were observed in the group at 2.59 g/L at day 360 and at day 576. An increase in the incidence of hepatocellular carcinoma was observed in the group at 2.59 g/L after 576 days. The incidence of liver foci per mouse in the group receiving intermittent treatment was similar to that in the group dosed continuously at 0.86 g/L after 576 days, but the incidence of hepatocellular

adenoma in the intermittent-treatment group was only 3 out of 34 versus 7 out of 28 in mice dosed continuously at 0.86 g/L. One hepatocellular carcinoma was observed in the intermittent-treatment group and in the group dosed continuously at 0.86 g/L (groups receiving equivalent total doses). [The Working Group noted that this study focused on liver; no other tissues were examined histopathologically.]

In an experiment that was designed primarily for the purpose of characterizing Ha-*ras* mutations in tumours induced by dichloroacetic acid, the incidence of hepatocellular carcinoma was 1 out of 39, 1 out of 25, and 23 out of 25 [$P < 0.001$] in female B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 0, 0.5, or 3.5 g/L, respectively, for 104 weeks (Schroeder *et al.*, 1997). Mice in the control group were given 1.5% acetic acid. The incidence of hepatocellular adenoma was not reported. [The Working Group noted the limited reporting of this experiment, and that histopathological examination was restricted to the liver.]

DeAngelo *et al.* (1999) conducted a 2-year study with interim kills in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 0 (n = 88), 0.5 (n = 55), 1 (n = 71), 2 (n = 55), or 3.5 g/L (n = 46). Dichloroacetic acid in the drinking-water was neutralized with NaOH. Water consumption was significantly reduced by dichloroacetic acid at the two higher doses over the first year of the study, but increased considerably in these groups during the second year and exceeded that of the other groups. The increase in water consumption during the second year was also noted at the lowest dose (0.5 g/L). A total of 35 mice in the control group and 30 mice from each of the groups receiving dichloroacetic acid were killed after 26, 52, and 78 weeks of treatment. Unscheduled deaths were reported for three mice in the control group, one mouse at 0.5 g/L, nine mice at 1 g/L, eleven mice at 2 g/L, and eight mice at 3.5 g/L. Thus 50, 24, 32, 14, and 8 mice remained at terminal kill (at 100 weeks), respectively. The number of mice per group for which pathological examination of the liver was performed was 85, 55, 65, 51, and 41, respectively. Data were reported as tumour prevalence and also as mean number of tumours per mouse, since multiple tumours are characteristic in mice given dichloroacetic acid at concentrations greater than 2 g/L (mean number of hepatocellular carcinomas at 0, 2, 3.5 and 5 g/L, respectively, was 0.3, 1.3, 2.5, 2.9, after 79–100 weeks of treatment). Hepatocellular carcinomas began to appear after 26 weeks of treatment in mice at 3.5 g/L. The prevalence of hepatocellular carcinoma was statistically significantly increased after 79–100 weeks in mice at 1, 2, or 3.5 g/L. [The Working Group noted that this was a group of studies presented together in one report. It was limited by the number of mice studied per group, inconsistent reporting, and limited pathology examination of tumour sites other than the liver. Data were reported as percentage of mice with tumours, and it was not always apparent what the effective number of mice was at terminal kill.]

A study attempted to determine the extent to which dichloroacetic acid and trichloroacetic acid contributed to liver tumours induced by trichloroethylene (Bull *et al.*, 2002). The only organ examined was the liver. Among other treatments, the study included assessment of the tumorigenic effects of drinking-water containing dichloroacetic acid at three concentrations (0.1, 0.5, and 2 g/L) in male B6C3F$_1$ mice. Dichloroacetic acid in the drinking-water was neutralized with NaOH. Mice were killed after 52 or 87 weeks of treatment and the data reported as combined incidence of liver hyperplastic nodules, hepatocellular adenoma or carcinoma. Increases in the incidence of liver hyperplastic nodules, hepatocellular adenoma, or carcinoma (combined) were observed in some groups of mice treated with dichloroacetic acid. [The Working Group noted that the study was limited by the examination of the liver only, the

short duration of exposure, the small number of mice remaining at 87 weeks, the uncertainty of reporting lesion prevalence (i.e. random selection of gross lesions for histopathology examination), and the issue of lesion grouping.]

Dichloroacetic acid has been tested in genetically modified mouse strains: the Tg.AC hemizygous and p53 haploinsufficient strains (NTP, 2007; Kissling *et al.*, 2009). Drinking-water containing dichloroacetic acid at concentrations of 0.5, 1, and 2 g/L was given to males and females of both strains. The duration of the studies was 41 weeks. While there was no evidence for induction of liver tumours, there was an increase in the incidence of bronchioloalveolar adenoma in male Tg.AC hemizygous mice (control group, 1 out of 10; 0.5 g/L, 2 out of 10; 1 g/L, 7 out of 10 ($P < 0.01$); 2 g/L, 3 out of 10).

3.1.2 Skin application

Male and female Tg.AC mice received dichloroacetic acid at a dose of 0, 31.25, 125, or 500 mg/kg bw applied to the skin (NTP, 2007; Kissling *et al.*, 2009). After 39 weeks, there was a statistically significant increase ($P < 0.01$) in the incidence of skin papilloma at the highest dose in males (8 out of 10 versus 0 out of 10 in the control group) and females (6 out of 10 versus 0 out of 10 in the control group). [The Working Group noted the small number of mice and the short duration of treatment used in this study.]

3.2 Rat

Oral administration

The two publications reporting studies in rats given drinking-water containing dichloroacetic acid (Richmond *et al.*, 1995; DeAngelo *et al.*, 1996) appeared to contain much of the same data. Since there were some inconsistencies in reporting of the two studies, the Working Group prepared a table (Table 3.2) to clarify how the data overlapped.

As the data were more completely reported for some groups in DeAngelo *et al.* (1996), the Working Group preferentially placed these data into the table when there were small discrepancies in reporting. The Working Group recognized these inconsistencies, but did not believe they affected the utility of the data for the evaluation of the carcinogenicity of dichloroacetic acid.

In a study of phenotypical changes in liver lesions according to duration of treatment and lesion type, male F344 rats (age, 28 days) were given drinking-water containing dichloroacetic acid at a concentration of 0 (drinking-water containing NaCl at 2.0 g/L to control for NaOH added to neutralize dichloroacetic acid), 0.05, 0.5, or 2.4 g/L for 45, 60, or 100–104 weeks (Richmond *et al.*, 1995). Results were only reported for the liver. All surviving rats in the group at the highest dose were killed at 60 weeks. After 45 weeks of treatment, a single adenoma was noted in the group at the highest dose. After 60 weeks, no lesions were observed in rats at the two lower doses, but in rats in the group at 2.4 g/L, 19 out of 27 had hyperplastic nodules ($P < 0.05$), 7 out of 27 had hepatocellular adenomas (not statistically significant), and 1 out of 27 (not statistically significant) had hepatocellular carcinomas. At terminal kill, in the control group, 1 out of 23 rats had hepatocellular adenoma; at 0.05 g/L, 0 out of 26 rats had any lesion; and at 0.5 g/L, 3 out of 29 had hyperplastic nodules, 6 out of 29 had hepatocellular adenoma, and 3 out 29 had hepatocellular carcinoma. [The Working Group noted that the limitations of this study were that only the liver was examined by histopathology, rats that died during the course of the experiment were not examined by histopathology, rats at 2.4 g/L were killed at 60 weeks, and the terminal kill of rats in the control group was at 104 weeks while that of rats in groups receiving dichloroacetic acid was at 100 weeks.]

The DeAngelo *et al.* (1996) study repeated part of the data set from Richmond *et al.* (1995), but added a water control and a single dose of dichloroacetic acid (neutralized with NaOH) that was given to male F344 rats in drinking-water at initial concentrations of 0 or 2.5 g/L [Study 2, Table 3.2] beginning at age 28–30 days (DeAngelo *et al.*, 1996). [The Working Group noted that the dose was reduced from 2.5 g/L to 1.5 g/L at 8 weeks, and then to 1 g/L at 26 weeks, resulting in a time-weighted average of 1.6 g/L over the study duration.] Prevalence data were provided for the terminal kill at 103 weeks. In the 33 rats remaining at termination in the control group, prevalences were: hyperplastic nodules, 3%; hepatocellular adenoma, 0%; and hepatocellular carcinoma, 3%. In the group receiving dichloroacetic acid, prevalences at termination were: hyperplastic nodules, 3.6%; hepatocellular adenoma, 10.7%; and hepatocellular carcinoma, 21.4%. [The Working Group noted that rats from interim kills of both studies did not appear to have been examined for neoplastic lesions, but were used to investigate mechanistic questions.] A renal tubular adenoma was found in the group receiving dichloroacetic acid, while none were observed in the control group. [The Working Group noted that the incidence of this tumour in historical controls in F344 rats was 10 out of 1352 (0.7%) (Haseman *et al.*, 1998).]

The DeAngelo *et al.* (1996) study indicated that mononuclear cell leukaemia was observed at a prevalence of 24% in the control group receiving NaCl, 20% in the group receiving dichloroacetic acid at 0.05 g/L, and 43% in the group receiving dichloroacetic acid at 0.5 g/L in the Richmond *et al.* (1995) study. Neither study indicated the prevalence of mononuclear cell leukaemia at 2.4 g/L. In the study by DeAngelo *et al.* (1996), the prevalence of mononuclear cell leukaemia was 9% in the control group receiving water, and 11% in the group receiving dichloroacetic acid at 1.6 g/L. [The Working Group noted that both experiments had limited statistical power. Although the reporting of the study by DeAngelo *et al.* (1996) was limited, with no data for individual animals, it nevertheless contained two separate experiments both reporting a positive response in the liver.]

3.3 Co-administration with known carcinogens or other modifying factors

In the Herren-Freund *et al.* (1987) initiation–promotion study in male B6C3F$_1$ mice, cited above (see Section 3.1.1), mice were initiated with an intraperitonal injection of *N*-ethyl-*N*-nitrosourea (ENU) at 2.5 mg/kg bw on postnatal day 15. From postnatal day 28 and continuing for 61 weeks, the mice were given drinking-water containing dichloroacetic acid at 0 (control; NaCl, 2 g/L), 2 or 5 g/L. The study focused on liver tumorigenesis. In the control group of mice initiated with ENU and maintained on water containing NaCl, 1 out of 22 mice had liver [hepatocellular] adenoma, and 1 out of 22 had hepatocellular carcinoma. In the group of mice initiated with ENU and subsequently treated with dichloroacetic acid at 2 g/L, 22 out of 29 ($P < 0.01$) mice had liver adenoma, and 19 out of 29 ($P < 0.01$) had hepatocellular carcinoma. In mice initiated with ENU, but treated with dichloroacetic acid at 5 g/L, incidences of these lesions were 31 out of 32 ($P < 0.01$) and 25 out of 32 ($P < 0.01$), respectively.

An initiation–promotion study assessed dichloroacetic acid as a promoter in female B6C3F$_1$ mice (Pereira & Phelps, 1996). Mice were initiated with an intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) at 25 mg/kg bw on postnatal day 15. Treatment with dichloroacetic acid began at age 7 weeks and was continued for 52 weeks. Dichloroacetic acid was administered in the drinking-water at concentrations of 2.0 ($n = 9$), 6.67 ($n = 9$), and 20.0 ($n = 24$) mM [i.e. 0.259, 0.86, and 2.59 g/L].

The study focused on liver tumorigenesis and data were expressed as numbers of lesions per mouse and percentage of mice with the indicated lesion. A "recovery" group (n = 12) was given dichloroacetic acid at 20 mM [2.59 g/L] for 31 weeks, after which the treatment was suspended, and the mice were killed at experimental week 52. After 52 weeks, there were increases in the incidences of hepatocellular adenoma and carcinoma in groups of MNU-initiated mice treated with dichloroacetic acid at 2.0, 6.7, or 20 mM relative to MNU-initiated controls (n = 39). The percentages of mice with hepatocellular adenoma were 10% (control), 40%, 20%, and 19.2%; and the percentages of mice with hepatocellular carcinoma were 17.5% (control), 20%, 10%, and 73.1%, for increasing doses. The percentages of mice with hepatocellular adenoma and hepatocellular carcinoma in the recovery group were 46.2% and 15.4%, respectively. [The Working Group noted the limited reporting of the study and the small number of mice used.]

Another study from the same group (Pereira *et al.*, 1997) examined the ability of mixtures of dichloroacetic acid and trichloroacetic acid to promote MNU-initiated liver tumours in female B6C3F$_1$ mice. All mice were initiated with an intraperitonal injection of MNU at 25 mg/kg bw on postnatal day 15. Nine groups were given dichloroacetic acid or trichloroacetic acid alone, or combinations of dichloroacetic acid and trichloroacetic acid. An additional control group was treated with MNU only. Dichloroacetic acid and trichloroacetic acid in the drinking-water were neutralized with NaOH. Treatments with dichloroacetic acid and trichloroacetic acid started at age 6 weeks and continued for 44 weeks. Survival (number surviving out of initial number of animals) was: controls, 29 out of 30; dichloroacetic acid, 1 g/L, 17 out of 20; dichloroacetic acid, 2 g/L, 19 out of 20; dichloroacetic acid, 3.2 g/L, 29 out of 30; trichloroacetic acid, 1 g/L, 20 out of 20; trichloroacetic acid, 4 g/L, 29 out of 30; dichloroacetic acid (3.2 g/L) + trichloroacetic acid (1 g/L), 21 out of 25; dichloroacetic acid (2 g/L) + trichloroacetic acid (1 g/L), 42 out of 45; dichloroacetic acid (1 g/L) + trichloroacetic acid (1 g/L), 22 out of 25; and trichloroacetic acid (4 g/L) + dichloroacetic acid (2 g/L), 19 out of 20. Dichloroacetic acid produced a dose-dependent increase in the incidence of hepatocellular adenoma per mouse relative to the MNU-initiated controls (MNU only, 0.07; MNU + dichloroacetic acid at 1 g/L, 0.06; MNU + dichloroacetic acid at 2 g/L, 0.32; MNU + dichloroacetic acid at 3.2 g/L, 1.8 [$P < 0.05$]). [The conclusions regarding interactions between dichloroacetic acid and trichloroacetic acid were discussed without presenting the detailed data.] A fixed dose of TCA at 1 g/L statistically significantly enhanced the yield of total proliferative lesions (liver foci and hepatocellular adenomas, combined) observed with dichloroacetic acid at 1 g/L, but effects were less than additive with treatments with dichloroacetic acid at 2 or 3.2 g/L. [The Working Group noted that the doses given were somewhat higher than those used in cancer bioassays, and probably affected consumption of drinking-water. As a consequence, the true dose received by the mouse may not have been linearly related to the concentration of dichloroacetic acid in the drinking-water.]

A study examined the interactions of three tumour promoters (dichloroacetic acid, trichloroacetic acid and carbon tetrachloride) in male B6C3F$_1$ mice initiated with vinyl carbamate (Bull *et al.*, 2004). Vinyl carbamate was administered at a dose of 3 mg/kg bw [administration route not reported] at age 2 weeks. Groups of 10 mice were treated with different doses of the individual promoters or mixtures for 18, 24, 30, or 36 weeks (70 different experimental groups in total). Macroscopically observable liver lesions were all sectioned, but only a subsample was randomly examined microscopically for diagnosis of hyperplastic nodules, hepatocellular adenoma or hepatocellular carcinoma. No attempt was made to differentiate between these lesions

in the analysis of the data. In mice receiving dichloroacetic acid at a concentration of 0.1, 0.5 or 2 g/L, the number and the size of liver lesions was increased compared with mice treated with vinyl carbamate only. There were significant interactions between the three agents that both enhanced or inhibited the development of liver lesions. The interactions between lesion size and number were frequently reciprocal in direction. [The Working Group noted the complexity of the data set, that only a representative sampling was submitted for histopathological analysis as a check on the gross observations, and that the size of individual groups was small.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Toxicokinetic studies of dichloroacetic acid have been detailed extensively in Volume 84 of the *IARC Monographs* (IARC, 2004). Therefore, this information is summarized here:

Dichloroacetic acid is readily absorbed from the gut and widely distributed systemically in humans and rodents. Dichloroacetic acid is metabolized to glyoxylate via the enzyme glutathione *S*-transferase zeta 1 (GST-zeta1) in humans and rodents. Glyoxylate is converted via lactate dehydrogenase to oxalate, which is excreted in the urine (Fig. 4.1). Transamination of glyoxylate in peroxisomes can produce glycine, which can be incorporated into protein. In rats and humans, dichloroacetic acid has been shown to inhibit its own metabolism by inhibiting GST-zeta1, the key enzyme responsible for its metabolism (Fig. 4.2).

The inhibitory effect of dichloroacetic acid on its own metabolism has been further explored in more recent studies in humans and in rodents:

In a stable-isotope study by Schultz & Shangraw (2006), the authors tested the effect of pretreatment with dichloroacetic acid on the pharmacokinetics of later doses of dichloroacetic acid in eight male and eight female volunteers. In the absence of pretreatment with dichloroacetic acid at a dose of 0.02 μg/kg bw per day for 14 days, there were no sex differences in the pharmacokinetics of dichloroacetic acid. Only women were affected by pretreatment with dichloroacetic acid, showing an increased area under the curve of concentration–time (AUC) for plasma dichloroacetic acid and a decreased rate of clearance.

Toxicokinetic studies in rodents (Saghir & Schultz, 2002; Schultz *et al.*, 2002, 2004) showed that dichloroacetic acid, even at environmental concentrations (0.2 g/L in drinking-water), inhibits its own metabolism via inhibition of GST-zeta1, slowing down the elimination of dichloroacetic acid and leading to increased potential for carcinogenicity in rodents. In mice, the ability of dichloroacetic acid to inhibit its own metabolism is greatest in the young (Schultz *et al.*, 2002, 2004). In another study by Saghir & Schultz (2005), rats were studied for the effects of depletion of GST-zeta1 on the elimination of mixtures of di- and tri-halogenated acidic acids. Pre-treatment with dichloroacetic acid (to deplete GST-zeta1) increased the elimination of tri-halogenated acetic acids.

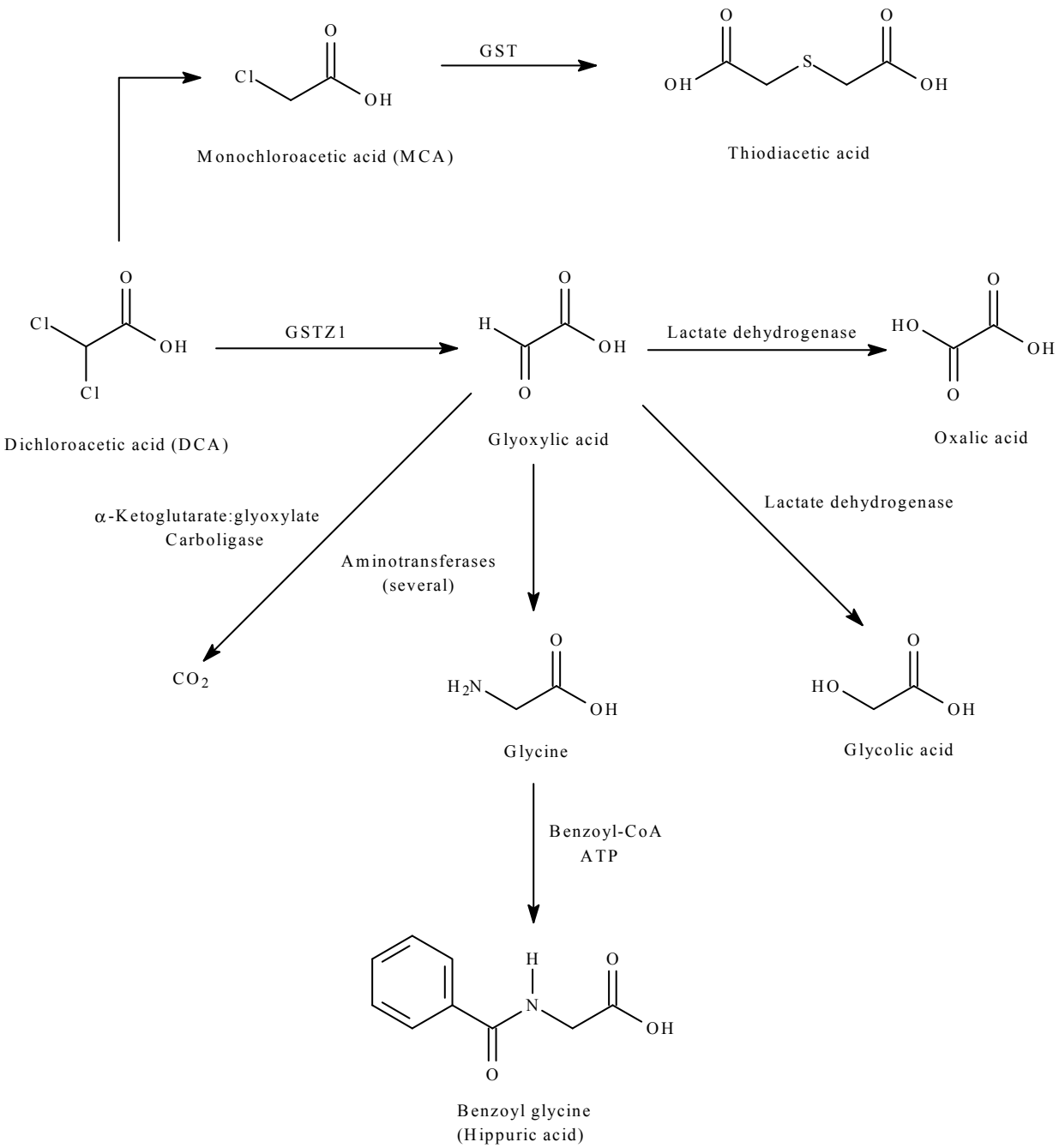
4.2 Genotoxicity and related effects

The results of tests for mutagenicity with dichloroacetic acid in mammalian systems are summarized in Table 4.1.

4.2.1 Humans

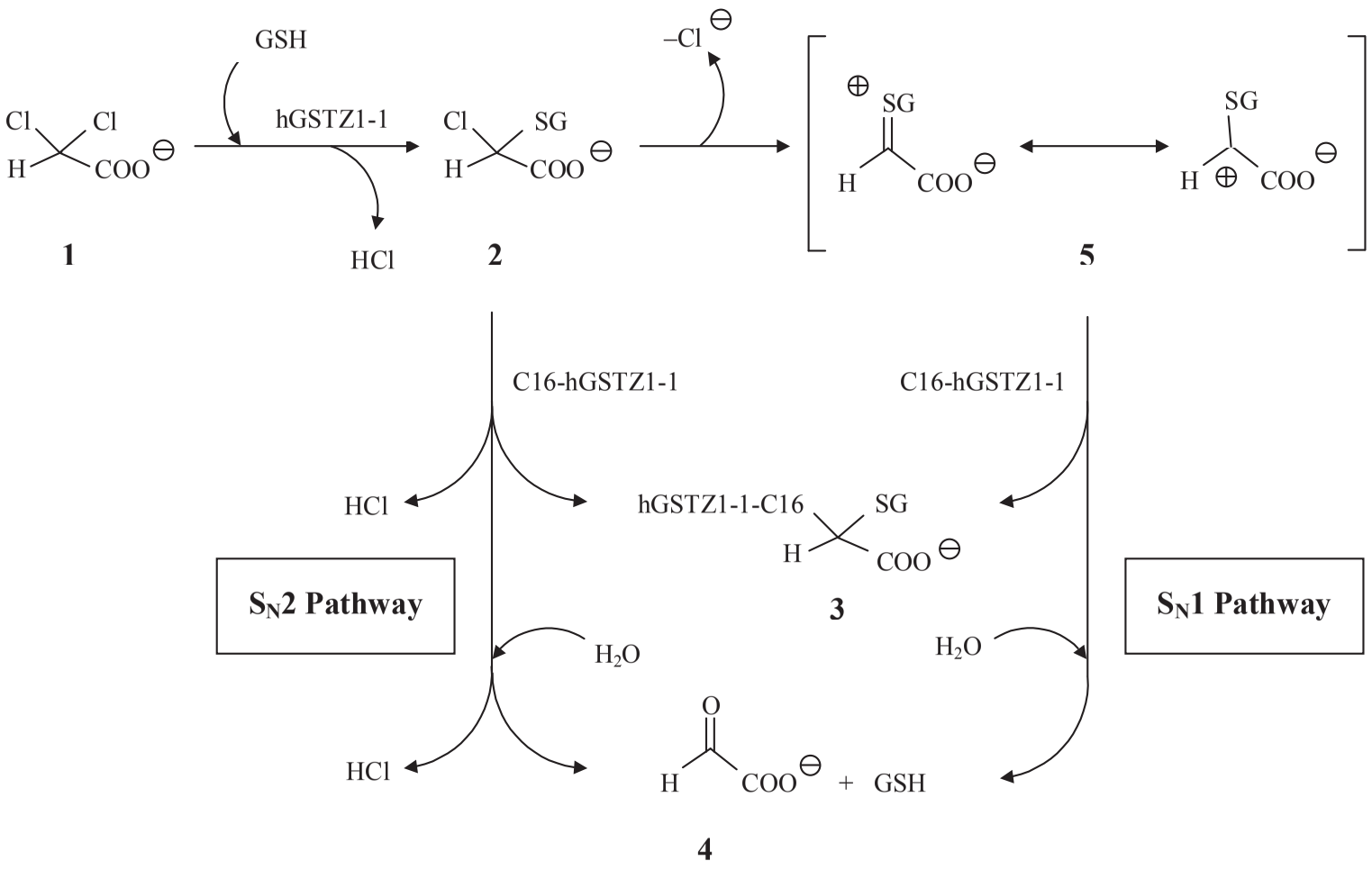
No DNA strand breaks were observed in human CCRF-CEM lymphoblastoid cells exposed to dichloroacetic acid *in vitro* (Chang *et al.*, 1992).

Fig. 4.1 Proposed metabolism of dichloroacetic acid



GST, glutathione-*S*-transferase; GSTZ1, GST-zeta1; P450, cytochrome P450
Prepared by the Working Group

Fig. 4.2 Mechanisms of human GSTZ1-catalysed biotransformation of dichloroacetic acid to glyoxylic acid and inactivation of GSTZ1 by dichloroacetic acid



1, dichloroacetic acid; 2, *S*-(α-chlorocarboxymethyl)glutathione; 3, human GSTZ1 covalently modified at cysteine-16; 4, glyoxylic acid; 5, sulfonium-carbocation intermediate
Adapted with permission from Anderson *et al.* (2002). Copyright (2002) American Chemical Society.

Table 4.1 Studies of genotoxicity with dichloroacetic acid in mammalian systems *in vitro* and *in vivo*

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
In vitro				
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells (single-cell gel electrophoresis assay)	3225	NT	–	Plewa *et al.* (2002)
DNA strand breaks, $B6C3F_1$ mouse hepatocytes	2580	NT	–	Chang *et al.* (1992)
DNA strand breaks, F344 rat hepatocytes	1290	NT	–	Chang *et al.* (1992)
Gene mutation, mouse lymphoma cell line L5178Y/$Tk^{+/-}$	5000	–	–	Fox *et al.* (1996a)
Gene mutation, mouse lymphoma cell line L5178Y/$Tk^{+/-}$–3.7.2C	400	NT	+	Harrington-Brock *et al.* (1998)
Gene mutation, Chinese hamster ovary cells, *HGPRT* [*Hprt*] gene mutation assay	129	NT	+	Zhang *et al.* (2010b)
Micronucleus formation, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	800	NT	–	Harrington-Brock *et al.* (1998)
Chromosomal aberrations, Chinese hamster ovary	5000	–	–	Fox *et al.* (1996a)
Chromosomal aberrations, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	600	NT	+	Harrington-Brock *et al.* (1998)
Aneuploidy, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	800	NT	–	Harrington-Brock *et al.* (1998)
DNA strand breaks, human CCRF-CEM lymphoblastoid cells	1290	NT	–	Chang *et al.* (1992)
In vivo				
DNA strand breaks, male $B6C3F_1$ mouse liver	13, oral, × 1	NT	+	Nelson & Bull (1988)
DNA strand breaks, male $B6C3F_1$ mouse liver	10, oral, × 1	NT	+	Nelson *et al.* (1989)
DNA strand breaks, male $B6C3F_1$ mouse liver	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse splenocytes	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse epithelial cells from stomach and duodenum	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse liver	5000, dw, × 7–14 days	NT	–	Chang *et al.* (1992)
DNA strand breaks, alkali-labile sites, cross linking, male $B6C3F_1$ mouse blood leukocytes (single-cell gel electrophoresis assay)	3500, dw, × 28 days	NT	+	Fuscoe *et al.* (1996)
DNA strand breaks, male Sprague-Dawley rat liver	30, oral, × 1	NT	+	Nelson & Bull (1988)
DNA strand breaks, male F344 rat liver	645, oral, × 1	NT	–	Chang *et al.* (1992)

Table 4.1 (continued)

Test system/end-point	Dosea (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
DNA strand breaks, male F344 rat liver	2000, dw, × 30 wk	NT	–	Chang *et al.* (1992)
Gene mutation, lacI transgenic male B6C3F$_1$ mouse liver assay	1000, dw, × 60 wk	NT	+	Leavitt *et al.* (1997)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 9 days	NT	+	Fuscoe *et al.* (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 28 days	NT	–	Fuscoe *et al.* (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 10 wk	NT	+	Fuscoe *et al.* (1996)
Micronucleus formation, male and female Crl:CD (Sprague-Dawley) BR rat bone-marrow erythrocytes	1100, i.v., × 3	NT	–	Fox *et al.* (1996a)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes	80 days	NT	–	Giller *et al.* (1997)

[a] Doses are in µg/mL for tests *in vitro*; mg/kg bw for tests *in vivo*, unless specified.

+, positive; –, negative; dw, drinking-water (in mg/L); HID, highest ineffective dose; i.v., intravenous injection; LED, lowest effective dose; NT, not tested; wk, week.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

Mutation frequencies were studied in male transgenic B6C3F$_1$ mice harbouring the bacterial *lacI* gene and given drinking-water containing dichloroacetic acid at 1.0 g/L or 3.5 g/L (Leavitt *et al.*, 1997). No statistically significant differences in mutation frequency were observed after 4 or 10 weeks of treatment at either dose when compared with controls. However, at 60 weeks, mice treated with dichloroacetic acid at 1.0 g/L showed a slight increase (1.3-fold) in mutation frequency compared with controls, and mice treated with dichloroacetic acid at 3.5 g/L showed an increase of 2.3-fold. Mutational spectrum analysis revealed that ~33% had G:C to A:T transitions and 21% had G:C to T:A transversions; this mutation spectrum was different to that seen in the untreated mice, indicating that the mutations were probably induced by treatment with dichloroacetic acid.

Harrington-Brock *et al.* (1998) evaluated dichloroacetic acid for mutagenic activity in L5178Y/*Tk*$^{+/-}$–3.7.2C mouse lymphoma cells. A dose-related increase in mutation frequency (and cytotoxicity) was observed at concentrations of 400–800 µg/mL. Most mutagenic activity of dichloroacetic acid at the *Tk* locus was due to the production of small-colony *Tk* mutants (indicating chromosomal mutations). There was no effect of pH on the induction of mutants.

Zhang *et al.* (2010a) tested the cytotoxic and genotoxic effects of dichloroacetic acid at 0, 200, 1000, 5000 or 10 000 µM [0, 129, 645 and 1290 µg/mL]) in a microplate-based test for cytotoxicity and an assay for HGPRT [*Hprt*] gene mutation with Chinese hamster ovary K1 cells, respectively. Two parameters were used to indicate long-term cytotoxicity: the lowest concentration at which cytotoxicity was apparent, and the percentage C1/2 value (the concentration at which cell density was reduced to 50% of values for negative controls). The lowest concentration at which dichloroacetic acid caused cytotoxicity was 2.87×10^{-3} M [370 µg/mL]. A statistically significant increase in the frequency of HGPRT mutation was observed at a concentration of 1000 µM [129 µg/mL].

(ii) Chromosomal aberration

Harrington-Brock *et al.* (1998) evaluated dichloroacetic acid for its potential to induce chromosomal aberration in mouse lymphoma cells treated with dichloroacetic acid at 0, 600, or 800 µg/mL). Results were clearly positive at both concentrations tested. However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to dichloroacetic acid (Fox *et al.*, 1996a).

(iii) Micronucleus formation

Fuscoe *et al.* (1996) investigated genotoxic potential *in vivo* in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid (pH-adjusted exposures, 0.5, 1, 2 and 3.5 g/L; available *ad libitum*, for up to 31 weeks). At the highest exposure tested, a statistically significant increase in the frequency of micronucleated erythrocytes was observed after exposure to dichloroacetic acid for 9 days, but not against a higher background at 28 days. A small but statistically significant increase was also observed after exposure for 10 weeks at the highest dose of dichloroacetic acid tested (3.5 g/L). The results of the alkaline single-cell gel electrophoresis (comet) assay are discussed below.

No statistically significant increase in micronucleus formation was observed in mouse lymphoma cells treated with dichloroacetic acid at 0, 600, or 800 µg/mL (Harrington-Brock *et al.*, 1998).

(iv) DNA damage

Fuscoe *et al.* (1996) also investigated genotoxic potential *in vivo* in bone marrow and blood leukocytes of male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid for up

to 31 weeks. DNA crosslinking was observed in blood leukocytes of mice exposed to dichloroacetic acid at 3.5 g/L for 28 days.

Nelson & Bull (1988) and Nelson *et al.* (1989) reported positive results for DNA unwinding with dichloroacetic acid, with Nelson *et al.* (1989) reporting the same response with dichloroacetic acid at 10 and 500 mg/kg bw in mice. Chang *et al.* (1992) conducted studies of DNA damage *in vitro* and *in vivo*, finding that primary rat (F344) hepatocytes and primary mouse hepatocytes treated with dichloroacetic acid for 4 hours did not exhibit DNA single-strand breaks as detected by the alkaline DNA unwinding assay. Similarly, analysis of DNA single-strand breaks in mice killed 1 hour after a single dose of dichloroacetic acid at 1, 5, or 10 mM/kg bw [129, 645, 1290 mg/kg bw] suggested that dichloroacetic acid did not cause DNA damage. There was no detectable DNA damage in F344 rats killed 4 hours after a single gavage dose of dichloroacetic acid (1–10 mM/kg bw [129–1290 mg/kg bw]).

(v) Mutational analyses of tumours

Anna *et al.* (1994) exposed male B6C3F$_1$ mice to drinking-water containing dichloroacetic acid at a concentration of 0 (50 animals) or 5 g/L (110 animals; about 900 mg/kg bw per day), 5 days per week, for 76 weeks. Dichloroacetic acid increased the incidence of hepatic adenoma (93% of exposed mice versus 8% of control mice had at least one adenoma), and hepatocarcinoma (74% of exposed mice versus 8% of control mice had at least one carcinoma). The frequency of mutation at H-*ras* codon 61 did not differ among dichloroacetic acid-induced and spontaneous hepatocellular tumours. However, significant changes were seen in the mutation spectra of H-*ras* [*Hras*] codon 61 after exposure to dichloroacetic acid. In the spontaneous tumours from the controls (study controls plus historical controls), the CAA of codon 61 became AAA in 58% of the tumours, CGA in 27% and CTA in 14%. In the dichloroacetic acid-exposed mice, H-*ras* codon 61 changes were AAA in 28%, CGA in 35% and CTA in 38%.

In a study by Ferreira-Gonzalez *et al.* (1995), male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at a concentration of 1.0 or 3.5 g/L (180 or 630 mg/kg bw per day) for 104 weeks. The incidence of liver carcinoma was 19%, 70.6% and 100% in the control group, and in the groups at 180 mg/kg bw per day and 630 mg/kg bw per day, respectively. DNA samples were examined from 32 spontaneous liver tumours from the control group, 13 tumours from the group at 180 mg/kg bw per day, and 33 tumours from the group at 630 mg/kg bw per day. Similar frequencies of mutation at H-*ras* proto-oncogene exon 2 were found in all three groups (spontaneous tumours, 58%; 180 mg/kg bw per day, 48%; and 630 mg/kg bw per day, 50%). Mutation frequencies in exons 1 and 3 were minimal. Comparative sequence analysis of exon 2 mutations in spontaneous and dichloroacetic acid-induced tumours revealed a substantial shift in the spectrum of base changes in codon 61. In spontaneous tumours, changes in codon 61 from CAA to AAA in 80% and CAA to CGA in 20% of the examined tumours were revealed, while no conversion of CAA to CTA was observed. In contrast, the frequency of conversion of CAA to AAA was 16% and 21% at doses of 180 and 630 mg/kg bw per day, respectively. Conversion of CAA to CGA was noted in 50% of the tumours from mice treated with dichloroacetic acid at 180 or 630 mg/kg bw per day, and conversion of CAA to CTA was observed in 34% and 29% in these two groups, respectively. Thus, although dichloroacetic acid-induced and spontaneous tumours involved similar levels of H-*ras* mutation, the mechanisms of tumour induction may be different. Differences in codon 61 mutation spectra between spontaneous and dichloroacetic acid-induced tumours in this study are similar to those reported in the study by Anna *et al.* (1994), in which there was also a lower number of CAA

Table 4.2 Studies of genotoxicity with dichloroacetic acid in bacterial systems

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
λ Prophage induction, *Escherichia coli* WP2s	2500	+	–	DeMarini *et al.* (1994)
SOS chromotest, *E. coli* PQ37	500	–	(+)	Giller *et al.* (1997)
Salmonella typhimurium, DNA repair-deficient strains TS24, TA2322, TA1950	31 000	–	–	Waskell (1978)
S. typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	NR	–	–	Herbert *et al.* (1980)
S. typhimurium TA100, reverse mutation	50	+	+	DeMarini *et al.* (1994)
S. typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	5000	–	–	Fox *et al.* (1996a)
S. typhimurium TA100, reverse mutation, liquid medium	100	+	+	Giller *et al.* (1997)
S. typhimurium RSJ100, reverse mutation	1935	–	+	Kargalioglu *et al.* (2002)
S. typhimurium TA104, reverse mutation, microsuspension	150 µg/plate	–	–	Nelson *et al.* (2001)
S. typhimurium TA98, reverse mutation	10 µg/plate	(+)	–	Herbert *et al.* (1980)
S. typhimurium TA98, reverse mutation	5160	–	+	Kargalioglu *et al.* (2002)
S. typhimurium TA100, reverse mutation	1935	+	+	Kargalioglu *et al.* (2002)
E. coli WP2*uvrA*, reverse mutation	5000	–	–	Fox *et al.* (1996a)

[a] Doses are in µg/mL for tests *in vitro*, unless specified.
+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NR, not reported

to AAA conversions and a higher number of CAA to CTA conversions in the dichloroacetic acid-induced tumours than in the spontaneous tumours.

Schroeder *et al.* (1997) examined dichloroacetic acid-induced tumours in female B6C3F$_1$ mice for mutations in H-*ras* codon 61. There was an H-*ras* mutation in only one of 22 tumours, revealing a CAA to CTA conversion.

(b) Bacterial and fungal systems: gene mutation

Studies to evaluate the mutagenicity of dichloroacetic acid in various strains of *S. typhimurium* and *E. coli* (Waskell, 1978; Herbert *et al.*, 1980; DeMarini *et al.*, 1994; Fox *et al.*, 1996a; Giller *et al.*, 1997; Nelson *et al.*, 2001; Kargalioglu *et al.*, 2002) are summarized in Table 4.2. Dichloroacetic acid was mutagenic in three strains of *S. typhimurium*: strain TA100 in three out of five studies, strain RSJ100 in a single study, and strain TA98 in two out of three studies. Dichloroacetic acid failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2*uvrA*. In one study, dichloroacetic acid caused a weak induction of SOS repair in *E. coli* strain PQ37 (Giller *et al.*, 1997).

4.3 Non-genotoxic mechanisms of carcinogenesis

4.3.1 Liver

The available evidence for non-genotoxic mechanisms for the induction by dichloroacetic acid of liver tumours in rodents (mouse) comprises the following: (a) epigenetic effects (especially DNA hypomethylation); (b) cytotoxicity and oxidative stress; (c) alteration of

proliferation and apoptosis, and clonal expansion; (d) PPARα activation; and (e) disruption of gap-junctional communication. Evidence supporting each of these non-genotoxic mechanisms from studies in humans and experimental animals is presented below.

(a) Epigenetic effects

Epigenetic events that have been studied primarily include studies of changes in DNA methylation, both of total DNA and of particular genes. Expression of the affected genes, and activity of DNA methyltransferases, has also been investigated.

(i) Humans

No dichloroacetic acid-specific data regarding alteration in DNA methylation from studies in humans were available to the Working Group.

(ii) Experimental systems

Hypomethylation of DNA may be related to the carcinogenicity of trichloroacetic acid and dichloroacetic acid in mice.

In female B6C3F_1 mice that received an intraperitoneal injection of MNU and were then given drinking-water containing trichloroacetic acid or dichloroacetic acid, DNA methylation in the resulting hepatocellular adenomas and carcinomas was about half that observed in non-tumour tissue from the same animal or from animals given only MNU (Tao *et al.*, 1998). Exposure of female B6C3F_1 mice to drinking-water containing trichloroacetic acid or dichloroacetic acid for 11 days also decreased total liver DNA methylation by 60% (Tao *et al.*, 1998). The same investigators (Tao *et al.*, 2004) also demonstrated hypomethylation of a region of the *Igf2* gene in liver and tumours from mice initiated with MNU and subsequently exposed to trichloroacetic acid or dichloroacetic acid. An association between hypomethylation and cell proliferation in liver of mice exposed to trichloroacetic acid or dichloroacetic acid was demonstrated by Ge *et al.* (2001). Hypomethylation of the internal cytosine of CCGG sites in the promoter region of the *Myc* gene began between 48 and 72 hours from the initiation of trichloroacetic acid or dichloroacetic acid exposure and continued to 96 hours. Pereira *et al.* (2001) investigated the effect of dichloroacetic acid treatment on hypomethylation and expression of the *Myc* gene and the promotion of liver tumours, in combination with chloroform. In a study by Pereira *et al.* (2001), female B6C3F_1 mice (age, 7–8 weeks) were given drinking-water containing chloroform at a concentration of 400, 800, or 1600 mg/L for 17 days. On the last 5 days of treatment, the mice were also given dichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Dichloroacetic acid decreased methylation and increased gene expression of *Myc* to a greater degree than did chloroform. Chloroform at doses greater than 800 mg/kg bw per day, co-administered with dichloroacetic acid, significantly reduced the ability of dichloroacetic acid to increase gene expression.

In a separate study, Pereira *et al.* (2004) gave female B6C3F_1 mice drinking-water containing dichloroacetic acid at a concentration of 3.2 g/L for 8 or 44 weeks. Dietary exposure to methionine (4 or 8 g/kg bw) abrogated DNA hypomethylation, reduced glycogen accumulation by 25% and was without effect on the increased liver/body weight ratio or peroxisome proliferation. Tumour multiplicity was decreased by methionine. The multiplicity of foci of altered hepatocytes was increased by methionine at the lower dose, and decreased by methionine at the higher dose, consistent with a slowing of progression of foci to tumours.

(b) Cytotoxicity and oxidative stress

(i) Humans

No studies on liver toxicity or oxidative stress in humans exposed to dichloroacetic acid were available to the Working Group.

(ii) Experimental systems

Histological examination of liver in most studies found little or no evidence of damage or of overt cytotoxicity.

Austin *et al.* (1996) investigated the potential for dichloroacetic acid to increase intercellular lipid peroxidation and the oxidation of DNA. Male B6C3F$_1$ mice were treated with a single oral dose of dichloroacetic acid (0, 30, 100, or 300 mg/kg bw). Nuclear DNA was extracted at various times to assess increases in relative guanosine hydroxylation. A statistically significant increase was seen in the group dosed at 300 mg/kg bw from 4 to 6 hours after dosing, but returned to near control levels at 8 hours after dosing. The level of hydroxylation appeared to be related to the ability to induce thiobarbituric acid-relative substances (TBARS), which is an indicator of lipid peroxidation. Statistically significant increases in lipid peroxidation have also been shown in cultured primary rat and mouse hepatocytes following exposure to dichloroacetic acid at concentrations as low as 0.5 mM [64.5 µg/mL] (in mice) and 1.0 mM [129 µg/mL] (in rats) (Everhart *et al.*, 1998).

(c) Alteration of cell proliferation and apoptosis, and clonal expansion

(i) Humans

No studies providing evidence of alteration of cell proliferation and apoptosis, or clonal expansion, after exposure to dichloroacetic acid in humans were available to the Working Group.

(ii) Experimental systems

Carter *et al.* (1995) gave male B6C3F$_1$ mice drinking-water containing dichloroacetic acid at 0, 0.5, or 5 g/L (0, 95, or 440 mg/kg bw per day, respectively) for up to 30 days. Significant, dose-related increases in absolute and relative (to total body weight) liver weights were seen at each 5-day interval. These trends increased with the length of exposure. Reduced thymidine incorporation (labelling index) and inhibition of mitosis was seen. Differences from the control group were statistically significant at 20 and 25 days, but not at 30 days. In mice in both treatment groups, hepatocytes had enlarged nuclei, consistent with polyploidy, and exhibited glycogen accumulation.

Tsai & DeAngelo (1996) examined responsiveness to growth factors in hepatocytes isolated from male B6C3F$_1$ mice given dichloroacetic acid. Inhibition of basal DNA synthesis was noted in cells isolated from mice exposed to dichloroacetic acid for 30, 60, or 90 days. However, this inhibition was reversed when cells from dichloroacetic acid-treated mice were treated in culture with growth factors.

Stauber *et al.* (1998) demonstrated that dichloroacetic acid increases cell proliferation of c-Jun-positive hepatocytes *in vitro*. Statistically significantly increased colony formation (no cytotoxicity) was seen in hepatocytes isolated from neonatal mice exposed to drinking-water containing dichloroacetic acid at 0.5 g/L. Colonies induced by dichloroacetic acid were positive for c-Jun, as were liver tumours induced in mice exposed *in vivo* (Stauber & Bull, 1997).

Male and female B6C3F$_1$ mice (age, 5 weeks) were given drinking-water containing dichloroacetic acid at 3.2 g/L, either alone, or together with chloroform at a concentration of 800 or 1600 mg/L (Pereira *et al.*, 2001). Before exposure to dichloroacetic acid, the mice were initiated with a single intraperitoneal dose of MNU at 300 mg/kg bw at age 15 days. The mice were killed at age 36 weeks. Greater numbers of hepatic foci were observed in dichloroacetic acid-treated animals (females more than in males). The tumour response was greater in males than in females. Chloroform in conjunction with dichloroacetic acid at both doses drastically reduced the incidence of adenoma and adenocarcinoma.

Snyder *et al.* (1995) examined the role of apoptosis (programmed cell death) suppression as a contributing factor to hepatocarcinogenicity induced by dichloroacetic acid. Regression

analysis revealed a statistically significant trend towards decreased apoptosis as the dose and duration of exposure increased. The lowest dose, 0.5 g/L, significantly ($P < 0.05$) decreased apoptosis at the earliest time-point (5 days) and also at days 15, 25, and 30. For the group at the highest dose, apoptosis was statistically significantly depressed when compared with controls for all time-points.

Walgren *et al.* (2005) demonstrated that in cultured hepatocytes from male Long-Evans rats, treatment with dichloroacetic acid at 0.01–1.0 mM [1.3–129 μg/mL] for 10–40 hours did not alter the incorporation of [^{3}H]thymidine. However, dichloroacetic acid synergistically enhanced proliferation induced by epidermal growth factor. Additionally, dichloroacetic acid significantly reduced the normal background cell loss, suggesting an inhibition of apoptosis.

In the study by Ge *et al.* (2001) discussed above, an increase in DNA replication (evidenced by increased proliferating cell nuclear antigen labelling index and mitotic labelling index) was observed 72 hours and 96 hours after the first daily gavage dose of either trichloroacetic acid or dichloroacetic acid.

A small initial increase in cell division has been reported in normal liver after treatment with dichloroacetic acid. In all cases, however, cell replication rates in normal liver decreased with long-term treatment (Stauber & Bull, 1997; Bull, 2000). Decreased rates of cell replication were paralleled by decreased rates of spontaneous apoptosis (Snyder *et al.*, 1995).

However, dichloroacetic acid increased cell replication rates in a dose-dependent statistically significant manner in altered hepatic foci and small tumours when long-term treatment was followed by continued administration of dichloroacetic acid at different doses (Stauber & Bull, 1997). These studies indicate that dichloroacetic acid has selective effects on cell replication. Another experiment, conducted *in vivo*, demonstrated that the growth of tumours, as measured by magnetic resonance imaging, slowed when treatment with dichloroacetic acid was suspended (Miller *et al.*, 2000). This effect was also demonstrated as increased growth of colonies when isolated anchorage-independent hepatocytes from B6C3F$_1$ mice were treated with dichloroacetic acid (Stauber *et al.*, 1998).

(d) Activation of peroxisome proliferator-activated receptor-α

The sections below review the evidence that dichloroacetic acid induces activation of peroxisome proliferator-activated receptor-α (PPARα).

(i) Humans

No studies were identified that addressed the dichloroacetic acid-induced activation of a PPARα mechanism in human liver. However, studies of transactivation *in vitro* have shown that human (and murine) versions of PPARα are activated by dichloroacetic acid (and trichloroacetic acid), while trichloroethylene is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Walgren *et al.* (2000a) showed that dichloroacetic acid did not increase oxidation of palmitoyl-coenzyme A in primary human hepatocyte cultures; the effects of dichloroacetic acid on cell proliferation in this study are addressed below.

(ii) Experimental systems

Direct evidence for activation of PPARα come from several studies of transactivation *in vitro*, which have shown that murine versions of PPARα are activated by both trichloroacetic acid and dichloroacetic acid, while tetrachloroethylene is relatively inactive. Activation of murine PPARα by chlorinated hydrocarbons in COS1 cells containing a murine PPARα reporter plasmid was tested (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Treatment with trichloroacetic acid and dichloroacetic acid for 24 hours resulted in activation of the reporter plasmid at concentrations of 1 mM [129 μg/mL]

and 5 mM [645 µg/mL] with a statistically significant concentration–response relationship. Walgren *et al.* (2000b) tested transactivation of murine PPARα using a reporter plasmid in HL8.5 cells cotransfected with mouse retinoic acid receptor α. Dichloroacetic acid caused an increase in activity (4 mM [516 µg/mL]), although the effect was not statistically significant.

Several studies have shown indirect evidence for PPARα activation by demonstrating that dichloroacetic acid is a peroxisome proliferator in mice and rats (Mather *et al.*, 1990; DeAngelo *et al.*, 1999). Induction of peroxisome proliferation has been associated repeatedly with long-term toxicity and carcinogenicity of dichloroacetic acid in the liver (DeAngelo *et al.*, 1989). Dichloroacetic acid induces peroxisome proliferation in the livers of both mice and rats, as indicated by increased activities of palmitoyl-coenzyme A oxidase and carnitine acetyl transferase, the appearance of a peroxisome proliferation-associated protein and increased volume density of peroxisomes after exposure to dichloroacetic acid for 14 days. With further treatment, peroxisome markers returned to control levels after 45–60 weeks (DeAngelo *et al.*, 1999).

Two reports suggest that the concentrations of dichloroacetic acid and trichloroacetic acid that result in peroxisome proliferation or PPARα activation are much higher than those that induce liver tumours (Bull, 2004; Bull *et al.*, 2004).

Indirect evidence for activation of PPARα comes from studies using enzyme markers. Laughter *et al.* (2004) reported that the induction of acyl-coenzyme A oxidase, palmitoyl-coenzyme A oxidase, and CYP4A by trichloroacetic acid and dichloroacetic acid was substantially diminished in PPARα-null mice.

Walgren *et al.* (2000a) found that both trichloroacetic acid and dichloroacetic acid (2 mM [258 µg/mL]), a concentration that was not cytotoxic) activated palmitoyl-coenzyme A oxidation in rat (LEH) and mouse ($B6C3F_1$) primary hepatocytes, and dichloroacetic acid was shown to be about twice as potent as trichloroacetic acid.

(e) Inhibition of intracellular communication

(i) Humans

No dichloroacetic acid-specific data on inhibition of gap-junctional communication in studies in humans were available to the Working Group.

(ii) Experimental systems

Benane *et al.* (1996) demonstrated an effect of dichloroacetic acid on gap-junctional communication in clone 9 cell cultures (normal rat hepatocytes). The shortest and lowest exposure to statistically significantly reduce dye transfer was 10 mM [1290 µg/mL] for 6 hours. The ability of dichloroacetic acid to disrupt communication was weaker (~5.8-fold) than other chlorinated compounds tested, including tetrachloroethylene, trichloroacetic acid, trichloroethanol, and chloral hydrate.

(f) Comparative analyses of liver tumours induced by dichloroacetic acid or trichloroacetic acid

Biomarkers of cell growth, differentiation, and metabolism in proliferative hepatocellular lesions promoted by dichloroacetic acid were investigated by Latendresse & Pereira (1997) to further determine differences between dichloroacetic acid and trichloroacetic acid in terms of mechanisms of carcinogenesis. Female $B6C3F_1$ mice were initiated with an intraperitoneal injection of MNU at age 15 days and treated with drinking-water containing dichloroacetic acid. More than half of tumours from dichloroacetic acid-treated mice expressed transforming growth factor-α, c-myc, CYP2E1, CYP4A1, and GST-π in more than 50% of cells. A different profile of histochemical markers was induced by trichloroacetic acid, supporting different mechanisms for these two haloacetic acids. Bull *et al.*

(2002) similarly observed that dichloroacetic acid-induced tumours often expressed c-jun, while trichloroacetic acid-induced tumours were uniformly lacking in c-jun expression.

Pereira (1996) studied the characteristics of lesions in female B6C3F$_1$ mice to evaluate differences between dichloroacetic acid and trichloroacetic acid. Foci of altered hepatocytes and tumours induced by dichloroacetic acid were reported to be predominantly eosinophilic. Foci induced by trichloroacetic acid were equally distributed between basophilic and eosinophilic, while hepatic tumours induced by trichloro-acetic acid were predominantly basophilic, including all observed hepatocellular carcinomas (n = 11), and lacked GST-π expression. These characteristics for trichloroacetic acid-induced tumours were also reported by Pereira *et al.* (1997). Tumours in control mice were also mostly basophilic, or mixed basophilic and eosinophilic. Since comparable numbers of the foci of trichloroacetic acid-treated mice were basophilic and eosinophilic, it suggested that the basophilic foci induced by treatment with trichloroacetic acid may be more likely to progress to tumours. Based on differences in the shape of the dose–response curves and staining characteristics of tumours, Pereira (1996) concluded that dichloroacetic acid and trichloroacetic acid act through different mechanisms. The characteristics of the foci and tumours induced by trichloroacetic acid were described as being consistent with the predominant basophilic staining observed in tumours induced by peroxisome proliferators, suggesting that this pathway might be involved in the observed hepatocarcinogenicity of trichloroacetic acid.

Similarly, Bull *et al.* (1990) also presented evidence that the mechanisms of carcinogenesis for trichloroacetic acid and dichloroacetic acid are different. In this study, dichloroacetic acid-treated mice showed marked cytomegaly, substantial glycogen accumulation, and necrosis of the liver. The dose–response relationship between proliferative liver lesions and dichloroacetic acid treatment followed a "hockey stick" pattern. In contrast, these effects were either minimal or absent in trichloroacetic acid-treated mice, and accumulation of lipofuscin (an indication of lipid peroxidation) was observed only in trichloroacetic acid-treated mice. In contrast to the dose–response relationship for dichloroacetic acid, the dose–response curve for trichloroacetic acid and proliferative lesions was linear.

4.3.2 Kidney

(a) Humans

No dichloroacetic acid-specific data from studies in humans were available to the Working Group.

(b) Experimental animals

Few studies have examined any effects, or potential mechanisms, of dichloroacetic acid in the kidney.

Mather *et al.* (1990) evaluated toxicological effects in groups of 10 male Sprague-Dawley rats given drinking-water containing dichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm [5000 µg/mL] for 90 days. At 500 and 5000 ppm [500 and 5000 µg/mL], relative kidney weights were statistically significantly ($P \leq 0.05$) increased when compared with controls. Changes in kidney histopathology (diffuse degeneration of the tubular epithelium and cells of the glomeruli) were observed in the group at 5000 ppm [5000 µg/mL].

In a follow-up study, Tao *et al.* (2005) treated B6C3F$_1$ mice with drinking-water containing dichloroacetic acid (3.2 g/L) for 7 days concurrently. In male, but not female mouse kidney, dichloroacetic acid decreased the methylation of DNA and the *c-myc* gene. To determine whether methionine co-administration would also prevent hypomethylation in the kidneys, male mice were fed diet containing methionine concurrently with drinking-water containing

dichloroacetic acid. Methionine prevented dichloroacetic acid-induced hypomethylation of the *c-myc* gene.

4.3.3 Other target tissues

Few studies have examined the effects of dichloroacetic acid in other target tissues, or their possible mechanisms. Madhok *et al.* (2010) demonstrated that dichloroacetic acid (20 mM [2580 µg/mL]) induces apoptosis and cell-cycle arrest in cancerous and non-cancerous cells of colorectal origin. Cancerous cells were more sensitive than non-cancerous cells to the growth-inhibitory effects of dichloroacetic acid.

4.4 Susceptibility data

4.4.1 Inter-individual variability

There were no data demonstrating that any particular human subpopulation is especially susceptible to the toxic effects of dichloroacetic acid. It has been suggested, however, that potential susceptibility may be related to polymorphisms in enzymes that are key to the metabolism of dichloroacetic acid.

For instance, the enzyme GST-zeta1 (*GSTZ1*) (Board *et al.*, 2001) is critical for dichloroacetic acid metabolism; it has been demonstrated that *Gstz1*-null mice fail to metabolize [^{13}C]-labelled dichloroacetic acid to [^{13}C]glyoxylate (Ammini *et al.*, 2003). In studies by Fang *et al.* (2006), a total of 10 single-nucleotide polymorphisms (SNPs) were identified in African, and Australian European subjects in a region 1.5 kb upstream of the *GSTZ1* start of transcription. Most recent studies suggest that there are four common polymorphic alleles of *GSTZ1*: 1a, 1b, 1c, and 1d (Board & Anders, 2011). *GSTZ1c* is the most common and is designated as the wild-type gene.

Dichloroacetic acid is an inactivator of *GSTZ1* in humans, rats, and mice. However, human *GSTZ1* is more resistant to inactivation than mouse or rat *Gstz1* (Tzeng *et al.*, 2000). The polymorphic variants of human *GSTZ1* differ in their susceptibility to inactivation, with 1a-1a being more resistant to inactivation than the other variants (Blackburn *et al.*, 2000; Blackburn *et al.*, 2001). A pharmacokinetic study (Li *et al.*, 2008) concluded that apparent inhibition of GSTZ-mediated metabolism of dichloroacetic acid is minimal at low doses (µg/kg bw per day), but may be significant for therapeutic doses of dichloroacetic acid and that polymorphisms of *GSTZ1* may help explain inter-individual variability in the plasma kinetics of dichloroacetic acid.

Short-term treatment of B6C3F$_1$ mice with dichloroacetic acid was shown to lead to an increase in activity of hepatic superoxide dismutase and catalase (Hassoun & Cearfoss, 2011). Because oxidative stress in the liver was suggested as one of the mechanisms of carcinogenesis by dichloroacetic acid (Austin *et al.*, 1995), polymorphisms in these protective enzymes may be of potential importance in protection against oxidative stress induced by dichloroacetic acid.

Individuals with glycogen storage disease (an inherited deficiency or alteration in any one of the enzymes involved in glycogen degradation) represent another group that may be more susceptible to toxicity caused by dichloroacetic acid. There is some evidence that alterations in glycogenolysis precede the development of many types of tumour (Bannasch, 1986; Bannasch *et al.*, 1986). The dose–response relationship for dichloroacetic acid-induced effects on hepatic glycogen is in the same range as that required for inducing liver tumours (Bull, 2000).

In addition, individuals with hyperoxaluria type 1, a rare genetic disorder, may be susceptible to elevated levels of glyoxylate originating from dichloroacetic acid metabolism. In this condition, the inability to convert glyoxylate to glycine leads to the formation and excretion of oxalate (Ribaya & Gershoff, 1982).

4.4.2 Life-stage susceptibility

The effect of dichloroacetic acid on its own metabolism is age-dependent in humans (Shroads *et al.*, 2008). Two randomized, double-blind, placebo-controlled clinical trials have been reported in which subjects received dichloroacetic acid at a dose of 12.5 mg/kg bw, twice per day for 6 months. In 43 children being treated for congenital lactic acid acidosis, no neurotoxicity was observed (Stacpoole *et al.*, 2006). In 30 adults, the trial had to be terminated prematurely because of the high incidence of symptomatic peripheral neuropathy. In studies by Shroads *et al.* (2008), nine patients were treated for 6 months with dichloroacetic acid at 25 mg/kg bw per day, and rats of varying ages were treated for 5 days with dichloroacetic acid at 50 mg/kg bw per day. Long-term administration of dichloroacetic acid showed a striking age-dependent decrease in plasma clearance in rats and humans. Monochloroacetate, a known neurotoxin, increased as a function of age in the urine of rats. This neurotoxin was detectable only in the plasma of older rats.

In female rats, exposure to dichloroacetic acid during gestation has been shown to result in the impairment of fetal maturation and soft-tissue anomalies (primarily of cardiac origin) indicating that the developing fetus may be uniquely susceptible to dichloroacetic acid-induced toxicity (Smith *et al.*, 1992). The study of Moser *et al.* (1999) provided additional limited evidence for increased susceptibility of rats to dichloroacetic acid-induced neurotoxicity when exposures begin shortly after weaning.

4.4.3 Sex differences

In a stable-isotope study by Schultz & Shangraw (2006), the effect of pretreatment with dichloroacetic acid on the pharmacokinetics of later doses of dichloroacetic acid was tested in eight male and eight female volunteers. In the absence of pretreatment with dichloroacetic acid (0.02 µg/kg bw per day for 14 days), there were no sex differences in the pharmacokinetics of dichloroacetic acid. Only women were affected by pretreatment, showing an increased AUC for plasma dichloroacetic acid and a decreased rate of clearance.

In a 26- and 39-week studies of carcinogenesis in Tg.AC hemizygous mice given dichloroacetic acid by dermal application (NTP, 2007), kidney nephropathy (observed in males) was the only non-cancer pathology to occur differently in males and females. This pathology was not observed in male or female mice of the same strain when dichloroacetic acid was given in the drinking-water, or in 26- and 41-week studies of carcinogenesis in p53 haplo-insufficient mice treated with dichloroacetic acid in drinking-water (NTP, 2007).

4.4.4 Effect of co-morbidities

The pharmacokinetics of dichloroacetic acid was evaluated in several small cohorts of humans with disease conditions. Most of the studies examined parameters of distribution and excretion.

In children (four boys and four girls, aged 1.5–10 years) with lactic acidosis caused by severe malaria, who were given dichloroacetic acid intravenously at a dose of 50 mg/kg bw, the average plasma half-life of dichloroacetic acid was 1.8 ± 0.4 hours, volume of distribution was 0.32 ± 0.09 L/kg, and the average AUC was 378 ± 65 mg/L per hour (Krishna *et al.*, 1995).

Two studies were conducted on the pharmacokinetics of dichloroacetic acid in patients with severe malaria. In one study that included 13 adults ([sex not reported]; average age, 27 ± 8 years) who were given dichloroacetic acid intravenously at a dose of 46 mg/kg bw over 30 minutes, the elimination half-life was 2.3 ± 1.8 hours, the clearance was 0.32 ± 0.16 L/h per kg and the volume of distribution was 0.75 ± 0.35 L/kg (Krishna *et al.*,

1994). In a second study, 11 adults (eight men and three women; average age, 32 ± 10 years) were given dichloroacetic acid intravenously at a dose of 46 mg/kg bw and a second dose (46 mg/kg bw) was given 12 hours later. The mean plasma half-life was 3.4 ± 2 hours after the first dose and 4.4 ± 2 hours after the second dose, the volume of distribution was 0.44 ± 0.2 L/kg and the plasma clearance was 0.13 ± 0.03 L/h per kg (Krishna *et al.*, 1996).

The effect of end-stage liver disease and liver transplantation on the pharmacokinetics of dichloroacetic acid was studied in 33 subjects [sex and age not reported] who were given dichloroacetic acid at a dose of 40 mg/kg bw by a 60-minute intravenous perfusion, then a second dose (40 mg/kg bw) by intravenous perfusion 4 hours later, before and during the anhepatic stage. The clearance of dichloroacetic acid during the paleohepatic, anhepatic and neohepatic stages was 1.0, 0.0 and 1.7 mL/kg per minute, respectively, indicating a major role of the liver in the metabolism of dichloroacetic acid (Shangraw & Fisher, 1996). The effect of cirrhosis on the pharmacokinetics of dichloroacetic acid was reported in six healthy volunteers (five men and one woman; age, 30 ± 3 years) and seven subjects with end-stage cirrhosis (five men and two women; age, 47 ± 3 years) who were given dichloroacetic acid at a dose of 35 mg/kg bw by intravenous perfusion over 30 minutes. The clearance of dichloroacetic acid was 2.14 mL/kg per minute in control subjects and 0.78 mL/kg per minute in patients with cirrhosis (Shangraw & Fisher, 1999).

The pharmacokinetics of dichloroacetic acid was studied in 111 patients with lactic acidosis (66 men; age, 56.0 ± 18.4 years), who received dichloroacetic acid (50 mg/kg bw) by intravenous perfusion over 30 minutes, then a second perfusion of 50 mg/kg bw, 2 hours after the beginning of the first. The pharmacokinetics were complex in the acutely ill patients studied and differed markedly from those observed in healthy volunteers. In healthy volunteers, the pharmacokinetics fitted a one-compartment model, while in the patients the data fitted one-, two- and three-compartment models. In the two-compartment model, the plasma half-life and plasma clearance were 18.15 ± 3.12 hours (mean ± standard error [SE]) and 0.041 L/kg per hour, respectively, after the first treatment, while the two values were 68.30 ± 14.50 hours (mean ± SE) and 0.017 L/kg per hour, respectively, after the second treatment. Plasma clearance of dichloroacetic acid tended to decrease as either the number of compartments or the number of treatments increased. The prolonged half-life and decreased plasma clearance indicate that repeated administration of dichloroacetic acid impairs its metabolism (Henderson *et al.*, 1997).

The pharmacokinetics of dichloroacetic acid was compared in healthy volunteers (27 subjects) and in patients with traumatic brain injury (25 subjects; average age, 52.8 ± 18.1 years). The healthy volunteers were given cumulative intravenous doses (two doses, 8 hours apart) of dichloroacetic acid at 45, 90 or 150 mg/kg bw; 16 patients with acute traumatic brain injury were given a single intravenous dose of dichloroacetic acid at 60, 100 or 200 mg/kg bw; six other patients were given three intravenous doses [dose not stated] of dichloroacetic acid at 24-hour intervals; and three patients were given six intravenous doses [dose not stated] at 12-hour intervals. The initial clearance of dichloroacetic acid (4.82 L/h) declined (1.07 L/h) after repeated doses in patients with traumatic brain injury.

4.5 Mechanistic considerations

Weak to moderate evidence suggested that dichloroacetic acid may be genotoxic. No induction of DNA strand breaks was observed in the only available study in a human lymphoblast cell line *in vitro*.

In mammalian systems, gene mutations were reported in experiments *in vivo* and limited

evidence existed for increased frequency of mutation after treatment with dichloroacetic acid *in vivo* and *in vitro*. Dichloroacetic acid clearly induced chromosomal aberrations in mouse lymphoma cells, but not in Chinese hamster ovary cells. With regard to micronucleus formation, results were conflicting *in vivo* and negative *in vitro* in mouse lymphoma cells. Inconsistent evidence existed to suggest that dichloroacetic acid could cause DNA damage (DNA unwinding) in studies *in vivo* in bone marrow and blood leukocytes in animals. In addition, several studies have found specific mutations in H-*ras* codon 61 in liver tumours after dichloroacetic acid administration, distinct from those in spontaneous tumours. In tests for genotoxicity in bacterial and fungal systems, only positive results were observed in assays for base substitution mutations in strains TA100 (three out of five tests), RSJ 100, and TA98.

Overall, the strength of evidence for the liver as a target organ is strong. Available mechanistic data come almost exclusively from studies in animals. Multiple mechanisms have been identified including epigenetic effects (global DNA hypomethylation and hypomethylation of the *Myc* gene promoter), oxidative stress (oxidative DNA damage and lipid peroxidation), effects on cell proliferation/apoptosis (a decrease in both cell proliferation and apoptosis, but selective enhancement of Jun-positive cells), induction of the peroxisome proliferation response (strong direct and indirect evidence for activation of PPARα in rodents, limited evidence for dichloroacetic acid as a ligand of human PPARα), disruption of gap-junctional intercellular communications (limited evidence from one study in a rat hepatocyte cell line *in vitro*). Because dichloroacetic acid is a metabolite of other chlorinated solvents, several studies have compared mutational and phenotypic profiles of liver tumours induced by various chlorinated solvents and concluded that little similarity exists.

Overall, the strength of evidence for the kidney as a target organ is weak. Some evidence of kidney toxicity has been reported in studies in animals. Several studies evaluated the effects of dichloroacetic acid in rodents and demonstrated increased relative kidney weight and effects on kidney histopathology in male rats exposed to high doses of dichloroacetic acid in drinking-water for 90 days. However, no similar effect was observed in mice. Hypomethylation of global DNA and of the *Myc* gene has been observed in kidney of male but not female mice.

Dichloroacetic acid is a sedative in animals and humans, and high doses have been shown to cause adverse effects on the central nervous system. In addition, peripheral neuropathy has been observed in humans (at therapeutic concentrations), and in rodents and dogs. There were no studies available that suggested a mechanism for these effects.

There is the potential for inter-individual variability in the adverse effects of dichloroacetic acid. GST-zeta1 is an important enzyme in the metabolism of dichloroacetic acid and common polymorphisms that result in differences in activation have been reported in humans. With respect to life-stage susceptibilities, neurotoxicity has been observed in adults, but not in children.

Dichloroacetic acid has been used in therapeutic studies for a variety of conditions related to impaired metabolism. Dichloroacetic acid activates pyruvate dehydrogenase. This effect has been suggested to be beneficial for human conditions associated with lactic acidosis, hypercholesterolaemia and hyperglycaemia. A suggestion of anti-cancer effects of dichloroacetic acid is based on its anti-proliferative effects and activation of pyruvate dehydrogenase which may in turn affect glycolysis, the major oxidative metabolic pathway in tumours.

5. Summary of Data Reported

5.1 Exposure data

Dichloroacetic acid is used as an intermediate in the production of glyoxylic acid, dialkoxy and diaroxy acids, sulfonamides and iron chelates. It is used to a lesser extent as a cauterizing agent and as a therapeutic agent for metabolic diseases. Dichloroacetic acid is readily transformed into dichloroacetate salts in aqueous solutions. Data on occupational exposure were only available for a small group of swimming-pool attendants who had very low levels in urine. Exposure of the general population to dichloroacetic acid occurs at the level of micrograms per litre in drinking-water (range, 10–40 μg/L) and from swimming pools (range, 10–100 μg/L) as a result of chlorine-based disinfection of water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Dichloroacetic acid has been evaluated for its carcinogenicity in seven studies with drinking-water (some involving more than one experiment) in male mice and two studies with drinking-water in female mice. Two studies with drinking-water (involving more than one experiment) were conducted in male rats. These studies varied significantly in quality and statistical power.

In all studies in male and female mice, there was an increase in the incidence of hepatocellular adenoma and/or hepatocellular carcinoma. In all studies in male rats, an increased incidence of hepatocellular adenoma and hepatocellular carcinoma was observed. The main deficiency of all these studies was that they uniformly focused on the development of liver tumours. As a result, they did not provide a basis for considering whether tumours in other organs might have been induced.

Dichloroacetic acid increased the incidence of bronchioloalveolar adenoma in female Tg.AC hemizygous mice after administration in drinking-water, and of skin papilloma in both males and females of the same strain after skin application.

The four initiation–promotion studies with dichloroacetic acid in mice provided positive results. Dichloroacetic acid was found to be an efficient promoter of *N*-ethyl-*N*-nitrosourea- and vinyl carbamate-initiated hepatocellular tumours.

5.4 Mechanistic and other relevant data

Major similarities exist between humans and laboratory animals with regard to the absorption, distribution, metabolism and excretion of dichloroacetic acid. Dichloroacetic acid has a very similar plasma half-life in humans and laboratory animals. Dichloroacetic acid is primarily metabolized through glutathione-*S*-transferase zeta 1 (GST-zeta1) to glyoxylic acid and then to oxalic and glycolic acids, glycine and CO_2. The minor metabolic pathway of dichloroacetic acid is to monochloroacetic acid with further processing to thiodiacetic acid. Dichloroacetic acid acts as an inhibitor of its own metabolism by inactivating GST-zeta1. Such inhibition has a major impact on plasma half-life depending on the duration of exposure. Repeated administration of dichloroacetic acid has been shown to increase plasma half-life in both humans and laboratory animals by about 10 times.

Weak to moderate experimental evidence was available to suggest that dichloroacetic acid is a genotoxic agent. Target organs for adverse health outcomes of dichloroacetic acid are liver, nervous system, and kidney. Cancer findings in

animals and toxicity findings in humans and laboratory animals designated liver as a major target organ for dichloroacetic acid. Available data suggested that dichloroacetic acid may also act through multiple non-genotoxic mechanisms in liver carcinogenesis. There is a potential for inter-individual variability in the adverse effects of dichloroacetic acid, because dichloroacetic acid is primarily metabolized through GST-zeta1; this enzyme is polymorphic, and such polymorphisms have been shown to have an impact ont the function of GST-zeta1.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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TRICHLOROACETIC ACID

Trichloroacetic acid was considered by previous IARC Working Groups in 1995 and 2004 (IARC, 1995, 2004). New data have since become available, and, together with information about sodium trichloroacetate (trichloroacetic acid, sodium salt), have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

(a) Trichloroacetic acid

Chem. Abstr. Serv. Reg. No.: 76-03-9

Chem. Abstr. Serv. Name: Trichloroacetic acid

IUPAC Systematic Name: 2,2,2-Trichloroacetic acid

Synonyms: TCA; TCA (acid); trichloracetic acid; trichloroethanoic acid; trichloromethane carboxylic acid

(b) Sodium trichloroacetate

Chem. Abstr. Serv. Reg. No.: 650-51-1

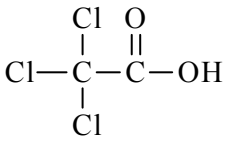
Chem. Abstr. Serv. Name: Sodium trichloroacetate

IUPAC Systematic Name: Sodium 2,2,2-trichloroacetate

Synonyms: TCA-sodium; TCA sodium salt; trichloroacetic acid, sodium salt

1.1.2 Structural and molecular formulae, and relative molecular mass

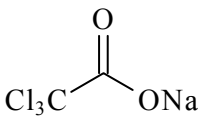
(a) Trichloroacetic acid



$C_2HCl_3O_2$

Relative molecular mass: 163.39

(b) Sodium trichloroacetate



$C_2Cl_3NaO_2$

Relative molecular mass: 185.37

1.1.3 Chemical and physical properties of the pure substance

(a) Trichloroacetic acid

Description: Very deliquescent crystals; slight, characteristic odour (O'Neil *et al.*, 2006)

Boiling-point: 196–197 °C (O'Neil *et al.*, 2006)

Melting-point: 57–58 °C (O'Neil *et al.*, 2006)

Density: 1.629 at 61 °C/4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared [2376], ultraviolet [1–6], nuclear magnetic resonance [6] and mass [1026] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

Solubility: Very soluble in water, ethanol, ethyl ether (O'Neil *et al.*, 2006) soluble in most organic solvents, including acetone, benzene, methanol and *o*-xylene (Morris & Bost, 1991)

Volatility: Vapour pressure, 1 kPa at 83.8 °C (Haynes, 2012)

Stability: Decomposes by heating with caustic alkalis into chloroform and alkali carbonate. Corrosive. Decomposition products are chloroform, hydrochloric acid, carbon dioxide and carbon monoxide (O'Neil *et al.*, 2006)

Octanol/water partition coefficient (P): log P, 1.33 (Hansch *et al.*, 1995)

Conversion factor: mg/m^3 = 6.68 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

(b) Sodium trichloroacetate

Description: Yellow powder (HSDB, 2012)

Melting-point: Decomposes at 165–200 °C (HSDB, 2012)

Density: 1.808 g/cm^3 (Guidechem, 2012)

Solubility: Soluble in water at 1.2 kg/L at 25 °C; soluble in ethanol; soluble in methanol at 232 g/L, acetone at 7.6 g/L, diethyl ether at 0.2 g/L, benzene at 0.07 g/L, carbon tetrachloride at 0.04 g/L, heptane at 0.02 g/L (all at 25 °C) (HSDB, 2012)

1.1.4 Technical products and impurities

Trichloroacetic acid and sodium trichloroacetate are marketed at various degrees of purity. Trichloroacetic acid is available as aqueous solutions, with concentrations ranging from 3% to 100% (w/v) (Spectrum Chemical, 2012). Sodium trichloroacetate is available as a granular powder at a purity of > 97% (Acros Organics, 2012).

Trade names for trichloroacetic acid include Aceto-Caustin and Amchem Grass Killer.

Trade names for sodium trichloroacetate include: Eribitox T95G, acp grasskiller, Antiperz; Antyperz, varitox weedmaster grass killer and Remazol SaltFD.

1.1.5 Analysis

Methods for the analysis of trichloroacetic acid have been reviewed by Delinsky *et al.* (2005). Selected methods for the analysis of trichloroacetic acid in water are identified in Table 1.1. A headspace gas chromatography-mass spectrometry method has been developed for measuring trichloroacetic acid in urine (Cardador & Gallego, 2010) and headspace gas chromatography methods have been developed for measuring trichloroacetic acid in blood and urine (Monster & Boersma,1975; Skender *et al.*, 1993).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Trichloroacetic acid was reported to have been first synthesized in 1840 by chlorination of acetic acid in sunlight (Beilstein Online, 2002). It is produced on an industrial scale by chlorination of acetic acid or chloroacetic acid at 140–160 °C. Calcium hypochlorite may be added as a chlorination accelerator, and metal catalysts (such as iron or copper compounds) have been used in some cases. Trichloroacetic acid is isolated from

Table 1.1 Methods for the analysis of trichloroacetic acid in water

Sample preparation	Assay procedure	Limit of detection	Reference
Extract methyl-*t*-butyl ether; derivatize to methyl ester; acidify; extract with methanol	GC/ECD	0.02 µg/L	EPA (2003) 552.3
Add ammonium chloride and ^{13}C-labelled internal standards; direct injection	IC-ESI-MS/MS	0.09 µg/L	EPA (2009) 557

ECD, electron capture detection; ESI-MS/MS, electrospray ionization tandem mass spectrometry; GC, gas chromatography; IC, ion chromatography

the crude product by crystallization (Koenig *et al.*, 2011).

Sodium trichloroacetate is produced industrially by neutralizing trichloroacetic acid with sodium hydroxide solution or sodium carbonate (Ullmans, cited in HSDB, 2012).

(b) Production volume

Trichloroacetic acid was produced by nine companies in India, two companies each in China, Germany and Mexico, and one company each in France, Israel, Italy, Japan, the Russian Federation, and Spain (Chemical Information Services, 2002a). It was formulated into pharmaceutical products by five companies in Italy, three companies in France, two companies in Poland and one company each in Argentina, Spain and Turkey (Chemical Information Services, 2002b).

Between the late 1940s and 1990, about 30 000 tonnes of trichloroacetate were applied as herbicide in Germany (Schöler *et al.*, 2003). [This amount may not all have been produced in Germany.] By 1993, only about 1000 tonnes per annum of trichloroacetic acid were produced in Germany (OECD–SIDS, 2000). HSDB (2012) reported production volumes of 5–230 tonnes per annum in the USA.

1.2.2 Use

The main application of trichloroacetic acid, usually as its sodium salt, has been as a selective herbicide. It was formulated as a water-soluble liquid or powder (Koenig *et al.*, 2011). Its main use was as grass killer for perennial grasses such as common Bermuda grass, quack grass and Johnson grass (Monaco *et al.*, 2002). Common crops on which it was used include sugar beet, sugar cane and canola.

Historically, trichloroacetate has been combined with 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to sterilize soil (Crafts, 1975) or for control of weeds. In Germany and Switzerland, the sale and import of sodium trichloroacetate as herbicide has been prohibited since 1989 (HSDB, 2012). In the USA, registrations for herbicidal products containing trichloroacetic acid were voluntarily cancelled by 1992 (California EPA, 1999), although existing stocks may have been used for some time after this date.

Trichloroacetic acid is also used as an etching or pickling agent in the surface treatment of metals, as a swelling agent and solvent in the plastics industry, as an auxiliary in textile finishing, as an additive to improve high-pressure properties in mineral lubricating oils and as an analytical reagent. Trichloroacetic acid and particularly its esters are important starting materials in organic syntheses (Koenig *et al.*, 1986; Morris & Bost, 1991; Clariant GmbH, 2002a, b, c).

Trichloroacetic acid can be used as a caustic agent on the skin or mucous membranes to treat local lesions and for the treatment of various dermatological diseases. There are reports of its use in removing tattoos, treating genital warts and in dermal peeling. It is also used as a precipitant of protein in the chemical analysis of body fluids and tissue extracts, and as a decalcifier and fixative in microscopy (Gennaro, 2000; Royal Pharmaceutical Society of Great Britain, 2002).

1.3 Occurrence

1.3.1 Natural occurrence

Trichloroacetic acid is not known to occur as a natural product.

1.3.2 Environmental occurrence

(a) Air

No data were available to the Working Group.

(b) Water

Trichloroacetic acid is produced as a by-product during the chlorination of water containing humic substances and may occur in drinking-water or swimming pools after chlorine-based disinfection of raw waters that contain natural organic substances (IARC, 2004).

Table 1.2 summarizes some recent levels of trichloroacetic acid found in surface waters, groundwater and drinking-water worldwide.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 35 124 employees in seven industries in the USA had potentially experienced occupational exposure to trichloroacetic acid (NIOSH, 1994). The estimate was based on a survey of companies and did not involve measurement of actual exposure.

Recently, occupational exposure of swimming-pool attendants to trichloroacetic acid in indoor and outdoor pools was evaluated by analysis of urine samples. After an exposure of 2 hours, the urine of 24 pool attendants contained trichloroacetic acid at a concentration of ~120 ng/L (range, < 60–163 ng/L). No trichloroacetic acid was detected in the urine of attendants at outdoor pools. Concentrations of trichloroacetic acid in the urine of pool attendants working indoors increased by 30% after the length of the shift doubled (4 hours) (Cardador & Gallego, 2011). [The Working Group noted that it was unclear by what route the pool attendants had been exposed.]

Because trichloroacetic acid is a major end-metabolite of trichloroethylene (IARC, 1976, 1979, 1987, 1995) and tetrachloroethylene (IARC, 1979, 1987, 1995) in humans, it has been used for many years as a biological marker of exposure to these compounds. It is also a metabolite of 1,1,1-trichloroethane (see IARC, 1979, 1987, 1999), and chloral hydrate (see *Monograph* on Chloral and Chloral Hydrate in this Volume; and IARC, 2004) is rapidly oxidized to trichloroacetic acid in humans. The levels of trichloroacetic acid reported in human blood and urine after occupational and environmental exposure to trichloroacetic acid, trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane are summarized in Table 1.3.

Raaschou-Nielsen *et al.* (2001) examined 2397 measurements of trichloroacetic acid in urine collected between 1947 and 1985 from workers in various industries in Denmark. The urine samples were usually taken after a request from the local labour-inspection agency or medical officer. The data showed that: (a) concentrations of trichloroacetic acid decreased by four times between 1947 and 1985; (b) the highest concentrations were observed in the iron and metal, chemical and dry-cleaning industries; (c) concentrations of trichloroacetic acid were twice as high in men than in women in the iron and metal, and dry-cleaning industries; (d) concentrations of trichloroacetic acid were higher in younger than in older workers; and (e) people working in an area in which trichloroethylene was used, but not working with trichloroethylene themselves, also showed urinary concentrations of trichloroacetic acid indicative of exposure.

Urinary concentrations of trichloroacetic acid in a large database of more than 3000 workers exposed to 1,1,1-trichloroethane, tetrachloroethylene and trichloroethylene in Finland showed a rapid 10-times decline in men between 1985 and 1992; the decline observed among women

Table 1.2 Concentrations of trichloroacetic acid in water

Country	Location	Concentration (µg/L)		Reference
		Mean or median[a]	Range	
Drinking-water				
Australia	Seven cities	NR	< 0.02–14	Simpson & Hayes (1998)
China	Eight typical water supplies	NR	8.4–30.9	Liu *et al.* (2011)
	Beijing	1.7	1.5–2.2	Wang & Wong (2005)
	Beijing	3.38	NR–20.10	Wei *et al.* (2010)
Greece	Athens	NR	3.5–17.9	Golfinopoulos & Nikolaou (2005)
	Mytilene (bottled water)	NR	NR–1.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	3.1[a]	1.5–5.0	Villanueva *et al.* (2012)
United Kingdom	England	9.8	4.1–18.5	Zhang *et al.* (2010a)
	Three large regions served by three water companies	12.7	NR–34	Malliarou *et al.* (2005)
		29.3	NR–95	
		21.1	NR–51	
	Scotland	20[a] [HAA]	11–134 [HAA]	Goslan *et al.* (2009)
Raw and surface water				
China	Eight typical water supplies	NR	33.6–488.5	Liu *et al.* (2011)
Republic of Korea	Four regions	46.6	43.5–55.8	Kim (2009)
Turkey	Country-wide (29 regions)	NR	18–149 [HAA]	Ates *et al.* (2007)
Swimming-pool water				
Republic of Korea	Seoul	156.4	19.7–636	Lee *et al.* (2010)
		17.4	1.3–85.8	
		97.2	1–413	

[a] Median
EGMO, electrochemically generated mixed oxidants; HAA, total halogenated acetic acids; NR, not reported

was less steep, such that the median exposure of women was higher than that of men (Anttila *et al.*, 1995).

Recent studies have shown that urinary concentrations of trichloroacetic acid in workers in electronic and related industries in China (Green *et al.*, 2004) are above those in dry cleaners in the USA (McKernan *et al.*, 2008).

1.3.4 Exposure of the general population

Concentrations of trichloroacetic acid in urine and blood in the general population are presented in Table 1.4.

Concentrations of trichloroacetic acid as a metabolite of trichloroethylene and tetrachloroethylene were determined in the urine of people living in the vicinity of dry-cleaning shops in Germany and the USA where tetrachloroethylene was used (Popp *et al.*, 1992; Schreiber *et al.*, 2002). Trichloroacetic acid has also been measured in adults with no known exposure to these chemicals (Ikeda & Ohtsuji, 1969; Hajimiragha *et al.*, 1986; Skender *et al.*, 1993; Calafat *et al.* 2003; Zhou *et al.*, 2012).

In China, trichloroacetic acid was measured in the urine of 418 male partners in couples seeking medical treatment for infertility (Xie *et al.*, 2011). Urinary creatinine-adjusted concentrations of trichloroacetic acid ranged from 0.4 to 43.1 mg/g creatinine, with a mean of 6.4 mg/g creatinine.

In a study of exposures at swimming pools, the average urinary concentrations of trichloroacetic

Table 1.3 Concentrations of trichloroacetic acid in blood and urine measured by biological monitoring in occupational settings with exposure to various chlorinated solvents

Country	Job/task	No. of subjects	Agent to which exposure occurred	Air levels Range [mg/m³][a]	Matrix	Concentrations in blood or urine (range)	Reference
China	Electronic and related industries	70	Trichloroethylene	32 ppm (range, 0.5–252 ppm) (estimated based on concentrations of TCA in urine)	Urine	1–386 mg/g creatinine	Green *et al.* (2004)
Denmark	Various industries	2397 measurements	Trichloroethylene	NR	Urine	78 mg/L 1947–53; n = 396) 57 mg/L (1954–59; n = 214) 58 mg/L (1960–64; n = 290) 49 mg/L (1965–69; n = 413) 49 mg/L (1970–74; n = 499) 28 mg/L (1975–79; n = 459) 14 mg/L (1980–85; n = 126)	Raaschou-Nielsen *et al.* (2001)
Finland	Solvent exposure	10 783 measurements from 3976	1,1,1-Trichloroethane, tetrachloroethylene, trichloroethylene	NR	Urine	Median: Women, 63 µmol/L Men, 48 µmol/L	Anttila *et al.* (1995)
The former state union of Serbia and Montenegro	Dry cleaning, degreasing	32	Trichloroethylene	NR	Blood Urine	0.43–154.92 µmol/L [0.07–25.3 mg/L] 0.58–42.44 mmol/mol creatinine [0.84–61 mg/g]	Skender *et al.* (1988)
The former state union of Serbia and Montenegro	Dry cleaning	10	Trichloroethylene	25–40 ppm [134–215 mg/m³]	Blood Urine	13.47–393.56 µmol/L [2.2–64 mg/L] 1.92–77.35 mmol/mol creatinine [2.8–112 mg/g]	Skender *et al.* (1991)
		18	Tetrachloroethylene	33–53 ppm [224–359 mg/m³]	Blood Urine	1.71–20.93 µmol/L [0.3–3.4 mg/L] 0.81–15.76 mmol/mol creatinine [1.2–23 mg/g]	
Germany	Printing and ceramics workshop	31	Trichloroethylene	5–70 ppm [26.9–376 mg/m³]	Urine	2.0–201.0 mg/g creatinine	Triebig *et al.* (1982)

Table 1.3 (continued)

Country	Job/task	No. of subjects	Agent to which exposure occurred	Air levels Range [mg/m³][a]	Matrix	Concentrations in blood or urine (range)	Reference
Germany	Dry cleaning (nine shops)	12	Tetrachloroethylene	NR	Urine	Mean, 682 µg/L; maximum, 1720 µg/L	Popp *et al.* (1992)
Japan	Workshop	85	Trichloroethylene	3–175 ppm [16.1–940 mg/m³]	Urine	9–297 mg/L	Ikeda *et al.* (1972)
Japan	Printing factory	46	1,1,1-Trichloroethane	4.3–53.5 ppm [23–289 mg/m³]	Urine	0.5–5.5 mg/L	Seki *et al.* (1975)
Japan	Automobile workshop	25	Trichloroethylene	1–50 ppm [5–269 mg/m³]	Urine	Average, 136 mg/g creatinine	Ogata *et al.* (1987)
Japan	Workshop	3	Trichloroethylene	NR	Urine	Range of means, 108–133 mg/L (trichloroacetate)	Itoh (1989)
Japan	Printing workshop	48	1,1,1-Trichloroethane	5–65 ppm [27–351 mg/m³]	Urine	2–5 mg/L	Kawai *et al.* (1991)
Republic of Korea	Solvent exposure	13	Tetrachloroethylene	0–61 ppm [0–414 mg/m³]	Urine	0.6–3.5 mg/L	Jang *et al.* (1993)
Spain	Swimming pool	24	Trichloroacetic acid	NR	Urine	< 60–163 ng/L	Cardador & Gallego (2011)
Sweden	Degreasing	31	Trichloroethylene	3–114 mg/m³	Urine	2–260 µmol/L [0.3–42.5 mg/L]	Ulander *et al.* (1992)
Sweden	Various industries	1670	Trichloroethylene	81% < 20 ppm (estimated from concentrations of TCA in urine)	Urine	81% < 50 mg/L	Axelson *et al.* (1994)
Switzerland	Metal degreasing	26	Trichloroethylene	10–300 ppm [54–1611 mg/m³]	Urine	57–980 mg/L	Boillat (1970)
USA	Metal degreasing	19	Trichloroethylene	170–420 mg/m³	Urine	3–116 mg/g creatinine	Lowry *et al.* (1974)
USA	Dry cleaning	18	Tetrachloroethylene	GM, 1.64 ppm; GSD, 3.26	Urine	Pre-shift: GM, 0.29 mg/g creatinine; GSD, 7.23 Post-shift: GM, 0.51 mg/g creatinine; GSD, 3.89	McKernan *et al.* (2008)

GM, geometric mean; GSD, geometric standard deviation; NR, not reported; TCA, trichloroacetic acid

[a] Converted by the Working Group, except where stated in mg/m³ in the article

Table 1.4 Concentrations of trichloroacetic acid in urine and blood of the general population with known or unknown exposures

Country Year of study	Subjects	No. of subjects	Concentration in urine		Concentration in blood		Reference
			Mean	Range	Mean	Range	
China	Male partners of subfertile couples	418	6.4	0.4–43.1 mg/g creatinine	NR	NR	Xie *et al.* (2011)
China	Pregnant women	398	13.4 µg/g creatinine	1–123 µg/g creatinine	NR	NR	Zhou *et al.* (2012)
Croatia 1993	No known exposure to solvents	39	NR	NR	45 µg/L	14–160 µg/L	Skender *et al.* (1993)
Germany	Unexposed	43	NR	NR	24 µg/L	5–221 µg/L	Hajimiragha *et al.* (1986)
Germany 1992	Living near dry-cleaning shops	29	105 µg/L	NR	NR	NR	Popp *et al.* (1992)
Japan	Students	66	ND–930 µg/g creatinine	NR	NR	NR	Ikeda & Ohtsuji (1969)
Spain	Swimmers, indoor pool	13	4390 ng/L	NR	NR	NR	Cardador & Gallego (2011)
	Child swimmers, indoor pool	6	6092 ng/L	NR	NR	NR	
	Swimmers, outdoor pool	8	4757 ng/L	NR	NR	NR	
USA	Living near dry cleaner	10	6.6 µg/g creatinine	NR	< 0.83–21	NR	Schreiber *et al.* (2002)
USA	NHANES	402	2.9 µg/L	< 0.5–25 µg/L	NR	NR	Calafat *et al.* (2003)

ND, not detected; NHANES, National Health and Nutrition Examination Survey; NR, not reported

Table 1.5 National regulations and guidelines for trichloroacetic acid

Country or region	Concentration (mg/m³)	Unit
Australia	6.7	TWA
Austria	5	TWA
Belgium	6.8	TWA
Canada, Quebec	6.7	TWA
Denmark	1	TWA
France	5	TWA
New Zealand	6.7	TWA
Singapore	6.7	TWA
Spain	6.8	TWA
Switzerland	7	TWA
USA, NIOSH	7	TWA

NIOSH, National Institute for Occupational Safety and Health; TWA, 8-hour time-weighted average
From GESTIS – database on hazardous substances (Information system on hazardous substances of the German Social Accident Insurance) (IFA, 2012)

acid were 4390 ng/L in 13 adults and 6092 ng/L in 6 children swimming in an indoor pool and 4757 ng/L in 8 adults using an outdoor pool (Cardador & Gallego, 2011). Exposure levels in the swimmers were one order of magnitude higher than those in the pool attendants (313 ng/L and 51 ng/L, respectively, for workers at indoor and outdoor pools after a 2-hour shift). [Exposures may be higher for swimmers than for pool attendants because the main route of exposure is through ingestion.]

1.4 Regulations and guidelines

1.4.1 Comparison of exposure limits worldwide

Trichloroacetic acid is classified by the United States Environmental Protection Agency as a possible human carcinogen based on evidence of carcinogenicity in experimental animals. Trichloroacetic acid is considered to be a confirmed carcinogen in experimental animals, with unknown relevance to humans by the American Conference of Industrial Hygienists (HSDB, 2012).

Table 1.5 summarizes current limits for occupational exposure.

2. Cancer in Humans

Trichloroacetic acid is a chemical that occurs in drinking-water and in swimming pools as part of a mixture of by-products resulting from water disinfection. The chemicals in water-disinfection by-products do not occur in an isolated manner and there is no epidemiological evidence on risk of cancer associated specifically with them. A detailed description of water-disinfection by-products and cancer risk is given in *IARC Monograph* Volume 101 (IARC, 2013).

3. Cancer in Experimental Animals

Studies in experimental animals exposed to trichloroacetic acid were limited by incomplete pathology examination, short durations of exposure, and small numbers of animals. Studies with such a design cannot show the full expression of carcinogenic response. For any study in which trichloroacetic acid is administered in the drinking-water, palatability is an issue; decreases in drinking-water consumption have been observed at high doses. Such decreased drinking-water consumption not only affects the dose

administered but also may affect the health of the animals.

See Table 3.1

3.1 Mouse

As part of an initiation–promotion study in male B6C3F$_1$ mice (age, 4 weeks), trichloroacetic acid was administered in a buffered solution of drinking-water at a concentration of 5 g/L (Herren-Freund *et al.*, 1987). Controls were given drinking-water containing sodium chloride at 2 g/L. The experiment was terminated at 61 weeks because of tumour induction. Histopathological examination was restricted to the liver. At termination of the experiment, mice treated with trichloroacetic acid were found to have an increased incidence of hepatocellular adenoma (2 out of 22 in controls versus 8 out of 22 in treated mice, [$P < 0.05$]) and hepatocellular carcinoma (0 out of 22 in controls versus 7 out of 22 in treated mice, $P < 0.01$). [The Working Group noted that the study was limited by observations only for the liver, the low statistical power due to the small numbers of mice studied, the short duration of exposure, and the use of a single dose.]

Three groups of male B6C3F$_1$ mice (age, 5 weeks) were given drinking-water containing trichloroacetic acid at a concentration of 0, 1, or 2 g/L for up to 52 weeks (Bull *et al.*, 1990). Solutions of trichloroacetic acid were neutralized with sodium hydroxide (NaOH). Five mice in the groups at 0 and 2 g/L were killed at 15, 24, 37 weeks. Eleven animals from each group had treatment suspended at 37 weeks and were killed at 52 weeks. Thirty-five mice in the control group, 11 mice treated with trichloroacetic acid at 1 g/L, and 24 mice treated with trichloroacetic acid at 2 g/L were killed after 52 weeks of continuous treatment. Only the liver was examined for macroscopic lesions, and histopathology was not conducted on all lesions. One mouse out of 35 in the control group was found to have a hyperplastic nodule at 52 weeks. Mice treated with trichloroacetic acid at 1 g/L for 52 weeks were found to have three hyperplastic nodules, two liver [hepatocellular] adenomas, and two hepatocellular carcinomas distributed among five of the eleven mice treated [$P < 0.05$ versus controls]. At 2 g/L, 10 hyperplastic nodules, 1 liver [hepatocellular] adenoma and 4 hepatocellular carcinomas were found among 19 of the 24 mice treated [$P < 0.05$ versus controls]. Mice for which treatment at 2 g/L was terminated at 37 weeks and that were killed at 52 weeks were found to have two hyperplastic nodules, no adenomas, and three carcinomas distributed among four of the eleven mice treated. [The Working Group noted that this study was limited by observations being restricted to the liver, limited histopathological confirmation of diagnoses, varying numbers of animals in control and treatment groups, and the short duration of the study.]

In another study, groups of female B6C3F$_1$ mice (age, 7 weeks) were given drinking-water containing trichloroacetic acid at 0 (controls; drinking-water contained sodium chloride at 20 mM to control for the neutralization of trichloroacetic acid with NaOH in treated groups; $n = 134$), 2.0 ($n = 93$), 6.67 ($n = 46$), or 20 mM ($n = 38$) for 360 or 576 weeks (Pereira, 1996). The concentrations of trichloroacetic acid in drinking-water corresponded to 0.327, 1.09, and 3.27 g/L. Histopathology examination was restricted to the liver. The incidence of tumours was reported as the number of tumour-bearing mice compared with the total number of mice examined at termination of the experiments. The number of mice examined varied greatly between treatment groups ($n = 18$–90). The results for the incidence of hepatocellular adenoma and of hepatocellular carcinoma are provided in Table 3.1. A statistically significant treatment-related increase in the incidence of hepatocellular adenoma and of hepatocellular carcinoma was observed. A greater response was reported at 567 days than at 360 days. [The Working Group

Table 3.1 Studies of carcinogenicity in experimental animals given trichloroacetic acid by oral administration

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	NaCl, 2 g/L; TCA, 5 g/L, in drinking-water 22/group	Hepatocellular adenoma: 2/22, 8/22* Hepatocellular carcinoma: 0/22, 7/22**	*[$P < 0.05$] **$P < 0.01$	Purity, > 99% Small number of mice. Histopathology restricted to the liver. Decreases in drinking-water consumption likely at this high dose. Short duration of exposure.
Mouse, B6C3F$_1$ (M) 52 wk Bull *et al.* (1990)	0, 1, 2 g/L, in drinking-water 35, 11, 24/group	Gross lesions: liver hyperplastic nodules, liver adenoma or hepatocellular carcinoma (combined): 2/35 [6%], 5/11* [45%], 19/24* [79%] Mice with confirmed hepatocellular carcinoma: 0, 2, 4	*[$P < 0.05$]	Analytical grade; purity, NR Histopathology restricted to the liver and not conducted on all gross lesions. Short exposure duration. Two groups of 10 females treated at 0 or 2 g/L were maintained until wk 52; no lesions were noted by the authors.
Mouse, B6C3F$_1$ (F) Up to 576 days Pereira (1996)	NaCl, 20 mM; TCA, 2.0, 6.67, 20.0 mM in drinking-water [0, 0.327, 1.09, and 3.27 g/L] 134, 93, 46, 38/group	*360 days* Hepatocellular adenoma: 1/40, 3/40, 3/19, 2/20 Hepatocellular carcinoma: 0/40, 0/40, 0/19, 5/20* *576 days* Hepatocellular adenoma: 2/90, 4/53, 3/27, 7/18* Hepatocellular carcinoma: 2/90, 0/53, 5/27*, 5/18*	*$P < 0.05$	Purity, NR Histopathology restricted to the liver.
Mouse, B6C3F$_1$ (M) 52 wk Bull *et al.* (2002)	0, 0.5, 2 g/L, in drinking-water 32, 20, 40/group	Liver adenoma: 0/20, 5/20*, 6/20* Hepatocellular carcinoma: 0/20, 3/20, 3/20 Hepatocellular adenoma or carcinoma (combined): 0/20, 6/20**, 8/20***	*[$P < 0.05$] ** [$P < 0.05$] *** [$P < 0.05$]	Purity, NR Study was focused on tumour phenotype and did not provide histopathology for all lesions.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 60 wk or 104 wk DeAngelo *et al.* (2008)	*Experiment 1 (60 wk):* NaCl, 2 g/L; TCA, 0.05, 0.5, 5 g/L in drinking-water 50/group *Experiment 2 (104 wk):* NaCl, 2 g/L; TCA, 4.5 g/L, in drinking-water 57, 58/group *Experiment 3 (104 wk):* acetic acid, 1.5 g/L; TCA, 0.05, 0.5 g/L, in drinking-water 72/group [In all experiments, all groups included interim kills]	*Experiment 1* Hepatocellular adenoma: 7% (n = 30), 15% (n = 27), 21% (n = 29), 38% (n = 29)* Hepatocellular carcinoma: 7% (n = 30), 4% (n = 27), 21% (n = 29), 38% (n = 29)* Hepatocellular adenoma or carcinoma (combined): 13% (n = 30), 15% (n = 27), 38% (n = 29)**, 55% (n = 29)*** *Experiment 2* Hepatocellular adenoma: 0% (n = 25), 59% (n = 36)* Hepatocellular carcinoma: 12% (n = 25), 78% (n = 36)* Hepatocellular adenoma or carcinoma (combined): 12% (n = 25), 89% (n = 36)* *Experiment 3* Hepatocellular adenoma: 21% (n = 42), 23% (n = 35), 51% (n = 37)* Hepatocellular carcinoma: 55% (n = 42), 40% (n = 35), 78% (n = 37)* Hepatocellular adenoma or carcinoma (combined): 64% (n = 42), 57% (n = 35), 87% (n = 37)*	* $P \leq 0.03$	The data set was reported as one study, but was actually three separate experiments, each with a different background tumour rate. The controls received different dosing solutions. There was limited reporting of lesions except for liver tumours. Some tumour data appears to have been mislabelled. The difference in data for 60-week and 104-week exposure could not be easily interpreted due to differences between the paradigms and in background tumour rate.
Rat, F344/N (M) 104 wk DeAngelo *et al.* (1997)	NaCl, 2 g/L; TCA, 0.05, 0.5, 5 g/L in drinking-water 50/group [All groups included interim kills]	Hepatocellular adenoma: 4.4% (n = 23), 4.2% (n = 24), 15% (n = 20), 4.6% (n = 22) Hepatocellular carcinoma: 0% (n = 23), 0% (n = 24), 0% (n = 20), 4.6% (n = 22)	NS	Purity, > 99% Small number of animals/group. Study focused on liver tumours, with limited histopathological examination and reporting of results. Historical background incidences of hepatocellular adenoma and carcinoma are low for this species and strain; the incidences of adenoma at the intermediate dose and carcinoma at highest dose are higher than in concurrent and historical controls.

F, female; M, male; NaCl, sodium chloride; NR, not reported; NS, not significant; TCA, trichloroacetic acid; wk, week

noted that this study was limited by examination being restricted to the liver.]

A similar paradigm was reported as part of an initiation–promotion study in female B6C3F$_1$ mice (age, 6 weeks) exposed for a shorter period (Pereira & Phelps, 1996). Groups of 10–15 mice were exposed for 31 weeks and groups of 19–40 mice were exposed for 52 weeks to trichloroacetic acid at the same concentrations used in Pereira (1996). The study gave negative results after 31 weeks of exposure to trichloroacetic acid alone. After 52 weeks, 4 out of 20 [$P < 0.05$] mice at 20.0 mM developed hepatocellular carcinoma (0.50 ± 0.18 tumour per mouse; $P < 0.05$, Mann–Whitney test) versus 0 out of 40 controls. [The Working Group noted that the study was limited by examination being restricted to the liver, and that the small number of animals studied and brief exposure period limited the power of the study to detect a carcinogenic response.]

A study was conducted to determine the extent to which trichloroacetic acid and dichloroacetic acid contribute to the carcinogenicity of trichloroethylene in B6C3F$_1$ mice. The study included groups that received treatment with trichloroacetic acid alone (Bull *et al.*, 2002). Histopathology was restricted to the liver. In these groups, male mice were treated with drinking-water containing trichloroacetic acid (neutralized with NaOH) at 0, 0.5 or 2 g/L for 52 weeks. Dose-dependent increases in the incidences of liver [hepatocellular] adenoma, and liver adenoma or hepatocellular carcinoma (combined) were observed in mice treated with trichloroacetic acid (Table 3.1). [The Working Group noted that the study was limited by examination being restricted to the liver, the short duration of exposure, the uncertainty of reporting lesion prevalence (i.e. random selection of gross lesions for histopathological examination), issues of lesion grouping, and the limited statistical power.]

The carcinogenicity of trichloroacetic acid and of several other chemicals was assessed in groups of 23–24 male and groups of 23–24 female neonatal B6C3F$_1$ mice (Von Tungeln *et al.*, 2002). In a first experiment, three sevenths of the total dose of trichloroacetic acid (2000 nmol per mouse, i.e. 327 µg per mouse) was administered by intraperitoneal injection on postnatal day 8 and four sevenths of the total dose on postnatal day 15. The mice were observed for 12 months. In a 20-month experiment, one third of the total dose (1000 nmol per mouse, i.e. 164 µg per mouse) was administered on postnatal day 8 and the remaining two thirds on postnatal day 15. [The dose in mg/kg bw was not reported and trichloroacetic acid was apparently not neutralized.] Histopathology was restricted to the liver. The incidences of hepatocellular adenoma, carcinoma and adenoma or carcinoma (combined) were not increased in either experiment. [The Working Group noted that the study was limited by histopathological examination being restricted to the liver, the small numbers of animals studied, the nonphysiological route of exposure, and the use of a low dose.]

In a study in male B6C3F$_1$ mice given drinking-water containing trichloroacetic acid (DeAngelo *et al.*, 2008), the three experiments reported were conducted in separate laboratories, as indicated by the very different background rates of tumour incidence. In these experiments, different doses were used with different control solutions. The first experiment involved mice treated with drinking-water containing trichloroacetic acid at concentrations of 0 (control), 0.05, 0.5 or 5 g/L for 60 weeks. The control group was given drinking-water containing sodium chloride at 2 g/L. In the second experiment, mice were treated with trichloroacetic acid at a concentration of 0 (control) or 4.5 g/L for 104 weeks. In this case, the control group received acetic acid at 1.5 g/L. In the third experiment, mice were treated with trichloroacetic acid at 0 (control), 0.05, or 0.5 g/L for 104 weeks. The control group for this experiment was given sodium chloride at 2 g/L. There was limited

reporting of lesions, except for liver tumours. The incidence of hepatocellular adenoma and of hepatocellular carcinoma in each experiment is presented in Table 3.1. In the first experiment, the incidences of hepatocellular adenoma and hepatocellular carcinoma were increased (relative to controls) in mice treated at 5 g/L for 60 weeks. The incidence of hepatocellular adenoma or carcinoma (combined) was increased in mice at 0.5 g/L. In the second experiment, substantial increases in the incidences of both tumour types were observed in mice treated with trichloroacetic acid at 4.5 g/L at 104 weeks relative to controls. In the third experiment, the incidences of both tumour types were also increased in mice treated with trichloroacetic acid at 0.5 g/L at 104 weeks relative to controls. [The Working Group noted that there was limited reporting of lesions except for liver tumours, that there was also no allowance of full expression of tumour response within 60 weeks, and that there was a very high background rate of incidence of liver tumours in control animals for the third experiment.]

3.2 Rat

Only one study in rats treated with trichloroacetic acid was available (DeAngelo *et al.*, 1997). Groups of 50 male F344/N rats (age, 28–30 days) were given drinking-water containing trichloroacetic acid at a concentration of 0, 0.05, 0.5, or 5 g/L. Trichloroacetic acid was neutralized with NaOH; to compensate for the resultant salt formation, control rats were given drinking-water containing sodium chloride at 2 g/L. Results were reported for 23, 24, 19, and 22 rats in the control group and groups at 0.05, 0.5, and 5.0 g/L, respectively, that were examined at terminal kill at 104 weeks, with one extra rat (from the group at the intermediate dose) examined for the liver tumour analysis. Six rats in the control group, and 8, 13, and 7 rats in the groups at the lowest, intermediate, and highest dose died during the course of the study. Interim kills of six rats per group were also carried out at 15, 30, 45, and 60 weeks for separate enzyme analysis. Complete gross pathological examinations were performed for rats in the group at the highest dose at 104 weeks; light microscopic examination was performed for kidney, liver, spleen, and testis only at the highest dose. [No indication was given as to whether a complete necropsy and pathological examination was performed for rats in the control group at terminal kill.] It was reported that trichloroacetic acid slightly decreased water consumption at all doses. Only data on liver tumours were presented, but not evaluated microscopically for rats that died early. The data were reported as the percentage of rats examined with hepatocellular adenoma (4.4%, 4.2%, 15%, and 4.6% for each group, respectively) and hepatocellular carcinoma (i.e. 0%, 0%, 0%, and 4.6%) as shown in Table 3.1. Although there were increases in the incidence of adenoma in the group receiving the intermediate dose, and in the incidence of carcinoma in the group receiving the highest dose, these increases were not statistically significant. [The Working Group noted that the historical control values for hepatocellular tumours, adenoma and especially carcinoma, are low in this species and strain, and that the percentage of rats with tumours exceeding the value for concurrent controls in this study also exceeds historical background levels (Haseman *et al.*, 1998). The Working Group also noted that this study was limited by the relatively small numbers of animals examined at 104 weeks and the limited histopathological examination and reporting of results.]

3.3 Administration with known carcinogens or other modifying factors

There have been several initiation–promotion studies with trichloroacetic acid, as well as studies of interactions with other carcinogenic

chemicals in which trichloroacetic acid consistently displayed hepatocellular tumour-promoting activity (Herren-Freund *et al.*, 1987; Pereira & Phelps, 1996; Pereira *et al.*, 1997, 2001; Bull *et al.*, 2004).

Two of these studies, which showed the most significant results in terms of promotion of hepatocellular tumours, are described here in more detail.

The Herren-Freund *et al.* (1987) study cited in Section 3.1 was an initiation–promotion study in male B6C3F$_1$ mice. Histopathological examination was restricted to the liver. Mice were initiated by intraperitoneal injection with *N*-ethyl-*N*-nitrosourea (ENU) at a dose of 2.5 or 10 mg/kg bw on postnatal day 15. Drinking-water containing trichloroacetic acid at 2 or 5 g/L was given from postnatal day 28 for 61 weeks. Controls treated with ENU only were given drinking-water containing sodium chloride at 2.5 g/L. The incidences of hepatocellular adenoma and carcinoma in mice treated only with ENU at 2.5 mg/kg bw were 1 out of 22, and 1 out of 22, respectively, and the incidences in mice treated only with ENU at 10 mg/kg bw were 9 out of 23, and 9 out of 23, respectively. The incidences in mice initiated with ENU at 2.5 mg/kg bw and subsequently treated with trichloroacetic acid at 2 g/L were 11 out of 33 ($P < 0.01$), and 16 out of 33 ($P < 0.01$), respectively. When mice were initiated with ENU at the same dose, but subsequently treated with trichloroacetic acid at 5 g/L, the incidences of these tumours were 6 out of 23 ($P < 0.01$), and 11 out of 23 ($P < 0.01$), respectively. The incidences in mice initiated with ENU at 10 mg/kg bw and treated with trichloroacetic acid at 5 g/L were 11 out of 28, and 15 out of 28, respectively.

In an initiation–promotion study with trichloroacetic acid in female B6C3F$_1$ mice (Pereira & Phelps, 1996), groups of mice were initiated with *N*-methyl-*N*-nitrosourea (MNU) as an intraperitoneal dose at 25 mg/kg bw on postnatal day 15. Treatment with drinking-water containing trichloroacetic acid began at age 7 weeks and was continued for 31 or 52 weeks. Histopathological examination was restricted to the liver. The data were expressed as numbers of hepatocellular tumours per mouse and percentage of mice with the indicated tumour were provided in parentheses. Concentrations of trichloroacetic acid were 2, 6.67, and 20.0 mM [i.e. 0.327, 1.09, and 3.27 g/L]. A "recovery" group was given trichloroacetic acid at 20 mM for 31 weeks, at which point treatment was suspended, and the mice were killed at week 52. Mice in the control group were given drinking-water containing sodium chloride at 2 mM. In MNU-initiated mice treated with trichloroacetic acid for 52 weeks, there were increases in the number of hepatocellular adenomas ($P < 0.05$) and hepatocellular carcinomas ($P < 0.05$) in the groups at 6.7 and 20 mM relative to controls treated with MNU only, i.e. hepatocellular adenoma: 0.28 ± 0.11 (17.5%), 2.00 ± 0.82 (83.3%), and 1.29 ± 0.24 (66.7%); hepatocellular carcinoma: 0.10 ± 0.05 (10%), 1.33 ± 0.42 (83.3%), and 2.79 ± 0.48 (83.3%); for the control group, and groups at 6.7 and 20 mM, respectively. The incidences of hepatocellular adenoma and hepatocellular carcinoma were also increased ($P < 0.05$) in the "recovery group": 0.91 ± 0.28 (63.6%), and 0.73 ± 0.33 (36.4%), respectively.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans and animals, trichloroacetic acid is rapidly absorbed dermally or orally, but is only slowly metabolized, accumulating to a steady-state after successive exposures. Most of the absorbed dose is excreted in the urine as the parent compound. Metabolism that does occur

is mainly oxidative through cytochrome P450 to dichloroacetic acid via a dichloroacetic acid radical (shown in Fig. 4.1). The results of several studies indicate that the urinary elimination or plasma clearance of trichloroacetic acid is slower in humans than in rodents. Also, protein binding in the plasma is greater in humans than in rodents.

4.1.1 Absorption

(a) Humans

Trichloroacetic acid is rapidly absorbed by the oral and dermal routes of exposure, based on the results of studies with drinking-water and in swimming pools treated with disinfectants, including trichloroacetic acid. In studies by Kim & Weisel (1998), subjects stood in swimming pools for 30 minutes and the content of trichloroacetic acid of the pool water was compared with that of urine samples taken before and after exposure. Urinary excretion of trichloroacetic acid, an indirect biomarker of exposure, increased with increasing exposure. No studies were available on the absorption of inhaled trichloroacetic acid.

(b) Experimental systems

Studies in animals also indicated that trichloroacetic acid is rapidly absorbed in the gut and is slowly metabolized (Larson & Bull, 1992a; Xu *et al.*, 1995). In studies by Larson & Bull (1992a), male F344 rats and B6C3F$_1$ mice were given single oral doses of ^{14}C-labelled trichloroacetic acid of between 5 and 100 mg/kg bw. Radiolabel recovered in the urine over 48 hours ranged from 57% to 72% of the administered dose, with 81–90% being parent compound. In expired air, radiolabelled carbon dioxide ($^{14}CO_2$) represented 4–8% of the administered dose. The plasma half-life of trichloroacetic acid was about 6 hours, and the volume of distribution was 365–485 mL/kg in rats and 335–555 mL/kg in mice. These data indicate the ready absorption of trichloroacetic acid.

4.1.2 Distribution

(a) Humans

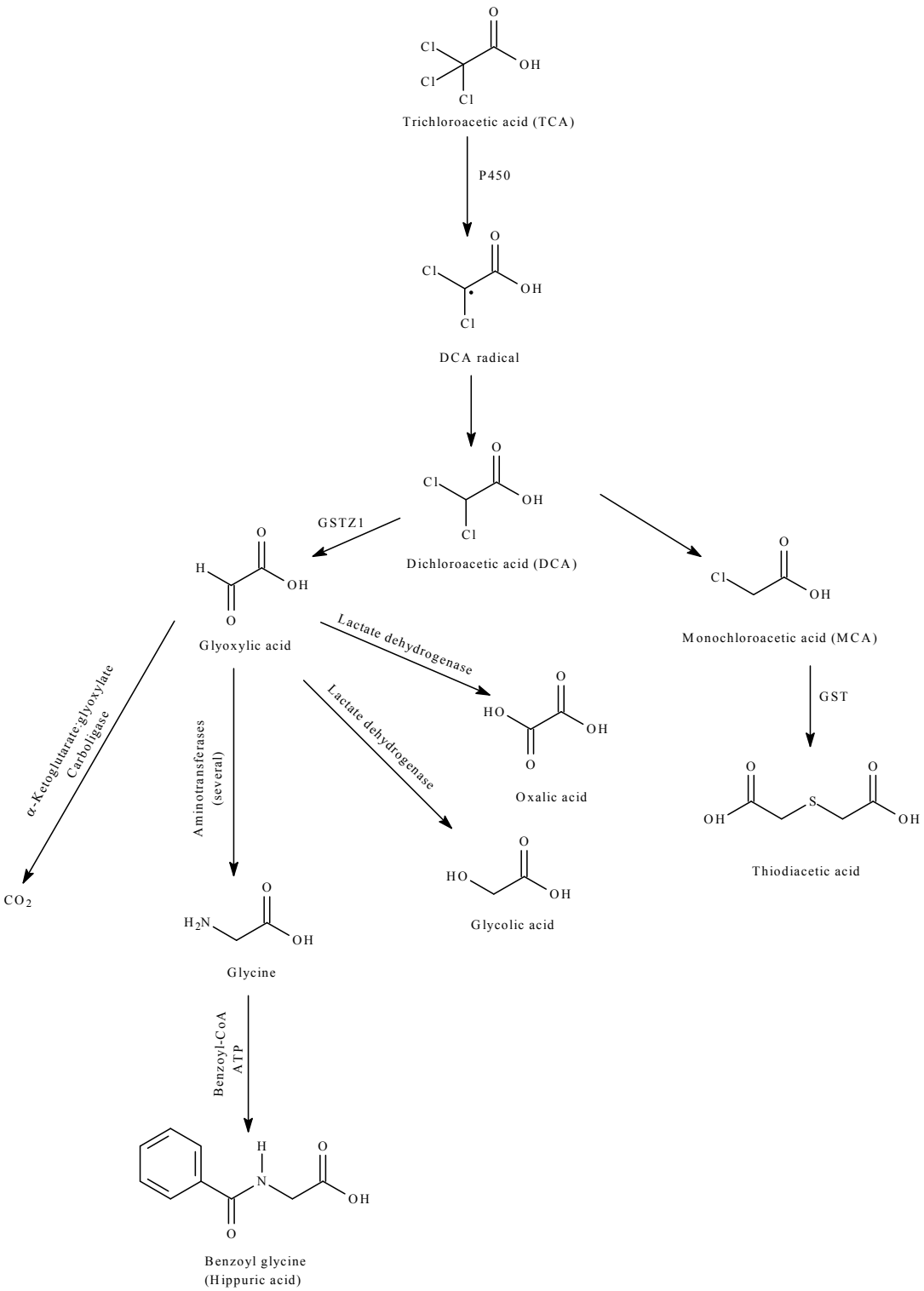
In a study by Müller *et al.* (1974), the half-life of trichloroacetic acid in plasma was approximately 50 hours and the volume of distribution was 115 mL/kg after a single oral dose of 3 mg/kg bw in healthy volunteers. The long half-life of plasma trichloroacetic acid is consistent with extensive binding to plasma proteins (Sellers & Koch-Weser, 1971; Stenner *et al.*, 1997, 1998). Templin *et al.* (1995) found that approximately 80% of ^{14}C-labelled trichloroacetic acid (dose, 6–61 nmol/mL [0.98–10 mg/mL]) in human plasma was bound to protein, a percentage that is higher than that observed in animals (see below).

A study by Lumpkin *et al.* (2003) used equilibrium dialysis to measure the binding of trichloroacetic acid at a range of concentrations to plasma of humans, rats and mice. Dissociation values did not vary between species, but the number of binding sites in plasma was higher in humans (2.97) than in rats (1.49) or mice (0.17). The greater plasma-protein binding in human plasma would be expected to increase the residence time for trichloroacetic acid in plasma and also reduce the amount of trichloroacetic acid available in other tissues.

(b) Experimental systems

The tissue distribution of absorbed trichloroacetic acid was reported by Yu *et al.* (2000). Male F344 rats were injected intravenously with ^{14}C-labelled trichloroacetic acid at doses of 0, 1, 10 or 50 mg/kg bw. Concentrations of trichloroacetic acid equivalents, based on detected radioactivity, were measured in plasma and in tissues at various times up to 24 hours after exposure. At early time-points, the highest concentrations of trichloroacetic acid equivalents were found in plasma, followed by kidney, erythrocytes, liver, skin, small intestine, muscle and fat. However, by 24 hours, the highest concentrations were found in the liver. Yu *et al.* (2000) hypothesized that

Fig. 4.1 Proposed metabolism of trichloroacetic acid



GST, glutathione-*S*-transferase; GSTZ1, GST-zeta1; P450, cytochrome P450
Prepared by the Working Group

trichloroacetic acid was cleared from the liver more slowly than from the plasma because of a concentration-transport process in hepatocyte plasma membranes.

Templin *et al.* (1995) measured the binding of ^{14}C-labelled trichloroacetic acid to protein in the plasma of the mouse, rat, and dog. At concentrations of 6–61 nmol/mL [0.98–10 mg/mL], the percentage bound was approximately 60% (dog) or 50% (mouse and rat).

4.1.3 Metabolism

According to data from studies in humans and animals, trichloroacetic acid is poorly metabolized, with most of the absorbed dose being excreted as the parent compound in the urine.

(a) Humans

In studies reported by Paykoc & Powell (1945), six patients received trichloroacetic acid in aqueous solution at a dose of between 1.5 and 3 g via an intravenous drip for 1 hour. By 10 days after dosing, approximately 75% of the dose had been excreted unchanged in the urine, indicating little metabolism. No metabolites were measured. The evidence for metabolites of trichloroacetic acid comes from studies in experimental animals, described below.

(b) Experimental systems

Fig. 4.1 shows a proposed scheme for metabolism based on the results of studies in experimental animals. In the study by Xu *et al.* (1995), male B6C3F$_1$ mice were given uniformly labelled ^{14}C-labelled trichloroacetic acid at a dose of 100 mg/kg bw. After 24 hours, the distribution of radioactivity, as a percentage of the administered dose, was 55% in urine, 5% in exhaled CO_2 and 5% in faeces. Small amounts of urinary radioactivity in both studies were found in the form of metabolites, dichloroacetate, monochloracetate, glyoxylate, glycolate, and oxalate. In the studies by Larson & Bull, the metabolism of trichloroacetic acid was studied in male Fischer 344 rats and male B6C3F$_1$ mice (Larson & Bull, 1992a). The animals were given ^{14}C-labelled trichloroacetic acid orally at a dose of 5, 20 or 100 mg/kg bw. Approximately 50% of any dose of trichloroacetic acid was excreted unchanged in the urine of rats and mice. The half-life of trichloroacetic acid in rats and mice given a dose of 20 or 100 mg/kg bw ranged from 4.2 to 7.0 hours, and the clearance ranged from 36 to 66 mL/kg per hour. The combined excretion of glyoxylic acid, oxalic acid and glycolic acid (known metabolites of dichloroacetic acid) in urine amounted to 4.9–10.8% of the administered dose. Dichloroacetic acid was detected in the urine of rats and mice, indicating the reduction of trichloroacetic acid. Trichloroacetic acid thus undergoes reduction to the dichloroacetyl radical (•CCl_2COOH), which may abstract a hydrogen atom to form dichloroacetic acid, or may react with oxygen to form a hydroperoxyl radical (•$OOCCl_2COOH$) that may yield oxalic acid (Larson & Bull, 1992a).

The metabolic fate of trichloroacetic acid has been investigated in male B6C3F$_1$ mice given [1,2-^{14}C]-labelled trichloroacetic acid at a dose of 100 mg/kg bw by gavage. About 5%, 55% and < 10% of the administered dose was eliminated in exhaled air as CO_2, in the urine, and in the faeces, respectively; about 25% was found in the carcass. Trichloroacetic acid, dichloroacetic acid, monochloroacetic acid, glyoxylic acid, glycolic acid, oxalic acid and unidentified metabolites accounted for 44.5%, 0.2%, 0.03%, 0.06%, 0.11%, 1.5%, and 10.2% of the urinary metabolites (Xu *et al.*, 1995).

The metabolism of trichloroacetic acid to dichloroacetic acid was studied in control and treated male B6C3F$_1$ mice given trichloroacetic acid intravenously at a dose of 100 mg/kg bw (Merdink *et al.*, 1998). In contrast with other reports (Larson & Bull, 1992a; Xu *et al.*, 1995), quantifiable concentrations of dichloroacetic acid were not detected in the blood of mice given

trichloroacetic acid. Although there is uncertainty about the metabolism of trichloroacetic acid to dichloroacetic acid, pharmacokinetic simulations indicate that dichloroacetic acid is probably formed as a short-lived metabolite of trichloroacetic acid and that its rapid elimination compared with its relatively slow formation prevents ready detection (Merdink *et al.*, 1998). The artefactual formation of dichloroacetic acid from trichloroacetic acid has also been noted: for example, trichloroacetic acid was converted to dichloroacetic acid in freshly drawn blood samples (Ketcha *et al.*, 1996).

The tissue disposition and elimination of ^{14}C-labelled trichloroacetic acid was studied in male Fischer 344 rats given trichloroacetic acid intravenously at a dose of 6.1, 61 or 306 µmol/kg bw [1, 10, or 50 mg/kg bw] (Yu *et al.*, 2000). The fraction of the initial dose excreted in the urine increased from 67% to 84% as the dose increased, and faecal excretion decreased from 7% to 4%. The elimination of trichloroacetic acid as CO_2 decreased from 12% to 8% of the total dose. The hepatic intracellular concentrations of trichloroacetic acid were significantly greater than the free plasma concentrations, indicating concentrative uptake by hepatocytes, and that trichloroacetic acid filtered at the glomerulus appears to be reabsorbed from either the renal tubular urine or the bladder (Yu *et al.*, 2000).

The biotransformation of trichloroacetic acid was studied in hepatic microsomal fractions isolated from control and pyrazole-treated male B6C3F$_1$ mice (Ni *et al.*, 1996). When trichloroacetic acid (5 mM [817 µg/mL]) was incubated with a microsomal fraction and a NADPH-generating system in the presence of the spin trap *N*-tert-butyl-α-phenyl-nitrone [the concentration of oxygen (O_2) in the closed reaction flasks was not stated], analysis by electron-spin resonance spectroscopy indicated the presence of a carbon-centred radical, which was not characterized (Ni *et al.*, 1996). Microsomal fractions from male B6C3F$_1$ mice or Fischer 344 rats were incubated with a NADPH-generating system, trichloroacetic acid (1 mM) [163.4 µg/mL], and the spin trap phenyl-*tert*-butyl nitroxide in an argon (anaerobic) atmosphere. Gas chromatographic-mass spectrometric analysis of methylated extracts of the reaction mixture revealed the formation of 2-*tert*-butyl-4,4-dichloro-3-phenylisoxazolidin-5-one derived from the dichloroacetate radical. The same product was formed when trichloroacetic acid was incubated with phenyl-*tert*-butyl nitroxide, ferrous sulfate and hydrogen peroxide (Fenton reaction system) (Merdink *et al.*, 2000).

Overall, the identified metabolites of trichloroacetic acid include dichloroacetic acid and its metabolites (CO_2, glyoxylic acid, oxalic acid, and glycolic acid). The extent of metabolism is not large, with identified metabolites accounting for less than 20% of the administered dose. Recovery of trichloroacetic acid in urine is around 50–75%, and the remaining fraction unidentified.

4.1.4 Excretion

(a) Humans

The major excretory route for trichloroacetic acid is the urine (see Section 4.1.3a).

Several studies have examined the elimination half-life of trichloroacetic acid in humans. The plasma half-life of trichloroacetic acid ranged from 4 to 5 days after oral ingestion of chloral hydrate at 15 mg/kg bw (Breimer *et al.*, 1974) and was 50.6 hours after oral administration of trichloroacetic acid at 3 mg/kg bw (Müller *et al.*, 1972, 1974). Paykoc & Powell (1945) reported a plasma half-life of 82 hours for trichloroacetic acid administered intravenously in six patients.

Froese *et al.* (2002) estimated the half-life of trichloroacetic acid in humans after drinking tap-water containing a range of disinfection products, including trichloroacetic acid. The intake of trichloroacetic acid was 20–82 µg/day during the 12-day study period. Although 10 volunteers (eight men, two women) were enrolled in the

study, useful elimination data were obtained for only three, in whom the elimination half-lives ranged from 2.3 to 3.67 days.

In the swimming pool studies of Kim & Weisel (1998) urinary excretion of trichloroacetic acid, in terms of μg per m^2 of surface area, increased with increasing exposures. Peak urinary trichloroacetic acid excretion rates occurred within 5 to 10 minutes after exposure and TCA was no longer detected after 3 hours. The data were consistent with rapid dermal absorption and subsequent urinary excretion of trichloroacetic acid.

Studies by the same group (Kim *et al.*, 1999), in which the content of trichloroacetic acid of chlorinated drinking-water ingested by subjects was compared to urinary trichloroacetic acid output, also indicated that trichloroacetic acid was rapidly absorbed from the gastrointestinal tract and was only slowly metabolized, accumulating to a steady-state after successive exposures. In additional studies of swimming pools, Cardador & Gallego (2011) analysed the urine of 49 swimmers (adults and children, male and female) after 1 hour of swimming and found trichloroacetic acid at an average concentration of 4400 ng/L. The major route of exposure was determined to be oral, with 5% exposure by inhalation and about 1% exposure via the dermal route.

Overall, available studies in humans indicated that trichloroacetic acid is slowly metabolized and excreted unchanged in urine, with a half-life estimated to be between 2 and 4 days (Paykoc & Powell, 1945; Müller *et al.*, 1972, 1974; Froese *et al.*, 2002).

(b) Experimental systems

In a study by Xu *et al.* (1995), male $B6C3F_1$ mice were given uniformly labelled ^{14}C-labelled trichloroacetic acid at a dose of 100 mg/kg bw. After 24 hours, the distribution of radioactivity, as a percentage of the administered dose, was 55% in urine, 5% in exhaled CO_2 and 5% in faeces.

Larson & Bull (1992a) studied excretory routes for trichloroacetic acid in male Fischer 344 rats and male $B6C3F_1$ mice. Results are detailed above in Section 4.1.3.

The kinetics of elimination were studied in male $B6C3F_1$ mice given trichloroacetic acid by gavage at a dose of 0.03, 0.12, or 0.61 mmol/kg bw [5, 20 and 100 mg/kg bw] (Templin *et al.*, 1993). The half-life ranged from 5.4 to 6.4 hours. A comparison of the area under the concentration–time curve for distribution of trichloroacetic acid to the blood and liver after exposure to trichloroethylene showed that distribution favoured the blood over the liver.

The half-lives for the elimination of trichloroacetic acid in male Fischer 344 rats given oral doses of 0.15 or 0.76 mmol/kg bw [24.5 and 124 mg/kg bw] were 7.9 and 13 hours, respectively, while those in male beagle dogs given oral doses of 0.15, 0.38, or 0.76 mmol/kg bw [24.5, 62, and 124 mg/kg bw] were 200, 175 and 238 hours, respectively (Templin *et al.*, 1995).

Stenner *et al.* (1997) reported similar concentrations of trichloroacetic acid in blood and bile of rats given trichloroacetic acid intravenously at a dose of 100 mg/kg bw, suggesting that enterohepatic recirculation may be occurring.

The kinetics of the elimination of trichloroacetic acid as a metabolite of inhaled trichloroethylene or after intravenous administration has been reported in pregnant rats and in lactating rats and nursing pups (Fisher *et al.*, 1989, 1990). In pregnant rats given trichloroacetic acid intravenously at a dose of 4 mg/kg bw on days 14–15 of pregnancy, the elimination rate constant was 0.045/h. Fetal exposure to trichloroacetic acid was estimated at 63–64% of the maternal dose (Fisher *et al.*, 1989). The elimination rate constant in lactating dams given trichloroacetic acid intravenously at 4.4 mg/kg bw was 0.086/hour.

4.2 Genotoxicity and related effects

Trichloroacetic acid has been studied in a variety of assays for genotoxic potential. Table 4.1, Table 4.2, and Table 4.3 summarize the studies carried out *in vivo*, *in vitro*, and in bacterial systems, respectively. [The Working Group noted that the evaluation of the studies of genotoxic potential *in vitro* must consider cytotoxicity and acidification of medium resulting in precipitation of proteins, as trichloroacetic acid is commonly used as a reagent to precipitate proteins.]

4.2.1 Human cell lines

Mackay *et al.* (1995) investigated trichloroacetic acid (as free acid) in an assay for chromosomal aberration *in vitro* using cultured human lymphocytes, both in the presence and absence of metabolic activation. Trichloroacetic acid induced chromosomal damage at concentrations (2000 and 3500 µg/mL) that significantly reduced the pH of the medium. However, neutralized trichloroacetic acid was without effect, even at a cytotoxic concentration of 5000 µg/mL. To further evaluate the role of pH changes in the induction of chromosomal damage, isolated liver-cell nuclei from $B6C3F_1$ mice were suspended in a buffer at various pH levels. A decrease in chromatin staining intensity was observed as pH decreased, suggesting that pH changes, independent of trichloroacetic acid exposure, can alter chromatin conformation. [The Working Group noted that it was possible that the reduced pH was responsible for clastogenicity induced by trichloroacetic acid in this study].

Chang *et al.* (1992) used the human lymphoblastic cell line, CCRF-CEM, to study the effect of trichloroacetic acid on DNA single-strand breaks. No effect was observed.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

Harrington-Brock *et al.* (1998) examined the potential of trichloroacetic acid at concentrations up to 2150 µg/mL without metabolic activation, and up to 3400 µg/mL with metabolic activation from S9 (9000 × *g* supernatant) to induce mutations in L5178Y/*Tk*+/−–3.7.2C mouse lymphoma cells. The mutation frequency doubled at concentrations of ≥ 2250 µg/mL with metabolic activation, including at several concentrations at which survival was > 10%. In the absence of metabolic activation, trichloroacetic acid increased the mutation frequency by twofold or greater only at concentrations of 2000 µg/mL with 11% survival rates. Both large- and small-colony mutants were observed, with small-colony mutants indicative of chromosomal damage. [The Working Group noted that no rigorous statistical analysis was conducted in this study.]

Trichloroacetic acid was tested in a microplate-based test for cytotoxicity, and an assay for HGPRT [*Hprt*] gene mutation in Chinese hamster ovary K1 cells (Zhang *et al.*, 2010b). Trichloroacetic acid was the least cytotoxic of seven haloacetic acids tested. Trichloroacetic acid, at concentrations of 0, 200, 1000, 5000 and 10 000 µM [33, 163, 817 and 1630 µg/mL], visibly increased mutation frequency, but did not show any statistically significant increase at any of the doses tested.

(ii) Chromosomal aberration

Chromosomal aberrations in bone marrow were observed *in vivo* after intraperitoneal or oral administration of trichloroacetic acid in mice (Bhunya & Behera, 1987), and after intraperitoneal administration of trichloroacetic acid in chickens (Bhunya & Jena, 1996).

Table 4.1 Studies of genotoxicity with trichloracetic acid *in vivo*

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
DNA strand breaks, B6C3F$_1$ mouse liver	1630, oral, × 1	+	Nelson & Bull (1988)
DNA strand breaks, B6C3F$_1$ mouse liver	500, oral, × 1	+	Nelson *et al.* (1989)
DNA strand breaks, B6C3F$_1$ mouse liver	500, oral, 10 repeats	–	Nelson *et al.* (1989)
DNA strand breaks, B6C3F$_1$ mouse liver and epithelial cells from stomach and duodenum	1630, oral, × 1	–	Chang *et al.* (1992)
DNA strand breaks, male B6C3F$_1$ mice	500 (neutralized) oral, × 1	–	Styles *et al.* (1991)
DNA strand breaks, male B6C3F$_1$ mouse liver	77, oral, 1/day, 13 wk	+	Hassoun *et al.* (2010a)
Micronucleus formation, Swiss mice	125, i.p., × 2	+	Bhunya & Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes	1300, i.p., × 2	–	Mackay *et al.* (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes	1080, i.p., × 2	–	Mackay *et al.* (1995)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes	80 µg/mL	+	Giller *et al.* (1997)
Chromosomal aberrations, Swiss mouse bone-marrow cells	125, i.p., × 1	+	Bhunya & Behera (1987)
	100, i.p., × 5	+	Bhunya & Behera (1987)
	500, oral, × 1	+	Bhunya & Behera (1987)
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow	200, i.p., × 1	+	Bhunya & Jena (1996)
Sperm morphology, Swiss mice, *in vivo*	125, i.p., × 5	+	Bhunya & Behera (1987)

[a] Doses are in mg/kg bw unless otherwise specified.

+, positive; –, negative; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; wk, week

(iii) Micronucleus formation

Trichloroacetic acid caused micronucleus formation in the bone marrow of mice (Bhunya & Behera, 1987) and chickens (Bhunya & Jena, 1996) exposed *in vivo via* intraperitoneal injection. A small increase in the frequency of micronucleated erythrocytes at 80 µg/mL in a test for micronucleus formation in the newt (*Pleurodeles waltl* larvae) was observed after exposure to trichloroacetic acid (Giller *et al.*, 1997). However, Mackay *et al.* (1995) found that trichloroacetic acid did not induce formation of micronuclei in bone marrow of C57BL mice given trichloroacetic acid intraperitoneally at doses of up to 1080 mg/kg bw per day for males and 1300 mg/kg bw per day for females for two consecutive days.

(iv) Other studies of DNA damage

The ability of trichloroacetic acid to induce single-strand DNA breaks (SSBs) has been examined in several studies, both *in vivo* and *in vitro* (see Table 4.1; Table 4.2; Nelson & Bull, 1988; Nelson *et al.*, 1989; Styles *et al.*, 1991; Chang *et al.*, 1992).

Nelson & Bull (1988) evaluated the ability of single oral doses of trichloroacetic acid to induce SSBs in Sprague-Dawley rats and B6C3F$_1$ mice *in vivo*. Dose-dependent increases in the frequency of SSBs were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of trichloroacetic acid to produce significant SSBs was 0.6 mmol/kg (98 mg/kg bw) in rats, but 0.006 mmol/kg (0.98 mg/kg bw) in mice.

A single oral dose of trichloroacetic acid at 500 mg/kg bw rapidly induced SSBs in the liver of male B6C3F$_1$ mice, but repair of SSBs also

Table 4.2 Studies of genotoxicity with trichloroacetic acid in mammalian systems *in vitro*

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
DNA strand breaks, B6C3F$_1$ mouse and F344 rat hepatocytes	1630	NT	–	Chang *et al.* (1992)
DNA damage, Chinese hamster ovary cells, comet assay	490	NT	–	Plewa *et al.* (2002)
DNA strand breaks, human CCRF-CEM lymphoblastic cells	1630	NT	–	Chang *et al.* (1992)
Gene mutation, mouse lymphoma L5178Y/*Tk*+/- cells	2250	(+)	?	Harrington-Brock *et al.* (1998)
Gene mutation, *HGPRT*, Chinese hamster ovary cells	1630 µM	NT	–	Zhang *et al.* (2010b)
Chromosomal aberration, human lymphocytes	5000 (neutralized)	NT	–	Mackay *et al.* (1995)

[a] Doses are in µg/mL, unless otherwise stated

+, positive; (+), weakly positive; –, negative; ?, inconclusive; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested

proceeded rapidly, with a return to control levels within 8 hours after dosing (Nelson *et al.*, 1989). However, in the follow-up study in male B6C3F$_1$ mice given 10 repeated doses of trichloroacetic acid at 500 mg/kg bw by oral gavage, there was no effect on SSBs in whole-liver homogenate (Nelson *et al.*, 1989).

In a follow-up experiment with a similar experimental design, Styles *et al.* (1991) tested trichloroacetic acid for its ability to induce strand breaks in male B6C3F$_1$ mice in the presence and absence of liver growth induction. The test animals were given one, two, or three daily doses of neutralized trichloroacetic acid (500 mg/kg bw) by gavage and killed 1 hour after the final dose. Additional mice were given a single dose of 500 mg/kg bw by gavage and killed 24 hours after treatment. No induction of SSBs was observed in DNA from the liver under the conditions of this assay.

Chang *et al.* (1992) gave B6C3F$_1$ mice single oral doses of trichloroacetic acid (1–10 mmol/kg [163–1630 mg/kg]) and reported no dose-related effect on DNA strand breaks as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats given trichloroacetic acid by gavage at up to 5 mmol/kg (817 mg/kg bw).

DNA SSBs were increased a dose-dependent manner in male B6C3F$_1$ mice treated with trichloroacetic acid by gavage for 4 weeks or 13 weeks. SSBs were increased in the liver by 75%, 125%, and 300% at doses of 77, 154, and 410 mg/kg bw per day at 4 weeks; and by 125%, 200%, and 310% at doses of 77, 154, and 410 mg/kg bw per day at 13 weeks.

Plewa *et al.* (2002) evaluated the induction of DNA strand breaks induced by trichloroacetic acid (1–3 mM [163–490 µg/mL]) in Chinese hamster ovary cells *in vitro* and did not observe genotoxicity.

Chang *et al.* (1992) used primary hepatocytes from mouse or rat to investigate the potential of trichloroacetic acid to induce DNA strand breaks. No effect of trichloroacetic acid was observed.

(b) Bacterial systems: gene mutation

Trichloroacetic acid has been evaluated in several test systems *in vitro*, including bacterial assays (Ames) using different strains of *S. typhimurium*, such as TA98, TA100, TA104, TA1535, and RSJ100 (see Table 4.3). The majority of

Table 4.3 Studies of genotoxicity with trichloroacetic acid in bacterial systems

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
λ Prophage induction, *E. coli* WP2s	10 000	–	–	DeMarini *et al.* (1994)
SOS chromotest, *E. coli* PQ37	10 000	–	–	Giller *et al.* (1997)
S. typhimurium TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	–	Shirasu *et al.* (1976)
S. typhimurium TA100, 98, reverse mutation	450 µg/plate	–	–	Waskell (1978)
S. typhimurium TA100, 1535, reverse mutation	4000 µg/plate	–	–	Nestmann *et al.* (1980)
S. typhimurium TA1537, 1538, 98, reverse mutation	2000 µg/plate	–	–	Nestmann *et al.* (1980)
S. typhimurium TA100, reverse mutation	520 µg/plate	NT	–	Rapson *et al.* (1980)
S. typhimurium TA100, 98, reverse mutation	5000 µg/plate	–	–	Moriya *et al.* (1983)
S. typhimurium TA100, reverse mutation	600	–	–	DeMarini *et al.* (1994)
S. typhimurium TA100, reverse mutation, liquid medium	1750	–	+	Giller *et al.* (1997)
S. typhimurium TA100, reverse mutation, liquid medium	3000	+	NT	Giller *et al.* (1997)
S. typhimurium TA104, reverse mutation, microsuspension	250 µg/plate	–	–	Nelson *et al.* (2001)
S. typhimurium TA100, RSJ100, reverse mutation	16 300	–	–	Kargalioglu *et al.* (2002)
S. typhimurium TA98, reverse mutation	13 100	–	–	Kargalioglu *et al.* (2002)
S. typhimurium TA1535, SOS DNA repair	NR	+	–	Ono *et al.* (1991)

[a] Doses are in µg/mL unless otherwise specified.
+, positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NR, not reported; NT, not tested

these studies did not report positive findings for genotoxicity.

However, trichloroacetic acid induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of metabolic activation from S9 (Ono *et al.*, 1991). Furthermore, Giller *et al.* (1997) reported genotoxic activity of trichloroacetic acid at noncytotoxic concentrations of 1750 to 2250 µg/mL in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9. The addition of S9 decreased the genotoxic response, with effects observed at 3000–7500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2500 and 10 000 µg/mL without and with microsomal activation, respectively.

4.3 Nongenotoxic mechanisms of carcinogenesis

4.3.1 Liver

The available evidence for nongenotoxic mechanisms for the rodent (mouse) liver tumours induced by trichloroacetic acid comprises the following: (i) epigenetic effects (especially DNA hypomethylation); (ii) cytotoxicity and oxidative stress; (iii) alteration of proliferation and apoptosis, and clonal expansion; (iv) PPARα activation; and (v) disruption of gap-junctional communication. Evidence from humans and from experimental animals supporting each of these nongenotoxic mechanisms of tumour

induction by trichloroacetic acid is presented below.

(a) Epigenetic effects

Epigenetic events that have been studied primarily include changes in methylation of total DNA or of particular genes. Expression of the affected genes and activity of DNA methyltransferases has also been investigated.

(i) Humans

No data on alterations in DNA methylation specific to trichloroacetic acid are available from studies in humans.

(ii) Experimental systems

The hypomethylation of DNA in response to exposure to trichloroacetic acid was investigated by Tao *et al.* (1998). Female B6C3F$_1$ mice (age, 15 days) received intraperitoneal injections of *N*-methyl-*N*-nitrosourea (MNU) at a dose of 25 mg/kg bw, and were subsequently given drinking-water containing trichloroacetic acid (neutralized to a concentration of 25 mmol/L [4085 mg/L]) for 44 weeks. After 11 days of exposure to trichloroacetic acid without pretreatment with MNU, the levels of 5-methylcytosine (5MeC) in total liver DNA was decreased (by about 60%) relative to untreated controls. After 44 weeks of exposure to trichloroacetic acid, levels of 5MeC were not different from those in controls that had received MNU only. No difference in DNA methylation was observed between the control groups in the short term (drinking-water control) and long-term (MNU only control) experiments. In hepatocellular adenomas and carcinomas promoted by trichloroacetic acid, the level of 5MeC in DNA was decreased by 40% and 51% when compared with non-tumour tissue from the same animal, or with liver tissue from control animals given only MNU, respectively. Cessation of treatment with trichloroacetic acid at 1 week before termination did not change the levels of 5MeC in either adenomas or carcinomas; however, levels remained lower than in non-tumour tissue. Levels of 5MeC in DNA from carcinomas were lower than in DNA from adenomas, suggesting that DNA methylation decreases with tumour progression.

Tao *et al.* (2000a) evaluated the methylation and expression of *c-jun* and *c-myc* proto-oncogenes in mouse liver after short-term exposure to trichloroacetic acid. Female B6C3F$_1$ mice were given water containing trichloroacetic acid (neutralized to pH 6.5–7.5 with sodium hydroxide) at a dose of 500 mg/kg bw per day by gavage for 5 days. Mice received methionine at a dose of 0, 30, 100, 300, or 450 mg/kg bw by intraperitonal injection, 30 minutes after the last exposure to trichloroacetic acid and were killed 70 minutes after dosing. Decreased methylation in the promoter regions of the *c-jun* and *c-myc* genes and increased levels of corresponding mRNA and proteins were found in the liver of mice exposed to trichloroacetic acid. Methionine prevented decreases in methylation of the two genes in a dose-dependent manner, with an effective dose of 100 mg/kg bw. At 450 mg/kg bw, methionine also prevented increases in the levels of mRNA and proteins from the two genes.

In another study, Tao *et al.* (2000b) examined the methylation of *c-jun* and *c-myc* genes, expression of both genes, and activity of DNA methyltransferase in liver tumours initiated by MNU and promoted by trichloroacetic acid in female B6C3F$_1$ mice. Mice aged 15 days were given either MNU at a dose of 25 mg/kg bw, or the saline vehicle control. Starting at age 6 weeks, the mice were given drinking-water containing neutralized trichloroacetic acid at a concentration of 20 mmol/L (3268 mg/L) continuously until age 52 weeks. The promoter regions of *c-jun* and *c-myc* in tumours were found to be hypomethylated relative to the promoter regions in non-tumour liver tissue from mice treated with trichloroacetic acid. The expression of mRNA and protein for each gene was also increased in trichloroacetic acid-promoted tumours relative

to non-tumour liver tissue. DNA methyltransferase activity was significantly increased in liver tumours from trichloroacetic acid-promoted mice when compared with non-tumour liver tissue from the same mice.

In a related study, Tao *et al.* (2004) reported hypomethylation of DNA and increased expression of the insulin-like growth factor II (*Igf2*) gene in liver tumours promoted by trichloroacetic acid. Specifically, the level of 5MeC in DNA from non-tumour liver tissue in mice exposed to trichloroacetic acid was decreased relative to that in DNA from mice initiated with MNU but not exposed to trichloroacetic acid. The level of 5MeC in trichloroacetic acid-promoted tumours was also decreased relative to the non-tumour liver tissue, indicating hypomethylation. Sequencing of the differentially methylated region-2 of the *Igf2* gene promoter revealed that 21–24 cytosine-guanine dinucleotide sites were methylated in initiated liver, compared with 15–17 sites in non-tumour liver tissue from mice with tumours promoted by trichloroacetic acid. Thus, exposure to trichloroacetic acid reduced the percentage of cytosine-guanine dinucleotide sites that were methylated from approximately 79% to 58%. The number of methylated cytosine-guanine dinucleotide sites was further reduced to approximately 11% in liver tumours promoted by trichloroacetic acid. Expression of mRNA was significantly increased (5.1 times) in liver tumours relative to non-tumour liver tissue from mice treated with trichloroacetic acid, but was not increased in non-tumour liver tissue from trichloroacetic acid-promoted mice when compared with the level of expression in MNU-initiated mice in the control group.

The temporal association of DNA methylation and cell proliferation (the latter discussed below) in mice exposed to trichloroacetic acid has been investigated by Ge *et al.* (2001). Female B6C3F_1 mice were given neutralized trichloroacetic acid by gavage at a dose of 500 mg/kg bw per day. Southern blot analysis indicated that the tumour promoter region of the *c-myc* proto-oncogene in the liver was hypomethylated at 72 hours and 96 hours.

Pereira *et al.* (2001) examined the effect of chloroform (a disinfection by-product present as a co-contaminant with trichloroacetic acid in drinking-water) on dichloroacetic acid- or trichloroacetic acid-induced hypomethylation and expression of the c-*myc* proto-oncogene in female B6C3F_1 mice. Both dichloroacetic acid and trichloroacetic acid decreased methylation in the promoter region of the *c-myc* gene and increased expression of *c-myc* mRNA. Coadministration of chloroform did not affect the extent of trichloroacetic acid-induced hypomethylation or mRNA expression, or the incidence or multiplicity of liver tumours promoted by trichloroacetic acid. By contrast, coadministration of chloroform prevented the hypomethylation and mRNA expression of the *c-myc* gene and the promotion of liver tumours by dichloroacetic acid.

(b) Cytotoxicity and oxidative stress

The available evidence for this mechanism includes both cytotoxicity and oxidative stress after exposure to trichloroacetic acid.

(i) Humans

No studies on liver toxicity or oxidative stress in humans exposed to trichloroacetic acid were identified.

(ii) Experimental systems

Cytotoxicity

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats given drinking-water containing neutralized trichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm (approximately 0, 4.1, 36.5, or 355 mg/kg bw per day) for 90 days. At 355 mg/kg bw per day, relative liver weights were statistically significantly ($P < 0.05$) increased (7%) compared with controls. Although hepatomegaly was observed in the group at the highest dose, no microscopic

lesions were observed. No consistent treatment-related effects were seen on clinical chemistry or immune-function parameters.

In a study by Bhat *et al.* (1991), groups of five male Sprague-Dawley rats were given one quarter of a median lethal dose (LD_{50}) of trichloroacetic acid in drinking-water for 90 days. One quarter of the reported LD_{50} for trichloroacetic acid, 3300 mg/kg bw, corresponds to an administered dose of approximately 825 mg/kg bw per day. Trichloroacetic acid induced minimal to moderate collagen deposition (an indication of liver injury) in portal triads and large central veins in four out of five rats (minimal collagen deposition was observed in one out of five controls). Morphological changes in the liver included portal vein dilation/extension of minimal to moderate severity in five out of five rats treated with trichloroacetic acid.

Acharya *et al.* (1995) evaluated liver toxicity caused by trichloroacetic acid as part of a study on the interactive toxicity of tertiary butyl alcohol and trichloroacetic acid. Groups of five to six young male Wistar rats (age, 50 days) were exposed to water containing trichloroacetic acid at 0 or 25 ppm (approximately 0 or 3.8 mg/kg bw per day, assuming water intake of 0.15 L/kg bw per day) for 10 weeks. Little, if any, trichloroacetic acid-induced liver toxicity was observed; relative liver weight was unaffected, and no significant changes were detected in the indicators of liver injury: aspartate and alanine aminotransferases, or alkaline and acid phosphatases. In contrast, indicators of lipid and carbohydrate homeostasis were affected by trichloroacetic acid. The activity of succinate dehydrogenase was increased by approximately 30% compared with controls. In the liver, levels of triglyceride and cholesterol were significantly decreased, while glycogen levels increased approximately eight times. Levels of serum cholesterol were also increased approximately twofold. There was little evidence for induction of oxidative stress in the liver. No increase in lipid peroxidation was observed in the liver.

In a follow-up study using the same exposure protocol (Acharya *et al.*, 1997), minimal hepatic alterations were observed in the group treated with trichloroacetic acid. Histopathological changes noted in the liver included centrilobular necrosis, hepatocyte vacuolation, loss of hepatic architecture, and hypertrophy of the periportal region.

Oxidative stress

The ability of trichloroacetic acid to induce oxidative-stress responses, such as lipid peroxidation and oxidative DNA damage, have been tested in a series of short-term or single-dose studies in mice (Larson & Bull, 1992a; Austin *et al.*, 1995, 1996; Parrish *et al.*, 1996). Trichloroacetic acid induced lipid peroxidation, as measured by induction of thiobarbituric acid-reactive substances (TBARS), and oxidative DNA damage, as measured by detection of 8-hydrodeoxyguanosine adducts (8-OHdG) after administration of single oral doses.

In a study by Austin *et al.* (1996), groups of six male $B6C3F_1$ mice were given a single oral dose of neutralized trichloroacetic acid at 0, 30, 100, or 300 mg/kg bw in water. Mice were deprived of food for 3 hours before dosing. A significant increase in 8-OHdG in nuclear DNA in the liver was observed in the group at 300 mg/kg bw at 8–10 hours after dosing. The maximum level of 8-OHdG was observed at 8 hours. Levels of 8-OHdG in groups at 30 or 100 mg/kg bw were not reported.

Austin *et al.* (1996) reported that the maximum concentration of trichloroacetic acid-induced TBARS in the liver occurred 9 hours after dosing in in mice. In an earlier study, Larson & Bull (1992b) also reported that the maximum concentration of TBARS in the liver occurred 9 hours after dosing in mice given trichloroacetic acid at a dose of 2000 mg/kg bw. In the study by Larson & Bull (1992b), it was reported that 9 hours after

a single oral dose of trichloroacetic acid at 100, 300, 1000, and 2000 mg/kg bw, levels of TBARS were 1.15, 1.7, 2, and 2.7 times greater than those of controls, respectively.

Parrish *et al.* (1996) evaluated the ability of haloacetic acids to induce oxidative DNA damage in mouse liver. Groups of six male B6C3F$_1$ mice were given drinking-water containing trichloroacetic acid at 0, 100, 500, or 2000 mg/L for either 3 or 10 weeks. Oxidative damage to DNA, as measured by 8-OHdG adducts, did not occur with prolonged treatment with trichloroacetic acid, although peroxisome proliferation was induced, as indicated by increased activity of palmitoyl-coenzyme A oxidase (palmitoyl-coA oxidase) and 12-hydroxylation of lauric acid.

Austin *et al.* (1995) tested the ability of trichloroacetic acid to induce markers of CYP450 after short-term treatments (the latter effects are discussed below). Male B6C3F$_1$ mice were given trichloroacetic acid at 0 or 1000 mg/L for 14 days. The following parameters were evaluated: (i) changes in microsomal 12-(ω) hydroxylation of lauric acid (an indicator for the activity of CYP4A); (ii) hydroxylation of *p*-nitrophenol (as an index of CYP2E1 activity); and (iii) protein levels for a panel of CYP450s. CYP4A activities doubled in mice treated with trichloroacetic acid, while no increase in CYP2E1 activity and no change in the overall amount of total liver microsomal P450 were found.

Hassoun & Ray (2003) investigated activation of cultured macrophages (J744A.1 cell line) *in vitro* by neutralized trichloroacetic acid (8–32 mM [1.3–5 mg/kg bw] for 24–60 hours). Reduced cell viability was observed at all concentrations and correlated well with increased activity of lactate dehydrogenase in media. After incubation for 24 hours, trichloroacetic acid caused increases in the levels of superoxide anion; however, incubations of 36 and 60 hours statistically significantly increased superoxide anion levels at 16, 24, and 32 mM [2.6; 4; 5 mg/mL] ($P < 0.05$). Superoxide dismutase activity was also affected by treatment with trichloroacetic acid. Significant increases in superoxide dismutase activity occurred at lower trichloroacetic acid concentrations (8–24 mM [1.3–4 mg/mL]) than in controls, but activity at the highest concentration (32 mM [5 mg/mL]) for 24–36 hours was similar to that of controls. Incubation of cells with trichloroacetic acid at 32 mM [5 mg/mL] for 60 hours resulted in 100% cell death. These results indicated that incubation with trichloroacetic acid at 8–32 mM [1.3–5 mg/mL] for 24–60 hours induces macrophage activation, which resulted in cytotoxicity due to oxidative stress.

The activation of phagocytic cells was supported by studies *in vivo* (Hassoun & Dey, 2008). Male B6C3F$_1$ mice were given trichloroacetic acid at 300 mg/kg bw by gavage and killed after 6 or 12 hours. At 12 hours, the superoxide anion increased by 62.5% in cells obtained by peritoneal lavage and 17.6% in hepatic tissue.

In further 4-week and 13-week studies, groups of male B6C3F$_1$ mice were given trichloroacetic acid at a dose of 7.7, 77, 154, or 410 mg/kg bw day by gavage for 4 and 13 weeks (Hassoun *et al.*, 2010a, b). These doses were comparable to those inducing hepatocarcinogenicity.

In the liver, dose- and time-dependent increases were seen in the production of superoxide anion (increases of up to 167% at 4 weeks, and up to 200% at 13 weeks), and lipid peroxidation (increases of up to 567% at 4 weeks, and up to 733% at 13 weeks). Trichloroacetic acid also induced dose-dependent increases in biomarkers of phagocytic activation in cells obtained by peritoneal lavage after 4, but not 13, weeks of exposure. The production of superoxide anion at 4 weeks increased up to 175% at doses of 77, 154, and 410 mg/kg bw per day, whereas at 13 weeks, the production increased significantly (60%) in the group treated at 77 mg/kg bw per day only. Similarly, the increase in myeloperoxidase activity was robust at 4 weeks, and modest at 13 weeks. TNF-α, released by peritoneal-lavage cells, increased dose-dependently up to ninefold

at 4 weeks, whereas at 13 weeks the increase (1.8-fold) was only found at 77 mg/kg bw per day.

In a 50-day study with trichloroacetic acid in drinking-water (Celik, 2007), female Sprague-Dawley rats (age, 4 months; numbers not reported) were given trichloroacetic acid at 2000 ppm (300 mg/kg bw per day, assuming a default water intake of 0.15 L/kg bw per day), while the control group received natural spring water. Trichloroacetic acid significantly increased the activity of serum aspartate aminotransferases, alanine aminotransferases, creatine phosphokinase, and acid phosphatase ($P < 0.05$) in treated rats. A slight but statistically insignificant increase in malondialdehyde was found in the erythrocytes and liver. The antioxidant enzymes, superoxide dismutase and catalase, were significantly increased. However, no changes in the activities of glutathione, glutathione reductase, or glutathione-*S*-transferase were found in any tissue.

(c) Alteration of cell proliferation and apoptosis, and clonal expansion

(i) Humans

No studies of altered cell proliferation and apoptosis, or clonal expansion, in humans exposed to trichloroacetic acid were identified.

(ii) Experimental systems

Several studies have observed hepatocyte proliferation in response to trichloroacetic acid in mice (Sanchez & Bull, 1990; Dees & Travis, 1994; Pereira, 1996; Stauber & Bull, 1997; DeAngelo *et al.*, 2008). For instance, Dees & Travis (1994) observed relatively small (two- to threefold), but statistically significant, increases in [^{3}H]thymidine incorporation in hepatic DNA in mice exposed for 11 days to trichloroacetic acid at doses (1 g/kg bw) that increased relative liver weight. Increased labelling of hepatic DNA was observed at doses lower than those associated with evidence of necrosis, suggesting that cell proliferation induced by trichloroacetic acid is not due to regenerative hyperplasia.

Miyagawa *et al.* (1995) examined the effect of trichloroacetic acid on replicative DNA synthesis in the liver of male B6C3F$_1$ mice. Mice were given a single dose by gavage of one-half of the maximum tolerated dose (250 mg/kg bw, as estimated from data provided by the authors), or the maximum tolerated dose (500 mg/kg bw), and incorporation of [^{3}H]thymidine in harvested hepatocytes was measured 24, 39, or 48 hours after dosing. For trichloroacetic acid, positive responses were observed at 250 mg/kg bw at 24 and 39 hours (6.5 and 4.9 times above controls) and at 500 mg/kg bw (9.8 times above controls).

Pereira (1996) evaluated cell proliferation in the liver of female B6C3F$_1$ mice treated with drinking-water containing trichloroacetic acid at 0, 2, 6.67, or 20 mM [327, 1090, or 3200 mg/L] for 5, 12, or 33 days by estimating the bromodeoxyuridine labelling index in hepatocytes. Cell proliferation was enhanced by 5 days exposure to trichloroacetic acid, but not for longer exposures of 12 or more days.

In a study reported by Stauber & Bull (1997), male B6C3F$_1$ mice were pretreated with drinking-water containing trichloroacetic acid at 2000 mg/L [480 mg/kg bw per day] for 50 weeks. The mice were then given drinking-water containing trichloroacetic acid at 0, 20, 100, 500, 1000, or 2000 mg/L [estimated doses of 0, 5, 23, 115, 230, or 460 mg/kg bw per day] for two additional weeks. Cell division rates in trichloroacetic acid-induced altered hepatic foci and tumours were high at all doses. Rates of cell division in altered hepatic foci and tumours remained high in mice for which exposure was terminated during the last 2 weeks of the study, indicating that these rates were independent of continued treatment with trichloroacetic acid.

Ge *et al.* (2001) exposed female B6C3F$_1$ mice to neutralized trichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Relative liver weights were significantly increased after

36, 72, and 96 hours. The proliferating cell nuclear antigen labelling index was significantly increased in liver cells at 72 and 96 hours, relative to controls. The mitotic index was significantly elevated in liver cells at 96 hours after the first dose.

A study *in vitro* by Channel & Hancock (1993) reported that exposure to medium containing trichloroacetic acid at 100 µg/mL decreased the rate of progression through S-phase of the cell cycle in WB344 cells (a nontumorigenic, epithelial, rat hepatocyte cell line).

(d) Activation of PPARα

The sections below review the evidence that trichloroacetic acid induces activation of PPARα.

(i) Humans

No studies were identified that addressed trichloroacetic acid-induced peroxisome proliferation or activation of PPARα in human liver. However, studies of transactivation *in vitro* have shown that human PPARα is activated by trichloroacetic acid and dichloroacetic acid. Maloney & Waxman (1999) demonstrated comparable transactivation potency against human and mouse PPARα with trichloroacetic acid at concentrations higher than 1 mM [163.39 µg/mL], and activation was dose-dependent up to 5 mM [817 µg/mL], tested. Walgren *et al.* (2000a) showed that neither trichloroacetic acid nor dichloroacetic acid had an effect on oxidation of palmitoyl-coenzyme A (palmitoyl-coA) in human hepatocyte cultures; however, no palmitoyl-coA oxidation activity was detected in control human hepatocytes in these experiments.

(ii) Experimental systems

Direct evidence for activation of PPARα

Several studies of transactivation *in vitro* have shown that murine versions of PPARα are activated by both trichloroacetic acid and dichloroacetic acid, while trichloroethylene itself is relatively inactive. Issemann & Green (1990) demonstrated transactivation by trichloroacetic acid of a mouse PPARα construct in mouse kidney COS1 cells, albeit with less potency than other known PPARα ligands.

Zhou & Waxman (1998) tested activation of mouse PPARα by chlorinated hydrocarbons using mouse kidney COS1 cells containing the murine PPARα reporter plasmid. Exposure to trichloroacetic acid for 24 hours resulted in activation of the reporter plasmid at concentrations higher than 1 mM [163.39 µg/mL]. In these experiments, trichloroacetic acid was about twice as potent as dichloroacetic acid. Trichloroethylene at concentrations of up to 5 mM had no effect.

In a similar study of transactivation with murine PPARα, a significant concentration–response relationship was obtained with trichloroacetic acid (Maloney & Waxman, 1999).

Walgren *et al.* (2000b) tested transactivation of murine PPARα using a reporter plasmid in HL8.5 cells co-transfected with mouse retinoic acid receptor α (mRXR). Trichloroacetic acid (4 mM [653 µg/mL]) showed a significant effect.

Indirect evidence for activation of PPARα: peroxisome proliferation

Elcombe (1985) examined peroxisome proliferation in male Wistar rats and male Swiss mice given corn oil containing trichloroacetic acid at a dose of 10–200 mg/kg bw per day by gavage for 10 consecutive days. Peroxisome volume densities were increased. Effects on other markers of peroxisome proliferation, presented below, demonstrated concomitant increases in β-oxidation activity.

DeAngelo *et al.* (1989) assessed relative species and strain sensitivities to the induction of hepatic peroxisome proliferation by chloroacetic acids. In the study in rats, male Sprague-Dawley, F344, and Osborne-Mendel rats received drinking-water supplemented with trichloroacetic acid at 0, 6, 12, or 31 mM (approximately 0, 212, 327, or 719 mg/kg bw per day) for 14 days. The volume fraction of cytoplasm from hepatic tissue

occupied by peroxisomes was decreased to less than half that seen in controls in this strain. [The Working Group noted that the reason for this paradoxical effect was not addressed.]

DeAngelo *et al.* (1989) also studied groups of six male mice of each of four strains ($B6C3F_1$, C3H, Swiss-Webster, and C57BL/6) that were exposed to drinking-water containing trichloroacetic acid at 0, 12, or 31 mM (approximately 0, 261, or 442 mg/kg bw per day) for 14 days. No effects were seen on body weight, but liver-to-body weight ratios were significantly increased at both dosages in all four strains as well as increases in the number and size of peroxisomes in the liver cytoplasm.

Indirect evidence for activation of PPARα: enzyme markers

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats dosed with drinking-water containing neutralized trichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm (approximately 0, 4.1, 36.5, or 355 mg/kg bw per day) for 90 days. At the highest dose, hepatic peroxisomal enzyme activity was statistically significantly increased (15%, $P < 0.05$) as measured by the activity of palmitoyl-coA oxidase.

Effects consistent with peroxisome proliferation (e.g. induction of lipid-metabolism enzymes, such as acyl-coA oxidase and palmitoyl-coA oxidase, increased liver weight) have been observed in male F344 rats exposed to trichloroacetic acid by gavage for 14 days (Goldsworthy & Popp, 1987), in male F344 rats exposed to drinking-water containing trichloroacetic acid for 14 days (DeAngelo *et al.*, 1989), or 104 weeks (DeAngelo *et al.*, 1997), in male Osborne-Mendel rats exposed via drinking-water for 14 days (DeAngelo *et al.*, 1989), and in male Sprague-Dawley rats exposed via drinking-water for 90 days (Mather *et al.*, 1990). In mice, peroxisome proliferation or changes consistent with peroxisome proliferation have been reported in male $B6C3F_1$mice exposed via drinking-water for 2–10 weeks (DeAngelo *et al.*, 1989; Sanchez & Bull, 1990; Austin *et al.*, 1995; Parrish *et al.*, 1996), in male $B6C3F_1$ mice exposed by gavage for 10 days (Goldsworthy & Popp, 1987), and in male C57BL/6 and Swiss-Webster mice exposed via drinking-water for 14 days (DeAngelo *et al.*, 1989). Furthermore, PPARα-null mice exposed to drinking-water containing trichloroacetic acid at 2 g/L for 7 days did not show the characteristic responses of acyl-coA oxidase, palmitoyl-coA oxidase, and CYP4A induction associated with PPARα activation and peroxisome proliferation in wild-type mice (Laughter *et al.*, 2004). In addition, the livers from wild-type mice, but not PPARα-null mice, exposed to trichloroacetic acid developed centrilobular hepatocyte hypertrophy, although no significant increase in relative liver weight was observed.

As discussed above, Elcombe (1985) demonstrated species differences in peroxisome proliferation in male Wistar rats and male Swiss mice given corn oil containing trichloroacetic acid at 10–200 mg/kg bw per day by gavage for 10 consecutive days. Dose-related increases in cyanide-insensitive palmitoyl-coA oxidation were observed in rats and mice after treatment with trichloroacetic acid. At doses of 200 mg/kg bw per day for 10 days, peroxisomal β-oxidation increased 6.5 times in Wistar rats and 4.8 times in Swiss mice. On the other hand, trichloroacetic acid had no effect on hepatic catalase activity. Dose-related increases in cyanide-insensitive palmitoyl-coA oxidation were also observed in cultured rat and mouse hepatocytes exposed to trichloroacetic acid; however, no stimulation of peroxisomal β-oxidation was observed in cultured human hepatocytes prepared from two samples of human liver and treated with trichloroacetic acid.

Goldsworthy & Popp (1987) assessed cyanide-insensitive activity of palmitoyl-coA oxidase in adult male F344 rats given corn oil containing trichloroacetic acid at 0 or 500 mg/kg bw per day by gavage for 10 consecutive days.

Hepatic peroxisomal enzyme activity increased statistically significantly ($P < 0.05$) in rats receiving trichloroacetic acid, resulting in levels of enzyme activity approximately 2.8 times greater than in controls. Liver-to-body weight ratios were also statistically significantly increased (41%, $P < 0.05$) relative to those in controls. Body weight gain was not changed.

DeAngelo *et al.* (1989) assessed relative species and strain sensitivities to the induction of hepatic peroxisome proliferation markers by chloroacetic acids. In the study in rats, male Sprague-Dawley, F344, and Osborne-Mendel rats received drinking-water supplemented with 0, 6, 12, or 31 mM [0, 212, 327, or 719 mg/kg bw per day] for 14 days. Activity of palmitoyl-coA oxidase was elevated in Osborne-Mendel rats by 2.4 times and in F344 rats by 1.6 times over control values at the highest dose. Although palmitoyl-coA oxidase activity was not affected in treated Sprague-Dawley rats at any dose, carnitine acetyl-coA transferase activity was increased by 321% above control values in Sprague-Dawley rats at the highest dose (significant increases were not observed at lower doses). The volume fraction of cytoplasm from hepatic tissue occupied by peroxisomes was decreased to less than half that seen in controls in this strain.

In the study of DeAngelo *et al.* (1989) in mice, groups of six male mice per each of four strains of mice ($B6C3F_1$, C3H, Swiss-Webster, and C57BL/6) were exposed drinking-water that contained trichloroacetic acid at 0, 12, or 31 mM [0, 261, or 442 mg/kg bw per day] for 14 days. No effects were seen on body weight, but liver-to-body weight ratios were significantly increased at both dosages in all strains. The activity of palmitoyl-coA oxidase was elevated in all four strains for all dose groups. Palmitoyl-coA oxidase levels were 276%, 325%, and 456% above controls at 12 mM and 648%, 644%, and 678% above controls at 31 mM for Swiss-Webster, C3H, and $B6C3F_1$ mice, respectively. Palmitoyl-coA oxidase activity in C57BL/6 mice was increased by 2100% and 2500% above control levels at the highest and lowest doses of trichloroacetic acid, respectively, indicating high strain sensitivity. DeAngelo *et al.* (1989) also reported that catalase activity was increased by 461% above controls in $B6C3F_1$ mice at the highest dose, with accompanying increases in the level of peroxisome proliferation-associated protein and the number and size of peroxisomes in liver cytoplasm described above. To summarize, the results of DeAngelo *et al.* (1989) indicated that mice, in general, are more sensitive than rats to the effects of trichloroacetic acid on peroxisome proliferation, as indicated by palmitoyl-coA oxidase activity.

Goldsworthy & Popp (1987) investigated induction of hepatic and renal peroxisome proliferation markers in adult male $B6C3F_1$ mice given trichloroacetic acid at a dose of 0 or 500 mg/kg bw per day in corn oil for 10 days via gavage. Hepatic peroxisomal-enzyme activity increased statistically significantly ($P < 0.05$) in mice receiving trichloroacetic acid, resulting in levels of enzyme activity that were 280% those of the controls. Liver-to-body weight ratios were also statistically significantly increased (40%; $P < 0.05$) relative to controls.

Austin *et al.* (1995) explored the relationship between trichloroacetic acid-induced lipid peroxidation and the ability of trichloroacetic acid to induce markers of peroxisome proliferation. Groups of 18 male $B6C3F_1$ mice were exposed to trichloroacetic acid at 0 or 1000 mg/L for 14 days. Mice pretreated with water or trichloroacetic acid were divided into groups of six and given a single dose of trichloroacetic acid of 300 mg/kg bw or an equivalent volume of distilled water by gavage (control). The mice were killed 9 hours after the single dose. The following end-points were evaluated: (1) lipid-peroxidation response, as measured by the production of TBARS; (2) indicators of peroxisome proliferation, as measured by increased activity of palmitoyl-coA oxidase and increased catalase activity. TBARS were measured after 14 days of pretreatment and after the

single dose. Mice treated with trichloroacetic acid had a lower mean concentration of TBARS compared with controls, but the difference was not statistically significant. In the single-dose experiment, mice pretreated with trichloroacetic acid exhibited a significant decrement in TBARS in liver homogenates after a single dose of trichloroacetic acid, when compared with mice that received the same single dose, but that had not been pretreated. In contrast, pretreatment with trichloroacetic acid caused increases in the activities of palmitoyl-coA oxidase and catalase by 4.5- and 1.7-fold, respectively.

Walgren *et al.* (2000b) assessed the effects of trichloroacetic acid on palmitoyl-CoA oxidation in rat (LEH) and mouse ($B6C3F_1$) primary hepatocytes and found that trichloroacetic acid (2 mM [327 µg/mL], a concentration that was not cytotoxic) activated palmitoyl-CoA oxidation in mouse and rat cells.

(e) Inhibition of intracellular communication

(i) Humans

No trichloroacetic acid-specific data on inhibition of gap-junctional communication were available from studies in humans.

(ii) Experimental systems

Experimental evidence that trichloroacetic acid inhibits gap-junctional communication was available from studies *in vitro*. Benane *et al.* (1996) assessed the effects of trichloroacetic acid on gap-junctional intercellular communication in clone 9 (ATCC CRL 1439), a normal liver epithelial cell line from a male Sprague-Dawley rat aged 4 weeks. The cells were grown in a nutrient mixture, plated, and exposed to trichloroacetic acid at concentrations of 0, 0.5, 1.0, 2.5, or 5 mM for varying times (1, 4, 6, 24, 48, or 168 hours). Lucifer yellow scrape-load dye transfer was used as a measure of gap-junctional intercellular communication. The lowest concentration and shortest time to reduce dye transfer was 1 mM over 1 hour. The reduction in dye transfer increased with higher concentrations and longer treatment time. Klaunig *et al.* (1989) performed a series of experiments to determine the effects of trichloroacetic acid on gap-junctional intercellular communication in primary cultured hepatocytes from male $B6C3F_1$ mice and F344 rats aged 6–8 weeks. Cells were exposed to trichloroacetic acid (dissolved in dimethyl sulfoxide) at 0, 0.1, 0.5, or 1 mM [0, 16.3, 82, 163 µg/mL] for up to 24 hours. Trichloroacetic acid inhibited Lucifer yellow dye transfer in mouse hepatocytes, either freshly plated or after 24 hours. Dye coupling was significantly reduced at all tested concentrations after 4 hours of treatment, but not after 8 or 24 hours. The inhibitory effect on dye transfer in mouse cells was unaffected by treatment with SKF-525A, an inhibitor of CYP450. In rat hepatocytes, dye transfer was unaffected by treatment with trichloroacetic acid at concentrations up to 1 mM [163 µg/mL] for as long as 24 hours or 6 hours in freshly plated cells. The results obtained in primary F344 rat hepatocytes by Klaunig *et al.* (1989) differed from those reported by Benane *et al.* (1996), who observed inhibition of dye transfer in cells from a Sprague-Dawley rat epithelial cell line treated with 1 mM [163 µg/mL] for durations of 1–168 hours. [The Working Group noted that the reason for the differential response in rat liver cells was unknown, but may be related to differences in the originating strain or in the type of cell tested (primary cultured hepatocytes versus established cell line)].

(f) Comparative analyses of liver tumours induced by trichloroacetic acid or dichloroacetic acid

See Section 4 of the *Monograph* on Dichloroacetic Acid in this Volume.

4.3.2 Kidney

Few studies had examined the effects of trichloroacetic acid on the kidney, or possible mechanisms.

Acharya *et al.* (1995) evaluated kidney toxicity in young male Wistar rats (age, 50 days) exposed to water containing trichloroacetic acid at 0 or 25 ppm [≈3.8 mg/kg bw per day, assuming water intake of 0.15 L/kg bw per day] for 10 weeks. While serum enzyme levels were unaffected by trichloroacetic acid, kidney, but not liver, levels of glutathione-*S*-transferase were decreased to approximately 66% of control values. No examination of kidney histology was performed in this study.

In a follow-up study using the same exposure protocol (Acharya *et al.*, 1997), histopathological changes were noted in the kidneys of animals treated with trichloroacetic acid, and included degeneration of renal tubules.

Goldsworthy & Popp (1987) assessed cyanide-insensitive activity of palmitoyl-coA oxidase in adult male F344 rats given trichloroacetic acid in corn oil at a dose of 0 or 500 mg/kg bw per day by gavage for 10 consecutive days. Activity of renal peroxisomal enzymes was statistically significantly ($P < 0.05$) increased by approximately 1.8 times over that in controls in rats. Kidney weights were not affected by treatment.

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats dosed with drinking-water containing neutralized trichloroacetic acid at concentrations up to 5000 ppm [355 mg/kg bw per day] for 90 days. At 355 mg/kg bw per day, relative kidney weights were statistically significantly ($P < 0.05$) increased (11%) compared with controls. There were no changes in kidney histopathology.

Pereira *et al.* (2001) examined kidney weight in B6C3F$_1$ mice injected with MNU at age 15 days and then exposed to drinking-water containing trichloroacetic acid (4.0 g/L) from age 4 weeks to age 36 weeks. No effect of trichloroacetic acid on kidney weight was found.

The temporal association of DNA methylation and cell proliferation (the latter discussed below) in mice exposed to trichloroacetic acid was investigated by Ge *et al.* (2001). Female B6C3F$_1$ mice were given neutralized trichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Trichloroacetic acid decreased methylation in the promoter region of the *c-myc* gene in the kidney and urinary bladder after 72 and 96 hours of treatment, but the response was less pronounced than in liver. Trichloroacetic acid had no effect on relative kidney weights.

In a follow-up study, Tao *et al.* (2005) treated B6C3F$_1$ mice with drinking-water containing trichloroacetic acid (4 g/L) for 7 days concurrently. In male, but not female mouse kidney, trichloroacetic acid decreased the methylation of DNA and of the *c-myc* gene. Coadministration of methionine in the diet prevented hypomethylation in the kidneys of male mice.

4.4 Susceptibility data

4.4.1 Inter-individual variability

It is not clear whether trichloroacetic acid is metabolized to dichloroacetic acid in any significant quantities (Bull, 2000; Lash *et al.*, 2000; Kim *et al.*, 2009). Genetic polymorphisms have been identified in glutathione *S*-transferase zeta 1 (*GSTZ1*), a key enzyme involved in the metabolism of dichloroacetic acid (Board *et al.*, 2001). *Gstz1*-null mice fail to metabolize ^{13}C-labelled dichloroacetic acid to [^{13}C]glyoxylate (Ammini *et al.*, 2003). Polymorphisms in *GSTZ1* would be relevant to susceptibility to trichloroacetic acid only if dichloroacetic acid is a metabolite of trichloroacetic acid, which is as yet unclear. Four polymorphic alleles of *GSTZ1* have been identified: 1a, 1b, 1c, and 1d (Board & Anders, 2011). *GSTZ1c* is the most common and is designated as the wild-type gene. Dichloroacetic acid is an inactivator of human, rat, and mouse GSTZ1. However, human *GSTZ1* is more resistant to inactivation than mouse or rat *Gstz1* (Tzeng *et al.*, 2000). The polymorphic variants of human *GSTZ1* differ in their susceptibility to

inactivation, with 1a-1a being more resistant to inactivation than the other variants.

Short-term treatment of $B6C3F_1$ mice with trichloroacetic acid was shown to induce hepatic activity of superoxide dismutase and catalase (Hassoun & Cearfoss, 2011). Because oxidative stress in the liver was suggested as one of the mechanisms of carcinogenesis by which trichloroacetic acid acts (Austin *et al.*, 1995), polymorphisms in these enzymes may be of importance in protection against trichloroacetic acid-induced oxidative stress.

Trichloroacetic acid also induces glycogen accumulation; prolonged glycogen accumulation can become irreversible (Kato-Weinstein *et al.*, 1998). Thus, individuals with glycogen storage disease, in the form of an inherited or spontaneous deficiency or alteration in any one of the enzymes involved in glycogen metabolism, may constitute another group that may be more susceptible than the general population to the toxicity of trichloroacetic acid.

4.4.2 Life-stage susceptibility

There is evidence that the developing fetus is highly susceptible to maternal exposure to trichloroacetic acid. Smith *et al.* (1989) examined developmental effects in pregnant Long-Evans rats given trichloroacetic acid at doses of up to 1800 mg/kg bw per day by gavage on days 6–15 of gestation. Maternal toxicity (reduced body-weight gain, increases in spleen and kidney weight) was evident in groups at doses exceeding 800 mg/kg bw per day. However, much more pronounced effects were observed in the fetuses. Dose-dependent increases in the frequency of fetus resorption, decreases in weight and length, and malformations in soft tissue, and skeletal and cardiac muscle were observed.

Singh (2006) examined the effect of trichloroacetic acid on the developing brain in Charles Foster rats given doses up to 1800 mg/kg bw per day by gavage on days 6–15 of gestation. A significant dose-dependent decrease in fetal brain weight was found at doses of 1200 mg/kg bw per day and higher.

Von Tungeln *et al.* (2002) investigated the potency of trichloroacetic acid in an assay for cancer in neonatal mice. In this study, male and female neonatal $B6C3F_1$ mice were given two intraperitoneal injections of trichloroacetic acid, with a total dose of 1000 or 2000 nmol [≈16 or 32 mg/kg bw], at age 8 and 15 days. The mice were killed and evaluated for liver tumours at age 12 months (higher dose) or 20 months (lower dose). The incidence of hepatic tumours in treated mice did not differ significantly from incidence in mice receiving the solvent only.

4.4.3 Sex differences

In one cancer bioassay, male and female mice were concurrently exposed to drinking-water containing trichloroacetic acid for 52 weeks (Bull *et al.*, 1990). A clear dose-related increase in the incidence of proliferative lesions (hyperplastic nodules, adenoma, or carcinoma) was observed in male $B6C3F_1$ mice, but not in females. Other available cancer bioassays in either males or females, conducted in separate laboratories, also suggested that males may be more sensitive than females to carcinogenicity induced by trichloroacetic acid. For example, Pereira *et al.* (2001) observed a tumour incidence of 25% in female $B6C3F_1$ mice exposed to drinking-water containing trichloroacetic acid at a dose of 784 mg/kg bw per day for 51 weeks. In studies by Bull and co-workers (Bull *et al.*, 1990; Bull, 2000), tumour incidences ranging from 55% to 83% have been reported in male mice exposed to drinking-water containing trichloroacetic acid at lower doses (309–480 mg/kg bw per day) for a comparable duration.

4.5 Mechanistic considerations

Evidence suggests that trichloroacetic acid is not genotoxic. In one study in human lymphocytes *in vitro*, no genotoxicity (chromosomal aberrations or DNA strand breaks) was observed with neutralized trichloroacetic acid. In mammalian systems *in vivo*, inconsistent evidence was available that trichloroacetic acid affects the induction of DNA strand breaks, micronucleus formation or chromosomal aberration. In mammalian studies *in vitro*, trichloroacetic acid had no genotoxic effects. Likewise, overwhelmingly negative results were obtained in bacterial and fungal test systems after exposure to trichloroacetic acid.

Major target organs for the adverse health outcomes associated with trichloroacetic acid are the liver and kidney.

Overall, the strength of evidence for liver as a target organ is strong. Multiple mechanisms are probably operative with regard to liver carcinogenesis in rodents. The following mechanisms have been identified, almost exclusively from studies in rodents or rodent test systems *in vitro*: epigenetic effects (global DNA hypomethylation and hypomethylation of the *c-myc* gene promoter), oxidative stress (oxidative DNA damage and lipid peroxidation, activation of phagocytic cells that may lead to generation of oxidants), increase in cell proliferation (an effect not observed in PPARα-null mice in a 7-day study), induction of the peroxisome proliferation response (strong direct and indirect evidence for activation of PPARα in rodents, limited evidence for trichloroacetic acid as a ligand of human PPARα), disruption of gap-junctional intercellular communications (limited evidence from several studies in rat cells *in vitro*). While there were consistent results in several rodent species and the overall body of evidence was coherent, no studies had been conducted to demonstrate that suppression of mechanistic processes leads to tumour suppression. Because trichloroacetic acid is a metabolite of other chlorinated solvents, several studies compared mutational and phenotypic profiles of liver tumours induced by various chlorinated solvents or their metabolites and concluded that little similarity exists. The strength of evidence for nongenotoxic mechanisms of liver cancer with trichloroacetic acid is moderate.

Some evidence suggests that kidney may also be a target organ for trichloroacetic acid. An increase in relative kidney weight has been reported in rats, but not mice. However, hypomethylation of global DNA and the *c-myc* gene has been observed in male but not female mouse kidney. The relevance of these observations to potential cancer hazard in kidney is unknown, given the absence of renal tumours.

There is weak evidence for potential inter-individual variability in the adverse effects of trichloroacetic acid. Glutathione *S*-transferase zeta 1 is an enzyme that has a major role in the metabolism of dichloroacetic acid, and common polymorphisms in the corresponding gene (*GSTZ1*) that result in differences in activation have been reported in humans (see the *Monograph* on Dichloracetic Acid in this Volume). Because dichloroacetic acid is a metabolite of trichloroacetic acid, it is not clear what relevance these polymorphisms may have to humans. It has been shown that trichloroacetic acid may cause oxidative stress in the liver in rodents, and that superoxide dismutase and catalase are induced; it is thus plausible that polymorphisms in these genes may have an impact on inter-individual variation in susceptibility. However, since this mechanism has not been confirmed in studies in humans, the relevance of such susceptibility is uncertain. With respect to life-stage susceptibilities, the developing fetus was suggested to be highly susceptible to trichloroacetic acid-induced toxicity. Males have been shown to be more prone to hepatocarcinogenicity after exposure to trichloroacetic acid in studies in rodents.

5. Summary of Data Reported

5.1 Exposure data

Trichloroacetic acid has been mainly used as the sodium salt as a selective herbicide. It is also used in the metal, plastics and textile industries and as an analytical reagent. It is used in the topical treatment of warts, cervical lesions and other dermatological conditions. The highest exposures to trichloroacetic acid result from it being a major end metabolite of several chlorinated organic solvents, in particular, trichloroethylene and tetrachloroethylene, and trichloroacetic acid in urine has therefore been widely used as a biological marker of exposure to these solvents. Widespread exposure also occurs at much lower levels in drinking-water and swimming pools as a by-product of chlorine-based water disinfection.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Several long-term bioassays (some including more than one experiment) have primarily focused on induction of liver tumours by trichloroacetic acid, with only limited pathology analyses of other tissues. Four drinking-water studies in male mice and two studies in female mice showed an increased incidence of hepatocellular adenoma and/or hepatocellular carcinoma. The only available study in rats given trichloroacetic acid in drinking-water had limited capacity to detect a carcinogenic response. Two initiation–promotion studies in mice showed that trichloroacetic acid is an efficient promoter of hepatocellular tumours initiated by *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea.

5.4 Mechanistic and other relevant data

Similarities exist between humans and laboratory animals with regard to the absorption, distribution and metabolism of trichloroacetic acid. Trichloroacetic acid has a much longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours), which is indicative of much slower excretion and metabolism in humans. The metabolism of trichloroacetic acid is rather slow, with the parent compound being the main urinary excretion product, and dichloroacetic acid being the main proximate metabolic product in all species studied. Dichloroacetic acid is further metabolized through GST-zeta1 to glyoxylic acid and then to oxalic and glycolic acids, glycine and carbon dioxide. The available evidence suggests that trichloroacetic acid is not a genotoxic agent. The available data in animals designate the liver as a major target organ for trichloroacetic acid. There is moderate evidence suggesting that trichloroacetic acid may act through multiple nongenotoxic mechanisms, leading to liver carcinogenesis.

Some data from studies in animals suggest that kidney may also be a target organ for trichloroacetic acid. The relevance of the apparent effects in the kidney to the cancer hazard potential of trichloroacetic acid in the kidney is unknown. There is a potential for inter-individual variability in adverse effects of trichloroacetic acid, because its major metabolite, dichloroacetic acid, is further metabolized by GST-zeta1. This enzyme is polymorphic, and such polymorphisms have been shown to have an impact on its function.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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CHLORAL AND CHLORAL HYDRATE

Chloral and chloral hydrate were considered by previous IARC Working Groups in 1995 and 2004 (IARC, 1995, 2004). New data have since become available and these have been taken into consideration in the present evaluation. Chloral and chloral hydrate are considered together since the two substances exist in equilibrium in aqueous solution.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

(a) Chloral

Chem. Abstr. Serv. Reg. No.: 75-87-6

Chem. Abstr. Serv. Name: Trichloroacetaldehyde

IUPAC Systematic Name: Chloral

Synonyms: Anhydrous chloral; 2,2,2-trichloroacetaldehyde; trichloro-ethanal; 2,2,2-tri-chloroethanal

(b) Chloral hydrate

Chem. Abstr. Serv. Reg. No.: 302-17-0

Deleted Chem. Abstr. Serv. Reg. No.: 109128-19-0

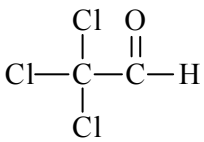
Chem. Abstr. Serv. Name: 2,2,2-Trichloro-1,1-ethanediol

IUPAC Systematic Name: Chloral hydrate

Synonyms: Chloral monohydrate; trichloroacetaldehyde hydrate; trichloroacetaldehyde monohydrate; 1,1,1-trichloro-2,2-dihydroxyethane

1.1.2 Structural and molecular formulae and relative molecular mass

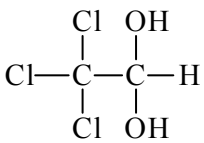
(a) Chloral



C_2HCl_3O

Relative molecular mass: 147.39

(b) Chloral hydrate



$C_2H_3Cl_3O_2$

Relative molecular mass: 165.40

1.1.3 Chemical and physical properties of the pure substances

(a) Chloral

Description: Oily liquid. Pungent, irritating odour (O'Neil *et al.*, 2006)

Boiling-point: 97.8 °C (O'Neil *et al.*, 2006)

Melting-point: –57.5 °C (O'Neil *et al.*, 2006)

Density: 1.510 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism [4626, 4426]), ultraviolet [5–3], nuclear magnetic resonance [8241] and mass [814] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

Solubility: Freely soluble in water, in which it is converted to chloral hydrate; soluble in diethyl ether and ethanol (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 10 kPa at 33.8 °C (Haynes, 2012)

Stability: Polymerizes under the influence of light and in presence of sulfuric acid to form a white solid trimer called metachloral (O'Neil *et al.*, 2006)

Conversion factor: mg/m^3 = 6.03 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa).

(b) Chloral hydrate

Description: Colourless, transparent, or white crystals with aromatic, penetrating and slightly acrid odour, slightly bitter, caustic taste. (O'Neil *et al.*, 2006)

Boiling-point: 96 °C, decomposes into chloral and water (Haynes, 2012)

Melting-point: 57 °C (O'Neil *et al.*, 2006)

Density: 1.9081 at 20 °C/4 °C (Haynes, 2012)

Spectroscopy data: Infrared (prism [5423]), nuclear magnetic resonance [10 362] and mass [1054] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

Solubility: Very soluble in water, olive oil. Freely soluble in acetone, methyl ethyl ketone (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 4.7 kPa at 20 °C; slowly evaporates on exposure to air (Jira *et al.*, 1986; O'Neil *et al.*, 2006)

Octanol/water partition coefficient (P): Log P, 0.99 (Hansch *et al.*, 1995)

Conversion factor: mg/m^3 = 6.76 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

1.1.4 Technical products and impurities

Technical-grade chloral ranges in purity from 94% to 99% by weight, with water being the main impurity. Other impurities can include chloroform, hydrogen chloride, dichloroacetaldehyde and phosgene (Jira *et al.*, 1986).

Trade names for chloral include Grasex and Sporotal 100.

Trade names for chloral hydrate include: Ansopal, Aquachloral, Chloradorm, Chloraldurat, Chloralix, Dormel, Elix-nocte, Escre, Hydral, Lanchloral, Lorinal, Medianox, Nervifene, Noctec, Novochlorhydrate, Nycton, Phaldrone, Rectules, Somnos, Suppojuvent Sedante, Tosyl, Trawotox and Welldorm.

The United States Pharmacopeia (USP) specifies that USP-grade chloral hydrate must contain not less than 99.5% chloral hydrate (US Pharmacopeial Convention, 2012). Chloral hydrate is available as a liquid-filled capsule containing 500 mg of chloral hydrate and as a syrup containing 500 mg/5 mL (PDR Network, 2012).

Table 1.1 Methods for the analysis of chloral hydrate in water

Sample preparation	Assay procedure	Limit of detection	Reference
Extract with methyl-*t*-butyl ether or pentane	GC/ECD	0.002 µg/L	EPA (1995)
Extract with ethyl acetate at pH 3.2	GC/MS	0.006 µg/L	Serrano *et al.* (2011)

ECD, electron capture detection; GC, gas chromatography; MS, mass spectrometry

1.1.5 Analysis

Methods for the analysis of chloral hydrate have been reviewed by Delinsky *et al.* (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of chloral hydrate in water are identified in Table 1.1. A biomonitoring method using gas chromatography-electron capture detection has been developed for measuring chloral hydrate in blood (Schmitt, 2002) and in urine (Garrett & Lambert, 1966).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Chloral was first synthesized by J. von Liebig in 1832 by chlorination of ethanol (Jira *et al.*, 2007).

Chloral is produced by chlorinating acetaldehyde or ethanol in acidic solution by gradually increasing the temperature from 0 °C to 90 °C (Jira *et al.*, 2007). Antimony trichloride is sometimes used as a catalyst. Chloral is distilled from the reaction mixture as the hydrate. The hydrate is then mixed with concentrated sulfuric acid, the heavier acid layer is drawn off, and chloral is distilled through a fractionating column of moderate height.

(b) Production volume

Estimated production and use of chloral in the Member States of the European Union in 1984 was 2500 tonnes (Environmental Chemicals Data and Information Network, 1993).

Chloral (anhydrous) is known to have been produced by 14 companies in China, seven companies in India and one company each in Brazil, France, Japan, Mexico, the Russian Federation and the USA. Chloral hydrate was produced by four companies in China, three companies in Germany, two companies in Japan and one company each in Mexico, the Russian Federation and Spain (Chemical Information Services, 2002).

Chloral hydrate has been produced for use as a hypnotic drug in relatively low and gradually declining volume for many years. As an indication of the scale, production in the USA for this purpose was about 135 tonnes in 1978 (Jira *et al.*, 1986).

1.2.2 Use

(a) Chloral

The principal historical use of chloral has been in the production of the insecticide dichlorodiphenyltrichloroethane (DDT) and, to a lesser extent, other insecticides such as methoxychlor, naled, trichlorfon, and dichlorvos and the herbicide trichloroacetic acid (Jira *et al.*, 2007). In the USA in 1975, about 40% of chloral was used in the manufacture of DDT, about 10% in the manufacture of other pesticides and about 50% in other applications (IARC, 1995). After the banning of DDT in many countries, demand for chloral for this use has declined dramatically.

Chloral has also been used in the production of rigid polyurethane foam (IARC 1995; Boĭtsov *et al.*, 1970) and to induce swelling of starch

granules at room temperature (Whistler & Zysk, 1978).

(b) Chloral hydrate

Chloral hydrate has been used as a hypnotic drug since the 1870s, principally for the short-term treatment of insomnia. It was also used to allay anxiety and to induce sedation and/or sleep post-operatively, before electroencephalogram evaluations, and to treat the symptoms of withdrawal of alcohol and other drugs such as opiates and barbiturates. For many years chloral hydrate was widely used for the sedation of children before diagnostic, dental or medical procedures, but although still in use, it has largely been replaced by newer drugs with a lower risk of overdose (Pershad *et al.*, 1999).

After oral administration, chloral hydrate is converted rapidly to trichloroethanol, which is largely responsible for its hypnotic action. Externally, chloral hydrate has a rubefacient action (producing redness of the skin) and has been used as a counter-irritant. It is administered by mouth as a liquid or as gelatin capsules. It has also been dissolved in a bland fixed oil and given by enema or as suppositories (Gennaro, 2000; Royal Pharmaceutical Society of Great Britain, 2002).

Chloral hydrate is also an ingredient in Hoyer's solution, which is used in microscopy to mount organisms such as bryophytes, ferns, seeds, and arthropods (Anderson, 1954).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Chloral and chloral hydrate are not known to occur as natural products.

1.3.2 Environmental occurrence

(a) Air

No data are available on human exposure to chloral or chloral hydrate in air. The low volatility of chloral hydrate from a water solution precludes significant exposure by inhalation (EPA, 2000).

(b) Water

When raw water, containing natural organic material such as humic, tannic or amino acids, is treated by chlorination for use as drinking-water or in swimming pools, by-products resulting from disinfection can be formed, such as chloral, which is rapidly transformed into chloral hydrate (Miller & Uden, 1983; Sato *et al.*, 1985; Trehy *et al.*, 1986; Italia & Uden, 1988). Chloral hydrate is primarily produced by the treatment of water with chlorine or chloramine, but the use of pre-ozonation before chlorination or chloramination can favour its formation (Richardson *et al.*, 2007).

Table 1.2 summarizes recent measurements of chloral hydrate in drinking-water and swimming pool water in several countries.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 11 278 employees in the USA were potentially exposed to chloral hydrate (NIOSH, 1994). The estimate is based on a survey of companies and did not involve measurement of actual exposures (IARC, 2004).

Chloral has been detected in the work environment during spraying and casting of polyurethane foam (Boĭtsov *et al.*, 1970). It has also been identified as an autoxidation product of trichloroethylene during extraction of vegetable oil (McKinney *et al.*, 1955), and detected in the output of etching chambers in semiconductor processing (Ohlson, 1986).

Table 1.2 Concentrations of chloral hydrate in drinking-water

Country	Location	Concentration (µg/L)		Reference
		Mean	Range	
Drinking-water				
Australia	Seven cities	NR	0.2–19	Simpson & Hayes (1998)
Canada	Country-wide (summer 1993)	6.1	< 0.1–18.9	Koudjonou *et al.* (2008)
		3.6	0.3–13.6	
		8.4	0.2–23.4	
China	Beijing	0.93	NR–10.44	Wei *et al.* (2010)
Greece	Athens	NR	0.2–12.5	Golfinopoulos & Nikolaou (2005)
	Mytilene	NR	NR–0.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	< 1[a]	< 1–12.1	Villanueva *et al.* (2012)
	Cordoba	NR	1.2–38	Serrano *et al.* (2011)
Swimming-pool water				
Republic of Korea	Seoul	16.9	5.1–34.9	Lee *et al.* (2010)
		3.6	ND–10.4	
		10.2	ND–23.4	
Spain	Cordoba	NR	53–340	Serrano *et al.* (2011)

[a] Median
ND, not detected; NR, not reported

In spite of the use of chloral as an intermediate in the synthesis of insecticides and herbicides, no specific measurement data from workers exposed during synthesis or formulation were available to the Working Group. Given the use of chloral hydrate as a sedative and hypnotic drug, workers could be exposed in the pharmaceutical industry during production. However no data on exposure measurement were identified by the Working Group.

1.3.4 Exposure in the general population

Chloral is an intermediate metabolite of trichloroethylene in humans, and chloral hydrate has been found in the plasma of people who have undergone anaesthesia with trichloroethylene (Cole *et al.*, 1975; Davidson & Beliles, 1991).

1.4 Regulations and guidelines

Chloral hydrate is a controlled substance in Canada and the USA, available by prescription only. Chloral hydrate is banned for marketing in India. There are no occupational limits for chloral or chloral hydrate.

2. Cancer in Humans

Chloral hydrate is a chemical that occurs in drinking-water and swimming pools as part of a mixture of by-products resulting from disinfection of drinking-water by chlorination. The chemicals in water-disinfection by-products do not occur in an isolated manner and there is no epidemiological evidence on risk of cancer associated specifically with these by-products. A detailed description of water-disinfection by-products and cancer risk is given in *IARC Monograph* Volume 101 (IARC, 2012a).

Only one epidemiological study has examined risk of cancer in humans exposed to chloral hydrate. Haselkorn *et al.* reported cancer morbidity among 2290 users of chloral hydrate within a cohort of 143 574 patients at Kaiser Permanente, USA, who had prescriptions filled

for the 215 most commonly used drugs between 1969 and 1973 (Haselkorn *et al.*, 2006). Study subjects in this cohort comprised an ethnically and socioeconomically diverse population, who had received both inpatient and outpatient care within the prepaid system. Cohort members were linked to the pharmacy records by each patient's unique medical record number. Cancer occurrence (1976–1998) for each study subject was ascertained via the local tumour registry and hospital records of all northern California hospitals in the Kaiser Permanente programme. Cancers diagnosed were verified by the review of medical records by a trained medical analyst. Expected numbers of overall and specific cancer were calculated based on age and sex standardized rates from the entire Kaiser Permanente cohort. By 1998, there were 285 cancer cases observed versus 258 expected [standardized incidence ratio, SIR, 1.1; 95% CI, 0.98–1.24]. Further, observed and expected numbers for cancers of mouth floor [SIR, 5.0; 95% CI, 1.03–14.6], stomach [SIR, 2.1; 95% CI, 1.2–3.3], lung [SIR, 1.3; 95% CI, 0.9–1.7], skin melanoma [SIR, 1.4; 95% CI, 0.7–2.4], prostate [SIR, 1.0; 95% CI, 0.7–1.4] and kidney [SIR, 0.2; 95% CI, 0.05–1.1] are reported. Relations between numbers of prescriptions of chloral hydrate and overall cancer, cancer of lung, stomach, prostate and melanomas were examined in a series of nested case–control studies, adjusted for potential confounders. No significant trends were observed. [The Working Group noted the relatively low power of this study to detect statistically significant deviations from unity for specific cancers].

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

Three groups of 20–35 male C57BL × $C3HF_1$ mice (age, 15 days) were given chloral hydrate in water intragastrically as a single dose at 0, 5, or 10 mg/kg body weight (bw). The mice were killed at various time intervals up to 92 weeks. Liver nodules were examined by histopathology and included hyperplastic nodules, hepatocellular adenoma and hepatocellular carcinoma [termed trabecular]. In mice killed between 48 and 92 weeks after dosing, a statistically significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) was observed in mice receiving chloral hydrate at a dose of 10 mg/kg bw (6 out of 8 versus 2 out of19 controls) (Rijhsinghani *et al.* (1986)). [The Working Group noted the small number of mice evaluated in this study and the low doses administered.]

The hepatocarcinogenicity of chloral hydrate was studied in two groups of 33–40 male $B6C3F_1$ mice given drinking-water containing chloral hydrate at a dose of 0 or 1 g/L (ingested dose, 166 mg/kg bw per day) for 104 weeks (Daniel *et al.*, 1992). Five animals per group were killed after 30 and 60 weeks of exposure. Microscopic examinations were performed on all gross lesions, liver, kidney, testis and spleen of mice surviving 104 weeks. Statistically significant increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were observed in treated mice surviving 104 weeks. Results are shown in Table 3.1. [The Working Group noted that a single dose was used, the group size was small, histopathological evaluation was limited to mice surviving at least 104 weeks, and the histopathological examinations were limited to the liver.]

The initial study by Daniel *et al.* (1992) was followed up with a study in groups of 72 male $B6C3F_1$ mice given repeated doses of chloral hydrate for 104 weeks (George *et al.*, 2000).

Table 3.1 Studies of carcinogenicity in experimental animals given chloral hydrate by oral administration

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C57BL×C3HF$_1$ (M) Up to 92 wk Rijhsinghani *et al.* (1986)	Single dose, by gavage at 0, 5, 10 mg/kg bw, to mice aged 15 days 35, 25, and 20/group	Hepatic nodules [hepatocellular adenoma or carcinoma (combined)]: 2/19, 2/9, 6/8*	*$P < 0.05$	Laboratory grade Small numbers of mice and low dose
Mouse, B6C3F$_1$ (M) 104 wk Daniel *et al.* (1992)	0, 1 g/L, in drinking-water 33, 40/group Interim kills of 10/group	Hepatocellular adenoma: 1/20, 7/24* Hepatocellular carcinoma: 2/20, 11/24** Hepatocellular adenoma or carcinoma (combined): 3/20, 17/24***	Fisher's exact test *$P \leq 0.05$ **$P \leq 0.03$ ***$P \leq 0.01$	Purity, > 95% Histopathological data only for the liver
Mouse, B6C3F$_1$ (M) 104 wk George *et al.* (2000)	0, 0.12, 0.58, 1.28 g/L, in drinking-water 72/group	Prevalence in mice surviving > 78 wk: Hepatocellular adenoma: 21.4 (n = 42), 43.5 (n = 46)*, 51.3 (n = 39)*, 50.0% (n = 32)* Hepatocellular carcinoma: 54.8 (n = 42), 54.3 (n = 46), 59.0 (n = 39), 84.4% (n = 32)* Hepatocellular adenoma or carcinoma (combined): 64.3 (n = 42), 78.3 (n = 46), 79.5 (n = 39)*, 90.6% (n = 32)*	*$P \leq 0.05$	Purity, > 99% Measured concentrations; histopathological data only for the liver; neoplasms observed in the kidney, spleen and testis reported not to exceed the incidences in control group or historical controls.
Mouse, B6C3F$_1$ (M) 12 or 20 months Von Tungeln *et al.* (2002)	*12 months* Two i.p. doses at age 8 and 15 days; total dose, 0 (DMSO control) or 2000 nmol per mouse 24, 24/group	*12 months* Hepatocellular adenoma: 1/24, 5/24	NS	Purity, NR Small numbers of mice; low total dose; histopathology restricted to the liver. Females were also injected with chloral hydrate and no hepatocellular tumours were observed.
	20 months Two i.p. doses at age 8 and 15 days; total dose, 0 (DMSO control) or 1000 nmol per mouse 23, 23/group	*20 months* Hepatocellular adenoma: 6/23, 9/23 Hepatocellular carcinoma: 2/23, 2/23 Hepatocellular adenoma or carcinoma (combined): 7/23, 10/23	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 104 wk NTP (2002a), Leakey *et al.* (2003)	Gavage 0, 25, 50, 100 mg/kg bw 5 days/wk by gavage to *ad libitum*-fed or dietary controlled mice. 48/group	*Fed* ad libitum Hepatocellular carcinoma: 4/48, 10/48, 10/47, 7/48 Hepatocellular adenoma or carcinoma (combined): 16/48, 25/48*, 23/47, 22/48 *Dietary controlled* Hepatocellular carcinoma: 2/48**, 5/48, 4/48, 8/48*** Hepatocellular adenoma or carcinoma (combined): 11/48****, 11/48, 14/48, 18/48	Poly-3 test *P = 0.0437 **P = 0.0371 (trend) ***P = 0.0422 ****P = 0.045 (trend)	Purity, > 99.5% No treatment-related reduction in survival
Mouse, B6C3F$_1$ (F) 104 wk NTP (2002b)	*Regimen A* Gavage; 0, 25, 50, 100 mg/kg bw, 5 days/wk 48/group	Pituitary gland (pars distalis) adenoma: 0/45*, 2/44, 0/47, 5/41** Malignant lymphoma: 9/48***, 7/48, 8/48, 15/48	Poly-3 test *P = 0.073 (trend) **P = 0.024 ***P = 0.0455 (trend)	Purity, 99% No exposure-related reduction in survival.
Rat, F344/N (M) 104 wk George *et al.* (2000)	0, 0.12, 0.58, 2.51 g/L, in drinking-water 78/group	Prevalence in rats surviving > 78 wk: Hepatocellular adenoma: 0 (n = 42), 7.1 (n = 44), 2.3 (n = 44), 4.5% (n = 42) Hepatocellular carcinoma: 2.4 (n = 42), 7.1 (n = 44), 0 (n = 44), 2.3% (n = 42) Hepatocellular adenoma or carcinoma (combined): 2.4 (n = 42), 14.3 (n = 44), 2.3 (n = 44), 6.8% (n = 42)	NS	Purity, > 99% Measured concentrations; histopathological data only for the liver; neoplasms observed in the kidney, spleen and testes reported not to exceed the incidences in control group or historical controls.

bw, body weight; F, female; i.p., intraperitoneal; M, male; mo, month; NR, not reported; NS, not significant; wk, week

Measured concentrations of chloral hydrate in the drinking-water were 0, 0.12, 0.58, and 1.28 g/L, corresponding to mean daily doses of 0, 13.5, 65, and 146.6 mg/kg bw. Six mice per group were killed after 26, 52, and 78 weeks. Histopathological examinations of the liver, kidney, spleen and testis were performed on mice surviving more than 78 weeks. Hepatocellular adenomas and carcinomas were observed as early as 52 weeks and incidence increased progressively with duration of treatment. In mice surviving more than 78 weeks, the incidence of hepatocellular adenoma was increased ($P \leq 0.05$) in all dose groups, the incidence of hepatocellular carcinoma was increased in the group at 1.28 g/L, and the incidence of hepatocellular adenoma or carcinoma (combined) was increased in the groups at 0.58 and 1.28 g/L. The incidences of neoplasms observed in the kidney, spleen, and testes were reported not to exceed those in the control group or in historical controls (data not shown). [The Working Group noted that the evaluations of tumour incidence were limited to the four organs examined microscopically.]

Groups of 24 male and 21 female neonatal B6C3F$_1$ mice were given intraperitoneal injections of chloral hydrate in dimethyl sulfoxide (DMSO) on postnatal day 8 (three sevenths of the total dose) and day 15 (four sevenths of the total dose) (Von Tungeln *et al.*, 2002). The total dose administered was 2000 nmol [0.33 mg] per mouse, and the mice were observed for 12 months. In a second experiment, groups of 23 males and 22 females were given intraperitoneal injections of chloral hydrate in DMSO on postnatal day 8 (one third of the total dose) and day 15 (the remaining two thirds of the total dose). In this experiment, the total dose administered was 1000 nmol [0.165 mg] per mouse and the groups were observed for 20 months. Control groups of 23–24 mice were injected with the vehicle (DMSO) only. Histopathological examination was restricted to the liver. In males, a non-statistically significant increase in the incidence of hepatocellular adenoma was observed after 12 months and 20 months (5 out of 24 versus 1 out of 24, and 9 out of 23 versus 6 out of 23, respectively). No hepatocellular tumours were observed in the control or treated groups of female mice. [The Working Group noted that only the liver was examined microscopically and that the total doses administered were much lower than those used in other long-term studies of this chemical.]

The National Toxicology Program performed two studies of the carcinogenic effects of chloral hydrate in B6C3F$_1$ mice (NTP, 2002a, b). The first study (NTP, 2002a; Leakey *et al.*, 2003) was an investigation of the influence of dietary restriction on the development of hepatocellular adenoma and hepatocellular carcinoma. Groups of 48 male B6C3F$_1$ mice were given chloral hydrate (dissolved in distilled water) by gavage at a dose of 0, 25, 50, or 100 mg/kg bw, 5 days per week for 104 weeks. The incidences of hepatocellular adenoma and hepatocellular carcinoma were much lower in mice with a controlled diet than in mice fed ad libitum. There was a statistically significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) in mice fed ad libitum at 25 mg/kg bw, but not at 50 or 100 mg/kg bw. There was a statistically significant increase in the incidence of hepatocellular carcinoma in mice with a controlled diet containing chloral hydrate at 100 mg/kg bw (Table 3.1). [The Working Group noted that the mice in this study were dosed for 5 days per week, while the doses in studies by Daniel *et al.* (1992) and George *et al.* (2000) were higher and were administered 7 days per week.]

In the second study (NTP, 2002b), female B6C3F$_1$ mice were given chloral hydrate (dissolved in distilled water) by gavage in two different regimens. In regimen A, groups of 48 mice were given chloral hydrate at a dose of 0, 25, 50, or 100 mg/kg bw, 5 days per week for 104 weeks (24 months). In regimen B, groups of 48 mice were given chloral hydrate at a dose of 0 (n = 24) or 100 mg/kg bw for 3, 6 or 12 months. Eight mice each from the

groups at 0 and 100 mg/kg bw were killed at 3, 6, or 12 months. The remaining mice were held until termination of the study at 24 months. For regimen A, an increasing trend in incidence of adenoma of the pituitary gland (pars distalis) in the group at the highest dose was observed in mice treated for 24 months. A statistically significant increasing trend in incidence of malignant lymphoma was also observed, but the incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma (combined) were not increased. For regimen B, tumour incidences were not increased in female mice exposed to chloral hydrate for 3, 6, or 12 months. [The Working Group noted that mice in this study were dosed for 5 days per week, while the studies by Daniel *et al.* (1992) and George *et al.* (2000) used higher doses that were administered 7 days per week.]

3.2 Rat

Oral administration

Groups of 50 male and 50 female Sprague-Dawley rats were given drinking-water containing chloral hydrate at nominal doses of 0, 0, 15, 45, and 135 mg/kg bw (Leuschner & Beuscher, 1998). The rats were examined after 124 weeks (males) or 128 weeks (females) of exposure. A statistically significantly increase in hepatic hypertrophy was reported in the group of male rats at the highest dose. It was reported that the incidence of neoplastic lesions was not increased in treated males or females compared with rats in the control groups. [The Working Group noted that the lack of incidence data in this paper hampered evaluation of the study.]

Groups of 78 male F344/N rats were given drinking-water containing chloral hydrate at measured concentrations of 0, 0.12, 0.58, or 2.51 g/L (George *et al.*, 2000). These treatments provided mean daily doses of 0, 7.4, 37.4, and 162.6 mg/kg bw. Six rats per group were killed after 13, 26, 52, and 78 weeks. Survivors were maintained on treatment for 104 weeks. Histopathological examination of the liver, kidney, spleen and testis was performed on rats surviving more than 78 weeks. There were no treatment-related increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma (combined). However, 3 out of 44 (7%) of rats receiving the lowest dose developed hepatocellular carcinomas, while the incidence of hepatocellular carcinomas for historical controls in this strain of rats was only 19 out of 2255 (0.8%) (Haseman *et al.*, 1998). The incidences of neoplasms of the kidney, spleen, and testis were reported not to exceed those observed in the control group or historical controls (data not shown). [The Working Group noted that the evaluations of tumour incidence were limited to the four organs examined microscopically.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Studies in humans have shown that chloral hydrate is rapidly absorbed after oral administration, with peak concentrations in blood occurring within 1 hour after dosing (Merdink *et al.*, 2008). The extent of oral absorption has not been measured precisely in humans, but is likely to be high due to the rapidity of absorption. Recovery of metabolites in urine, which represents a lower boundary on absorption, has been reported to be between 47% and 60% for subjects followed for 1 week after administration of a single oral dose (Müller *et al.*, 1974; Merdink *et al.*, 2008). It should be noted that 1 week is

insufficient for complete urinary excretion of the metabolites of chloral hydrate. With repeated oral dosing, Owens & Marshall (1955) reported high inter-individual variation in recovery, with average daily excretion ranging from 7% to 94% of the daily dose of chloral hydrate.

No data were available to the Working Group on absorption in humans via other routes of exposure.

(b) *Experimental systems*

Orally administered chloral hydrate is rapidly absorbed in rats and mice, with peak concentrations reported at 15 minutes after administration (Beland *et al.*, 1998). The extent of oral absorption has not been measured precisely in experimental systems, but is likely to be high due to the rapidity of absorption. The percentage absorption was not estimated since no studies on urinary excretion or mass balance for orally administered chloral hydrate were available to the Working Group.

No experimental data on absorption via other routes of exposure were available to the Working Group.

4.1.2 Distribution

(a) Humans

In humans, orally administered chloral hydrate enters the liver where it undergoes extensive metabolism (see Section 4.1.3); only a limited amount enters the systemic circulation (Merdink *et al.*, 2008). Chloral hydrate in the blood is rapidly eliminated by metabolism, as shown by Zimmermann *et al.* (1998), who reported a half-life of less than 1 hour, and Merdink *et al.* (2008), who reported a rapid initial decline with a terminal half-life of about 10 hours. [Although no data on distribution in human tissues were available, the Working Group noted that due to the extensive first-pass effect, it was likely that concentrations of chloral hydrate were much lower in extra-hepatic tissues than in the liver of humans exposed orally.]

No data on distribution via other routes of administration were available to the Working Group.

(b) *Experimental systems*

In rats and mice, orally administered chloral hydrate enters the liver where it undergoes extensive metabolism (see Section 4.1.3); only a limited amount enters the systemic circulation (Beland *et al.*, 1998). Chloral hydrate in the blood is rapidly eliminated, probably by metabolism, with levels reported to be below the limit of detection within 3 hours after oral administration (Beland *et al.*, 1998). [Although no data on tissue distribution were available, the Working Group noted that due to the extensive first-pass effect, it was likely that concentrations of chloral hydrate in extra-hepatic tissues were much lower than those in the liver of rodents exposed orally.]

The half-life of chloral hydrate after intravenous administration has been reported to be about 5–20 minutes in mice and rats (Abbas *et al.*, 1996; Merdink *et al.*, 1999), indicating rapid elimination from the systemic circulation. Abbas *et al.* (1996) reported concentrations of chloral hydrate in the liver to be about one quarter to one half of concentrations in the blood. No data on distribution to other tissues after intravenous administration were available to the Working Group.

No data on distribution via other routes of administration were available to the Working Group.

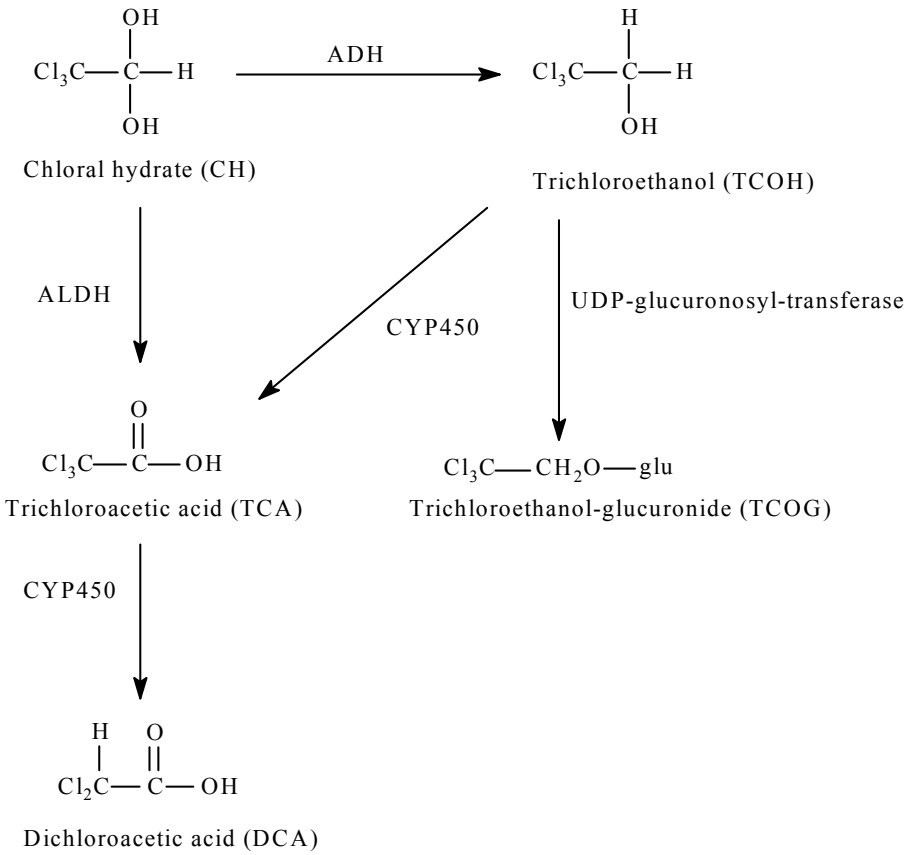
4.1.3 Metabolism

(a) Humans

The metabolic pathways of chloral hydrate and its metabolites in humans are depicted in Fig. 4.1.

Multiple studies in humans have reported that the metabolites of chloral hydrate include trichloroethanol, its glucuronide, and trichloroacetic acid. For instance, Owens & Marshall

Fig. 4.1 Metabolism of chloral hydrate



ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP450, enzyme of the cytochrome P450 family

(1955), Breimer *et al.* (1974), and Merdink *et al.* (2008) measured free trichloroethanol, total trichloroethanol (free plus glucuronidated), and trichloroacetic acid in blood and/or urine after administration of chloral hydrate to human volunteers. The terminal half-lives of trichloroethanol and trichloroacetic acid after oral exposure to chloral hydrate have been measured to be about 8–13 hours for trichloroethanol and 4–5 days for trichloroacetic acid (Breimer, 1977; Zimmermann *et al.*, 1998; Merdink *et al.*, 2008).

Merdink *et al.* (2008) also examined the importance of enterohepatic recirculation, by which trichloroethanol-glucuronide is excreted with bile into the intestine, where trichloroethanol is regenerated and reabsorbed in the gut. In this study, the subjects consumed a high-fat meal 4 hours after dosing with chloral hydrate, to "synchronize" the secretion of bile and subsequent excretion of trichloroethanol-glucuronide into the intestine. The resulting cyclic variation in the amount of trichloroethanol-glucuronide and the complex kinetic behaviour of plasma trichloroacetic acid (e.g. two distinct peak concentrations) are consistent with enterohepatic recirculation.

Considerable amounts of dichloroacetic acid were reported as a urinary metabolite of chloral hydrate in children (Henderson *et al.*, 1997a). However, only trace amounts were detected after administration of chloral hydrate to adults (Merdink *et al.*, 2008). [The Working Group noted that due to uncertainties as to artefactual (*ex vivo*) formation of dichloroacetic acid

in biological samples (Ketcha *et al.*, 1996), it was unclear whether this difference was due to life stage or to the analytical methodologies used.]

Bronley-DeLancey *et al.* (2006) used cryogenically preserved human hepatocytes to simultaneously evaluate the kinetics of the metabolism of chloral hydrate and alcohol dehydrogenase/aldehyde dehydrogenase (ADH/ALDH) genotype. Thirteen samples of human hepatocytes were examined, and large inter-individual variation in the V_{max} values for formation of trichloroethanol and trichloroacetic acid was reported. In this sample of limited size, no correlation with ADH/ALDH genotype was apparent. Furthermore, despite the large variation in V_{max} values between individuals, disposition of chloral hydrate into downstream metabolites was found to be relatively constant.

(b) Experimental systems

A similar spectrum of metabolites has been reported in experimental studies. Studies in rats, mice, and dogs have all identified trichloroacetic acid, trichloroethanol-glucuronide, and trichloroethanol as the major metabolites of chloral hydrate (Breimer *et al.*, 1974; Abbas *et al.*, 1996; Beland *et al.*, 1998; Merdink *et al.*, 1998, 1999).

The importance of enterohepatic recirculation in the kinetics of chloral hydrate metabolites has been examined by Merdink *et al.* (1999) through comparison between intact and bile-cannulated rats given chloral hydrate, trichloroethanol, and trichloroacetic acid via the jugular vein. A statistically significant difference in the kinetics of trichloroethanol and its glucuronide was reported for between intact and bile-cannulated rats given trichloroethanol. Kinetic differences for chloral hydrate, trichloroethanol and its glucuronide, or trichloroacetic acid after administration of chloral hydrate were evident only at the highest dose of chloral hydrate (192 mg/kg bw, compared with 12 and 48 mg/kg bw). Moreover, chloral hydrate, trichloroethanol and its glucuronide, and trichloroacetic acid were all detected in bile, with trichloroethanol-glucuronide exhibiting the highest peak concentrations. Therefore, while the data are consistent with enterohepatic recirculation of trichloroethanol and its glucuronide occurring in rats, this process appears to have little impact on the kinetics of chloral hydrate or its metabolites in rats, except at higher doses.

Abbas *et al.* (1996) reported detecting dichloroacetic acid in mice given chloral hydrate by oral administration, but it was later determined that these data were probably confounded by artefactual formation of dichloroacetic acid during sample preparation (Ketcha *et al.*, 1996; Abbas & Fisher, 1997; Merdink *et al.*, 1998). Later studies (Beland *et al.*, 1998; Merdink *et al.*, 1998, 1999) reported only trace or undetectable amounts of dichloroacetic acid in mice or rats given chloral hydrate. Merdink *et al.* (1998) have suggested that it is likely that some dichloroacetic acid is being formed as a short-lived intermediate, but that the extremely rapid elimination kinetics of dichloroacetic acid relative to its formation do not allow for accumulation (and detection) of dichloroacetic acid in the blood.

Chloral hydrate was shown to be an inhibitor of ALDH (Wang *et al.*, 1999), suggesting that production of trichloroacetic acid from chloral hydrate may not increase in a linear fashion with dose. An inhibitory effect of chloral hydrate on liver ADH was also reported in studies in mice (Sharkawi *et al.*, 1983). In a short-term study in rats, Poon *et al.* (Poon *et al.*, 2002) showed that exposure to drinking-water containing chloral hydrate led to statistically significant reduction in activity of liver ALDH, while the activity of liver aniline hydroxylase (a marker for CYP2E1) was significantly elevated in males and females receiving chloral hydrate at 200 ppm. In the same study, the findings of Wang *et al.* (1999) were confirmed, showing that chloral hydrate is a potent inhibitor of liver ALDH *in vitro*, with an IC_{50} of 8 μM, while trichloroacetic acid was weakly inhibitory, and trichloroethanol was without effect.

4.1.4 Excretion

(a) Humans

The primary known excretion route for chloral hydrate in humans is as the metabolites trichloroethanol-glucuronide and trichloroacetic acid in urine (Owens & Marshall, 1955; Merdink *et al.*, 2008). Owens & Marshall (1955) found that recovery of urinary metabolites was not complete, with average daily excretion ranging from 7% to 94% of daily doses of chloral hydrate, according to individual. Therefore, it is possible that other excretion routes exist that have not been well characterized. For instance, low concentrations of chloral hydrate have been found in breast milk. Although breastfeeding infants may be sedated by chloral hydrate in breast milk, the highest concentration measured in milk (about 15 µg/mL) is considerably lower than that measured in blood after administration of chloral hydrate at a clinically active dose (100 µg/mL) (Bernstine *et al.*, 1956; Wilson, 1981).

(b) Experimental systems

In mice and rats, chloral hydrate appears to be excreted primarily in urine as the metabolites trichloroethanol-glucuronide and trichloroacetic acid (Merdink *et al.*, 1998, 1999); however, there were no comprehensive studies of mass balance available to the Working Group. Therefore, the extent of recovery represented by urinary excretion is unknown, although it is often assumed to be 100% (Beland *et al.*, 1998).

Few notable differences have been found in the excretion of chloral hydrate in rats and mice. While Beland *et al.* (1998) noted statistically significant differences in the half-lives of trichloroethanol and its glucuronide in rats and mice, all estimated half-lives were very short (< 1 hour). Beland *et al.* (1998) also reported that with the same regime of repeated doses (12 doses of chloral hydrate at 50 or 200 mg/kg bw over 16 days), the area under the concentration–time curve (AUC) of trichloroacetic acid in rats was greater than that in mice.

4.2 Genotoxicity and related effects

4.2.1 Humans

Studies on the genotoxic effects of chloral hydrate in humans *in vivo* and *in vitro* are presented in Table 4.1.

Ikbal *et al.* (2004) assessed the frequencies of micronucleus formation and sister-chromatid exchange in cultured peripheral blood lymphocytes of 18 infants (age range, 31–55 days) before and after administration of a single dose (50 mg/kg bw) of chloral hydrate in breast milk or formula administered for the purposes of sedation before a hearing test. There was a statistically significant increase in the mean frequency of micronucleus formation (2.57 ± 0.20/1000 cells before treatment versus 3.56 ± 0.17/cell after treatment; $P = 0.004$), as well as in the mean frequency of sister-chromatid exchange (7.03 ± 0.18/cell before treatment versus 7.90 ± 0.19/cell after treatment; $P < 0.001$). On an individual level, 15 out of 18 individuals showed an increase in the frequency of micronucleus formation with treatment, and 18 out of 18 individuals showed an increase in the frequency of sister-chromatid exchange with treatment. Sister-chromatid exchange was also assessed by Gu *et al.* (1981) in human lymphocytes exposed *in vitro*, with inconclusive results.

The ability of chloral hydrate to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmokers (Sbrana *et al.*, 1993). Cells were exposed to chloral hydrate at doses of 50–250 µg/mL for 72 hours or 96 hours. No increases in the percentage of cells with hyperdiploidy, tetraploidy, or endoreduplication were observed when cells were exposed for 72 hours at any dose tested. Although no dose–response relationships was observed after 96 hours of exposure, there was a statistically

Table 4.1 Studies of genoxicity with chloral hydrate in humans *in vitro* and *in vivo*

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
In vitro				
DNA damage (comet assay), TK6 cells	16.5	NT	+	Liviac *et al.* (2010)
DNA damage (comet assay), HepG2 cells	3.3	NT	+	Zhang *et al.* (2012)
DNA SSB, lymphoblastoid cells	1650	NT	–	Chang *et al.* (1992)
Gene mutation, *TK* and *HPRT* locus, lymphoblastoid cells	1000	NT	+	Beland (1999)
SCE, lymphocytes	54	NT	(+)	Gu *et al.* (1981)
Micronucleus formation, lymphocytes	100	–	+	Van Hummelen & Kirsch-Volders (1992)
Micronucleus formation, lymphoblastoid AHH-1 cell line	100	NT	+	Parry *et al.* (1996)
Micronucleus formation, lymphoblastoid maximum contaminant level-5 cell line	500	NT	–	Parry *et al.* (1996)
Micronucleus formation (kinetochore-positive), diploid LEO fibroblasts	120	NT	+	Bonatti *et al.* (1992)
Micronucleus formation, TK6 cells	413	NT	–	Liviac *et al.* (2010)
Aneuploidy (double Y induction), lymphocytes	250	NT	+	Vagnarelli *et al.* (1990)
Aneuploidy (hyperdiploidy and hypodiploidy), lymphocytes	50	NT	+	Sbrana *et al.* (1993)
Polyploidy, lymphocytes	137	NT	+	Sbrana *et al.* (1993)
C-Mitosis, lymphocytes	75	NT	+	Sbrana *et al.* (1993)
In vivo				
Micronucleus formation, infants, peripheral lymphocytes	50, oral	NT	+	Ikbal *et al.* (2004)

[a] Doses are in µg/mL for tests *in vitro*, and mg/kg bw for tests *in vivo*.

+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; SCE, sister-chromatid exchange; SSBs, single strand breaks.

significant increase in the percentage of hyperdiploid cells at 150 μg/mL, and in the percentage of tetraploid cells at 137 μg/mL.

4.2.2 Experimental systems

Chloral hydrate has been evaluated for genotoxic potential in a variety of assays in experimental systems (see Table 4.2, 4.3 and 4.4).

(a) DNA binding and damage

There has been limited analysis of the DNA-binding potential of chloral hydrate (Keller & Heck, 1988; Ni *et al.*, 1995; Von Tungeln *et al.*, 2002). Keller & Heck (1988) conducted experiments in $B6C3F_1$ mice *in vitro* and in *vivo*. The mice were pretreated by gavage with trichloroethylene at a dose of 1500 mg/kg bw per day for 10 days, and then given [^{14}C]-labelled chloral intraperitoneally at a dose of 800 mg/kg bw. No detectable covalent binding of the radiolabel to DNA in the liver was observed.

Keller & Heck (1988) investigated the potential of chloral hydrate to form DNA–protein crosslinks in rat liver nuclei. No statistically significant increase in the frequency of DNA–protein crosslinks was observed with chloral hydrate at concentrations of 25, 100, or 250 mM [3.7, 14.7, 36.8 mg/mL]. DNA and RNA isolated from the nuclei treated with [C^{14}]-labelled chloral did not have any detectable bound radiolabel; however, concentration-dependent binding of the radiolabel to proteins from choral-treated nuclei was observed.

Incubation of chloral hydrate with liver microsomes from male $B6C3F_1$ mice resulted in increases in the amounts of lipid-peroxidation products (malondialdehyde and formaldehyde). This effect was inhibited by free radical scavengers, α-tocopherol or menadione (Ni *et al.*, 1994). Ni *et al.* (1995) subsequently observed malondialdehyde adducts in calf thymus DNA in the presence of chloral hydrate and liver microsomes from male $B6C3F_1$ mice. In another study in $B6C3F_1$ mice, exposure *in vivo* to nonradiolabelled chloral hydrate at a concentration of 2000 nmol [330 μg] resulted in an increase in malondialdehyde-derived adducts and 8-oxo-2′-deoxyguanosine adducts in liver DNA, indirect indicators of oxidative DNA damage (Von Tungeln *et al.*, 2002).

Kiffe *et al.* (2003) carried out the single-cell gel electrophoresis assay (comet assay) in Chinese hamster ovary K5 cells under standard assay conditions or with modifications involving collection of all cells, concurrent treatment with ethyl methanesulfonate (for detecting crosslinking properties) and/or analysis of subcellular DNA breakage. Chloral hydrate gave negative results except at the highest concentration (5000 μg/mL), at which cell viability was about 30%. Zhang *et al.* (2012) conducted the single-cell gel electrophoresis assay in HepG2 cells to assess the DNA-damaging potential of chloral hydrate. A statistically significant increase ($P < 0.01$) in DNA damage was reported after treatment with chloral hydrate at 20 μM [3.3 μg/mL] for 4 hours, with cell viability exceeding 75%. Cell viability decreased below 75% at concentrations of 80 μM [13.2 μg/mL] and higher. Liviac *et al.* (2010) carried out the single-cell gel electrophoresis assay in TK6 cells and reported statistically significant increases in DNA damage at concentrations of 100 μM [16.5 μg/mL] and higher, with no change in cell viability up to the highest concentration tested (10 mM) [1654 μg/mL]. Liviac *et al.* (2010) also examined DNA-repair kinetics, reporting that induced DNA damage was repaired after 45 minutes. Therefore, the degree of DNA damage appears to depend heavily on the type of cell used in the experimental system.

(b) Mutations

Chloral hydrate induced gene mutation in *Salmonella typhimurium* TA100 and TA104 strains, but not in most other strains assayed. Four out of six studies of reverse mutation in *S. typhimurium* TA100 and two out of two

Table 4.2 Genotoxicity of chloral hydrate in bacterial, yeast, and fungal systems

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
SOS chromotest, *Escherichia coli* PQ37	10 000	–	–	Giller *et al.* (1995)
Salmonella typhimurium TA1535, TA98, reverse mutation	10 000	–	–	Waskell (1978)
S. typhimurium TA100, reverse mutation	2000 µg/plate	+	+	Ni *et al.* (1994)
S. typhimurium TA100, reverse mutation, liquid medium	300	+	–	Giller *et al.* (1995)
S. typhimurium TA100, TA104, reverse mutation	1000 µg/plate	+	+	Beland (1999)
S. typhimurium TA104, reverse mutation	1000 µg/plate	+	+	Ni *et al.* (1994)
S. typhimurium TA1535, reverse mutation	1850	–	–	Leuschner & Leuschner (1991)
S. typhimurium TA1535, TA1537 reverse mutation	6667	–	–	Haworth *et al.* (1983)
S. typhimurium TA1535, reverse mutation	10 000	–	–	Beland (1999)
S. typhimurium TA98, reverse mutation	7500	–	–	Haworth *et al.* (1983)
S. typhimurium TA98, reverse mutation	10 000 µg/plate	–	+	Beland (1999)
A.nidulans, diploid strain 35X17, mitotic crossover	1650	NT	–	Crebelli *et al.* (1985)
A. nidulans, diploid strain 30, mitotic crossover	6600	NT	–	Käfer (1986)
A. nidulans, diploid strain NH, mitotic crossover	1000	NT	–	Kappas (1989)
A. nidulans, diploid strain P1, mitotic crossover	990	NT	–	Crebelli *et al.* (1991)
A. nidulans, diploid strain 35X17, nondisjunction	825	NT	+	Crebelli *et al.* (1985)
A. nidulans, diploid strain 30, aneuploidy	825	NT	+	Käfer (1986)
A. nidulans, haploid conidia, aneuploidy, polyploidy	1650	NT	+	Käfer (1986)
A. nidulans, diploid strain NH, nondisjunction	450	NT	+	Kappas (1989)
A. nidulans, diploid strain P1, nondisjunction	660	NT	+	Crebelli *et al.* (1991)
A. nidulans, haploid strain 35, hyperploidy	2640	NT	+	Crebelli *et al.* (1991)
Saccharomyces cerevisiae, meiotic recombination	3300	NT	Inconclusive	Sora & Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	2500	NT	+	Sora & Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	3300	NT	+	Sora & Agostini Carbone (1987)
S. cerevisiae, D61.M, mitotic chromosomal malsegregation	1000	NT	+	Albertini (1990)
Drosophila melanogaster, somatic mutation wing spot test	825	NT	+	Zordan *et al.* (1994)
D. melanogaster, induction of sex-linked lethal mutation	37.2 (feed)	NT	Inconclusive	Beland (1999)
D. melanogaster, induction of sex-linked lethal mutation	67.5(injected)	NT	–	Beland (1999)

[a] Doses are in µg/mL for tests *in vitro* unless otherwise specified.

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

Table 4.3 Genotoxicity of chloral hydrate in mammalian systems *in vitro*

Test system/end-point	Dose [a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
DNA–protein crosslinks, rat nuclei	41 250	NT	–	Keller & Heck (1988)
DNA SSB, rat primary hepatocytes	1650	NT	–	Chang *et al.* (1992)
DNA damage (several variants of comet assay), Chinese hamster ovary (CHO K5) cells	5000	NT	–/+	Kiffe *et al.* (2003)
Gene mutation, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	1000	NT	(+)	Harrington-Brock *et al.* (1998)
Gene mutation, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	165	NT	–	Liviac *et al.* (2011)
Gene mutation, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	562	NT	(+)	Fellows *et al.* (2011)
SCE, Chinese hamster ovary cells	100	+	+	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells	165	NT	+	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells	250	NT	–	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells	400	NT	+	Parry *et al.* (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells	400	NT	+	Lynch & Parry (1993)
Micronucleus formation, Chinese hamster V79 cells	316	NT	+	Seelbach *et al.* (1993)
Micronucleus formation, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	1300	NT	–	Harrington-Brock *et al.* (1998)
Micronucleus formation, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	500	NT	+	Nesslany & Marzin (1999)
Chromosomal aberration, Chinese hamster CHED cells	20	NT	+	Furnus *et al.* (1990)
Chromosomal aberration, Chinese hamster ovary cells	1000	+	+	Beland (1999)
Chromosomal aberration, mouse lymphoma L5178Y/*Tk*$^{+/-}$ cells	1250	NT	(+)	Harrington-Brock *et al.* (1998)
Aneuploidy, Chinese hamster CHED cells	10	NT	+	Furnus *et al.* (1990)

Table 4.3 (continued)

Test system/end-point	Dose [a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
Aneuploidy, primary Chinese hamster embryonic cells	250	NT	+	Natarajan *et al.* (1993)
Aneuploidy, Chinese hamster LUC2p4 cells	250	NT	+	Warr *et al.* (1993)
Aneuploidy, mouse lymphoma L5178Y/*Tk*$^{+/-}$	1300	NT	–	Harrington-Brock *et al.* (1998)
Tetraploidy and endoredupliation, Chinese hamster LUC2p4cells	500	NT	+	Warr *et al.* (1993)
Cell transformation, Syrian hamster embryo cells (24-hour treatment)	350	NT	+	Gibson *et al.* (1995)
Cell transformation, Syrian hamster embryo cells (7-day treatment, conditioned media without X-ray irradiated feeder layer)	5	NT	+	Pant *et al.* (2008)
Cell transformation, Syrian hamster dermal cell line (24-hour treatment)	50	NT	+	Parry *et al.* (1996)

[a] Doses are in µg/mL for tests *in vitro*.

+, positive; (+), weakly positive; –, negative; –/+, some variants of test gave negative results, some gave positive results; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; SCE, sister-chromatid exchange; SSB, single-strand break

Table 4.4 Genotoxicity of chloral hydrate in mammalian systems *in vivo*

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
DNA SSB, male Sprague-Dawley rat liver	300, oral	+	Nelson & Bull (1988)
DNA SSB, male F344 rat liver	1650, oral	–	Chang *et al.* (1992)
DNA SSB, male B6C3F$_1$ mouse liver	100, oral	+	Nelson & Bull (1988)
DNA SSB, male B6C3F$_1$ mouse liver	825, oral	–	Chang *et al.* (1992)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes (raised in water containing chloral hydrate)	200	+	Giller *et al.* (1995)
Micronucleus formation, *Carassius auratus gibelio* (crucian carp) blood, gill, and fin cells (raised in water containing chloral hydrate)	400	+	Arkhipchuk & Garanko (2005)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	–	Leuschner & Leuschner (1991)
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	–	Russo & Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo & Levis (1992b)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo *et al.* (1992)
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	–	Leopardi *et al.* (1993)
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen *et al.* 1994
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini *et al.* (1994)
Micronucleus formation, B6C3F$_1$ mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley *et al.* (1996)
Micronucleus formation, B6C3F$_1$ mouse spermatids after meiotic cell treatment	413, i.p.	–	Nutley *et al.* (1996)
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	–	Grawé *et al.* (1997)
Micronucleus formation, male B6C3F$_1$ mouse bone-marrow erythrocytes	500, i.p., × 3	+	Beland (1999)
Chromosomal aberration, male and female F1 mouse bone-marrow cells	600, i.p.	–	Xu & Adler (1990)
Chromosomal aberration, male and female Sprague-Dawley rat bone-marrow cells	1000, oral	–	Leuschner & Leuschner (1991)
Chromosomal aberration, BALB/c mouse spermatogonia treated	83, i.p.	–	Russo & Levis (1992b)

Table 4.4 (continued)

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
Chromosomal aberration, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo *et al.* (1984)
Chromosomal aberration, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	–	Marrazzini *et al.* (1994)
Chromosomal aberration, ICR mouse oocytes	600, i.p.	–	Mailhes *et al.* (1993)
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	–	Xu & Adler (1990)
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller & Adler (1992)
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	–	Leopardi *et al.* (1993)
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini *et al.* (1994)

[a] Doses are in mg/kg bw for tests *in vivo*.

+, positive; –, negative; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; SSB, single-strand breaks

studies in *S. typhimurium* TA104 gave positive results (Haworth *et al.*, 1983; Ni *et al.*, 1994; Giller *et al.*, 1995; Beland, 1999). Waskell (1978) studied the effect of chloral hydrate (dose range, 1.0–13 mg/plate) on gene mutation in different *S. typhimurium* strains (TA98, TA100, TA1535) in the Ames assay. No revertant colonies were observed in strains TA98 or TA1535 either in the presence and absence of metabolic activation by S9 (9000 × *g* rat liver supernatant). Similar results were obtained by Leuschner & Leuschner (1991); however, in TA100, a dose-dependent statistically significant increase in the frequency of revertant colonies was obtained in the presence and absence of metabolic activation. It should be noted that the chloral hydrate used (obtained from Sigma), recrystallized one to six times from chloroform, was described as "crude." However, this positive result was consistent with those of other studies in this strain, as noted above (Waskell, 1978). Giller *et al.* (1995) studied the genotoxicity of chloral hydrate in three short-term tests. Chloral induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth *et al.* (1983). [The Working Group noted that these results were consistent with those of several studies with trichloroethylene, in which low, but positive, responses were observed in the TA100 strain in the presence of metabolic activation from S9, even when genotoxic stabilizers were not present.]

A statistically significant increase in the frequency of mitotic segregation was observed in *Aspergillus nidulans* treated with chloral hydrate at a concentration of 5 [827 µg/mL] or 10 mM [1654 µg/mL] (Crebelli *et al.*, 1985). Studies of mitotic crossing-over in *A. nidulans* gave negative results, while these same studies gave positive results for aneuploidy (Crebelli *et al.*, 1985, 1991; Käfer, 1986; Kappas, 1989).

Two studies in *Saccharomyces cerevisiae* investigated chromosomal malsegregation after exposure to chloral hydrate (Sora & Agostini Carbone, 1987; Albertini, 1990). Chloral hydrate (1–25 mM) [165–4135 µg/mL] was dissolved in the sporulation medium and the frequencies of various meiotic events such as recombination and disomy were analysed. Chloral hydrate inhibited sporulation as a function of dose and increased the frequency of diploid and disomic clones. Chloral hydrate was also tested for mitotic chromosome malsegregation in *S. cerevisiae* D61.M (Albertini, 1990). The test strain was exposed at a dose range of 1–8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed after exposure to chloral hydrate.

Two studies of mutagenicity with chloral hydrate were performed in *Drosophila* (Zordan *et al.*, 1994; Beland, 1999). In these two studies, chloral hydrate gave positive results in the wing spot test for somatic mutation (Zordan *et al.*, 1994), while the results of a test for induction of sex-linked lethal mutation were equivocal when chloral hydrate was administered in the feed, but negative when chloral hydrate was administered by injection (Beland, 1999).

In a mammalian system, Harrington-Brock *et al.* (1998) noted that increases in mutant frequency observed in *Tk* $^{+/-}$ mouse lymphoma cell lines treated with chloral hydrate were not statistically significant. The mutants were primarily small-colony *Tk* mutants, indicating that most mutants induced by chloral hydrate resulted from chromosomal mutation rather than point mutation. It should be noted that cytotoxicity was observed at most concentrations tested (350–1600 µg/mL). Percentage cell survival ranged from 96% to 4%. Fellows *et al.* (2011) and Liviac *et al.* (2011) also tested for mutagenicity with chloral hydrate in *Tk* $^{+/-}$ mouse lymphoma cell lines without metabolic activation from S9. Fellows *et al.* (2011) reported a statistically significant increase in mutant frequency at the highest tested concentration of 3.4 mM [562 µg/mL], at which a high degree of cytotoxicity was observed. Liviac *et al.* (2011) tested chloral hydrate at lower concentrations of 1 µM to 1 mM [0.16–165 µg/mL], at which

relative total growth was at least 66%, and did not observe any statistically significant increases in mutant frequency. [The Working Group noted that reported increases in mutant frequencies in mammalian systems were not statistically significant, except at highly cytotoxic concentrations.]

(c) Chromosomal effects

Exposure to chloral hydrate induces the formation of micronuclei in most test systems, including assays *in vitro* and *in vivo* in mammalian species (Degrassi & Tanzarella, 1988; Leuschner & Leuschner, 1991; Bonatti *et al.*, 1992; Russo *et al.*, 1992; Russo & Levis, 1992a, b; Van Hummelen & Kirsch-Volders, 1992; Leopardi *et al.*, 1993; Lynch & Parry, 1993; Seelbach *et al.*, 1993; Allen *et al.*, 1994; Marrazzini *et al.*, 1994; Giller *et al.*, 1995; Nutley *et al.*, 1996; Parry *et al.*, 1996; Grawé *et al.*, 1997; Harrington-Brock *et al.*, 1998; Beland, 1999; Nesslany & Marzin, 1999; Ikbal *et al.*, 2004; Arkhipchuk & Garanko, 2005; Liviac *et al.*, 2010). For instance, chloral hydrate has been shown to induce micronucleus formation, but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by non-clastogenic agents are generally believed to represent intact chromosomes that have failed to segregate into either daughter-cell nucleus at cell division (Xu & Adler, 1990; Russo *et al.*, 1992). Furthermore, micronuclei induced by chloral hydrate in mouse bone-marrow cells (Russo *et al.*, 1992) and in cultured mammalian cells (Degrassi & Tanzarella, 1988; Bonatti *et al.*, 1992) have been shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescence methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division (Degrassi & Tanzarella, 1988; Hennig *et al.*, 1988; Eastmond & Tucker, 1989). In contrast, Allen *et al.* (1994) harvested spermatids from male C57B1/6J mice given chloral hydrate by intraperitoneal administration 49 days before harvesting, and found statistically significantly increased frequencies of kinetochore-negative micronuclei; however, no dose–response relationship was observed. The results of this study contrast with those of studies described above (Degrassi & Tanzarella, 1988; Bonatti *et al.*, 1992) which demonstrated predominantly kinetochore-positive micronuclei.

Chloral hydrate induced aneuploidy *in vitro* in multiple Chinese hamster cell lines (Furnus *et al.*, 1990; Natarajan *et al.*, 1993; Warr *et al.*, 1993) and in human lymphocytes (Vagnarelli *et al.*, 1990; Sbrana *et al.*, 1993), but not in mouse lymphoma cells (Harrington-Brock *et al.*, 1998). Studies performed *in vivo* in various mouse strains led to increased aneuploidy in spermatocytes (Russo *et al.*, 1984; Liang & Pacchierotti, 1988; Miller & Adler, 1992), but not in oocytes (Mailhes *et al.*, 1993) or bone-marrow cells (Xu & Adler, 1990; Leopardi *et al.*, 1993).

The potential of chloral hydrate to induce aneuploidy in mammalian germ cells has been of particular interest since Russo *et al.* (1984) first demonstrated that treatment of male mice with chloral hydrate results in statistically significant increases in the frequencies of hyperploidy in metaphase II cells. This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division, and may be a consequence of chloral hydrate interfering with spindle formation [reviewed by Russo *et al.* (1984) and Liang & Brinkley (1985)]. Chloral hydrate also causes meiotic delay, which may be associated with aneuploidy (Miller & Adler, 1992).

Several studies have included analysis of chromosomal aberration *in vitro* and *in vivo* after exposure to chloral hydrate; there have been some positive results *in vitro* (Furnus *et al.*, 1990; Harrington-Brock *et al.*, 1998; Beland, 1999). In mouse lymphoma cell lines and Chinese hamster embryo cells treated with chloral hydrate, there was no significant increase in chromosomal aberration (Harrington-Brock *et al.*, 1998; Furnus

et al., 1990). Other studies of chromosome aberration *in vivo* have mostly reported negative results (Liang & Pacchierotti, 1988; Xu & Adler, 1990; Leuschner & Leuschner, 1991; Russo & Levis, 1992a, b; Mailhes *et al.*, 1993), with the exception of one study (Russo *et al.*, 1984) in mice of an F1 cross, C57B1/Cne × C3H/Cne.

Positive results for sister-chromatid exchange were observed by Beland (1999) in Chinese hamster ovary cells exposed *in vitro* with and without an exogenous metabolic activation system.

(d) Cell transformation

Chloral hydrate gave positive results in three studies designed to measure cellular transformation in Syrian hamster cells (dermal and/or embryo) exposed to chloral hydrate (Gibson *et al.*, 1995; Parry *et al.*, 1996; Pant *et al.*, 2008).

4.3 Non-genotoxic mechanisms of carcinogenesis

4.3.1 Mechanisms related to liver carcinogenesis

(a) Cell proliferation

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Rijhsinghani *et al.* (1986) reported increases in the mitotic index in liver cells of male C56BL × C3HF1 mice (age, 15 days), 24 hours after receiving chloral hydrate as a single dose at 5 or 10 mg/kg bw by gavage. The increase was only statistically significant at 5 mg/kg bw. No necrosis was observed. George *et al.* (2000) measured the hepatocyte labelling index in male F344 rats and male B6C3F$_1$ mice given drinking-water containing chloral hydrate for 13 (rats only), 26, 52, and 78 weeks. Except for the intermediate dose (0.58 g/L) at 26 weeks, no increases in labelling index were reported in either species. No changes in liver necrosis were observed in either species at any dose or time-point. [The Working Group noted that the lack of significant liver necrosis suggests that the small, transient proliferative responses observed were not due to cytotoxicity. The transient nature of any proliferative responses suggested that cell proliferation induced by chloral hydrate is not an important mechanistic contributor to carcinogenicity caused by chloral hydrate.]

(b) Cell communication

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Chloral hydrate has been reported to reduce gap-junction communication in rat clone 9 cells *in vitro* (Benane *et al.*, 1996; Zhang *et al.*, 2011). There are no data as to whether cell communication is altered *in vivo*.

(c) Activation of peroxisome proliferator-activated receptor α (PPARα)

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Male Sprague-Dawley rats given drinking-water containing chloral hydrate for 7 days exhibited increased activity of the hepatic peroxisomal enzyme palmitoyl-coenzyme A oxidase, which is indicative of PPARα activation (Poon *et al.*, 2000). Male B6C3F$_1$ mice given drinking-water containing chloral hydrate for 26 weeks did not show evidence of PPARα activation, as measured by activity of hepatic cyanide-insensitive palmitoyl-coenzyme A oxidase (George *et al.*, 2000). Similarly, Leakey *et al.* (2003) reported that male B6C3F$_1$ mice given chloral hydrate by

gavage for 15 months showed no induction of the markers of PPARα activation lauric acid ω-hydroxylase activity and CYP4A immunoreactive protein when fed *ad libitum*, but increases in these markers were reported at the highest dose, (100 mg/kg bw) in dietary-controlled mice.

There were increases in the incidence of liver tumours in mice fed *ad libitum* and dietary-controlled mice (George *et al.*, 2000; Leakey *et al.*, 2003). Sprague-Dawley rats have not been tested for carcinogenicity, but a long-term bioassay in male F344 rats did not report increased incidences of liver tumours (George *et al.*, 2000). The lack of association between PPARα activation and liver tumorigenicity across experiments suggests that PPARα activation is not an important mechanistic contributor to carcinogenicity attributable to chloral hydrate.

4.3.2 Mechanisms related to lung carcinogenesis

(a) Cytotoxicity

(i) Humans

No data on mechanisms for lung carcinogenesis of chloral hydrate in humans were available to the Working Group.

(ii) Experimental systems

No data on mechanisms of lung carcinogenesis of chloral hydrate in animals were available to the Working Group. Odum *et al.* (1992) reported that exposure of mice to chloral hydrate at a concentration of 100 ppm [100 µg/mL] by inhalation for 6 hours led to pulmonary cytotoxicity in the form of bronchiolar lesions, alveolar necrosis, desquamation of the epithelium, and alveolar oedema. These effects appeared to be a direct effect of chloral hydrate, rather than a result of metabolism, since exposures to trichloroethanol and trichloroacetic acid did not result in any pulmonary effects. Odum *et al.* (1992) reported that the bronchiolar lesions appeared identical to those caused by exposure to trichloroethylene by inhalation.

4.4 Susceptibility data

4.4.1 Inter-individual variability

Early studies of sedative effects of chloral hydrate have demonstrated that its effects on the central nervous system are strain-dependent in rats (Riley *et al.*, 1979) and mice (McIntyre & Alpern, 1985), similar to that reported for ethyl alcohol. These observations suggested similarities in pathways of metabolism for chloral hydrate and ethyl alcohol. Indeed, Lipscomb *et al.* (Lipscomb *et al.*, 1997) showed that CYP2E1 is a key pathway for oxidative metabolism of trichloroethylene to chloral hydrate, that there exists up to 10-fold difference in CYP2E1 activity among humans, and that human inter-individual variability in microsomal formation of chloral hydrate correlates with CYP2E1 activity and protein levels. This study did not address the potential for these inter-individual differences in formation of chloral hydrate to affect its toxicity or carcinogenicity.

Chloral hydrate is further metabolized to trichloroacetic acid by ALDH, and to trichloroethanol by ADH. ALDH and ADH are known to be polymorphic in humans, and these polymorphisms are well known to have a major impact on cancer susceptibility in people who consume beverages containing ethanol, especially in Asian countries (IARC, 2010, 2012b, 2012c; Chang *et al.*, 2012). It has therefore been suggested that subpopulations with certain polymorphisms in the ALDH and ADH metabolic pathways may have greater than expected formation of trichloroacetic acid and thus an enhanced risk of adverse health effects after exposure to chloral hydrate or other chlorinated solvents.

The effects of chloral hydrate on the metabolism of alcohol and acetaldehyde have been suggested as a mechanism for the dramatic

effects of coexposure to chloral hydrate (as well as other chlorinated solvents) and ethyl alcohol. Firstly, such coexposures lead to more than additive sedative effects in rodents (Sharkawi *et al.*, 1983) and humans (Sellers *et al.*, 1972). Secondly, adverse health effects indicative of elevated blood levels of acetaldehyde have been described as "degreaser's flush" (Stewart *et al.*, 1974). Thus, an additional factor for individual susceptibility to adverse health effects of chloral hydrate may be coexposure to ethyl alcohol.

4.4.2 Life-stage susceptibility

Several clinical reports document adverse health effects in neonates or infants who underwent anaesthesia by chloral hydrate (Reimche *et al.*, 1989). It has been suggested that accumulation of trichloroacetic acid and trichloroethanol in the tissues of infants may be the reason for the enhanced frequency of toxic effects. Because of the immaturity of hepatic metabolism, particularly the glucuronidation pathway, and decreased glomerular filtration, the half-life of trichloroethanol is longer in infants (pre-term and full-term) than in adults. There is indirect evidence of competition for hepatic glucuronidation for bilirubin with trichloroethanol in pre-term infants. In addition, it has been shown that enzymes participating in alcohol metabolism are expressed at lower levels in infants than in adults (Tran *et al.*, 2007).

Neuroapoptosis has been detected in the infant mouse brain after exposure to chloral hydrate (Cattano *et al.*, 2008).

4.4.3 Sex differences

With regard to differences in tumour incidence between males and females in studies of long-term exposure to chloral hydrate, some studies reported differential susceptibility in males and females (see Section 3). However, major inconsistencies exist between studies for target tissue and tumour incidence, which makes it difficult to conclude with certainty that either males or females may be more susceptible to carcinogenesis induced by chloral hydrate.

[The Working Group noted that although male laboratory rodents seem to be more sensitive than females to the hepatic effects of chloral hydrate, there was no evidence for differences between men and women in their sensitivity to the sedative or adverse health effects of chloral hydrate at the recommended clinical doses.]

4.5 Toxic non-cancer effects

4.5.1 Liver toxicity, including hepatomegaly, necrosis, and enzyme changes

(a) Humans

One study has shown that sedative doses of chloral hydrate to newborns increase the likelihood of hyperbilirubinaemia (Lambert *et al.*, 1990).

(b) Experimental systems

Long-term (104 weeks) exposure of male mice to chloral hydrate at a dose equivalent to 166 mg/kg bw per day resulted in no changes in organ weights except in the liver, which showed an approximate 40% increase in absolute liver weight and in the liver-to-body weight ratio (Daniel *et al.*, 1992a). The same type of study was conducted in male and female rats with the only non-cancer effect reported as focal hepatocellular necrosis in 2 out of 10 male rats (Daniel *et al.*, 1992b).

Similar studies in mice and rats by George *et al.* (2000) also showed no effects in rats and only proliferative lesions in the liver of mice at doses up to approximately 150 mg/kg bw per day. The National Toxicology Program (NTP, 2002a) conducted studies of long-term exposure in male and female mice given chloral hydrate at doses of up to 71 mg/kg bw per day, and

observed no non-neoplastic effects. A long-term (approximately 125 weeks) study by Leuschner & Beuscher (1998) in rats given chloral hydrate at doses of up to 135 mg/kg bw per day showed no increase in non-neoplastic lesions.

The short-term toxicity of chloral hydrate has been studied in CD1 mice and Sprague-Dawley rats. In mice, administration of chloral hydrate at daily doses of 14.4 or 144 mg/kg bw by gavage for 14 consecutive days resulted in an increase in relative liver weight and a decrease in spleen size. No other changes were seen. Mice given drinking-water containing chloral hydrate at a concentration of 0.07 or 0.7 mg/mL for 90 days showed dose-related hepatomegaly in males only and significant changes in hepatic microsomal enzymes in both males and females, indicative of hepatic toxicity (Sanders *et al.*, 1982).

Male Sprague-Dawley rats were given drinking-water containing chloral hydrate at a concentration of 0.13, 1.35, or 13.5 mg/L for 7 days (Poon *et al.*, 2000). No changes were observed in body or organ weights.

4.5.2 Central nervous system

(a) Humans

Choral hydrate has long been used in both human and veterinary medicine as a sedative and hypnotic drug. The effects on the central nervous system are due to the metabolite, trichloroethanol (Shapiro *et al.*, 1969; Miller & Greenblatt, 1979).

(b) Experimental systems

Exposure of female CD1 mice to chloral at a dose of 100 ppm [603 µg/L] for 6 hours induced deep anaesthesia, which was fully reversible on cessation of exposure (Odum *et al.*, 1992).

4.5.3 Kidney toxicity

(a) Humans

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

(b) Experimental systems

The National Toxicity Program (NTP, 2002a) reported an increase in the incidence of glomerulosclerosis in mice fed *ad libitum* with diets containing chloral hydrate at a concentration of 25 or 100 mg/kg. Other long-term studies did not report any lesions in the kidney (Daniel *et al.*, 1992a; Leuschner & Beuscher, 1998).

4.6 Synthesis of mechanistic considerations

A comprehensive database exists to characterize the absorption, distribution, metabolism and excretion of chloral hydrate in humans and experimental animals; this database supports the conclusion that major similarities are evident between humans and rodents. Chloral hydrate is rapidly metabolized to trichloroethanol and trichloroacetic acid, which have been detected in blood and urine. Trichloroacetic acid that is formed from the metabolism of chloral hydrate is slowly metabolized and has a longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours). The major excretion pathway for metabolites of chloral hydrate is in the urine. The major enzymes responsible for the metabolism of chloral hydrate are ADH and ALDH. This metabolic pathway is very similar in humans and rodents.

Strong evidence suggests that chloral hydrate is genotoxic, both *in vivo* and *in vitro*, in mammalian and other test systems (including the standard genotoxicity battery, with and without metabolic activation). The types of genotoxic damage detected encompassed mutations, chromosomal aberrations, and micronucleus

formation. One study found that in infants exposed orally to chloral hydrate, a significant increase in micronucleus formation in peripheral blood lymphocytes was observed.

Adverse health outcomes of chloral hydrate have been reported for the liver, kidney, and central nervous system, which suggests that these organs are potential targets for this chemical.

The strength of evidence for liver as a target tissue is strong; in addition to cancer in mice, some evidence of liver toxicity (hyperbilirubinaemia) has been reported in infants exposed to chloral hydrate. Available data on non-genotoxic mechanisms in the liver are almost exclusively from studies in animals. Multiple mechanisms have been suggested to operate, including a transient increase in cell proliferation, induction of the peroxisome proliferation response (inconclusive evidence for activation of PPARα in both studies in rodents, with poor correlation with tumours), and disruption of gap-junction intercellular communications (limited evidence from one study *in vitro*). The strength of evidence for non-genotoxic mechanisms in liver cancer is weak.

The strength of evidence for the central nervous system as a target tissue is strong. Chloral hydrate is a sedative in humans and in animals. The relevance of neurotoxicity to cancer is unknown.

The strength of evidence for kidney as a target tissue is weak. Some evidence of kidney toxicity (glomerular sclerosis) has been reported in studies in animals.

There is strong potential for inter-individual variability in the adverse effects of chloral hydrate. ADH and ALDH are the major metabolic enzymes for chloral hydrate, and common polymorphisms that result in differences in alcohol metabolism have been reported in humans. Consumption of alcoholic beverages may also be a susceptibility factor because of effects on alcohol metabolism enzymes that are important for the biotransformation of chloral hydrate.

5. Summary of Data Reported

5.1 Exposure data

Chloral is a chlorinated aldehyde that is readily converted to chloral hydrate in the presence of water. It can be formed as a disinfection by-product as a result of chlorine-based disinfection of water. Chloral has been used historically in the production of dichlorodiphenyltrichloroethane (DDT) and, to a lesser extent, other insecticides and pharmaceuticals. The use of chloral has declined steadily since the use of DDT has been restricted. Chloral hydrate is used as a sedative during medical procedures and to reduce anxiety related to withdrawal from drugs. Exposure to chloral hydrate may occur at microgram-per-litre levels through consumption of chlorinated drinking-water and ingestion of swimming-pool water.

5.2 Human carcinogenicity data

Only one epidemiological study has examined risk of cancer in humans after exposure to chloral hydrate. Relationships between the number of prescriptions for chloral hydrate and morbidity for all cancers, and cancers of the lung, stomach, prostate, and melanoma were examined in nested case–control analyses based on records from a health plan in California, USA, and no significant associations were observed. The single available study of carcinogenicity in humans provides no support for relationship between exposure to chloral hydrate and risk of cancer.

5.3 Animal carcinogenicity data

Chloral hydrate was evaluated for carcinogenicity in male mice in two studies with drinking-water and in two gavage studies. In the studies with drinking-water, chloral hydrate

induced a significant increase in the incidence of hepatocellular adenoma and hepatocellular carcinoma. In the gavage studies, an increased incidence in hepatocellular adenoma or carcinoma (combined) was observed.

In another study in male dietary-controlled mice treated by gavage, chloral hydrate increased the incidence and the trend in incidence of hepatocellular carcinoma.

In the single study in female mice treated by gavage, exposure to chloral hydrate was associated with increases in the trend and in the incidence of malignant lymphoma and in the incidence of adenoma of the pituitary gland.

Chloral hydrate in drinking-water was evaluated for carcinogenicity in one study in male and female rats, and in a second study in male rats. No dose-related increases in tumour incidence were reported. In one of the studies, however, the incidence of hepatocellular carcinoma in one treatment group of male rats was higher than that in historical controls. Several design and reporting deficiencies limited the power of both studies to adequately evaluate the carcinogenic potential of chloral hydrate in rats.

5.4 Mechanistic and other relevant data

Major similarities exist between humans and experimental animals with regard to the absorption, distribution and metabolism of chloral hydrate. In all species studied, rapid metabolism of chloral hydrate by alcohol and aldehyde dehydrogenases results in formation of trichloroacetic acid and trichloroethanol). However, trichloroacetic acid has a much longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours), which is indicative of much slower excretion or metabolism of this metabolite in humans. Strong evidence is available from studies in humans *in vivo*, animals *in vivo* and *in vitro*, and from bacterial and other test systems, to support the conclusion that chloral hydrate is a genotoxic agent. Cancer findings in animals and toxicity findings in humans and animals designate liver as a major target organ for chloral hydrate. In the liver, non-genotoxic mechanisms may participate in carcinogenesis. Some data on kidney toxicity in rodents suggest that kidney may also be a target organ. The central nervous system is also a potential target organ because chloral hydate has a sedative effect in humans and animals. The relevance of the apparent effects on the kidney and central nervous system to the potential cancer hazard of chloral hydrate in these organs is unknown. There is potential for inter-individual variability in the adverse effects of chloral hydrate because it is metabolized through alcohol and aldehyde dehydrogenases; polymorphisms in these enzymes have been shown to have a major impact on their function. Consumption of beverages containing alcohol may also be a susceptibility factor due effects on these same enzymes.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of chloral and chloral hydrate.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of chloral and chloral hydrate.

6.3 Overall evaluation

Chloral and chloral hydrate are *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In reaching the evaluation, the Working Group considered the following:

- Chloral hydrate is absorbed, distributed and metabolized similarly in humans and in rodents;
- Chloral hydrate is genotoxic in numerous assays for genotoxicity;
- Evidence exists that chloral hydrate is genotoxic in exposed humans, supporting the conclusion that a genotoxic mechanism also operates in humans.

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1,1,1,2-TETRACHLOROETHANE

1,1,1,2-Tetrachloroethane was considered by previous IARC Working Groups in 1986, 1987, and 1998 (IARC, 1986, 1987, 1999). New data have since become available and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

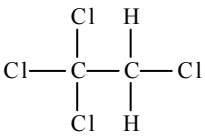
Chem. Abstr. Serv. Reg. No.: 630-20-6

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

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

Synonym: (Chloromethyl)trichloromethane

1.1.2 Structural and molecular formulae, and relative molecular mass



$C_2H_2Cl_4$

Relative molecular mass: 167.85

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless heavy liquid (HSDB, 2012)

Boiling-point: 130.2 °C (Haynes, 2012)

Melting-point: –70.2 °C (Haynes, 2012)

Density: 1.5406 g/cm^3 at 20 °C (Haynes, 2012)

Solubility: Slightly soluble in water (1.07 g/L at 25 °C); soluble in acetone, benzene, chloroform; miscible in diethyl ether and ethanol (Haynes, 2012)

Volatility: Vapour pressure, 1 kPa at 17 °C (Haynes, 2012)

Octanol/water partition coefficient (P): log P, 2.93 (HSDB, 2012)

Conversion factor: mg/m^3 = 6.87 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa).

1.1.4 Technical products and impurities

1,1,1,2-Tetrachloroethane is available in research quantities at a purity of > 99% (Sigma Aldrich, 2012).

Trade names for 1,1,1,2-tetrachloroethane include: Freon 130a, F130a and HCC 130a (Springer Materials, 2013).

Table 1.1 Methods for the analysis of 1,1,1,2-tetrachloroethane

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Air collected in specially prepared canister; desorb on cold trap	GC/MS	NR	EPA (1999a)
		GC/ECD	NR	
		GC/FID	NR	
		GCPID	NR	
	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
		GC/ECD	NR	
		GC/FID	NR	
		GCPID	NR	
Water	Purge with inert gas and trap; desorb to GC	GC/PID	NR	EPA (1988)
		GC/HECD	0.01 µg/L	EPA (1995a)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.05 µg/L	EPA (1988), EPA (1995b)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	NR	EPA (1996a)
		GC/HECD	0.005 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 (ground water)	EPA (1996b)

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

1.1.5 Analysis

Methods for the analysis of volatile organic compounds have been reviewed by Delinsky *et al.* (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of 1,1,1,2-tetrachloroethane in various matrices are identified in Table 1.1.

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

1,1,1,2-Tetrachloroethane is a by-product in industrial chlorination reactions, mainly from the production of 1,1,1-trichloroethane from 1,1-dichloroethane, 1,1,2-trichloroethane and 1,1,2,2-tetrachloroethane from 1,2-dichloroethane. It can be prepared in a highly purified form by isomerization of 1,1,2,2-tetrachloroethane or by chlorination of 1,1-dichloroethylene at approximately 40 °C in the liquid phase. Aluminium chloride is used in both reactions as a Lewis catalyst (HSDB, 2012).

(b) Production volume

Between 1 and 10 million pounds [454–4540 tonnes] of 1,1,1,2-tetrachloroethane were produced in or imported into the USA in 2002 (EPA, 2008).

1.2.2 Use

A major use of 1,1,1,2-tetrachloroethane was as a solvent in the manufacture of insecticides, herbicides, soil fumigants, bleaches, paints and varnishes. In the 1990s, 1,1,1,2-tetrachloroethane was used primarily as a feedstock for the production of solvents such as trichloroethylene and tetrachloroethylene (HSDB, 2012). It was used in World War II to impregnate clothing as a defence against mustard gas (Norman *et al.*, 1981).

Table 1.2 Concentrations of 1,1,1,2-tetrachloroethane in air

Location	Concentration		Comments	Reference
	Mean	Range		
Outdoor air				
Remote				
Eight sites; north and south Atlantic	NR	0.2–1.7 ppt	Background tropospheric levels	Class & Ballschmiter (1986)
Hamburg, Germany	NR	0.006–0.3 µg/m³	12 sites	Bruckmann *et al.* (1988)
Phoenix, AZ, USA	8.5 ppt	NR		Singh *et al.* (1981)
Oakland, CA, USA	4.2 ppt	NR		Singh *et al.* (1981)
Los Angeles, CA, USA	3.7 ppt	NR		Singh *et al.* (1981)
Throughout country, USA	0.071 ppt	ND–3.1 ppt	43 sites	HSDB (2012)
Throughout country, USA	2.2 ppt	ND–63	602 sites	HSDB (2012)
Kuwait City, Kuwait	39 µg/m³	ND–708	1994–95	Bouhamra (1997)[a]
Indoor air				
Kuwait	216 µg/m³	ND–2557 µg/m³	1994, 1995	Bouhamra (1997)[a]

[a] [The Working Group noted that the median reported was higher than the mean, which raises doubt about the validity of the data]
ND, not detected; NR, not reported; ppt, parts per trillion

1.3 Occurrence and exposure

1.3.1 Natural occurrence

1,1,1,2-Tetrachloroethane is not known to occur as a natural product.

1.3.2 Environmental occurrence

1,1,1,2-Tetrachloroethane may be formed incidentally during the manufacture of other chlorinated ethanes and released into the environment as air emissions or in wastewater. It has been detected at low levels in urban air, ambient air, drinking-water, ambient water, groundwater, wastewater and soil samples (HSDB, 2012).

(a) Air

Table 1.2 presents some recent data on levels of 1,1,1,2-tetrachloroethane in air.

(b) Water

Data on the levels of 1,1,1,2-tetrachloroethane in water were available from one study in Maryland, USA, which reported a maximum concentration of 3 µg/L in river water near a military testing area (Burton *et al.*, 2002).

1.3.3 Exposure

No data were available on levels of 1,1,1,2-tetrachloroethane in occupational settings or in the general population.

1.4 Regulations and guidelines

There are no exposure limits for 1,1,1,2-tetrachlorethane.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

Table 3.1 Studies of carcinogenicity in experimental animals given 1,1,1,2-tetrachloroethane by gavage

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 103 wk or 65 wk NTP (1983)	0, 250, 500 mg/kg bw per day, 5 days/wk, in corn oil 50/group	Hepatocellular adenoma: 6/48, 14/46, 21/50 Hepatocellular carcinoma: 12/48, 13/46, 6/50 Hepatocellular adenoma or carcinoma (combined): 18/48, 27/46, 27/50	Life-table test $P < 0.001$ (trend) $P = 0.021$ (lower dose) $P < 0.001$ (higher dose) $P = 0.012$ (trend) $P = 0.010$ (higher dose) $P < 0.001$ (trend) $P = 0.035$ (lower dose) $P < 0.001$ (higher dose)	Purity, > 99.4% Toxicity made it necessary to kill mice at higher dose at 65 wk, which reduced the sensitivity of the study.
Mouse, B6C3F$_1$ (F) 103 wk or 65 wk NTP (1983)	0, 250, 500 mg/kg bw per day, 5 days/wk, in corn oil 50/group	Hepatocellular adenoma: 4/49, 8/46, 24/48 Hepatocellular carcinoma: 1/49, 5/46, 6/48 Hepatocellular adenoma or carcinoma (combined): 5/49, 13/46, 30/48	Life-table test $P < 0.001$ (trend) $P < 0.001$ (higher dose) $P < 0.001$ (trend) $P = 0.008$ (higher dose) $P < 0.001$ (trend) $P = 0.011$ (lower dose) $P < 0.001$ (higher dose)	Purity, > 99.4% Toxicity made it necessary to kill mice at higher dose at 65 wk, which reduced the sensitivity of the study.
Rat, F344/N (M) 103 wk NTP (1983)	0, 125, 250 mg/kg bw per day, 5 days/wk, in corn oil 50/group	Liver neoplastic nodules or hepatocellular carcinoma (combined): 0/49, 1/49, 3/48	$P = 0.058$ (trend)	Purity, > 99.4% Reduced survival in the group at the higher dose reduced the sensitivity of study.
Rat, F344/N (F) 103 wk NTP (1983)	0, 125, 250 mg/kg bw per day, 5 days/wk, in corn oil 50/group	Mammary gland fibroadenoma: 6/49, 15/49, 7/46	$P \leq 0.024$ (lower dose)	Purity, > 99.4%

bw, body weight; F, female; M, male; wk, week

3.1 Mouse

Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice were given 1,1,1,2-tetrachloroethane at a dose of 0, 250, or 500 mg/kg bw per day by gavage in a corn oil vehicle, 5 days per week, for a scheduled duration of 103 weeks (NTP, 1983). Owing to toxicity in the central nervous system, male and female mice in the group receiving the higher dose were killed after 65 weeks of exposure. Mice in the group receiving the lower dose and in the control group were killed after 103 weeks. In males, there was a statistically significant treatment-related increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined). There was a positive trend in the incidence of hepatocellular carcinoma, with the incidence in male mice at the higher dose being statistically significantly increased [based on life-table analysis that adjusted for early mortality.] In females, statistically significant increases in the incidence of hepatocellular adenoma and of hepatocellular carcinoma were observed in mice at the higher dose. A positive trend with daily dose was still evident for both tumour types, despite female mice in the group at the higher dose being killed at 65 weeks. [The Working Group noted that termination at 65 weeks reduced the ability of the study to detect late developing tumours.]

3.2 Rat

Oral administration

Groups of 50 male and 50 female F344/N rats were given 1,1,1,2-tetrachloroethane at a dose of 0, 125, and 250 mg/kg bw per day by gavage in a corn oil vehicle, 5 days per week, for 103 weeks (NTP, 1983). Survival of male rats was significantly reduced relative to controls at the end of the experiment: control group, 29 out of 50; lower dose, 25 out of 50; and higher dose, 21 out of 50. Survival was not significantly reduced in female rats.

A marginal positive trend ($P = 0.058$) in the combined incidence of liver neoplastic nodules or hepatocellular carcinoma was observed in male rats (0 out of 49, 1 out of 49, 3 out of 48). The one hepatocellular carcinoma observed in this study was in the group at the higher dose.

A statistically significant increase ($P \leq 0.024$) in the incidence of fibroadenoma of the mammary gland in female rats was observed in the group receiving the lower dose relative to controls (6 out of 49, 15 out of 49, 7 out of 46). The incidences of other neoplasms in treated rats were not statistically different from controls. However, a rare renal tubular cell adenoma and a rare transitional cell papilloma of the bladder were observed in the group of male rats at the higher dose, a rare renal tubular cell adenoma was observed in the group of female rats at the higher dose, and six uncommon mesotheliomas of the tunica vaginalis or the peritoneum were observed in male rats at the lower dose (3 out of 50) and higher dose (3 out of 48). No such tumours were observed in controls. [The Working Group noted that the high mortality among male rats given the high dose reduced the sensitivity of this study.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Absorption

(a) Humans

No direct data on the absorption of 1,1,1,2-tetrachloroethane in humans exposed by inhalation, or by oral or dermal administration, were available. Estimates of the human

blood:air partition coefficient *in vitro* for this compound averaged about 30 (Meulenberg & Vijverberg, 2000), indicating respiratory uptake of 1,1,1,2-tetrachloroethane from inhaled air under equilibrium conditions.

(b) Experimental systems

Gargas & Andersen (1989) conducted experiments in rats exposed by closed-chamber, whole-body inhalation. Chamber concentrations declined with time, which was consistent with an equilibrium alveolar gas–exchange model, in which uptake by inhalation is largely determined by the partition coefficient. Estimates of the rat blood:air partition coefficient *in vitro* for this compound averaged about 42 (Meulenberg & Vijverberg, 2000), indicating considerable respiratory uptake of 1,1,1,2-tetrachloroethane from inhaled air under equilibrium conditions.

In a study by Mitoma *et al.* (1985), groups of male Osborne-Mendel rats were exposed to 1,1,1,2-tetrachloroethane at a dose of 25 or 200 mg/kg bw and groups of B6C3F$_1$ mice were exposed to 1,1,1,2-tetrachloroethane at 100 or 400 mg/kg bw, 5 days per week, for 4 weeks. The animals were then given a single dose of radiolabeled compound; 65% of the administered dose in rats and 84% in mice was recovered as metabolites in exhaled breath and urine over 48 hours.

4.1.2 Distribution

(a) Humans

No direct data on the tissue distribution of 1,1,1,2-tetrachloroethane in humans were available to the Working Group. However, Meulenberg & Vijverberg (2000) used empirical regression models to predict tissue:air partition coefficients based on measured saline:air and oil:air partition coefficients. Based on their predictions, tissue:blood partition coefficients in humans were estimated to range from 1.4 (kidney) to 56 (fat), depending on the lipid content of the tissues. These values suggest that 1,1,1,2-tetrachloroethane would be widely distributed to tissues after systemic delivery.

(b) Experimental systems

No direct data on the tissue distribution of 1,1,1,2-tetrachloroethane were available to Working Group. However, Gargas & Andersen (1989) reported tissue:blood partition coefficients in the range of 0.7 (muscle) to 26.5 (fat), suggesting that 1,1,1,2-tetrachloroethane would be widely distributed to tissues after systemic delivery.

4.1.3 Metabolism

(a) Humans

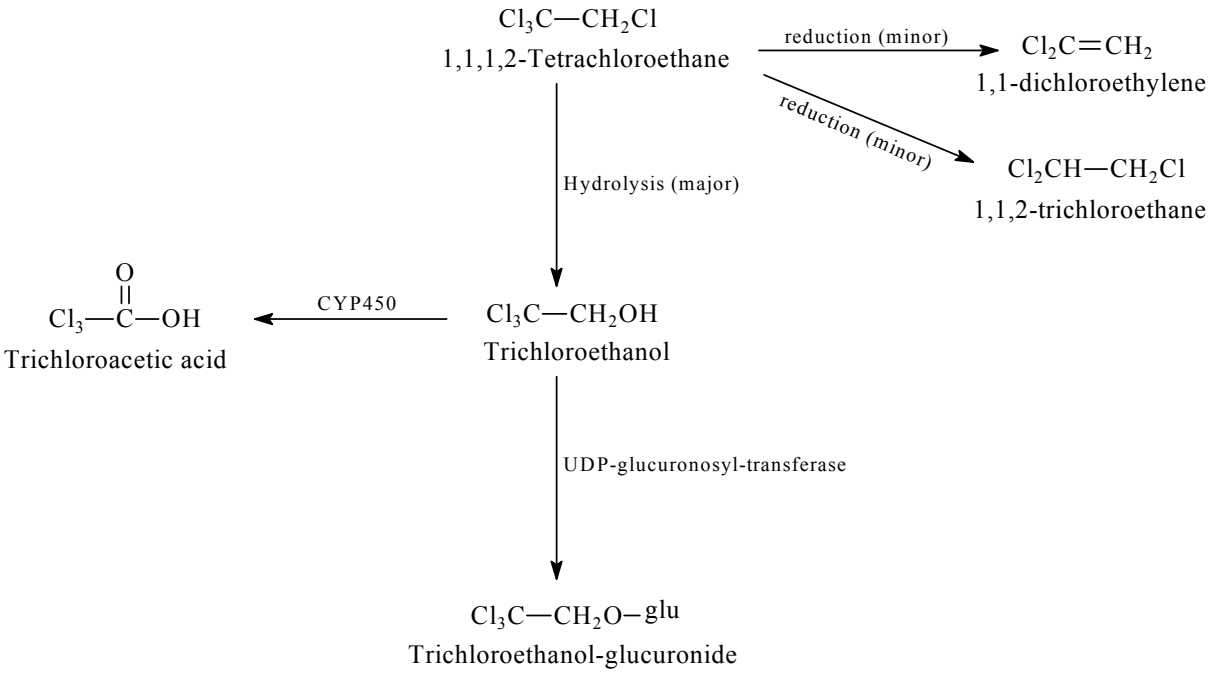
No data were available to the Working Group.

(b) Experimental systems

The metabolism of 1,1,1,2-tetrachloroethane is extensive in rodents. Yllner (1971) reported that between 19% and 56% of the administered dose was recovered as urinary metabolites in mice injected subcutaneously with ^{14}C-labelled tetrachloroethane. Mitoma *et al.* (1985) characterized the overall metabolic disposition of several chlorinated hydrocarbons, including 1,1,1,2-tetrachloroethane, in Osborne-Mendel rats and B6C3F$_1$ mice given either 25% or 100% of the maximum tolerated dose. Mitoma *et al.* (1985) reported that a small fraction was expired as carbon dioxide (1% in rats and 2% in mice), and a larger fraction was excreted in exhaled air and urine as metabolites (60% in rats and 77% in mice), after repeated oral dosing for 4 weeks.

The major urinary metabolite in the rat, guinea-pig, and rabbit (Truhaut & Nguyen-Phu-Lich, 1973), and mouse (Yllner, 1971) was trichloroethanol, with smaller amounts of trichloroacetic acid. Yllner (1971) considered that the possibility that trichloroethylene was an intermediate in the metabolism of 1,1,1,2-tetrachlorethane was inconsistent with the very small fraction (0.02%) of the administered dose expired

Fig. 4.1 Proposed metabolism of 1,1,1,2 tetrachlorethane



Prepared by the Working Group

as trichloroethylene. Therefore, Yllner (1971) hypothesized that trichloroethanol is formed via hydrolytic removal of chlorine (see Fig. 4.1).

Anaerobic incubation of 1,1,1,2-tetrachloroethane with microsomes from rat liver showed extensive reductive metabolism to 1,1-dichloroethylene and 1,1,2-trichloroethane at a ratio of about 25:1 (Thompson *et al.*, 1984). The results of these experiments *in vitro* were consistent with evidence of 1,1-dichloroethylene and 1,1,2-trichloroethane in the blood of rats treated with 1,1,1,2-tetrachloroethane *in vivo* (Thompson *et al.*, 1984). However, these metabolites are likely to be minor, because the trace amounts of trichloroethanol and trichloroacetic acid resulting from metabolism of 1,1-dichloroethylene and 1,1,2-trichloroethane (ATSDR 1989, 1994) cannot account for the trichloroethanol and trichloroacetic acid found in the urine after administration of 1,1,1,2-tetrachloroethane.

4.1.4 Excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In experimental animals, 1,1,1,2-tetrachloroethane is eliminated primarily as metabolites in urine, with small amounts as carbon dioxide in expired air. At higher doses (g/kg bw), the parent compound is excreted in expired air (Mitoma *et al.*, 1985; Yllner, 1971). The patterns of elimination in rats and mice are qualitatively similar (Mitoma *et al.* 1985). Elimination is fairly rapid, with a large fraction excreted in the first 24 hours, but significant amounts of trichloroacetic acid are present in the urine at 48–72 hours after exposure (Yllner 1971).

4.2 Genotoxicity and related effects

4.2.1 Humans

No studies on the genotoxicity of 1,1,1,2-tetrachloroethane in humans were available to the Working Group.

4.2.2 Experimental systems

Several studies examining the potential genotoxicity of 1,1,1,2-tetrachloroethane were available. These are summarized in Table 4.1 and are discussed below. While the results of the various tests were not always consistent, most suggested that 1,1,1,2-tetrachloroethane is genotoxic, and that metabolic activation is generally required to elicit genotoxicity.

(a) Covalent binding to DNA

In a study by Colacci *et al.* (1989), covalent binding to DNA, RNA, and proteins of liver, lung, kidney, and stomach was assessed in organs of male Wistar rats and BALB/c mice 24 hours after administration of 1,1,1,2-tetrachloroethane by intraperitoneal injection. Binding to DNA *in vivo* was generally higher in mouse organs than in rat organs. The covalent binding index (CBI) was calculated as 82 for mouse liver DNA, and 40 for rat liver DNA, thus classifying 1,1,1,2-tetrachloroethane as a weak to moderate initiator.

Paolini *et al.* (1990) studied covalent binding of radiolabelled 1,1,1,2-tetrachloroethane to DNA and specifically assessed the influence of the presence of reduced purine nucleotides and cytochrome P450 activity on the responses. Binding of [^{14}C]-labelled 1,1,1,2-tetrachloroethane to DNA *in vitro*, as mediated by microsomes from mouse liver, was increased 4.4-fold upon addition of both NADPH and NADH. They also observed a significant enhancement with addition of the purine nucleotides in mutagenesis experiments in the diploid D7 strain of *Saccharomyces cerevisiae*. Specifically, the frequencies of mitotic gene conversion and reverse point mutation were both enhanced. No evidence of mutagenesis was observed without addition of NADH.

(b) Mutation and cytogenetic effects

As shown in Table 4.1, 1,1,1,2-tetrachloroethane has given negative results in tests for mutagenicity in several strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, TA98), with and without exogenous metabolic activation. Positive results have been reported by Strubel & Grummt (1987), in TA97 (with metabolic activation), and in TA98 or TA100 with and without metabolic activation; the results for TA104 were borderline positive in this study. In another published report of tests, however, 1,1,1,2-tetrachloroethane at higher exposures was reported to lack mutagenicity in the TA98 and TA100 strains (Haworth *et al.* 1983).

Bronzetti *et al.* (1989) found that 1,1,1,2-tetrachloroethane induced recombination but not mutation in *S. cerevisiae*.

Whittaker *et al.* (1990) assessed the mutagenic activity of 1,1,1,2-tetrachloroethane and 11 other organic solvents. Induced mitotic chromosome loss was assayed using the diploid yeast strain *S. cerevisiae* D61.M; the assay relied on uncovering expression of multiple recessive markers reflecting presumptive loss of the chromosome VII homologue carrying the corresponding wild-type alleles. In this assay, 1,1,1,2-tetrachloroethane did not induce chromosome loss, but elicited high degrees of respiratory deficiency, reflecting anti-mitochondrial activity.

Foureman *et al.* (1994) found that sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*.

Crebelli *et al.* (1988) tested a series of halogenated solvents for induction of mitotic segregation in *Aspergillus nidulans* diploid strain P1. 1,1,1,2-Tetrachloroethane significantly increased the frequency of morphologically abnormal colonies that produced euploid whole-chromosome segregants (haploids and non-disjunctional

Table 4.1 Studies of genotoxicity with 1,1,1,2-tetrachloroethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Salmonella typhimurium, forward mutation, arabinose resistance	–	–	150	Roldán-Arjona *et al.* (1991)
S. typhimurium TA100, reverse mutation	–	–	166	Haworth *et al.* (1983)
S. typhimurium TA100, reverse mutation	+	+	5	Strubel & Grummt (1987)
S. typhimurium TA104, reverse mutation	(+)	(+)	25	Strubel & Grummt (1987)
S. typhimurium TA1535, reverse mutation	–	–	166	Haworth *et al.* (1983)
S. typhimurium TA1537, reverse mutation	–	–	166	Haworth *et al.* (1983)
S. typhimurium TA98, reverse mutation	–	–	166	Haworth *et al.* (1983)
S. typhimurium TA98, reverse mutation	+	+	125	Strubel & Grummt (1987)
S. typhimurium TA97, reverse mutation	–	+	5	Strubel & Grummt (1987)
Saccharomyces cerevisiae strain D7, gene conversion, *trp* locus	+	–	168	Bronzetti *et al.* (1989)
Aspergillus nidulans strain P1, genetic crossing-over	+	NT	400	Crebelli *et al.* (1988)
S. cerevisiae, reverse mutation, *ilv* locus	–	–	1 679	Bronzetti *et al.* (1989)
S. cerevisiae strain D7, reverse mutation	–	+	839–1679	Paolini *et al.* (1990)
S. cerevisiae strain D61.M, aneuploidy	–	NT	1340	Whittaker *et al.* (1990)
Aspergillus nidulans strain P1, aneuploidy	+	NT	200	Crebelli *et al.* (1988)
Drosophila melanogaster, sex-linked recessive lethal mutations	–	NT	1500 µg/mL inj	Foureman *et al.* (1994)
Gene mutation, mouse lymphoma L5178Y cells, $Tk^{+/-}$ locus *in vitro*	–	+	200	McGregor *et al.* (1988)
Gene mutation, mouse lymphoma L5178Y cells, $Tk^{+/-}$ locus *in vitro*	–	?	200	Sofuni *et al.* (1996)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	–	248	Galloway *et al.* (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	506	Galloway *et al.* (1987)
Chromosomal aberrations, Chinese hamster lung fibroblasts *in vitro*	–	–	200	Matsuoka *et al.* (1996)
Aneuploidy, Chinese hamster lung fibroblasts *in vitro*	+	+	100	Matsuoka *et al.* (1996)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Cell transformation, BALB/c-3T3 mouse cells	–	NT	250	Tu *et al.* (1985)
Binding (covalent) to calf thymus DNA *in vitro*	NT	+	9.6	Colacci *et al.* (1989)
Binding (covalent) to calf thymus DNA *in vitro*	(+)	+	NR	Paolini *et al.* (1990)
Binding (covalent) to DNA, BALB/c mouse lung, liver, kidney and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1989)
Binding (covalent) to DNA, Wistar rat lung, liver, kidney and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1989)

[a] +, positive; (+), weakly positive; –, negative; ?, inconclusive

[b] Tests in vitro, µg/mL; tests in vivo, mg/kg bw per day

HID, highest ineffective dose; inj, injection; i.p., intraperitoneal; LED, lowest effective dose; NR, not reported; NT, not tested

diploids). A borderline increase in crossing-over frequency was also observed, suggesting the involvement of non-DNA targets in the induction of aneuploidy. Conclusive evidence for induction of aneuploidy as a primary genetic event was provided by experiments with haploid strain 35.

As summarized in Table 4.1, 1,1,1,2-Tetrachloroethane induced gene mutations in the *Tk*$^{+/-}$ assay in mouse lymphoma cells only in the presence of an exogenous metabolic activation system. It did not increase the frequency of chromosomal aberration in Chinese hamster lung fibroblasts or ovary cells, but did induce sister chromatid exchange in Chinese hamster ovary cells and aneuploidy in Chinese hamster lung fibroblasts in the absence of exogenous metabolic activation. 1,1,1,2-Tetrachloroethane did not induce cell transformation in BALB/c-3T3 cells.

4.3 Nongenotoxic mechanisms of carcinogenesis

4.3.1 Mechanisms related to carcinogenesis in the liver

(a) Cytotoxicity

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

The National Toxicology Program (NTP, 1983) conducted 2-year bioassays with 1,1,1,2-tetrachloroethane in male and female F344 rats and B6C3F$_1$ mice. Rats were given 1,1,1,2-tetrachloroethane at 0, 125, or 250 mg/kg bw per day in corn oil, 5 days per week for 103 weeks. Significant reduction in survival was observed in males at the higher dose. Hepatic clear-cell changes were observed in female rats. Mice were given 1,1,1,2-tetrachloroethane at 0, 250, or 500 mg/kg bw per day in corn oil, 5 days per week for 103 weeks (control, lower dose) or 65 weeks (higher dose). A large proportion of mice at the higher dose were found to be moribund at 65 weeks and were killed. Increased incidence of alterations in liver cellular structure, characterized by inflammation, necrosis, fatty metamorphosis, and hepatocytomegaly, were observed at the higher dose.

In a study of reproductive toxicity (Truhaut *et al.*, 1974), rats were given 1,1,1,2-tetrachloroethane as a single dose at 300 mg/kg bw per day, 5 days per week, for 10 months. Adult rats and pups had hepatic fatty vacuolization, and adults also had centrilobular necrosis.

(b) Promotion of γ-glutamyltranspeptidase-positive foci

(i) Humans

No data on this mechanism in humans were located by the Working Group.

(ii) Experimental systems

Story *et al.* (1986) assessed the differences between a series of halogenated hydrocarbons and phenobarbital in induction of pre-neoplastic foci in rat liver. The assay for foci in rat liver was taken as evidence of initiating or promoting potential. Young adult male Osborne-Mendel rats were given partial hepatectomies 24 hours before receiving a single intraperitoneal dose of 1,1,1,2-tetrachloroethane or one of several other chemicals tested. One week later, the rats were started either on a diet containing phenobarbital at 0.05% (w/w), or on daily treatment with 1,1,1,2-tetrachloroethane in corn oil by gavage, for 7 weeks. Rats were killed 1 week after the end of treatment and livers were assayed for γ-glutamyltranspeptidase as a putative pre-neoplastic marker. 1,1,1,2-Tetrachloroethane was without significant effect during the initiation protocol at the maximum tolerated dose and was without effect in the promotion protocol.

4.3.2 Mechanisms related to toxicity in the kidney

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

The National Toxicology Program (NTP, 1983) conducted 2-year bioassays of 1,1,1,2-tetrachloroethane in male and female F344 rats and $B6C3F_1$ mice. Rats were given a dose of 0, 125, or 250 mg/kg per day in corn oil, 5 days per week for 103 weeks. A significant reduction in survival was observed in males at the higher dose. Male rats also showed a treatment-related increase in the incidence of mineralization of the kidneys.

The National Toxicology Program conducted a short-term study of renal toxicity with 1,1,1,2-tetrachloroethane in male F344 rats (NTP, 1996). In rats given two daily doses of (1.24 mmol/kg per day) for 21 days, hyaline droplet nephropathy was observed. An increased renal-cell labelling index, indicating replicative DNA synthesis, was also observed.

4.4 Susceptibility

No data were available to the Working Group.

4.5 Synthesis of mechanistic considerations

There is weak evidence to suggest that 1,1,1,2-tetrachloroethane is genotoxic. The overall database of studies of genotoxicity is limited. Most studies of mutagenesis in bacteria reported mixed, mostly negative, results. A few studies have indicated possible clastogenic effects, and one study showed DNA binding.

The target organs of adverse health outcomes associated with 1,1,1,2-tetrachloroethane are the liver, kidney, and central nervous system. The liver appears to be a major target organ for 1,1,1,2-tetrachloroethane based on cancer bioassays and toxicity data. In the liver, suggested nongenotoxic mechanisms, exclusively from studies in rodents, include cytotoxicity (shown in rats and mice in a 2-year bioassay), hepatomegaly, and steatosis (fatty vacuolation reported in rats). One study examined the potential for 1,1,1,2-tetrachloroethane to promote γ-glutamyltranspeptidase-positive foci in rat liver and showed no effect. Overall, the evidence for mechanisms of carcinogenesis in the liver is weak.

Evidence of kidney toxicity in male rats was found in two (long- and short-term) studies by the National Toxicology Program. The changes included mineralization, hyaline droplet nephropathy and increased cell proliferation.

5. Summary of Data Reported

5.1 Exposure data

1,1,1,2-Tetrachloroethane has been used as a solvent in insecticides, herbicides, varnishes and other products. It now has no known commercial use, other than as an intermediate in the manufacture of chlorinated solvents. Very low levels have been reported in ambient and indoor air, and in surface water near contaminated areas.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,1,1,2-Tetrachloroethane was evaluated for carcinogenicity in a 2-year study in mice and rats treated by gavage in a corn oil vehicle. Toxicity resulted in termination of the male and female mice at the higher dose after only 65 weeks of exposure. The group receiving the lower dose and the control group continued on study for the

full treatment period. Based on survival-adjusted analyses, statistically significant increases in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or hepatocellular carcinoma (combined) were observed in male and female mice. Survival was also reduced in male rats at the higher dose. A slight increase in the trend in the incidence of liver neoplastic nodules or hepatocellular carcinoma (combined) was observed in male rats. In female rats, an increase in the incidence of fibroadenoma of the mammary gland was observed at the lower dose.

5.4 Mechanistic and other relevant data

Little information was available from studies in humans or experimental animals on the absorption and distribution of 1,1,1,2-tetrachloroethane; however, it is likely that it would be absorbed readily and distributed widely throughout the body. No data on metabolism or excretion in humans were available. In rodents, metabolism produces tetrachloroethanol and then its glucuronide and trichloroacetic acid, which would be excreted primarily in the urine. 1,1,-dichloroethylene and 1,1,2-trichloroethylene may also be formed from 1,1,1,2-tetrachloroethane, albeit in lesser amounts. There is weak evidence to suggest that 1,1,1,2-tetrachloroethane is a genotoxic agent. Based on data on cancer and toxicity in animals, the liver appears as a major target organ for 1,1,1,2-tetrachloroethane. In the liver, the evidence for non-genotoxic mechanisms of carcinogenesis is weak.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1,1,1,2-tetrachlorethane.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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1,1,2,2-TETRACHLOROETHANE

1,1,2,2-Tetrachloroethane was considered by previous IARC Working Groups in 1979, 1987, and 1998 (IARC, 1979, 1987, 1999). New data have since become available and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

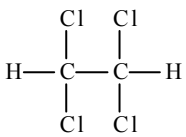
Chem. Abstr. Serv. Reg. No.: 79-34-5

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

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

Synonym: Acetylene tetrachloride, *sym*-tetrachloroethane

1.1.2 Structural and molecular formulae, and relative molecular mass



$C_2H_2Cl_4$

Relative molecular mass: 167.85

1.1.3 Chemical and physical properties of the pure substance

Description: Nonflammable, heavy, mobile liquid with sweetish, suffocating, chloroform-like odour (O'Neil, *et al.*, 2006)

Boiling-point: 146.5 °C (O'Neil *et al.*, 2006)

Melting-point: –44 °C (O'Neil *et al.*, 2006)

Density: 1.587 at 25 °C, relative to H_2O at 4 °C (O'Neil, *et al.*, 2006)

Solubility: Very sparingly soluble in water (1 g/350 mL at 25 °C); Soluble in acetone, benzene and chloroform, miscible with methanol, ethanol, diethyl ether, petroleum ether, carbon tetrachloride, carbon disulfide, dimethylformamide and oils. Has the highest solvent power of the chlorinated hydrocarbons (Haynes, 2012; O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 1 kPa at 32.4 °C (Haynes, 2012)

Octanol/water partition coefficient (P): log P, 2.39 (Haynes, 2012)

Conversion factor: mg/m^3 = 6.87 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

Table 1.1 Methods for the analysis of 1,1,2,2-tetrachloroethane

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Air collected in specially prepared canister; desorb on cold trap	GC/MS	0.09–0.28 ppm	EPA (1999a)
		GC/ECD	NR	
		GC/FID	NR	
		GCPID	NR	
	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
		GC/ECD	NR	
		GC/FID	NR	
		GCPID	NR	
Water	Purge with inert gas and trap; desorb to GC	GC/PID	NR	EPA (1988)
		GC/HECD	0.01 µg/L	EPA (1995a)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.04 µg/L	EPA (1988), EPA (1995b)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	NR	EPA (1996a)
		GC/HECD	0.01 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 (ground water)	EPA (1996b)

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

1.1.4 Technical products and impurities

1,1,1,2-Tetrachloroethane is found as an impurity in technical-grade 1,1,2,2-tetrachloroethane (HSDB, 2012).

1,1,2,2-Tetrachloroethane is available in research quantities at purities of > 98% and > 95% (Sigma Aldrich, 2012).

Trade names for 1,1,2,2-tetrachloroethane include Freon 130, F130 and HCC 130 (Springer Materials, 2013).

1.1.5 Analysis

Methods for the analysis of volatile organic compounds have been reviewed by Delinsky *et al.*, (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of 1,1,2,2-tetrachloroethane in various matrices are identified in Table 1.1.

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

1,1,2,2-Tetrachloroethane is manufactured by chlorination of ethylene; by catalytic chlorination of ethane; or by chlorination of 1,2-dichloroethane (O'Neil *et al.*, 2001). When ethylene is used as a feedstock, 1,1,2,2-tetrachloroethane is not usually isolated initially, but is thermally cracked to produce other products (Mertens, 1993). To produce 1,1,2,2-tetrachloroethane of high purity, chlorination of acetylene has been used.

(b) Production volume

In the 1960s, production of 1,1,2,2-tetrachloroethane in the USA was > 100 000 tonnes per year (OECD SIDS, 2002). Because 1,1,2,2-tetrachloroethane is no longer used as a solvent, production has since dramatically decreased (ATSDR, 2008). Production worldwide has been reported to be

Table 1.2 Concentration of 1,1,2,2-tetrachloroethane in air

Location	Concentration		Comments	Reference
	Mean	Range		
Outdoor air				
Eight remote sites; North & South Atlantic	NR	0.2–1.7 ppt	Background tropospheric levels	Class & Ballschmiter (1986)
Tarragona, Spain	0.1 µg/m³	NR–0.7 µg/m³	Near large industrial complex	Ramírez *et al.* (2012)
Hamburg, Germany	NR	0.08–0.6 µg/m³	12 sites	Bruckmann *et al.* (1988)
Dallas, TX, USA	0.3[a] ppb	0.2 – 0.4 ppb	Ambient air near natural gas wells	Rich (2011)
Missoula, MT, USA	< 0.06[a] ng/m³	ND–0.3 ng/m³	Ambient outdoor air	Ward *et al.* (2009)
MN, USA	0.06 µg/m³	NR–6.87 µg/m³	25 outdoor sites in state (1991–1998)	Pratt *et al.* (2000)
Country-wide, USA	5.4 ppt	ND–4800 ppt	853 urban/suburban sites	ATSDR (2008)
Los Angeles, CA, USA	16.6 ppt	NR	Outdoor air	Singh *et al.* (1981)
Phoenix, AZ, USA	17.0 ppt	NR	Outdoor air	Singh *et al.* (1981)
Oakland, CA, USA	7.1 ppt	NR	Outdoor air	Singh *et al.* (1981)
Kuwait City, Kuwait	1188 µg/m³	0–17 604 µg/m³	Outdoor air	Bouhamra (1997)
Indoor air				
Missoula, MT, USA	< 0.06[a] ng/m³	ND–2.0 ng/m³	Indoor air, 80 homes	Ward *et al.* (2009)
Knoxville, TN, USA	13.0 µg/m³	NR	Indoor air, 8 homes	ATSDR (2008)
Kuwait City, Kuwait	3458 µg/m³	0–26 521 µg/m³	Indoor air	Bouhamra (1997)

[a] Median
ND, not detected; NR, not reported

between 10 000 and 100 000 tonnes per year, and most production occurred in China (OECD SIDS, 2002).

1.2.2 Use

In the past, 1,1,2,2-tetrachloroethane was used as a solvent, for degreasing metals, in paint removers, varnishes, lacquers, photographic film, rust removers, resins and waxes, extraction of oils and fats, and as an alcohol denaturant, in organic synthesis (Lewis, 1993). It has also been used in the manufacture of cyanogen chloride, polymers, and tetrachloro-alkylphenol, and as a solvent in the preparation of adhesives (Mackison *et al.*, 1981). Previously it was used in soil sterilization, as a weed killer, and in insecticide formulations, but is currently not registered in the USA for any of these purposes (ATSDR, 2008). Less common uses included in the determination of theobromine in cacao, as an immersion fluid in crystallography, in the biology laboratory to produce pathological changes in the gastrointestinal tract, liver and kidneys (O'Neil *et al.*, 2001), in the estimation of the water content of tobacco and many drugs, and as a solvent for impregnation of furs with chromium chloride (Sittig, 1985).

The primary current use of 1,1,2,2-tetrachloroethane is as a feedstock or chemical intermediate in the manufacture of trichloroethylene, tetrachloroethylene, and 1,2-dichloroethylene (ATSDR, 2008).

1.3 Occurrence

1.3.1 Natural occurrence

1,1,2,2-Tetrachloroethane is not known to occur as a natural product.

Table 1.3 Regulations and guidelines worldwide for 1,1,2,2-tetrachlorethane

Country or region	Concentration (mg/m³)	Interpretation	Carcinogenicity
Australia	6.9	TWA[a]	–
Austria	7	TWA	–
Belgium	7	TWA	–
Canada, Quebec	6.9	TWA	–
Denmark	7	TWA	–
France	7	TWA	–
Germany	7	TWA	TRGS 905 K3[b], MAK Category 3B[c]
New Zealand	6.9	TWA	–
Poland	5	TWA	–
Singapore	6.9	TWA	–
Spain	7	TWA	–
Switzerland	7	TWA	–
USA			–
NIOSH	7	TWA	–
OSHA	35	TWA	–
EPA	–	–	Group C[d]
ACGIH	–	–	A3[e]
NTP	–	–	Not listed

[a] Eight-hour time-weighted average
[b] Substances that are possibly carcinogenic for humans and thus give cause for concern.
[c] Substances that are proven/possibly carcinogenic and therefore give reason for concern. There are clues for carcinogenic effects which, however, are not enough for allocation into a different category.
[d] Possible human carcinogen
[e] Confirmed animal carcinogen with unknown relevance to humans.
ACGIH, American Conference of Industrial Hygienists; EPA, United States Environmental Protection Agency; MAK, [Maximale Arbeitsplatz-Konzentration] Maximum Workplace Concentration; NTP, National Toxicology Program; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; TRGS, Technical Rules for Hazardous Substances
From GESTIS-database on hazardous substances (IFA, 2012)

1.3.2 Environmental occurrence

1,1,2,2-Tetrachloroethane is primarily released to the atmosphere and to surface water as fugitive emissions during its production or use as a chemical intermediate (ATSDR, 2008). It was estimated that 1.4 tonnes of 1,1,2,2-tetrachloroethane were released to the atmosphere from 20 manufacturing and processing facilities in the USA in 2005 (ATSDR, 2008).

(a) Air

Table 1.2 presents some recent data on levels of 1,1,2,2-tetrachloroethane in air.

(b) Water

A nationwide study in the USA reported a mean concentration of 1,1,2,2-tetrachloroethane of 0.6 µg/L (range, 0.1–25 µg/L) in ground and surface water (ATSDR, 2008). Another study in the USA reported a range of 9000–17 000 µg/L in a river near a military testing site in the state of Maryland (Burton *et al.*, 2002).

1.3.3 Occupational exposure

In a large cross-sectional survey of small- to medium-scale industries in Japan in 1995–96, no use of 1,1,2,2-tetrachloroethane was reported (Ukai *et al.*, 1997). Two more recent (2009–2010) cross-industry surveys on use of organic solvents

in almost 1500 workplaces and 1900 laboratories in Japan confirmed this finding (Nagasawa *et al.*, 2011a, b).

1.3.4 Exposure of the general population

In Mexico City, 90 volunteers who lived near air-pollution monitoring stations wore personal samplers for 24 hours. 1,1,2,2-Tetrachlorethane was only detected in 3 out of 105 personal airspace monitors (Serrano-Trespalacios *et al.*, 2004). In studies in the USA of 1235 people in 2003–04 and 3131 people in 2005–06, 1,1,2,2-tetrachloroethane was not detected in blood (CDC, 2013).

1.4 Regulations and guidelines

Several countries have set time-weighted average (TWA) doses of 7 mg/m^3, including Australia, Austria, Belgium, Canada, Denmark, France, Germany, Japan, New Zealand, Singapore, Spain, Switzerland, and the National Institute for Occupational Safety and Health in the USA (Table 1.3). In the USA, the Occupational Safety and Health Administration has set a TWA dose of 35 mg/m^3.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In one study conducted by the US National Cancer Institute, male and female B6C3F$_1$ mice were given 1,1,2,2-tetrachloroethane by gavage in corn oil for 78 weeks, and observed for up to 90 weeks (NCI, 1978). Male mice were initially given 1,1,2,2-tetrachloroethane at a dose of 0 (n = 20), 100 (n = 50), or 200 (n = 50) mg/kg bw per day, 5 days per week, for 18 weeks. At week 19, the lower and higher doses were increased to 150 and 300 mg/kg bw per day, respectively, for 3 weeks, then increased again to 200 and 400 mg/kg bw, for 5 weeks, and finally decreased to 150 and 300 mg/kg bw for the remaining 52 weeks of treatment. The TWA doses were 0, 142, and 282 mg/kg bw per day. A significant dose-dependent trend in the incidence of hepatocellular carcinoma was observed at termination of the study. The incidence rates in the groups at the lower and higher doses were higher than in the controls. [The Working Group noted the alteration in the doses administered over time, the small number of mice in the control group, the limited duration of exposure, the increased mortality due to tubular nephrosis in weeks 69–70 in the group at the higher dose, and that the mice were housed in the same room as animals treated with several other volatile agents.]

The initial doses to female mice were 0 (n = 20), 100 (n = 50), and 200 (n = 50) mg/kg bw. At week 19, the lower and higher doses were increased to 150 and 300 mg/kg bw per day, respectively, for 3 weeks, then increased again to 200 and 400 mg/kg bw per day for 5 weeks, and finally decreased to 150 and 300 mg/kg bw per day for the remaining 52 weeks of treatment. The TWA doses were the same as for males. A significant positive trend in the incidence of hepatocellular carcinoma was observed in female mice at termination of the study. The incidence of hepatocellular carcinoma was higher at both doses

Table 3.1 Studies of carcinogenicity in experimental animals given 1,1,2,2-tetrachloroethane by gavage

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 90 wk NCI (1978)	0, 142, 282 mg/kg bw per day[a], in corn oil, for 78 wk 20, 50, 50/group	Hepatocellular carcinoma: 1/18, 13/50, 44/49	Cochran–Armitage trend test and Fisher exact test $P < 0.005$ (lower dose), $P < 0.001$ (higher dose), $P < 0.001$ (trend)	Purity, > 90% Variation of dose with time. Mice housed in same room where other volatile agents were studied. High mortality in higher-dose group due to tubular nephrosis (wk 69–70).
Mouse, B6C3F$_1$ (F) 90 wk NCI (1978)	0, 142, 282 mg/kg bw per day[a], in corn oil, for 78 wk 20, 50, 50/group	Hepatocellular carcinoma: 0/20, 30/48, 43/47	Cochran Armitage trend test and Fisher exact test $P < 0.001$ (lower dose), $P < 0.001$ (higher dose), $P < 0.001$ (trend)	Purity, > 90% Variation of dose with time. Mice housed in same room where other volatile agents were studied. High mortality in high-dose group (cause not identified).
Rat, Osborne-Mendel (M) 110 wk NCI (1978)	0, 62, 108 mg/kg bw per day[a], in corn oil, for 78 wk 20, 50, 50/group	Liver neoplastic nodules [liver adenoma] or hepatocellular carcinoma (combined): 0/20, 0/50, 3/49 [carcinoma, 2/49] Haemangiosarcoma: 0/20, 2/50, 3/49	NS	Purity, > 90% Variation of dose with time. Rats housed in same room where other volatile agents were studied. Early mortality at higher dose. The liver tumour incidence in high-dose animals was increased compared to historical controls.
Rat, Osborne-Mendel (F) 110 wk NCI (1978)	0, 43, 76 mg/kg bw per day[a], in corn oil, for 78 wk 20, 50, 50/group	Endometrial stromal polyp: 0/20, 8/50, 4/48	NS	Purity, > 90% Variation of dose with time. Rats housed in same room where other volatile agents were studied. Early mortality at both doses.

[a] Time-weighted average doses (see also text)

F, female; M, male; NS, not significant; wk, week

than in controls. [The Working Group noted the alteration in the doses administered over time, the small number of mice in the control group, the limited duration of exposure, the increased mortality due to unidentified causes in the group at the higher dose, and that the mice were housed in the same room as animals treated with several other volatile agents.]

3.2 Rat

Oral administration

In one study conducted by the US National Cancer Institute, male and female Osborne-Mendel rats were given 1,1,2,2-tetrachloroethane by gavage in corn oil for 78 weeks, and observed for up to 110 weeks (NCI, 1978). Male rats were initially given 1,1,2,2-tetrachloroethane at 0 (*n* = 20), 50 (*n* = 50), and 100 (*n* = 50) bw per day, 5 days per week. At week 15, the lower and higher doses were increased to 65 and 130 mg/kg bw per day, respectively. At week 33, treatment of rats at the higher dose was suspended for 1 week, then continued for a further 4 weeks. This dosing cycle of "1 week off" and "4 weeks on" was maintained until the end of treatment at week 78. The TWA doses were 0, 62, and 108 mg/kg bw per day. An increase in the incidence of liver neoplastic nodules [adenoma] or hepatocellular carcinoma (combined) was observed at the higher dose (0 out of 20, 0 out of 50, 3 out of 49 [carcinoma, 2 out of 49]). This increase was not statistically significant when compared with concurrent controls, but was significant compared with historical controls. The incidence of liver neoplastic nodules [adenoma] or hepatocellular carcinoma (combined) in male Osborne-Mendel rats among historical controls was 9 out of 975 (0.9% [carcinoma: 2 out of 975, 0.2%]; Goodman *et al.*, 1980). The increase in the incidence of haemangiosarcoma (0 out of 20, 2 out of 50, 3 out of 49) was not statistically significant compared with concurrent controls or historical controls (25 out of 975 [2.6%]; Goodman *et al.*, 1980). [The Working Group noted that the power of this study was reduced by the frequent changes in the doses administered, the small number of rats in the control group, the limited duration of exposure, and that the rats were housed in the same room as animals treated with several other volatile agents.]

Like the males, the female rats initially were given 1,1,2,2-tetrachloroethane at a dose of 0 (*n* = 20), 50 (*n* = 50), or 100 (*n* = 50) mg/kg bw per day (NCI, 1978). At week 26, the lower and higher doses were reduced to 40 and 80 mg/kg bw per day, respectively. Beginning at week 33, dosing was suspended for 1 week and then continued for a further 4 weeks. This dosing cycle of 1 week off and 4 weeks on was maintained until the end of treatment at week 78. The TWA doses were 0, 43, and 76 mg/kg bw per day. Survival was found to be reduced with increasing dose. No treatment-related increase in the incidence of liver tumours was noted, but there was an increase in the incidence of endometrial stromal polyps at the lower dose (0 out of 20, 8 out of 50, 4 out of 48). This increase was not statistically significant when compared with concurrent controls, but was significant compared with the incidence in historical controls. The incidence of endometrial stromal polyps in female Osborne-Mendel rats among historical controls was 43 out of 970 (4.4%; Goodman *et al.*, 1980). [The Working Group noted the frequent changes in the doses administered, the increased mortality in both dose groups, that the rats were housed in the same room as animals treated with several other volatile agents, and that the group of rats receiving the higher dose was reduced by 10 in the first 5 weeks of the study (including 8 rats with pneumonia). Reduced survival, the small number of rats in the control group, and the limited duration of exposure reduced the sensitivity of this study to adequately characterize the carcinogenic potential of the test agent.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Absorption

(a) Humans

No data on absorption of orally administered 1,1,2,2-tetrachloroethane in humans were identified by the Working Group. However, systemic toxicities after ingestion indicated absorption from the gastrointestinal tract in humans. The extent of absorption has not been characterized. One study reported on the absorption of 1,1,2,2-tetrachloroethane administered by inhalation (Morgan *et al.*, 1970). Volunteers inhaled ^{38}Cl-labelled 1,1,2,2-tetrachloroethane from a bulb in a single breath, which they held for 20 seconds. Approximately 97% of the inhaled dose was absorbed systemically, while only 3% was excreted as the parent compound in exhaled breath.

Estimates of the human blood:air partition coefficient *in vitro* averaged about 114 (Meulenberg & Vijverberg, 2000), indicating considerable respiratory uptake of 1,1,2,2-tetrachloroethane from inhaled air under equilibrium conditions.

(b) Experimental systems

Numerous studies indicated that 1,1,2,2-tetrachloroethane is readily absorbed after exposure by either oral or inhalation in rodents, but fewer data are available on the quantitative extent of absorption. Hanley *et al.* (1988) exposed male Osborne-Mendel rats and B6C3F$_1$ mice to ^{14}C-labelled 1,1,2,2-tetrachloroethane at a dose of 10 ppm (68.7 mg/m^3) as vapour for 6 hours, and recovered between 92% and 98% of the body burdens of radioactivity as metabolites, indicating very high uptake in both species at this level of exposure.

Gargas & Andersen (1989) conducted experiments on closed-chamber, whole-body inhalation in rats, and found a decline in chamber concentrations with time, consistent with an equilibrium alveolar gas-exchange model in which uptake by inhalation was largely determined by the partition coefficient. Estimates of the blood:air partition coefficient in rats *in vitro* averaged about 142 (Meulenberg & Vijverberg, 2000), indicating considerable respiratory uptake of 1,1,2,2-tetrachloroethane from inhaled air under equilibrium conditions. While the estimated blood:air partition coefficient for rats was slightly higher than that for humans, absorption would be expected to be similar since > 99% of the compound reaching the alveolar region would be absorbed into the blood in both species.

Hanley *et al.* (1988) gave male Osborne-Mendel rats and B6C3F$_1$ mice a single oral dose of radiolabelled 1,1,2,2-tetrachloroethane at 150 mg/kg in corn oil. Only 4–6% of the radiolabel was recovered in the faeces 72 hours after exposure, while > 90% was found as metabolites in both species, indicating complete absorption in rats and mice within 72 hours. Mitoma *et al.* (1985) exposed groups of male Osborne-Mendel rats to 1,1,2,2-tetrachloroethane at a dose of 25 or 100 mg/kg bw per day, and B6C3F$_1$ mice at 50 or 200 mg/kg bw per day, 5 days per week, for 4 weeks, followed by a single dose of radiolabelled compound, and recovered 79% of the administered dose as metabolites in rats and 68% in mice in 48 hours. Because metabolite recovery may not be complete, the results of this study were consistent with the virtually complete absorption estimated by Hanley *et al.* (1988).

Absorption also occurs via the dermal route. When 0.5 mL or 1 mL of 1,1,2,2-tetrachloroethane was applied to the skin of mice or guinea-pigs and the dose site occluded to prevent evaporation, all of the applied dose was absorbed within 30 minutes (Tsuruta, 1975; Jakobson *et al.*, 1982).

4.1.2 Distribution

(a) Humans

No data on tissue distribution of 1,1,2,2-tetrachloroethane were available to the Working Group. Meulenberg & Vijverberg (2000) used empirical regression models to predict tissue:air partition coefficients of volatile organic compounds based on measured saline:air and oil:air partition coefficients. Based on their predictions, tissue:blood partition coefficients in humans were estimated to range from 1.1 (kidney) to 38 (fat), depending on the lipid content of the tissues. These values suggested that 1,1,2,2-tetrachloroethane would be widely distributed to tissues after systemic delivery.

(b) Experimental systems

No data on the tissue distribution of 1,1,2,2-tetrachloroethane were available to the Working Group. Gargas *et al.* (1989) measured tissue:blood partition coefficients in the range of 0.7 (muscle) to 40 (fat), suggesting that 1,1,2,2-tetrachloroethane would be widely distributed to tissues after systemic delivery.

4.1.3 Metabolism

(a) Humans

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

(b) Experimental systems

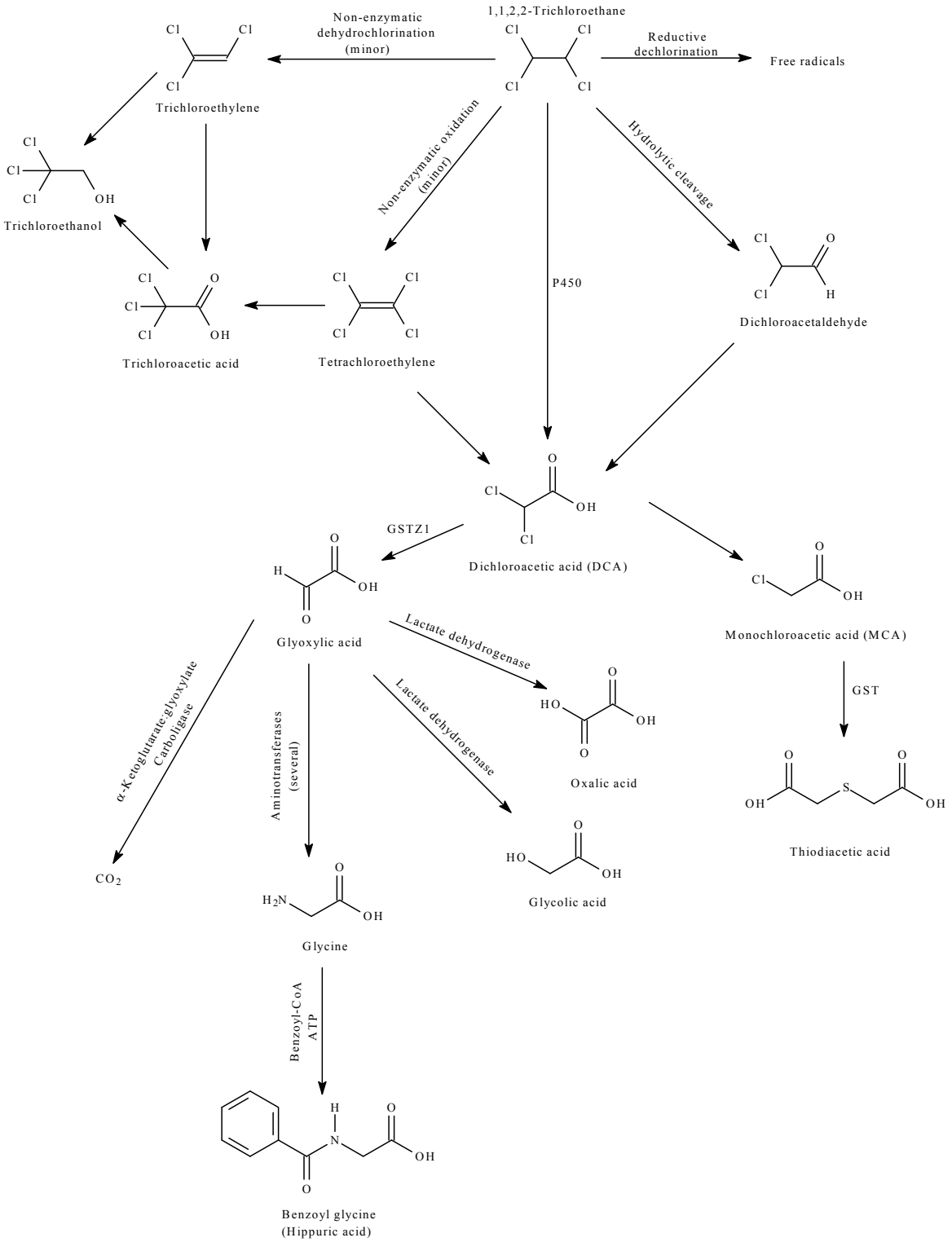
The metabolism of 1,1,2,2-tetrachloroethane is extensive in rodents, with 68–96% of the administered dose recovered as metabolites in mice and rats (Yllner, 1971; Mitoma *et al.*, 1985; Hanley *et al.*, 1988). Hanley *et al.* (1988) conducted quantitative recovery studies of 1,1,2,2-tetrachloroethane administered orally or by inhalation in rats and mice and showed that > 90% of the absorbed dose was metabolized.

The major pathway appears to be via oxidation to dichloroacetic acid, the major urinary metabolite detected in mice after intraperitoneal injection (Yllner, 1971) and in studies *in vitro* with rat liver microsomal and nuclear cytochrome P450 enzymes (Halpert & Neal, 1981; Halpert, 1982; Casciola & Ivanetich, 1984). The expiration of carbon dioxide (CO_2) and the presence of glyoxylic acid and oxalic acid in urine reported by Yllner (1971) are consistent with this pathway, since dichloroacetic acid can be further metabolized to glyoxylic acid to form oxylic acid and CO_2 (Fig. 4.1).

Yllner (1971) and Mitoma *et al.* (1985) reported trichloroethanol and/or trichloroacetic acid as urinary excretion products. Yllner (1971) also reported small amounts (0.2% to 0.4% of dose) of trichloroethylene and tetrachloroethylene in expired air. Yllner (1971) found that small amounts of 1,1,2,2-tetrachloroethane undergo non-enzymatic degradation to trichloroethylene in neutral aqueous solution, and suggested this pathway to explain the presence of trichloroethylene. Yllner (1971) suggested that a minute amount of 1,1,2,2-tetrachloroethane can be non-enzymatically converted to tetrachloroethylene. Trichloroethylene formation may explain the presence of trichloroethanol in urine, and either trichloroethylene or tetrachloroethylene could explain the presence of trichloroacetic acid. However, the source of trichloroethanol and trichloroacetic acid has not been conclusively demonstrated.

Multiple cytochrome P450 isozymes (CYP) are likely to be involved in the formation of dichloroacetic acid, as demonstrated by studies reporting increased metabolism following pretreatment with phenobarbital (Halpert 1982; Casciola & Ivanetich, 1984), xylene (Halpert 1982), or ethanol (Sato *et al.* 1980). Isozymes induced by these chemicals include members of the CYP subfamilies, CYP2A, CYP2B, CYP2E, and CYP3A (Omiecinski *et al.*, 1999).

Fig. 4.1 Metabolism of 1,1,2,2-tetrachloroethane



The reader is referred to the *Monographs* on trichloroethylene, tetrachloroethylene, trichloroacetic acid, dichloroacetic acid and 1,1,1,2-tetrachloroethane in this Volume for additional details on the metabolic pathway of 1,1,2,2-trichloroethylene.

Among several halogenated aliphatic hydrocarbons tested for their ability to decrease hepatic CYP content in rats, 1,1,2,2-tetrachloroethane was the second most active after carbon tetrachloride (Vainio *et al.*, 1976). Further evidence for the importance of CYP in the metabolism of 1,1,2,2-tetrachloroethane was obtained by Halpert (1982), who demonstrated covalent binding of a radiolabelled 1,1,2,2-tetrachloroethane metabolite to rat liver microsomes or a reconstituted mono-oxygenase system. On the basis of recovery of metabolites, 1,1,2,2-tetrachloroethane is metabolized by CYP to dichloroacetyl chloride, which can covalently bind to various nucleophilic groups or be hydrolysed to release dichloroacetic acid.

Halpert *et al.* (1986) also showed that incubation of a reconstituted system containing the phenobarbital-inducible form of CYP with 1,1,2,2-tetrachloroethane resulted in destruction of the haeme moiety. This provided further evidence of the role of CYP in the metabolism of 1,1,2,2-tetrachloroethane.

Tomasi *et al.* (1984) used electron spin resonance spectroscopy with spin trapping to demonstrate the formation of free radical intermediates in incubations of isolated hepatocytes from rats with eight aliphatic haloalkanes, including 1,1,2,2-tetrachloroethane. Formation of free radicals was demonstrated for 1,1,2,2-tetrachloroethane under both normoxic and hypoxic conditions.

Paolini *et al.* (1992) provided further evidence that 1,1,2,2-tetrachloroethane undergoes CYP-dependent oxidative metabolism to generate free radical intermediates that may cause lipid peroxidation and oxidative injury to the liver. Male and female mice were given a single oral dose of 1,1,2,2-tetrachloroethane (300 or 600 mg/kg bw). Analysis by electron spin resonance spectroscopy with spin trapping showed formation of a trichloroethyl free radical, the structure of which was not confirmed.

Thompson *et al.* (1985) investigated the reductive metabolism of several haloethanes, including 1,1,2,2-tetrachloroethane, by rat liver microsomes. Metabolism was NADPH-dependent, occurred only under anaerobic conditions, and led to formation of 1,2-dichloroethylene. Thus, depending on oxygenation status, CYP can catalyse both the oxidative and reductive metabolism of 1,1,2,2-tetrachloroethane.

Bolt (1987) compared metabolic rates of 1,1,2,2-tetrachloroethane between mice and rats, and found higher metabolic rates in mice. However, Mitoma *et al.* (1985) reported greater total metabolism in rats compared with mice (79% versus 68%).

4.1.4 Excretion

(a) Humans

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

(b) Experimental systems

In experimental animals, 1,1,2,2-tetrachloroethane is eliminated primarily as expired CO_2 and metabolites in urine, with a small amount expired unchanged (Yllner 1971; Ikeda & Ohtsuji, 1972; Mitoma *et al.* 1985; Hanley *et al.* 1988). Of the metabolites, major fractions (25–50%) were exhaled as CO_2, approximately 20% excreted in urine and approximately 5% excreted in faeces (Hanley *et al.*, 1988). Yllner (1971) reported similar findings in mice given an intraperitoneal injection of ^{14}C-labelled tetrachloroethane (about half the dose expired as CO_2, and about 30% excreted in urine). A small percentage (4%) was expired unchanged, and the remaining was retained in the carcass. Mitoma *et al.* (1985) characterized the overall metabolic disposition of several chlorinated hydrocarbons, including 1,1,2,2-tetrachloroethane, in Osborne-Mendel rats and B6C3F$_1$ mice after administration of 25% or 100% of the maximum tolerated dose. In

their study, a smaller fraction was expired as CO_2 (2% in rats and 10% in mice), and a larger fraction excreted (46% in rats and 30% in mice), after repeated oral dosing for 4 weeks. The patterns of elimination in rats and mice were qualitatively similar (Mitoma *et al.* 1985; Hanley *et al.* 1988). Elimination was fairly rapid, with a large fraction excreted in the first 24 hours, but significant amounts remained in the urine and expired air at 48–72 hours after exposure (Yllner, 1971).

4.2 Genotoxicity and related effects

4.2.1 Humans

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

4.2.2 Experimental systems

Several studies on genotoxicity with 1,1,2,2-tetrachloroethane were available to the Working Group. These are summarized in Table 4.1 and discussed below.

(a) Covalent binding

Colacci *et al.* (1987) found significant radiolabelling of DNA, RNA, and proteins from the liver, kidney, lung and stomach of male Wistar rats and BALB/c mice after intraperitoneal injection of radiolabelled 1,1,2,2-tetrachloroethylene.

Eriksson & Brittebo (1991) gave intravenous injections of ^{14}C-labelled 1,1,2,2-tetrachloroethane to C57B1 mice. Autoradiography showed selective localization of radiolabel in the nasal olfactory mucosa, epithelia of the trachea, bronchi and bronchioles, and squamous epithelia of the oral cavity, tongue and oesophagus. Homogenates of olfactory mucosa and liver were compared for their ability to activate radiolabelled 1,1,2,2-tetrachloroethane and bound radiolabel. For both processes, the capacity of the olfactory mucosa was higher than that of the liver. In addition, the effects of a CYP inhibitor, metyrapone, were consistent with 1,1,2,2-tetrachloroethane undergoing oxidative metabolism.

[The Working Group noted that the binding observed may be the result of a free radical formed via reductive dechlorination; as discussed above, free radical intermediates have been detected by spin-trapping techniques (Tomasi *et al.*, 1984; Paolini *et al.*, 1992; ATSDR, 1996). Alternatively, it may be the result of metabolic incorporation after metabolism through dichloroacetic acid to glycine.]

(b) DNA damage

Mirsalis *et al.* (1989) conducted *in vivo* to *in vitro* assays of DNA repair in hepatocytes and measurements of S-phase synthesis activity with 24 chemicals, including 1,1,2,2-tetrachloroethane. In the assay for DNA repair in hepatocytes, F344 rats or B6C3F$_1$ mice were given 1,1,2,2-tetrachloroethane by oral gavage and hepatocytes were isolated and incubated with radiolabelled thymidine. 1,1,2,2-Tetrachloroethane failed to induce unscheduled DNA synthesis in either rats or mice, and produced equivocal results with respect to S-phase synthesis activity in mice.

(c) Mutation and cytogenetic effects

Sofuni *et al.* (1996) reported on a collaborative study involving 42 Japanese laboratories to assess and compare a large battery of chemicals in the assay for clastogens in mouse lymphoma cells *in vitro*, using the microwell method. The results for 1,1,2,2-tetrachloroethane were inconclusive, with positive, negative, and unacceptable [high background] results reported.

As shown in Table 4.1, 1,1,2,2-tetrachloroethane has given negative results in several strains of *Salmonella typhimurium* (TA100, TA1530, TA104, TA1535, TA1537, TA1538, TA98) with and without exogenous metabolic activation. A few positive results have been reported by Strubel & Grummt (1987), in the TA98, TA97 or TA100 strains with and without metabolic activation.

Table 4.1 Genetic and related effects of 1,1,2,2-tetrachloroethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli pol A, differential toxicity (spot)	+	NT	16 000/disc	Brem *et al.* (1974); Rosenkranz (1977)
Salmonella typhimurium, forward mutation, arabinose resistance	–	–	150	Roldán-Arjona *et al.* (1991)
Salmonella typhimurium TA100, reverse mutation	–	–	2000	Nestmann *et al.* (1980)
Salmonella typhimurium TA100, reverse mutation	–	–	500	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	(+)	+	125	Strubel & Grummt (1987)
Salmonella typhimurium TA1530, reverse mutation	+	NT	1680/disc	Brem *et al.* (1974); Rosenkranz (1977)
Salmonella typhimurium TA104, reverse mutation	–	(+)	500	Strubel & Grummt (1987)
Salmonella typhimurium TA1535, reverse mutation	–	NT	1680/disc	Brem *et al.* (1974); Rosenkranz (1977)
Salmonella typhimurium TA1535, reverse mutation	–	–	2000	Nestmann *et al.* (1980)
Salmonella typhimurium TA1535, reverse mutation	–	–	500	Haworth *et al.* (1983)
Salmonella typhimurium TA1537, reverse mutation	–	–	2000	Nestmann *et al.* (1980)
Salmonella typhimurium TA1537, reverse mutation	–	–	500	Haworth *et al.* (1983)
Salmonella typhimurium TA1538, reverse mutation	–	NT	1680/disc	Brem *et al.* (1974); Rosenkranz (1977)
Salmonella typhimurium TA1538, reverse mutation	–	–	2000	Nestmann *et al.* (1980)
Salmonella typhimurium TA98, reverse mutation	–	–	2000	Nestmann *et al.* (1980)
Salmonella typhimurium TA98, reverse mutation	–	–	500	Haworth *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	(+)	+	5	Strubel & Grummt (1987)
Salmonella typhimurium TA97, reverse mutation	+	+	5	Strubel & Grummt (1987)
Saccharomyces cerevisiae strain D7, gene conversion, *trp*5 locus	+	NT	875	Callen *et al.* (1980)
Saccharomyces cerevisiae strain D7, homozygosis, *ade*2 locus	+	NT	875	Callen *et al.* (1980)
Aspergillus nidulans strain P1, genetic crossing-over	–	NT	640	Crebelli *et al.* (1988)
Saccharomyces cerevisiae strain D7, reverse mutation, *ilv*1 locus	+	NT	875	Callen *et al.* (1980)
Aspergillus nidulans strain P1, aneuploidy	+	NT	320	Crebelli *et al.* (1988)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–	NT	1500 ppm, feed	Woodruff *et al.* (1985)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	56	Galloway *et al.* (1987)
Sister chromatid exchange, BALB/c 3T3 cells *in vitro*	+	+	500	Colacci *et al.* (1992)
Chromosomal aberration, Chinese hamster ovary cells *in vitro*	–	–	653	Galloway *et al.* (1987)
Chromosomal aberration, mouse lymphoma assay *in vitro*	+/–	+/–	100–600 µg/mL	Sofuni *et al.* (1996)

Table 4.1 (continued)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, BALB/c 3T3 mouse cells	–	NT	250	Tu *et al.* (1985)
Cell transformation, BALB/c 3T3 mouse cells	(+)	+	125	Colacci *et al.* (1990)
Cell transformation, BALB/c 3T3 mouse cells	NT	+	62.5	Colacci *et al.* (1992)
Unscheduled DNA synthesis, B6C3F_1 mouse hepatocytes *in vivo*	–	NT	1000 p.o. × 1	Mirsalis *et al.* (1989)
DNA binding (covalent), calf thymus DNA *in vitro*	–	+	10	Colacci *et al.* (1987)
DNA and RNA binding, male BALB/c mouse liver, kidney, lung and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1987)
DNA and RNA binding, male Wistar rat liver, kidney, lung and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1987)
Binding to protein, male BALB/c mouse lung, liver, kidney and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1987)
Binding to protein, male Wistar rat lung, liver, kidney and stomach *in vivo*	+	NT	1.46 i.p. × 1	Colacci *et al.* (1987)
Rat-liver foci assay for tumour initiation and promotion	+	NT	100	Milman *et al.* (1988)

[a] +, positive; (+), weakly positive; –, negative

[b] Unless otherwise specified, tests *in vitro*, μg/mL; tests *in vivo*, mg/kg bw per day.

HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; NT, not tested; p.o., oral

However, 1,1,2,2-tetrachloroethane was reported to lack mutagenicity in the TA98 and TA100 strains in two other published reports of tests at much higher exposures (Nestmann *et al.*, 1980; Haworth *et al.*, 1983). 1,1,2,2-Tetrachloroethane also gave positive results in the TA1530 strain without metabolic activation (Brem *et al.*, 1974).

1,1,2,2-Tetrachloroethane did not induce chromosomal aberrations in Chinese hamster ovary cells (Galloway *et al.*, 1987). 1,1,2,2-Tetrachloroethane induced sister chromatid exchanges in Chinese hamster ovary cells and mouse BALB/c 3T3 cell cultures *in vitro* (Galloway *et al.*, 1987; Colacci *et al.*, 1992).

Brem *et al.* (1974) assessed the ability of a series of haloalkanes, haloethanols, and haloacetaldehydes to induce mutations in *S. typhimurium* or to inhibit growth of DNA polymerase-deficient (pol A^+/pol A^-) *Escherichia coli*. 1,1,2,2-Tetrachloroethane was weakly mutagenic in both assays.

Roldán-Arjona *et al.* (1991) examined the association between mutagenicity of 16 halogenated aliphatic hydrocarbons, including 1,1,2,2-tetrachloroethane, in the Ames test in *S. typhimurium* and their documented carcinogenicity. 1,1,2,2-Tetrachloroethane was not mutagenic either in the presence or absence of metabolic activation from rat liver S9 fraction (9000 × *g* supernatant), and caused concentration-dependent lethality.

Callen *et al.* (1980) studied the mutagenicity of several halogenated aliphatic hydrocarbons, including 1,1,2,2-tetrachloroethane, in *Saccharomyces cerevisiae*. 1,1,2,2-Tetrachloroethane induced mitotic gene convertants and recombinants when incubated with yeast cells in log phase.

Crebelli *et al.* (1988) tested a series of halogenated solvents for induction of mitotic segregation in *Aspergillus nidulans* diploid strain P1. 1,1,2,2-Tetrachloroethane significantly increased the frequency of morphologically abnormal colonies that produced euploid whole-chromosome segregants (haploids and non-disjunctional diploids), but not the frequency of genetic crossing-over.

Woodruff *et al.* (1985) found that 1,1,2,2-tetrachloroethane did not increase the frequency of sex-linked recessive lethal mutation in *Drosophila melanogaster*.

(d) Cell transformation

Tu *et al.* (1985) found that 1,1,2,2-tetrachloroethane gave negative results at concentrations up to 250 µg/mL in an assay for the induction of transformation of BALB/c-3T3 cells *in vitro* in the absence of exogenous metabolic activation.

Colacci *et al.* (1990) demonstrated that 1,1,2,2-tetrachloroethane is capable of inducing transformation of BALB/c 3T3 cells *in vitro* either in the presence or absence of metabolic activation from rat liver S9 fraction. The highest concentration of 1,1,2,2-tetrachloroethane, 1000 µg/mL, induced transformation in a S9-independent manner, while lower concentrations (< 500 µg/mL) exhibited a requirement for S9 fraction that was inversely related to concentration. These results were consistent with metabolic activation being necessary for transformation, exogenous activation being necessary at lower concentrations and endogenous activation being adequate at higher concentrations.

In a subsequent study, BALB/c 3T3 cells transformed by exposure to 1,1,2,2-tetrachloroethane gave positive results in an assay for chemoinvasion in athymic mice (Colacci *et al.*, 1993).

4.3 Nongenotoxic mechanisms of carcinogenesis

4.3.1 Mechanisms related to liver carcinogenesis

(a) Cytotoxicity

(i) Humans

Zheng *et al.* (2012) evaluated the effects of exposure to 1,1,2,2-tetrachloroethane in 18 workers in a factory processing plastic products. Measurements of several parameters in serum considered indicative of liver damage, including alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, and γ-glutamyltranspeptidase (GGT), showed increases of 3- to 25-fold on admission to the hospital. Histological findings were also indicative of liver injury, including crowded hepatic plates that were lobular with swollen, fatty and ballooned degeneration of hepatocytes. Lymphocytes and neutrophils were found in the hepatic sinusoids. Infiltration of Kupffer cells was found in the majority of samples.

(ii) Experimental systems

In long-term studies, rats and mice were given diets containing 1,1,2,2-tetrachloroethane at doses up to 108 mg/kg bw per day (rats) or 284 mg/kg bw per day (mice) for 78 weeks (NCI, 1978). Hepatic fatty metamorphosis was observed in male rats at the highest dose, but not in female rats, or either species of mouse tested.

In a study of toxicity, male and female F344/N rats and B6C3F$_1$ mice were given feed containing 1,1,2,2-tetrachloroethane (enclosed in starch microcapsules to minimize evaporation) at dietary concentrations of up to 4600 ppm (rats) or 9100 ppm (mice) for 14 weeks (NTP, 2004). Body weights were significantly decreased compared with controls in rats at doses of up to 1180 ppm, and in mice at doses up to 2300 ppm. The liver was the primary target for toxicity. In rats, histological changes in the liver (cytoplasmic vacuolization, hepatocellular hypertrophy and necrosis, and hepatocellular mitotic alterations) and alterations in plasma enzymes indicative of decreased liver function were observed.

Dahlström-King *et al.* (1990) used suspensions of isolated rat hepatocytes to develop a model to study the cytotoxicity of chlorinated hydrocarbons *in vitro*. To validate the model, the acute cytotoxicity of four compounds known to be hepatotoxic *in vivo* and four compounds known to be not hepatotoxic *in vivo* was determined by measuring release of alanine aminotransferase from cells incubated for 30–180 minutes. Acute cytotoxicity in the model *in vitro* was found to be poorly correlated with hepatotoxicity *in vivo*. Thus, although carbon tetrachloride is the most potent hepatotoxicant *in vivo* of the eight chlorinated hydrocarbons tested, 1,1,2,2-tetrachloroethane was actually more potent than carbon tetrachloride in the isolated cell suspension.

In the study by Cottalasso *et al.* (1998), male Sprague-Dawley rats received a single dose of 1,1,2,2-tetrachloroethane (574 mg/kg bw) and were killed after 5, 15, 30, or 60 minutes. The activity of serum aspartate aminotransferase and alanine aminotransferase, and concentrations of hepatic triglycerides were significantly higher in exposed rats than in controls, while the activity of microsomal glucose 6-phosphatase was decreased.

(b) Oxidative stress

Gavino *et al.* (1984) incubated slices of various rat tissues with one of several halogenated hydrocarbons and measured release of total ethane and pentane as a measure of lipid peroxidation. Rats were also made either vitamin E-deficient or given excess iron (iron overload) to prime the oxidative-stress response. Bromotrichloromethane produced the largest release of total ethane and pentane from the liver. Release of total ethane and pentane from 1,1,2,2-tetrachloroethane was somewhat lower,

but equivalent to that from carbon tetrachloride and 1,1,2,2-tetrabromoethane, and was higher than that from tetrachloroethylene. Release of total ethane and pentane occurred from several tissues, with intestine, brain and kidney producing greater releases than the liver.

Paolini *et al.* (1992) detected conjugated dienes in male and female mice given 1,1,2,2-tetrachloroethane as a single oral dose (300 or 600 mg/kg bw), providing evidence for 1,1,2,2-tetrachloroethane-induced lipid peroxidation *in vivo* as part of the mechanism of hepatotoxicity.

(c) Promotion of GGT-positive foci

Story *et al.* (1986) examined the ability of 1,1,2,2-tetrachloroethane to induce liver tumours, using GGT activity as a preneoplastic marker in the liver of young male Osborne-Mendel rats, with or without phenobarbital induction. 1,1,2,2-Tetrachloroethane increased the formation of GGT-positive foci only when rats were first induced with phenobarbital. The ability of 1,1,2,2-tetrachloroethane to initiate or promote tumour formation in livers of young adult male Osborne Mendel rats was investigated (Story *et al.*, 1986; Milman *et al.*, 1988). After partial hepatectomy, 1,1,2,2-tetrachloroethane was given at the maximum tolerated dose in either the initiation phase followed by phenobarbital, or in the promotion phase preceded by diethylnitrosamine. 1,1,2,2-Tetrachloroethane was found to modestly increase the number of GGT-positive foci during the initiation phase and strongly increase the number of GGT-positive foci in the promotion protocol.

4.4 Other adverse effects

4.4.1 Kidney

In long-term studies by the National Cancer Institute, rats and mice were given diets containing 1,1,2,2-tetrachloroethane at a dose of up to 108 mg/kg bw per day (rats) or 284 mg/kg bw per day (mice) for 78 weeks(NCI, 1978). In mice, the toxic effects observed in the kidney were acute tubular nephrosis in males, hydronephrosis in females, and chronic kidney inflammation in both sexes.

4.4.2 Central nervous system

1,1,2,2-Tetrachloroethane has a sedative effect in humans and animals (IARC, 1999; ATSDR, 2007). The relevance of neurotoxicity to cancer hazard is unknown.

4.5 Mechanistic considerations

Limited information is available to characterize the absorption, distribution, metabolism and excretion of 1,1,2,2-tetrachloroethane in humans and experimental animals. High absorption was shown experimentally in humans. High absorption is also suggested by the high blood:air partition coefficient that has been measured in human blood. In animals, several studies have shown that 1,1,2,2-tetrachloroethane is readily absorbed orally or by inhalation; dermal absorption has also been shown in mice. Although no direct data on distribution were available, estimated tissue:blood partition coefficients indicate that 1,1,2,2-tetrachloroethane would be widely distributed in humans and animals, especially to tissues with a high lipid content. No data on metabolism or excretion in humans were available. In animals, the major metabolite is dichloroacetic acid (via cytochrome P450-mediated oxidation). Glyoxylic acid and CO_2, metabolites of dichloroacetic acid, have been measured in exposed animals, further supporting formation of dichloroacetic acid. Trichloroethylene and tetrachloroethylene are minor metabolites formed via a non-enzymatic pathway. Formation of a carbon-centred radical has been reported in two studies; however, the identity and quantity of this radical species have not been characterized.

Although some positive results for genotoxicity were reported, most studies of mutagenesis in bacteria gave negative results. A few studies have indicated possible clastogenic effects and one study showed binding to DNA and protein.

Evidence for liver toxicity is available from studies in humans and animals, including studies with human hepatocytes *in vitro*. Suggested mechanisms for liver carcinogenesis in rodents include cytotoxicity (*in vivo* and *in vitro*) and oxidative stress (*in vivo* and *in vitro*). One study showed the potential for 1,1,2,2-tetrachloroethane to promote GGT-positive foci in rat liver. Other target tissues for the adverse health outcomes associated with 1,1,2,2-tetrachloroethane are the liver, kidney, and central nervous system.

5. Summary of Data Reported

5.1 Exposure data

1,1,2,2-Tetrachloroethane has been used in small amounts in many applications, mainly as a solvent and for degreasing metals. The primary current use is as a chemical intermediate in the manufacture of chlorinated solvents. Very low concentrations have been reported in ambient and indoor air, and in surface water near contaminated areas.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,1,2,2-Tetrachloroethane was evaluated for carcinogenicity in one study in rats and in one study in mice treated by gavage in corn oil. Survival was reduced in male and female mice, and in female rats. There was an increase in the trend and the incidence of hepatocellular carcinoma in male and female mice. The incidence of hepatocellular tumours in exposed male rats was greater than that in historical controls.

5.4 Mechanistic and other relevant data

Little information was available from studies on absorption and distribution in humans. In experimental animals, it is likely that 1,1,2,2-tetrachloroethane is absorbed readily and distributed widely throughout the body. No data on metabolism or excretion in humans were available; in rodents, metabolism produces dichloroacetic acid and then glyoxylic acid which, along with several other metabolites, including CO_2, are excreted in the urine and breath. Trichloroethylene and tetrachloroethylene may also be formed from 1,1,2,2-tetrachloroethane, albeit in lesser amounts. 1,1,2,2-Tetrachloroethane is weakly genotoxic. Major target tissues for adverse health outcomes are the liver, kidney, and the central nervous system. Based on cancer findings in animals and toxicity findings in animals and humans, the evidence for liver as a target tissue is strong. The evidence for nongenotoxic mechanisms of carcinogenesis in the liver is moderate. 1,1,2,2-Tetrachloroethane is also toxic to the kidney and has a sedative effect in humans and animals. No data on potential inter-individual variability in response to the adverse effects of 1,1,2,2-tetrachloroethane were available.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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LIST OF ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
5MeC	5-methylcytosine
8-OHdG	8-hydrodeoxyguanosine adducts
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ALT	alanine transferase
AST	aspartate transferase
AUC	area under the concentration–time curve
BEI	biological exposure index
bw	body weight
CAREX	CARcinogen EXposure
CBI	covalent binding index
CCBL	cysteine-conjugate β-lyase
CI	confidence interval
coA	coenzyme A
CYP450	cytochrome P450
DCVCS	*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide
DCVT	*S*-(1,2-dichlorovinyl)-thiol
DDT	dichlorodiphenyltrichloroethane
DMSO	dimethyl sulfoxide
ECD	electron capture detection
ENU	*N*-ethyl-*N*-nitrosourea
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FID	flame ionization detection
FMO	flavin-containing monooxygenase
GC	gas chromatography
GGT	γ-glutamyltranspeptidase OR γ-glutamyltransferase???
GSH	glutathione
GST	glutathione-*S*-transferase
GTK	glutamine transaminase K
HDL	high-density lipoprotein
HECD	Hall electrolytic conductivity detection

HR	hazard ratio
LD_{50}	median lethal dose
LOH	loss of heterozygosity
MCD	microcoulometric detection
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
MNU	*N*-methyl-*N*-nitrosourea
MS	mass spectrometry
NA	not applicable
NAcDCVC	*N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAG	*N*-acetylglucosaminidase
ND	not detected
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NR	not reported
NS	not significant
NTP	National Toxicology Program
OEL	occupational exposure limit
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PBN	phenyl-*tert*-butyl nitroxide
PID	photoionization detection
PPARα	peroxisome proliferator-activated receptor alpha
ppm	parts per million
ppt	parts per trillion
RR	relative risk
S9	9000 × *g* supernatant
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SSB	single-strand DNA break
SSCP	single-stand conformation polymorphism
TBARS	thiobarbituric acid-reactive substances
TLV	threshold limit value
TWA	time-weighted average
UDS	unscheduled DNA synthesis
USP	United States Pharmacopeia
vs	versus

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IARC MONOGRAPHS

This Volume of the *IARC Monographs* provides an assessment of the carcinogenic hazards associated with exposure to seven chlorinated solvents, including trichloroethylene, tetrachloroethylene, and their metabolites (dichloroacetic acid, trichloroacetic acid, and chloral hydrate). All these agents were previously assessed by IARC Working Groups more than 10 years ago, and new epidemiological and mechanistic evidence has been considered in this reevaluation.

Trichloroethylene has been used in several industries, such as manufacture and repair of aircraft and automobiles, and in screw-cutting, while tetrachloroethylene is widely used in dry-cleaning and as a feedstock for the production of chlorinated chemicals.

The *IARC Monographs* Working Group relied on epidemiological evidence, carcinogenicity bioassays, and mechanistic and other relevant data to evaluate the carcinogenic hazards to humans exposed to these agents.