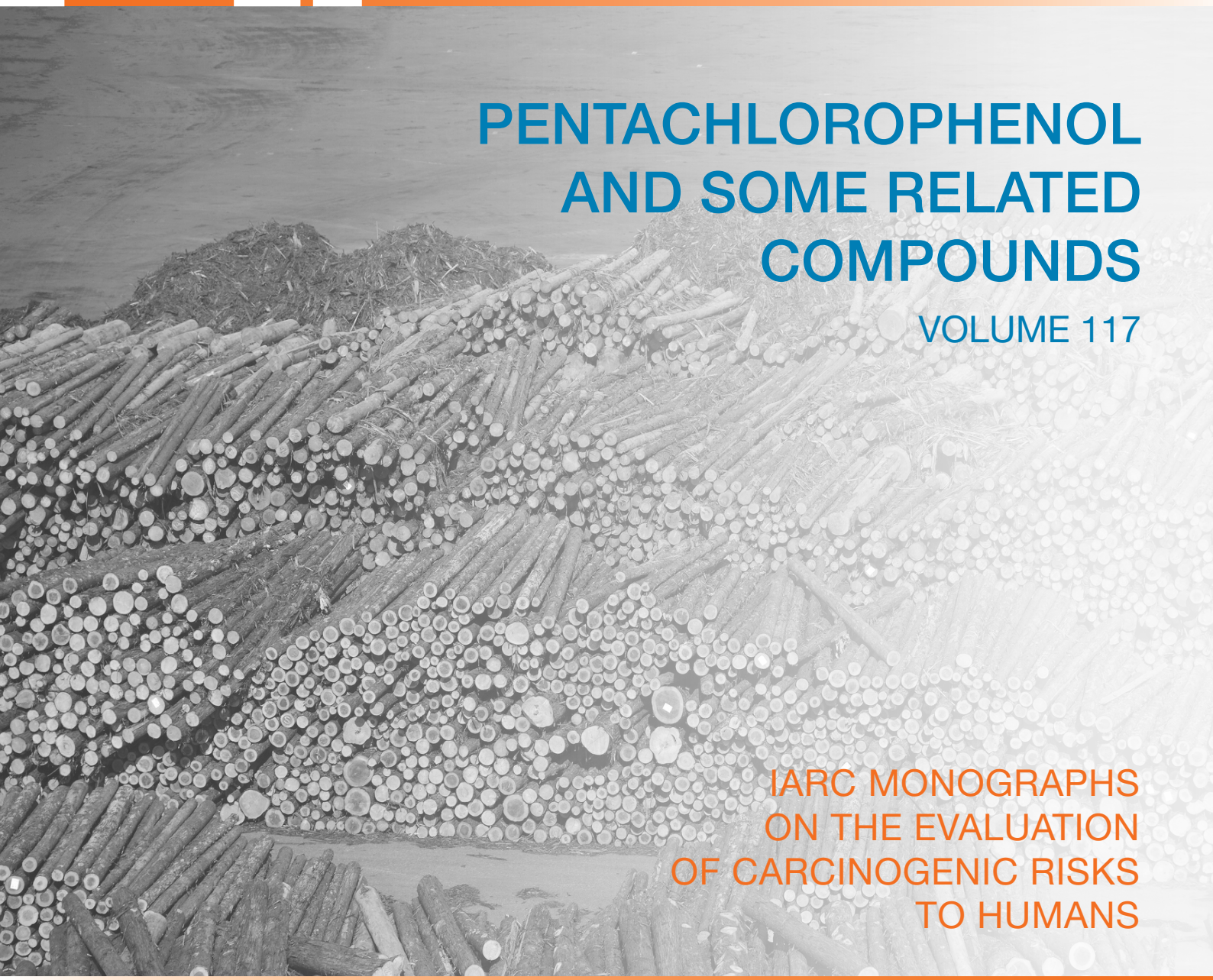
IARC MONOGRAPHS



PENTACHLOROPHENOL AND SOME RELATED COMPOUNDS

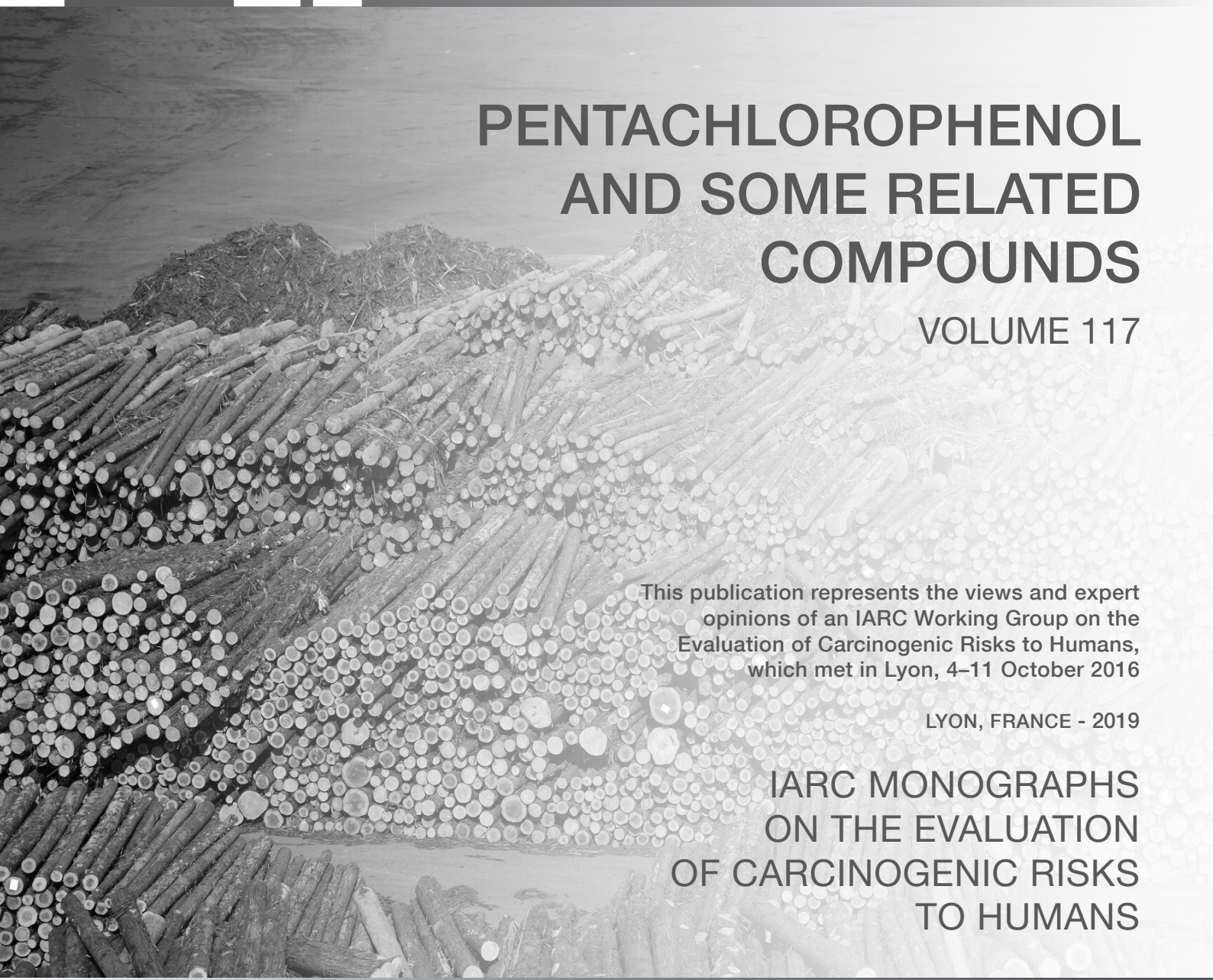
VOLUME 117

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



PENTACHLOROPHENOL AND SOME RELATED COMPOUNDS

VOLUME 117

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, 4–11 October 2016

LYON, FRANCE - 2019

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic 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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[3] 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.

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PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

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

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

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

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

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

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

which may give rise to different impurities, are described.

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

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

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

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

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

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

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

(b) Quality of studies considered

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

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

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

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

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

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

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

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

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

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

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

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

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

(b) Quantitative aspects

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

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

(c) Statistical analyses

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

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

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

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

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

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

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

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

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

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

(c) Other data relevant to mechanisms

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

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

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

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

***Sufficient evidence of carcinogenicity*:**

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

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

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

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

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

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

(b) Carcinogenicity in experimental animals

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

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

***Sufficient evidence of carcinogenicity*:**

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

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

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

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

(c) Mechanistic and other relevant data

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

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

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

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

(d) Overall evaluation

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

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

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

Group 1: The agent is carcinogenic to humans.

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

Group 2.

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

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

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

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

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

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

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

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

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

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

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

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

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

(e) Rationale

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

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GENERAL REMARKS

This one-hundred-and-seventeenth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of pentachlorophenol, 2,4,6-trichlorophenol, 3,3′,4,4′-tetrachloroazobenzene, aldrin, and dieldrin.

Pentachlorophenol, aldrin, and dieldrin are classified as persistent organic pollutants under the Stockholm Convention. Aldrin and dieldrin had been previously evaluated as *not classifiable as to* their *carcinogenicity to humans* (Group 3) in Supplement 7 (IARC, 1987), and combined exposures to polychlorophenols or to their sodium salts were evaluated as *possibly carcinogenic to humans* (Group 2B) in Volume 71 (IARC, 1999) of the *IARC Monographs*. 3,3′,4,4′-Tetrachloroazobenzene was not previously evaluated by the *IARC Monographs* programme. A summary of the findings of this volume appears in *The Lancet Oncology* (Guyton et al., 2016).

Pentachlorophenol

Impurities of chlorophenols include polychlorinated dibenzo-*para*-dioxins, as well as polychlorinated dibenzofurans, polychlorinated phenoxyphenols, polychlorinated diphenyl ethers, polychlorinated benzenes, and polychlorinated biphenyls. However, the pattern of excess cancers seen with pentachlorophenol differed from that observed in populations that are highly exposed to dioxins. In addition, the pattern of tumours in experimental animals exposed to pentachlorophenol was similar across three test agents of different purity. Similarly, test agents varying in purity induced mechanistic effects that are different from those exhibited by dioxins. These mechanistic studies provided strong evidence of multiple key characteristics of carcinogens (Smith et al., 2016).

3,3′,4,4′-Tetrachloroazobenzene

3,3′,4,4′-Tetrachloroazobenzene is not manufactured commercially but is formed during the production and degradation of chloroanilide herbicides. 3,3′,4,4′-Tetrachloroazobenzene bears structural similarity to dioxins and is highly lipophilic but is rapidly metabolized, with extensive azo reduction in the gut and liver to give 3,4-dichloroaniline metabolites that are readily eliminated. The spectrum of rodent tumours induced by 3,3′,4,4′-tetrachloroazobenzene encompasses those observed with other aryl hydrocarbon receptor (AhR) agonists previously evaluated as *carcinogenic to humans* (Group 1) (e.g. dioxins, dioxin-like polychlorinated biphenyls, and 2,3,4,7,8-pentachlorodibenzofuran). In addition, 3,3′,4,4′-tetrachloroazobenzene activates AhR and/or induces multiple non-neoplastic effects that are consistent with, or are

hallmarks of, AhR activation in various species, such as rodents, rabbits, chicken, and zebrafish (Poland et al., 1976; NTP, 1998, 2010; Xiao et al., 2016). 3,3′,4,4′-Tetrachloroazobenzene was classified as *probably carcinogenic to humans* (Group 2A) because it belongs, based on mechanistic considerations, to a class of agents that activate AhR, and some members of this class have previously been evaluated as Group 1 or Group 2A.

Aldrin and dieldrin

Aldrin and dieldrin each induced hepatocellular carcinomas in studies of carcinogenicity in experimental animals. For aldrin, epidemiological data were inadequate and mechanistic data were sparse. For dieldrin, epidemiological studies provided *limited evidence* in humans for breast cancer, whereas the evidence was *inadequate* for non-Hodgkin lymphoma and other cancers. Mechanistic studies provided moderate evidence for multiple key characteristics of carcinogens (Smith et al., 2016). Because aldrin rapidly converts to dieldrin in the body, exposure to aldrin inevitably entails internal exposure to dieldrin. Dieldrin is slowly excreted in humans, due to inefficient metabolism and sequestration in fat. Dieldrin, and aldrin metabolized to dieldrin, was evaluated as *probably carcinogenic to humans* (Group 2A).

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PENTACHLOROPHENOL

1. Exposure Data

1.1 Identification of the agent

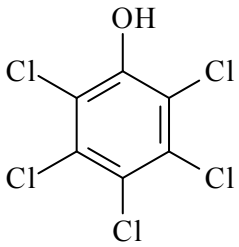
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 87-86-5

Chem. Abstr. Serv. Name: Pentachlorophenol

IUPAC Systematic Name: Pentachlorophenol

Synonyms: Chlorophenasic acid; Chlorophen; PCP; penchlorol; penta; pentachlorophenate; 2,3,4,5,6-pentachlorophenol; 1-hydroxy-2,3,4,5,6-pentachlorobenzene



Molecular formula: C_6HCl_5O

Relative molecular mass: 266.34

1.1.2 Chemical and physical properties of the pure substance

Description: Colourless to light brown needle-like crystals with characteristic phenolic odour (Budavari, 1996; IARC, 1999; NTP, 1999)

Boiling point: 310 °C (decomposes) (Lide, 1997; IARC, 1999)

Melting point: 191 °C (anhydrous) (EPA, 2010a)

Density: 1.978 g/mL (at 22 °C/4 °C) (EPA, 2010a)

Solubility: Slightly soluble in water (80 mg/L at 20 °C); soluble in acetone and benzene; very soluble in diethyl ether, ethanol, and methanol (EPA, 2010a)

Vapour pressure: 1.1×10^{-4} mm Hg (0.02 Pa) at 20 °C; relative vapour density (air = 1), 9.20 (EPA, 2010a; PubChem, 2018)

Log K_{ow}: 5.01 (EPA, 2010a)

Conversion factor: 1 ppm = 10.9 mg/m^3 (air), at normal temperature (25 °C) and pressure (1 atm) (EPA, 2010a)

Dissociation constant (pK_a): 4.7 at 25 °C (WHO, 2003)

1.1.3 Technical products and impurities

(a) Some trade names

Acutox; Chem-Penta; Chem-Tol; Cryptogil ol; Dowicide 7; Dowicide EC-7; Dow Pentachlorophenol DP-2 Antimicrobial; Durotox; Fungifen; Fungol; Glazd Penta; Grundier Arbezol Lauxtol; Lauxtol A; Liroprem; Moosuran; Penta; Pentacon; Penta-Kil; Pentasol; Penwar; Peratox; Permacide; Permagard; Permasan; Permatox; Priltox; Permite; Santophen; Santophen 20; Sinituho; Term-i-Trol; Thompson's Wood Fix; Weedone; Witophen P (NTP, 1999).

(b) Impurities

Pentachlorophenol is manufactured in a multistage chlorination process that results in contamination with dioxins, furans, and other chlorophenols. Consequently, the formulation that is used and that people are exposed to is a chemical grade, commonly referred to as the technical or commercial grade, which is composed of approximately 90% pentachlorophenol and 10% impurities. Depending on the specific synthesis process, the level of these impurities may vary with differing grades of manufactured pentachlorophenol (EPA, 2010a). In general, technical-grade pentachlorophenol contains 85–90% pentachlorophenol, 4–10% tetrachlorophenol, ~5% chlorinated diphenyl ethers, and < 1% trichlorophenol. Trace amounts to thousands of parts per million of polychlorinated dibenzo-*para*-dioxins (PCDDs) and chlorinated dibenzofurans can be detected. Grades described as analytical or pure are generally ≥ 98% pentachlorophenol, and the concentrations of dioxins and furans are low to non-detectable (IARC, 1991; NTP, 1999; EPA, 2010a).

The impurities consist of several chlorophenol congeners, PCDDs, and polychlorinated dibenzofurans (PCDFs). Of the PCDD and PCDF contaminants, the higher chlorinated congeners are predominantly found as impurities within technical-grade pentachlorophenol: isomers of hexachlorodibenzo-*para*-dioxin (HxCDD), heptachlorodibenzo-*para*-dioxin (HpCDD), and octachlorodibenzo-*para*-dioxin (OCDD), and isomers of tetrachlorodibenzofuran (TCDF), pentachlorodibenzofuran (PeCDF), hexachlorodibenzofuran (HxCDF), heptachlorodibenzofuran (HpCDF), and octachlorodibenzofuran (OCDF) (McLean et al., 2009). An analytical study in 1973 on 19 samples of commercial pentachlorophenol or pentachlorophenol sodium salt products from Switzerland reported concentration ranges of PCDD contaminants as 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) (< 0.01–0.25 ppm), pentachlorodibenzo-*para*-dioxin (PeCDD) (< 0.03–0.08 ppm), HxCDD (< 0.03–10 ppm), HpCDD (0.3–240 ppm), and OCDD (1.2–370 ppm); for PCDF contaminants, the ranges were TCDF (< 0.02–0.45 ppm), PeCDF (< 0.03–0.65 ppm), HxCDF (< 0.03–39 ppm), HpCDF (< 0.1–320 ppm), and OCDF (< 0.1–300 ppm) (Buser & Bosshardt, 1976). Dioxin and dioxin-like polychlorinated biphenyl (PCB) impurities have also been measured in agrochemical formulations of pentachlorophenol produced in Japan during the 1960s and early 1970s. Four pentachlorophenol samples exhibited dioxin impurity concentrations in a wide range from 14 to 24 000 µg/g active ingredient (Masunaga et al., 2001). The presence of dioxin and dioxin-like congeners and several other impurities have also been measured in most samples of the pesticides studied that were derived from chlorophenols in the USA in the early 1970s (Woolson et al., 1972; Plimmer, 1973). In addition to PCDD and PCDF contaminants, hexachlorobenzene and chlorophenoxy constituents may also be present in technical-grade pentachlorophenol (United Nations, 2010). 2,4,6-Tetrachlorophenol was also reported to be a by-product in the manufacture of pentachlorophenol (Kauppinen et al., 1994).

Table 1.1 gives a detailed list of contaminants of concern measured in Canadian products containing pentachlorophenol (United Nations, 2010), and their conversion to TCDD toxicity equivalence factors (TEFs), also called TCDD-equivalents (EPA, 2010b).

1.2 Production and use

1.2.1 Production process

Pentachlorophenol is produced via two pathways, either by stepwise chlorination of phenols in the presence of catalysts (anhydrous aluminium chloride or ferric chloride) or alkaline hydrolysis of hexachlorobenzene. Use of the analytical grade of pentachlorophenol requires a

Table 1.1 Contaminants of concern in Canadian products containing pentachlorophenol

Compound	CAS No.	TEF[a]	Concentration (ng/g)		Concentration (ng TCDD-eq/g)	
			Minimum	Maximum	Minimum	Maximum
Polychlorinated dibenzo-para-dioxins (PCDDs)						
2,3,7,8-TCDD	1746-01-6	1	0.028	0.175	0.028	0.175
1,2,3,7,8-PeCDD	40321-76-4	1	0.247	1.08	0.247	1.08
1,2,3,4,7,8-HxCDD	39227-28-6	0.1	1.1	86.8	0.11	8.68
1,2,3,6,7,8-HxCDD	57653-85-7	0.1	232	344	23.2	34.4
1,2,3,7,8,9-HxCDD	19408-74-3	0.1	14.8	203	1.48	20.3
1,2,3,4,6,7,8-HpCDD	35822-46-9	0.01	4570	13 500	45.7	135
OCDD	3268-87-9	0.0003	34 000	130 000	10.2	39
Polychlorinated dibenzofurans (PCDFs)						
2,3,7,8-TCDF	51207-31-9	0.1	0.022	0.068	0.0022	0.0068
1,2,3,7,8-PeCDF	57117-41-6	0.03	0.099	0.309	0.00297	0.00927
2,3,4,7,8-PeCDF	57117-31-4	0.3	0.431	2.74	0.1293	0.822
1,2,3,4,7,8-HxCDF	70648-26-9	0.1	176	577	17.6	57.7
1,2,3,6,7,8-HxCDF	57117-44-9	0.1	12	38.2	1.2	3.82
2,3,4,6,7,8-HxCDF	60851-34-5	0.1	34.9	245	3.49	24.5
1,2,3,7,8,9-HxCDF	72918-21-9	0.1	31.1	178	3.11	17.8
1,2,3,4,6,7,8-HpCDF	67562-39-4	0.01	3140	17 700	31.4	177
1,2,3,4,7,8,9-HpCDF	55673-89-7	0.01	681	3150	6.81	31.5
OCDF	39001-02-0	0.0003	54 400	283 000	16.32	84.9
Sum					*161*	*637*

[a] TCDD-equivalents are derived from EPA (2010b)
PCDDs are: tetra- (TCDD), penta- (PeCDD), hexa- (HxCDD), hepta- (HpCDD), and octachlorodibenzo-*para*-dioxin (OCDD)
PCDFs are: tetra- (TCDF), penta- (PeCDF), hexa- (HxCDF), hepta- (HpCDF), and octachlorodibenzofuran (OCDF)
TEF, toxic equivalency factor
Courtesy of Annemiek van der Zande. Adapted from: https://www.unece.org/fileadmin/DAM/env/documents/2013/air/PCP.pdf

purification process to remove the contaminants that were created during the manufacture of pentachlorophenol (EPA, 2010a).

1.2.2 Production volume

The worldwide production of pentachlorophenol in 1981 was estimated to be 90 000 tonnes per year (United Nations, 2010). No more recent global information was available to the Working Group.

Production volume in the USA was 45 million pounds [~20 400 tonnes] in 1983 and decreased to 24 million pounds [~10 900 tonnes] in 1987 (ATSDR, 2001). The production volume in the USA was 9100 tonnes in 1996 and 7257 tonnes in 2009 (United Nations, 2010). In 2010, one company was still manufacturing pentachlorophenol at three facilities, located in Alabama and Kansas, USA, and in Mexico (United Nations, 2010).

Production volume for pentachlorophenol in Canada for 1981 (last year of production) was reported as 2200 tonnes. Canada imported 472 tonnes from the USA and Mexico in 2007 (CAREX Canada, 2009).

There is no known current European production of pentachlorophenol since it ceased in 1992 in most countries (OSPAR, 2004). Before then, production occurred in Poland, Germany, the Netherlands, Switzerland, the United

Kingdom, Spain, and France. Spain stopped production in 2003 (United Nations, 2010).

China still produces pentachlorophenol with an annual production volume of 5000 tons [~4536 tonnes] reported in 2010 (United Nations, 2010).

1.2.3 Use

Pentachlorophenol was first introduced for use as wood preservative in the 1930s, and this use remains by far the major application (United Nations, 2010). The salt, sodium pentachlorophenate (Na-PCP) (Chemical Abstracts Service (CAS) No. 131-52-2), was used for similar purposes as pentachlorophenol and readily degrades to pentachlorophenol. The ester, pentachlorophenyl laurate (CAS No. 3772-94-9), was used in textiles. The environmental behaviour of all three substances is quite similar (United Nations, 2010).

In the USA, pentachlorophenol was widely used as an herbicide, algicide, defoliant, wood preservative, germicide, fungicide, and molluscicide, and could be found in ropes, paints, adhesives, canvas, leather, insulation, and brick walls (NTP, 1999; EPA, 2010a). The common use of chlorophenols including pentachlorophenol in tanneries was reported in Tuscany, Italy, and in Sweden, from the early 1950s to the late 1980s (Seniori-Costantini et al., 1989; Mikoczy et al., 1994; Mikoczy & Hagmar, 2005). Pentachlorophenol was used as a molluscicide for fish-pond cleaning for schistosomiasis vector control in China (Zheng et al., 2012). Pentachlorophenol was also used for the production of pentachlorophenol laurate, used in textiles and other fabrics (United Nations, 2010).

In 1984 in the USA, indoor applications of pentachlorophenol were prohibited (NTP, 1999; EPA, 2010a). In 1986 in the USA, approximately 97% of pentachlorophenol usage was as a wood preservative, 1% as a general herbicide, and the remainder for miscellaneous smaller applications (IARC, 1991). Pentachlorophenol is also no longer contained in wood-preserving solutions or insecticides and herbicides available for home and garden use, because it is a restricted-use pesticide (ATSDR, 2001). Currently, application of pentachlorophenol (and its sodium salt) is limited to industrial areas (e.g. utility poles, cross arms, railroad cross ties, wooden pilings, fence posts, and lumber/timbers for construction) in Canada and the USA; products containing pentachlorophenol remain registered for heavy-duty wood preservation, predominantly to treat utility poles and cross arms (CAREX Canada, 2009; EPA, 2010a).

The marketing and use of pentachlorophenol and its compounds was prohibited in the European Union in 1994, except for the treatment of wood, impregnation of fibres and heavy-duty textiles not intended for clothing, as an ingredient in chemical synthesis and, under individual authorizations, treatment in situ of buildings of cultural or historic interest (OSPAR, 2004; INERIS, 2011). In 2001 in Europe (mainly France, Portugal, and Spain), pentachlorophenol and its derivatives, Na-PCP and pentachlorophenyl laurate, were used to control sap stain in green lumber. It was also used on millwork to prevent the growth of mould and fungi, and as a preservative for waterproof materials (i.e. tarpaulins) that are used in outdoor applications (IARC, 1999; OSPAR, 2004). In the European Union, pentachlorophenol was no longer used for wood preservation by 2009 (United Nations, 2010).

Current use of pentachlorophenol for wood preservation is mainly in North America (United Nations, 2010). Several companies were registered as manufacturing pentachlorophenol in 2016: USA (10 companies), Mexico (2), Germany (2), Canada (1), Hong Kong Special Administrative Region (1), South Africa (1), Switzerland (2), India (1), the United Kingdom (3), Israel (1), the Netherlands (1), China (1), and Japan (1) (Chem Sources, 2016).

1.3 Analytical methods

Analytical methods for most biological and environmental media mostly rely on gas chromatography–mass spectrometry or high-performance liquid chromatography–ultraviolet methods, which are described in detailed elsewhere (IARC, 1991; ATSDR, 2001; WHO, 2003; INERIS, 2011).

1.4 Occurrence and exposure

1.4.1 Occupational exposure

Occupational exposure to pentachlorophenol may occur during the manufacture of pentachlorophenol and formulations containing pentachlorophenol (as main ingredient and as contaminant), during mixing or spraying of pentachlorophenol-containing formulations for agricultural use, during treatment of wood products with pentachlorophenol-containing formulations, or during handling of or contact with the treated wood products. Pentachlorophenol exposure may also occur in workers employed in waste incineration, during treatment of materials such as textiles, leathers, or pelts, or in handling the treated materials (Karci, 2014). Dermal contact with pentachlorophenol formulations and treated products is expected to be a main exposure route.

(a) Air

In a wood-treatment plant in the USA that used pentachlorophenol, average air concentrations ranged from 263 to 1888 ng/m^3 (Wyllie et al., 1975). Area air concentrations for two workers in United States wood-treatment plants involved in brush application of pentachlorophenol in enclosed spaces had mean air concentrations of 230–430 µg/m^3 (Casarett et al., 1969). For lumber mill workers exposed to pentachlorophenol-containing wood preservatives, mean tetrachlorophenol air concentrations ranged from 31 to 498 ppb [317–5000 µg/m^3]; pentachlorophenol concentrations were below the detection limit of 0.5 µg/m^3. Canadian sawmill workers exposed to chlorophenol wood preservatives had personal air pentachlorophenol concentrations of 5–6 ppb [54.5–65.4 µg/m^3] (Embree et al., 1984). In a Finnish sawmill that used 2,3,4,6-tetrachlorophenol containing 10–20% 2,4,6-trichlorophenol and 5% pentachlorophenol, mean chlorophenol air concentrations (measured as the sum of tetrachlorophenol and pentachlorophenol) were highest in the vicinity of machine stacking (75 µg/m^3), preparation of treatment solution (66 µg/m^3), indoor vat-dipping (64 µg/m^3), and trough dipping (55 µg/m^3) (Kauppinen & Lindroos, 1985).

Workers involved in the production of pentachlorophenol at one plant in the USA in the 1980s had an overall mean pentachlorophenol concentration in personal air samples of 1.26 mg/m^3 (Marlow, 1986). These workers also experienced air exposure to hexachlorobenzene (range, < 0.0003 to 0.015 mg/m^3), HpCDD (mean, 0.038 µg/m^3), and OCDD (mean, 0.336 µg/m^3) (Marlow, 1986). At a pentachlorophenol-production plant in Germany, 10 of 67 area air samples in the production area exceeded 0.5 mg/m^3 and 18 were less than 0.1 mg/m^3 (Bauchinger et al., 1982). In the area of the same facility where Na-PCP was produced, 8 of 55 area air measurements exceeded 0.5 mg/m^3, and 7 were less than 0.1 mg/m^3. In another pentachlorophenol-production facility in Germany, air measurements of pentachlorophenol ranged from 1.2 to 180 µg/m^3 (Ziemsen et al., 1987).

(b) Biological markers and intake

Pentachlorophenol has been measured in the urine, and occasionally blood, of agricultural workers, wood-processing workers, electrical utility workers, hazardous and municipal waste incinerator workers, harbour workers involved in river dredging, sawmill workers, and workers involved in treating wood products (Table 1.2). Urinary and blood pentachlorophenol

concentrations were generally highest in workers directly involved in treating wood or lumber with pentachlorophenol-containing formulations or who had direct contact with the treated product, with mean urinary and blood concentrations often reported to be > 100 µg/L. Urinary pentachlorophenol concentrations in hazardous and municipal waste incinerator workers were similar to those in unexposed workers. No information on pentachlorophenol concentrations in textile or leather workers was available to the Working Group.

Pentachlorophenol-exposed workers were often monitored for serum concentrations of PCDDs and PCDFs, which are impurities in the pentachlorophenol. In these studies, concentrations of some, but not all congeners, were higher in pentachlorophenol-exposed workers than in unexposed workers. Workers in a Michigan plant that manufactured 2,4,5-trichlorophenol and pentachlorophenol, and formulated chlorophenol-based products had serum concentrations of several dioxin, furan, and PCB congeners that increased with increasing years of employment in a 2,4,5-trichlorophenol- or pentachlorophenol-exposed job (Collins et al., 2007, 2008; Burns et al., 2008). Mean concentrations of TCDD in the pentachlorophenol-only, 2,4,5-trichlorophenol-only, and the community reference group were 8.0, 15.9, and 3.3 pg/g lipid, respectively, and mean WHO 2005-TEQs (Van den Berg et al., 2006) were 56.7, 51.3, and 33.0 toxic equivalency (TEQ), respectively (Collins et al., 2008). 2,4,5-Trichlorophenol workers have a relatively simple congener profile, consisting primarily of elevated TCDD. In contrast, pentachlorophenol workers have a more complex profile, with significantly higher percentages of the contribution to the TEQ for the following congeners compared with the community reference: 1,2,3,4,7,8-HxCDD (2.6% vs 2.2%), 1,2,3,6,7,8-HxCDD (26.3% vs 20.5%), 1,2,3,7,8,9-HxCDD (3.6% vs 2.3%), 1,2,3,4,6,7,8-HpCDD (3.3% vs 2.0%), and OCDD (0.4% vs 0.1%) percentages (Collins et al., 2008). No significant difference was observed between pentachlorophenol workers and the community referents for TCDD (12.2% vs 15.0%).

A consistent congener profile was seen in former sawmill workers in New Zealand after exposure to pentachlorophenol-based anti-sapstain fungicides or to commercial-grade pentachlorophenol (McLean et al., 2009). The highest mean serum concentrations were observed for OCDD (309.25 pg/g lipid). The mean concentration of TCDD in exposed workers was similar to never-exposed workers (1.88 vs 1.48 pg/g lipid, respectively). The serum dioxin levels of these workers remained elevated 20 years after they had been exposed compared with never-exposed workers, with mean WHO2005-TEQs of 13.67 and 9.56, respectively. Their serum dioxin concentrations increased with both employment duration and estimated exposure intensity, with a mean TEQ of 14.1 in those with more than 10 years of exposure to pentachlorophenol.

Former, retired workers at a plant manufacturing pentachlorophenol, and nearby residents in Taiwan, China, had levels of 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and total TEQ levels that were significantly higher than those of the reference groups. Their 2,3,7,8-TCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HxCDF, and OCDF levels were significantly lower than those of the reference groups. Mean WHO98-TEQs (Van den Berg et al., 1998) were 95.8–109.6 TEQ/g lipid in the retired workers who had been exposed to Na-PCP, and 22.9 TEQ/g lipid in the general population (Chang et al., 2012).

Serum PCDD/F concentrations of hazardous waste incinerator workers did not show significant differences between workplace groups after 1, 3, 8, and 12 years of operation compared with baseline concentrations or non-occupationally exposed subjects (Schuhmacher et al., 2002; Agramunt et al., 2003; Mari et al., 2009, 2013).

Table 1.2 Concentrations of pentachlorophenol in biological samples from occupationally exposed workers

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Blood	USA, NR	Wood treatment	Treatment of lumber, furniture, and other wood products	18	Mean, 2190–5140 µg/L	190–14 000 µg/L	Begley et al. (1977)
Blood	Portugal, NR	Wood transformation unit	Mean, 6 years of exposure	11	Mean, 2273 µg/L	133–6884 µg/L	Ferreira et al. (1997)
Plasma	Italy, NR	Wooden strip board factory	Applied PCP by brush to wooden boards	14	Mean, 288.7 µg/L; median, 67 µg/L	2–1442 µg/L	Colosio et al. (1993)
Plasma	Italy, NR	Wooden strip board factory	Handled treated wood and other indirect exposure	18	Mean, 144.7 µg/L; median, 130 µg/L	14–350 µg/L	Colosio et al. (1993)
Plasma	Italy, NR	Wooden strip board factory	Unexposed	37	Mean, 8.9 µg/L; Median, 5.6 µg/L	0–76 µg/L	Colosio et al. (1993)
Plasma	United Kingdom, 1982–1983	PCP manufacturing	Formulation of PCP-containing fluids	29	Mean, 1.3 mmol/L [3.46×10^5 µg/L]	0.4–4.8 mmol/L [1.06×10^5–12.8×10^5 µg/L]	Jones et al. (1986)
Plasma	United Kingdom, 1982–1983	Wood treatment	Remedial sprayers working on house timber treatment	108	Mean, 6.0 mmol/L [16×10^5 µg/L]	0.2–29 mmol/L [0.5×10^5–77.2×10^5 µg/L]	Jones et al. (1986)
Plasma	United Kingdom, 1982–1983	Wood treatment	Handled treated wood in fabrication of pallets, roof trusses	68	Mean, 4.8 mmol/L [12.8×10^5 µg/L]	0.3–45 mmol/L [0.8×10^5 to 120×10^5 µg/L]	Jones et al. (1986)
Plasma	United Kingdom, 1982–1983	Wood treatment	Timber-treatment operators (unexposed)	9	Mean, 0.7 mmol/L [1.8×10^5 µg/L]	0.3–1.8 mmol/L [0.8×10^5 to 4.8×10^5 µg/L]	Jones et al. (1986)
Plasma	United Kingdom, 1982–1983	Wood treatment	Furniture joiners	61	Mean, 0.2 mmol/L [0.5×10^5 µg/L]	0.1–0.6 mmol/L [0.3×10^5 to 1.6×10^5 µg/L]	Jones et al. (1986)
Serum	Canada, NR	Sawmill workers	Tasks involving dermal contact with treated lumber	5	Mean, 714 ± 383 µg/L	NR	Embree et al. (1984)
Serum	Canada, NR	Sawmill workers	Close proximity to treated lumber, but no dermal contact	5	Mean, 241 ± 232 µg/L	NR	Embree et al. (1984)
Serum	USA, 1967–1973	Agricultural workers and wood processing workers	Farmers or pest control operators	280	Mean, 250 µg/L	< 10–8400 µg/L	Klemmer et al. (1980)

Table 1.2 (continued)

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Serum	USA, 1967–1973	Agricultural workers and wood processing workers	Workers involved in dipping wood products in a 5% PCP mixture	22	Mean, 3780 µg/L	150–17 400 µg/L	Klemmer et al. (1980)
Serum	USA, 1967–1973	Agricultural workers and wood processing workers	Workers involved in pressure treatment of wood products	24	Mean, 1720 µg/L	20–7700 µg/L	Klemmer et al. (1980)
Serum	USA, 1967–1973	Agricultural workers and wood processing workers	Unexposed	32	Mean, 320 µg/L	20–7200 µg/L	Klemmer et al. (1980)
Serum	Finland, NR	Sawmill workers	Moving lumber that was dipped in chlorophenol solution containing 23% TCP, 74% tetrachlorophenol, 3% PCP	7	Mean, (p.m.) 0.85 µmol/L [226 µg/L]	NR	Pekari et al. (1991)
Serum	USA, 1972	Wood treatment plant	Plant workers, including managers, loaders, labourers, pressure treaters	6	Monthly mean, 769.1–2215.8 µg/L	NR	Wyllie et al. (1975)
Serum	USA, 1972	Wood treatment plant	Chemists	1	NA	38–68 µg/L	Wyllie et al. (1975)
Urine	Germany, NR	Municipal waste incinerator	Municipal waste workers	53	Mean, 2.60 µg/g creatinine	0.43–8.88 µg/g creatinine	Angerer et al. (1992)
Urine	Germany, NR	Municipal waste incinerator	Unexposed	248	Mean, 3.21 µg/g creatinine	< 0.8–67.79 µg/g creatinine	Angerer et al. (1992)
Urine	Germany, 1990–1993	PCP-exposed construction painters; bricklayers	Painters, 40% reported exposure to wood preservatives (assumedly PCP free) at least once per week	189	Median, 2.4 µg/g creatinine	< 0.2–52 µg/g creatinine	Bader et al. (2007)
Urine	Germany, 1990–1993	PCP-exposed construction painters; bricklayers	Bricklayers, < 10% reported contact with wood preservatives once per month	148	Median, 1.8 µg/g creatinine	< 0.2–25 µg/g creatinine	Bader et al. (2007)

Table 1.2 (continued)

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Urine	USA, NR	Wood treatment	Treatment of lumber, furniture, and other wood products	18	Mean, 590–1360 µg/L	30–3600 µg/L	Begley et al. (1977)
Urine	Spain, 1999–2011	Hazardous waste incinerator	Plant workers, including incinerator operators; boiler maintenance, furnace maintenance, and control panel workers; and waste-gas-washing operators	16	Annual means, 0.1–1.9 µg/g creatinine	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Urine	Spain, 1999–2011	Hazardous waste incinerator	Laboratory workers	6	Annual means, 0.1–2.7 µg/g creatinine	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Urine	Spain, 1999–2011	Hazardous waste incinerator	Administrative/ management workers	5	Annual means, 0.4–2.0 µg/g creatinine	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Urine	USA, NR	Wood treating plant	PCP application to the lumber – vat dipping	11	Mean, 2600 µg/L	NR	Casarett et al. (1969)
Urine	USA, NR	Wood treating plant	PCP application to the lumber – tank	11	Mean, 1600 µg/L	NR	Casarett et al. (1969)
Urine	Italy, NR	Wooden strip board factory	Applied PCP by brush to wooden boards	14	Mean, 127.3 µg/L; median, 69.5 µg/L	2–324 µg/L	Colosio et al. (1993)
Urine	Italy, NR	Wooden strip board factory	Handled treated wood and other indirect exposure	18	Mean, 154 µg/L; median, 125 µg/L	31–363 µg/L	Colosio et al. (1993)
Urine	Italy, NR	Wooden strip board factory	Unexposed	37	Mean, 4.7 µg/L; median, 3.7 µg/L	0–27 µg/L	Colosio et al. (1993)
Urine	Spain, 1999–2000	Hazardous waste incinerator	Plant workers	19	Annual means 0.5–1.9 µg/g creatinine	NR	Domingo et al. (2001); Schuhmacher et al. (2002)
Urine	Spain, 1999–2000	Hazardous waste incinerator	Laboratory workers	3	Annual means, 0.14–1.9 µg/g creatinine	NR	Domingo et al. (2001); Schuhmacher et al. (2002)

Table 1.2 (continued)

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Urine	Spain, 1999–2000	Hazardous waste incinerator	Administrative workers	1	Annual means, 0.51–1.7 µg/g creatinine	NR	Domingo et al. (2001); Schuhmacher et al. (2002)
Urine	Spain, 1999–2000	Hazardous waste incinerator	Pre-employment baseline	28	0.45 µg/g creatinine	0.03–1.40 µg/g creatinine	Domingo et al. (2001); Schuhmacher et al. (2002)
Urine	Canada, NR	Sawmill workers	Close proximity to treated lumber, but no dermal contact	3	Mean, 45 ± 15 µg/L	NR	Embree et al. (1984)
Urine	Canada, NR	Sawmill workers	Dermal contact with treated lumber	3	Mean, 105 ± 18 µg/L	NR	Embree et al. (1984)
Urine	Portugal, NR	Wood transformation unit	Mean, 6 years of exposure	11	Mean, 1200 µg/L	70–5566 µg/L	Ferreira et al. (1997)
Urine	USA, NR	Wood treatment	Wood treaters	88	Mean, 174 ± 342 µg/L	NR	Gilbert et al. (1990)
Urine	USA, NR	Wood treatment	Unexposed	61	Mean, 35 ± 53 µg/L	NR	Gilbert et al. (1990)
Urine	United Kingdom, NR	PCP manufacturing	Formulation of PCP-containing fluids	26	Mean 39.6 nmol/mmol creatinine	7.4–300 nmol/mmol creatinine	Jones et al. (1986)
Urine	United Kingdom, NR	Wood treatment	Remedial sprayers working on house timber treatment	112	Mean, 274 nmol/mmol creatinine	11–1260 nmol/mmol creatinine	Jones et al. (1986)
Urine	United Kingdom, NR	Wood treatment	Handled treated wood in fabrication of pallets, roof trusses	54	Mean, 74 nmol/mmol creatinine	5–655 nmol/mmol creatinine	Jones et al. (1986)
Urine	United Kingdom, NR	Wood treatment	Timber-treatment operators (unexposed)	9	Mean, 35.5 nmol/mmol creatinine	10.3–151.4 nmol/mmol creatinine	Jones et al. (1986)
Urine	USA, NR	Lumber mill	Unexposed workers from a lumber mill engaged in wood treatment with PCP	114	Monthly geometric mean, 32.2 µg/L	3–137 µg/L	Kalman (1984)
Urine	USA, 1981–1982	Lumber mill	Sapstain preparation and treatment	88	Monthly means, 69–103 µg/L	4–636 µg/L	Kleinman et al. (1986)
Urine	USA, 1981–1982	Lumber mill	Unexposed	38	Monthly means, 29–39 µg/L	NR	Kleinman et al. (1986)

Table 1.2 (continued)

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Urine	USA, 1967–1973	Agriculture and wood processing	Farmers or pest control operators	210	Mean, 10 µg/L	< 10- 400 µg/L	Klemmer et al. (1980)
Urine	USA, 1967–1973	Agriculture and wood processing	Workers involved in dipping wood products in a 5% PCP mixture	18	Mean, 950 µg/L	< 10–7800 µg/L	Klemmer et al. (1980)
Urine	USA, 1967–1973	Agriculture and wood processing	Workers involved in pressure treatment of wood products	23	Mean, 270 µg/L	< 10–2400 µg/L	Klemmer et al. (1980)
Urine	USA, 1967–1973	Agriculture and wood processing	Unexposed	32	Mean, 30 µg/L	< 10–1000 µg/L	Klemmer et al. (1980)
Urine	Finland, NR	Sawmill workers	Contact with chlorophenols	35	NR	< LOD–15.9 µg/g creatinine	Kontsas et al. (1995)
Urine	Finland, NR	Sawmill workers	Unexposed	17	NR	< LOD–13.7 µg/g	Kontsas et al. (1995)
Urine	Finland, 1980–1981	Sawmill workers	Primarily dermal contact with chlorophenols	112	Median, 7.8 µmol/L [2 × 10^3 µg/L]	0.1–211 µmol/L [26–56 × 10^3 µg/L]	Lindroos et al. (1987)
Urine	Finland, 1980–1981	Sawmill workers	Skin and respiratory exposure to chlorophenols	84	Median, 1.4 µmol/L [373 µg/L]	0.1–48 µmol/L [26–13 × 10^3 µg/L]	Lindroos et al. (1987)
Urine	Finland, 1980–1981	Sawmill workers	Primarily respiratory exposure to chlorophenols	34	Median, 0.9 µmol/L [240 µg/L]	0.1–13 µmol/L [26–3.5 × 10^3 µg/L]	Lindroos et al. (1987)
Urine	Finland, NR	Sawmill workers	Moving lumber that was dipped in chlorophenol solution containing 23% trichlorophenol, 74% tetrachlorophenol, 3% PCP	7	Mean, 0.34 µmol/L [90 µg/L]	0.2–0.9 µmol/L [53–240 µg/L]	Pekari et al. (1991)
Urine	Germany, 1997	Harbor workers	River dredging	83	Median, 1.4 µg/g creatinine	0.1–18.1 µg/g creatinine	Radon et al. (2004)
Urine	Germany, 1997	Harbor workers	Office workers	80	Median, 1.0 µg/g creatinine	0.1–8.1 µg/g creatinine	Radon et al. (2004)
Urine	Canada, 1989	Electrical utility	Linemen	23	Geometric mean, 29.6 µg/g creatinine	NR	Thind et al. (1991)
Urine	Canada, 1989	Electrical utility	Administrative workers	5	Geometric mean, 10.2 µg/g creatinine	NR	Thind et al. (1991)
Urine	USA, 1972	Wood treatment plant	Plant workers, including managers, loaders, labourers, pressure treater	6	Monthly mean, 84–312 µg/L	NR	Wyllie et al. (1975)

Table 1.2 (continued)

Sample matrix	Country, year	Occupation	Work tasks, type of worker, or specific exposure	No. of workers	Exposure		Reference
					Level	Range	
Urine	USA,1972	Wood treatment plant	Chemists	1	NA	2.6–4.3 µg/L	Wyllie et al. (1975)
Urine	Canada, 1987	Sawmills	67 sawmill jobs	225	Mean, 99 µg/L; geometric mean, 43 µg/L	2–989 µg/L GSD, 3.6	Teschke et al. (1989)
Urine	Germany, NR	PCP production facility	PCP production area	8	2380 ± 1910 µg/L	NR	Bauchinger et al. (1982)
Urine	Germany, NR	PCP production facility	Na-PCP sacking area	14	840 ± 650 µg/L	NR	Bauchinger et al. (1982)
Blood	Germany, NR	PCP production facility	PCP production area	8	4730 ± 3410 µg/L	NR	Bauchinger et al. (1982)
Blood	Germany, NR	PCP production facility	Na-PCP sacking area	14	2230 ± 1510 µg/L	NR	Bauchinger et al. (1982)
Blood	Germany, NR	PCP production facility	Transported, weighed raw materials for PCP production	9	Mean, 58 µg/L	23–116 µg/L	Ziemsen et al. (1987)
Blood	Germany, NR	PCP production facility	Handled finished PCP solutions	11	Mean, 330 µg/L	59–775 µg/L	Ziemsen et al. (1987)

GSD, geometric standard deviation; LOD, limit of detection; Na-PCP, sodium pentachlorophenate; NA, not applicable; NR, not reported; PCP, pentachlorophenol

1.4.2 Community exposure

Pentachlorophenol is a persistent organic pollutant (EPA, 2008; United Nations, 2010). Community exposure may continue long after the cessation of pentachlorophenol use. The general population may be exposed from proximity to pentachlorophenol-treated wood products, and from food, land, air, and water contaminated with pentachlorophenol. Exposure may also occur from dermal contact with leathers and textiles treated with pentachlorophenol, such as leather car seats in hot weather (Favaro et al., 2008). The largest sources of pentachlorophenol emissions are wood preservation and hazardous waste handling of pentachlorophenol-treated wood products. In the USA in 2008, an estimated 172 kg of pentachlorophenol was released to the air, 513 kg to the water, and 1865 kg was placed in landfills (United Nations, 2010).

Dietary exposure to pentachlorophenol has been estimated to account for nearly all non-occupational human exposure because pentachlorophenol partitions mainly into the soil (96.5%) and accumulates in the food chain, especially in fruits, vegetables, and grains (Hattemer-Frey & Travis 1989; Coad & Newhook, 1992); however, a more recent study suggested that inhalation exposure may account for 43–54% of pentachlorophenol exposure in children aged 3 years (Wilson et al., 2010).

(a) Water

Pentachlorophenol has low water solubility (14 mg/L at 25 °C) (Choudhary et al., 2013). Tap and well water concentrations of pentachlorophenol in China were on average 0.01–0.12 µg/L, with a maximum of 0.77 µg/L (Zheng et al., 2012). Drinking-water samples in Poland had mean pentachlorophenol concentrations ranging from 0.70 to 3.27 µg/L, and river-water samples had pentachlorophenol concentrations ranging from 0.03 to 640 µg/L (Michałowicz et al., 2011), and examples of private drinking-water sources being contaminated from new installations of pentachlorophenol-treated utility poles have been reported (Karlsson et al., 2013). In surface water samples in China, average pentachlorophenol concentrations ranged from not detected to 7.4 µg/L, varying by water type, location, year, and use of pentachlorophenol (Zheng et al., 2012). Freshwater and marine water samples from Belgium, France, Germany, the Netherlands, and the United Kingdom contained pentachlorophenol at concentrations that typically ranged from 0.01 to 0.17 µg/L, with maximum average concentrations up to 1.5 µg/L (Muir & Eduljee, 1999).

(b) Sediment and soil

In China, mean soil and sediment concentrations of pentachlorophenol were < 10 µg/kg dry weight (dw) for 29 of the 37 locations tested, between 10–63 µg/kg dw for 7 locations, and the remaining location had a mean concentration of 15 850 µg/kg dw (Zheng et al., 2012). Pentachlorophenol concentrations in the soil of rice fields in Japan decreased by half from the 1980s (0.72–41 ng/g dw; mean, 10 ng/g) to the 2000s (not detected to 21 ng/g dw; mean, 4.9 ng/g dw); however, concentrations of PCDD/Fs remained steady (Kobayashi et al., 2008). The observed PCDD/F congeners were consistent with impurities in pentachlorophenol and in 2,4,5-trichlorophenyl-4′-nitrophenylether (chlornitrofen). In Belgium, France, Germany, the Netherlands, and the United Kingdom, pentachlorophenol concentrations in freshwater sediments fell from levels of 200 µg/kg in 1991 to 15 µg/kg in 1997 (Muir & Eduljee, 1999).

(c) Air

Detectable concentrations of pentachlorophenol were present in 29 of 30 air samples collected in Canada, with mean air concentrations of 0.23 ng/m^3 in Waskesiu, 0.30 ng/m^3 in Regina, and 1.53 ng/m^3 in Yellowknife (Cessna et al., 1997). Pentachlorophenol was present in

7 of 11 air samples from two Canadian cities, with concentrations ranging from 0.2 to 6.8 ng/m^3 (Waite et al., 1998). In the same study, pentachlorophenol concentrations in the air of rural sites ranged from 0.1 to 1.5 ng/m^3 and were detected less frequently. Pentachlorophenol was also found in all air samples collected adjacent to a utility-pole storage site with concentrations ranging from 0.7 to 1233 ng/m^3 (Waite et al., 1998). Pentachlorophenol was detected in the air in all seven precipitation samples collected during rain events in Portland, Oregon, USA, in 1984, with a mean concentration of 54 ng/L; pentachlorophenol was not detected in the concurrently collected air samples (Leuenberger et al., 1985).

(d) Residential exposure

Pentachlorophenol was detected in 94% of household dust samples taken from rooms in which children spent the most time, in California, USA, in 2001–2006, with arithmetic and geometric mean concentrations of pentachlorophenol of 199 ng/g and 77 ng/g, respectively (Ward et al., 2009).

Pentachlorophenol was detected in more than 50% of the indoor air (median, 1.2–2.1 ng/m^3), outdoor air (median, 0.22–0.91 ng/m^3), and dust samples (median, 35–81 ng/g) from the homes and day-care facilities in North Carolina and Ohio, USA, in 2000–2001 (Wilson et al., 2007). Household dust concentrations in homes in Germany had low concentrations of pentachlorophenol, with a 95th percentile of 2.8 mg/kg in 1990/91 and 1.2 mg/kg in 1997/98 (Heudorf et al., 2000). The median pentachlorophenol concentration in household dust samples in homes in Germany with wooden panelling to which wood preservatives had been applied earlier was 5.0 ppm, with 72% of samples less than 25 ppm and 5% greater than 100 ppm (Meissner & Schweinsberg, 1996). Pentachlorophenol was detected in 96% of 861 vacuum-dust samples from German homes, with a median concentration of 0.3 µg/g (range, < 0.03–30.9 µg/g) (Seifert et al., 2000).

(e) Food

Pentachlorophenol was used for fish-pond cleaning in China for control of the schistosomiasis vector via molluscicide activity (Zheng et al., 2012). A review found low concentrations of pentachlorophenol in aquatic organisms, ranging from < 0.02 to 172 µg/kg wet weight (ww) (Zheng et al., 2012). Seafood samples contained pentachlorophenol at concentrations ranging from 37.7 ng/g ww in fish to 146 ng/g ww in crab (Basheer et al., 2004). Common carp contained pentachlorophenol at concentrations of < 0.5–61 µg/kg ww (Ge et al., 2007).

Pentachlorophenol was detected in fewer than 21% of the solid food samples of 257 children in the USA (Wilson et al., 2007). The proportion of pork samples containing pentachlorophenol at > 0.1 ppm dropped from 32% in 1981–82 to 6.6% in 1987–88 (MacNeil et al., 1990). For animals exposed to pentachlorophenol in wood shavings, serum pentachlorophenol concentrations ranged from 0.08 to 5.26 ppm in bovines, and liver pentachlorophenol concentrations ranged from 0.02 to 2.16 ppm in chickens (MacNeil et al., 1990). Pentachlorophenol concentrations in eggs of hens reared on pentachlorophenol-contaminated wood shavings was 500 ng/g whole weight (Brambilla et al., 2009). Egg concentrations dropped after pentachlorophenol-contaminated shavings were removed. Pentachlorophenol concentrations in the threshing floor material of henhouses in Poland were 11 ± 2.8 µg/kg (Piskorska-Pliszczynska et al., 2016).

Pentachlorophenol concentrations ranging from 0.054 to 0.11 µg/g were detected in 5 of 12 recycled paper/paperboard food packaging samples, but none was detected in 16 virgin paper products (Ozaki et al., 2004).

Chlorophenols have been used during the production of bark cork, and may inadvertently

form from the use of hypochlorite solutions to clean cork stoppers and wooden barrels (Ozhan et al., 2009). Pentachlorophenol has been measured in oak barrels that are used to age wine and other spirits, with concentrations ranging from 5 to 120 µg/g (Pizarro et al., 2006). Pentachlorophenol concentrations in red wine varied from 12 to 123 ng/L and were correlated with trichlorophenol concentrations in the cork (Ozhan et al., 2009).

In northern Bavaria, Germany, the mean concentration of pentachlorophenol in the diet was 13.9 ± 8.0 µg/kg, with a range of 2.7 to 27.6 µg/kg, excluding one high value of 516 µg/kg (Geyer et al., 1987).

(f) Biological markers

Pentachlorophenol has been measured in the urine and blood of populations of varying ages and geographical locations over the past several decades (Table 1.3). The proportion of samples with detectable concentrations of pentachlorophenol ranged from ≈50 to 100%. Reported mean and median urinary concentrations ranged from 1 to 14 µg/g creatinine, and from < 1 to 25 µg/L, respectively. In a literature review of studies from China, Zheng et al. (2012) found urinary pentachlorophenol concentrations ranging from < 0.1 to 2523 µg/L, with significantly higher concentrations in areas where schistosomiasis is epidemic and where Na-PCP was used as a biocide than in control areas (mean, 111 vs 0.35 µg/L, respectively). In the epidemic areas, the mean concentration of pentachlorophenol in body fluids was 253 µg/L (Zheng et al., 2012).

In the USA, mean serum pentachlorophenol concentrations were higher in people living in pentachlorophenol-treated homes than in conventional homes (not treated with pentachlorophenol) (420 vs 40 ppb [µg/L]) (Cline et al., 1989).

In Sweden, breast milk was found to have median concentrations of pentachlorophenol of 20 pg/g (range, 10–570 pg/g) (Guvenius et al., 2003). Concentrations of pentachlorophenol in breast milk in China ranged from 0.32 to 13 ng/g (mean, 2.2 ng/g) (Hong et al., 2005) and from 2 to 3 µg/L (Zheng et al., 2012).

In 17 males aged 16–87 years in northern Bavaria, Germany, mean concentrations of pentachlorophenol were 80 µg/kg, 50 µg/kg, 50 µg/kg, 20 µg/kg, 14 µg/kg, 25 µg/L, and 6.9 µg/L, respectively, in liver, kidney, brain, spleen, adipose, blood, and urine (Geyer et al., 1987). Similar concentrations were observed in four females.

In a meta-analysis of data from various geographical regions, pentachlorophenol levels in human blood decreased exponentially between 1978 and 2008 (Zheng et al., 2011). Worldwide blood concentrations were predicted to be 2.5–7 µg/L between 1995 and 2003, and 39–90 µg/L between 1967 and 1979. Highest body burdens of pentachlorophenol in the 1980s were observed in North America (geometric mean, 123.26 µg/L), but after 1995 pentachlorophenol body burdens in North America and Europe were similar (mean, 1.15–3.14 µg/L). The geometric mean for Sweden during 1976–2001 was only 5.34 µg/kg, reduced by 80% compared with 21.7 µg/kg in the 1970s and 1980s. Mean pentachlorophenol concentrations in breast milk and adipose samples were 14 µg/kg and 11 µg/kg, respectively. The rate of decline in pentachlorophenol concentrations was slower in blood than in urine, with a weak decreasing trend in lipid samples (Zheng et al., 2011).

The long-term average daily intake of pentachlorophenol in the 1980s was estimated to be 16 µg/day (Hattemer-Frey & Travis, 1989). Estimated total daily pentachlorophenol exposure in the Canadian general population was estimated to be 99, 105, 50, and 28 ng/kg body weight (bw) per day in infants, toddlers, children, and adults, respectively (Coad & Newhook, 1992). In the same study, aboriginal subsistence fishermen were estimated to have a daily pentachlorophenol intake of 58 ng /kg bw per day. The median measured aggregate potential dose was

7–9 ng/kg bw per day in children aged 3 years and younger, and was estimated to be 37–51% from the diet, 43–54% from inhalation, and 6–9% from other sources (Wilson et al., 2010). Estimated pentachlorophenol intake of infants from breast milk ranged from 0.09 to 3.73 mg/infant-year in China (Hong et al., 2005).

1.5. Regulations and guidelines

A list of regulations and guidelines for occupational exposure to pentachlorophenol in air is provided in Table 1.4.

Before 2014, recommended American Conference of Governmental Industrial Hygienists (ACGIH) limits for biological measures of exposure were 2 mg/g creatinine in urine (before last shift of work week) and 5 mg/L in plasma (end of shift) (ATSDR, 2001). In 2014, those recommended limits were removed; ACGIH currently recommends monitoring in urine for occupationally exposed individuals without recommending any particular limit (ACGIH, 2014).

There are additional restrictions and requirements regarding pentachlorophenol in food packaging and additives, transportation, hazardous waste, and releases to the environment in the USA, and some states within the USA impose additional restrictions (ATSDR, 2001).

Pentachlorophenol is also regulated as a potential water contaminant in some regions. For example, in the USA the maximum allowable concentration for pentachlorophenol in drinking-water (bottled or tap water) is 1 µg/L (EPA, 2016). The World Health Organization (WHO) has a provisional guideline value of 9 µg/L in drinking-water (WHO, 2003).

Pentachlorophenol and its salts and esters are listed in Annex A of the Stockholm Convention on Persistent Organic Pollutants, under which parties must take steps to eliminate production and use unless they have registered for an exemption (Stockholm Convention, 2008).

As of 2009, pentachlorophenol may not be placed on the market or used as a substance, or used in a concentration equal to or greater than 0.1% by weight in substances or preparations placed on the market in the European Community (European Commission, 2014). According to the European Union harmonized classification and labelling system, pentachlorophenol is "suspected of causing cancer (Carc. 2)" [H351], "fatal if inhaled (Acute Tox. 2)" [H330], "toxic if swallowed (Acute Tox. 3)" [H301], "toxic in contact with skin (Acute Tox. 3)" [H311], "very toxic to aquatic life (Aquatic Acute 1)" [H400], and "very toxic to aquatic life with long lasting effects (Aquatic Chronic 1)" [H410], and "causes serious eye irritation (Eye Irrit. 2)" [H319], "causes skin irritation (Skin Irrit. 2)" [H315], and "may cause respiratory irritation (STOT SE 3)" [H335] (ECHA, 2016). Before this European Community directive, Austria, Denmark, Finland, Germany, the Netherlands, Norway, Sweden, and Switzerland had more restrictive policies or bans on the use of pentachlorophenol (OSPAR, 2004). France, Ireland, Portugal, Spain, and the United Kingdom were directed to phase out the use of pentachlorophenol in treatment of wood and certain heavy-duty textiles between 2006 and 2009 (European Commission, 2007).

Pentachlorophenol-containing products including treated wood and glue cannot be produced, used or imported into Japan, where pentachlorophenol is listed as a class I specified chemical due to its persistence, potential for bioaccumulation, and toxicity (Ministry of Health, Labour and Welfare, 2016).

The sale and use of pesticides containing pentachlorophenol are restricted to limited commercial uses in the USA (ATSDR, 2001).

Table 1.3 Concentrations of pentachlorophenol in biological samples from the general population

Country, year	Age (years)	Sample matrix	No. of samples	Exposure		Comments	Reference
				Level	Range, % detects		
Japan, 1974	28–79	Adipose tissue	25	Mean, 0.14 ppm	< 0.005–0.57 ppm, 76% detects		Ohe (1979)
Belgium, NR	21–74	Spinal fluid	16	Mean, 0.75 ± 0.49 µg/L	0.24–2.03 µg/L, 100% detects	No correlation with serum PCP levels; 3 subjects had previously used PCP-containing wood preservatives	Jorens et al. (1991)
Sweden, 2000–2001	Mean, 32	Breast milk	15	20 pg/g fresh weight	10–570 pg/g fresh weight, 100% detects		Guvenius et al. (2003)
		Maternal plasma	15	2830 pg/g fresh weight	1360–13 200 pg/g fresh weight, 100% detects		
		Cord blood plasma	15	1960 pg/g fresh weight	820–7580 pg/g fresh weight, 100% detects		
Canada, 1993–96	21–74	Umbilical cord plasma	30	Median, 1670 pg/g wet weight	628–7680 pg/g wet weight, 100% detects		Sandau et al. (2002)
Germany, 1998	0–62	Plasma	623	Mean, 2.4 µg/L; Median, 1.7 µg/L	< LOD–59.3 µg/L		Heudorf et al. (2000)
Norway	48–62	Plasma	281	Mean, 958 ng/L; Median, 711 ng/L	< LOD–7686 ng/L, 94% detects	Women	Rylander et al. (2012)
Nigeria	Adults	Blood	29	NR	< trace–21.3 ppb, [trace–0.23 µg/L] 100% detects		Atuma & Okor (1985)
Spain, 2001–2003	4	Serum	66	Mean, 6.4 µg/L	1.5–35 µg/L, NR	Children in the area of a large factory producing organochlorine solvents	Carrizo et al. (2008)
Spain, 2001–2003	4	Serum	131	Mean, 0.61 µg/L	< 0–4.7 µg/L, NR	Children in a rural environment not exposed to high HCB or PeCB inputs	Carrizo et al. (2008)
USA, 1980–86	NR	Serum	34	Mean, 40 µg/L; median, 40 µg/L	15–75 µg/L, 100% detects	Higher in those living in PCP-treated homes than in conventional homes (means 420 vs 40 ppb [µg/L], respectively)	Cline et al. (1989)
Portugal, NR	Adults	Serum	10	Mean, 15 µg/L	3–17 µg/L, 100% detects		Ferreira et al. (1997)
Spain, 1985	Adults	Serum	50	Mean, 21.9 µg/L	2.5–116.5 µg/L, 100% detects		Gómez-Catalán et al. (1987)

Table 1.3 (continued)

Country, year	Age (years)	Sample matrix	No. of samples	Exposure		Comments	Reference
				Level	Range, % detects		
Belgium, NR	21–74	Serum	16	Mean, 22 ± 16 µg/L	4 to 60 µg/L, 100% detects	Three patients had previously used PCP-containing wood preservatives	Jorens et al. (1991)
Nigeria, NR	Adults	Urine	35	NR	< 0.025–0.23 ppm, [25–230 µg/L] 100% detects		Atuma & Okor (1985)
USA, 1980–86	NR	Urine	143	Mean, 3.4 µg/L; median, 3.0 µg/L	1–17 µg/L, 100% detects		Cline et al. (1989)
Portugal, NR	Adults	Urine	10	Mean, 6 µg/L	1–31 µg/L, 100% detects		Ferreira et al. (1997)
Spain, 1985	Adults	Urine	50	Mean, 25 µg/L	4–136 µg/L, 100% detects	Correlation with tetrachlorophenol, r = 0.883	Gómez-Catalán et al. (1987)
USA, 1976–80	12–74	Urine	6990	Median detected, 6 µg/L	< 2–2670 µg/L, 72% detects		Kutz et al. (1992)
Germany, 1990–1998	25–69	Urine	1895	GM, 2.7 µg/L (1990/92), 1 µg/L (1998)	< 1–13 (95th percentile) µg/L, 82% detects (1990/92), 50% detects (1998)	No difference by presence of wood preservatives at home	Schulz et al. (2007)
Canada, NR	6–87	Urine	69	Mean, 0.75 µg/L; median, 0.5 µg/L	0.05–3.6 µg/L, 94% detects	24-h urine collection, 0.5–20.2 nmol/day	Treble & Thompson (1996)
USA, 1998–2001	Pregnant women	Urine	361	Median, 7.3 µg/g creatinine	10–90th percentile: 1.1–67 µg/g creatinine		Berkowitz et al. (2003)
USA, 1999–2002	Pregnant women	Urine	747	NR	< 0.9–29.5 µg/L, 2–4% detects; NHANES: < 0.5–15.2 µg/L, 10% detects	Agricultural area, California	Castorina et al. (2010)
USA, NR	2–6	Urine	197	Median, 14 µg/g creatinine	> 1 (minimum NR) –330 µg/g creatine, 100% detects		Hill et al. (1989)
USA, 1988–94	20–59	Urine	886	Median, 1.2 µg/g creatinine	< 1–29 µg/g creatinine, 59% detects		Hill et al. (1995)
USA, 2001	Adults	Urine	106	Mean, 0.7 µg/g creatinine; median, 0.4 µg/g creatinine	< 0.2–6.4 µg/g creatinine, 96% detects		Morgan (2015)
Canada, 1993	36–76	Urine	31	Mean, 1.3 µg/L	< 2–3.2 µg/L	Sport fish consumers from three Great Lakes	Anderson et al. (1998)

Table 1.3 (continued)

Country, year	Age (years)	Sample matrix	No. of samples	Exposure		Comments	Reference
				Level	Range, % detects		
Germany, 1990–1992	18–79	Urine	1295	GM, 2.67 µg/L (adults)	95th percentile, 12.8 µg/L		Seifert et al. (2000)
Germany 1990–1992	6–14	Urine	695	GM, 4.15 µg/L	95th percentile, 14.9 µg/L		Seifert et al. (2000)
USA, NR	1.5–5	Urine	254	Mean, 0.61–1.27 µg/L; GM, 0.63, 2.20 µg/L	< 0.1–23.8 µg/L, 94% detect		Wilson et al. (2007)

GM, geometric mean; HCB, hexachlorobenzene; NHANES, National Health and Nutrition Examination Survey; NR, not reported; PCP, pentachlorophenol; PeCB, pentachlorobenzene; TCP, trichlorophenol

Table 1.4 Regulations and guidelines for occupational limits for pentachlorophenol in air

Country or region	Concentration (mg/m³)	Value
Australia	0.5	TWA
Belgium	0.5	TWA
Canada, Ontario	0.5	TWA
Canada, Quebec	0.05	TWA
Denmark	0.05	TWA
Denmark	0.1	STEL
Finland	0.5	TWA
Finland	1.5	15-min STEL
Hungary	0.001	TWA
Ireland	0.5	TWA
Ireland	1.5	15-min STEL
Japan, JSOH	0.5	TWA
New Zealand	0.5	TWA
China	0.3	TWA
Poland	0.5	TWA
Poland	1.5	STEL
Singapore	0.5	TWA
Republic of Korea	0.5	TWA
Spain	0.5	TWA
Sweden	0.5	TWA
Sweden	1.5	15-min STEL
Switzerland	0.05	TWA inhalable aerosol
United Kingdom	0.5	TWA
United Kingdom	1.5	STEL
USA		
ACGIH (TLV)	0.5	TWA
ACGIH[a]	1	STEL
NIOSH (REL)	0.5	10-h TWA
NIOSH (IDLH)	2.5	TWA
OSHA (PEL)	0.5	TWA

[a] Confirmed animal carcinogen with unknown relevance to humans (A3)

ACGIH, American Conference of Governmental Industrial Hygienists; IDLH, immediately dangerous to life or health; IRIS, Integrated Risk Information System; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; STEL, short-term exposure limit; TLV, threshold limit value; TWA, 8-hour time-weighted average (unless otherwise specified)

From ATSDR (2001); IFA (2016)

2. Cancer in Humans

Several epidemiological studies have examined risk of cancer associated with exposure to pentachlorophenol. A series of population-based case–control studies conducted in Sweden, New Zealand, and the USA have investigated associations between a range of chlorophenols and phenoxy herbicides and lymphatic and haematopoietic cancers and soft tissue sarcoma. In addition, a case–control study nested within a cohort identified from an international register of occupationally exposed workers also examined risk of non-Hodgkin lymphoma (NHL) and soft tissue sarcoma. There have been four informative studies in occupational cohorts that have included exposure assessment techniques designed to separate the effects attributable to pentachlorophenol from those associated with the other chlorophenols or phenoxy herbicides and their dioxin contaminants. Studies that reported results only for chlorophenols in general (see Section 1.3.2 for a list of studies) were judged to be uninformative and were not considered further by the Working Group.

2.1 Cohort studies

See Table 2.1.

Kogevinas et al. (1995) conducted two case–control studies of soft tissue sarcoma and NHL nested within an international register of workers exposed to phenoxy herbicides, chlorophenols, and dioxins, which had previously been used for a cohort study of mortality, coordinated by IARC. The IARC cohort consisted of more than 21 000 workers from 24 cohorts in 11 countries: 11 cases of soft tissue sarcoma and 32 cases of NHL were identified. Five controls per case, matched for age, sex, and country of residence were selected from the cohort. Quantitative estimates of exposure of all participants to 21 chemicals or mixtures were developed by a panel of industrial hygienists on the basis of information obtained from company exposure questionnaires and company records combined with individual job history. Study participants were categorized as non- or ever-exposed, and the ever-exposed assigned to low, medium, or high exposure categories. Conditional logistic regression analyses were used to calculate odds ratios (OR) and 95% confidence intervals (CI) for each chemical. No cases of soft tissue sarcoma were observed in those exposed to pentachlorophenol, while there were three pentachlorophenol-exposed cases of NHL, giving an elevated but not statistically significant risk estimate (OR , 2.75; 95% CI, 0.45–17.00; 3 cases). In the analyses by level of exposure (lagged by 5 years), all three cases of NHL with exposure to pentachlorophenol were in the high exposure category (OR, 4.19; 95% CI, 0.59–29.59); however, the cases were all from one British cohort originally assembled to investigate a cluster of lymphoma cases at the plant. Several other exposures were also associated with a non-statistically significant excess risk of NHL including "any dioxin or furan" and TCDD. Exposure–response relationships of increasing risk with increasing exposure to "any dioxin or furan" and TCDD were observed. [The Working Group noted that this was a large cohort, with objective exposure assessment methods. The limitations included that only mortality was assessed, that exposures to several compounds were highly correlated, and that only three cases of NHL were exposed to pentachlorophenol and all were from the same plant.]

Demers et al. (2006) conducted an extended follow-up of mortality and cancer incidence in a cohort of about 27 000 male workers employed for at least 1 year between 1950 and 1995 in 14 sawmills in British Columbia, Canada. This cohort had previously been studied by Hertzman et al. (1997). Eleven of these sawmills had used chlorophenates as antifungal wood treatments (either tetrachlorophenol or pentachlorophenol, or a mixture of both), while the remaining three

Table 2.1 Cohort studies on cancer and exposure to pentachlorophenol

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1995) Australia, Denmark, Finland, Germany, Netherlands, New Zealand, Sweden, and United Kingdom 1939–1992 Nested case–control	Cases: 32 NHL; 11 STS; International Register of Workers Exposed to Phenoxy Herbicides Controls: 158 NHL; 55 STS; Incidence density sampling (5 controls per case matched for age, sex, country) Exposure assessment method: company records; review by industrial hygienists to estimate exposure to 21 chemicals	NHL (incidence)	All PCP-exposed workers	3	2.75 (0.45–17.00)	Age, sex, country of residence when employed	Strengths: large study; objective exposure assessment methods; estimates of exposure to PCP, phenoxy herbicides, dioxins and furans Limitations: no quantitative exposure information; exposures to several compounds highly correlated; low power
			High cumulative PCP exposure relative to non-exposed	3	4.19 (0.59–29.59)		
		STS (incidence)	Ever exposed	0	–	Age, sex, country	
Demers et al. (2006) British Columbia, Canada 1950–1995 Cohort	25 685; 27 464 men employed ≥ 1 year in 14 sawmills, 1950–1995; 25 685 men included for incidence analysis Exposure assessment method: company records; job history combined with historical records on type of chlorophenol used by time period and estimates from senior workers on intensity of dermal exposure	All cancers combined	Sawmill workers			Age, calendar period	This cohort was previously studied by Hertzman et al. (1997) Strengths: large cohort; completeness and duration of follow-up; exposure assessment discriminated between PCP and TCP Limitations: limited power for rare cancers
			Mortality	1495	1 (0.95–1.05)		
			Incidence	2571	0.99 (0.95–1.04)		
		NHL (incidence)	PCP exposure-years			Age, time period, race	
			Years of exposure in quartiles: sawmill workers	92	0.99 (0.81–1.21)		
			< 1	38	1		
			1–2	13	1.33 (0.7–2.52)		
			2–5	24	1.88 (1.08–3.28)		
			5+	17	1.71 (0.91–3.24)		
			Trend-test *P*-value: 0.03				
		MM (incidence)	PCP exposure-years			Age, time period, race	
			Years of exposure in quartiles: sawmill workers	25	0.8 (0.52–1.18)		
			< 1	6	1		
			1–2	4	2.09 (0.57–7.61)		
			2–5	4	1.3 (0.34–4.98)		

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Demers et al. (2006) (cont.)			5+	11	4.18 (1.36–12.9)		
			Trend-test *P*-value: 0.02				
		Kidney (incidence)	PCP exposure-years			Age and time period	
			Sawmill workers	79	1.1 (0.88–1.38)		
			< 1	32	1		
			1–2	9	1.03 (0.49–2.18)		
			2–5	22	1.79 (0.99–3.24)		
			5+	16	1.66 (0.85–3.23)		
			Trend-test *P*-value: 0.07				
		Lung (incidence)	PCP exposure-years			Age and time period	
			Sawmill workers	519	1.02 (0.93–1.11)		
			< 1	216	1		
			1–2	78	1.11 (0.86–1.45)		
			2–5	119	1.07 (0.84–1.36)		
			5+	106	1.12 (0.87–1.44)		
			Trend-test *P*-value: 0.45				
		Liver/HCC (incidence)	PCP exposure-years			Age and time period	
			Sawmill workers	21	0.79 (0.49–1.21)		
			< 1	3	1		
			1–2	4	4.09 (0.89–18.76)		
			2–5	12	8.47 (2.21–32.45)		
			5+	2	1.41 (0.21–9.22)		
			Trend-test *P*-value: 0.18				
		STS (incidence)	PCP exposure-years			Age and time period	
			Sawmill workers	13	0.84 (0.49–1.44)		
			< 1	18	1		
			1–2	3	0.64 (0.18–2.2)		
			2–5	2	0.18 (0.04–0.85)		
			5+	0	–		
			Trend-test *P*-value: 0.11				

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2009) Dow Chemical Co., Michigan, USA 1950–1985 Cohort	773 men; subcohort of a larger dioxin-exposed cohort, selected on the basis of employment in departments with known exposure to PCP Exposure assessment method: company records	All cancers combined	All PCP workers	94	1 (0.8–1.2)	Age, calendar period	Strengths: complete ascertainment of vital status Limitations: small cohort size; men only
			PCP (no TCP)	71	1 (0.8–1.3)		
		NHL	All exposed workers	8	2.4 (1–4.7)		
			PCP (no TCP)	7	2.8 (1.1–5.7)		
			High cumulative exposure to TCDD, > 0.825 ppb-years	3	3.1 (0.6–9.1)		
			High cumulative exposure to HxCDD, > 8 ppb-years	5	5.3 (1.7–12.4)		
			High cumulative exposure to HpCDD, > 142 ppb-years	4	4.6 (1.3–11.8)		
			High cumulative exposure to OCDD, > 470 ppb-years	4	4.7 (1.3–12)		
		Kidney	All PCP workers	4	1.7 (0.5–4.4)		
			PCP (no TCP)	4	2.3 (0.6–5.8)		
		Lung	All exposed workers	30	1 (0.6–1.4)		
			PCP (no TCP)	25	1.1 (0.7–1.6)		

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ruder &Yiin (2011) USA 1936–2005 Cohort	2122 members of NIOSH Dioxin Registry, Exposure assessment method: work in exposed jobs	All cancers combined	All exposed workers	326	1.17 (1.05–1.31)	Age (5 year categories), sex, race	Strengths: length of follow-up from first exposure Limitations: most had exposure to multiple chemicals
			PCP + TCP	88	1.01 (0.81–1.24)		
			PCP (no TCP)	238	1.25 (1.09–1.42)		
		NHL	All exposed workers	17	1.77 (1.03–2.84)		
			PCP + TCP	8	2.5 (1.08–4.93)		
			PCP (no TCP)	9	1.41 (0.64–2.67)		
		MM	All exposed workers	7	1.5 (0.6–3.1)		
			PCP + TCP	1	0.72 (0.02–3.99)		
			PCP (no TCP)	6	1.84 (0.68–4)		
		Kidney	All exposed workers	8	1.2 (0.52–2.37)		
			PCP + TCP	4	1.8 (0.49–4.61)		
			PCP (no TCP)	4	0.9 (0.25–2.31)		
		Lung	All exposed workers	126	1.36 (1.13–1.62)		
			PCP + TCP	27	0.91 (0.6–1.33)		
			PCP (no TCP)	99	1.56 (1.27–1.9)		

CI, confidence interval; HCC, hepatocellular carcinoma; HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*para*-dioxin; HxCDD, 1,2,3,4,7,8-hexachlorodibenzo-*para*-dioxin; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NIOSH, National Institute for Occupational Safety and Health; OCDD, octachlorodibenzo-*para*-dioxin; PCP, pentachlorophenol; STS, soft tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin; TCP, 2,4,6-trichlorophenol

mills had not. Cumulative dermal exposure to chlorophenate was calculated by combining historical use records and job title-based exposure patterns with duration of employment, and historical records on the chlorophenate formulations used in each mill at different time periods, which were used to assign separate indices of exposure to tetrachlorophenol and pentachlorophenol. In a validation study, the results of urinary chlorophenate measurements in 226 workers in one sawmill currently exposed to pentachlorophenol were strongly correlated with the estimates made by groups of raters including hygienists and senior workers. Urinary chlorophenate levels ranged from 2 to 989 μg/L, with a geometric mean of 43 μg/L, and a geometric standard deviation of 3.6. In comparisons between the overall sawmill cohort and the general population of British Columbia, there was no excess in all cancer mortality (SMR, 1.0; 95% CI, 0.95–1.05) or incidence (standardized incidence ratio, SIR, 0.99; 95% CI, 0.95–1.04). There was a moderate elevation in kidney cancer mortality (standardized mortality ratio, SMR, 1.31; 95% CI, 0.98–1.73), but not incidence (SIR, 1.10; 95% CI, 0.88–1.38). Internal analyses based on quartiles of cumulative exposure to pentachlorophenol, after adjustment for age, calendar period, and race, showed significant positive trends in mortality from kidney cancer ($P = 0.02$) and multiple myeloma ($P = 0.03$). There were significant positive trends in incidence of NHL ($P = 0.03$) and multiple myeloma ($P = 0.02$). When the pentachlorophenol exposures were lagged by 10 or 20 years, the statistically significant positive trends for incidence of both NHL and multiple myeloma remained, as did the trend for kidney cancer with a 20 year lag. By contrast, internal analyses by exposure to tetrachlorophenol showed no significant dose–response relationship for either mortality or incidence of NHL or multiple myeloma, although there was a significant positive trend ($P = 0.04$) for mortality from kidney cancer, and latency analyses also showed no significant trends apart from cancer of the rectum. [The Working Group noted that the strengths of this study included the large sample size, the completeness of follow up, the high-quality exposure assessment that discriminated between pentachlorophenol and tetrachlorophenol, the conduct of internal analyses, and the examination of both mortality and incidence. A limitation was that the effect estimates for kidney cancer were not adjusted for tetrachlorophenol, but exposure to pentachlorophenol and tetrachlorophenol were not strongly correlated.]

Collins et al. (2009) extended for an additional 9 years the mortality follow-up of a small cohort ($n = 773$) of pentachlorophenol production workers employed between 1937 and 1980 by a chemical company in Michigan, USA. This cohort consisted of employees who had worked at any time in any department in which exposure to pentachlorophenol could have occurred, and was a subset of a cohort of 2192 workers with exposure to PCDDs that had been assembled previously. Exposure estimates in the form of ordinal rankings of intensity of exposure to pentachlorophenol, to TCDD, and to the higher chlorinated dioxins that are characteristic of the pattern of congeners found as contaminants in pentachlorophenol. Exposure estimates were based on job history and a combination of historical occupational hygiene and process data (Ramlow et al., 1996). Results of a survey of serum dioxin levels in a small group of workers ($n = 128$) were also used to define separate exposure categories for TCDD and for the specific pentachlorophenol-related HxCDDs, HpCDDs, and OCDD. A subset of 577 workers who were determined to have been exposed to pentachlorophenol but not to 2,4,6-trichlorophenol were also identified from historical records. In comparisons of the overall cohort with the general population of the USA, no excess risk of mortality from all cancers (SMR, 1.0; 95% CI, 0.8–1.2) was observed, although mortality from NHL was significantly elevated in both the overall cohort

(SMR, 2.4; 95% CI, 1.0–4.7; 8 deaths) and in the pentachlorophenol-only cohort (SMR, 2.8; 95% CI, 1.1–5.7; 7 deaths). There was also a non-statistically significant increase in the SMR for kidney cancer among all workers exposed to pentachlorophenol (SMR, 1.7; 95% CI, 0.5–4.4; 4 deaths), and in the pentachlorophenol-only cohort (SMR, 2.3; 95% CI, 0.6–5.8; 4 deaths). Internal analyses stratified on the basis of cumulative exposure to the dioxin congeners tested showed a statistically significant increase in mortality from NHL at the highest tertile of exposure to the pentachlorophenol-related congeners, but not to TCDD [which suggests a stronger association with pentachlorophenol (and the characteristic dioxin congeners) than with dioxins per se]. Mortality from NHL was elevated but not significantly in the high-exposure category for TCDD (SMR, 3.1; 95% CI, 0.6–9.1; 3 deaths), but the elevation was higher and significant for HxCDD (SMR, 5.3; 95% CI, 1.7–12.4; 5 deaths), HpCDD (SMR, 4.6; 95% CI, 1.3–11.8; 4 deaths), and OCDD (SMR, 4.7; 95% CI, 1.3–12.0; 4 deaths). [The Working Group noted that the strengths of this study included complete ascertainment of vital status, and the exposure assessment discriminated between pentachlorophenol, trichlorophenol, and dioxin congeners allowing the effects to be attributed with more confidence to pentachlorophenol. Limitations were the size of cohort and the examination of mortality only.]

Ruder & Yiin (2011) identified a cohort of 2122 individuals who had ever worked in a pentachlorophenol-production department at one of four chemical manufacturing plants in the USA from the NIOSH Dioxin Registry, and compared mortality with that of the general population. Most (90%) of the cohort members had recognized exposure to other chemicals produced at these plants, and more than 40% were also exposed to 2,4,5-trichlorophenol (and therefore to TCDD as a contaminant). Standardized mortality ratios were calculated for the entire cohort, and separately for those exposed to both 2,4,5-trichlorophenol and pentachlorophenol (n = 720) and to pentachlorophenol only (n = 1402). All cancer mortality was significantly elevated in both the full cohort (SMR, 1.17; 95% CI, 1.05–1.31) and in the pentachlorophenol-only cohort (SMR, 1.25; 95% CI, 1.09–1.42), but not elevated in the 2,4,5-trichlorophenol/pentachlorophenol cohort (SMR, 1.01; 95% CI, 0.81–1.24). Mortality from lung cancer was also significantly elevated in the full cohort (SMR, 1.36; 95% CI, 1.13–1.62) and the pentachlorophenol-only cohort (SMR, 1.56; 95% CI, 1.27–1.90), but not in the 2,4,5-trichlorophenol/pentachlorophenol cohort (SMR, 0.91; 95% CI, 0.60–1.33). The significant elevation in mortality from lung cancer occurred in one plant only, and it was noted that more than 70% of those dying from lung cancer had worked for less than a year in departments with exposure to pentachlorophenol. Non-Hodgkin lymphoma was significantly elevated in the full cohort (SMR, 1.77; 95% CI, 1.03–2.84; 17 deaths) and in the 2,4,5-trichlorophenol/pentachlorophenol cohort (SMR, 2.50; 95% CI, 1.08–4.93; 8 deaths), and elevated but not significantly in the pentachlorophenol-only cohort (SMR, 1.41; 95% CI, 0.64–2.67; 9 deaths). There was no clear association between duration of exposure to pentachlorophenol and mortality overall or from either lung cancer or NHL. [The Working Group noted that the strengths of the study included the length of follow up, and the good-quality exposure assessment, while the limitations were that most study participants had exposures to multiple chemicals and that the study examined mortality only.]

2.2 Case–control studies

See Table 2.2.

Seven case–control studies from Sweden, New Zealand, and California, USA have reported data relevant to exposure to pentachlorophenol and cancer risk, and are summarized below.

Table 2.2 Case–control studies on cancer and exposure to pentachlorophenol

Reference, location enrolment/follow-up	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pearce et al. (1986a) New Zealand 1977–1981	Cases: 83; cancer registry Controls: 396; 168 cancer patients from cancer registry; 228 general population controls Exposure assessment method: questionnaire; job-title based	NHL	Ever exposed to fencing as a farmer			Age, respondent type (proxy/ direct), sex	Strengths: population-based study; good response rates Limitations: limited exposure assessment
			Cancer controls	33	1.9 [1.1–3.4]		
			General population controls	33	1.9 [1.0–3.7]		
			Ever exposed to fencing as a contractor				
			Cancer controls	4	1.4 [0.6–3.3]		
			General population controls	4	6.1 [0.9–40.2]		
			Pooled estimate - farmer and/ or fencing contractor	37	2.0 [1.2–3.4]		
Hardell et al. (1994) Umea, Sweden 1974–1978	Cases: 105; oncology department records Controls: 335; national population registry and death registry Exposure assessment method: self-administered questionnaire; next-of-kin proxy respondents for deceased cases and controls; lifetime work history recorded	NHL	Exposure duration > 1 week continuously or 1 month in total			Sex, age, place of residence and vital status	Strengths: use of population registries for ascertainment of both cases and controls Limitations: potential for recall bias in self-reported exposure; little information was provided on the exposure assessment methods
			"High grade" PCP exposure	15	8.8 (3.4–24.0)		

Table 2.2 (continued)

Reference, location enrolment/follow-up	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell et al. (2002) Sweden; four northern counties and three counties in mid-Sweden 1987–1992	Cases: 515; Swedish cancer registry Controls: 1141; national population registry Exposure assessment method: questionnaire; self-report; 43% of NHL cases had proxy and only living HCL cases were recruited; minimum exposure, 1 day; exposures lagged 1 year	NHL and HCL combined	All exposed workers	64	1.40 (0.99–1.98)	Study, study area and vital status	Pooled analysis of earlier studies of NHL and HCL Overlaps with Nordström et al. (1998), Hardell & Eriksson (1999), Hardell et al. (2002), Eriksson et al. (2008). Strengths: large population-based study; high response rates Limitations: potential for recall bias with high proportion of proxy respondents.; limited power for chlorophenol exposure
Pearce et al. (1986b) New Zealand 1977–1981	Cases: 76; cancer registry Controls: 315; other cancer patients from cancer registry Exposure assessment method: questionnaire; Telephone interview with clarification of circumstances for certain occupations	MM	Ever worked as a fencer	29	1.6 (0.9–2.7)	Age, sex, respondent type (proxy/direct)	Strengths: population-based study, good response rates Limitations: limited exposure assessment
Smith et al. (1984) New Zealand 1976–1980	Cases: 82; cancer registry Controls: 92; cancer registry Exposure assessment method: questionnaire	STS	Fencing as a farmer Fencing contractor Sawmill worker or Timber merchant Potential chlorophenol exposure at sawmill	20 5 12 3	0.8 (0.4–1.5) 1.9 (0.5–8.6) 1.3 (0.6–2.9) 0.7 (0.1–2.7)	Age	Strengths: population-based study Limitations: limited exposure assessment; use of cancer subjects as controls

Table 2.2 (continued)

Reference, location enrolment/follow-up	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell et al. (1995) Sweden 1970–1980	Cases: 434; from four previous studies identified through cancer registries Controls: 948; population controls from same four studies Exposure assessment method: questionnaire; self or proxy report of exposure; minimum exposure time, 1 day; exposures lagged by 5 years	STS (Soft tissue sarcoma)	All exposed workers	27	2.8 (1.5–5.4)	Age, vital status, and study	Strengths: cases from cancer registries and controls from population registers; study size and power Limitations: potential for recall bias in self-report
Ward et al. (2009) California, USA 2001–2006	Cases: 184; children aged < 7 years from 9 paediatric clinics Controls: 212; birth certificates matched on age, sex, race, ethnicity and maternal residence Exposure assessment method: environmental monitoring; analysis of carpet dust	ALL	PCP concentration (ng/g) in carpet dust			Age, sex, race/ ethnicity, income, year and season of the interview/dust collection	Strengths: quantitative assessment of residential exposure Limitations: lower response rate in controls
			Low (< 32.2)	38	1		
			32.2 to < 75.8	46	1.28 (0.68–2.4)		
			75.8 to < 164.7	47	1.46 (0.78–2.74)		
			164.7–22 676	31	0.84 (0.43–1.65)		
			Trend-test *P*-value: 0.476				
		ALL	PCP loading (ng/m^2) in carpet dust				
			< 32.7	51	1		
			32.7 to < 82.2	34	0.56 (0.29–1.08)		
			82.2 to < 272.5	43	0.78 (0.42–1.47)		
			≥ 272.5	32	0.47 (0.24–0.92)		
			Trend-test *P*-value: 0.045				

ALL, acute lymphoblastic/lymphocytic leukaemia; CI, confidence intervals; HCL, hairy cell leukaemia; NHL, non-Hodgkin lymphoma; PCP, pentachlorophenol

A series of case–control studies were conducted in Sweden using similar methods to examine associations between phenoxyacetic acids, chlorophenols and organic solvents and NHL, hairy cell leukaemia, and soft tissue sarcoma (Hardell et al., 1994, 1995, 2002). In all of these studies, cases were identified from either oncology departments or cancer registries, with living controls identified from the national population registry and deceased controls identified from a national death registry for matching to deceased cases. A self-administered questionnaire was used to obtain data on demographics, lifestyle factors, and a lifetime work history, as well as occupational and recreational exposure to specific substances. Proxy interviews with next of kin were conducted for deceased cases or controls, and where answers from either living or proxy study participants were incomplete or unclear, a telephone interview was used to clarify the information. For pentachlorophenol (assessed separately), exposure was classified as low grade where it was used for less than 1 week continuously or less than 1 month in total; if exposure was greater than this, it was classified as high grade. Data were analysed by the specific self-reported exposures, with odds ratios and 95% confidence intervals calculated after stratification by age, vital status, and study. The relevant studies are summarized below. [The Working Group noted that a strength of these studies was that they were population-based and used national registers for identification of study participants. The limitations included the use of self-reported exposure, and the large proportion of proxy respondents, increasing concerns about recall bias.]

Two cancer registry-based case–control studies of multiple myeloma and NHL examined associations with exposure to phenoxy herbicides and chlorophenols in New Zealand (Pearce et al., 1986a, b). Both studies identified cases from the national cancer registry, and recruited controls with other cancers from the register, and in the case of the NHL study supplemented these with controls from the general population, recruited from the electoral roll. Interviews were conducted by telephone, with stem questions leading to more specific questions on certain occupations determined a priori to entail exposure to pentachlorophenol. [The Working Group noted that the strengths of these studies were that they were population-based studies, identifying cases from tumour registries, while the limitations included the crude exposure assessment and potential for co-exposure to other chlorophenols, phenoxy herbicides, and wood preservatives.]

Smith and colleagues conducted a case–control study of cases of soft tissue sarcoma (International Classification of Diseases, ninth revision, ICD-9, 171) in men diagnosed in New Zealand between 1976 and 1980 and identified from the national cancer registry (Smith et al., 1984). [The Working Group noted that this study had several limitations, namely that other cancer patients, with cancer sites not stated, were used as controls; that the majority of interviews were conducted with proxies; and the low power to detect an excess risk. The conduct of follow-up interviews was a strength.]

A population-based case–control study in California, USA, examined risk of childhood leukaemia and examined associations with a range of persistent organochlorine pesticides measured in carpet dust (Ward et al., 2009). [The Working Group noted that the strengths included the quantitative assessment of residential exposure, while the limitations were the relevance of exposure measurements in dust to exposure in individuals, and the low response rate in controls.]

2.2.1 Non-Hodgkin lymphoma

In a study in New Zealand, Pearce et al. (1986a) compared 83 cases of NHL (ICD, 202) recruited from the national cancer registry with 168 controls with other types of cancer recruited

from the same register and 228 general population controls recruited from the electoral roll. In telephone interviews, participants reported occupational history, with more specific information on work circumstances sought from those who had held certain occupations. As there was potential for exposure to chemicals used to treat wood products used for fencing, fencing as a farmer or work as a fencing contractor was examined. A significantly elevated risk was observed in both jobs in comparison with the general population controls, and in a pooled estimate combining both occupations. It was acknowledged that these associations may be with either pentachlorophenol used as an antifungal treatment on all wood products used for fencing or with the chromated copper arsenate treatment applied to timber for outside use or ground contact. [The Working Group noted that there is no known association between exposure to arsenic or hexavalent chromium and NHL.] No excess risk was observed among sawmill workers, many of whom are known to be exposed to pentachlorophenol (OR, 0.9; [95% CI, 0.0–2.7]).

Hardell et al. (1994) re-analysed data from a case–control study of malignant lymphoma conducted previously in which a 6-fold risk of NHL had been observed in people exposed to phenoxyacetic acids or chlorophenols in Sweden (Hardell et al., 1981). The study compared 105 men aged 25–85 years with histopathologically verified NHL who had been admitted to an oncology department between 1974 and 1978 with 335 controls matched for sex, age, place of residence, and vital status. When the data was analysed by occupation no significant elevation of risk was observed. Significantly elevated risks were observed for estimated high grade (OR, 8.8; 95% CI, 3.4–24.0) pentachlorophenol exposure.

Hardell et al. (2002) also reported the results of a pooled analysis of two case–control studies on 404 cases of NHL and 111 cases of hairy cell leukaemia recruited between 1987 and 1992 in Sweden. Response rates were 91% for cases and 84% for controls in the study on NHL, and 91% for cases and 83% for controls in the study on hairy cell leukaemia. Conditional logistic regression was used to calculate odds ratios and 95% confidence intervals for each exposure. In the combined analysis of NHL and hairy cell leukaemia, an odds ratio of 1.40 (95% CI, 0.99–1.98; 64 cases) was reported for exposure to pentachlorophenol. When applying different latency periods, the highest risk associated with exposure to pentachlorophenol was observed with an induction period of 20–30 years (OR, 2.13; 95% CI, 1.07–4.25). [The Working Group noted discrepancies in the number of participants reported in different analyses in this paper.]

Pearce et al. (1986b) conducted a case–control study of multiple myeloma and farming in which they recruited 102 male public hospital patients aged less than 70 years and compared these with 4 cancer patient controls for each case matched on year of registration and within 2 years of birth date. In telephone interviews that were similar to those in the study on NHL, information was sought on work history, with extra questions for specific occupations. A non-significant association with fencing work, which has the potential for exposure to both pentachlorophenol and chromated copper arsenate was observed (OR, 1.6; 90% CI: 0.9–2.7). [The Working Group noted that exposures to chromium, copper, and arsenic are not known to be risk factors for multiple myeloma.]

2.2.2 Soft tissue sarcoma

In light of early reports of an association between exposure to phenoxy herbicides or chlorophenols and soft tissue sarcoma in Sweden, Smith et al. (1984) conducted a case–control study in New Zealand.

Smith and colleagues conducted a case–control study of cases of soft tissue sarcoma (ICD-9, 171) in men diagnosed in New Zealand

between 1976 and 1980 and identified from the national cancer registry. In the study by Smith et al. (1984), cases were histologically reviewed by a pathologist. One control per case, with the same year of registration and within 2 years of age, was randomly selected from among other cancer patients in the registry. After excluding ineligible participants, 82 cases (84%) and 111 controls (83%) were included. Data on activities with a potential for exposure to chlorophenoxy herbicides and chlorophenols were collected in telephone interviews with patients or next of kin. This study found no evidence of any association with several occupations known to have potential exposure to wood treatment compounds containing pentachlorophenol.

Hardell et al. (1995) conducted a pooled analysis of data from four earlier studies in Sweden to examine associations between exposure to pesticides and soft tissue sarcoma in men, including 434 cases and 948 controls. In total, 63% of cases in the three studies where this was reported were deceased. A significant excess risk (OR, 2.8; 95% CI, 1.5–5.4; 27 cases) was observed in those ever exposed to pentachlorophenol.

2.2.3 Childhood acute lymphocytic leukaemia

In the study by Ward et al. (2009), noted above, analyses focused on a subset of cases aged 7 years or younger that were ascertained from nine major paediatric clinics in the study area. Controls were individually matched to cases on age, sex, race, Hispanic ethnicity, and maternal residence, and were selected from birth certificate files. The distribution of pentachlorophenol was categorized into quartiles based on the measured values in household carpet dust among controls. The concentration of pentachlorophenol in carpet dust was not associated with increased risk, and a significant inverse trend in risk of acute lymphocytic leukaemia with increased chemical loading of pentachlorophenol in carpet dust was observed.

2.3 Exposure assessment and biological markers in epidemiological studies

Individual exposure to pentachlorophenol has been assessed in epidemiological studies using several different methods. The simplest method, commonly used in case–control studies, uses retrospective interviews or questionnaires to ascertain whether each individual worked in particular jobs for which investigators had determined that exposure to chlorophenols was likely, e.g. wood treatment or chlorophenol manufacturing. Some studies on chlorophenols did not obtain sufficient information to distinguish jobs using pentachlorophenol from jobs using other chlorophenols (e.g. Woods et al., 1987; Ali et al., 2004). Job classifications may be adequate to detect some differences in cancer risk ('t Mannetje & Kromhout, 2003), but may also be surrogates for a variety of co-exposures in addition to pentachlorophenol.

Several population-level studies collected more detailed information that could be used to distinguish jobs exposed to pentachlorophenol. A series of case–control studies in New Zealand used retrospective telephone interviews with patients or next of kin to determine whether each individual had worked in particular jobs for which investigators had determined that exposure to phenoxy herbicides or chlorophenols was likely (Smith et al., 1984; Pearce et al., 1986a, b). Initial questions used a pre-specified list of occupations, and for those who reported having worked in those occupations subsidiary questions were asked regarding the specific nature of work and potential for exposure to specific chemicals. A series of Swedish case–control studies obtained complete occupational histories, and included questions regarding duration of use of specific

chemicals, including pentachlorophenol and classes of chemicals including chlorophenols, phenoxy acids, and organic solvents (Hardell et al., 1994, 1995, 2002). This allowed for additional epidemiological analyses comparing "low grade" (exposure duration greater than 1 week continuously or 1 month in total) to "high grade" exposures (Hardell et al., 2002). There was some evidence that workers in stable careers can reliably report on past production methods and use of frequently handled chemicals (Friesen et al., 2015; IARC, 2017). For example, orchardists in one study showed good consistency in recalling commonly used pesticides and pesticide categories for repeated exposure questionnaires after 21–25 years (Engel et al., 2001). However, recall for infrequently used chemicals can be poor (Engel et al., 2001), and the use of next-of-kin proxies for deceased participants may exacerbate exposure misclassification and the potential for recall bias (Nam et al., 2005).

A mortality study with the National Institute for Occupational Safety and Health (NIOSH) dioxin registry used work records from four pentachlorophenol production facilities and used detailed company-specific information and expert judgment to determine whether workers had been exposed to 21 chemicals and mixtures, including pentachlorophenol and trichlorophenol (Ruder & Yiin, 2011). Duration of work in pentachlorophenol departments was the primary exposure metric. It is likely that the use of work records in this study provided more accurate exposure assignments than self or proxy reports.

One case–control study of childhood leukaemia in California, USA, used home carpet-dust samples to characterize exposure to pentachlorophenol and other persistent organochlorines (Ward et al., 2009). Dust samples were obtained from the room most often used during waking hours for participants who lived in the same residence at time of diagnosis and at the time of sampling (often several years after diagnosis, in 2001–2006). [The Working Group noted that house dust is a major exposure source for young children in older homes, so carpet dust measurements might be a good surrogate for childhood exposure] (Ward et al., 2009). A different study found moderate to high intraclass correlations (0.37–0.95) for pesticides in repeated home-dust samples collected over approximately 2 years (Deziel et al., 2013). However, a study in young children in North Carolina, USA, estimated that dust and soil ingestion contributed only about 6–9% of their total pentachlorophenol exposure, based on dust, diet, and air samples collected in 2003–2005, with the remaining portion from indoor and outdoor air inhalation (43–54% of total exposure), and diet (37–51%) (Wilson et al., 2010).

Studies that used company work records and study-specific job–exposure matrices (JEMs) to assess pentachlorophenol (or total chlorophenol) exposures include a nested case–control study in an international register of workers (Ott et al., 1993; Kauppinen et al., 1994; Kogevinas et al., 1995), and records-based cohort studies of chemical-plant workers in Michigan, USA (Collins et al., 2007, 2009), and Canadian sawmill workers (Hertzman et al., 1997; Heacock et al., 2000; Demers et al., 2006). For JEM-based exposure assignments, exposure intensity scores are assigned for each job (often department- and plant-specific) over time; exposure intensities are then multiplied by job duration and summed across all jobs to calculate a cumulative exposure score for each worker (e.g. Ott et al., 1993; Collins et al., 2007; Cooper & Jones, 2008). The quality of JEM-based exposure assignments thus depends on the accuracy of the intensity score assigned to each job, the variability in personal exposures within each job, and the completeness and validity of the work records linking individual workers to specific jobs or tasks. A wide variety of methods are used to estimate exposure intensity scores, but the scores are most reliable when supported by routine biomonitoring or personal exposure measurements throughout

the duration of exposure. In practice, exposure measurements are often only available for part of the exposure period or not at all, in which case investigators rely on models and/or judgment to assign exposure intensity scores during some or all periods. For example, the Kogevinas et al. (1995) case–control study relied on a team of three industrial hygienists (who were blind to health outcomes) to assign a unitless exposure intensity for each job as the product of judgment-based subscores on innate job tasks, emissions of agents, average daily contact time of the workers with the contaminants, the use of personal protective equipment, and "certain other factors" (Kauppinen et al., 1994). Without direct measurements of pentachlorophenol exposure for the jobs in Kogevinas et al. (1995), non-differential exposure misclassification is likely, resulting in attenuation of epidemiological effect estimates towards a null association.

The studies of Canadian sawmill workers (Hertzman et al., 1997; Demers et al., 2006) and their offspring (Heacock et al., 2000) used JEMs for which exposure intensities were assigned based on hours of annual dermal exposure to chlorophenol, obtained by averaging values gained from interviews of randomly sampled groups of long-term workers (Teschke et al., 1996). Relatively stable intensity estimates with intraclass correlations of 0.78–0.88 were obtained when exposure scores were averaged across raters, whereas scores from individual raters were often discordant regarding the degree of chlorophenol exposure for any job at any particular time (Teschke et al., 1996). Exposure intensities were also validated against several hundred urinary chlorophenate concentrations collected over two seasons in 1986 (Hertzman et al., 1988); the average of the two urine concentrations for each individual was well correlated (r = 0.72) with cumulative exposure scores for total chlorophenols produced by a JEM (Hertzman et al., 1988). The Demers et al. (2006) study refined the previous JEMs by incorporating the percentages of pentachlorophenol and tetrachlorophenol in the specific fungicides used in each plant over time, creating a more specific measure of exposure less prone to attenuation (Friesen et al., 2007).

Occupational exposure to pentachlorophenol is often concomitant with exposure to other polychlorophenols, dibenzodioxins, dibenzofurans, and other chemicals (IARC, 1997, 1999; and Section 1.1.3(b)). This can make it difficult to attribute epidemiological associations with adverse health effects to any one chemical in the mixture, particularly in studies that did not distinguish pentachlorophenol from other chlorophenols, dioxins, or contaminants. Some studies have included quantitative assessments of selected co-exposures, providing a basis for disaggregating their putative effects. For example, the epidemiological study of Canadian sawmill workers used detailed plant records to determine both the pentachlorophenol and tetrachlorophenol content of the products used at any particular time (Demers et al., 2006). Because the formulations changed over time and individual workers worked at different times and in different job tasks, the cumulative exposure scores for the two chemicals were only moderately correlated (r = 0.45) (Demers et al., 2006; as per Cooper & Jones, 2008).

Similarly, cumulative exposures to pentachlorophenol and several dioxins were assessed using study-specific JEMs in an epidemiological study of chemical-plant workers in Michigan, USA (Ramlow et al., 1996; Collins et al., 2009). These studies assigned each job to a pentachlorophenol exposure intensity score of 1–3 based on advice of veteran employees with experience in pentachlorophenol production and industrial hygiene monitoring with sampling primarily in the 1960s and 1970s. TCDD and hexa- to octachlorinated congeners (HxCDD/OCDD) exposure intensities were also assigned values of 0–4 and 0–2, respectively (Ramlow et al., 1996). Later in 2004 and 2005, serum samples were collected from 412 workers and analysed for dioxins

and dibenzofurans; TCDD was elevated among 2,4,5-trichlorophenol workers but not among pentachlorophenol workers (Collins et al., 2007). For the later epidemiological analysis (Collins et al., 2009) a one-compartment pharmacokinetic model was used to calibrate dioxin and dibenzofuran exposure intensities in the JEMs to available serum measurements, and to predict annual and cumulative serum concentrations for each congener (Flesch-Janys et al., 1996; Collins et al., 2009). Although the availability of independent exposure assignments for pentachlorophenol, dioxins, and dibenzofurans would have allowed for mutual adjustment of these co-exposures in epidemiological models, Collins et al. (2009) only evaluated associations with cumulative TCDD-TEQ (a weighted sum of cumulative dioxins and dibenzofuran exposures; EPA, 2010b), with and without exclusion of workers exposed to 2,4,5-trichlorophenol.

Urinary measures of pentachlorophenol and its glucurono-conjugate can be used as a biomarker of short-term exposure. As reviewed in Section 4.1.5, terminal urinary excretion half-lives in humans range from 10 to 20 days, with some evidence of biphasic elimination and more rapid excretion during the first few days (ATSDR, 2001). Other chlorophenols (tetra- and tri-) have been reported to have shorter urinary excretion half-lives (Pekari et al., 1991). Although the short half-lives for chlorophenols limits their utility for chronic exposure assessment, they can be used in validation studies for exposure assignments based on other methods such as JEMs, as in the Canadian sawmill studies (Hertzman et al., 1988).

3. Cancer in Experimental Animals

See Table 3.1.

[In line with IARC (1991), the Working Group noted that a study in mice (United States National Technical Information Service, 1968; Innes et al., 1969) and a study in rats (Schwetz et al., 1978) were inadequate for the evaluation because of some deficiencies in the study design, including the variable combination of small number of animals, dosage used, unknown purity of the compound, and absence of histopathology data. These studies are not included in Table 3.1.]

3.1 Mouse

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 9 weeks) were fed diets containing technical-grade pentachlorophenol (purity, 90.4%) at a concentration of 100 or 200 ppm, or commercial-grade pentachlorophenol (purity, 91%; containing a smaller amount of dioxins and furans than the technical-grade pentachlorophenol) at a concentration of 100, 200, or 600 ppm for 2 years. Two groups of 35 male and 35 female mice were fed control diets. The mice were killed after 112 weeks (NTP, 1989; McConnell et al., 1991). For the most part, mean body weights, food consumption, and survival of mice exposed to pentachlorophenol were comparable to those of controls; however, the survival of females at the lowest dose was significantly reduced after 628 days with the commercial-grade formulation.

In male mice, significant dose-related increase in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) was observed with either formulation of pentachlorophenol. The incidence of adrenal pheochromocytoma increased significantly in male mice exposed to both formulations. In female mice exposed to the commercial formulation, there was a significant dose-related increase in the incidence of hepatocellular adenoma, and the incidence of adrenal pheochromocytoma increased significantly at the highest dose. At the highest doses of either formulation, significantly higher incidence of haemangiosarcoma of the spleen [mainly] and/

Table 3.1 Studies of carcinogenicity in experimental animals exposed to pentachlorophenol

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 9 wk 112 wk McConnell et al. (1991); NTP (1989)	Oral PCP (technical grade), 90.4% Diet 0, 100, 200 ppm, daily 35, 50, 50 12, 24, 22	*Liver* Hepatocellular adenoma: 5/32*, 20/47**, 33/48*** Hepatocellular carcinoma: 2/32*, 10/47, 12/48** Hepatocellular adenoma or carcinoma (combined): 7/32*, 26/47**, 37/48*** *Adrenal gland* Pheochromocytoma: 0/31*, 10/45**, 23/45***	* $P < 0.001$ (trend) ** $P < 0.05$ *** $P < 0.001$ * $P < 0.05$ (trend) ** $P < 0.05$ * $P < 0.001$ (trend) ** $P = 0.015$ *** $P < 0.001$ * $P < 0.001$ (trend) ** $P < 0.05$ *** $P < 0.001$	Strengths: GLP study Impurities: TCP, 0.01%; tetrachlorophenol, 3.8%; HCB, 50 ppm; TCDD, ND; HxCDD, 10.1 ppm; HpCDD, 296 ppm; OCDD, 1386 ppm; PeCDD, 1.4 ppm; HxCDF, 9.9 ppm; HpCDF, 88 ppm; OCDF, 43 ppm
Full carcinogenicity Mouse, B6C3F$_1$ (F) 9 wk 112 wk McConnell et al. (1991); NTP (1989)	Oral PCP (technical grade), 90.4% Diet 0, 100, 200 ppm, daily 35, 50, 50 28, 41, 30	*Liver* Hepatocellular adenoma: 3/33, 8/49, 8/50 Hepatocellular carcinoma, 0/33, 1/49, 1/50 *Vascular system* Haemangiosarcoma: 0/35*, 3/50, 6/50**	NS Tumour incidence: 0/33, 1/49, 1/50 * $P < 0.05$ (trend) ** $P < 0.05$	Strengths: GLP study Impurities: TCP, 0.01%; tetrachlorophenol: 3.8%; HCB: 50 ppm; TCDD, ND; HxCDD, 10.1 ppm; HpCDD, 296 ppm; OCDD, 1386 ppm; PeCDF, 1.4 ppm; HxCDF, 9.9 ppm; HpCDF, 88 ppm; OCDF, 43 ppm

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 9 wk 112 wk McConnell et al. (1991); NTP (1989)	Oral PCP (commercial grade), 91% Diet 0, 100, 200, 600 ppm, daily 35, 50, 50, 50 25, 28, 29, 35	*Liver* Hepatocellular adenoma: 5/35*, 13/48, 17/48**, 32/49*** Hepatocellular carcinoma: 1/35, 7/48, 7/48*, 9/49* Hepatocellular adenoma or carcinoma (combined): 6/35*, 19/48**, 21/48***, 34/49**** *Adrenal gland* Pheochromocytoma, 0/34*, 4/48, 21/48**, 44/49** Pheochromocytoma (malignant): 1/34, 0/48, 0/48, 3/49	*P < 0.001 (trend) **P < 0.01 ***P < 0.001 *P < 0.05 *P < 0.001 (trend) **P = 0.015 ***P = 0.001 ****P < 0.001 *P < 0.001 (trend) **P < 0.001 NS	Strengths: GLP study Impurities: TCP, 0.007%; tetrachlorophenol: 9.4%; HCB: 65 ppm; TCDD, < 0.04 ppm; HxCDD, 0.19 ppm; HpCDD, 0.53 ppm; OCDD, 0.69 ppm; PeCDF, ND; HxCDF, 0.13 ppm; HpCDF, 0.15 ppm; OCDF, ND
Full carcinogenicity Mouse, B6C3F$_1$ (F) 9 wk 112 wk McConnell et al. (1991); NTP (1989)	Oral PCP (commercial grade), 91% Diet 0, 100, 200, 600 ppm, daily 35, 50, 50, 50 29, 28, 38, 39	*Liver* Hepatocellular adenoma: 1/34*, 3/50, 6/49, 30/48** Hepatocellular carcinoma: 0/34, 1/50, 0/49, 2/48 *Vascular system* Haemangiosarcoma: 0/35*, 1/50, 3/50, 8/49** *Adrenal gland* Pheochromocytoma: 0/35*, 1/49, 2/46, 38/49**	*P < 0.001 (trend) **P < 0.001 NS *P (trend) < 0.001 **P = 0.016 *P (trend) < 0.001 **P < 0.001	Strengths: GLP study Impurities: TCP, 0.007%; tetrachlorophenol: 9.4%; HCB: 65 ppm; TCDD, < 0.04 ppm; HxCDD, 0.19 ppm; HpCDD, 0.53 ppm; OCDD, 0.69 ppm; PeCDF, ND; HxCDF, 0.13 ppm; HpCDF, 0.15 ppm; OCDF, ND

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, *Nrf2*$^{+/+}$ (M) 7 wk 60 wk Tasaki et al. (2014)	Oral PCP, 98.6% CRF-1 diet 0, 600, 1200 ppm Ad libitum 15, 15, 20 80%, 27%, 0%	*Liver* Cholangiocarcinoma: 0/15, 0/15, 3/20 Hepatocellular adenoma: 2/15, 1/15, 2/20 Hepatocellular carcinoma, 1/15, 0/15, 0/20	 NS NS NS	
Full carcinogenicity Mouse, *Nrf2*$^{-/-}$ (M) 7 wk 60 wk Tasaki et al. (2014)	Oral PCP, 98.6% CRF-1 diet 0, 600, 1200 ppm Ad libitum 15, 15, 20 53%, 13%, 0%	*Liver* Cholangiocarcinoma, 0/15, 2/15, 6/20* Hepatocellular adenoma, 0/15, 2/15, 4/20* Hepatocellular carcinoma: 1/15, 0/15, 0/20	 *$P < 0.05$ *$P < 0.05$ NS	
Full carcinogenicity Mouse, Tg.AC (F) 14 wk 26 wk Spalding et al. (2000)	Skin PCP, 99% Acetone 0, 0.75, 1.5, 3.0 mg 5×/wk for 20 wk 15, 13, 13, 14 13, 8, 10, 12	Skin papilloma: 1/15, 1/13, 8/13*, 14/14** No. of tumours per total animals per group: 0.07, 0.08, 0.85, 11.6	*[$P < 0.005$, Fisher exact test]; **[$P < 0.0001$, Fisher exact test] NR	Limitations: small number of animals per group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Mouse, B6C3F$_1$ (M) 6 wk 25 wk Umemura et al. (1999)	Oral PCP, 98.6% Diet 0, 300, 600 ppm, daily 20, 20, 20 NR, NR, NR	*Liver* Hepatocellular adenoma, 4/15, 10/15*, 13/18**	*$P < 0.05$, Fisher exact test **$P < 0.01$, Fisher exact test	Mice were given drinking-water containing NDEA at 20 ppm for 13 wk, and after a 4 wk interval, were given basal diet or diet with PCP for 25 wk In another experiment in the same study, when PCP was tested as an initiator followed by drinking-water containing phenobarbital, no hepatocellular tumours were produced
		Hepatocellular carcinoma, 0/15, 2/15, 4/18	NS	
		Hepatocellular adenoma or carcinoma (combined): 4/15, 10/15*, 13/18**	*$P < 0.05$, Fisher exact test **$P < 0.01$, Fisher exact test	
		No. of tumours per total animals per group: 0.33 ± 0.62, 1.27 ± 1.89*, 2.22 ± 3.32*	*$P < 0.05$, Student *t*-test	
Initiation–promotion (tested as promoter) Mouse, B6C3F$_1$ (M) 6 wk 23 wk Umemura et al. (2003a)	Oral PCP, 98.6% Diet 0, 300, 600 ppm, daily 15, 15, 15 NR, NR, NR	*Liver* Hepatocellular adenoma: 1/15, 4/15, 11/15*	*$P < 0.01$, Fisher exact test	Mice were given drinking-water containing NDEA at 20 ppm for 8 wk, and after a 4 wk interval, were given basal diet or diet with PCP for 23 wk
		Hepatocellular carcinoma: 0/15, 1/15, 3/15	NS	
		Cholangioma, 0/15, 1/15, 9/15*	*$P < 0.01$, Fisher exact test	
		Cholangiocarcinoma, 0/15, 0/15, 8/15*	*$P < 0.01$, Fisher exact test	
Initiation–promotion (tested as promoter) Mouse, B6C3F$_1$ (M) 6 wk 25 wk Umemura et al. (2003b)	Oral PCP, 98.6% Diet 0, 600 ppm, daily 20, 20 NR, NR	*Liver* Cholangioma, 0/15, 12/18*	*$P < 0.01$, Fisher exact test	Mice were given drinking-water containing NDEA at 20 ppm for 13 wk, and after a 4 wk interval, were given basal diet or diet with PCP for 25 wk
		Cholangiocarcinoma, 0/15, 11/18*	*$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 2 years Chhabra et al. (1999); NTP (1999)	Oral PCP, 99% Diet 0, 200, 400, 600, 1000 ppm, daily 50, 50, 50, 50, 50 12, 16, 21, 31, 27	*Tunica vaginalis* Malignant mesothelioma, 1/50, 0/50, 2/50, 0/50, 9/50* *Nose* Nasal squamous cell carcinoma, 1/50, 3/50, 1/50, 0/50, 5/50	*$P = 0.014$ (Poly-3 test) NS	Strengths: GLP study The group at 1000 ppm received PCP in the feed for 52 wk and control feed thereafter (stop-exposure group); historical control incidence of nasal squamous cell carcinoma (feeding studies): 5/1341 (0.4 ± 1.0%); range, 0–4%
Full carcinogenicity Rat, F344 (F) 6 wk 2 years Chhabra et al. (1999); NTP (1999)	Oral PCP, 99% Diet 0, 200, 400, 600, 1000 ppm, daily 50, 50, 50, 50, 50 28, 33, 34, 28, 28	Any tumour type	NS	Strengths: GLP study The group at 1000 ppm received PCP in the feed for 52 weeks and control feed thereafter (stop-exposure group)
Co-carcinogenicity Rat, MRC-Wistar (M) 6–8 wk 86–88 wk Mirvish et al. (1991)	Oral PCP (technical grade), 86% Diet 0 (HENU alone), 500 (HENU+PCP), 500 (PCP alone) ppm, daily NR, NR, NR NR, NR, NR	*Bone marrow, lymph node* Acute myelocytic leukaemia, 4/20, 9/15*, 0/5 B-cell lymphoma, 5/20, 2/15, 0/5 *Bone* Osteosarcoma, 5/20, 4/15, 0/5	*[$P < 0.05$, 2-tail Fisher exact test; vs HENU alone] NS NS	Drinking-water containing HENU at 75 mg/L was given 4 days/wk for 40 wk, 2 wk after PCP treatment Impurities: TCDD, 25 µg/kg; and TCDF, 670 µg/kg

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Co-carcinogenicity Rat, MRC-Wistar (F) 6–8 wk 86–88 wk Mirvish et al. (1991)	Oral PCP (technical grade), 86% Diet 0 (HENU alone), 500 (HENU+PCP), 500 (PCP alone) ppm daily NR, NR, NR NR, NR, NR	*Bone marrow, lymph node* Acute myelocytic leukaemia, 3/19, 3/15, 0/9 B-cell lymphoma, 3/19, 3/15, 0/9 *Bone* Osteosarcoma, 0/19, 1/15, 0/9 *Liver* Adenoma, 1/19, 5/15, 6/9	 NS NS NS NS	Drinking-water containing HENU at 75 mg/L was given 4 days/wk for 40 wk, 2 wk after PCP treatment; no untreated control group Impurities: TCDD, 25 µg/kg; and TCDF, 670 µg/kg

F, female; GLP, good laboratory practice; HCB, hexachlorobenzene; HENU, 2-hydroxyethylnitrosourea; HpCDD, heptachlorodibenzo-*para*-dioxin; HpCDF, heptachlorodibenzofuran; HxCDD, hexachlorodibenzo-*para*-dioxin; HxCDF, hexachlorodibenzofuran; M, male; ND, not detected; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; OCDD, octachlorodibenzo-*para*-dioxin; OCDF, octachlorodibenzofuran; PCP, pentachlorophenol; PeCDD, pentachlorodibenzo-*para*-dioxin; ppm, parts per million; TCDD, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TCP, trichlorophenol; vs, versus; wk, week

or liver was also seen in female mice (McConnell et al., 1991; NTP, 1989). [The study complied with the requirements of good laboratory practice (United States Food and Drug Administration, GLP regulations).]

In a study by Tasaki et al. (2014), 50 male wildtype (*Nrf2*$^{+/+}$) and 50 male *Nrf2*-deficient (*Nrf2*$^{-/-}$) mice (age, 7 weeks) were divided into three groups of 15–20 animals and fed diets containing pentachlorophenol (purity, 98.6%) at concentrations of 0, 600, or 1200 ppm for 60 weeks. The survival rates at concentrations of 0, 600, or 1200 ppm were 80%, 27%, and 0%, respectively, in *Nrf2*$^{+/+}$ mice, and 53%, 13%, and 0%, respectively, in *Nrf2*$^{-/-}$ mice. Statistically significant decreases in body-weight gain were observed from week 11 at the higher pentachlorophenol dose for both genotypes, and from week 16 in *Nrf2*$^{-/-}$ mice and from week 36 in *Nrf2*$^{+/+}$ mice treated at the lower dose.

Cholangiocarcinoma, characterized by infiltrative atypical epithelial cells scattered into the stroma or multilayered with mitotic cells, was observed in all treated mice except in *Nrf2*$^{+/+}$ mice treated with the lower dose of pentachlorophenol; the incidence of cholangiocarcinoma in *Nrf2*$^{-/-}$ mice treated with the higher dose of pentachlorophenol was significantly increased. Hepatocellular adenoma or hepatocellular carcinoma was observed in all groups, but only the incidence of hepatocellular adenoma in *Nrf2*$^{-/-}$ mice treated with pentachlorophenol at the higher dose was significantly increased compared with controls (Tasaki et al., 2014).

Groups of 10 male and 10 female *p53*$^{+/-}$ mice (age, 8–11 weeks) were fed diets containing pentachlorophenol (purity, 99%) at a concentration of 0, 100, 200, or 400 ppm for 26 weeks. No significant increase in tumour incidence was observed in exposed mice (Spalding et al., 2000). [This study is not included in Table 3.1.]

3.1.2 Skin application

Groups of 15 female hemizygous Tg.AC mice (age, 14 weeks) were treated with pentachlorophenol (purity, 99%) as a topically applied dose at 0, 0.75, 1.50, or 3.0 mg per mouse daily for 20 weeks. The initial doses were 0 (control), 3.0, 6.0, or 12.0 mg/mouse, but due to toxicity after the first application, the two higher doses were reduced to 0.75 mg and 1.5 mg, respectively. At 26 weeks, the intermediate dose and highest dose significantly increased the incidence of skin papilloma compared with controls (Spalding et al., 2000).

3.1.3 Initiation–promotion studies

Three groups of 20 male B6C3F$_1$ mice (age, 6 weeks) were fed diets containing pentachlorophenol (purity, 98.6%) at a concentration of 0, 600, or 1200 ppm for 13 weeks, with subsequent administration of drinking-water containing phenobarbital at a concentration of 500 ppm for 29 weeks. Three other groups were initiated with drinking-water containing *N*-nitrosodiethylamine (NDEA) at 20 ppm for 13 weeks and, after a 4-week recovery interval, fed diets containing pentachlorophenol at 0, 300, or 600 ppm for 25 weeks. The incidence of hepatocellular adenoma, and incidence and multiplicity of hepatocellular adenoma or carcinoma (combined) in mice treated with pentachlorophenol after NDEA initiation were significantly increased compared with those in mice given NDEA only. In contrast, in mice given pentachlorophenol as an initiator followed by phenobarbital, no significant enhancement of neoplastic lesions occurred (Umemura et al., 1999).

Groups of 15 male B6C3F$_1$ mice (age, 6 weeks) were given drinking-water containing NDEA at 20 ppm for 8 weeks, and after a 4-week interval were fed diets containing pentachlorophenol (purity, 98.6%) at a concentration of 0 (basal diet), 300, or 600 ppm for 23 weeks. Exposure

to pentachlorophenol at 600 ppm significantly promoted the induction of hepatocellular adenoma by NDEA, and also caused significant progression of NDEA-induced cystic biliary hyperplasia to cholangioma and cholangiocarcinoma (Umemura et al., 2003a).

Groups of 20 male B6C3F$_1$ mice (age, 6 weeks) were given drinking-water containing NDEA at 20 ppm for 13 weeks and then, after a 4-week interval, given diets containing pentachlorophenol (purity, 98.6%) at a concentration of 0 or 600 ppm for 25 weeks. In mice initiated with NDEA followed by treatment with pentachlorophenol, the incidences of cholangioma and cholangiocarcinoma were significantly increased compared with mice receiving NDEA only (Umemura et al., 2003b).

Four groups of 10 female CD-1 mice (age, 6 weeks) were treated with a single skin application of 7,12-dimethylbenz[*a*]anthracene in acetone as an initiator. One week later, three groups received pentachlorophenol by skin application at a dose of 2.5, 50, or 1000 µg twice per week for 24 weeks. The fourth group was treated with acetone only for 19 weeks, and served as negative control group. The incidence of skin papilloma was 0/10, 1/10, 3/10, and 3/10 in the groups treated with pentachlorophenol at a dose of 0, 2.5, 50, and 1000 µg, respectively (Chang et al., 2003). [The Working Group judged the study inadequate for the evaluation because of the shorter duration of the study for negative controls compared with the duration for pentachlorophenol-treated groups. This study is not included in Table 3.1.]

3.1.4 Co-carcinogenicity

[Three studies in mice investigated co-carcinogenicity, as described below; these studies are not included in Table 3.1.]

Eight groups of 36 adult female CD-1 mice (age, not reported; average initial weight, 25 g) were fed diets containing 1′-hydroxysafrole at a concentration of 0.14% or 0.27%, or safrole at a concentration of 0.13% or 0.25%, with or without pentachlorophenol (purity, > 99%) at 0.05% for 12 months, and thereafter control diet for 4 months. Concurrent administration of pentachlorophenol with 1′-hydroxysafrole or safrole significantly decreased the incidence and multiplicity of hepatomas of type (a), (b), or mixed types (a) and (b) [hepatocellular tumours], as compared with those induced by 1′-hydroxysafrole or safrole alone (Boberg et al., 1983).

In an intraperitoneal injection study, groups of 23–42 male B6C3F$_1$ mice were given pentachlorophenol (purity, > 99%) at a dose of 0.04 µmol/g bw either on postnatal day 12 or on postnatal days 8 and 12. The mice were then injected with either 1′-hydroxysafrole (0.05, 0.1, or 0.2 µmol/g bw) or NDEA (0.01 or 0.02 µmol/g bw) and killed 10 or 9 months later, respectively. Prior treatment with pentachlorophenol did not affect hepatocarcinogenicity induced by NDEA, but decreased hepatocarcinogenicity induced by 1′-hydroxysafrole (Boberg et al., 1983).

Groups of 35 female CD-1 mice (age, 6 weeks) were fed diets containing *N*-*N*-dimethyl-4-aminoazobenzene at 0.02% or 0.04%, or 4-aminoazobenzene (AB) at 0.018% or 0.035%, with or without pentachlorophenol (purity, > 99%) at 0.05% for 10 months, and thereafter control diet for up to 7–8 months. Co-treatment with pentachlorophenol significantly decreased the incidence and multiplicity of hepatomas of type (a) or mixed types (a) and (b) [hepatocellular tumours] induced by *N*-*N*-dimethyl-4-aminoazobenzene or 4-aminoazobenzene (Delclos et al., 1986).

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female F344/N rats (age, 6 weeks) were fed diets containing pentachlorophenol (purity, 99%) at a concentration of

0 (control), 200, 400, or 600 ppm for 2 years. A stop-exposure group of 50 male and 50 female rats received diet containing pentachlorophenol at 1000 ppm for 52 weeks, and control feed thereafter for the remainder of the 2-year study. Survival was greater than that of controls in males at 600 ppm and at 1000 ppm, but similar to that of controls in all other exposed groups. Mean body weights were generally lower than those of controls in rats at 400 and 600 ppm. Despite a transitory body-weight reduction, mean body weights of males and females of the stop-exposure group were similar to those of controls by the end of the study (Chhabra et al., 1999; NTP, 1999).

At 2 years, malignant mesothelioma originating from the tunica vaginalis of the testis was present in nine males in the group fed 1000 ppm for 52 weeks (18%) compared with one male in the control group (2%) ($P = 0.014$). The incidence of malignant mesothelioma in historical controls in feeding studies was 40/1354 (3.0 ± 2.3%; range, 0–8%) (Chhabra et al., 1999; NTP, 1999). The range for background incidence of tunica vaginalis mesothelioma is 0.2–5% (Maronpot et al., 2016). [The Working Group considered that the significantly increased incidence in the stop-exposure group of males compared with the matched controls was highly suggestive of a treatment-related effect.] Nasal squamous cell carcinomas were present in five males fed 1000 ppm for 52 weeks (10%) compared with one male in the control group (2%); this was not a significant increase in incidence, but exceeded the range in historical controls for this neoplasm (0–4%; incidence, 5/1341). No carcinogenic activity of pentachlorophenol was seen in male or female rats fed diets containing pentachlorophenol at 200, 400, or 600 ppm for 2 years, or in female rats in the stop-exposure group (Chhabra et al., 1999; NTP 1999). [The study was conducted under the requirements of good laboratory practice (United States Food and Drug Administration, GLP regulations).]

3.2.2 Co-carcinogenicity

Groups of male and female Wistar (MRC-W) rats (age, 8–10 weeks) were fed diets containing pentachlorophenol (technical-grade pentachlorophenol: purity, 86%; containing TCDD at 25 µg/kg and TCDF at 670 µg/kg) for 2 weeks before (and during) treatment with tap water or drinking-water containing 2-hydroxyethylnitrosourea (HENU) [a carcinogen] at a concentration of 75 mg/L (4 days per week) at 0 or 500 ppm for 86–88 weeks. The effective numbers of rats surviving more than 11 weeks were: 20, 15, and 5 in males, and 19, 15, and 9 in females in the groups receiving HENU only, HENU + pentachlorophenol, and pentachlorophenol only, respectively. All survivors were killed at age 94 weeks. HENU alone induced B-cell lymphoma and skeletal osteosarcoma, with higher incidences of both tumour types in males than in females, but incidences of these tumour types were not increased by co-treatment with pentachlorophenol. However, pentachlorophenol acted synergistically with HENU to significantly [$P < 0.05$] increase the incidence of acute myelocytic leukaemia in males (Mirvish et al., 1991).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

Oral absorption of pentachlorophenol is relatively rapid and extensive in all species studied. It distributes throughout the whole body, but preferentially in the viscera. In the blood, pentachlorophenol is extensively bound to plasma proteins, which partly explains its slow elimination. The observed elimination is slowest in humans, faster

in rats, and fastest in mice. Pentachlorophenol is mostly eliminated as glucurono- or sulfo-conjugates, and as tetrachlorohydroquinone (TCHQ) and its conjugates (IARC, 1991). Further oxidation to benzoquinones and their semiquinones has been demonstrated (Lin et al., 1997, 1999). The TCHQ (oxidative) pathway is minor in humans, and about as important as the conjugation pathway in rodents (Reigner et al., 1991, 1992a, c, 1993). For the same exposure dose, mice experience a 4-fold greater amount of protein adducts in liver nuclei than rats, whereas rats experience a 3-fold greater amount in liver cytosol (Lin et al., 1997, 1999). Published physiologically based pharmacokinetic models for pentachlorophenol were not available to the Working Group.

4.1.2 Absorption

(a) Humans

Oral absorption (in a case of acute poisoning) was rapid, with a plateau blood concentration reached by 2–4 hours after ingestion (Haley, 1977; Young & Haley, 1978). About 90% of the ingested dose was recovered in the urine (Braun et al., 1979; Reigner et al., 1992a). Absorption after inhalation is similarly high (Proudfoot, 2003).

Absorption through the skin is well documented qualitatively, but quantitative information is scarce. Although extensive, absorption through skin occurs to a lesser extent and more slowly than after ingestion (Williams, 1982; Proudfoot, 2003). However, in human cadaver skin in vitro, only 1–4% of the dose applied in acetone or soil was recovered in the skin or passed through it (Wester et al., 1993).

(b) Experimental systems

In the rhesus monkey (*Macaca mulatta*), absorption of pentachlorophenol through the skin was approximately 30% and 25% of the dose applied in acetone or soil, respectively, after 24 hours (Wester et al., 1993). After a single oral dose of 10 mg/kg of [^{14}C]-labelled pentachlorophenol, peak plasma concentrations (10–30 µg/g) were attained within 12–24 hours after administration (Braun & Sauerhoff, 1976). The three male monkeys seemed to absorb pentachlorophenol more slowly (absorption rate constant, 0.2 per hour) than the three females (rate constant, 0.4 per hour), but inter-subject variability was of the order of a factor of 2 [thus, the difference was probably not statistically significant.]

In male B6C3F$_1$ mice, a dose of 15 mg/kg of pentachlorophenol administered by gastric intubation was completely absorbed and peak concentrations were seen after 1 or 2 hours (Reigner et al., 1992c).

In male Sprague-Dawley rats, a bolus injection of 1–3 mg (the exact dose was not given) of [^{14}C]-labelled pentachlorophenol in the duodenum resulted in a peak portal-vein plasma concentration after 20 minutes, most of the substance being transported by the portal vein to the liver (Jandacek et al., 2009). In the same strain and sex, after oral administration of pentachlorophenol at 2.5 mg/kg, the peak plasma concentration occurred between 1.5 and 2 hours, with an absorption half-life ranging from 0.25 to 1.5 hours, and a bioavailability of 90% (Reigner et al., 1991). In Fisher 344 rats, the absorption of pentachlorophenol from the gastrointestinal tract after gavage doses of 9.5 and 38 mg/kg was first order with an absorption half-life of about 1.3 hours, and a bioavailability of more than 80% (Yuan et al., 1994). Similar results were obtained by Braun et al. (1977).

The permeability of pork skin [which resembles human skin] to pentachlorophenol has been studied with various solvents, showing that pentachlorophenol in aqueous solutions is fairly well and rapidly absorbed (10% absorption, with a peak at 4 or 5 hours) (Baynes et al., 2002).

4.1.3 Distribution

(a) Humans

Blood and urine levels in occupationally exposed people and the general population have been extensively measured (see also Section 1.4; IARC, 1991; Bader et al., 2007; Carrizo et al., 2008). Pentachlorophenol has also been measured in breast milk and umbilical cord blood (Sandau et al., 2002; Guvenius et al., 2003; Hong et al., 2005; Park et al., 2008). Pentachlorophenol found in the blood is extensively bound to plasma proteins: at least 96% is bound, according to Uhl et al. (1986), and 99.5% according to Reigner et al. (1993). This explains for the most part the long half-life of pentachlorophenol in humans (Reigner et al., 1993). Post-mortem tissues from 21 people from the general population of northern Bavaria, Germany, showed pentachlorophenol (in decreasing order of concentration) in the liver, kidney, brain, spleen, and fat (Grimm et al., 1981; Proudfoot, 2003). In a fatal case of pentachlorophenol poisoning, the highest concentrations of pentachlorophenol were found in the bile and renal tissue, with lower concentrations in the lung, liver, and blood (Ryan et al., 1987; Proudfoot, 2003).

(b) Experimental systems

In two female rhesus monkeys, radioactivity was measured in the major organs 15 days after the oral administration of a single dose of [^{14}C]-labelled pentachlorophenol at 10 mg/kg. About 10% of the administered dose was recovered (the rest was excreted in the urine and faeces). The liver, small intestine, and large intestine contained the largest fractions of radioactivity (1%, 5%, and 2%, respectively) (Braun & Sauerhoff, 1976). In monkeys, about 99% of pentachlorophenol in blood plasma is bound to proteins (Reigner et al., 1993).

In female NMRI mice given a subcutaneous or intraperitoneal injection of [^{14}C]pentachlorophenol at 15–37 mg/kg bw, the highest specific activity was found in the gall bladder and its contents, the wall of the stomach fundus, the contents of the gastrointestinal tract, and the liver. Only traces (less than 0.05%) were detected in exhaled air (Jakobson & Yllner, 1971). In male B6C3F$_1$ mice, plasma protein binding was high (98.8%), but lower than in the other species tested (rat, monkey, human, cow, by increasing order of binding) and the blood to plasma concentration ratio was about 0.6 (Reigner et al., 1992c, 1993). After intravenous injection or stomach intubation with pentachlorophenol at 15 mg/kg, blood plasma kinetics were well described by a one-compartment model (Reigner et al., 1992c).

In rats (strain, not reported), 40 hours after a single oral administration of [^{14}C]pentachlorophenol at 31–40 mg/kg, the highest levels of radioactivity were found in the liver, kidney, and blood (Larsen et al., 1972). Similar results were obtained in female Sprague-Dawley rats after oral administration of a single dose of radiolabelled pentachlorophenol at 10 or 100 mg/kg, with plasma concentration peaking after 4 to 6 hours (Braun et al., 1977). Distribution to tissues was rapid and no distribution phase was observed (Braun et al., 1977; Reigner et al., 1991; Yuan et al., 1994). Plasma protein binding of pentachlorophenol in rats was about 99%, higher than in mice, but lower than in humans (Schmieder & Henry, 1988; Reigner et al., 1993). Modelling studies of the kinetics of pentachlorophenol in rats show that a one-compartment model with zero-order input and kinetic parameters estimated after intravenous administration adequately predicted pentachlorophenol concentrations in the plasma during long-term exposures to drinking-water containing pentachlorophenol (such as during carcinogenicity experiments) (Reigner et al., 1992b; Yuan, 1993, 1995).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

See Fig. 4.1

(i) Humans

Conjugation with glucuronic acid is the major route of metabolism in humans, with about 80–90% of the administered dose (regardless of its value) being found as glucuronide in the urine (Uhl et al., 1986; Reigner et al., 1992a). An earlier study [with pentachlorophenol of unspecified purity] found much less glucuronide in the urine (Braun et al., 1979). [The Working Group noted that the discrepancy is likely due to the analytical method and the choice of volunteers with low blood concentrations of pentachlorophenol before controlled exposure, as discussed by Reigner et al. (1992a).] Dechlorination is a (minor) route of metabolism in humans (Ahlborg et al., 1974) and the formation of TCHQ and its glucuronide represents probably no more than 10–20% of the administered dose. Here also, discrepancies between studies are best explained by differences in analytical methods (Reigner et al., 1992a). Palmitoylpentachlorophenol, a conjugate of pentachlorophenol with palmitic acid, has been found in human abdominal adipose tissue; quantitatively, it is a minor metabolite: for exposures in a typical range of 10–100 µg/day (Reigner et al., 1992a), the level in one individual was of the order of 0.2 µg/g (Ansari et al., 1985). The formation of the metabolites is slow (50% of them are formed in about 5–10 days), mostly because of the high plasma-protein binding (Reigner et al., 1992a) and possible enterohepatic cycling [which the Working Group considered probable] (Braun et al., 1979).

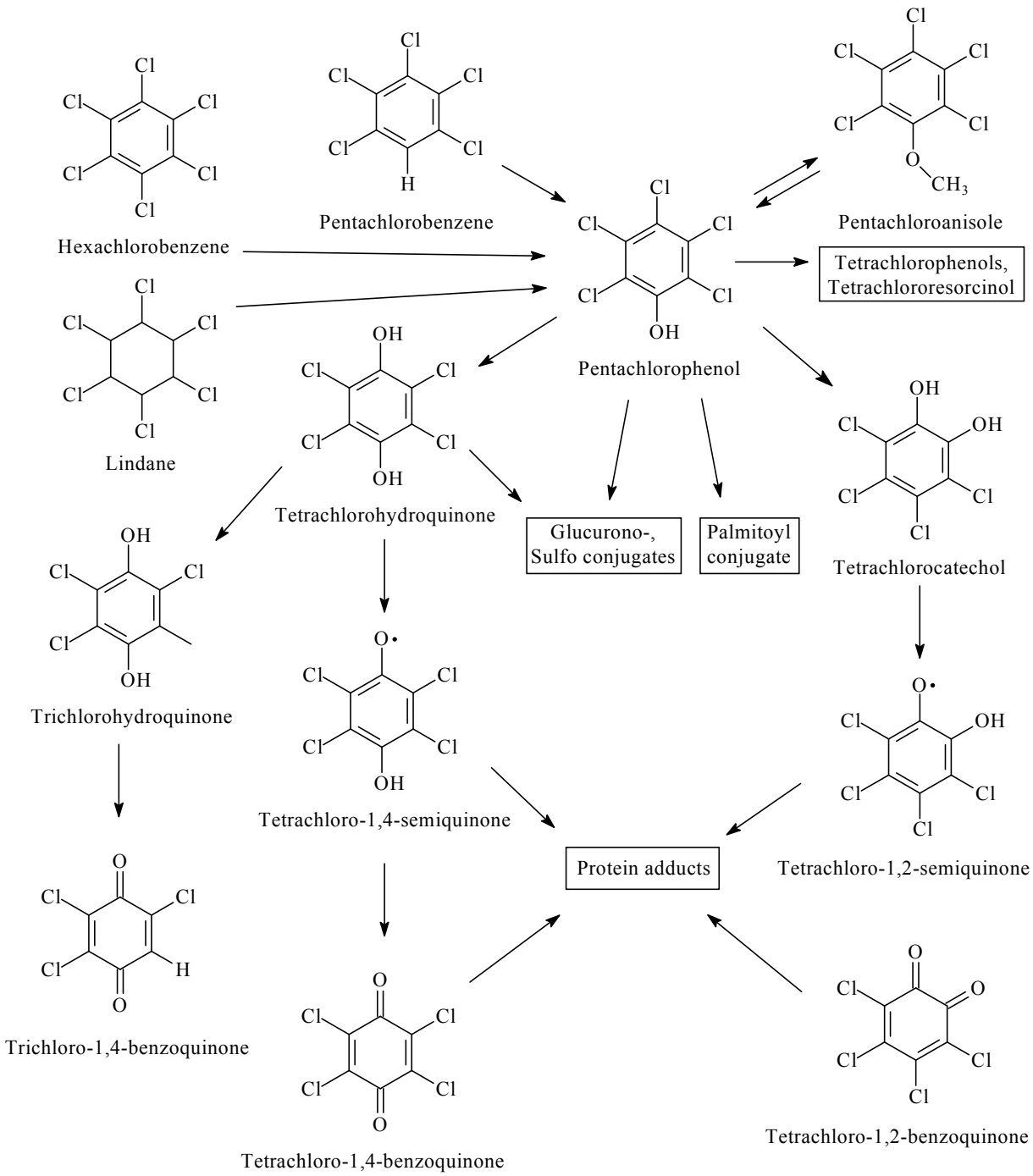
Human liver microsomes in vitro were able to metabolize pentachlorophenol of unspecified purity into TCHQ (Juhl et al., 1985) and glucurono-conjugates (Lilienblum, 1985). Oxidation may involve cytochrome P450 3A4 (Proudfoot, 2003). Pentachlorophenol and other chlorinated phenols are also substrates for purified human hydroxysteroid sulfotransferase hSULT2A1 (Gulcan et al., 2008).

(ii) Experimental systems

In mice, most early studies did not control well for hydrolysis and degradation of the oxidized and conjugated metabolites, making these studies difficult to interpret. The most definitive study, by Reigner et al. (1992c) in male B6C3F$_1$ mice, showed that TCHQ was formed together with glucurono- and sulfo-conjugates of both pentachlorophenol and TCHQ (Reigner et al., 1992c). In B6C3F$_1$ mice treated with pentachlorophenol at a dose of 20 mg/kg bw by gavage, liver protein adducts were formed by reactions with the pentachlorophenol metabolites tetrachloro-1,4-benzoquinone (tetrachloro-*para*-benzoquinone) and tetrachloro-1,2-benzoquinone (tetrachloro-*ortho*-benzoquinone) (Lin et al., 1997). Quantitative time courses were reported.

In rats (mainly Sprague-Dawley), the presence of free pentachlorophenol and TCHQ and their glucurono- or sulfo-conjugates was demonstrated in the urine after pentachlorophenol exposure (Ahlborg et al., 1974; Reigner et al., 1991). The rapid oxidative dechlorination of pentachlorophenol to TCHQ is mediated by liver microsomal enzymes. TCHQ can be further dechlorinated to trichlorohydroquinone (Ahlborg et al., 1980). The metabolites isolated from rat urine and identified were: 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, tetrachlorocatechol (tetrachloro-*ortho*-hydroquinone), tetrachloro-resorcinol, trichlorohydroquinone, TCHQ, and traces of trichloro-1,4-benzoquinone, and tetrachloro-1,4-benzoquinone (Renner & Hopfer, 1990). Rat liver microsomes also convert pentachlorophenol to its glucuronide, but not very efficiently (Lilienblum, 1985). In vitro, pentachlorophenol can be esterified with palmitic acid by rat liver microsomes in the

Fig. 4.1 Metabolism of pentachlorophenol based on human and animal observations



Compiled by the Working Group

presence of coenzyme A (Leighty & Fentiman, 1982). In the same system, oxidative dechlorination of pentachlorophenol forms TCHQ and tetrachlorocatechol, which are oxidized to tetrachloro-1,4-benzoquinone and tetrachloro-1,2-benzoquinone, respectively (van Ommen et al., 1986; Lin et al., 1997, 1999). Five cysteinyl adducts of haemoglobin and albumin have been identified in the blood of rats given pentachlorophenol at a dose of up to 40 mg/kg bw. Those adducts were formed by reactions with the pentachlorophenol metabolites tetrachloro-1,4-benzoquinone and its semiquinones (Waidyanatha et al., 1996).

Pentachlorophenol is a metabolite of hexa- and pentachlorobenzene in rat, mouse, guinea-pig, laying hen, and rainbow trout. It has also been identified as a urinary metabolite of lindane in rats and rabbits (Ahlborg et al., 1980; Umegaki & Ichikawa, 1989).

Pentachlorophenol can be methylated by some fungi and bacteria to form pentachloroanisole (Vodicnik et al., 1980). Pentachloroanisole, in turn, is rapidly demethylated to pentachlorophenol in rats and mice (Yuan et al., 1993).

(b) Modulation of metabolic enzymes

(i) Humans

Pentachlorophenol is a strong inducer of cytochrome P450 enzymes, especially CYP3A, in cultured human hepatoma cells (Dubois et al., 1996). Also in vitro, pentachlorophenol inhibited acetylcholinesterase activity in the membrane of human erythrocytes (Matsumura et al., 1997). It decreased the expression of mRNA of several enzymes (CYP11A, CYP17, CYP19, 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase) involved in steroidogenesis in the human adrenocortical carcinoma cell line H295R in vitro (Ma et al., 2011).

(ii) Experimental systems

Pentachlorophenol is an inhibitor of *O*-acetyltransferase and sulfotransferase family 1 enzymes (Mulder & Scholtens, 1977; Shinohara et al., 1986) and has been used as such in many experimental systems. In female Wistar rats fed diets containing pentachlorophenol, liver cytochrome P450 was induced (Vizethum & Goerz, 1979).

4.1.5 Excretion

(a) Humans

Renal excretion of unconjugated pentachlorophenol is a minor pathway of elimination in humans, partly because of the extensive binding of pentachlorophenol to plasma proteins, leaving only a small fraction available for renal filtration (Reigner et al., 1992a). After a single oral dose, most of the administered dose is found in the urine as glucurono-conjugates, with a plasma and urinary excretion half-life of about 10–20 days (Uhl et al., 1986; Reigner et al., 1992a, 1993). This leads to significant accumulation in the body after repeated doses: for a given quantity absorbed per day, the quantity found in blood plasma is about six times higher at steady state than for a single dose (Reigner et al., 1992a).

(b) Experimental systems

In monkeys exposed orally to a single dose of [^{14}C]pentachlorophenol at 10 mg/kg, about 70–80% of the radioactivity was recovered in the urine after 15 days and 10–20% in the faeces, with linear kinetics and a half-life values for plasma clearance of about 80 hours for both males and females (Braun & Sauerhoff, 1976). Faecal excretion was steady, indicating that enterohepatic circulation [of the glucuronide, most probably] was occurring. Up to 30% of an oral dose of [^{14}C] pentachlorophenol of 50 mg/kg was excreted in the bile of rhesus monkeys during 1 day (Rozman, et al., 1982). Given the extensive enterohepatic cycling, pentachlorophenol is likely to be mainly

eliminated by glucurono-conjugation, but the sample preparation methods used by Braun & Sauerhoff (1976) did not prevent lysis of the conjugates and did not permit its observation in the urine (Reigner et al., 1993).

In male B6C3F$_1$ mice, after either intravenous or oral administration of pentachlorophenol, the elimination half-life from blood plasma was about 5–6 hours. After 48 hours, only 60–70% of the dose administered (15 mg/kg) was recovered in the urine and faeces. [The Working Group noted that the remainder was most likely retained in the body]. In the urine, 7–9% of the dose administered was excreted as free pentachlorophenol, 3–6% as free TCHQ, 1% as pentachlorophenol glucuronide, 1–3% as tetrachlorohydroquinone glucuronide, 15% as pentachlorophenol sulfate and 15% as tetrachlorohydroquinone sulfate. In the faeces, 6–9% of the dose administered was pentachlorophenol (free and conjugates), and less than 1% was TCHQ (free and conjugates) (Reigner et al., 1992c).

In Sprague-Dawley rats (three male and three females) given a single oral dose of [^{14}C] pentachlorophenol at 10 or 100 mg/kg, urine and faeces were collected at 24-hour intervals, and the animals killed after 8 or 9 days (Braun et al., 1977). Most of the radioactivity (80%), except for females at the higher dose (55%), was recovered from the urine within 8 or 9 days. Most of the remainder (20%) (40% for females at the higher dose) was recovered from the faeces. [The Working Group noted the small number of animals and the variability of the results.] Collection of the expired air at 12-hour intervals from the rats receiving the lower dose showed that less than 1% of the administered dose was excreted as [^{14}C]CO_2. Elimination of radioactivity from the plasma was biphasic with a first half-life of about 15 hours – similar to that found by Larsen et al. (1972) – and a second one that was poorly identified (except in females at the higher dose, for which elimination was monophasic with a half-life of 30 hours). In another study in Sprague-Dawley rats (males only), a biphasic elimination profile from plasma was observed with improved analytical and statistical analyses (Reigner et al., 1991). The first elimination phase had a half-life of 6–8 hours after treatment with pentachlorophenol at a dose of 2.5 mg/kg by intravenous injection or gavage. Those results were coherent with those obtained in Fischer 344 rats after treatment with pentachlorophenol by injection or in the diet at a dose of 5, 9.5, or 38 mg/kg (Yuan et al., 1994). In male Sprague-Dawley rats, a terminal half-life of 35 hours was observed after administration of [^{14}C]pentachlorophenol at a dose of 20 mg/kg by intravenous injection. Irrespective of exposure route, about 60–70% of the 2.5 mg/kg dose was recovered in the urine after 72 hours (pentachlorophenol, 5%; TCHQ, 1%; conjugated pentachlorophenol, 20%; conjugated TCHQ, 30%). After either injection or gavage, 10% of the dose was recovered in faeces after chemical hydrolysis (9% pentachlorophenol and 1% TCHQ), indicating that biliary excretion and [most likely] enterohepatic cycling contribute to elimination (Reigner et al., 1991).

4.2 Mechanisms of carcinogenesis

This section summarizes in the following order the available evidence for the key characteristics of carcinogens (Smith et al., 2016), concerning whether pentachlorophenol induces oxidative stress; is genotoxic; modulates receptor-mediated effects; alters cell proliferation, cell death, or nutrient supply; induces chronic inflammation; and is immunosuppressive. For the other key characteristics of human carcinogens, insufficient data were available for evaluation.

4.2.1 Oxidative stress

(a) Humans

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

Michałowicz (2010) reported that pentachlorophenol (0.01 μg/mL and higher) caused small increases in concentrations of reactive oxygen species (ROS) in human lymphocytes isolated from four healthy nonsmoking donors (see Section 4.2.2).

A series of studies examined the role of TCHQ, a known metabolite of pentachlorophenol, in oxidative stress induced by pentachlorophenol (see Section 4.2.2). TCHQ caused DNA strand breaks (Witte et al., 1985) that were suppressed by desferrioxamine, an iron chelator (Carstens et al., 1990). In follow-up studies, desferrioxamine inhibited TCHQ-induced DNA damage by scavenging the reactive tetrachlorosemiquinone radical (Witte et al., 2000). In HepG2 cells, tetrachlorobenzoquinone was genotoxic (Dong et al., 2014). Tetrachlorobenzoquinone also increased phosphorylation of histone γH2AX, and increased 8-hydroxy-2′-deoxyguanosine (8-OHdG) and ROS in these cells. *N*-acetylcysteine attenuated both the oxidative-stress markers and the genotoxicity induced by tetrachlorobenzoquinone.

(b) Non-human mammalian experimental systems

(i) Studies on pentachlorophenol in vivo

Sai-Kato et al. (1995) reported dose-dependent increases in 8-OHdG in the liver but not in the kidney or spleen of mice exposed to pentachlorophenol. Prior exposure to vitamin E, but not to vitamin C or to β-carotene, attenuated the pentachlorophenol-induced hepatic 8-OHdG.

Umemura and colleagues examined the role of oxidative stress in toxicity attributable to pentachlorophenol (purity, 98.6%) in a series of studies by evaluating 8-OHdG as measured by high-performance liquid chromatography–electrochemical detection. In mice treated for 2 and 4 weeks, pentachlorophenol (300, 600, and 1200 ppm in the diet) caused dose-dependent increases in hepatic 8-OHdG, liver weights, hepatotoxicity, and the 5-bromo-2′-deoxyuridine (BrdU) labelling index (Umemura et al., 1996). When given alone for 8 weeks, pentachlorophenol increased hepatic 8-OHdG in a dose-dependent manner (Umemura et al., 1999). In an initiation–promotion study (with diethylnitrosamine as initiator), green tea decreased the number of mice with adenomas and the average number of tumours per mouse only at the highest dose of pentachlorophenol (Umemura et al., 2003a, b). The lowest and the highest doses of pentachlorophenol increased 8-oxodeoxyguanosine (8-OHdG) levels in liver DNA and the labelling index in both hepatocytes and extrabiliary epithelial cells. Green tea decreased the pentachlorophenol-induced increases in 8-OHdG and the increases in labelling indices (Umemura et al., 2003b). The decrease in labelling indices induced by green tea may result from the attenuation of pentachlorophenol-induced hepatotoxicity.

Umemura and colleagues have also evaluated the role of oxidative stress in the toxicity and carcinogenicity of pentachlorophenol using a variety of transgenic mice. In Umemura et al. (2006), mice deficient in nuclear factor erythroid 2-related factor (*Nrf2*$^{-/-}$) and their heterozygote (*Nrf2*$^{-/+}$) and homozygote (*Nrf2*$^{+/+}$) controls were given diets containing pentachlorophenol at 150–1200 ppm for 4 weeks. End-points included measures of oxidative stress (hepatic 8-OHdG and thiobarbituric acid-reactive substances), hepatotoxicity (increased liver weight, serum biochemistry, and cell proliferation), and changes in expression of NAD(P):quinone oxidoreductase 1, UDP-glucuronosyltransferase, and CYP1A2. At the highest dose, pentachlorophenol increased levels of hepatic 8-OHdG and thiobarbituric acid-reactive substances only in the *Nrf2*$^{-/-}$ knockout mice. Increases in hepatocyte proliferation were observed at all doses in the *Nrf2*$^{-/-}$ mice and in the *Nrf2*$^{-/+}$ heterozygotes. Pentachlorophenol increased hepatocyte proliferation in the wildtype mice at all doses except at 150 ppm (Umemura et al., 2006). In a separate

study of *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice, long-term exposure to pentachlorophenol increased the incidence of cholangiofibrosis in mice of either genotype (at concentrations of 600 and 1200 ppm *Nrf2*$^{-/-}$, and at 1200 ppm in *Nrf2*$^{+/+}$ mice) (Tasaki et al., 2014; see also Section 3.1).

The guanine-hypoxanthine phosphoribosyl transferase (*gpt*) delta transgenic mouse model was also used to evaluate the role of pentachlorophenol in oxidative stress and genotoxicity (Tasaki et al., 2013). The *gpt* delta animal model can detect point mutations within the *gpt* gene and deletion mutations within the *red/gam* (Spi$^-$) gene (Masumura et al., 2003; Hibi et al., 2011). Tasaki et al. crossed *p53*$^{-/-}$ mice with *gpt* transgenic mice and reported that exposure to pentachlorophenol for 13 weeks increased levels of 8-OHdG and NAD(P):quinone oxidoreductase 1 in the liver in *p53* wildtype and *p53*$^{-/-}$ mice. No increases in the frequency of *gpt* and *red/gam* mutations were observed in either the *p53* wildtype or *p53*$^{-/-}$ mice (Tasaki et al., 2013; see also Table 4.3, Section 4.2.2).

Bordelon et al. (2001) injected mice (age, 15 days) with pentachlorophenol as a single dose of up to 100 mg/kg bw. No 8-OHdG adducts were detected in the liver using [^{32}P]-postlabelling. No signs of toxicity were observed in the infant mice; the median lethal dose (LD_{50}) for pentachlorophenol in adult mice in this study was 50 mg/kg bw.

Lin et al. (2002) reported increased 8-OHdG adduct formation using high-performance liquid chromatography–electrochemical detection in the liver of male rats exposed to pentachlorophenol at 60 mg/kg bw per day by gavage for 27 weeks; no 8-OHdG adducts were detected after a shorter exposure (5 days) at this dose or at 120 mg/kg per day. Two major DNA adducts were detected using [^{32}P]-postlabelling after nuclease P1 adduct enrichment. One co-migrated with adducts formed by the metabolite tetrachloro-1,4-benzoquinone. This adduct appeared to be formed in parallel with 8-OHdG in the chronically exposed rats only, at levels 10 times lower than those of 8-OHdG adducts (Lin et al., 2002). In another study in male rats, 8-OHdG lesions and chromosomal aberrations were not induced in the liver after exposure to an intraperitoneal dose of pentachlorophenol at 10 mg/kg per day for 5 days, but the frequency of sister-chromatid exchange was significantly increased (Daimon et al., 1997) (see also Table 4.3, Section 4.2.2).

(ii) Studies on metabolites of pentachlorophenol in vivo

In mice treated with pentachlorophenol (purity, not reported; 40 mg/kg bw) or TCHQ (20 mg/kg bw) by intraperitoneal administration and necropsied 6 hours after exposure, glutathione (GSH) levels in the liver were depleted by 65% by TCHQ, but were unaltered by pentachlorophenol (Wang et al., 1997). In rats given a single intraperitoneal injection of pentachlorophenol (40 mg/kg bw) or TCHQ (15 mg/kg bw), with or without a 2-hour pretreatment with vitamin E (100 mg/kg bw), there was an increase in urinary levels of 8-epi-prostaglandin F2α, a major F2-isoprostane that is increased by free-radical mediated arachidonic acid oxidation (Wang et al., 2001). The increase in 8-epi-prostaglandin F2α was associated with increases in serum alanine aminotransferase and aspartate aminotransferase, and was attenuated by co-administration of vitamin E. TCHQ was more effective than pentachlorophenol in all cases (Wang et al., 2001).

Mice fed diet containing TCHQ (300 mg/kg bw per day) for 2 weeks had increased hepatic 8-OHdG (measured by liquid chromatography–electrochemical detection) (Dahlhaus et al., 1994). No increases in oxidative stress were observed in mice given a single intraperitoneal dose of TCHQ (20 or 50 mg/kg bw), 6 or 24 hours after exposure (Dahlhaus et al., 1994; see also Section 4.2.2).

(iii) Studies on pentachlorophenol metabolites in vitro

In splenocytes isolated from male ICR mice and exposed to pentachlorophenol (purity, > 98%; 25, 50, and 100 μM) or TCHQ (12.5, 25, and 50 μM), ROS were increased by TCHQ in a dose-dependent manner as measured using dichlorodihydrofluorescein diacetate (DCFH-DA). Viability began decreasing at 15 minutes, falling to 50–60% at the lowest dose, and 20–40% at the higher doses at 6 hours (Chen et al., 2014).

Siraki et al. (2004) evaluated a variety of *para*-benzoquinones, including tetrachloro-*para*-benzoquinone [tetrachloro-1,4-benzoquinone], in rat primary hepatocytes and pheochromocytoma (PC12) cells. Tetrachloro-*para*-benzoquinone induced ROS at concentrations that were 10 times lower than those for the half-maximal response (EC_{50}) for GSH depletion in rat hepatocytes or the PC12 cells. Of the 14 benzoquinone derivatives, tetrachloro-*para*-benzoquinone was the most potent for cytotoxicity and ROS formation. The potency of tetrachloro-*para*-benzoquinone for GSH depletion was similar to that of five other benzoquinone derivatives.

In mouse embryonic fibroblast NIH 3T3 cells exposed to TCHQ (5–50 μM) for 30 minutes, cell viability was significantly decreased in a dose-dependent manner and this was attenuated by co-treatment with *N*-acetylcysteine. In the same cells, apoptosis was observed after exposure to TCHQ (50 μM) for 8 hours (Wang et al., 1997; see Section 4.2.4).

TCHQ induced 8-OHdG adducts in Chinese hamster V79 lung fibroblasts (Dahlhaus et al., 1995, 1996; see also Section 4.2.2). Dahlhaus et al. (1996) observed that pentachlorophenol and tetrachloro-*ortho*-hydroquinone (also named tetrachlorocatechol) did not induce 8-OHdG, whereas 8-OHdG was increased by tetrachloro-*para*-hydroquinone, tetrachloro-*para*-benzoquinone [tetrachloro-1,4-benzoquinone], and tetrachloro-*ortho*-benzoquinone [tetrachloro-1,2-benzoquinone]. Tetrachloro-*para*-hydroquinone is the main metabolite of pentachlorophenol, while tetrachloro-*para*-benzoquinone and tetrachloro-*ortho*-hydroquinone are minor metabolites in rats and humans (Juhl et al., 1985).

(c) Non-mammalian experimental systems

In various species of fish, exposure to pentachlorophenol increased oxidative stress, decreased the glutathione/oxidized glutathione (GSH/GSSG) ratio, and altered genes and proteins involved in the response to oxidative stress (Thomas & Wofford, 1984; Zhang et al., 2008; Luo et al., 2009). Pentachlorophenol (purity, > 98%) was shown to be an uncoupler of oxidative phosphorylation in zebrafish embryos (Xu et al., 2014). No effect of pentachlorophenol (3.75–75 μM) on lipid peroxidation was seen in the digestive gland from mussels (Milowska et al., 2003). Pietsch et al. (2014) demonstrated effects of pentachlorophenol or TCHQ on ROS, superoxide dismutase, and cell viability in rainbow trout liver RTL-W1 cells that were shown to metabolize pentachlorophenol to TCHQ.

In yeast, pentachlorophenol increased concentrations of superoxide dismutase in *Humicola lutea* 110 (Angelova et al., 1995).

The antioxidants butylated hydroxytoluene and butylated hydroxyanisole increased toxicity and delayed cell growth in *Pseudomonas fluorescens* bacteria co-treated with pentachlorophenol (Trevors et al., 1981).

(d) Acellular systems

Naito et al. (1994) reported 8-OHdG in calf thymus DNA co-exposed to TCHQ and Cu(II) (20 μM) (see Section 4.2.2). DNA damage was attenuated by copper chelators and H_2O_2 scavengers, suggesting that Cu(I) and hydrogen peroxide (H_2O_2) were involved in the mechanism of DNA damage. Incubation of TCHQ with H_2O_2 produced hydroxyl radicals (·OH), which could not be inhibited by the presence of several iron chelators. [The Working Group noted that

TCHQ can react directly with H_2O_2 to produce hydroxyl radicals in a reaction independent of the classic Fenton system (Zhu et al., 2000; Zhu & Shan, 2009)]. However, in *Escherichia coli*, the presence of copper can enhance the cytotoxicity of pentachlorophenol (Zhu & Chevion, 2000; Zhu et al., 2001).

4.2.2 Genetic and related effects

Studies on pentachlorophenol have been carried out in exposed humans, in human cells in vitro, in other mammals in vivo, and in non-mammalian systems, as summarized in Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5, and Table 4.6. If purity was not reported in the study, pentachlorophenol was considered to be of technical grade (approximately 90% pentachlorophenol and 10% contaminants).

(a) Humans

(i) Exposed humans

See Table 4.1.

Several studies of genetic effects in humans occupationally exposed to pentachlorophenol were available. Bauchinger et al. (1982) (also reported in Schmid et al., 1982) reported increases in chromosomal aberrations, but not in sister-chromatid exchanges, in 22 male workers in a pentachlorophenol-producing factory (14 workers exposed to Na-PCP and 8 to pentachlorophenol). All workers were smokers, and duration of exposure ranged from 1 to 30 years. The matched control group was of 22 unexposed workers (9 smokers) from similar employment settings; however, pentachlorophenol was measured in the blood and urine of pentachlorophenol-factory workers but not in the controls. Increases in the frequencies of chromosome-type aberrations (i.e. dicentric chromosomes and acentric fragments) were not influenced by smoking habits. There was no effect on the frequency of sister-chromatid exchange when smoking was controlled for.

Ziemsen et al. (1987) also measured chromosomal aberrations and sister-chromatid exchange in 20 workers exposed for 3–34 years during production of wood preservatives that consisted of pentachlorophenol and Na-PCP. Exposure was estimated by measurement of serum concentration of pentachlorophenol. No association was found between frequency of chromosomal aberrations or sister-chromatid exchange in peripheral lymphocytes and duration of employment, age, smoking status, type of exposure (pentachlorophenol or Na-PCP), or serum concentration of pentachlorophenol.

Another small study reported no significant increase in the frequency of chromosomal aberrations in six workers from a pentachlorophenol wood-treatment plant; four unmatched controls were used for comparisons (Wyllie et al., 1975). Exposure was estimated by measurement of concentration of pentachlorophenol in the serum and urine.

(ii) Human cells in vitro

See Table 4.2.

Several studies in human cells in vitro reported using the comet assay to detect DNA strand breaks (Michałowicz, 2010; Michałowicz & Majsterek, 2010; Stang & Witte, 2010; Tisch et al., 2005; Ozaki et al., 2004; Jin et al., 2012). Michałowicz (2010) reported a significant, dose-dependent increase in percentage DNA damage as measured by the alkaline comet assay in primary lymphocytes exposed to pentachlorophenol (purity, 99.5%) at 0.2, 1.0, or 5.0 µg µg/mL for 1 hour. These concentrations also increased levels of ROS detectable by the fluorescent probe 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), but cell viability was only decreased at ≥125 µg/mL. Michałowicz & Majsterek (2010) repeated these findings using repair enzymes to detect oxidized DNA bases in the comet assay. Stang & Witte, (2010) reported that pentachlorophenol (purity, not reported) induced dose-dependent DNA

Table 4.1 Genetic and related effects of pentachlorophenol in exposed humans

End-point	Tissue	Cell type if specified	Description of exposure and controls	Mean exposure level	Response, significance[a]	References
Chromosomal aberrations	Blood,	Lymphocytes	22 male workers at a PCP plant (8: PCP; 14: Na-PCP) and 22 male controls	< 0.1 to > 0.5 mg/m^3 (TWA)	+ (P = 0.004)	Bauchinger et al. (1982); Schmid et al. (1982)
Sister-chromatid exchange					–	
Chromosomal aberrations	Blood	Lymphocytes	20 workers at PCP wood-preservative production plant	180 µg/m^3	–	Ziemsen et al. (1987)
Sister-chromatid exchange					–	
Chromosomal aberrations	Blood	NR	6 PCP wood-treatment plant workers and 4 unexposed controls; Idaho	1887.9 ng/m^3 for 5 mo	–	Wyllie et al. (1975)

[a] +, positive; –, negative

mo, month; Na-PCP, sodium pentachlorophenate ; NR, not reported; PCP, pentachlorophenol; TWA, time-weighted average

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

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	References
		Without metabolic activation	With metabolic activation			
DNA strand breaks, alkaline comet assay	Lymphocytes (primary)	+	NT	0.2 µg/mL	Purity, 99.5%	Michałowicz (2010)
DNA strand breaks, comet assay + Fpg and Endo III enzymes	Lymphocytes (primary)	+	NT	0.2 µg/mL	Purity, 99.5%	Michałowicz & Majsterek (2010)
DNA strand breaks, high-throughput comet assay	Lymphocytes (primary)	–	+	0.5 mM [133 µg/mL]	Purity, NR	Stang & Witte (2010)
DNA strand breaks, high-throughput comet assay	Liver, HepG2	+	NT	1 mM [266 µg/mL]	Purity, NR	Stang & Witte (2010)
	Fibroblast, NHDF-p	–	+	1.25 mM [333 µg/mL]		
	Cervical carcinoma, HeLa	–	+	1.15 mM [306 µg/mL		
DNA strand breaks, comet assay and microgel electrophoresis	Cervical carcinoma, HeLa	–	NT	50 µM [13.5 µg/mL]	Purity, NR	Jin et al. (2012)
Chromosomal aberrations, sister-chromatid exchange	Nasal mucosal epithelial cells (primary)	+	NT	1.2 M [3.2×10^5 µg/mL]	Purity, > 99.5%	Tisch et al. (2001, 2005)
	Lymphocytes (primary)	–	NT	90 µg/mL	Na-PCP, technical grade; purity, 85%	Ziemsen et al. (1987)

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

Endo III, endonuclease III; Fpg, formamidopyrimidine DNA glycosylase; HIC, highest ineffective concentration; LEC, lowest effective concentration; Na-PCP, sodium pentachlorophenate; NR, not reported; NT, not tested

damage in a high-throughput comet assay in HepG2 cells without S9, and in primary lymphocytes, fibroblasts, and HeLa cells (and in CHO V79 cells, described below) in the presence of S9. Pentachlorophenol (purity, not reported) gave negative results in a separate study in HeLa cells (Jin et al., 2012), and DNA damage induced by pentachlorophenol (purity, > 99%) in the human promyelocytic leukaemia cell line HL-60 was attributed to high toxicity (51% viable cells) (Ozaki et al., 2004). Concentration-dependent induction of DNA damage in primary mucosal epithelial cells isolated from human nasal conchae was induced by pentachlorophenol (purity, > 99.5%) (Tisch et al., 2001, 2005).

Technical-grade Na-PCP (purity, 85%; up to the cytotoxic concentration of 90 µg/mL) did not increase the frequency of chromosomal aberrations or sister-chromatid exchange in primary lymphocytes from healthy donors, exposed in vitro (Ziemsen et al., 1987).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

Studies that also reported on 8-OHdG and other end-points relevant to oxidative stress (Sai-Kato et al., 1995; Daimon et al., 1997; Tasaki et al., 2013) are discussed in Section 4.2.1.

No increase in the frequency of micronucleus formation was observed in mouse or rat bone marrow after intraperitoneal injection of pentachlorophenol (purity, 91.6%) every 24 hours for 3 days. The highest dose in mice (150 mg/kg bw) and rats (75 mg/kg bw) was lethal (NTP, 1999).

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Pentachlorophenol (purity, > 99.5%) did not induce forward (Jansson & Jansson, 1986) or reverse (Helleday et al., 1999) *Hprt* mutations in Chinese hamster V79 lung fibroblasts. Pentachlorophenol (purity, not reported; 6.66 µg/mL) did not induce DNA strand breaks (or 8-OHdG) in Chinese hamster V79 cells (Dahlhaus et al., 1996). However, significant DNA strand breakage was detected by the comet assay in another study in Chinese hamster V79 cells treated with pentachlorophenol at a higher concentration (266 µg/mL) and with S9 (Stang & Witte, 2010). In Chinese hamster ovary cells, DNA strand breaks were not detected after exposure to pentachlorophenol at 10 µg/mL (Ehrlich, 1990), but a marginal induction of chromosomal aberrations (80 µg/mL; only with S9) and sister-chromatid exchange (30 µg/mL; only without S9) was reported at slightly higher concentrations of pentachlorophenol (purity, 91.6%) (NTP, 1999).

(iii) Non-mammalian experimental systems in vivo

See Table 4.5.

In zebrafish, analytical-grade pentachlorophenol induced point mutations in the *Tp53* gene (Yin et al., 2006) and DNA adduct formation (Fang et al., 2015).

Pentachlorophenol produced chromosomal aberrations and/or micronuclei in freshwater fish (Farah et al., 2003, 2006) (purity, 99%), catfish (Ahmad et al., 2002) (purity, 99%), snails (Pavlica et al., 2000), and mussels (Pavlica et al., 2000; Villela et al., 2006), but not in frogs exposed as larvae (Venegas et al., 1993). Pentachlorophenol induced DNA strand breaks in mussels in vivo (Pavlica et al., 2001; Villela et al., 2006) and in vitro (Milowska et al., 2003), as well as in earthworms (Klobučar et al., 2011).

Pentachlorophenol did not induce nondisjunction or chromosome loss in *Drosophila melanogaster* (Ramel & Magnusson, 1979).

In the onion, pentachlorophenol (purity, 99%) induced chromosomal aberrations in one study (Ateeq et al., 2002), but not in another (Venegas et al., 1993). Micronucleus formation was observed in the onion (Repetto et al., 2001).

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

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regiment	Comments	References
DNA adducts (8-OHdG, HPLC-ECD)	Mouse, B6C3F$_1$ (M)	Liver	+	60 mg/kg	Gavage, single dose/6 h	Purity, 98.6%	Sai-Kato et al. (1995)
DNA adducts,^{32}P-postlabelling	Rat, F344/Du Crj (M)	Liver	–	10 mg/kg bw	i.p., 5 days	Purity, NR	Daimon et al. (1997)
Sister-chromatid exchanges	Rat, F344/Du Crj (M)	Liver	+				
Chromosomal aberrations			–				
Mouse spot test	Mouse, *p53*$^{+/+}$ or *p53*$^{-/-}$ C57BL/6 *gpt delta* (M)	Liver	–	6000–12 000 ppm	p.o., 13 wk		Tasaki et al. (2013)
Micronucleus formation	Mouse, B6C3F$_1$ (M)	Bone marrow (PCE)	–	150 mg/kg bw	i.p., 1×/day, 3 days	Purity, 91.6% HID was lethal	NTP (1999)
Micronucleus formation	Rat, F344/N, (M)	Bone marrow (PCE)	–	75 mg/kg bw	i.p., 1×/day, 3 days	Purity, 91.6% HID was lethal	NTP (1999)

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

bw, body weight; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; HPLC-ECD, high-performance liquid chromatography–electrochemical detection; LED, lowest effective dose; M, male; NR, not reported; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCE, polychromatic erythrocytes; p.o., oral; ppm, parts per million; wk, week

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

End-point	Cell line	Results[a]		Concentration (LEC or HIC)	Comments	References
		Without metabolic activation	With metabolic activation			
DNA strand breaks, alkaline elution assay	Chinese hamster ovary	–	NT	10 µg/mL	Purity, NR	Ehrlich (1990)
DNA strand breaks, alkaline elution assay	Chinese hamster fibroblast V79	–	NT	25 µM [6.66 µg/mL]	Purity, NR	Dahlhaus et al. (1996)
DNA strand breaks, high-throughput comet assay	Chinese hamster fibroblast V79	–	+	1 mM [266 µg/mL]	Purity, NR	Stang & Witte (2010)
Hprt mutation	Chinese hamster fibroblast V79	–	NT	50 µg/mL	Purity, > 99.5%	Jansson & Jansson (1986)
Hprt mutation	Chinese hamster fibroblast V79	–	NT	35 µg/mL (V79SPD8 clone), 40 µg/mL (V79Sp5 clone)	Purity, NR	Helleday et al. (1999)
Chromosomal aberrations	Chinese hamster ovary	–	(+)	80 µg/mL	Purity, 91.6%	Galloway et al. (1987); NTP (1989, 1999)
Sister-chromatid exchange		(+)	–	30 µg/mL		

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

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

Table 4.5 Genetic and related effects of pentachlorophenol in non-mammalian experimental systems in vivo

End-point	Species, strain, tissue	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Tp53 gene mutation	*Tuebingen* zebrafish, liver	+	5 µg/L (10 days)	Purity, > 98%	Yin et al. (2006)
DNA adducts (8-OHdG, ELISA)	*Danio rerio* zebrafish, AB strain embryos	+	30 µg/L (6 days post fertilization)	Purity, > 99% 8-OHdG lesions in larvae	Fang et al. (2015)
Chromosomal aberrations	*Channa punctatus*, kidney	+	0.6 ppm [600 µg/L] (96 h)	Purity, 99%	Farah et al. (2006)
Micronucleus formation	*Channa punctatus*, erythrocytes	+	0.2 ppm [200 µg/L] (96 h)	Purity, 99%	Farah et al. (2003)
Micronucleus formation	*Heteropneustes fossilis*, erythrocytes	+	0.1 ppm [100 µg/L] (96 h)	Purity, 99%	Ahmad et al. (2002)
Micronucleus formation	*Caudiverbera caudiverbera* larvae, erythrocytes	–	1.5 ppm [1500 µg/L] (6 days)	Purity, NR	Venegas et al. (1993)
Micronucleus formation	*Planorbarius corneus*, haematocytes	+	100 µg/L (7 days)	Technical grade	Pavlica et al. (2000)
DNA strand breaks, comet assay	*Dreissena polymorpha*, haematocytes	+	80 µg/L (7 days)	Technical grade	Pavlica et al. (2001)
Micronucleus formation	*Dreissena polymorpha*, haematocytes	+	10 µg/L (up to 14 days)	Technical grade	Pavlica et al. (2000)
DNA strand breaks, comet assay	*Limnoperna fortune*, haematocytes	+	100 µg/L (2 h)	Purity, NR	Villela et al. (2006)
Micronucleus formation		+	10 µg/L (24 or 48 h)		
DNA strand breaks, comet assay	*Eisenia fetida,* coelomocytes	+	0.125 µg/cm^2 (24 h)	Purity, NR	Klobučar et al. (2011)
Aneuploidy, nondisjunction and loss of sex chromosomes	*Drosophila melanogaster*	–	400 ppm [400 µg/mL]	Purity, NR	Ramel & Magnusson (1979)
Chromosomal aberrations	*Allium cepa* (onion)	–	1.5 ppm [1.5 µg/mL] (6 days)	Purity, NR	Venegas et al. (1993)
Chromosomal aberrations	*Allium cepa* (onion)	+	0.5 ppm [0.5 µg/mL]	Purity, 99%	Ateeq et al. (2002)
Micronucleus formation	*Allium cepa* (onion)	+	5 µM [1.33 µg/mL]	Purity, NR	Repetto et al. (2001)

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

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCR, polymerase chain reaction; ppm, parts per million

(iv) Non-mammalian experimental systems in vitro

See Table 4.6.

Pentachlorophenol gave positive results in lower eukaryotic non-mammalian systems. Pentachlorophenol induced forward gene conversion (Fahrig, 1974; Fahrig et al., 1978), mutation (Fahrig et al., 1978), and mitotic recombination (Waters et al., 1982) in various strains of *Saccharomyces cerevisiae*.

In prokaryotic non-mammalian systems, pentachlorophenol did not induce reverse mutations in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, TA1537, or YG1024 in nearly all studies identified (Waters et al., 1982; Donnelly et al., 1990; George et al., 1991; Markiewicz et al., 1996; Donnelly et al., 1998; Gichner et al., 1998; NTP, 1999). Two studies reported positive results with exogenous S9 in TA98 (Nishimura & Oshima, 1983; Gopalaswamy & Nair, 1992).

Pentachlorophenol did not induce reverse mutations or DNA damage in two strains of *Escherichia coli* (Waters et al., 1982). DNA strand breaks were detected using the Microscreen prophage-induction assay in *Escherichia coli* after exposure to pentachlorophenol (purity, 92%) (DeMarini et al., 1990). Results were positive with exogenous S9 and marginally positive in the absence of S9. DNA damage was also detected in two strains of *Bacillus subtilis*, one wildtype (Ozaki et al., 2004) and one recombination-deficient (Waters et al., 1982); Ozaki et al. used pentachlorophenol with a purity of > 99%.

(v) Acellular systems

See Table 4.6.

Van Ommen et al. (1986) reported the formation of both DNA and protein adducts in calf thymus DNA and microsomal proteins from rats after exposure to pentachlorophenol, but Witte et al. (1985) did not detect adduct formation in calf thymus or bacteriophage DNA in the absence of metabolic activation.

Dai et al. reported the formation of pentachlorophenol–DNA adducts with calf thymus DNA (Dai et al., 2005) or excess deoxyguanosine (Dai et al., 2003) in the presence of peroxidase. Adducts were detected using liquid chromatography-mass spectrometry with nuclear magnetic resonance spectral analysis. The oxidation of pentachlorophenol by the peroxidases (horseradish and myeloperoxidase) yielded chlorophenoxyl radicals that formed oxygen adducts that were specific to the C8 of deoxyguanosine; adducts were not formed with the three other deoxynucleosides. These chlorophenoxyl radicals were also able to self-pair to form an electrophilic 1,4-benzoquinone; this derivative can also react with deoxyguanosine to form 4′′-hydroxy-1,N^2-benzetheno-deoxyguanosine adducts (Dai et al., 2005). These chlorophenoxyl radicals were specific to pentachlorophenol oxidation by peroxidases; when the same experiment was conducted using rat liver microsomes, different DNA adducts formed from the electrophilic benzoquinone metabolites were observed (Dai et al., 2003).

(c) Genetic and related effects of pentachlorophenol metabolites

See Table 4.7.

Several studies investigated the genetic and related effects of the major metabolites of pentachlorophenol, including TCHQ, tetrachlorocatechol (also named tetrachloro-*ortho*-hydroquinone), tetrachloro-1,2-benzoquinone (tetrachloro-*ortho*-benzoquinone), and tetrachloro-1,4-benzoquinone (tetrachloro-*para*-benzoquinone) (see also Section 4.2.1).

(i) TCHQ

TCHQ induced DNA damage measured by the comet assay in the human fibroblast GM 5757 cell line (Witte et al., 2000; Stang & Witte, 2010), and by alkaline elution in Chinese hamster ovary cells (Ehrlich, 1990). TCHQ induced mutations at the *Hprt* locus (but not at the Na/K-ATPase

Table 4.6 Genetic and related effects of pentachlorophenol in non-mammalian and acellular systems in vitro

End-point	Species, strain	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Mitotic gene conversion	*Saccharomyces cerevisiae* D4	+	NT	0.19 mM [50.6 µg/mL]	Purity, NR Vehicle, DMSO	Fahrig (1974)
Mitotic gene conversion	*Saccharomyces cerevisiae* MP-1	+	NT	400 µg/mL	Purity, 99% Survival, 59%	Fahrig et al. (1978)
Forward mutation	*Saccharomyces cerevisiae* MP-1	+	NT	400 µg/mL	Purity, 99% Survival, 59%	Fahrig et al. (1978)
Mitotic recombination	*Saccharomyces cerevisiae* D3	+	+	10 mg/plate	Technical grade	Waters et al. (1982)
Reverse mutation	*Salmonella typhimurium* TA100	–	–	0.04 µmol/plate [11 µg/plate]	Purity, NR	Nishimura & Oshima (1983)
Reverse mutation	*Salmonella typhimurium* TA98	–	+	0.04 µmol/plate [11 µg/plate]	Purity, NR	Nishimura & Oshima (1983)
Reverse mutation	*Salmonella typhimurium* TA98	NT	–	50 µg/plate	Purity, NR	Donnelly et al. (1990)
Reverse mutation	*Salmonella typhimurium* TA98, TA100	–	–	100 µg/plate		George et al. (1991)
Reverse mutation	*Salmonella typhimurium* TA98	–	+	100 µg/plate	Purity, NR	Gopalaswamy & Nair (1992)
Reverse mutation	*Salmonella typhimurium* TA98	–	–	100 µg/plate	Purity, NR	Markiewicz et al. (1996)
Reverse mutation	*Salmonella typhimurium* TA97a, TA98 and TA100	–	–	200 µg/plate	Purity, > 98%	Donnelly et al. (1998)
Reverse mutation	*Salmonella typhimurium* YG1024	–	–	200 µM [53.3 µg/mL]	Purity, NR	Gichner et al. (1998)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	–	–	30 µg/plate	Purity, 91.6%	Haworth et al. (1983); NTP (1989, 1999)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	–	–	10mg/plate	Technical grade	Waters et al. (1982)
Prophage λ induction	*Escherichia coli* WP2s (λ)	+	+	12.71 µM [3.4 µg/mL]	Purity, 92%	DeMarini et al. (1990)
Differential toxicity	*Escherichia coli* p3478 (polA-)	–	NT	10 mg/plate	Technical grade	Waters et al. (1982)

Table 4.6 (continued)

End-point	Species, strain	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Reverse mutation	*Escherichia coli* WP2	–	–	10 mg/plate	Technical grade	Waters et al. (1982)
Differential toxicity	*Bacillus subtilis* M45 (*recA*-)	+	NT	10 mg/plate	Technical grade	Waters et al. (1982)
Differential toxicity	*Bacillus subtilis* M45 (*recA*–, *recA*+)	+	NT	3 µg/plate (*recA*–), 6 µg/plate (*recA*+)	Purity, > 99%	Ozaki et al. (2004)
DNA adducts, covalent binding	Calf thymus DNA	–	NT	100 mM [26.6×10^3 µg/mL]	Purity, NR	Witte et al. (1985)
DNA adducts, covalent binding	Calf thymus DNA	NT	+	100 µM [26.63 µg/mL]	Purity, NR	van Ommen et al. (1986)
C8-dG O-adduct, LC-MS	Bacteriophage PM2 DNA	–	NT	100 mM [26.6×10^3 µg/mL]	Purity, NR	Witte et al. (1985)
C8-dG O-adduct, LC-MS	2′-Deoxyguanosine	+	–	100 µM [26.63 µg/mL]	Purity, NR Horseradish peroxidase/ H_2O_2 Alternative adduct formed in presence of S9	Dai et al. (2003)
DNA strand breaks, quantitative gel electrophoresis	Calf thymus DNA	+	NT	100 µM [26.63 µg/mL]	Purity, NR In the presence of horseradish peroxidase/ H_2O_2.	Dai et al. (2005)

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

C8-dGO, C-8-2′-deoxyguanosine; DMSO, dimethyl sulfoxide; HIC, highest ineffective concentration; LC-MS, liquid chromatography-mass spectrometry; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × g supernatant

Table 4.7 Genetic and related effects of metabolites of pentachlorophenol

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	References
Tetrachlorohydroquinone					
DNA strand breaks, comet assay	Human fibroblasts, GM 5757	+	6.5 µM [1.6 µg/mL]	Purity, NR	Witte et al. (2000)
DNA strand breaks, high throughput comet assay	Human fibroblasts, NHDF-p	+	NR	Purity, "grade II"	Stang & Witte (2010)
DNA strand breaks, alkaline elution assay	Chinese hamster ovary	+	2 µg/mL	Purity, NR	Ehrlich (1990)
DNA strand breaks, alkaline elution assay	Chinese hamster fibroblast, V79	+	25 µM [6.2 µg/mL]	Purity, NR	Dahlhaus et al. (1995)
Hprt mutation Na/K-ATPase locus, ouabain-resistant mutants	Chinese hamster fibroblast, V79	+ –	20 µM [5 µg/mL] 60 µM [15 µg/mL]	Purity, > 99%	Jansson & Jansson (1991)
Micronucleus formation	Chinese hamster fibroblast, V79	+	10 µM [2.5 µg/mL]	Purity, > 99%.	Jansson & Jansson (1992)
DNA adducts, covalent binding	Calf thymus DNA	+	50 mM [12.5 µg/mL]	Purity, NR	Witte et al. (1985)
DNA adducts, covalent binding	Calf thymus DNA	–	200 µM [50 µg/mL]	Purity, NR. Positive with 20 µM Cu(II)	Naito et al. (1994)
DNA strand breaks, quantitative gel electrophoresis	Bacteriophage PM2 DNA	+	5 µM [1.3 µg/mL]	Purity, NR	Witte et al. (1985)
DNA strand breaks, ^{32}P-labelled DNA fragments, electrophoresis	Plasmid DNA	–	200 µM [50 µg/mL]	Purity, NR Positive with 20 µM Cu(II)	Naito et al. (1994)
*Tetrachlorocatechol (tetrachloro-*ortho*-hydroquinone)*					
DNA strand breaks, comet assay + Fpg and Endo III	Human lymphocytes (primary)	+	0.02 µg/mL	Purity, 99%	Michałowicz & Majsterek (2010)
DNA strand breaks, alkaline comet assay	Human lymphocytes (primary)	+	0.2 ppm [0.2 µg/mL]	Purity, 99.5%	Michałowicz (2010)
Aldehydic DNA lesions, aldehyde reactive slot-blot assay (ASB assay)	Human breast cancer, MCF-7	+	500 µM [124 µg/mL]	Only positive with prior GSH depletion	Lin et al. (2005)
Na/K-ATPase locus, ouabain resistant mutant; *Hprt* mutation	Chinese hamster fibroblasts, V79	–	120 µM [30 µg/mL]	Purity, > 99%	Jansson & Jansson (1991)
Aldehydic DNA lesions, ASB assay	Calf thymus DNA	–	100 µM [25 µg/mL]	Positive with 20 µM Cu(II) + 100 µM NAD(P)H	Lin et al. (2005)
Tetrachlorobenzoquinone					
DNA strand breaks, comet assay	Human liver, HepG2	+	6.25 µM [1.5 µg/mL]	Isomer of TCBQ not specified; purity, NR	Dong et al. (2014)

Table 4.7 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	References
DNA strand breaks for γ-H2AX; micronucleus formation	Human liver, HepG2	+	12.5 µM [3 µg/mL]	Isomer of TCBQ not specified; purity, NR	Dong et al. (2014)
DNA strand breaks, alkaline elution assay	Chinese hamster fibroblast, V79	–	25 µM [6.2 µg/mL]	TC*o*BQ; purity, NR	Dahlhaus et al. (1996)
DNA strand breaks, alkaline elution assay	Chinese hamster fibroblast, V79	+	25 µM [6.2 µg/mL]	TC*p*BQ; purity, NR	Dahlhaus et al. (1996)
DNA adducts, Cl_2BQ-dG, HPLC-MS-NMR	2′-Deoxyguanosine	+	0.062 mmol/20 mL [3.1 mM] [762 µg/mL]	Isomer of TCBQ, not specified	Nguyen et al. (2005)

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

Cl_2BQ-dG, dichlorobenzoquinone-deoxyguanosine; GSH, glutathione; HIC, highest ineffective concentration; HPLC-MS-NMR, high-performance liquid chromatography-mass spectrometry-nuclear magnetic resonance; LEC, lowest effective concentration; NR, not reported; TCBQ, tetrachlorobenzoquinone; TCoBQ, tetrachloro-*ortho*-benzoquinone (tetrachloro-1,2-benzoquinone); TCpBQ, tetrachloro-*para*-benzoquinone (tetrachloro-1,4-benzoquinone)

gene locus) (Jansson & Jansson, 1991), micronuclei (Jansson & Jansson, 1992), 8-OHdG adducts, and DNA strand breaks (Dahlhaus et al., 1996; 1995; see Section 4.2.1) in Chinese hamster V79 lung fibroblasts. TCHQ induced DNA adducts in calf thymus DNA and DNA strand breaks in bacteriophage PM2 DNA (Witte et al., 1985). Naito et al. (1994) also reported DNA strand breaks in plasmid DNA and DNA adducts in calf thymus DNA, but only after co-exposure to Cu(II) (20 μM) plus TCHQ.

(ii) Tetrachlorocatechol

Tetrachlorocatechol (also named tetrachloro-*ortho*-hydroquinone) induced oxidized base damage (Michałowicz & Majsterek, 2010) and DNA strand breaks (Michałowicz, 2010) in human primary lymphocytes. Tetrachlorocatechol induced aldehydic DNA lesions, or abasic (apurinic/apyrimidinic) sites, in human breast cancer MCF-7 cells depleted of GSH, and in calf thymus DNA with the addition of Cu(II) and NAD(P)H (Lin et al., 2005). However, in V79 Chinese hamster lung fibroblasts, tetrachlorocatechol did not induce mutations at *Hprt* or the Na/K-ATPase gene loci (Jansson & Jansson, 1991), and did not increase the frequency of DNA strand breaks or 8-OHdG adducts (Dahlhaus et al., 1996; see Section 4.2.1).

(iii) Tetrachloro-1,2-benzoquinone and tetrachloro-1,4-benzoquinone

Both tetrachloro-1,2-benzoquinone (tetrachloro-*ortho*-benzoquinone) and tetrachloro-1,4-benzoquinone (tetrachloro-*para*-benzoquinone) formed 8-OHdG adducts in Chinese hamster V79 lung fibroblast cells, but only tetrachloro-1,4-benzoquinone (the metabolite of TCHQ) induced DNA damage (Dahlhaus et al., 1996). In HepG2 cells, tetrachloro-1,4-benzoquinone increased DNA strand breaks (as measured by the comet assay), histone γ-H2AX phosphorylation, 8-OHdG adducts, and micronucleus formation (Dong et al., 2014). Nguyen et al. (2005) reported the formation of tetrachlorobenzoquinone adducts to 2′-deoxyguanosine; it was not specified whether the *ortho* or *para* form of tetrachlorobenzoquinone was used.

4.2.3 Receptor-mediated effects

(a) Exposed humans

No data were available to the Working Group.

(b) Human and other mammalian cells in vitro

The literature on receptor-mediated effects was sparse; however, high-throughput data (discussed in Section 4.3) suggest interaction with several nuclear receptor subtypes, including estrogen receptors and the aryl hydrocarbon receptor (AhR).

Pentachlorophenol exhibited antagonism for estrogen receptors in human HELN cells expressing estrogen-receptor subtypes ERα and ERβ (Lemaire et al., 2006). Other studies in fish, discussed below, indicated predominantly anti-estrogenic activity (Petit et al., 1997; Lemaire et al., 2006; Zhao et al., 2006a, b). However, pentachlorophenol was shown to be estrogenic in the human MCF-7 cell proliferation assay in a single study (Suzuki et al., 2001). In this study, pentachlorophenol at concentrations in the nanomolar range was estrogenic, and when tested as a binary mixture with estradiol (E2), synergistic effects were detected (Suzuki et al., 2001). [The Working Group noted that the divergent results were most likely the consequence of the different cell types and assay methods used, together with effects of pentachlorophenol that are unrelated to binding with and activation of estrogen receptors.]

(c) Non-human mammals in vivo

The developmental neurotoxicity of pentachlorophenol was associated with decreases in circulating thyroxine (T4) in the dam and the pups. Decreases in plasma T4 were also observed in rats exposed perinatally to pentachlorophenol (Kawaguchi et al., 2008). Ewe lambs or their dams

were fed pentachlorophenol at a dose of 1 mg/kg bw per day from conception to age 67 weeks (Beard & Rawlings, 1999). Serum levels of free T4 and total T4 were decreased in the offspring when measured on weeks 65–66; smaller decreases were observed for triiodothyronine (T3). In addition, exposure to pentachlorophenol blunted the T4 and T3 increases in response to endogenous thyroid-stimulating hormone (Beard & Rawlings, 1999). The decrease in T4 was associated with increased scrotal circumference, seminiferous tubule atrophy, and reduced epididymal sperm density (Beard et al., 1999). A multigenerational study in minks exposed to pentachlorophenol at 1 mg/kg per day reported decreased serum T4 concentrations in the F_2 males and F_3 males and females (Beard & Rawlings, 1998).

(d) Non-mammalian experimental systems

In cultures of juvenile goldfish (*Carassius auratus*) hepatocytes, pentachlorophenol failed to induce an estrogenic effect as measured by vitellogenin concentrations in the media and was cytotoxic at very low concentrations (< 1.21 µg/mL) (Zhao et al., 2006a, b). Co-culturing pentachlorophenol with 17β-estradiol in this in-vitro model significantly reduced the estrogenic activity of 17β-estradiol, with a potency similar to that of the anti-estrogen tamoxifen (Zhao et al., 2006a). Similarly, the anti-estrogenic effects of pentachlorophenol were corroborated by results from a reporter-gene assay in yeast expressing rainbow trout estrogen-receptor, in which pentachlorophenol inhibited estrogen-dependent cell growth (Petit et al., 1997).

Pentachlorophenol (1 and 10 µg/L) increased mRNA expression of thyroid hormone receptors α and β (Thrα and Thrβ) in zebrafish embryo cultures (Cheng et al., 2015). In contrast, *Thrβ* gene expression was decreased by exposure to pentachlorophenol (27 µg/L) in the brain of male but not female zebrafish (age, 4 months) (Yu et al., 2014). In a study in vitro on purified transthyretin from Japanese quail, treatment with pentachlorophenol displaced radiolabelled T3 from transthyretin, but was without effect on thyroid hormone receptor (Ishihara et al., 2003) suggesting that the effects of pentachlorophenol are limited to displacement of thyroid hormones from serum carrier proteins.

4.2.4 Altered cell proliferation or death

(a) Humans

No data were available to the Working Group.

In the HepG2 human hepatoma cell line, TCHQ and pentachlorophenol altered the expression of several apoptosis-relevant genes, including *BCL-2*. *BAX*, heat shock protein (HSP) expression, and cellular apoptosis susceptibility (CAS) gene while PCP altered *BCL-2* and *BAX* expression but not HSP and CAS (Wang et al., 2001). TCHQ -induced apoptosis and DNA laddering, but cell death induced by pentachlorophenol appeared to be more characteristic of necrosis. TCHQ, but not pentachlorophenol, induced apoptosis and DNA fragmentation, and decreased *CAS* gene expression in human T-24 bladder cells. Neither compound exhibited these effects in Chang human liver cells (with HeLa markers) (Wang et al., 2000). In both cell lines, TCHQ, but not pentachlorophenol, decreased BCL-2/BAX protein expression.

Both pentachlorophenol and TCHQ markedly increased apoptotic cell number and induced DNA fragmentation in Jurkat human T cells, although TCHQ was more potent (Wispriyono et al., 2002). TCHQ but not pentachlorophenol increased the phosphorylation of all mitogen-activated protein kinases (MAPKs) examined [i.e. extracellular signal-regulated protein kinase (ERK), p38, and c-Jun NH(2)-terminal kinase (JNK)]. Apoptosis by pentachlorophenol or TCHQ was mildly (but significantly) suppressed by a MAPK/ERK kinase inhibitor (U0126), markedly suppressed by a p38 inhibitor (SB203580), and almost completely suppressed when both inhibitors were given at the same

time. LL-Z1640–2, an inhibitor of JNK phosphorylation, did not affect apoptosis induced by either TCHQ or pentachlorophenol.

A study in vitro reported that pentachlorophenol at 60 µg/mL induced a slowdown of cell proliferation in human lymphocytes from normal healthy donors (Ziemsen et al., 1987).

(b) Experimental systems

Chen et al. (2015) reviewed effects of pentachlorophenol and TCHQ in mice, rats, and in mammalian cells in vitro, noting that TCHQ induced apoptosis/necrosis both in vivo and in vitro. Antioxidants attenuated cytotoxicity, apoptosis/necrosis, and other effects induced by pentachlorophenol and/or TCHQ. In addition, a role for MAPK in pentachlorophenol/TCHQ-triggered cytotoxicity was shown by the finding that higher doses of TCHQ could lead to necrosis of freshly isolated splenocytes through marked increases in ROS and sustained ERK activation (Chen et al., 2014).

In studies detailed in Section 4.2.1, increased hepatocyte cell proliferation was reported in B6C3F$_1$ male mice exposed to pentachlorophenol at 600 or 1200 ppm for 8 weeks (Umemura et al., 1999). Liver weights were increased in mice exposed to pentachlorophenol (600 ppm for 2 or 4 weeks) (Umemura et al., 1996, 2003a). Pentachlorophenol (600 ppm in the diet for 2 weeks) increased cell proliferation in epithelial cells of intrahepatic bile ducts as well as hepatocytes in exposed B6C3F$_1$ mice (Umemura et al., 2003b). Furthermore, hepatic cell proliferation caused by pentachlorophenol was enhanced in *Nrf2*-deficient mice (*Nrf2*$^{-/-}$ or *Nrf2*$^{+/-}$) compared with *Nrf2*$^{+/+}$ mice, whereas the effects of pentachlorophenol on relative liver weights was diminished in *Nrf2*$^{-/-}$ and *Nrf2*$^{+/-}$ mice compared with *Nrf2*$^{+/+}$ mice (Umemura et al., 2006).

In an initiation–promotion study on skin tumours, dermally administered pentachlorophenol and TCHQ (2.5, 50, or 1000 µg, twice per week for 25 weeks, 1 week after initiation with dimethylbenz[*a*]anthracene) enhanced mice skin epidermal hyperplasia and proliferating cell nuclear antigen labelling index in the epidermis, with TCHQ showing greater effects (Chang et al., 2003).

In male B6C3F$_1$ mice treated with pentachlorophenol (300 or 600 ppm in the diet), there was a dose-related inhibition of gap-junctional intercellular communication in hepatocytes, associated reductions in connexin32 (Cx32) plaques in the plasma membrane, and increased cell proliferation index. These effects were attenuated by pre-and co-treatment with green tea extract (Sai et al., 2000).

Pentachlorophenol, but not TCHQ, inhibited gap-junctional intracellular communication in rat liver epithelial cells (WB cells) (Sai et al., 1998). Pentachlorophenol treatment of v-myc-transfected rat liver epithelial cells inhibited gap-junctional intercellular communication and associated apoptosis induced by serum deprivation (Sai et al., 2001).

In vitro, cell proliferation was enhanced in pentachlorophenol-treated AML 12 mouse hepatocyte cells (Dorsey et al., 2004, 2006). TCHQ affected proliferation and differentiation in two stroma-free murine bone marrow culture models, a multipotent progenitor cell line (factor-dependent cell Paterson-, FDCP-mix), and primary lineage-depleted bone marrow cells (Henschler et al., 2001).

4.2.5 Inflammation and immunosuppression

(a) Exposed humans

Exposure to pentachlorophenol has been associated with inflammation as well as cellular and humoral immunodeficiency in several cohort studies (Klemmer et al., 1980; Cooper & Macauley, 1982; Daniel et al., 1995, 2001), but not in a case–control study (Colosio et al., 1993). In cohort studies, increased prevalence rates for inflammation and low-grade infections of the skin and subcutaneous tissue, mucous

membranes of the eyes and upper respiratory tract (Klemmer et al., 1980) and more frequent respiratory tract infections (Daniel et al., 2001) have been documented in workers occupationally exposed to pentachlorophenol. T-lymphocyte dysfunction and increased circulating concentrations of cytokines including interleukin-8 (IL-8) have also been documented in workers (n = 188) exposed to pentachlorophenol (Daniel et al., 1995), whereas exposure to pentachlorophenol for more than 6 months was negatively associated with circulating concentrations of IL-2, soluble IL-2R, IL-6, IL-10, interferon-gamma (IFN-γ), tumour necrosis factor-α (TNF-α), transforming growth factor-β2 (TGF-β2), soluble IL-1R antagonist, and soluble intercellular adhesion molecule-1 (ICAM-1) (Daniel et al., 2001). Exposure to pentachlorophenol was associated with a blunted proliferative response to mitogens in those with the highest exposure. Pentachlorophenol exposure has also been linked with pancreatitis in a single study (Cooper & Macauley, 1982). In the only available case–control study (Colosio et al., 1993), no effect was observed on serum immunoglobulins, complement fractions, autoantibodies, or on absolute or differential counts of peripheral blood mononuclear cells.

Human lymphocytes were collected from people living in log homes treated with pentachlorophenol as a preservative, and compared with cells collected from a control group of people not living in log homes (McConnachie & Zahalsky, 1991). Exposed individuals had lower proliferative response to a variety of antigens. In addition, there was an increase in natural-killer cell activity, but only in exposed females.

(b) Human cells in vitro

A variety of studies used isolated human lymphocytes to evaluate the effects of pentachlorophenol on markers of immune response.

In one study in vitro, the lytic function of human natural killer cells was decreased by exposure to pentachlorophenol (10 μM) for 24 hours or more. Lower concentrations of pentachlorophenol required longer incubations to produce similar effects (Nnodu & Whalen; 2008). Similar results were also found in another study that showed that pentachlorophenol (5 μM) decreases the lytic effects of natural killer cells (Reed et al., 2004).

In a study using human peripheral blood lymphocytes treated with pentachlorophenol for 1, 2, and 6 days, pentachlorophenol (10 μM) decreased natural-killer cell binding function (34.6%), and CD11a (21.7%) and CD56 (26.2%) cell-surface proteins (Hurd et al., 2012), indicative of immune suppression. In another study, pentachlorophenol (40–200 μM; either technical or analytical grade) increased cell proliferation in response to antigen, although higher concentrations decreased cell proliferation in isolated lymphocytes from healthy donors (Lang & Mueller-Ruchholtz, 1991). In addition, lymphokine production and immunoglobulin secretion was significantly decreased by both technical- and analytical-grade pentachlorophenol.

(c) Mammalian experimental systems

In mice, a single oral dose of pentachlorophenol (0, 10, 30, or 100 mg/kg) activated the interferon signalling gene network in the liver within 24 hours (Kanno et al., 2013). No effect on inflammation was observed in Mexican hairless dogs treated topically for 7 days with pentachlorophenol (Kimura et al., 1998).

Palmitoylpentachlorophenol, a putative metabolite of pentachlorophenol, induced pancreatic toxicity in rats after a single exposure at 100 mg/kg by gavage (Ansari et al., 1987). The pancreatic lesions observed consisted of focal, spotty vacuolization, loss of pancreatic acini, and acute inflammatory infiltrate.

Several studies evaluated and compared the immunosuppressive effects of technical- and analytical-grade pentachlorophenol in rodents (Kerkvliet et al., 1982a, b, 1985a, b; White & Anderson, 1985; Holsapple et al., 1987; Blakley

et al., 1998). A more recent study by Chen et al. (2013) observed no significant immunosuppressive effects of pentachlorophenol in mice. Others have observed cytokine changes in mice exposed to TCHQ but not to pentachlorophenol, with no changes in immune function (Chang et al., 2003). Elevated serum tumour necrosis factor-α (TNF-α) was observed in mice treated with TCHQ for 25 weeks, but not at earlier time points or in those treated with pentachlorophenol. Neither pentachlorophenol- nor TCHQ-treated mice exhibited changes in serum interleukin-1β (IL-1beta) levels. [The Working Group noted that the immunosuppressive effects of technical-grade pentachlorophenol may be attributable to dioxin contaminants.]

A few studies examined the effects of technical-grade pentachlorophenol in cattle and pigs (Forsell et al., 1981; Hillam & Greichus, 1983; Hughes et al., 1985). No observed effects occurred in lactating cattle exposed to pentachlorophenol for 135 days (Forsell et al., 1981). Technical-grade pentachlorophenol induced a broad spectrum of toxicity in bull calves (Hughes et al., 1985). Histological lesions reported were cortical atrophy in the thymus and squamous metaplasia and hyperkeratous changes in the Meibomian gland of the eyelid. These effects were not observed in animals receiving the purified pentachlorophenol. Pigs exposed to pentachlorophenol at 5, 10, or 15 mg/kg bw for 30 days had decreased lymphocyte counts, and decreased serum gamma globulin and IgG (Hillam & Greichus, 1983).

A pentachlorophenol metabolite, TCHQ, interacts with murine haematopoietic progenitor cells, stimulating the formation of macrophages (Henschler et al., 2001).

(d) Non-mammalian experimental systems

In goldfish (*Carassius auratus*), pentachlorophenol (0.053 and 0.13 mg/L in the water for 14 days) decreased serum IgM concentrations (Chen et al., 2004). In macrophages isolated from goldfish, pentachlorophenol (1–50 µg/mL) decreased *IL-1β* and *TNF-α* mRNA expression and suppressed IgM production in co-cultured B cells at cytotoxic concentrations (Chen et al., 2005).

Technical-grade pentachlorophenol and, to a lesser extent, analytical-grade pentachlorophenol inhibited the respiratory burst of the isolated leukocytes from *Fundulus heteroclitus* (Atlantic killifish) (Roszell & Anderson, 1993). In contrast, analytical-grade pentachlorophenol (0.1–1 µg/L for 14 days) had no effect on immune function in rainbow trout (Shelley et al., 2009).

Treatment with pentachlorophenol (100–1000 ppm) enhanced the resistance of pathogenic bacteria to antibiotics (Chandra & Sankhwar, 2011).

4.3 Data relevant to comparisons across agents and end-points

High-throughput screening data generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) were considered in the assessment of the five chemicals reviewed in *IARC Monographs* Volume 117 (pentachlorophenol, 2,4,6- trichlorophenol, 3,3′,4,4′-tetrachloroazobenzene, aldrin and dieldrin) as well as two metabolite isomers of 2,4,6-trichlorophenol (2,4,5- trichlorophenol and 2,3,6- trichlorophenol). The United States Environmental Protection Agency (EPA) has systematically analysed concentration–response sample-assay pairs from ToxCast and Tox21. The resulting concentration–response models and activity calls have been publicly released via the interactive Chemical Safety for sustainability (iCSS) ToxCast Dashboard (EPA, 2015a, b). Summary matrix files, the ToxCast data analysis pipeline (tcpl) R package and connected database (invitrodb_v1) are also available (EPA, 2015c).

The tcpl R package and associated database enables access to all of the underlying concentration–response data, the analysis decision logic and methods, concentration–response model outputs, activity calls, and activity caution flags.

The Tox21 and ToxCast research programmes have tested more than 8000 and 1800 chemicals, respectively. ToxCast, specifically, has tested 1000 chemicals across the full assay battery in conjunction with ToxCast Phase I and II. The remaining 800 chemicals were tested as part of an endocrine profiling effort that resulted in a subset of assays being tested. For the present volume of the *IARC Monographs*, one chemical had no testing data (3,3′,4,4′-tetrachloroazobenzene), one was tested only in Tox21 assay components, and the remaining chemicals were tested in both ToxCast and Tox21 assays.

Data on the current publicly released ToxCast assay battery, including the Tox21 assays run at the United States National Institutes of Health (NIH), comprise 1192 assay end-points derived from 762 assay components (i.e. readouts) and 359 assays (i.e. experiments). The 359 assays were sourced from 12 vendors or collaborators spanning diverse technological and biological space, including more than 350 gene targets. Roughly a third of the final assay end-points were analysed from biochemical (cell-free) assay formats, with the remainder being cell-based (cell lines, primary cells, and co-cultures) or whole embryo (zebrafish larvae). The biochemical assays have no xenobiotic metabolism capacity, while the cell-based assays have a variable biotransformation capability varying from very limited to moderate. Thus, chemical effects requiring biotransformation to active metabolites may be missed in some or all of the assays in vitro. Relatively uniform testing concentration ranges were used, from low nanomolar up to approximately 100–200 micromolar. Compounds of very low relative molecular mass generally have only low affinity for biomolecular interactions due to limited free energy for binding (Hopkins et al., 2004). Hence screening in vitro at the concentrations used in ToxCast and Tox21 may be insufficient to detect molecular interactions of receptor-type interactions. These compounds of very low relative molecular mass may also have high vapour pressure, which could lead to loss of sample during testing and, thus, failure to reach effective active concentrations.

The Tox21 and ToxCast assays in vitro were selected to cover a broad range of potential toxicity mechanisms and are not specifically focused on carcinogenesis. Therefore, the Working Group of *IARC Monograph*s Volume 112 mapped the assay end-points available at that time to the key characteristics of human carcinogens (IARC, 2017; Smith et al., 2016). The consensus assignments resulted in 263 assay end-points mapped to 7 of 10 "key characteristics" (IARC, 2017). Subsequently, the Working Groups for *IARC Monograph*s Volumes 113, 115, and the present Volume 117 updated these "mappings," including reviewing the additional assay end-points added to Tox21 and ToxCast data since the initial determination. As a result, 25 assay end-points were added to the initial 263 that were mapped to key characteristics, resulting in 288 in total. The assay end-points used, the activity call, and the mapping to "key characteristics" are available as supplemental material to the present volume (Annex 1). The key characteristics listing number of assays included and a brief description are given below.

1. *Is electrophilic or can be metabolically activated*: 31 assay end-points consisting of CYP biochemical activity, and aromatase, which regulates conversion of androgens to estrogens. [The Working Group noted that these assays largely indicate inhibition of CYP activity, and do not directly measure metabolic activation or electrophilicity.]
2. *Is genotoxic*: 10 assay end-points consisting of cellular TP53 induction and DNA repair-sensitive cellular assays. [The Working Group

noted that *TP53* activation can occur in response to a variety of cell stresses in addition to DNA damage.]

3. *Alters DNA repair or causes genomic instability*: 0 assay end-points
4. *Induces epigenetic alterations*: 14 assay end-points including biochemical assays targeting histone deacetylases and other enzymes modifying chromatin, as well as assays for cellular transcription factors involved in epigenetic regulation. [The Working Group noted these end-points have not been extensively validated with reference compounds for epigenetic alterations.]
5. *Induces oxidative stress*: 18 assay end-points, all cellular assays, targeting nuclear erythroid-related factor 2/antioxidant response element (NRF2/ARE), other stress-related transcription factors, and protein upregulation in response to ROS.
6. *Induces chronic inflammation*: 45 assay end-points, mostly using primary human cells, measuring protein expression levels indicative of inflammatory responses, including cytokines, cell adhesion molecules, and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB).
7. *Is immunosuppressive*: 0 assay end-points.
8. *Modulates receptor-mediated effects*: 93 assay end-points targeting nuclear receptors (e.g. AhR, androgen receptor, estrogen receptor, farnesoid X receptor, peroxisome proliferator-activated receptor, pregnane X receptor, retinoic acid receptor, among others) in cellular assays for transactivation, receptor dimerization, and nuclear translocation, as well as biochemical radioligand-binding assays and coregulatory recruitment assays.
9. *Causes immortalization*: 0 assay end-points.
10. *Alters cell proliferation, cell death, or nutrient supply*: 88 assay end-points measuring cell cycle markers, proliferation, cytotoxicity, and mitochondrial toxicity, using a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

For each chemical, the results of the in-vitro assays that represent each "key characteristic" can be compared with the results for a larger compendium of substances with similar in-vitro data, so that a particular chemical can be aligned with other chemicals with similar toxicological effects. Nonetheless, the available assays do not cover the full spectrum of relevant targets, and metabolic capacity in many of the assays is limited, which could account for any absence of bioactivity. Conversely, the presence of bioactivity alone does not definitively imply that the agent exhibits that key characteristic, as the assay data are considered along with other information, both in vivo and in vitro.

Each chemical was assigned an "active" or "inactive" call within each assay end-point based on the normalized concentration–response data in the ToxCast database using methods published previously (Sipes et al., 2013). ToxCast/Tox21 tested a broad range of screening concentrations designed to identify whether compounds elicited bioactivity and at what potency. In the analysis by the Working Group, each "active" was given a value of 1, and each "inactive" was given a value of 0. Thus, by assigning all active compounds a value of 1, the "potency" estimates from the concentration–response data were not explicitly used for all subsequent analyses.

A brief summary of potentially significant outcomes for each of the substances relevant to the present volume follows (see also Table 4.8).

4.3.1 Specific effects across the "key characteristics" based on data from high-throughput screening in vitro

A summary is given below for each relevant compound (see also Table 4.8).

Table 4.8 Summary of activity of compounds reviewed in *IARC Monographs* Volume 117 and tested in ToxCast high-throughput screening assays

Key characteristic (No. of assay end-points)	No. of active end-points / No. of end-points tested					
	Pentachlorophenol	2,4,6-Trichlorophenol	2,4,5-Trichlorophenol	2,3,6-Trichlorophenol	Aldrin	Dieldrin
Characteristic (1) *Is electrophilic or can be metabolically activated* (31 end-points)						
CYP inhibition (29 end-points)	0/9	0/9	1/9	NT	0/9	2/9
Aromatase inhibition (2 end-points)	1/2	0/2	0/2	0/1	0/2	1/2
Characteristic (2) *Is genotoxic* (10 end-points)						
P53 activation (9 end-points)	6/8	0/8	1/8	0/6	3/8	0/8
DNA damage (1 end-point)	0/1	0/1	0/1	0/1	0/1	0/1
Characteristic (4) *Induces epigenetic alterations* (14 end-points)						
DNA binding (7 end-points)	5/7	1/7	3/7	0/7	1/7	0/7
Transformation catalyst (7 end-points)	0/6	0/6	0/6	NT	0/6	0/6
Characteristic (5) *Induces oxidative stress* (18 end-points)						
Oxidative stress (7 end-points)	0/5	0/5	3/5	0/1	4/5	1/5
Oxidative stress marker (6 end-points)	4/6	3/6	4/6	1/6	3/6	3/6
Metalloproteinase (5 end-points)	NT	NT	NT	NT	1/5	NT
Characteristic (6) *Induces chronic inflammation* (45 end-points)						
NFκB (2 end-points)	1/2	0/2	0/2	0/2	2/2	0/2
Cell adhesion (14 end-points)	0/14	0/14	0/14	NT	0/14	0/14
Cytokines (29 end-points)	0/29	0/29	1/29	NT	0/29	1/29
Characteristic (8) *Modulates receptor-mediated effects* (93 end-points)						
ER (18 end-points)	2/18	2/18	4/18	0/6	4/18	4/18
AHR (2 end-points)	2/2	1/2	0/2	1/2	0/2	0/2
Other nuclear receptors (29 end-points)	3/29	0/29	6/29	0/20	4/29	3/29
RAR (6 end-points)	2/6	0/6	3/6	0/4	2/6	3/6
PPAR (12 end-points)	5/12	3/12	3/12	2/8	1/12	1/12
PXR (7 end-points)	4/7	1/7	2/7	1/6	3/7	3/7
AR (12 end-points)	3/12	0/12	2/12	0/9	1/12	3/12
FXR (7 end-points)	2/7	0/7	0/7	0/3	2/7	3/7

Table 4.8 (continued)

Key characteristic (No. of assay end-points)	No. of active end-points / No. of end-points tested					
	Pentachlorophenol	**2,4,6-Trichlorophenol**	**2,4,5-Trichlorophenol**	**2,3,6-Trichlorophenol**	**Aldrin**	**Dieldrin**
Characteristic (10) ***Alters cell proliferation, cell death, or nutrient supply*** (88 end-points)						
Cytotoxicity (49 end-points)	18/41	2/40	14/40	0/21	20/40	10/40
Mitochondrial toxicity (14 end-points)	1/10	1/10	2/10	1/2	2/10	1/10
Cell cycle marker (21 end-points)	5/18	1/18	7/18	0/6	4/18	2/18
Proliferation (4 end-points)	0/4	0/4	0/4	NT	0/4	0/4

AHR, aryl hydrocarbon receptor; AR, androgen receptor; CYP, cytochrome; ER, estrogen receptor; FXR, farnesoid X receptor; NT, not tested; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; TCoBQ, tetrachloro-*ortho*-benzoquinone (tetrachloro-1,2-benzoquinone); TCpBQ, tetrachloro-*para*-benzoquinone (tetrachloro-1,4-benzoquinone)

(a) Pentachlorophenol

Pentachlorophenol (CAS No. 87-86-5) was tested across the full assay suite of ToxCast and Tox21, with data available on 870 assay end-points. The results for the 255 assay end-points mapped to key characteristics are summarized in Table 4.8. The assays with most activity were related to TP53 in human liver or intestinal cell lines, transcription factor activation indicative of DNA-binding, transcription factors that are markers of oxidative stress (in particular NRF2), a variety of receptor-mediated effects, and cytotoxicity and cell cycle markers. The activity across multiple nuclear receptor assays is difficult to interpret because of inconsistency across assay platforms (e.g. Attagene (ATG) vs Nova screen (NVS) vs Tox21). However, two assays for anti-estrogenic activity were consistent, and corroborate data on receptor-mediated effects (Section 4.2.3). In addition, pentachlorophenol showed activity in many cytotoxicity assays in cell lines as well as in primary human cells, which may have confounded results either directly through cell death or indirectly through generation of lipid peroxidation products. Finally, it cannot be ruled out that the activity in the AhR assay might be related to dioxin contamination.

(b) 2,4,6-Trichlorophenol and metabolites

2,4,6-Trichlorophenol (CAS No. 88-06-2) and one of its metabolites, 2,4,5-trichlorophenol (CAS No. 95-95-4) were tested across the full assay suite of ToxCast and Tox21, with data available on 883 assay end-points for both compounds. Another metabolite, 2,3,6-trichlorophenol (CAS No. 933-75-5), was tested in a more limited suite of assays, with data available on 276 assay end-points. The results for the assay end-points mapped to key characteristics are summarized in Table 4.8. Of the three isomers, 2,4,5-trichlorophenol was the most active, specifically in assays related to transcription-factor activation indicative of DNA binding, oxidative stress responses, as well as transcription factors that are markers of oxidative stress, a variety of receptor-mediated effects, and cytotoxicity and cell cycle markers. 2,4,6-Trichlorophenol was less active, but many of its effects overlapped with those of 2,4,5-trichlorophenol. 2,3,6-Trichlorophenol was the least active. Of particular note, were assays related to oxidative stress, since in several cases the "inactive" calls were for earlier time points in assays that gave "active" calls at later time points. Such a time delay is consistent with the need for metabolic activation. In addition, 2,4,5-trichlorophenol was active in all the oxidative stress assays in which 2,4,6-trichlorophenol was active. The activity across multiple nuclear-receptor assays was difficult to interpret because most active calls were for ATG assays, while many of the NVS binding assays and the corresponding Tox21 assays gave inactive calls for the same receptor. In addition, 2,4,5-trichlorophenol, which had the most activity across these assays, also exhibited the most activity across cytotoxicity assays in cell lines as well as in primary human cells. Thus it is possible that the results for modulation of nuclear receptors may have been confounded either directly through cell death or indirectly through generation of lipid peroxidation products.

(c) 3,3′,4,4′-Tetrachloroazobenzene

3,3′,4,4′-Tetrachloroazobenzene (CAS No. 14047-09-7) was not tested.

(d) Aldrin and dieldrin

Aldrin (CAS No. 309-00-2) and dieldrin (CAS No. 60-57-1) were tested across the full assay suite of ToxCast and Tox21, with data available on 879 and 878 assay end-points, respectively. The results for the assay end-points mapped to key characteristics are summarized in Table 4.8. Both compounds were active in assays related to oxidative stress responses, as well as transcription factors that are markers of oxidative stress, a variety of receptor mediated

effects, and cytotoxicity and cell cycle markers. Of particular note were assays related to oxidative stress, since several of the "inactive" calls were for earlier time points in assays that gave "active" calls or were active at later time points, or were active for aldrin. Aldrin and dieldrin were active in three out of six assays for transcription factor markers of oxidative stress, two of which were common to the agents. The activity across multiple nuclear-receptor assays were difficult to interpret because most of the activity was in ATG assays, whereas the corresponding NVS binding assays and the corresponding Tox21 assays for the same receptors were either inactive or active for the opposite direction (antagonism vs agonism). In addition, aldrin, and to a lesser extent dieldrin, also exhibited the most activity across cytotoxicity assays in cell lines as well as primary human cells. Thus it is possible that the results for modulation of nuclear receptors may have been confounded either directly through cell death or indirectly through generation of lipid peroxidation products.

4.3.2 Integrating effects across end-points and chemicals

To integrate the data across individual assay end-points into the cumulative score for each key characteristic, the toxicological prioritization index (ToxPi) approach (Reif et al., 2010) and associated software (Reif et al., 2013; Filer et al., 2014) were used. In the Working Group's analyses, the ToxPi score provides a measure of the relative potential for a chemical to be associated with a "key characteristic". ToxPi is a dimensionless index score that integrates multiple different assay results and displays them visually. Within each subset of end-points ("slice"), data are translated into ToxPi slice-wise scores for all compounds as detailed below and in the publications describing the approach and the associated software package (Reif et al., 2013). Within each individual slice for a given chemical, the distance from the origin represents the relative chemical-elicited activity of the component assays (i.e. slices extending farther from the origin were associated with "active" calls on more assays). The overall score for a chemical, visualized as a radial ToxPi profile, is the aggregation of all slice-wise scores.

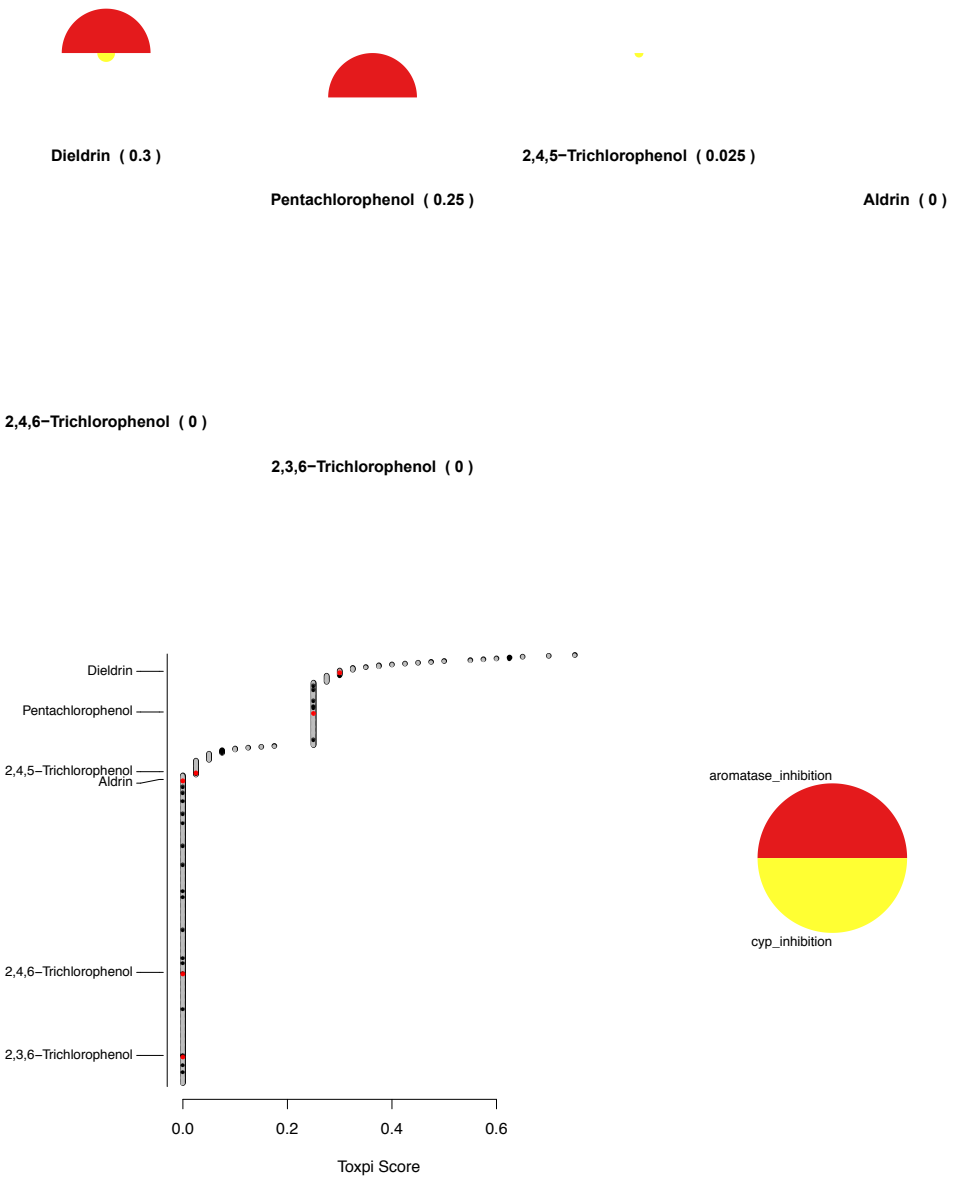
The relative effects of pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin were compared with those of 489 (out of more than 800 total) chemicals previously evaluated by the *IARC Monographs* for which Tox21/ToxCast assay end-point data were available (not including chemicals in the present Volume 117 that have been evaluated previously). Of these 489 chemicals, 30 are classified in Group 1 (*carcinogenic to humans*), 47 are in Group 2A (*probably carcinogenic to humans*), 163 are in Group 2B (*possibly carcinogenic to humans)*, 248 are in Group 3 (*not classifiable as to its carcinogenicity to humans*), and 1 is in Group 4 (*probably not carcinogenic to humans).*

The results are presented in a dot plot as a rank order of all compounds in the analysis arranged in the order of their relative activity. The relative positions of pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin (i.e. all chemicals evaluated in Volume 117) in the ranked list are also shown on the *y*-axis. The legend key (lower right graphic in each plot) lists components of the ToxPi chart as subcategories that comprise assay end-points in each characteristic. The ToxPi profile and numeric score for each of the chemicals evaluated in Volume 117 are shown above the ranking chart.

Specific observations across chemicals are as follows:

- Characteristic (1) *Is electrophilic or can undergo metabolic activation* (Fig. 4.2): Dieldrin and pentachlorophenol ranked the

Fig. 4.2 ToxPi rankings using ToxCast assay end-points mapped to metabolic activation

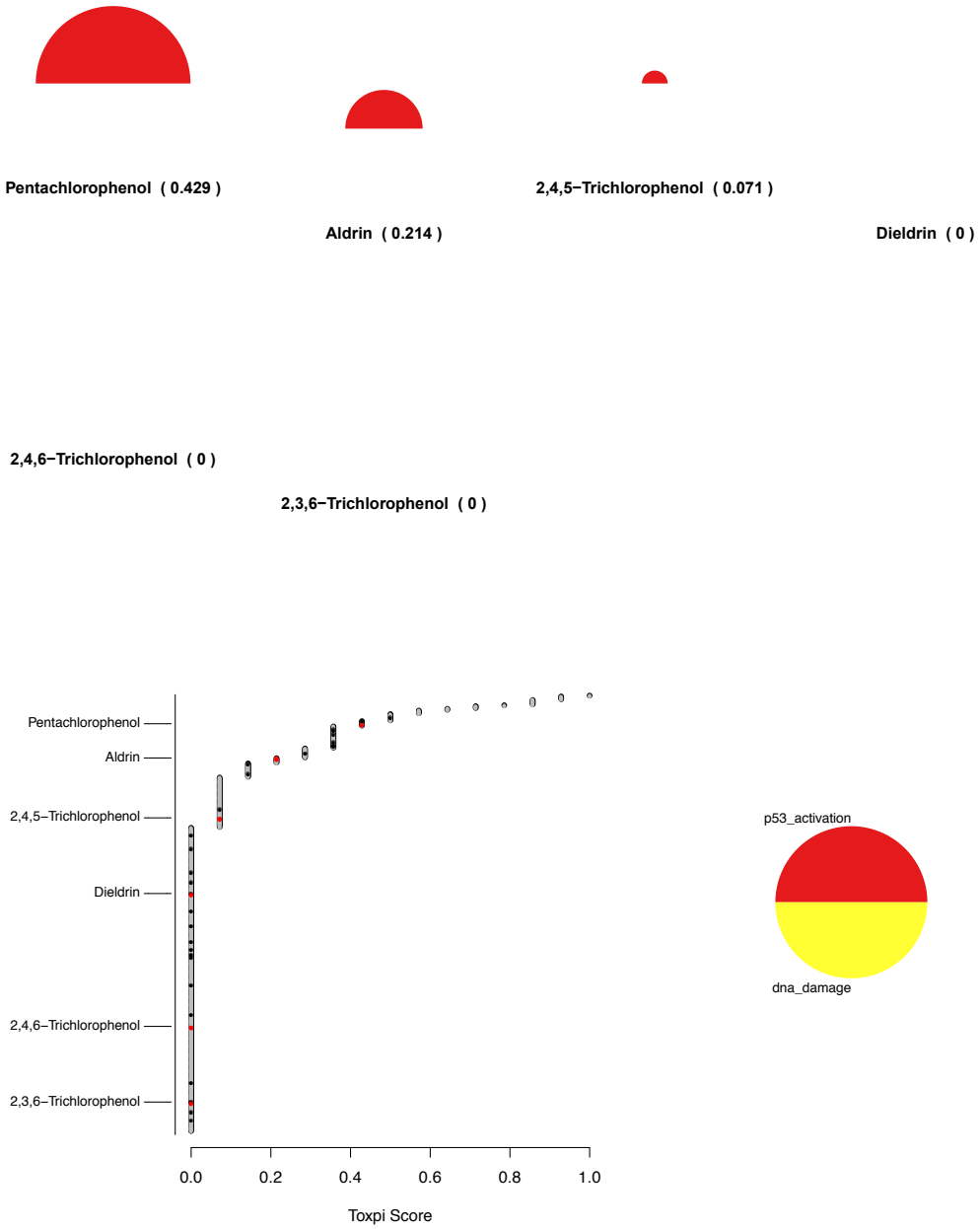


Across the top, the ToxPi shapes and scores for characteristic 1 (is electrophilic or can undergo metabolic activation) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

highest among the chemicals in Volume 117, with the other chemicals being evaluated having minimal activity. However, even for dieldrin and pentachlorophenol, the ToxPi score was in the lower half of the range across all the chemicals evaluated by IARC. The highest ranking chemical is malathion, with a ToxPi score of 0.75.

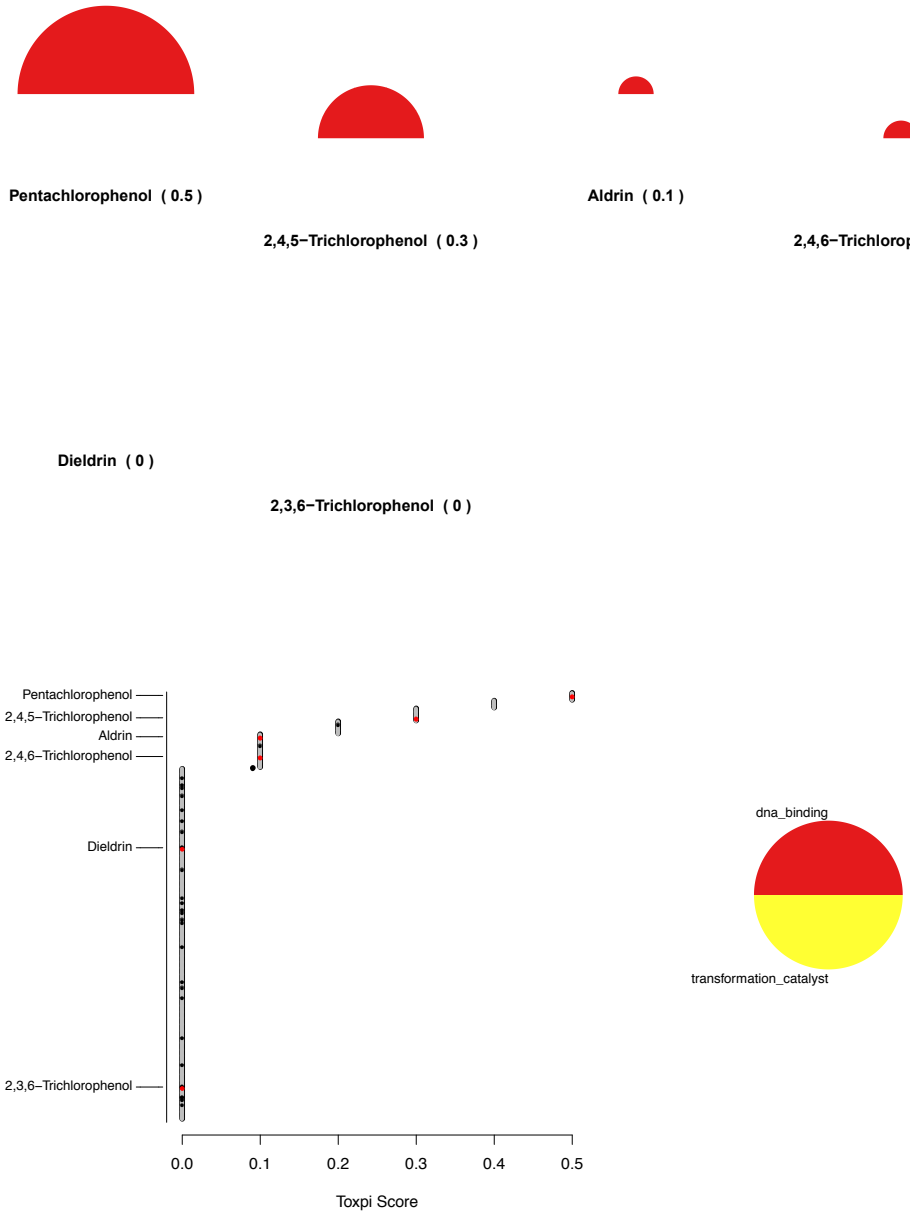
- Characteristic (2) *Is genotoxic* (Fig. 4.3): Pentachlorophenol and aldrin ranked the highest among the chemicals in Volume 117, with the other chemicals being evaluated having minimal activity. However, all the ToxPi scores for chemicals evaluated in this volume were in the lower half of the range across all the chemicals evaluated by IARC. The highest ranking chemical is Michler's ketone, with a ToxPi score of 1.0.
- Characteristic (4) *Induces epigenetic alterations* (Fig. 4.4): Pentachlorophenol and 2,4,5-trichlorophenol ranked the highest among the chemicals in Volume 117, with the other chemicals being evaluated having minimal activity. In addition, pentachlorophenol had the highest ranking ToxPi score of 0.5, jointly with eight other chemicals previously evaluated by IARC. Seven of these other eight chemicals additionally had the same ToxPi shape, with all the activity related to DNA binding, rather than a transformation catalyst.
- Characteristic (5) *Induces oxidative stress* (Fig. 4.5): Aldrin and 2,4,5-trichlorophenol ranked the highest among the chemicals in Volume 117, but dieldrin, pentachlorophenol, and 2,4,6-trichlorophenol also showed activity. Aldrin was ranked sixth, and 2,4,5-trichlorophenol ranked twenty-third overall among IARC chemicals, with chlordane having the highest score of 0.58. From the ToxPi shape, it is clear that most of the chemicals showed activity for oxidative stress markers, but aldrin and 2,4,5-trichlorophenol had relatively high activity in measures of stress responses.
- Characteristic (6) *Induces chronic inflammation* (Fig. 4.6): Aldrin and pentachlorophenol ranked the highest among the chemicals in Volume 117, with the other chemicals being evaluated having minimal activity. The highest ranked chemical was tris(2,3-dibromopropyl) phosphate with a ToxPi score of 0.83, more than double the score for any other chemical evaluated by IARC.
- Characteristic (8) *Modulates receptor-mediated effects* (Fig. 4.7): Pentachlorophenol, dieldrin, 2,4,5-trichlorophenol, and aldrin were all highly ranked in terms of activity in assay end-points mapped to receptor-mediated effects, with pentachlorophenol having the second highest ToxPi score among chemicals evaluated by IARC. Aldrin is at the top 95th percentile of ToxPi scores, with a ranking of 26 out of 495 compounds. Moreover, the relative promiscuity of these compounds is evident from the shape of the ToxPi, which shows relatively high activity across multiple categories of receptors. The overall highest ranking chemical is kepone (chlordecone), with a ToxPi score of 0.582, only slightly higher than that for pentachlorophenol.
- Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply* (Fig. 4.8): 2,4,5-trichlorophenol, aldrin, and pentachlorophenol ranked the highest among the chemicals in Volume 117, all above the top 95th percentile rankings. These chemicals also had similar ToxPi shapes, with most of the activity related to cytotoxicity and cell cycle markers, less activity in mitochondrial toxicity, and none in proliferation. Dieldrin had a somewhat lower score (but similar shape), and there was minimal activity for 2,4,6-trichlorophenol and 2,3,6-trichlorophenol. The highest ranked chemical overall is 3,3′,5,5′-tetrabromobisphenol A, with a

Fig. 4.3 ToxPi rankings using ToxCast assay end-points mapped to genotoxicity



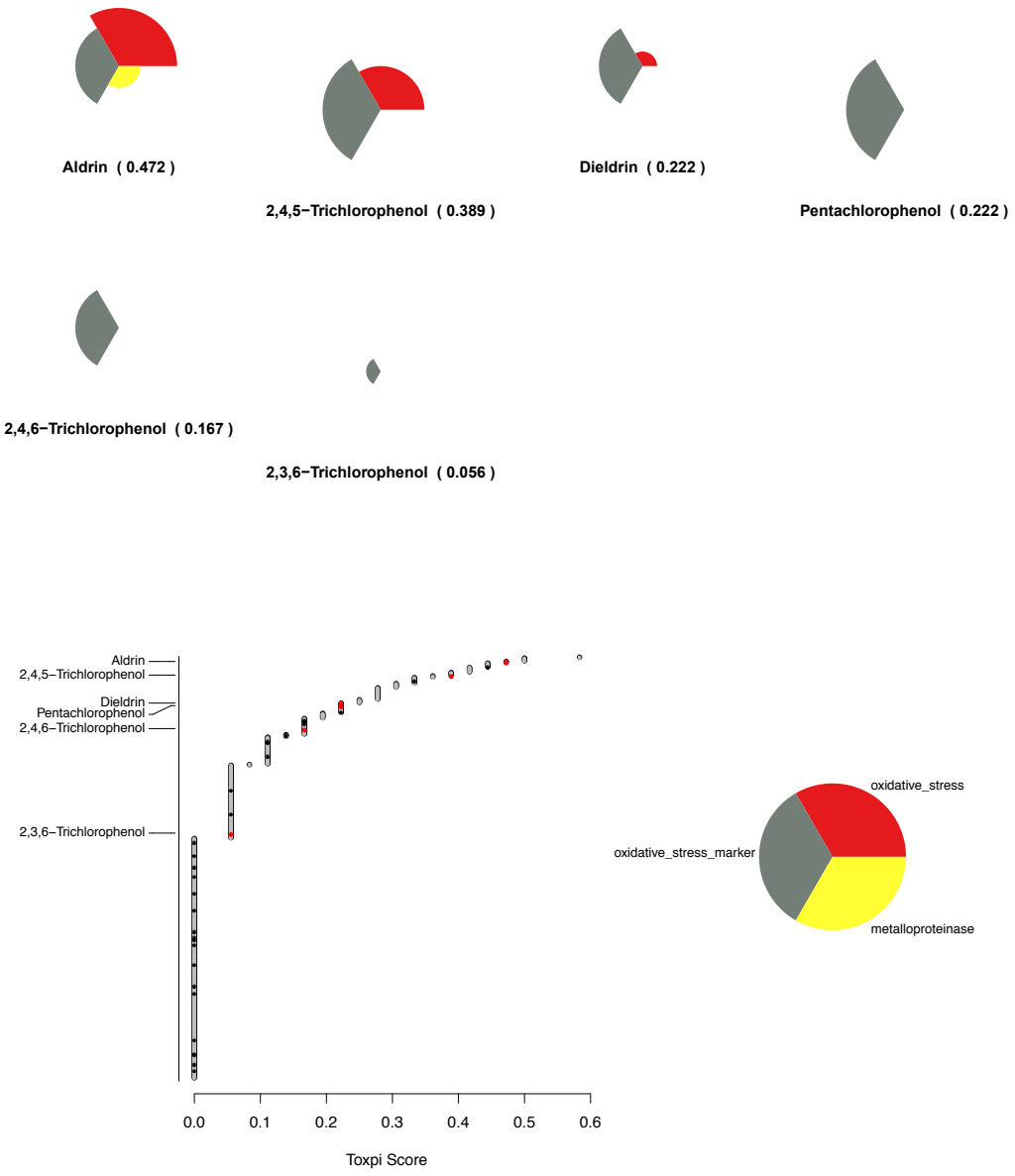
Across the top, the ToxPi shapes and scores for characteristic 2 (is genotoxic) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

Fig. 4.4 ToxPi rankings using ToxCast assay end-points mapped to epigenetic alterations



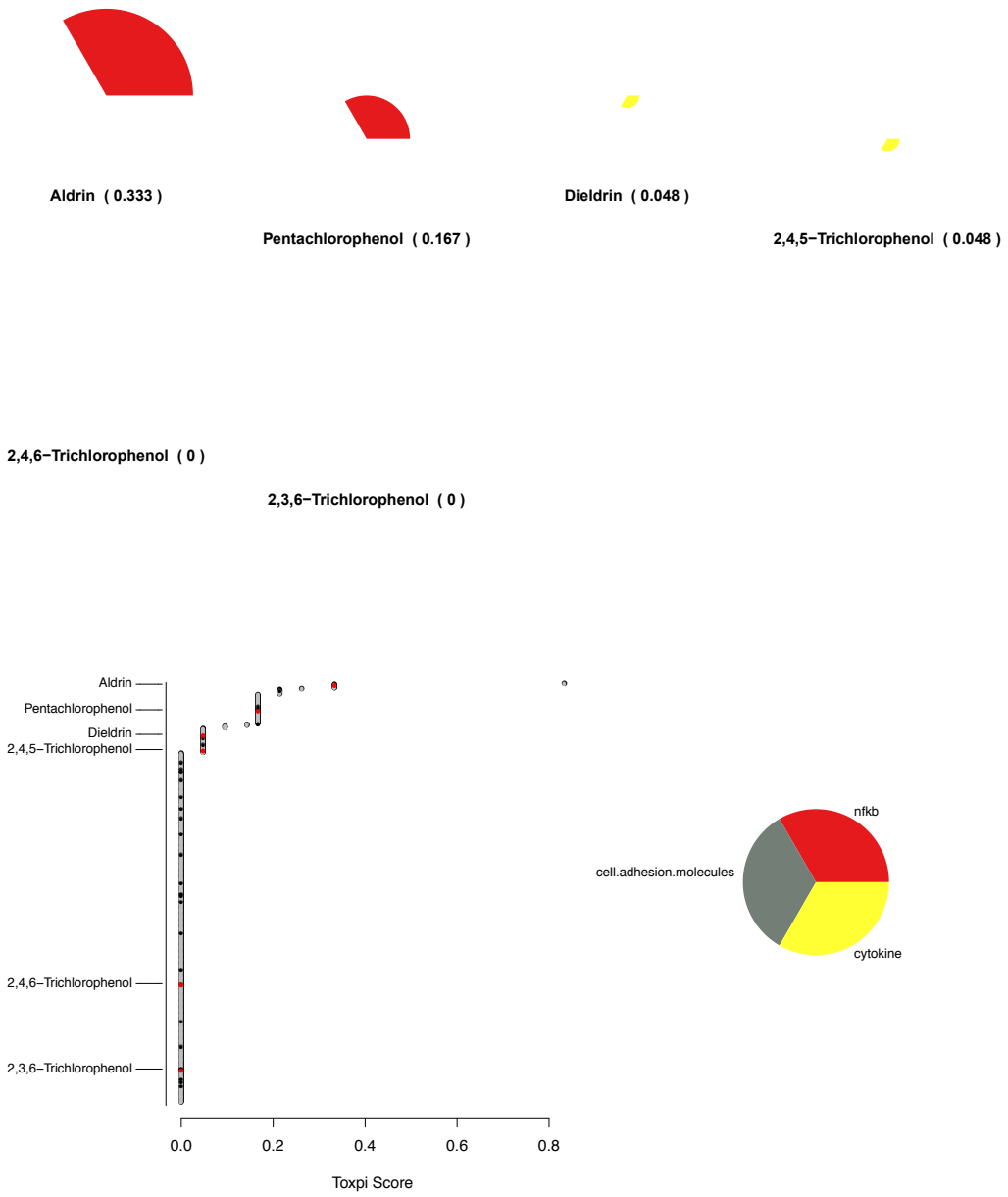
Across the top, the ToxPi shapes and scores for characteristic 4 (induces epigenetic alterations) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

Fig. 4.5 ToxPi rankings using ToxCast assay end-points mapped to oxidative stress markers



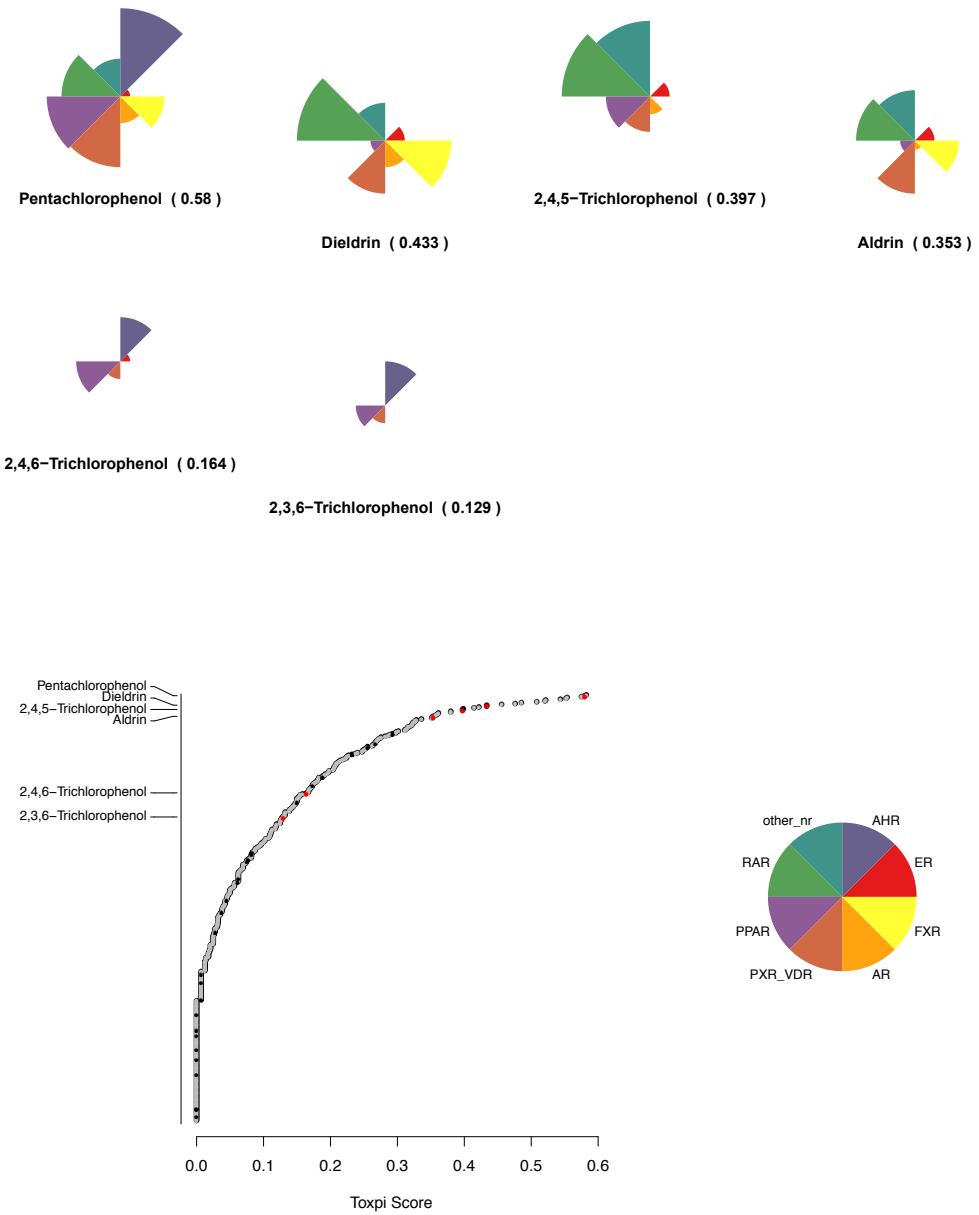
Across the top, the ToxPi shapes and scores for characteristic 5 (induces oxidative stress) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

Fig. 4.6 ToxPi rankings using ToxCast assay end-points mapped to chronic inflammation



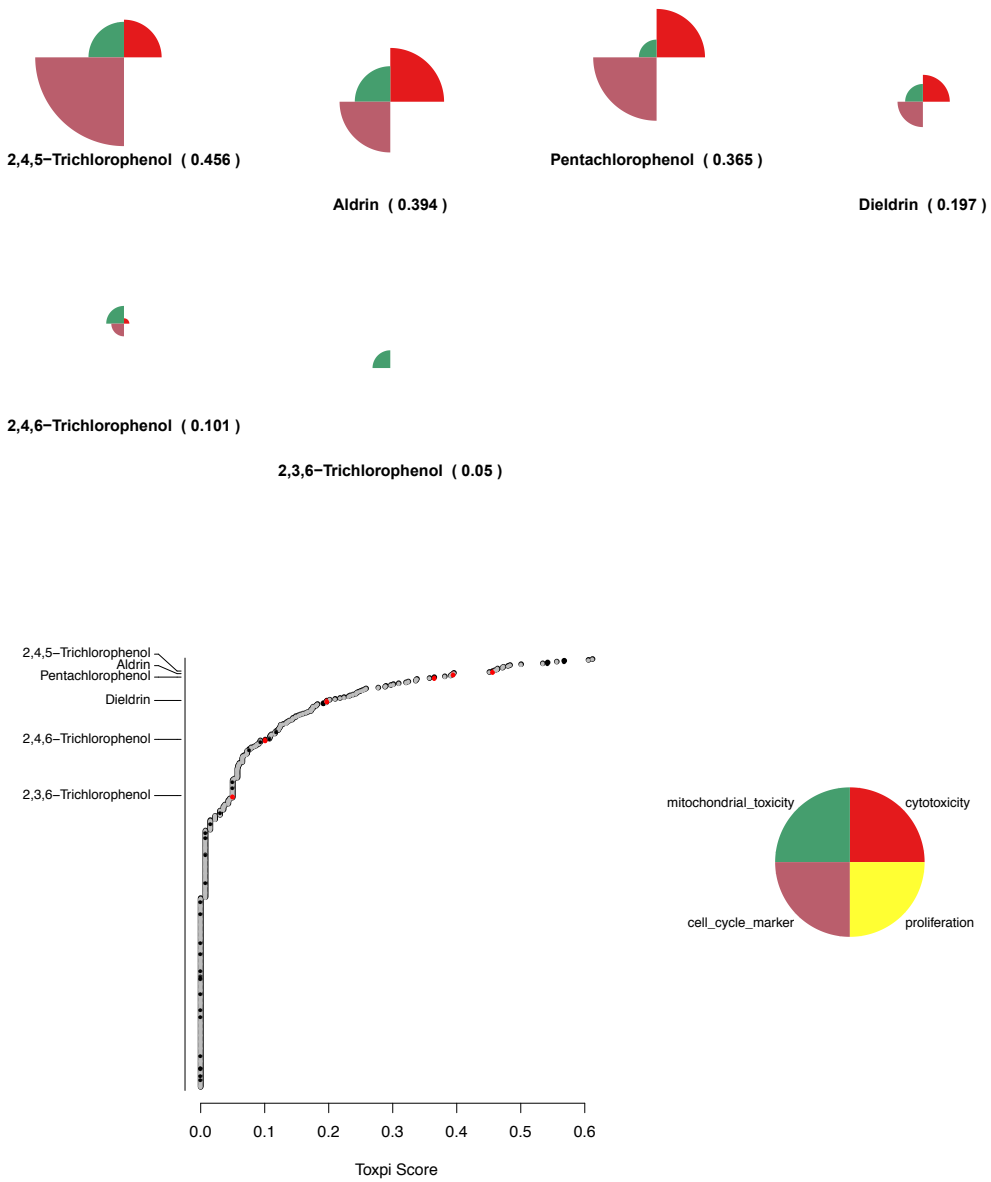
Across the top, the ToxPi shapes and scores for characteristic 6 (induces chronic inflammation) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

Fig. 4.7 ToxPi rankings using ToxCast assay end-points mapped to modulation of receptor-mediated effects



Across the top, the ToxPi shapes and scores for characteristic 7 (modulates receptor-mediated effects) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

Fig. 4.8 ToxPi rankings using ToxCast assay end-points mapped to cell proliferation, death, or nutrient supply



Across the top, the ToxPi shapes and scores for characteristic 10 (alters cell proliferation, cell death, or nutrient supply) are shown from the six chemicals pentachlorophenol, 2,4,6-trichlorophenol (and its metabolite isomers 2,4,5-trichlorophenol and 2,3,6-trichlorophenol), aldrin, and dieldrin tested in the ToxCast programme that are in the present *IARC Monographs* Volume 117. On the lower left, the relative ranks of these chemicals (red dots) are shown (*y*-axis) with respect to their ToxPi scores (*x*-axis) compared with the 489 chemicals previously evaluated by IARC (grey dots, with chemicals classified in Group I [carcinogenic to humans] as black dots). On the lower right, the subcategories of the ToxPi chart, as well as their respective colour coding are shown.

score of 0.61, and also a similar shape in that cytotoxicity and cell cycle markers make the greatest contribution, with additional contribution from mitochondrial toxicity.

Whereas examining each chemical's activity individually gives a sense of "absolute" activity, this comparison across chemical provides important context with respect to "relative" activity. Overall, this comparison across chemicals demonstrates that several chemicals evaluated in the present volume – pentachlorophenol, aldrin, dieldrin, and 2,4,5-trichlorophenol, a metabolite of 2,4,6-trichlorophenol – rank very highly with respect to assays mapped to the key characteristics of cytotoxicity, receptor modulation and, to a lesser extent, oxidative stress. However, the results of receptor modulation need to be interpreted with caution, since in some cases the results are not consistent across assay platforms for the same receptor, and because they can be confounded by cytotoxicity.

4.4 Susceptibility

No studies in humans were available to the Working Group.

One study in experimental animals examined the role of oxidative stress in the carcinogenicity of pentachlorophenol, using *Nrf2* knockout mice (Tasaki et al., 2014). Alterations in the Nrf2 pathway in mice affected development of cholangiocarcinoma after dietary exposure to pentachlorophenol at 1200 ppm. The wildtype mice did not develop cholangiocarcinomas.

4.5 Other adverse effects

4.5.1 Humans

Several epidemiological studies have examined non-cancer health effects in populations exposed to pentachlorophenol. Several studies have reported haematological effects in populations exposed to pentachlorophenol (Roberts, 1983; McConnachie & Zahalsky, 1991; Colosio et al., 1993). These effects range from aplastic anaemia to increased activation of T cells, increased incidence of autoimmunity, and immunosuppression and B-cell dysregulation. Neurological effects, such as nausea, lethargy, and peripheral neuropathies, have also been reported (Jorens & Schepens, 1993). Reported hepatic effects include increases in serum bile acids (Colosio et al., 1993). Begley et al. (1977) found depressed creatinine clearance and phosphorus reabsorption values in 18 workers exposed to pentachlorophenol, suggesting reduced glomerular filtration rate and tubular function. A few small case reports describing acute poisonings to pentachlorophenol identified a wide range of symptoms, including fever, hepatotoxicity and neurological symptoms (Wood et al., 1983; Walls et al., 1998). Chloracne was also a common finding in workers exposed to pentachlorophenol (Lambert et al., 1986; O'Malley et al., 1990; Leet & Collins, 1991).

Dahlgren et al. (2003) reported increases in the prevalence of bronchitis and asthma in a population of residents near a wood treatment plant who had sustained prolonged low-level exposure to pentachlorophenol and other wood-processing waste chemicals. These results may suggest impacts on the immune system.

Dimich-Ward et al. (1996) followed children of fathers who had worked in British Columbia sawmills for 1 year or more. The population consisted of 19 675 children of 9512 fathers. The study found an increased risk of congenital anomalies of the eye, with no associations for low birth weight, prematurity, still births, or neonatal deaths.

4.5.2 Experimental systems

In rats and mice, the liver is a target organ for pentachlorophenol, with a range of effects reported, including increased liver weight,

hepatocellular hypertrophy, and vacuolization (Kerkvliet et al., 1982a, b; Umemura et al., 1996; NTP, 1999). Necrosis, periportal fibrosis, and hepatocellular degeneration were seen at high doses of pure or technical-grade pentachlorophenol in rodents (Kerkvliet et al., 1982a, b; NTP, 1999). Mild to moderate renal toxicity (e.g. increased kidney weights and blood urea nitrogen) has been observed in rodents with long-term exposure to pure or technical-grade pentachlorophenol (Kimbrough & Linder, 1978; Nishimura et al., 1980; Blakley et al., 1998) but is infrequently accompanied by histopathological changes in the kidney.

5. Summary of Data Reported

5.1 Exposure data

Technical-grade pentachlorophenol is composed of approximately 90% pentachlorophenol and 10% contaminants, including other chlorophenols and various dioxin and furan congeners (primarily hexa-, hepta-, and octa- congeners). Pentachlorophenol and its salts have been widely used as herbicide, algicide, defoliant, wood preservative, germicide, fungicide, and molluscicide. Pentachlorophenol has been classified as a persistent organic pollutant under the Stockholm Convention, which requires parties to take measures to eliminate its production and use. Pentachlorophenol is banned for most uses in North America and Europe, but exceptions exist for heavy duty wood preservation, such as treating utility poles. Continued use in other parts of the world has been reported, such as for cleaning fish ponds to control schistosomiasis vectors in Asia.

Occupational exposure to pentachlorophenol has been measured in workers involved in the manufacture of pentachlorophenol and other chlorophenols, sawmill workers, agricultural workers, workers involved in treating wood products, electrical-utility workers, and waste-incinerator workers. Pentachlorophenol exposures were generally highest in workers directly involved in treating wood or who had direct contact with the treated product, with mean urinary concentrations often exceeding 100 µg/L. The general population may be exposed to pentachlorophenol from proximity to treated wood products, from contaminated food and waters, from incinerator emissions, and from contact with leather and textiles treated with chlorophenols. Median urinary concentrations of pentachlorophenol measured between the 1970s and 2000s in the general population ranged from < 1 to 25 µg/L.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to pentachlorophenol, the Working Group identified four reports from occupational cohorts and seven reports from population-based case–control studies. It was noted that interpretation of the results of all studies with respect to the carcinogenicity of pentachlorophenol was complicated by contamination with dioxin and furan, as well as co-exposures to other chlorophenols. Of particular interest was the contaminant 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), an IARC Group 1 carcinogen, which is not found in significant levels in pentachlorophenol; however, a number of the other higher chlorinated dioxins of substantially lower potency are characteristic of the pattern of contamination, including a range of hexachlorodibenzo-*para*-dioxin (HxCDD), heptachlorodibenzo-*para*-dioxin (HpCDD), and octachlorodibenzo-*para*-dioxin (OCDD) congeners. The studies that the Working Group found most informative were cohort studies that dealt with this issue by using high-quality exposure assessment techniques. These techniques included estimation of cumulative dermal

exposure to pentachlorophenol in a cohort study of Canadian sawmill workers, and measurement of the profile of dioxin congeners in serum to differentiate between chemicals in a cohort study of chemical-company workers. Three or more independent studies reported results for evaluation of risk of non-Hodgkin lymphoma (NHL), multiple myeloma, kidney, soft tissue sarcoma, and cancer of the lung, while cancer of the liver was reported in only one study. The cohort study of sawmill workers was considered to be a key investigation because of its relatively large size and high-quality exposure assessment, and the analysis of both mortality and incidence. Although considerably smaller and including only mortality follow-up, the chemical-company and National Institute for Occupational Safety and Health (NIOSH) cohort studies were both considered informative due to the quality of the exposure assessment and the length of follow-up.

5.2.1 NHL and other haematopoietic cancers

An elevated risk of NHL after exposure to pentachlorophenol was reported in all four cohort studies, and in three independent case–control studies, two from Sweden and one from New Zealand. In most studies, the increased risks for the most highly exposed workers were statistically significant and at least 2-fold. In the study from Canada, a statistically significant trend with estimated cumulative dermal exposure to pentachlorophenol was observed. In the chemical-company cohort analyses, risk in the subcohort exposed to pentachlorophenol (but not 2,4,5-trichlorophenol) was significantly elevated. In addition, a statistically significant risk was observed in the entire cohort in those with high exposure to the HxCDD, HpCDD, and OCDD congeners that can be considered as markers of exposure to pentachlorophenol as used in industry. An elevated but not statistically significant risk was observed for exposure to TCDD, although TCDD levels were lower in the pentachlorophenol subcohort than in the 2,4,5-trichlorophenol subcohort. A non-significantly increased risk of mortality due to NHL was found in the NIOSH study of pentachlorophenol-manufacturing workers exposed to pentachlorophenol but not trichlorophenol. All three case–control studies reported excess risks of NHL with exposure to pentachlorophenol, although there was less clarity on the extent to which the risk can be attributed solely to exposure to pentachlorophenol in these studies due to the use of job titles or self-reported exposure.

The Canadian sawmill study found a similar statistically significant trend in incidence of multiple myeloma with increasing cumulative dermal exposure to pentachlorophenol, and non-significant excess risks were observed in both the NIOSH cohort and in New Zealand fencing workers, but not in the chemical-company cohort.

Because of the consistent associations observed in several studies in different countries, and the observation of either exposure–response trends or the highest risk in the highest exposure category in two occupational cohort studies with high-quality exposure assessments, the Working Group concluded that the data demonstrated a causal association between NHL and exposure to pentachlorophenol, such that chance, bias and confounding can be ruled out with reasonable confidence. While the numbers were small in studies of multiple myeloma, which is now classified as a subtype of NHL, the increased risks observed in three studies lend support to this conclusion.

5.2.2 Other cancers

For other cancer sites, the results observed were generally not statistically significant or not consistent across studies. Elevated risk of cancer of the kidney was reported in three cohort studies, with a significant trend in Canadian sawmill workers; however, the numbers of cases

were small in all studies. Excess risk of cancer of the lung was observed in the pentachlorophenol-only subcohort in the NIOSH study, but there was no excess in either the Canadian sawmill workers or chemical-company cohorts. A statistically significant excess of soft tissue sarcoma was observed in one case–control study in Sweden, but no excess was observed in either the Canadian cohort or a New Zealand case–control study. Mortality from cancer of the liver was investigated in the Canadian cohort and, although numbers were small, a substantial excess risk was observed; however, there was no other support for this finding.

Overall, the Working Group concluded that there was scarce and inconsistent evidence of carcinogenicity after exposure to pentachlorophenol for these other cancer sites.

5.3 Animal carcinogenicity data

In mice, there were six studies of carcinogenicity with pentachlorophenol: five feeding studies, and one skin application study in transgenic females. The five feeding studies included two studies in males and females, two studies in transgenic males, and one study in transgenic males and females. There were three initiation–promotion studies with pentachlorophenol tested as a promoter in males. There were three co-carcinogenicity studies in males or females.

In mice, in one feeding study cited in one report, technical-grade pentachlorophenol increased the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), and pheochromocytoma of the adrenal gland in males; and of haemangiosarcoma of the vascular system in females. In the same report, in a second feeding study, commercial-grade pentachlorophenol (with a smaller concentration of dioxins and furans compared with technical-grade pentachlorophenol) increased the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), and pheochromocytoma of the adrenal gland in males; and of haemangiosarcoma of the vascular system, hepatocellular adenoma, and pheochromocytoma of the adrenal gland in females.

In one feeding study in male transgenic mice, there was an increase in the incidence of hepatocellular adenoma and cholangiocarcinoma of the liver. In the skin application study in female transgenic mice, there was an increase in the incidence of skin papilloma.

In three initiation–promotion studies in mice, pentachlorophenol by oral administration (feeding) promoted the development of hepatocellular adenoma or carcinoma (combined) in one study, hepatocellular adenoma in two studies, and cholangioma and cholangiocarcinoma of the liver in two studies.

In rats, there was one feeding study and one co-carcinogenicity study in males and in females. In the feeding study, pentachlorophenol increased the incidence of malignant mesothelioma of the tunica vaginalis of the testis in males. In the co-carcinogenicity study, pentachlorophenol increased the incidence of acute myelocytic leukaemia in males.

5.4 Mechanistic and other relevant data

Absorption of pentachlorophenol via oral and dermal exposure is rapid and extensive in all species studied, including humans, monkeys, mice, and rats. Pentachlorophenol distributes widely in the body via blood circulation. Pentachlorophenol is extensively bound to plasma proteins, with the greatest binding in humans, which leads to slow direct elimination of the parent compound. Metabolism involves both conjugation to glucurono- or sulfo-conjugates subsequently excreted in the urine, as well as oxidation to reactive metabolites,

including benzoquinones and semiquinones. Both pathways are active across the species studied, but the oxidation pathway is predominant in rodents while the conjugation pathway predominates in humans. Pentachlorophenol is also a strong inducer of cytochrome P450 enzymes, particularly CYP3A, and is an inhibitor of *O*-acetyltransferase and sulfotransferase enzymes. Excretion half-life is 10–20 days in humans and shorter in other mammalian species, such as monkeys (~80 hours), rats (~35 hours), and mice (~6 hours). There is *strong* evidence of metabolic activation to electrophilic benzoquinones and redox-cycling semiquinones.

In addition, there were data available on other key characteristics of carcinogens to evaluate whether pentachlorophenol induces oxidative stress, is genotoxic, modulates receptor-mediated effects, induces inflammation, is immunosuppressive, and alters cell proliferation, cell death, or nutrient supply.

There is *strong* evidence that pentachlorophenol induces oxidative stress and genotoxicity that can operate in humans. No studies of oxidative stress in exposed humans were available. Numerous studies in human cells, in mammalian systems in vivo or in vitro, and in non-mammalian experimental systems have reported increases in reactive oxygen species, oxidative stress markers, and DNA adducts associated with oxidative damage. Moreover, many studies across different species and experimental systems also demonstrated that these effects can be attenuated with co-exposure to a variety of antioxidants. These effects have been observed with treatment using either pentachlorophenol or metabolites such as tetrachlorohydroquinone (TCHQ) and tetrachlorobenzoquinone. TCHQ is the most studied metabolite, and appears to be more potent than pentachlorophenol, consistent with the need for metabolic activation of pentachlorophenol to induce oxidative stress. In addition, studies in *Nrf2*-knockout mice demonstrated that dysregulation of antioxidant expression increased pentachlorophenol-induced oxidative damage, cholangiofibrosis, and cholangiocarcinomas. In addition, multiple studies demonstrated genotoxicity consistent with oxidative damage in the form of 8-hydroxy-2′-deoxyguanosine (8-OHdG) formation in the livers of mice (and to a lesser extent, rats) treated in vivo. However, in a study using transgenic mice, pentachlorophenol exposure increased 8-OHdG, but did not induce *gpt* reporter gene mutations in the liver of *Tp53* wildtype or *Tp53*$^{-/-}$ mice. Pentachlorophenol also caused DNA strand breaks in multiple human cell types. Pentachlorophenol did not induce reverse mutations in the Ames test, whereas positive results were found in yeast and other bacterial assays that are more sensitive to oxidative DNA damage. Studies in acellular systems also reported DNA damage and/or adducts caused by pentachlorophenol in the presence of metabolic activation. Positive results have been reported for pentachlorophenol metabolites such as TCHQ, including mutation, micronucleus formation, and DNA strand breaks in multiple experimental systems. Evidence for induction of chromosomal aberrations, micronucleus formation, and sister-chromatid exchange, which includes studies in exposed humans and in multiple experimental mammalian systems, is mixed.

There is *strong* evidence that pentachlorophenol modulates receptor-mediated effects that can operate in humans with respect to anti-estrogenic activity. There are consistent results from studies in vitro using complementary techniques, including in human cells and in high-throughput screening data from Tox21. Several studies in mammals reported modulation of thyroid hormones after developmental exposures to pentachlorophenol, while the results of studies in vitro were ambiguous.

There is *strong* evidence that pentachlorophenol alters cell proliferation, cell death, or nutrient supply that can occur in humans. Pentachlorophenol and TCHQ induce apoptosis

in multiple experimental systems in vitro and in vivo, including in several human cell lines. Pentachlorophenol increases cell proliferation in mouse hepatocytes, intrahepatic bile duct epithelia, and skin, and alters proliferation and differentiation in mouse bone marrow culture, but decreased cell proliferation in one study in human lymphocytes in vitro. In several different experimental systems in vitro and in vivo, inhibition of gap-junction intercellular communication was observed after treatment with pentachlorophenol.

There is *moderate* evidence that pentachlorophenol induces chronic inflammation and is immunosuppressive. One study in exposed humans suggested increases in the frequency of mild infections and inflammation in skin, eye membrane and mucosa. Multiple studies in human cells and mammalian systems in vitro and in vivo suggest disruption of cytokines and/or deficiencies in cellular or humoral immunity as a result of treatment with pentachlorophenol. However, dioxin contamination present in technical-grade pentachlorophenol may have contributed to observations of immune suppression, as some effects were not observed with analytical-grade pentachlorophenol.

In the ToxCast/Tox21 high-throughput testing programmes of the United States government, pentachlorophenol was active for multiple assay end-points measuring markers of oxidative stress and *TP53* activation, consistent with the strong evidence for oxidative stress and associated genotoxicity discussed above. Pentachlorophenol was also active for many assay end-points related to modulation of receptor-mediated effects; however, these effects may be related to cytotoxicity, which was also observed across many assay end-points in cell lines and primary human cells.

There were no data on cancer susceptibility in humans. In experimental animals, one study in *Nrf2*-knockout mice suggested that dysregulation of antioxidant expression can increase susceptibility to pentachlorophenol-induced carcinogenicity.

Pentachlorophenol has been associated with haematological effects in some human studies, and effects on thyroid function, reproduction, toxicity in liver, and kidney in experimental animals.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of pentachlorophenol. Pentachlorophenol causes non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

The Working Group attributed the cancers observed in studies in humans and experimental animals to exposure to pentachlorophenol, and not to impurities in pentachlorophenol, based on the following considerations:

- Measured impurities in pentachlorophenol are dominated by higher chlorinated dioxins and furans, which are much less potent than 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD).
- The pattern of excess cancers observed in studies of occupational exposure to

pentachlorophenol differed from those observed in studies with high exposure to dioxins (i.e. excesses of all cancers combined, cancer of the lung, and soft tissue sarcoma, in addition to non-Hodgkin lymphoma).

- The pattern of excess cancers observed in experimental animals was similar for technical-grade pentachlorophenol (purity, 90.4%), commercial-grade pentachlorophenol (purity, 91%; with lower content of dioxin and furan), and analytical-grade pentachlorophenol (purity, ≥ 98%).
- Mechanistic studies with technical- and analytical-grade pentachlorophenol observed a wide spectrum of effects that differed from those observed with dioxins and furans.

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2,4,6-TRICHLOROPHENOL

1. Exposure Data

1.1 Identification of the agent

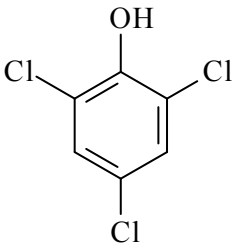
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 88-06-2

Chem. Abstr. Serv. Name: 2,4,6-Trichlorophenol

IUPAC Systematic Name: 2,4,6-Trichlorophenol

Synonyms: 2,4,6-TCP, Trichlorfenol



Molecular formula: $C_6H_3Cl_3O$

Relative molecular mass: 197.46

1.1.2 Chemical and physical properties of the pure substance

Description: Colourless to yellow crystals with a strong phenolic odour (Budavari, 1996; IARC, 1999; NTP, 2016)

Boiling-point: 246 °C (Lide, 1997; IARC, 1999)

Melting-point: 69 °C (Lide, 1997; IARC, 1999)

Solubility: Soluble in water (438–1200 mg/L at 25 °C) (Choudhary et al., 2013); soluble in acetone, acetic acid, diethyl ether, benzene, carbon tetrachloride, toluene, and ethanol (Lewis, 1993; Lide, 1997; IARC, 1999; NTP, 2016)

Vapour pressure: 133 Pa at 76.5 °C (United States National Library of Medicine, 1997; IARC, 1999)

Octanol/water partition coefficient: log K_{ow}, 3.69 (NTP, 2016)

Conversion factor: 1 ppm = 8.08 mg/m^3, at normal temperature (25 °C) and pressure (1 atm)

Dissociation constant (pK_a): 6.23 at 25 °C (NTP, 2016).

1.1.3 Technical products and impurities

(a) Trade names

Omal, Dowicide 2S, Phenaclor (NTP, 1979; ATSDR, 1999)

(b) Impurities

Technical-grade 2,4,6-trichlorophenol (2,4,6-TCP) has been found to contain 2,3,7-trichlorodibenzo-*para*-dioxin, 1,3,6,8-tetrachlorodibenzo-*para*-dioxin, 1,3,7,9-tetrachlorodibenzo-*para*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and other tetra-, penta-, and hexachlorodibenzofurans (WHO, 1989; INERIS, 2005). In the USA, 1,3,6,8-tetrachlorodibenzo-*para*-dioxin and 2,3,7-trichlorodibenzo-

para-dioxin were found in a commercial sample of 2,4,6-TCP at levels of 49 and 93 mg/kg, respectively (Firestone et al., 1972). In a Swedish sample of 2,4,6-TCP, 2,3,7,8-tetrachlorodibenzofuran was found at 1.5 mg/kg, penta-, hexa-, and heptachlorodibenzofurans at 17.5, 36, and 4.8 mg/kg, respectively, and polychlorinated dibenzo-*para*-dioxins (PCDDs) at < 3 mg/kg (Rappe et al., 1979).

1.2 Production and use

1.2.1 Production process

2,4,6-TCP was prepared by Laurent in 1836 by the chlorination of phenol, and this method has been used in the USA. In Japan, 2,4,6-TCP is co-produced during the manufacture of *ortho*- or *para*-chlorophenol, in a process also involving the chlorination of phenol (IARC, 1979). Distillation allows separation of 2,4,6-TCP from 2,3,4,6-tetrachlorophenol and pentachlorophenol, which are also formed during the reaction (INERIS, 2005).

1.2.2 Production volume

Commercial production of technical-grade 2,4,6-TCP in the USA was first reported in 1950 (IARC, 1979). In 1975, production was discontinued by the only manufacturer in the USA because of the high cost of removing PCDDs (NTP, 2016). In the USA, imports of 2,4,6-TCP totalled 2200 lb [~1000 kg] in 1976, 600 lb [~272 kg] in 1978, and 550 lb [~250 kg] in 1980 (IARC, 1979; NTP, 2016). In 2000, the United States Environmental Protection Agency (EPA) reported that 2,4,6-TCP was no longer used commercially (EPA, 2000).

In 2009, 2,4,6-TCP was produced by one manufacturer each in China, India, and Europe, and was available from 27 suppliers worldwide, including 16 suppliers in the USA (NTP, 2016).

In 2016, several companies were registered as manufacturing 2,4,6-TCP (mostly analytical grade): USA (11 companies), Canada (1), Germany (1), Switzerland (2), United Kingdom (2), China (1), Hong Kong Special Administrative Region, China (1), and Japan (2) (Chem Sources, 2016).

1.2.3 Use

2,4,6-TCP has been used primarily in various pesticide formulations and as a wood preservative. It has also been used as a fungicide, glue preservative, insecticide, bactericide, defoliant, herbicide, and anti-mildew agent for textiles (NTP, 2016). According to reports from New Zealand and Sweden, chlorophenols including 2,4,6-TCP were used from the 1970s until the late 1980s for the treatment of pelts (Glover et al., 1975; Pearce et al., 1988; Mikoczy et al., 1994). Chlorophenols have been used during the production of bark cork, and may inadvertently form from the use of hypochlorite solutions to clean cork stoppers and wooden barrels (Ozhan et al., 2009).

2,4,6-TCP is an intermediate for the synthesis of several chemicals such as pentachlorophenol, 2,3,4,6-tetrachlorophenol, and their sodium salts (INERIS, 2005).

Although most uses of 2,4,6-TCP were cancelled in the USA, 2,4,6-TCP continues to be used in the synthesis of some fungicides (NTP, 2016).

1.3 Analytical methods

Analytical methods for 2,4,6-TCP in different media have been described elsewhere (ATSDR, 1999; INERIS, 2005).

1.4 Occurrence and exposure

1.4.1 Occupational exposure

Occupational exposure to 2,4,6-TCP may occur in workers involved in the manufacture of 2,4,6-TCP and other chlorophenols, formulations containing 2,4,6-TCP, and chemicals that use 2,4,6-TCP as an intermediate (e.g. higher chlorinated phenols, phenolic resins, dyes, and drugs). 2,4,6-TCP is a common by-product in manufacturing pentachlorophenol, 2,4-dichlorophenol, tetrachlorophenol, and their salts, so workers exposed to those substances may also be exposed to 2,4,6-TCP (Kogevinas et al., 1995).

Occupational exposure also occurs in workers who apply formulations containing 2,4,6-TCP (e.g. sawmill workers), and workers exposed to 2,4,6-TCP as a by-product or contaminant (e.g. hazardous-waste incinerator workers) (ATSDR, 1999). Exposure may also occur in workers using 2,4,6-TCP as a biocide for treating textiles and leathers, or in workers handling the treated materials (de Souza Silveira et al., 2012; Karci, 2014). For example, 2,4,6-TCP was widely adopted for use as a fungicide for cured lamb pelts in New Zealand (Glover et al., 1975). Exposure to 2,4,6-TCP often occurs concurrently with other chlorophenol compounds, such as pentachlorophenol, and with PCDDs and polychlorinated dibenzofurans (PCDFs) (see Section 1.1.3).

(a) Air

In a Finnish sawmill that had regularly used 2,3,4,6-tetrachlorophenol containing 10–20% 2,4,6-TCP and 5% pentachlorophenol since the 1940s, median area air concentrations of 2,4,6-TCP ranged from 13 to 18 μg/m^3 for workers involved in outdoor vat-dipping, spraying lumber bundles, and trough-dipping lumber, to 68 μg/m^3 for workers involved in machine-stacking of lumber. Exposure of short duration to 2,4,6-TCP at a median air concentration of 610 μg/m^3 could occur inside kilns during drying. No 2,4,6-TCP was detected near workers who were trimming, grading, and packaging lumber (Kauppinen & Lindroos, 1985).

(b) Biological markers

Data on concentrations of 2,4,6-TCP in the urine have been collected in several studies in humans (Table 1.1). 2,4,6-TCP has been measured in the urine of hazardous- and municipal-waste incinerator workers, sawmill workers, and harbour workers involved in river dredging. Mean concentrations were typically < 4 μg/g creatinine. Urinary concentrations of 2,4,6-TCP ranged from 0.1 to 5.5 μg/g of creatinine in harbour workers and controls in Europe (Radon et al., 2004), from < 3 to 3.1 μg/g of creatinine in sawmill workers in Finland (Kontsas et al., 1995), and from 0.04 to 8.73 μg/g of creatinine in hazardous-waste incinerator workers in Europe (Domingo et al., 2001; Agramunt et al., 2003; Mari et al., 2013). No information on urinary concentrations of 2,4,6-TCP in textile or leather workers was found.

1.4.2 Community exposure

The general population may be exposed to 2,4,6-TCP as a result of proximity to 2,4,6-TCP-treated wood products, from dermal contact with 2,4,6-TCP-treated leathers and textiles, from use of wood preservatives that may contain 2,4,6-TCP, or from food and water contaminated with 2,4,6-TCP. Air exposure to 2,4,6-TCP may occur from the incineration of chlorinated compounds in municipal and hazardous waste, coal, and wood (ATSDR, 1999). 2,4,6-TCP can also be formed inadvertently when water containing phenol or some aromatic acids is treated with hypochlorite, such as during the bleaching process in pulp and paper mills, and during the disinfection of drinking-water sources (NTP, 2016; ToxNet, 2016).

Table 1.1 Concentrations of 2,4,6-trichlorophenol in urine samples from occupationally exposed workers

Country, year	Occupation	Work task or type of worker	No. of workers	Exposure[a]		Reference
				Level	Range	
Germany, 1997	Harbour workers	River dredging	83	Median, 0.36	0.1–3.8	Radon et al. (2004)
Germany, 1997	Harbour workers	Office workers	80	Median, 0.30	0.1–5.5	Radon et al. (2004)
Germany, 1999–2000	Hazardous-waste incinerator workers	Baseline, pre-employment	28	Mean, 0.86	0.04–8.73	Domingo et al. (2001); Schuhmacher et al. (2002)
Germany, 1999–2001	Hazardous-waste incinerator workers	Plant workers	19	Annual mean, 1.1–3.5	NR	Domingo et al. (2001); Schuhmacher et al. (2002)
Germany, 1999–2002	Hazardous-waste incinerator workers	Laboratory workers	3	Annual mean, 0.15–1.0	NR	Domingo et al. (2001); Schuhmacher et al. (2002)
Germany, 1999–2003	Hazardous-waste incinerator workers	Administrative worker	1	Annual mean range, 0.3–0.6	NR	Domingo et al. (2001); Schuhmacher et al. (2002)
Spain, 1999–2011	Hazardous-waste incinerator workers	Plant workers, including incinerator operators, boiler maintenance, furnace maintenance, control panel, and waste-gas-washing operators	16	Annual mean range, 0.3–3.5	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Spain, 1999–2012	Hazardous-waste incinerator workers	Laboratory workers	5	Annual mean range, 0.05–1.00	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Spain, 1999–2013	Hazardous-waste incinerator workers	Administrative workers	5	Annual mean range, 0.1–1.4	NR	Agramunt et al. (2003); Mari et al. (2009); Mari et al. (2013)
Germany, NR	Municipal waste incinerator	Municipal waste workers	53	Median, 0.85	0.30–3.86	Angerer et al. (1992)
Germany, NR	Municipal waste incinerator	Unexposed	248	Median, 0.6	< 1.2–10.6	Angerer et al. (1992)
Finland, NR	Sawmill workers	Tasks involving contact with chlorophenols	35	NR	One sample, 3.1; remainder, < 3	Kontsas et al. (1995)
Finland, NR	Sawmill workers	Unexposed	17	NR	All, < 3	Kontsas et al. (1995)
Finland, NR	Sawmill workers	Moving lumber that had been dipped in chlorophenol solution	7	Mean, 5.04 µmol/L [995 µg/L]		Pekari et al. (1991)

[a] Urinary concentrations are presented in µg/g creatinine unless otherwise indicated

NR, not reported

Compiled by the Working Group

(a) Water

2,4,6-TCP in water biodegrades in 8–14 days and absorbs readily to solids and sediments (ToxNet, 2016). 2,4,6-TCP concentrations in water were higher downstream (< 3.2 µg/L) than upstream (≤ 0.08 µg/L) from a Finnish pulp and paper mill (Oikari et al., 1985). 2,4,6-TCP was detected in 54% of surface water samples collected from Chinese rivers; the median concentration of 2,4,6-TCP was 2.0 ng/L, with substantially higher concentrations observed in rivers in northern China (maximum, 28 650 ng/L) than in southern China (Gao et al., 2008). In Poland, mean concentrations of 2,4,6-TCP ranged from 0.06 to 0.89 µg/L in river-water samples, and from 0.09 to 0.83 µg/L in drinking-water samples (Michałowicz et al., 2011). In river-water samples in the Republic of Korea, the median concentration of 2,4,6-TCP was 3.6 ng/L, and the maximum was 22 ng/L (Sim et al., 2009).

(b) Sediment and soil

Release of 2,4,6-TCP to soil may occur from disposal of manmade wastes, atmospheric deposition, and accidental releases (ATSDR, 1999). In river-sediment samples in the Republic of Korea, 2,4,6-TCP concentrations ranged from < 0.15 to 3.8 ng/g dry weight (Sim et al., 2009).

(c) Air

2,4,6-TCP exists as a vapour in the air and is degraded, with a half-life of 24 days, by reaction with photochemically produced hydroxyl radicals (ToxNet, 2016). In Portland, Oregon, USA in 1984, 2,4,6-TCP was detected in the air in five out of seven measured rain events, with a mean concentration (in samples in which 2,4,6-TCP was detected) of 0.15 ng/m^3 in the air samples and 1.4 ng/L in the precipitation samples (Leuenberger et al., 1985).

(d) Residues in food, and dietary intake

2,4,6-TCP has been measured at concentrations of up to 0.042 µg/g in coffee (Spadone et al., 1990). 2,4,6-TCP concentrations in red wine varied from 13 to 42 ng/L and were correlated with 2,4,6-TCP concentrations in the cork of the bottle (Ozhan et al., 2009), while oak barrels used to age wine and other spirits contained 2,4,6-TCP at concentrations ranging from 0.3 to 0.8 µg/g (Pizarro et al., 2006). 2,4,6-TCP has been measured at concentrations of up to 0.075 µg/g in semi-bleached-paper dishes and napkins (Ozaki et al., 2004).

(e) Household exposure

No measurements of 2,4,6-TCP in samples collected in homes were available to the Working Group.

(f) Biological markers

2,4,6-TCP has been measured in the urine in the general population (Table 1.2). The proportion of samples with detectable concentrations of 2,4,6-TCP ranged from 0% to 88%. Median concentrations were < 5 µg/L.

In the National Health and Nutrition Examination Survey (NHANES) of 1999–2004 in the USA, children aged < 15 years had urinary concentrations of 2,4,6-TCP ranging from 0.16 to 1772 µg/g of creatinine (Xu et al., 2011). In the NHANES and Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohorts of pregnant women, urinary concentrations of 2,4,6-TCP ranged from 0.4 to 142 µg/L (Castorina et al., 2010). In one study in the USA, < 5% of breast-milk samples contained 2,4,6-TCP at detectable levels when the limit of detection was 1.2 µg/L (Ye et al., 2006).

Table 1.2 Concentrations of 2,4,6-trichlorophenol in urine samples from the general population

Country, year	Age (years)	No. of samples	Exposure		Comments	Reference
			Level	Range, % detects		
Republic of Korea, 2009	18–69	1865	Median, 0.47 µg/g creatinine	< 0.05–127 µg/g creatinine, 88% detects	Higher in rural residents	Kim et al. (2014)
USA, 1988–94	20–59	867	Median, < 2 µg/g creatinine	< 2–28 µg/g creatinine, 9.5% detects		Hill et al. (1995)
USA, 1999–2002	NR	523	Median, 1.4 µg/L	< 0.6–142 µg/L, 56% detects	Pregnant women, 13 weeks gestation; California agricultural area; CHAMACOS cohort	Castorina et al. (2010)
USA, 1999–2002	NR	479	Median, 4.5 µg/L	0.4–62 µg/L, 74% detects	Pregnant women, 26 weeks gestation; California agricultural area; CHAMACOS cohort	Castorina et al. (2010)
USA, 1999–2002	NR	223	Median, 1.8 µg/L	< 1.3–68 µg/L, 60% detects	Pregnant women NHANES	Castorina et al. (2010)
USA, NR	2–6	197	Median, < 1 µg/g creatinine	< 1–34 µg/g creatinine, 21% detects		Hill et al. (1989)
USA, 2003–2010	6 to > 60	10 423	Median, < 1 µg/g creatinine	95th percentile, 0.9–5.20 µg/g creatinine, < 50% detects	NHANES	NHANES (2015)
Canada, 1993	36–76	31	NR	All < 2 µg/g creatinine	Sport fish consumers from three great lakes	Anderson et al. 1998
Germany, 1998	18–69	692	Median, 0.3 µg/g creatinine	0.2–4.1 µg/g creatinine	General population of Germany	Becker et al. (2003)

CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; NHANES, National Health and Nutrition Examination Survey; NR, not reported
Compiled by the Working Group

1.5 Regulations and guidelines

Occupational exposure limits for 2,4,6-TCP in air included an 8-hour average air concentration of 0.5 mg/m^3 in Denmark and Sweden, and short-term air concentrations of 1.0 mg/m^3 in Denmark and 1.5 mg/m^3 (for 15 minutes) in Sweden (IFA, 2016).

The World Health Organization (WHO) has established an international drinking-water guideline for 2,4,6-TCP of 200 µg/L (WHO, 1993).

The United States EPA has established ambient water quality criteria of 1.4 µg/L on the basis of seafood (fish or shellfish) and water consumption, 2.4 µg/L on the basis of seafood consumption only, and 2.0 µg/L on the basis of organoleptic-effect criteria (NTP, 2016).

In the USA, there are additional restrictions and requirements regarding transportation, presence in ambient air and hazardous waste, and releases to the environment (ATSDR, 1999). For example, 2,4,6-TCP is listed as a hazardous air pollutant under the Clean Air Act, and as a hazardous substance under the Clean Water Act (NTP, 2016), triggering a variety of requirements regarding pollutant monitoring, emissions control, record keeping, and reporting by major source.

The United States EPA has classified 2,4,6-TCP as Group B2, a "probable human carcinogen" (EPA, 1999). Under the harmonized classification and labelling system of the European Union, 2,4,6-TCP is "suspected of causing cancer (Carc. 2)" [H351] and has been determined to be "very toxic to aquatic life (Aquatic Acute 1)" [H400] and "very toxic to aquatic life with long lasting effects (Aquatic Chronic 1)" [H410], to be "harmful if swallowed (Acute Tox. 4)" [H302], to "cause serious eye irritation (Eye Irrit. 2)" [H319], and to "cause skin irritation (Skin Irrit. 2)" [H315] (ECHA, 2016).

2. Cancer in Humans

While many studies have examined the risk of cancer among workers exposed to 2,4,5-trichlorophenol, with a focus on contamination by 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), very few studies have provided results for the 2,4,6 isomer of trichlorophenol (2,4,6-TCP). The Working Group reviewed all the available epidemiological studies with relevant results for the evaluation of 2,4,6-TCP. All studies focused on occupational exposure. One case–control study nested in an occupational cohort (Kogevinas et al., 1995) and several case–control studies provided pertinent data. Studies in New Zealand, where 2,4,6-TCP was used for the treatment of sheep pelts (Glover et al., 1975), are reviewed below (Smith et al., 1984; Pearce et al., 1986b, 1988). Two case–control studies in Sweden reported associations between exposure to phenoxy herbicides or chlorophenols and soft tissue sarcoma and non-Hodgkin lymphoma (NHL) (Eriksson et al., 1981; Hardell et al., 1981), but did not present specific results for 2,4,6-TCP and were not considered further. 2,4,6-TCP has been used in tanneries, as has pentachlorophenol, but cohort studies of tannery workers did not specify which chlorophenol was in use; these studies were therefore not reviewed by the Working Group.

2.1 Cohort studies

See Table 2.1.

The IARC international register of workers exposed to phenoxy herbicides, chlorophenols, and dioxins included some workers exposed to 2,4,6-TCP (Saracci et al., 1991; Kogevinas et al., 1995). The pooled cohort consisted of 21 183 workers from 24 cohorts in 11 countries in Europe, North America, and Oceania. Work history records, detailed company exposure questionnaires, and company reports were used

Table 2.1 Epidemiological studies of cancer and exposure to 2,4,6-trichlorophenol

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1995) Australia, Denmark, Finland, Germany, Netherlands, New Zealand, Sweden, United Kingdom 1939–1992 Nested case–control	Cases: 32 NHL; 11 STS; cohort was identified from the International Register of Workers Exposed to Phenoxy Herbicides Controls: 158 NHL; 55 STS; incidence density sampling (5 controls per case matched for age, sex, country) Exposure assessment method: company records reviewed by industrial hygienists to estimate cumulative exposure to 21 chemicals	NHL STS	2,4,6-TCP (ever use) 2,4,6-TCP (ever use)	2 1	0.8 (0.08–8.04) 5.0 (0.31–79.94)	Matching factors: sex, age, country of residence at time of employment, country of cohort	Strengths: large study; objective exposure assessment methods; estimates of exposure to PCP, phenoxy herbicides, dioxins and furans; cancer incidence data Limitations: no quantitative exposure information; exposures to several compounds highly correlated; low power
Smith et al. (1984) New Zealand 1976–1980 Case–control	Cases: 82; male cases reported to the national cancer registry Controls: 92; one cancer control per case, matched by year of registration and age from the registry Exposure assessment method: questionnaire; work in pelt departments in meat works or in tanneries where 2,4,6-TCP was used; telephone interview	STS	Pelt department workers in meat works Tannery workers Pelt department or tannery workers	4 3 6	[4.7 (0.26–85.6)] – [7.2 (0.79–65.82)]	Country, sex, age	0 controls exposed for tannery workers; matching was not retained in the analysis Strengths: population based study with good participation rates; cases histologically confirmed; the questionnaire asked specifically about work in pelt departments Limitations: certainty of assignment of exposure to 2,4,6-TCP unclear; low power

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pearce et al. (1986b) New Zealand 1977–1981 Case–control	Cases: 83; Cancer registry Controls: 396; 168 cancer patients from cancer registry 228 general population controls Exposure assessment method: questionnaire; job-title based	NHL	Employment in pelt department in meat works:			Age, respondent type (proxy/ direct), sex	Strengths: population-based study; good response rates Limitations: limited exposure assessment
			Other cancer controls	4	2.3 (0.6–9.5)		
			General population controls	4	4.1 (0.9–18.6)		
			Pooled controls	4	2.7 (0.9–8.5)		
Pearce et al. (1988) New Zealand 1977–1981 Case–control	Cases: 183; NHL; cancer registry, histologically confirmed, and diagnosed under the age of 70 yr Controls: 338; patients reported to the national cancer registry with other types of cancers, randomly selected, within 2 yr of age to the case and population controls randomly selected from the 1982 New Zealand electoral roll Exposure assessment method: questionnaire; work in pelt departments in meat works or in tanneries where 2,4,6-TCP was used	NHL: ICD-9, 200 and 202	Pelt department workers	10	[1.9 (0.9–4)]	Respondent type (proxy/direct), age, sex, decade of birth	Strengths: population based study with good participation rates Limitations: level of exposure not known; low power
			Tannery workers	2	[0.5 (0.1–2.8)]		

2,4,6-TCP, 2,4,6-trichlorophenol; CI, confidence intervals; ICD, International Classification of Disease; NHL, non-Hodgkin lymphoma; PCP, pentachlorophenol; STS, soft tissue sarcoma; 2,4,6-TCP, 2,4,6-trichlorophenol; yr, year(s)

by three industrial hygienists (blinded to the disease status of the workers) to reconstruct exposure to 21 chemicals or mixtures. Kauppinen and colleagues reported a prevalence of exposure to 2,4,6-TCP of 6% within the pooled cohort, with a mean duration of employment of 4.8 years (Kauppinen et al., 1994).

Kogevinas and colleagues conducted nested case–control studies of soft tissue sarcoma and NHL among the pooled cohort of workers within the register (Kogevinas et al., 1995). Analyses were conducted using conditional logistic regression with a 5-year exposure lag. Only one exposed case of soft tissue sarcoma, (odds ratio, OR, 5.0; 95% confidence interval, CI, 0.31–79.94) and two exposed cases of NHL (OR, 0.80; 95% CI, 0.08–8.04) were identified. [Overall, this study had a strong design and exposure assessment; however, because of the low prevalence of exposure, it was underpowered for studying the association between 2,4,6-TCP and NHL or soft tissue sarcoma.]

2.2 Case–control studies

Smith and colleagues conducted a case–control study of men with soft tissue sarcoma (International Classification of Disease, ICD-9, 171) diagnosed in New Zealand between 1976 and 1980 (Smith et al., 1984). Cases were reported by public hospitals, which contributed 95% of population coverage in New Zealand to the national cancer registry, in 1976–1980. Cases were reviewed histologically by a pathologist. Controls were randomly selected from the same registry. A telephone interview was conducted with questions on activities with the potential for exposure to chlorophenoxy herbicides and chlorophenols. The questionnaire asked specifically about pelt departments in view of their use of 2,4,6-TCP for treating sheepskins. Proxy interviews were conducted with 57% of cases and 64% of controls. Four cases and one control reported working in pelt departments of meat works (OR, 4.7; [95% CI, 0.3–86]), while three cases and no controls reported working in areas of tanneries where exposure to 2,4,6-TCP may also have occurred. When these two groups were combined (six exposed cases in total), the odds ratio was 7.2 ([95% CI, 0.8–66]). Further interviews provided conflicting information on dates when 2,4,6-TCP was used, casting doubt on the exposure of four of the six cases. For example, the tannery where the exposed cases worked was closed, but a similar tannery owned by the same company in New Zealand reported that 2,4,6-TCP was used only after 1962, whereas pentachlorophenol was used only before 1962. [This study had several limitations. Other cancer patients (cancer sites not stated) were used as controls. Most of the interviews were conducted with proxies. There was low power to detect an excess risk. Although the fact that follow-up interviews were conducted was a strength, no results were presented in which cases for whom exposure to 2,4,6-TCP was doubtful were excluded.]

A similarly designed case–control study of NHL in New Zealand also provided relevant results for this evaluation (Pearce et al., 1986b, 1988). Initially, only men with NHL (ICD-9, 202 only), excluding lymphosarcoma and reticulosarcoma (ICD-9, 200), were recruited from a national cancer registry (Pearce et al., 1986b). Two matched controls per case were randomly selected from the registry. An additional control group comprised 300 men randomly selected from the 1982 electoral roll for New Zealand. During a telephone interview, participants were asked about activities with a potential for exposure to phenoxy herbicides and chlorophenols. Odds ratios were adjusted for decade of birth and whether the subject or a relative was interviewed. Odds ratios for work in pelt departments in meat works where 2,4,6-TCP was used were 2.3 [95% CI, 0.6–9.5] when using other cancer controls, and 4.1 [95% CI, 0.9–18.6] when using general population controls. The results when both sets of controls were pooled were similar

(OR, 2.7, [95% CI, 0.9–8.5]). When potentially exposed participants were further interviewed it was discovered that two out of four cases and four out of ten controls had performed tasks during which they were unlikely to have been exposed, but a revised analysis was only reported for all chlorophenols combined. No excess was observed among tannery workers when either cancer or population controls were used.

This case–control study was later expanded to include lymphosarcoma and reticulosarcoma (ICD-9, 200) and subsequently reported results for all NHL combined (ICD-9, 200, 202) (Pearce et al., 1988). A target sample of 121 cases of lymphosarcoma and reticulosarcoma was identified (100 participated, 83%) and an expanded set of 338 cancer controls (81% participation) and the population controls were not used in the further analyses. The odds ratio for work in pelt departments in meat works was 2.2 ([95% CI, 0.8–6.3]; based on 6 cases) for lymphosarcoma and reticulosarcoma. The odds ratio for NHL was 1.9 ([95% CI, 0.8–4.6]; based on 10 cases). [In this analysis, the cases and controls with questionable exposure appeared to have been removed.] The results for pelt department workers were similar to those for all meat-works employment, and the highest risk was observed among men who had worked at meat works and in fencing. No excess risk was observed for tannery workers (OR, 0.5; [95% CI, 0.1–2.8]; based on 2 exposed cases). [This study had limited precision and levels of exposure were not known. There was also the potential for exposure to pentachlorophenol and other potentially carcinogenic exposures related to fencing for participants who had worked in both jobs.]

2.3 Exposure assessment in epidemiological studies

Few epidemiological studies had evaluated exposure to 2,4,6-TCP. Several epidemiological studies relying on job classification were conducted in New Zealand (Smith et al., 1984; Pearce et al., 1986a, b, 1988). These studies used retrospective telephone interviews with patients or next of kin to determine whether each individual had worked in particular jobs for which the investigators had determined that exposure to phenoxy herbicides or chlorophenols was likely. Initial questions used a pre-specified list of occupations, and people who reported having worked in those occupations were asked subsidiary questions regarding the specific nature of the work and the potential for exposure to specific chemicals. Exposure was treated as a dichotomous variable (yes/no for each particular job) in the epidemiological analyses. Pelt departments of meat works were identified a priori as an occupation of interest due to the known use of 2,4,6-TCP in treating sheep pelts; however, Pearce et al. (1986b) reported that 6 of the 14 study participants initially reporting having worked in a pelt department had actually worked in a fellmongery removing wool before sheep skins were treated, and were thus unlikely to have been exposed to 2,4,6-TCP. Meat works employees were likely to have been exposed to other chemicals as well as 2,4,6-TCP (Pearce et al., 1986b).

Only one epidemiological study evaluating the magnitude of exposure to 2,4,6-TCP was identified (Kogevinas et al., 1995). For this study, three industrial hygienists (blinded to health status of the workers) reviewed general work processes and conditions for cancer cases and controls sampled from IARC's international register of more than 21 000 workers exposed to phenoxy herbicides, chlorophenols, and contaminants, from 11 countries in Europe, North America, and Oceania. By 1990, 19 cohorts were enrolled, including sprayers of

phenoxy herbicides and workers from companies manufacturing or formulating phenoxy acids or chlorophenols (Saracci et al., 1990). On the basis of company questionnaires regarding chemical production and use characteristics as well as the general literature, unit-less job-specific exposure intensities specific to this study were assigned for a variety of phenoxy herbicides, dioxins, and chlorophenols, including 2,4,6-TCP, based on the product of a subscore for each job task (ranging from 1 to 10, and assumed to be constant over time and equivalent across plants) and modifying factors (including for emissions of agents, average daily contact time of the workers with the contaminants, and the use of personal protective equipment) (Kauppinen et al., 1994). [Although the authors noted that dermal exposure might be important for many of these compounds, the extent to which specific exposure routes were considered in scoring was unclear.] Exposure intensities were then multiplied by job duration and summed across all jobs in each individual's work history to calculate a cumulative exposure score for each worker. [Although this method may be adequate for detecting strong contrasts in 2,4,6-TCP exposure between cases and controls, the exposure assignments probably suffer from substantial non-differential measurement error due to an apparent lack of direct measurements of 2,4,6-TCP in the workplace, resulting in attenuation of epidemiological effect estimates towards null association.]

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

In a study by the United States National Cancer Institute (NCI), groups of 50 male B6C3F_1 mice (age, 6 weeks) were fed diets containing 2,4,6-TCP (purity, 96–97%; with 17 minor contaminants [not further specified]) at a concentration of 5000 or 10 000 ppm for 105 weeks. Groups of 50 female B6C3F_1 mice (age, 6 weeks) were fed diets containing 2,4,6-TCP at 10 000 (lower dose) or 20 000 (higher dose) ppm for 38 weeks, at which time the doses were reduced to 2500 (lower dose) and 5000 (higher dose) ppm because of markedly reduced body-weight gain in the treated animals. After this change in doses, the study continued for a further 67 weeks. Time-weighted average doses for females were 5214 (lower dose) and 10 428 (higher dose) ppm. The control groups comprised 20 untreated male mice and 20 untreated female mice. There was no dose-related trend in mortality in males or females. Survival of males was 16/20 for controls, 44/50 at the lower dose, and 45/50 at the higher dose. Survival of females was 17/20 for controls, 44/50 at the lower dose, and 40/50 at the higher dose. At the end of the study, body weights of treated groups of males and females were lower than those of controls. The incidence of hepatocellular adenoma was significantly increased in treated males, and in females at the higher dose. There was a significant positive trend in the incidence of hepatocellular carcinoma in females. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in males (in both treated groups, with a significant positive trend) and in females (at the higher dose, with a significant positive trend) (NTP, 1979). [The Working Group noted that the number of concurrent controls was small, and that the PCDD content of the diet was not determined.]

Stoner et al. (1986) evaluated tumours of the lung in the A/J mouse after administration of 2,4,6-TCP (reagent grade) in tricaprylin by gavage or by intraperitoneal injection three times per week for 8 weeks. Groups of 16 male and 16 female A/J mice (age, 6–8 weeks), were given total doses of 2,4,6-TCP of 0 (control) or 1200 mg/kg bw by gavage, or 0 (control) 240, 600, or 1200 mg/kg bw by intraperitoneal injection.

Table 3.1 Studies of carcinogenicity with 2,4,6-trichlorophenol in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 105 wk NTP (1979)	Oral 2,4,6-TCP, 96–97% Diet 0, 5000, 10 000 ppm Ad libitum 20, 50, 50 16, 44, 45	*Liver* Hepatocellular adenoma: 3/20, 22/49*, 32/47** Hepatocellular carcinoma: 1/20, 10/49, 7/47 Hepatocellular adenoma or carcinoma (combined): 4/20, 32/49*, 39/47**	 NR (trend), *[$P < 0.05$] **[$P < 0.0001$] NS $P < 0.001$ (trend); *$P = 0.001$; **$P < 0.001$	17 (unspecified) minor impurities; PCDD content was not determined Limitations: small number of controls
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 105 wk NTP (1979)	Oral 2,4,6-TCP, 96–97% Diet 0, 5214, 10 428 ppm (TWA) Ad libitum 20, 50, 50 17, 44, 40	*Liver* Hepatocellular adenoma: 1/20, 12/50, 17/48* Hepatocellular carcinoma: 0/20, 0/50, 7/48 Hepatocellular adenoma or carcinoma (combined): 1/20, 12/50, 24/48*	 NR (trend), *[$P < 0.02$] $P = 0.005$ (trend) $P < 0.001$ (trend); *$P < 0.001$	Dietary levels: 38 wk at 10 000 or 20 000 ppm, then 67 wk at 2500 or 5000 ppm, resulting in TWA of 5214 and 10 428 ppm, respectively 17 (unspecified) minor impurities; PCDD content was not determined Limitations: small number of controls
Full carcinogenicity Rat, F344 (M) 6 wk 106–107 wk NTP (1979)	Oral 2,4,6-TCP, 96–97% Diet 0, 5000, 10 000ppm Ad libitum 20, 50, 50 18, 35, 34	*Haematopoietic system* Malignant lymphoma: 1/20, 2/50, 0/50 Monocytic leukaemia: 3/20 (15%), 23/50 (46%)*, 28/50 (56%)** Monocytic leukaemia or malignant lymphoma (combined): 4/20, 25/50*, 29/50**	 NS $P = 0.003$ (trend); *$P = 0.013$; **$P = 0.002$ $P = 0.006$ (trend); *$P = 0.019$; **$P = 0.004$	Limitations: small number of controls 17 (unspecified) minor impurities; PCDD content was not determined Historical control incidence at laboratory, leukaemia: 11/255 (4%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (F) 6 wk 106–107 wk NTP (1979)	Oral 2,4,6-TCP, 96–97% Diet 0, 5000, 10 000 ppm Ad libitum 20, 50, 50 14, 39, 39	*Haematopoietic system* Malignant lymphoma: 0/20, 0/50, 2/50 Monocytic leukaemia: 3/20, 11/50, 10/50 Monocytic leukaemia or malignant lymphoma (combined): 3/20 (15%), 11/50 (22%), 13/50 (26%)	 NS NS NS	17 (unspecified) minor impurities; PCDD content was not determined Historical control incidence at laboratory, leukaemia or malignant lymphoma (combined): 42/420 (10%) Limitations: small number of controls

F, female; M, male; NR, not reported; NS, not significant; PCDD, polychlorinated dibenzo-*para*-dioxin; ppm, parts per million; 2,4,6-TCP, 2,4,6-trichlorophenol; TWA, time-weighted average; wk, week(s)

There was no increase in the incidence or multiplicity of tumours of the lung in treated mice when compared with vehicle controls (Stoner et al., 1986). [The Working Group noted the short duration of the experiment.]

One study in mice was judged inadequate for the evaluation by the Working Group because of some deficiencies in the study design, including the variable combination of small number of animals, dosage used, unknown purity of the compound, and absence of histopathology data (NCI, 1968; Innes et al., 1969).

3.2 Rat

In a study by the NCI, groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given diets containing 2,4,6-TCP (purity, 96–97%; 17 minor contaminants [not further specified]) at a concentration of 5000 or 10 000 ppm for 106–107 weeks. Groups of 20 males and 20 females served as controls. There was no dose-related trend in mortality in males or females. Survival of males was 18/20 for the controls, 35/50 at the lower dose, and 34/50 at the higher dose, and survival of females was 14/20, 39/50, and 39/50, respectively. Throughout the study, body weights of treated groups of males and females were lower than those of controls. The incidence of monocytic leukaemia was significantly increased in both groups of treated males (controls, 3/20; lower dose, 23/50; and higher dose, 28/50) with a significant positive trend. The incidence of this neoplasm in historical controls at the laboratory was 11/255 (4%). Other adverse effects observed at 2 years in exposed males and females included leukocytosis of the peripheral blood and bone marrow hyperplasia. There was no significant increase in the incidence of tumours in treated females (NTP, 1979). [The Working Group noted the small number of concurrent controls, and that the PCDD content was not determined (although the neoplasms observed had not previously been associated with exposure to dioxin).]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

[The Working Group noted that absorption, distribution, metabolism, and excretion have been much less studied for 2,4,6-TCP than for pentachlorophenol.]

4.1.2 Absorption

No data on the absorption of 2,4,6-TCP in humans or experimental systems were available to the Working Group. On the basis of analogy to other chlorophenols, 2,4,6-TCP is likely to be rapidly absorbed.

4.1.3 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In male Wistar rats, peak concentrations were observed in all tissues 30 minutes after a single intraperitoneal dose of 2,4,6-TCP (25 mg/kg bw). The highest concentration (approximately 65 mg/kg) was found in the kidneys, followed by blood, liver, fat, muscle, and brain. After 10 hours, only trace amounts of 2,4,6-TCP remained in the blood and tissues (Pekari et al., 1986).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

See Fig. 4.1.

(i) Humans

No data were available to the Working Group. Lindane (γ-hexachlorocyclohexane) is metabolized in part to 2,4,6-TCP by human liver microsomes (Fitzloff et al., 1982).

(ii) Experimental systems

In male Wistar or Sprague-Dawley rats dosed daily for 15 days with radiolabelled 2,4,6-TCP (25 µg) by gavage, unconjugated urinary 2,4,6-TCP and isomers represented 63% of the total administered dose. Conjugates eliminated in the urine (80% of which were conjugates with glucuronic acid) accounted for 28% of the total administered dose, and an additional 6% of the total administered dose was eliminated in the faeces (Bahig et al., 1981). Male Wistar rat liver microsomes can metabolize 2,4,6-TCP into 2,6-dichloro-1,4-hydroquinone, the 2,6-dichloro-1,4-semiquinone free radical, and two isomers of hydroxy-pentachlorodiphenyl ether (Juhl et al., 1989). Horseradish peroxidase can catalyse hydrogen peroxide (H_2O_2)-dependent oxidative 4-dechlorination of 2,4,6-TCP, leading to the formation of 2,6-dichloro-1,4-benzoquinone (Ferrari et al., 1999). [The Working Group considered that the formation in vivo of 2,6-dichloro-1,4-benzoquinone was likely, by analogy to pentachlorophenol.]

Lindane (γ-hexachlorocyclohexane) is metabolized in part to 2,4,6-TCP by male Wistar rats in vivo (Baliková et al., 1989), and by rat liver microsomes (Fitzloff et al., 1982). 2,4,6-TCP is also produced by the conversion of the α-isomer of hexachlorocyclohexane (purity, 95%) in male Wistar rats (Macholz et al., 1982), and by the conversion of prochloraz in male Sprague-Dawley rats (Laignelet et al., 1992).

(b) Modulation of metabolic enzymes

(i) Humans

2,4,6-TCP inhibited acetylcholinesterase activity in the human erythrocyte membrane in vitro (Matsumura et al., 1997). It also decreased the expression of mRNA of several enzymes involved in steroidogenesis in the human adrenocortical carcinoma cell line H295R in vitro (Ma et al., 2011; see Section 4.2.4).

(ii) Experimental systems

In male adult Sprague-Dawley rats given 2,4,6-TCP (purity, unspecified; oral doses of up to 400 mg/kg for 14 days), no significant effects were observed on *O*-ethyl *O*-*para*-nitrophenyl phenylphosphonothioate (EPN) detoxification (which involves mixed-function oxidases and arylesterase) or on uridine 5′-diphospho (UDP)-glucuronyltransferase (Carlson, 1978). In vitro, with microsomal fractions from the same rats, 2,4,6-TCP inhibited EPN detoxification and methylation of *para*-nitroanisole, but not UDP-glucuronyltransferase (Carlson, 1978).

4.1.5 Excretion

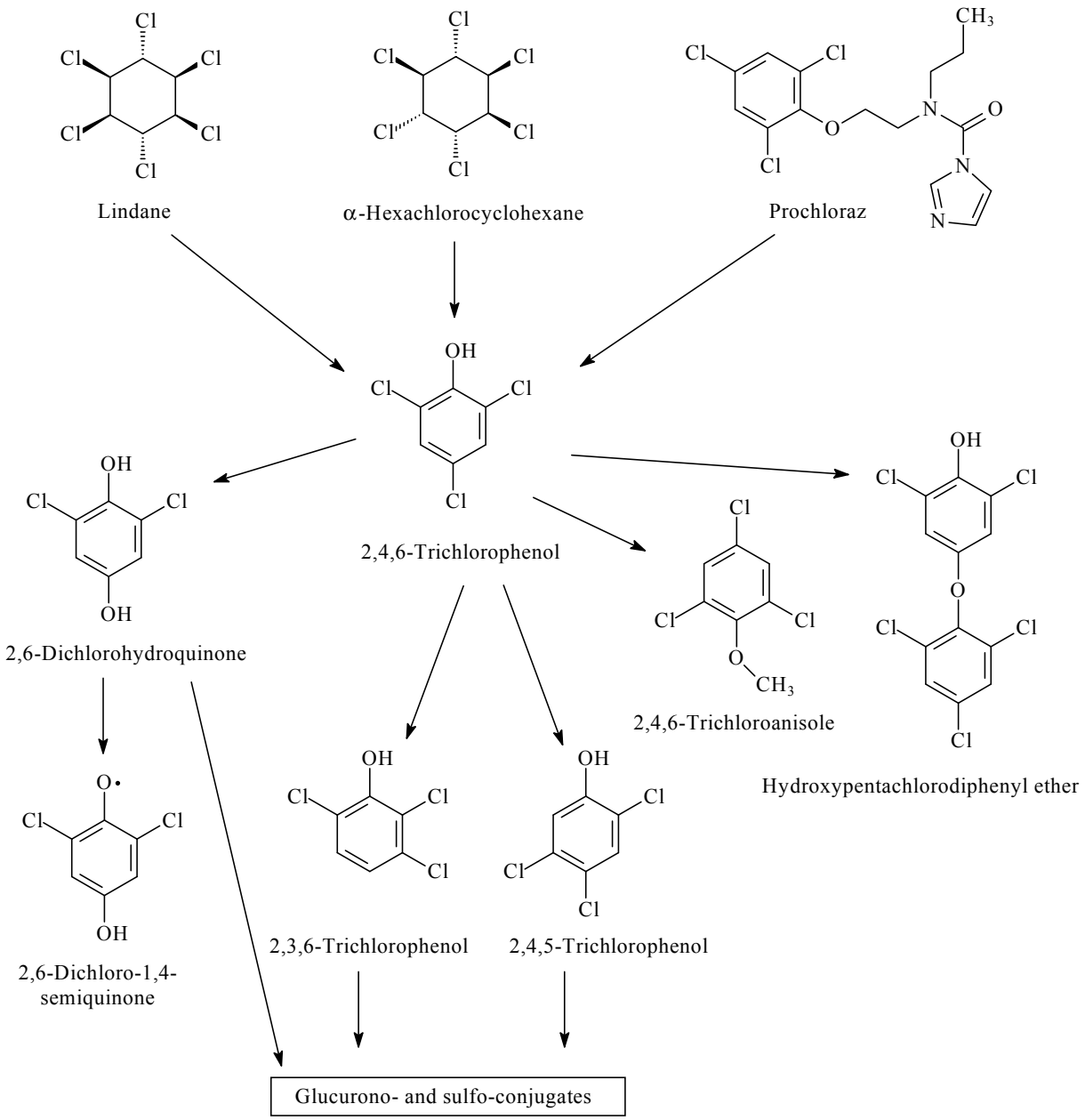
(a) Humans

2,4,6-TCP was detected in the urine of children in a study of parent-reported attention deficit hyperactivity disorder (Xu et al., 2011).

(b) Experimental systems

Half-lives for trichlorophenols range from hours to days, compounds with higher chlorine content having longer half-lives (IARC, 1986). In male Wistar or Sprague-Dawley rats dosed daily for 15 days with 25 µg of radiolabelled 2,4,6-TCP by gavage, the excretion of radiolabel in the urine and faeces reached a plateau after 2 days and decreased sharply to a few percent of the administered dose within 3 days after exposure. About 6% of the administered dose was excreted in the faeces, and the rest in the urine (Bahig

Fig. 4.1 Metabolism of 2,4,6-trichlorophenol



Compiled by the Working Group

et al., 1981). In male Wistar rats given a single intraperitoneal dose of 2,4,6-TCP at 25 mg/kg bw, 90% of the administered dose was excreted between 4 and 6 hours. The half-life of 2,4,6-TCP in all the tissues studied ranged from 1.4 to 1.8 hours. The half-life of conjugated 2,4,6-TCP in the blood was also 1.4 hours (Pekari et al., 1986).

4.2 Mechanisms of carcinogenesis

This section summarizes in the following order the available evidence for the key characteristics of carcinogens (Smith et al., 2016), concerning whether 2,4,6-TCP is genotoxic; induces oxidative stress; alters cell proliferation, cell death, or nutrient supply; or modulates receptor-mediated effects. For the other key characteristics of carcinogens, insufficient data were available for evaluation.

4.2.1 Genetic and related effects

(a) Humans

See Table 4.1.

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

In the human promyelocytic leukaemia cell line HL-60, 2,4,6-TCP (50 µg/mL) increased DNA damage in the comet assay (Ozaki et al., 2004).

(b) Experimental systems

No data from mammalian experimental systems in vivo were available to the Working Group.

The assay for forward mutation in L5178Y *Tk*$^{+/-}$ mouse lymphoma cells gave positive results with 2,4,6-TCP (80 µg/mL) (McGregor et al., 1988). Although 2,4,6-TCP (100 µg/mL) failed to induce mutation at the *Hprt* locus or chromosomal aberrations in Chinese hamster fibroblast V79 cells in the absence of metabolic activation (Jansson & Jansson, 1986, 1992), 2,4,6-TCP (30 µg/mL or higher) induced statistically significant, dose-related increases in the frequency of hyperdiploidy and micronucleus formation (Jansson & Jansson, 1992). Armstrong et al. (1993) also observed hyperdiploidy in V79 cells.

2,4,6-TCP did not induce structural chromosomal aberrations or sister-chromatid exchanges in Chinese hamster ovary (CHO) cells (Galloway et al., 1987). However, when the protocol was adjusted to extend the recovery period after treatment before harvest, chromosomal aberrations in CHO and V79 cells were induced by 2,4,6-TCP (600 µg/mL) both in the presence (S9) and absence of metabolic activation in CHO cells (Armstrong et al., 1993).

An elevated frequency of point mutations in the *Tp53* gene in the liver genome was seen in zebrafish exposed to 2,4,6-TCP (5 µg/L) for 10 days (Yin et al., 2009).

2,4,6-TCP induced forward mutation, but not gene conversion, in *Saccharomyces cerevisiae* (Fahrig et al., 1978). 2,4,6-TCP did not induce reverse mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (Haworth et al., 1983). 2,4,6-TCP (50 µg/disc) caused DNA damage, as detected by the recombinant (*rec*) assay in *Bacillus subtilis* (Ozaki et al., 2004). DNA strand breaks were detected using the Microscreen prophage-induction assay in *Escherichia coli* exposed to 2,4,6-TCP (DeMarini et al., 1990).

DNA strand breaks were induced after microsomal activation of 2,4,6-TCP (1 mM) and incubation with bacteriophage PM2 DNA plasmid (Juhl et al., 1989). 2,4,6-TCP (10 µM) formed deoxyguanosine adducts after bioactivation by a representative peroxidase system (Dai et al., 2005).

Table 4.1 Genetic and related effects of 2,4,6-trichlorophenol

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC/HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Human leukaemia, HL-60	+	NT	50 µg/mL	Purity, > 97%	Ozaki et al. (2004)
Tk mutation	Mouse lymphoma, L5178Y cells	+	NT	80 µg/mL	Purity, NR	McGregor et al. (1988)
Hprt mutation	Chinese hamster fibroblasts, V79	–	NT	100 µg/mL	Purity, > 99.5% Cell survival, 53%	Jansson & Jansson (1986)
Hprt mutation	Chinese hamster fibroblasts, V79	–	NT	180 µg/mL	Purity, 99.7% Cell survival, 14%	Jansson & Jansson (1992)
Chromosomal aberrations	Chinese hamster fibroblasts, V79	–	NT	60 µg/mL	Purity, 99.7%	Jansson & Jansson (1992)
Aneuploidy, micronucleus formation	Chinese hamster fibroblasts, V79	+	NT	30 µg/mL	Purity, 99.7%	Jansson & Jansson (1992)
Chromosomal aberrations	Chinese hamster ovary, CHO-W-B1	–	–	500 µg/mL	Purity, NR	Galloway et al. (1987)
Sister-chromatid exchanges	Chinese hamster ovary, CHO-W-B1	–	–	500 µg/mL	Purity, NR	Galloway et al. (1987)
Chromosomal aberrations	Chinese hamster ovary, CHO-WBL	+	+	600 µg/mL	Purity, > 99.7%	Armstrong et al. (1993)
Chromosomal aberrations	Chinese hamster fibroblasts, V79	+	NT	600 µg/mL	Purity, > 99.7%	Armstrong et al. (1993)
Aneuploidy	Chinese hamster fibroblasts, V79	+	NT	50 µg/mL		Armstrong et al. (1993)
Tumour suppressor gene (*Tp53*) mutation	Zebrafish liver in vivo	+	NA	5 µg/L, 10 d	Purity, > 98%	Yin et al. (2009)
Forward mutation	*Saccharomyces cerevisiae* MP-1	+	NT	400 mg/L	Purity, 99%	Fahrig et al. (1978)
Gene conversion	*Saccharomyces cerevisiae* MP-1	–	NT	400 mg/L	Purity, 99%	Fahrig et al. (1978)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	–	–	666 µg/plate	Purity, "practical" grade	Haworth et al. (1983)
Differential toxicity	*Bacillus subtilis* M45 (*recA*$^-$)	+	NT	3 µg/plate	Purity, > 97%	Ozaki et al. (2004)
Differential toxicity	*Bacillus subtilis* H17 (*recA*$^+$)	+	NT	6 µg/plate	Purity, > 97%	Ozaki et al. (2004)
Prophage λ induction	*Escherichia coli* WP2s	+	+	32 µM [6.3 µg/mL]	Purity, "practical grade" Toxicity + S9 at 255 µM [50.3 µg/mL]	DeMarini et al. (1990)

Table 4.1 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC/HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Bacteriophage PM2 DNA	NT	+	1 mM [200 µg/mL]	More strand breaks observed with S9 from induced rats than from non-induced rats	Juhl et al. (1989)
DNA adducts, C8-dG O-adducts, LC/MS	2′-Deoxyguanosine	–	+	10 µM [2 µg/mL]	Horseradish peroxidase/H_2O_2 system	Dai et al. (2005)

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

C8-dG O-adducts, C8-deoxyguanosine O-adduct; DMSO, dimethyl sulfoxide; HIC, highest ineffective concentration; *Hprt*, hypoxanthine guanine phosphoribosyl transferase gene; LC-MS, liquid chromatography-mass spectrometry; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; *Tk*, thymidine kinase gene

4.2.2 Oxidative stress

No studies in exposed humans, in human cells, or in rodents in vivo were available to the Working Group.

2,4,6-TCP (1.0 mM) induced oxidative stress in mouse embryonic fibroblasts, as shown by an upregulation of nuclear-E2-related factor 2 (*Nrf2*) and haem oxygenase 1 (*Hmox-1*) mRNA expression, the nuclear translocation of Nrf2 protein, and an upregulation of reactive oxygen species evaluated with dichlorodihydrofluorescein diacetate (DCFH) by flow cytometry (Zhang et al., 2014). [The Working Group noted the recognized limitations of DCFH as a marker of oxidative stress (Bonini et al., 2006; Kalyanaraman et al., 2012).]

In studies in non-mammalian systems, electron paramagnetic resonance demonstrated free-radical generation and oxidative stress in goldfish (*Carassius auratus*) liver after intraperitoneal injection with 2,4,6-TCP at a concentration of 5 mg/kg (Ji et al., 2007; Li et al., 2007). 2,4,6-TCP (10 µM) increased malondialdehyde content and the activities of peroxidase and superoxide dismutase in one plant species, *Arabidopsis* (Li et al., 2015).

The peroxidation of 2,4,6-TCP yielded 2,6-dichloro-1,4-benzoquinone (Ferrari et al., 1999), and a 2,4,6-trichlorophenoxyl radical intermediate was demonstrated by electron spin resonance analysis (Sturgeon et al., 2011; Sumithran et al., 2012).

4.2.3 Cell proliferation, cell death, and nutrient supply

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

In long-term studies, 2,4,6-TCP (0.5% in the diet) significant increased the incidence of leukocytosis in the peripheral blood of male rats, and of hyperplasia of the bone marrow in male and female rats (NTP, 1979). In male and female mice fed diets containing 2,4,6-TCP (0.5% in the diet), focal and nodular areas of hepatocyte hyperplasia were present (NTP, 1979; Huff, 2012).

2,4,6-TCP promoted differentiation of mouse primary lineage-depleted bone marrow cells into granulocytes (at 300 µM), macrophages (at 100 µM), and erythrocytes (at 10 µM) (Henschler et al., 2001).

Liao et al. demonstrated that in monkey kidney Vero cells, 2,4,6-TCP (0.25 µg/mL) induced cell membrane damage, as shown by flow cytometry analysis and propidium iodide staining (Liao et al., 2010a, b, 2011). 2,4,6-TCP (1.0 mM) induced apoptosis in mouse embryonic fibroblasts, as demonstrated by annexin V–fluorescein isothiocyanate/propidium iodide staining and flow cytometry analysis (Zhang et al., 2014).

4.2.4 Receptor-mediated effects

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

Unlike a parent compound of 2,4,6-TCP, prochloraz (an imidazole fungicide), 2,4,6-TCP (50 µM) did not inhibit the response induced by R1881 (an androgen-receptor agonist) in an androgen-receptor reporter-gene assay (Vinggaard et al., 2002). As noted in Section 4.1, 2,4,6-TCP decreased the expression of mRNA of several enzymes involved in steroidogenesis in H295R cells (Ma et al., 2011).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays carried out by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) programmes of the government of the USA, see Section 4.3 of the *Monograph* on pentachlorophenol in the present volume.

In a microarray study in the female rare minnow, 2,4,6-TCP (at 10 μg/L) altered levels of mRNA encoding proteins related to endocrine and metabolic pathways (Fang et al., 2014).

4.4 Cancer susceptibility data

No data were available to the Working Group.

4.5 Other adverse effects

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

5. Summary of Data Reported

5.1 Exposure data

2,4,6-Trichlorophenol (2,4,6-TCP) has been used primarily in various pesticide formulations and as a wood preservative. It has also been used as a fungicide, glue preservative, insecticide, bactericide, defoliant, and herbicide, as well as an anti-mildew agent for textiles, leather, and pelts. 2,4,6-TCP is used as an intermediate for the synthesis of several chemicals, including pentachlorophenol and 2,3,4,6-tetrachlorophenol, and their sodium salts. Commercial production of technical-grade 2,4,6-TCP in the USA was first reported in 1950, with production reduced in the mid-1970s because of the high cost of removing toxic impurities such as polychlorinated dibenzo-*para*-dioxins. Although most uses of 2,4,6-TCP were subsequently cancelled in the USA, it continues to be used in the synthesis of some fungicides. There were several registered manufacturers in North America, Europe, and Asia, but production levels were not available. Data on the environmental persistence of 2,4,6-TCP were sparse.

Occupational exposure to 2,4,6-TCP has been measured in two studies of sawmill workers and in several studies of hazardous-waste incinerator workers. The highest observed occupational exposures occurred in sawmill workers who were moving wood products that had been dipped in a chlorophenol solution (mean, 5.04 μmol/L [995 μg/L]), but this was based on only seven workers.

The general population may be exposed to 2,4,6-TCP from proximity to chlorophenol-treated wood products, from air emissions from waste incinerators, and from food and water contaminated with chlorophenols. Median urinary concentrations of 2,4,6-TCP in the general population were generally < 2 μg/g creatinine.

5.2 Human carcinogenicity data

Few studies of cancer in humans have been conducted that provide results relevant to evaluation of the carcinogenicity of 2,4,6-TCP. Two population-based case–control studies conducted in New Zealand provided results for men exposed occupationally, either in meat works or tanneries. One found an increased risk of soft tissue sarcoma, while the other found an increased risk of non-Hodgkin lymphoma, neither of which was statistically significant. Both studies were based on small numbers of exposed cases, and the role for other potentially confounding factors could not be ruled out. A large, international pooled cohort of workers exposed to phenoxy herbicides, chlorophenols, and dioxins included a small proportion of workers exposed to 2,4,6-TCP, among whom there was one case of soft tissue sarcoma and two cases of non-Hodgkin lymphoma. In light of the small number of studies available for each cancer site, and the very small numbers of cases exposed to 2,4,6-TCP in each study, the Working Group concluded that there were insufficient data to draw a conclusion regarding the carcinogenicity of 2,4,6-TCP.

5.3 Animal carcinogenicity data

There was one study of carcinogenicity in male and female mice fed diets containing 2,4,6-TCP. 2,4,6-TCP increased the incidence of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined) (with a significant positive trend) in males and females. There was also a significant positive trend in the incidence of hepatocellular carcinoma in females.

There was one study of carcinogenicity in male and female rats fed diets containing 2,4,6-TCP. 2,4,6-TCP increased the incidence of monocytic leukaemia (with a significant positive trend) in males. No significant increase in tumour incidence was reported in females.

One study in A/J mice treated by gavage and one study in A/J mice treated by intraperitoneal administration gave negative results.

5.4 Mechanistic and other relevant data

Data on the absorption, distribution, metabolism, and excretion of 2,4,6-TCP were sparse. On the basis of analogy to other chlorophenols, 2,4,6-TCP is likely to be rapidly absorbed, widely distributed in the body by blood circulation, and predominantly metabolized to conjugates that are excreted in the urine. Excretion after a single intraperitoneal administration in rats was rapid, with 90% of the administered dose excreted within 6 hours.

Regarding the key characteristics of carcinogens, adequate data were available to evaluate whether 2,4,6-TCP is genotoxic and induces oxidative stress.

There is *moderate* evidence that 2,4,6-TCP is genotoxic. One study in human cells in vitro and several studies in bacteria reported DNA strand breaks after administration of 2,4,6-TCP. Several studies in Chinese hamster cells in vitro observed effects on chromosomes, such as chromosomal aberrations, micronucleus formation, and hyperdiploidy, but not sister-chromatid exchanges. Mutations were observed in yeast, the mouse lymphoma assay, and zebrafish, but not in bacteria or Chinese hamster fibroblasts.

There is *moderate* evidence that 2,4,6-TCP induces oxidative stress. No studies in vivo were available; however, all available studies in vitro in mouse embryonic fibroblasts, fish, and plants reported increased generation of free radicals (including reactive oxygen species) and/or increased antioxidant activities. Additionally, a phenoxyl radical intermediate had been identified.

In the Toxicity Forecaster (ToxCast) and Toxicity Testing in the 21st Century (Tox21) high-throughput testing programmes of the government of the USA, 2,4,6-TCP was largely inactive, except for in a few assays related to oxidative stress.

There were no data on cancer susceptibility and few data on other adverse effects.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2,4,6-trichlorophenol.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,4,6-trichlorophenol.

6.3 Overall evaluation

2,4,6-Trichlorophenol is *possibly carcinogenic to humans* (*Group 2B*).

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3,3′,4,4′-TETRACHLOROAZOBENZENE

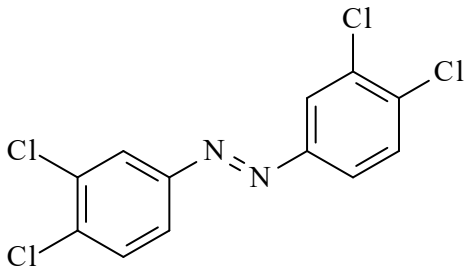
1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 14047-09-7

Chem. Abstr. Serv. Name: 3,4,3′,4′-Tetrachloroazobenzene

Synonyms: Bis(3,4-dichlorophenyl)diazene; azobenzene, 3,3′,4,4′-tetrachloro-; diazene, bis(3,4-dichlorophenyl)-; diazene, bis(3,4-dichlorophenyl)- (9Cl); TCAB



Molecular formula: $C_{12}H_6Cl_4N_2$

Relative molecular mass: 320

In the *trans* configuration, 3,3′,4,4′-tetrachloroazobenzene (TCAB) can assume a planar conformation with a molecular shape similar to that of 2,3,7,8-tetra-chlorodibenzo-*para*-dioxin (TCDD) (Poland et al., 1976; NTP, 2010)

Description: Bright orange, crystalline solid

Melting point: 158 °C

Solubility: water solubility: 6.72×10^{-3} mg/L at 25 °C

Vapour pressure: 1.56×10^{-7} mm Hg (2.07×10^{-5} Pa) at 25 °C

Hazardous decomposition: When heated to decomposition it emits toxic fumes of chlorine and oxides of nitrogen

Octanol/water partition coefficient: log K_{ow}, 5.53 (Hashimoto et al., 1994; NTP, 1998)

Stability: TCAB is stable as a bulk chemical when stored at room temperature (NTP, 2010)

Conversion factor: 1 ppm = 13.1 mg/m^{3,} at normal temperature (25 °C) and pressure (1 atm).

1.2 Production and use

1.2.1 Production

TCAB is not commercially manufactured but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives, which include propanil (3′,4′-dichlorophenylpropionanilide; CAS No., 709-98-8), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; CAS No., 330-55-2), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea; CAS No., 330-54-1), and neburon (1-butyl-3-(3,4-dichlorophenyl)-1-methylurea; CAS No., 555-37-3) (Poland et al., 1976; Sundström et al., 1978; Bunce et al., 1979; Hill et al., 1981).

In the late 1980s in the USA, when the production volume of propanil was 10 million pounds [~4536 tonnes] per year, the resultant annual production of TCAB for propanil alone may have been as high as 12 000 kg (McMillan et al., 1991). Likewise, with a production volume of 100 000–1 000 000 pounds [45–454 tonnes] of 3,4-dichloroaniline per year, the resultant annual production of TCAB may have been as high as 3900 kg (EPA, 1985). Because 3,4-dichloroaniline is used as a precursor in dye manufacture and, to a limited extent, as a heat transfer fluid, in addition to its use in the manufacture of herbicides (EPA, 1985), TCAB could be present in products other than herbicides (NTP, 2010).

1.2.2 Use

No known direct use of TCAB has been reported.

In 2007, the use of propanil and diuron in the USA was estimated to range from 4 million to 6 million pounds [1814–2722 tonnes] and from 2 million to 4 million pounds [907–1814 tonnes], respectively (EPA, 2011).

In California, USA, the use of several anilide pesticides including propanil was reported to have increased from < 1000 pounds in 1998 to ~2 million pounds [< 0.45 to ~907 tonnes] in 2014 (OEHHA, 2016).

1.3 Methods of analysis

No data were available to the Working Group.

1.4 Occurrence and exposure

Concentrations of TCAB in technical grades ranged from 0.1 to 9.9 µg/g for propanil, 5.7 to 12 µg/g for diuron, 6.7 to 28 µg/g for linuron, and 1.9 to 23 µg/g for neburon (Di Muccio et al., 1984). Hill et al. (1981) found TCAB at concentrations of 1000–1400 µg/g in propanil, 9–51 µg/g in 3,4-dichloroaniline, 28 µg/g in diuron, and 9 µg/g in linuron; no detectable TCAB was reported in 1,2-dichlorobenzene or neburon (Hill et al. 1981). Singh & Bingley (1991) found TCAB at concentrations of 1–30 µg/g in commercial herbicides containing propanil (Singh & Bingley, 1991). Call et al. (1983) found TCAB at a concentration of 670 µg/g in technical-grade propanil (Call et al., 1983).

In addition, environmental contamination by TCAB occurs from the degradation of chloroanilide herbicides (acylanilides, phenylcarbamates, and phenylureas) in soil by peroxide-producing microorganisms (Bartha et al., 1968; Bartha & Pramer, 1969; Lay & Ilnicki, 1974). TCAB is also formed by the photolysis and biolysis of 3,4-dichloroaniline (Miller et al., 1980; NTP, 2010).

Workers who manufacture or work with other products that have 3,4-dichloroaniline as a precursor (e.g. dyes) or as a heat transfer fluid may also be exposed to TCAB (EPA, 1985; NTP, 2010).

1.4.1 Occupational exposure

No measurements of TCAB exposure in workers were available to the Working Group. Occupational exposure may occur in workers involved in the manufacture of aniline herbicides, applicators who spray or mix aniline herbicide-containing formulations, and farm workers engaged in re-entry tasks. TCAB exposure would vary depending on the aniline herbicides used or produced (see Section 1.2).

1.4.2 Community exposure

The general population may be exposed to TCAB from residues on food, or from living near areas where aniline herbicides are applied.

(a) Sediment and soil

TCAB sorbs very strongly to soils, and has been detected in the top 10 cm of soil up to 2 years after application of propanil (Kearney et al., 1970). TCAB was found in 6 of 99 soil samples from rice-growing areas of the USA, with concentrations ranging from 0.01 to 0.05 ppm (Kearney et al., 1970; Carey et al., 1980). TCAB formation in soil varies with pH, with negligible formation in soils that are more alkaline than pH 6.0, and measurable levels in soils with a pH range of 4.0 to 5.5 (Hughes & Corke, 1974).

(b) Food

No data were available on TCAB measurements in the food supply; however, TCAB uptake in the food chain was observed experimentally in non-mammalian systems. Proportional increases in TCAB body burden were seen in Japanese medaka (*Oryzias latipes*) exposed to diets containing increasing concentrations of TCAB (0.5–2500 ppm) (Allinson & Morita, 1995a). The aquatic snail Indohiramakigai (*Indoplanorbis exustus*) was found to bioaccumulate TCAB from its environment during controlled exposures (Allinson & Morita, 1995b). Uptake of TCAB was also observed experimentally with soybeans (Worobey, 1984), carrots (Worobey, 1988), and rice plants (Still, 1969).

(c) Air, water, and residential dust

No data were available to the Working Group.

(d) Biological markers

No data on concentrations of TCAB in the general population were available to the Working Group.

1.5. Regulations and guidelines

In July 2012, California, USA, listed TCAB as a known carcinogen under the Safe Drinking Water and Toxic Enforcement Act (Proposition 65), based on findings from the National Toxicology Program (NTP) (OEHHA, 2012).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were given TCAB (purity, ≥ 99.8%) at doses of 0 (control), 3, 10, or 30 mg/kg body weight (bw), in corn oil:acetone (99:1) by gavage, 5 days per week for 104 weeks (NTP, 2010). Survival was significantly decreased in males at 10 and 30 mg/kg bw, and in females at 30 mg/kg bw. At 0, 3, 10, and 30 mg/kg bw, survival was 35/50, 31/50, 5/50, and 0/50 in males, and 35/50, 30/50, 32/50, and 20/50 in females, respectively. Mean body weights of males at 10 and 30 mg/kg bw were 10% and 8% less than those of the vehicle controls at the last weighing at weeks 101 and 73, respectively. Mean body weights of females at 3 mg/kg bw were 7% greater than those of the vehicle controls after week 64.

The incidence of transitional cell carcinoma of the urethra was significantly increased (with a significant positive trend) in all treated groups of males. Two such neoplasms were also observed in females at 30 mg/kg bw (2/50; 4%). One male at 10 mg/kg bw and one female at 30 mg/kg bw had transitional cell carcinoma of the ureter. There was a significantly increased incidence of bronchioloalveolar adenoma of the lung in all treated groups of males, with a significant positive trend, and a significantly increased incidence of bronchioloalveolar carcinoma of the lung in females at 30 mg/kg bw, with a significant positive trend.

Table 3.1 Studies of carcinogenicity with 3,3′,4,4′-tetrachloroazobenzene (TCAB) in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 5–6 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 3, 10, 30 mg/kg bw 10 mL/kg, 5 d/wk 50, 50, 50, 50 35, 31, 5, 0	*Urethra* Transitional cell carcinoma: 0/50*, 32/50**, 46/49**, 49/50**	*P (trend) < 0.001, **P < 0.001; poly-3 test	Significant decrease in survival at 10 and 30 mg/kg bw Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for bronchioloalveolar carcinoma (corn-oil gavage studies): 13/200 (6.5 ± 2.5%); range, 4–10%
		Lung Bronchioloalveolar adenoma: 5/50*, 16/50**, 12/49***, 6/50****	*P (trend) = 0.014, **P = 0.002, ***P = 0.006, ****P = 0.037; poly-3 test	
		Bronchioloalveolar adenoma or carcinoma (combined): 7/50*, 17/50**, 15/49***, 6/50	*P (trend) = 0.014, **P = 0.007, ***P = 0.003; poly-3 test	
		Bronchioloalveolar carcinoma: 3/50, 1/50, 4/49, 0/50	NS	
		Forestomach Squamous cell carcinoma: 0/50*, 1/50, 1/49, 3/50**	*P (trend) = 0.012, **P = 0.023; poly-3 test	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5–6 wk 105 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 3, 10, 30 mg/kg 10 mL/kg, 5 d/wk 50, 50, 50, 50 35, 30, 32, 20	*Urethra* Transitional cell carcinoma: 0/50, 0/50, 0/50, 2/50	NS	Significant decrease in survival at 30 mg/kg bw Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for cystic keratinizing epithelioma was 0/196 for corn-oil gavage studies and 0/1496 for all routes
		Lung Bronchioloalveolar carcinoma: 0/49*, 2/50, 1/50, 4/50**	*P (trend) = 0.031,**P = 0.042; poly-3 test	
		Bronchioloalveolar adenoma or carcinoma (combined): 3/49*, 8/50, 5/50, 10/50**	*P (trend) = 0.028, **P = 0.015; poly-3 test	
		Cystic keratinizing epithelioma: 0/49, 0/50, 0/50, 2/50	NR	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5–6 wk 105 wk NTP (2010) (cont.)		*Forestomach* Squamous cell carcinoma: 0/50*, 1/50, 1/50, 4/50**	*P (trend) = 0.011, **P = 0.040; poly-3 test	
		Skin Fibrosarcoma: 1/50*, 6/50, 5/50, 8/50**	*P (trend) = 0.023, **P = 0.008; poly-3 test	
		Fibrosarcoma or malignant schwannoma: 2/50*, 8/50, 7/50, 12/50**	*P (trend) = 0.004, **P = 0.001; poly-3 test	
		Spleen/lymphatic tissue Malignant lymphoma: 2/50, 5/50, 8/50*, 7/50**	*P = 0.049, **P = 0.050; poly-3 test	
Full carcinogenicity Rat, Harlan Sprague-Dawley (M) 5 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 10, 30, 100 mg/kg 2.5 mL/kg, 5 d/wk 50, 50, 50, 50 28, 9, 4, 2	*Lung* Cystic keratinizing epithelioma: 0/50*, 14/50**, 31/50**, 37/50**	*P (trend) < 0.001, **P < 0.001; poly-3 test	Significant decrease in survival in all treated groups Principal strengths: the duration of exposure and observation was adequate; as was the schedule of exposure; GLP study
		Oral mucosa Gingival squamous cell carcinoma: 1/50, 5/50*, 4/50, 5/50**	*P = 0.046, **P = 0.033; poly-3 test	
		Liver Cholangiocarcinoma: 0/50*, 4/50**, 4/50***, 6/50****	*P (trend) = 0.007, **P = 0.030, ***P = 0.026, ****P = 0.003; poly-3 test	
		Thyroid Follicular cell adenoma: 0/50*, 3/50, 4/50**, 4/50***	*P (trend) = 0.037, **P = 0.025, ***P = 0.021; poly-3 test	
		All organs Malignant schwannoma: 0/50*, 0/50, 1/50, 3/50	*P (trend) = 0.010; poly-3 test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Harlan Sprague-Dawley (F) 5 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 10, 30, 100 mg/kg 2.5 mL/kg, 5 d/wk 50, 50, 50, 50 25, 30, 18, 17	*Lung* Cystic keratinizing epithelioma: 0/50*, 6/50**, 26/49***, 39/49***	* P (trend) < 0.001, **P = 0.014; poly-3 test, ***P < 0.001	Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for cholangiocarcinoma (corn-oil gavage studies): 1/473 (0.2 ± 0.7%); range, 0–2% Historical control incidence for gingival squamous cell carcinoma: 4/473 (0.8 ± 1.0%); range, 0–2% Historical control incidence for forestomach squamous cell papilloma: 0/473; forestomach squamous cell carcinoma: 2/473 (0.4 ± 0.8%) [range, 0–2%]
		Liver Cholangiocarcinoma: 1/50, 1/50, 1/49, 3/49	NS	
		Oral mucosa Gingival squamous cell carcinoma: 0/50*, 0/50, 4/50, 6/50**	*P (trend) = 0.002, **P = 0.015; poly-3 test	
		Forestomach Squamous cell papilloma: 0/50, 0/50, 0/50, 3/50	NS	
		Squamous cell carcinoma: 0/50, 1/50, 0/50, 1/50	NS	
		Squamous cell papilloma or squamous cell carcinoma (combined): 0/50*, 1/50, 0/50, 4/50	*P (trend) = 0.009; poly-3 test	

* Significance is indicated using asterisks

d, day(s); F, female; GLP, Good Laboratory Practice; M, male; NR, not reported; NS, not significant; TCAB, 3,3′,4,4′-tetrachloroazobenzene; wk, week(s)

A significantly increased incidence of bronchioloalveolar adenoma or carcinoma (combined) was observed in males at 3 and 10 mg/kg bw, and in females at 30 mg/kg bw. However, the incidence of bronchioloalveolar carcinoma was not increased in treated males compared with concurrent controls, or compared with the range for historical controls for corn-oil gavage studies for this neoplasm (incidence, 13/200; range, 4–10%).

In the forestomach, the incidence of squamous cell carcinoma in males and females at 30 mg/kg bw was significantly increased (3/50 and 4/50, respectively), with a significant positive trend, compared with that in the control groups receiving vehicle only (0/50 in males and females).

In females, the incidence of malignant lymphoma was significantly increased at 10 and 30 mg/kg bw. The incidence of fibrosarcoma, and of fibrosarcoma or malignant schwannoma (combined) of the skin was significantly increased in females at 30 mg/kg bw, with a significant positive trend. One occurrence of a single cystic keratinizing epithelioma and one occurrence of multiple cystic keratinizing epithelioma of the lung were reported in females at 30 mg/kg bw (2/50); the incidence of cystic keratinizing epithelioma in historical controls was 0/196 for corn-oil gavage studies and 0/1496 for all routes.

[The Working Group noted that in this study that complied with good laboratory practice (GLP), the duration of exposure and observation, and the schedule of exposure, were adequate.]

3.2 Rat

Groups of 50 male and 50 female Harlan Sprague-Dawley rats (age, 5 weeks) were given TCAB (purity, ≥ 99.8%) at doses of 0 (control), 10, 30, or 100 mg/kg bw, in corn oil:acetone (99:1) by gavage, 5 days per week, for 104 weeks (NTP, 2010). Survival of all treated groups of males (9/50, 4/50, 2/50) was significantly less than that of the controls receiving vehicle only (28/50). The number of females surviving to study termination was 30/50, 18/50, and 17/50 in the treated groups, respectively, compared with controls receiving vehicle only (25/50). Mean body weights of males at 100 mg/kg bw were less than those of males in the vehicle-control group throughout the study. Mean body weights of males at 30 mg/kg bw were 6% less than those of males in the vehicle-control group after week 24, and those of males at 10 mg/kg bw were 7% less than those of males in the vehicle-control group after week 80. Mean body weights of females at 100 mg/kg bw were less than those of females in the vehicle-control group throughout the study, and those of females at 30 mg/kg bw were 6% less than those of females in the vehicle-control group after week 36.

The incidence of multiple cystic keratinizing epithelioma and of cystic keratinizing epithelioma (including multiple) of the lung in males and females was significantly increased, with a positive trend, in all treated groups compared with controls, except for multiple cystic keratinizing epithelioma in females at 10 mg/kg bw.

In males, the incidence of cholangiocarcinoma of the liver in all treated groups was significantly greater than that in the control group, with a positive trend. In females, the incidence of cholangiocarcinoma of the liver in the group at the highest dose (3/49) was above the upper bound of the range for historical controls (incidence, 1/473; range, 0–2%).

The incidence of gingival squamous cell carcinoma of the oral mucosa was increased in treated males and females compared with controls: the increases in males at 10 and 100 mg/kg bw and in females at 100 mg/kg bw were significantly greater than those in the controls, and the increase in females at 30 mg/kg bw (6/50) exceeded the upper bound of the range for historical controls (incidence, 4/473; range, 0–2%), with a significant

positive trend in the incidence of this tumour in females.

There was a significant increase in the incidence of follicular cell adenoma of the thyroid gland in males at 30 or 100 mg/kg bw, with a significant positive trend.

There was a significant positive trend in the incidence of forestomach squamous cell papilloma or carcinoma (combined) in females. Two single and one multiple squamous cell papilloma of the forestomach occurred in females at 100 mg/kg bw; the incidence of this tumour in historical controls in females treated by gavage (corn oil) was 0/473. Single instances of forestomach squamous cell carcinoma occurred in males and females at 10 mg/kg bw, and in females at 100 mg/kg bw; the incidence of this tumour in historical controls in females was 2/473 (range, 0–2%).

In males, there was a significant positive trend in the incidence of malignant schwannoma in the thoracic cavity, with an incidence of 0/50 (control), 0/50, 1/50, and 3/50, respectively.

[In this GLP study, the duration of exposure and observation, and the schedule of exposure, were adequate.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

TCAB is a halogenated aryl hydrocarbon that is isosteric to TCDD, and is highly lipophilic. Like TCDD, TCAB binds to the aryl hydrocarbon receptor (AhR) and is a potent inducer of hepatic aryl hydrocarbon hydroxylase. In contrast to TCDD, however, TCAB is readily eliminated from the body (Pillai et al., 1996). TCAB is metabolized more readily than TCDD, and therefore does not bioaccumulate; however, the metabolic products of TCAB have structural alerts that suggest potential toxicity. Several studies have examined the toxicokinetics of TCAB in rats (Burant & Hsia, 1984; Pillai et al., 1996; NTP, 2010), TCAB is readily metabolized and two studies permit a biotransformation pathway to be compiled from the available data (Hsia & Kreamer, 1981; Pillai et al., 1996).

4.1.2 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Studies in non-human mammalian models indicated that TCAB is absorbed either by inhalation or by dermal routes. For example, inhalation by rats (strain not specified), and dermal application in male albino rabbits, resulted in various systemic toxic effects of TCAB, indicating absorption (EPA OTS, 1983; EPA, 1985).

Several studies in rodents have shown that TCAB is readily absorbed via the oral route (Burant & Hsia, 1984; Pillai et al., 1996; NTP, 2010). In male Sprague-Dawley rats treated with [^{14}C]-labelled TCAB (single dose, 10 mg/rat) by gavage, a substantial amount of radiolabel (66% of the administered dose) was excreted in the urine and faeces after 24 hours, and a marked distribution of radiolabel into adipose tissue was found (Burant & Hsia, 1984). The oral bioavailability of TCAB (32 mg/kg) in male Fischer rats was calculated to be 30% when blood concentration–time curve (AUC) values were compared after oral and intravenous dosing regimens (Pillai et al., 1996). After oral administration, extensive azo reduction of TCAB (presumably by gut microbes) and first-pass metabolism may contribute to reduced systemic absorption, limiting the amount of TCAB that reaches the systemic circulation (Pillai et al., 1996). When TCAB was given orally to male Fischer rats as a

single dose (32 mg/kg bw), the apparent first-order absorption rate constant (K_a) and lag time for absorption (T_{lag}) were estimated to be 0.44 hour^{-1} and 1.5 hour, respectively (Pillai et al., 1996). By the end of a 3-month oral dosing regimen in female Sprague-Dawley rats (dose levels, 0, 0.1, 3, or 100 mg/kg bw), high concentrations of TCAB were found in the blood and tissues, with the highest amount found in adipose tissue (NTP, 2010). Together, these findings indicated that TCAB is absorbed via the gastrointestinal tract and widely distributed in experimental animals.

4.1.3 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

The distribution of TCAB in rats has been evaluated in acute and chronic studies. In female Sprague-Dawley rats given TCAB as a single intravenous dose (2.87 mg/kg bw), half-lives of elimination ($t_{1/2\alpha}$ and $t_{1/2\beta}$) from the blood were 1 hour and 5.6 hours, respectively (NTP, 2010). The volume of distribution (V_{ss}) was 743 mL/kg bw, systemic clearance (CL_s) was 352 mL/hour per kg bw, and mean residence time (*MRT*) was 2.1 hours. [The Working Group noted that these data indicate that TCAB is rapidly distributed to tissues.] The terminal elimination half-life of ~6 hours indicated that TCAB is cleared relatively rapidly from the blood. Furthermore, prior exposure to TCAB (daily gavage doses of 3 mg/kg bw for 7 days) did not affect the distribution and elimination kinetics of TCAB.

Pillai et al. (1996) reported on the tissue distribution of [^{14}C]-labelled TCAB in male Fischer rats given single doses orally (3.2 and 32 mg/kg bw) or intravenously (3.2 mg/kg bw). Only 6% of the administered radiolabel remained in tissues 96 hours after dosing. Tissue distribution was similar after either oral or intravenous administration of TCAB. Adipose tissue exhibited by far the highest tissue-to-blood ratio, followed by kidney, with brain exhibiting the lowest ratio.

Distribution in rats after long-term exposure was also reported (NTP, 2010). TCAB levels in blood and tissue were monitored after the last dose of a 3-month study of TCAB (0.1, 3, or 100 mg/kg bw, by gavage) in female Sprague-Dawley rats. TCAB was mostly undetectable in blood of rats at the lowest dose. For the groups at 3 and 100 mg/kg bw, respectively, C_{max} was 192.3 and 619.8 ng/mL, and dose-normalized AUC values in blood were 332.8 and 28.7 ng•hour•kg/mL per mg, indicating a decrease in the relative amount of absorbed TCAB with increasing dose. Concentrations in adipose tissue were ~100 times those in liver and lung, and gradually declined, with half-lives in adipose tissue of 115, 81, and 86 hours for the groups at 0.1, 3, and 100 mg/kg bw, respectively. In general, similar half-lives were found in liver and lung. After a 3-month exposure, TCAB was mainly distributed to adipose tissue, where it was moderately persistent, whereas the extent of distribution to other tissues was more limited.

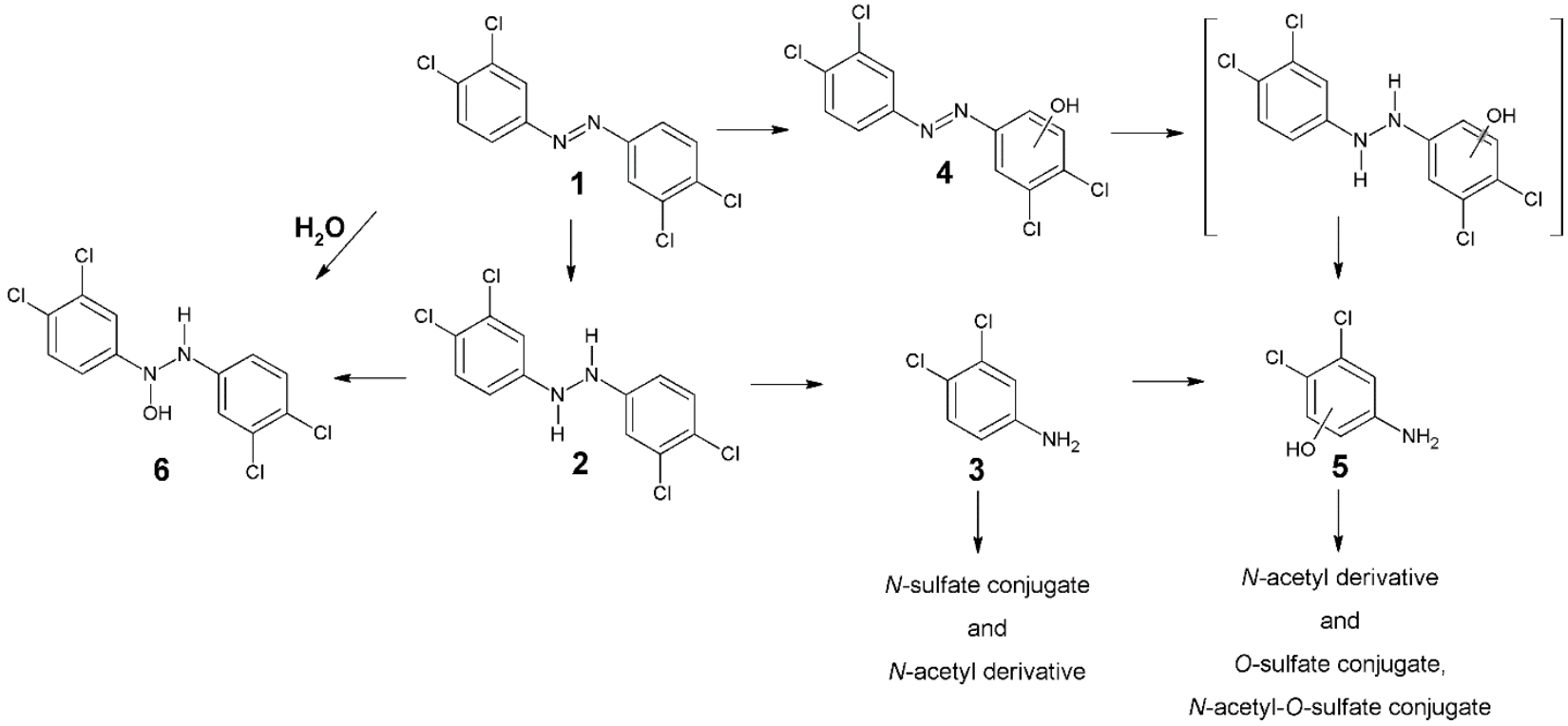
Collectively, the concentrations of TCAB in rat tissues increased in a dose-dependent manner regardless of route of administration. After parenteral administration, TCAB was distributed rapidly into tissues ($t_{1/2\alpha}$, 1 hour), mainly adipose tissue. Terminal elimination of TCAB from the blood was also fairly rapid ($t_{1/2\beta}$, 4–6 hours), whereas elimination of TCAB from adipose tissue was slower (Pillai et al., 1996; NTP, 2010).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Humans

No data were available to the Working Group.

Fig. 4.1 Biotransformation of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in the rat



(1) 3,3′,4,4′-tetrachloroazobenzene (TCAB); (2) 3,3′,4,4′-tetrachlorohydrazobenzene; (3) 3,4-dichloroaniline; (4) TCAB phenol; (5) hydroxylated 3,4-dichloroaniline; (6) *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene. The structure in square brackets is a putative intermediate, because it has not been isolated. Putative dechlorination reactions are not shown. Adapted with permission from: 3,3′,4,4′-Tetrachloroazobenzene absorption, disposition, and metabolism in male Fischer 344 rats, Drug Metab Dispos, 24(2):238–244, ©ASPET (1996) (Pillai et al., 1996), with information from Hsia & Kreamer (1981).

(b) Experimental systems

The azo linkage in TCAB makes it highly susceptible to metabolic biotransformation. The metabolic pathway for TCAB given in Fig. 4.1 is based on data obtained in rats in vivo and in vitro (Hsia & Kreamer, 1981; Pillai et al., 1996). Extensive azo reduction of TCAB (1, Fig. 4.1) to 3,4-dichloroaniline metabolites (3, Fig. 4.1) via 3,3′,4,4′-tetrachlorohydrazobenzene (2, Fig. 4.1) after oral administration in rats, presumably by gut microbes, decreases the systemic absorption of TCAB (Pillai et al., 1996). [The Working Group noted that the dichloroaniline metabolites have structural alerts that suggest potential toxicity.] Azo reduction also probably occurs in rat liver, because rat liver microsomes could catalyse this reaction via cytochrome P450 monooxygenases (Hsia & Kreamer, 1981). In addition to reductive metabolism, TCAB can be oxidized by cytochrome P450s to a major metabolite named TCAB phenol (4, Fig. 4.1) (Hsia & Kreamer, 1981). It is likely that TCAB phenol undergoes further azo reduction, giving hydroxylated chloroaniline derivatives (5, Fig. 4.1). This is supported by the identification of several derivatives of dichloroaniline and their sulfate conjugates in the urine of rats given an oral dose of [^{14}C]-labelled TCAB, including an *O*-sulfate conjugate of ring-hydroxylated *N*-acetyl-3,4-dichloroaniline that accounted for about 25% of the total radiolabel in the urine (Pillai et al., 1996). [The Working Group noted that compared with the lipophilic TCAB, the dichloroaniline conjugates would be rapidly eliminated from the body.] Monochloroaniline derivatives were also detected in rat urine, indicating dechlorination (Pillai et al., 1996). In addition to the urinary metabolites, the main metabolite in rat bile was putatively identified as *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene (6, Fig. 4.1); [the Working Group noted that this metabolite could be produced either by the hydration of the azo linkage in TCAB or by oxidation of the hydroazo linkage in 3,3′,4,4′-tetrachlorohydrazobenzene] (2; Fig. 4.1) (Hsia & Kreamer, 1981). During incubation of rat liver microsomes with [^{14}C]-labelled TCAB, a portion of the radiolabel (6% after 60 minutes) was irreversibly bound to the microsomal pellet, suggesting covalent modification of macromolecules by a TCAB-derived reactive metabolite (Hsia & Kreamer, 1981). Covalent binding was dependent on nicotinamide adenine dinucleotide phosphate reduced form (NADPH) and could be inhibited by monooxygenase inhibitors. The reactive metabolite was not identified (Hsia & Kreamer, 1981).

TCAB interacts with the AhR with a binding affinity (K_d) of 1.1 nM (Poland et al., 1976). TCAB was further shown to be a potent inducer of hepatic aryl hydrocarbon hydroxylase in chicken embryos (Poland et al., 1976). In male Sprague-Dawley rats, TCAB (25 mg/kg bw per day, for 5 days) increased liver-to-body weight ratios, and increased hepatic cytochrome P450 content (2.7-fold vs control animals) in a dose-dependent manner (Hsia & Kreamer, 1979a). Consequently, TCAB has been used as an experimental tool to induce hepatic cytochrome P450 activities in animals (Saint-Ruf et al., 1979; Keys et al., 1985; Shaddock et al., 1989; McMillan et al., 1990). Furthermore, the TCAB congener 3,3′,4,4′-tetrachloroazoxybenzene was also shown to be an effective inducer of hepatic monooxygenase activity (McMillan et al., 1990). Receptor-mediated effects involving the AhR pathway are further discussed in Section 4.2.1(a). No studies were found to indicate that TCAB is a ligand for the xenobiotic receptors pregnane X receptor (PXR) or constitutive androstane receptor (CAR).

4.1.5 Excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

TCAB-derived metabolites are excreted in the faeces and urine (Burant & Hsia, 1984; Pillai et al., 1996). Male Sprague-Dawley rats treated with [^{14}C]-labelled TCAB (10 mg/rat, by gavage) had excreted 55% of the administered dose in the faeces and 27% in the urine over a 48-hour period (Burant & Hsia, 1984). Male Fischer rats treated with [^{14}C]-labelled TCAB (3.2 and 32 mg/kg bw, by gavage) also excreted significant amounts of radiolabel in the faeces (53–56% of the administered dose) and the urine (39–45% of the administered dose) over a 48-hour period (Pillai et al., 1996). In the urine, no parent TCAB residue was found, and the *O*-sulfate conjugate of ring-hydroxylated *N*-acetyl-3,4-dichloroaniline accounted for 25% of the radiolabel. Modest differences between rat strains were noted in urinary excretion; over a 24-hour period, Sprague-Dawley rats had excreted 20% (Burant & Hsia, 1984) and Fischer rats had excreted 30–40% (Pillai et al., 1996) of the administered dose.

Faecal elimination of [^{14}C]-labelled TCAB equivalents was mainly due to biliary excretion of a [^{14}C]-labelled TCAB metabolite into the gastrointestinal tract and its subsequent excretion in the faeces (Pillai et al., 1996). After intravenous administration of [^{14}C]-labelled TCAB (3.2 mg/kg bw), 33% of the administered dose was excreted in the bile within 6 hours, whereas only 21% was eliminated in the faeces by 24 hours. [The Working Group noted that the difference was due to enterohepatic recirculation of [^{14}C]-labelled TCAB equivalents.] The main biliary metabolite was putatively identified as *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene (6, Fig. 4.1; Pillai et al., 1996). No unchanged TCAB was detected in faecal extracts after intravenous administration of [^{14}C]-labelled TCAB; the fraction of faecal radiolabel attributable to unchanged (and putatively unabsorbed) [^{14}C]-labelled TCAB after an oral dose was not determined (Pillai et al. 1996).

Together the data indicated that TCAB metabolites are excreted readily and that both urine and faeces are important routes of excretion.

4.2 Mechanisms of carcinogenesis

This section summarizes in the following order the available evidence for the key characteristics of carcinogens (Smith et al., 2016), concerning whether TCAB modulates receptor-mediated effects; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; and is genotoxic. For the other key characteristics of carcinogens, insufficient data were available for evaluation.

4.2.1 Receptor-mediated effects

(a) Aryl hydrogen receptor pathway

(i) Humans

There is no direct evidence that TCAB binds to the human AhR; however, several studies were available on chloracne (a skin condition characterized by comedones and retention cysts), which is pathognomonic for AhR activation in humans (Poland et al., 1976). Several series of chloracne cases have been reported among workers at plants where dichloroaniline herbicides were produced (Taylor et al., 1977; Morse et al., 1979; Scarisbrick & Martin, 1981; McDonagh et al., 1993). One plant produced methazole, one produced propanil and carbamate pesticides, two others produced dichloroaniline and diuron, and another plant was described only as manufacturing dichloroaniline derivatives. In addition to the end products, TCAB and 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB) were reported as contaminants and other chemicals used in production were present. Workers were apparently exposed to chemicals as a result of an accident in one plant and through poor housekeeping practices in others. However, neither individual exposure data for the chloracne cases nor quantitative data

on the environmental levels of TCAB or any other agent in the plants were reported. [Consequently, the Working Group noted that although development of chloracne was associated with exposure to TCAB, the possibility that other chemicals were involved could not be ruled out.]

No other data from humans were available to the Working Group, including on human AhRs, or concerning AhR activation in human cells in vitro.

(ii) Non-human mammalian experimental systems in vivo

Poland et al. (1976) reported that TCAB and TCAOB induced hepatic aryl hydrocarbon hydroxylase activity, a marker of CYP1A1 activity and AhR activation, in male C57/Bl6 mice. TCAB and TCAOB were, respectively, approximately 20 and 8000 times less potent than TCDD.

Several studies in rodents examined non-neoplastic effects that have been associated with activation of the AhR. In $B6C3F_1$ mice exposed by oral gavage for 13 weeks, TCAB increased liver weights and thymic atrophy (NTP, 1998, 2010; van Birgelen et al., 1999). Chronic non-neoplastic effects of TCAB in female Sprague-Dawley rats included hyperplastic and proliferative lesions in the liver, thyroid gland, forestomach, oral mucosa, and adrenal cortex (NTP, 2010), similar to those observed with AhR agonist chemicals (NTP, 2006a, b, c).

In male Sprague-Dawley rats fed diets containing TCAB for up to 120 days, decreased body-weight gains, increased liver and spleen weights, and decreased testis weights were reported (Hsia et al., 1980, 1982). The increased liver weights were accompanied by increases in hepatic cytochrome P448 and aryl hydrocarbon hydroxylase activity (Hsia et al., 1980). In Sprague-Dawley and Fisher 344/N rats exposed by oral gavage for 13 weeks, TCAB induced hepatic CYP1A1 and CYP1A2, in association with increased liver weights and thymic atrophy (NTP, 1998). Weanling male Sprague-Dawley rats given TCAB as two weekly intraperitoneal doses (25 mg/kg bw) for up to 28 days developed a wasting syndrome and thymic atrophy (Hsia & Kreamer, 1985). Thymic atrophy, increased liver weights, depressed levels of hepatic gluconeogenic enzymes, and increased levels of total hepatic cytochrome P450 were also seen in male Sprague-Dawley rats given intraperitoneal injections of TCAB twice per week for 7 and 28 days (Hsia et al., 1982; Hsia & Kreamer, 1985). In immature male Wistar rats, TCAB (300 µg/kg bw, intraperitoneal) and other halogenated hydrocarbons induced hepatic testosterone 7α-hydroxylase, inhibited other testosterone hydroxylases, and decreased androstenedione formation (Keys et al., 1985). These effects on testosterone metabolism were correlated with decreased body weight.

In a study of long-term toxicity and carcinogenicity in female Sprague-Dawley rats, TCAB induced cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa (NTP, 2010). These effects were observed in similar studies with TCDD, 2,3,4,6,7-pentachlorodibenzofuran, and 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126), all of which are AhR agonists (NTP, 2006a, b, c, 2010).

[The Working Group noted that multiple studies in mice and rats have reported effects that are hallmarks of, or consistent with, AhR activation.]

As noted below, there were several neoplastic and non-neoplastic findings with TCAB that were not observed in any of the bioassays with AhR agonist chemicals (NTP, 2006a, b, c, 2010).

(iii) Non-human mammalian experimental systems in vitro

Poland et al. (1976) first reported that TCAB and TCAOB bound the murine AhR from C57BL/6J mouse liver cytosol with an equilibrium dissociation constant about one fifth that of TCDD.

Xiao et al. (2016) evaluated the ability of TCAB to induce ethoxyresorufin-*O*-deethylase (EROD) activity, a marker for CYP1A1 and AhR activation, in a rat hepatoma cell line (H4IIE cells). TCAB induced EROD activity, like TCDD did, but was ~1.2×10^{-5} times as potent.

(iv) Non-mammalian experimental systems

Poland et al. (1976) reported an increased incidence of aryl hydrocarbon hydroxylase activity, a marker for CYP1A1 and AhR activation, in chicken embryos exposed to TCAB. In a rainbow trout liver cell line (RTL-W1 cells), TCAB induced EROD activity, like TCDD did, but was ~8.7×10^{-4} times as potent (Xiao et al., 2016). In a test for toxicity in the zebrafish embryo, TCAB induced a variety of cardiovascular disorders including heart oedema and heart malformations as well as yolk malformations, which have also been observed with AhR agonists (Xiao et al., 2016).

(b) Other receptors

TCAB (1000 µg/L) produced responses of less than 10% of maximum in the estrogen receptor (ER) and androgen receptor (AR)-CALUX assays (reporter cell lines derived from human osteosarcoma U2OS cells) (Xiao et al., 2016).

TCAB decreased circulating thyroxine concentrations, but had no effect on triiodothyronine and thyroid stimulating hormones, in Fischer 344/N (NTP, 1998) and Sprague-Dawley rats (NTP, 2010) after 13 weeks of exposure. These effects on thyroid hormones are similar to those reported for TCDD (NTP, 2006a). Decreased thyroxine concentrations were seen in male offspring in the NTP evaluation of developmental neurotoxicity of TCAB in Sprague-Dawley rats (dams exposed before mating, and male offspring exposed on postnatal days 4–21) (Harry et al., 2014). The decreased thyroxine concentrations were associated with histopathological changes in the hippocampus, suggesting that the decreases in circulating hormones resulted in developmental neurotoxicity (Harry et al., 2014).

4.2.2 Inflammation and immunosuppression

(a) Humans

As noted above (see Section 4.2.1), several case series of chloracne have been reported among workers at plants where dichloroaniline herbicides were produced and where TCAB was one of the exposures. Chloracne is an inflammatory process that leads to keratinous plugs in the skin pores resulting in cysts and dark pustules.

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

(b) Experimental systems

TCAB induced acnegenic effects in the rabbit ear bioassay (Hill et al., 1981). Solutions (0.1 mL) containing TCAB were painted onto the left ear of rabbits daily for 5 days (the right ear was used as the untreated control). Chloracne-like lesions were observed in $B6C3F_1$ mice in a study of long-term toxicity and carcinogenicity by the NTP (2010). The findings consisted of gross inflammatory skin lesions, characterized histologically by inflammation, fibrosis, hyperplasia, and ulcers (NTP, 2010). Chronic active inflammation of the ureter (males) and the lung (females) were also observed in mice exposed to TCAB for 2 years (Ramot et al., 2009; NTP, 2010). Inflammation of the thyroid, blood vessels, pancreas, and nose were observed in rats (Ramot et al., 2009; NTP, 2010). Neoplastic effects occurred in many of the tissues in which inflammation or chronic active inflammation was also observed.

4.2.3 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In Fisher 344/N (NTP, 1998) and Sprague-Dawley (NTP, 2010) rats, and in B6C3F$_1$ mice (NTP, 1998) exposed for 13 weeks, TCAB induced hyperplasia of the forestomach in males and females. In male and female rats, TCAB increased the incidence of oral gingival hyperplasia and of hyperplasia of the zona fasciculata of the adrenal cortex (NTP, 2010). Hyperplasia of the follicular cells in the thyroid gland was seen in male rats (NTP, 1998; NTP, 2010). An increased incidence of haematopoietic cell proliferation in the spleen was observed in TCAB-exposed male and female rats and mice (NTP, 1998, 2010; van Birgelen et al., 1999). In male and female mice, epidermal hyperplasia as well as glandular stomach focal epithelial hyperplasia and urinary bladder transitional cell hyperplasia were observed (NTP, 2010). Neoplastic effects occurred in many of the tissues in which hyperplasia was also observed.

With bromodeoxyuridine labelling, no alterations in cell proliferation were observed in the liver of Sprague-Dawley rats treated with TCAB for 13 weeks (NTP, 2010). Ramot et al. (2012) found a dose-related increase in the incidence of gingival squamous cell hyperplasia and of gingival cystic keratinizing hyperplasia in all treated Sprague-Dawley rats (but not of cystic keratinizing hyperplasia in males at the highest dose), using proliferating cell nuclear antigen staining as a marker of proliferation.

4.2.4 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Bhusari et al. (2014) evaluated a subset of the tumours reported by NTP (2010) (see Section 3) for alterations in *Kras* and *Tp53*, two genes involved in human cancers. Urethral tumours from male and female mice had transforming point mutations in *Kras* (38%) and *Tp53* (63%). Similar rates of these mutations were observed in the mouse pulmonary carcinomas (*Kras*, 36%; *Tp53*, 55%). The mutations were not observed in the two pulmonary tumours that occurred in untreated animals. [The Working Group noted that a small subset of the tumours was available for analysis, and only two pulmonary carcinomas and no urethral tumours from control animals were examined. In addition, spontaneous or chemically induced transitional cell carcinomas of the urethra or ureter of B6C3F$_1$ mice were not reported in any other 2-year NTP cancer bioassays (approximately 600 studies were available). The increase in frequency of point mutations in *Kras* and *Tp53* suggested that TCAB or its metabolites may target guanine or cytosine bases.]

In B6C3F$_1$ mice, TCAB gave negative results in a test for micronucleus formation in the bone marrow in male mice after 3 days of intraperitoneal exposure at doses as high as 200 mg/kg bw per day (Witt et al., 2000). Increases in the frequency of micronucleated normochromatic erythrocytes were observed in male mice after 13 weeks of exposure at 10 and 30 mg/kg bw per day (NTP, 1998; Witt et al., 2000). In female mice exposed to TCAB for 13 weeks, there was a significant increasing trend in the frequency of micronucleated normochromatic erythrocytes, but results for the individual dose levels were not statistically significantly different from those of controls (NTP, 1998; Witt et al., 2000).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

In rat primary hepatocytes isolated from untreated male Sprague-Dawley rats, overnight treatment with TCAB did not significantly increase unscheduled DNA synthesis (McMillan et al., 1988). TCAB also did not induce unscheduled DNA synthesis in hepatocytes isolated from naive rats in a separate study (Shaddock et al.,

Table 4.1 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Tp53 and *Kras* mutation	Mouse, B6C3F$_1$ (M, F)	Urethral (M) and pulmonary carcinomas (M, F)	+	10 mg/kg bw	i.g., 2 yr, 3, 10, or 30 mg/kg bw, 5 d/wk	Bhusari et al. (2014)
Micronucleus formation	Mouse, B6C3F$_1$ (M)	Bone marrow (PCE)	–	200 mg/kg	i.p., 3×, 50, 100, 150, or 200 mg/kg	NTP (1998); Witt et al. (2000)
	Mouse, B6C3F$_1$ (M, F)	Peripheral blood erythrocytes	+	10 mg/kg	i.g., 13 wk, 0.1, 1, 3, 10, or 30 mg/kg, 5 d/wk	

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

bw, body weight; d, day; F, female; HID, highest ineffective dose; i.g. intragastric; i.p., intraperitoneal; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; wk, week(s)

Table 4.2 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	–	–	6.4 µg/mL		McMillan et al. (1988)
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	–	+	3.2 µg/mL		Shaddock et al. (1989)
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	(+)	NT	10 µM	Short (3 h) incubation, unclear whether triplicates were from the same or different samples	Hsia & Kreamer (1979b)
Hgprt mutation	Chinese hamster, CHO-K1 ovary cell line	–	–	14.4 µg/mL		McMillan et al. (1988)

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

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; Hgprt, hypoxanthine guanine phosphoribosyl transferase

Table 4.3 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in bacteria (*Salmonella typhimurium*)

Strain	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
TA97	Reverse mutation	–	+	50 µg/plate	NTP (1998)
TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	10 000 µg/plate	NTP (1998)
TA97, TA98, TA100, TA104	Reverse mutation	–	–	250 µg/plate	McMillan et al. (1988)
TA98, TA100	Reverse mutation	+	+	100 µg/plate	Gilbert et al. (1980)

[a] +, positive; –, negative; the level of significance was set at $P < 0.05$ in all cases
HIC, highest ineffective concentration; LEC, lowest effective concentration

1989). However, TCAB induced unscheduled DNA synthesis when the rats were pretreated with metabolic enzyme inducers, giving positive results at concentrations of 3.2 µg/mL (phenobarbital pretreated) or 6.4 µg/mL (Aroclor 1254 and TCAB pretreated) or higher (Shaddock et al., 1989). TCAB was not mutagenic in the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) assay in Chinese hamster ovary cells with or without metabolic activation (S9) (McMillan et al., 1988).

(iii) Non-mammalian systems

See Table 4.3.

TCAB gave positive results in *Salmonella typhimurium* strain TA97 in the presence of metabolic activation (S9), but not in strains TA98, TA100, TA1535, and TA1537 with or without metabolic activation (NTP, 1998). McMillan et al. (1988) reported negative results for TCAB in *S. typhimurium* strains TA97, TA98, TA100, and TA104 with or without metabolic activation. Gilbert et al. (1980) found that TCAB gave positive results in TA98 and TA100 with and without metabolic activation.

4.3 Data relevant to comparisons across agents and end-points

TCAB was not tested in high-throughput screening assays carried out by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) programmes of the government of the USA; for relevant results for other chemicals reviewed in the present volume, see Section 4.3 of the *Monograph* on pentachlorophenol in the present volume.

4.4 Cancer susceptibility data

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

With the exception of chloracne, described above, no data were available to the Working Group.

4.5.2 Experimental systems

Long-term exposure to TCAB in rodents resulted in a broad range of adverse effects across many tissues (NTP, 2010). In addition to those noted above (see Section 4.2), atrophy

was observed in the lymph nodes, spleen, and pancreas in male and female rats, and in the clitoral gland, ovaries, thymus, and spleen in mice (NTP, 2010).

5. Summary of Data Reported

5.1 Exposure data

3,3′,4,4′-Tetrachloroazobenzene (TCAB) is not commercially manufactured, but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives. TCAB has been measured at concentrations up to 1400 μg/g in propanil, and at up to 28 μg/g in linuron, diuron, or neburon formulations. Environmental contamination by TCAB occurs from the degradation of chloroanilide herbicides in the soil by peroxide-producing microorganisms, and by the photolysis and biolysis of 3,4-dichloroaniline. The use of propanil and other chloroanilide herbicides has increased substantially over the past two decades; current annual use in the USA is estimated to exceed 6 million pounds [~2700 tonnes]. However, no measurements of TCAB exposure in occupational settings or in the general population were reported. Occupational exposure may include workers involved in the manufacture of aniline herbicides, applicators who spray or mix aniline herbicide-containing formulations, and farm workers who enter fields after spraying. The general population may be exposed to TCAB from residues on food, or from living near areas where aniline herbicides are applied. TCAB sorbs strongly to soils and has been detected in the top 10 cm of soil up to 2 years after application of propanil.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

TCAB was tested for carcinogenicity in one gavage study in mice and one gavage study in rats.

In male mice, there was an increase in the incidence of transitional cell carcinoma of the urethra, bronchioloalveolar adenoma of the lung, and squamous cell carcinoma of the forestomach.

In female mice, there was an increase in the incidence of fibrosarcoma of the skin, malignant schwannoma or fibrosarcoma (combined) of the skin, bronchioloalveolar carcinoma of the lung, bronchioloalveolar adenoma or carcinoma (combined) of the lung, squamous cell carcinoma of the forestomach, and malignant lymphoma. There were also two instances of the rare tumour transitional cell carcinoma of the urethra in females at the highest dose.

In male rats, there was an increase in the incidence of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, gingival squamous cell carcinoma of the oral mucosa, and follicular cell adenoma of the thyroid gland, and a positive trend in the incidence of malignant schwannoma.

In female rats, there was an increase in the incidence of cystic keratinizing epithelioma of the lung, gingival squamous cell carcinoma of the oral mucosa, and squamous cell papilloma or carcinoma (combined) of the forestomach. Rare cholangiocarcinomas of the liver were reported in treated females.

5.4 Mechanistic and other relevant data

No data were available on the absorption of TCAB in humans after oral, dermal, or inhalation exposures. The bioavailability of a bolus oral dose of TCAB given to rats is ~30% of the administered dose. Adipose tissue is a main storage depot after distribution of TCAB. TCAB

is rapidly metabolized, with extensive azo reduction in the gut and liver to give 3,4-dichloroaniline metabolites. TCAB metabolites are excreted readily in the urine and faeces.

With respect to the key characteristics of carcinogens, adequate data were available to evaluate whether TCAB modulates receptor-mediated effects; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; and is genotoxic.

There is *strong* evidence that TCAB modulates receptor-mediated effects, but data in exposed humans and human cells are sparse. Chloracne has been reported in four case series of workers involved in the production of dichloroaniline herbicides, with exposures to TCAB, 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB), and other chemicals. Chloracne is pathognomonic for activation of the aryl hydrocarbon receptor (AhR) and has been observed in experimental studies of rabbits and mice treated with TCAB. TCAB activates the AhR in vivo in rats, mice, and chicken embryos. In long-term studies in rodents, exposure to TCAB induced cytochrome P450s (CYP1A1 and CYP1A2), caused wasting syndrome, increased liver weights, decreased circulating thyroxine concentrations, and induced thymic atrophy. These effects are consistent with or are hallmarks of AhR activation, and are observed after AhR agonist exposures. TCAB activates the AhR in vitro in mice, rats, and rainbow trout.

There is *strong* evidence that TCAB induces chronic inflammation, but data in exposed humans and human cells are sparse. Chloracne, which is in part an inflammatory response, has been observed in the dichloroaniline-herbicide production workers mentioned previously, as well as in experimental studies of rabbits and mice treated with TCAB. Chronic inflammation was observed in numerous tissue types in rats and mice exposed to TCAB for up to 2 years. These inflammatory responses are consistent with those induced by AhR agonists in long-term studies.

There is *strong* evidence that TCAB alters cell proliferation, cell death, or nutrient supply. In experimental animals, long-term exposure to TCAB induces hyperplasia in numerous tissue types.

There is *weak* evidence that TCAB is genotoxic. In mice, 13 weeks of dietary exposure to TCAB induced increases in the frequency of micronucleus formation in male and female mice. However, short-term exposure to TCAB in male mice did not alter the frequency of micronucleus formation. There are conflicting findings for genotoxicity in assays for bacterial mutagenesis with TCAB.

No evidence was available concerning cancer susceptibility.

Long-term exposure to TCAB resulted in a broad range of non-neoplastic adverse effects across many tissues in mice and rats.

In sum, TCAB activates the AhR in experimental systems in vitro and in vivo. TCAB displays a wide variety of effects that are also induced by AhR agonists, including pathognomonic effects such as chloracne.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 3,3′,4,4′-tetrachloroazobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,3′,4,4′-tetrachloroazobenzene.

6.3 Overall evaluation

3,3′,4,4′-Tetrachloroazobenzene is *probably carcinogenic to humans* (*Group 2A*).

6.4 Rationale

3,3′,4,4′-Tetrachloroazobenzene is *probably carcinogenic to humans (Group 2A)* on the basis of its belonging to the class of agents that activate the aryl hydrocarbon receptor (AhR), including dioxins, polychlorinated biphenyls, and polybrominated biphenyls, that are categorized as Group 1 or Group 2A carcinogens. The rationale for this evaluation is as follows:

- In vitro, 3,3′,4,4′-tetrachloroazobenzene binds to the mouse AhR, and activates rat and rainbow trout AhRs.
- 3,3′,4,4′-Tetrachloroazobenzene induces a spectrum of tumours in rats and mice that includes those observed with other AhR agonists that are categorized in Group 1, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyl 126 (PCB-126), and 2,3,4,7,8-pentachlorodibenzofuran.
- 3,3′,4,4′-Tetrachloroazobenzene induces multiple non-neoplastic effects in mice, rats, rabbits, chickens, and zebrafish consistent with AhR activation, including chloracne (a response pathognomonic for AhR-mediated toxicity) in mice and rabbits.

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ALDRIN AND DIELDRIN

1. Exposure Data

1.1 Identification of the agents

1.1.1 Nomenclature

(a) Aldrin

Chem. Abstr. Serv. Reg. No.: 309-00-2

IUPAC Systematic Name:

(1*R*,4*S*,4a*S*,5*S*,8*R*,8a*R*)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene (HHDN)

Synonyms:1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimethanonaphtalene; HHDN (ATSDR, 2002)

"Aldrin" is most commonly used to mean HHDN with a purity of > 95%, except in Denmark and the countries of the former Soviet Union, where it is the name given to pure HHDN (IPCS, 1989, WHO, 2003).

(b) Dieldrin

Chem. Abstr. Serv. Reg. No.: 60-57-1

IUPAC Systematic Name:

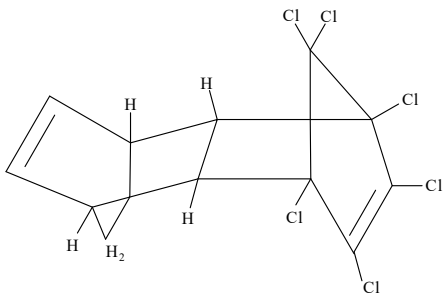
(1*R*,4*S*,4a*S*,5*R*,6*R*,7*S*,8*S*,8a*R*)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene (HEOD)

Synonyms: 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa-hydro-1,4-*endo*,exo-5,8-dimethanonaphtalene; HEOD

"Dieldrin" is most commonly used to mean HEOD with a purity of > 85%, except in Denmark and the countries of the former Soviet Union, where it is the name given to pure HEOD (IPCS, 1989; WHO, 2003).

1.1.2 Chemical and physical properties of the pure substances

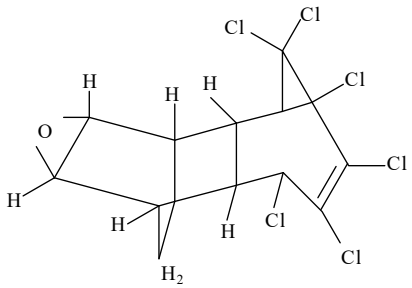
(a) Aldrin



Molecular formula: $C_{12}H_8Cl_6$

Relative molecular mass: 364.91

(b) Dieldrin



Molecular formula: $C_{12}H_8Cl_6O$

Relative molecular mass: 380.91

Table 1.1 summarizes the chemical and physical properties of aldrin and dieldrin.

Chemical reactivity: Aldrin is stable to heat, and in the presence of inorganic and organic bases, hydrated metal chlorides, and mild acids. Epoxidation of aldrin with peracetic or perbenzoic acid forms the 6,7-epoxy derivative, dieldrin. The unchlorinated ring is attacked by oxidizing agents and strong acids (IARC, 1974).

1.1.3 Technical products and impurities

(a) Aldrin

Some trade names: Aldrec; Aldrex; Drinox; Octalene; Seedrin; Compound 118 (ATSDR, 2002)

Impurities: Octachlorocyclopentene, hexachlorobutadiene, toluene, and polymerization products (IPCS, 1989; WHO, 2003)

In 1967, the composition of technical aldrin was reported to be as follows: hexachloro-hexahydro-dimethano-naphthalene, 90.5%; other polychloro-hexahydro-dimethano-naphthalene (isodrin), 3.5%; hexachloro-tetrahydro-methano-indene (chlordane), 0.5%; hexachlorocyclopentadiene, 0.6% hexachlorobutadiene, 0.2%; octachlorocyclopentene, 0.5%; hexachloroethane, < 0.1%; HHDN diadduct, 0.1%; bicycloheptadiene, < 0.1%; toluene, 0.3%; and other compounds (primarily a complex mixture of compounds formed by polymerization of hexachlorocyclopentadiene and bicycloheptadiene during the aldrin reaction), 3.6% (IARC, 1974).

In the 1960s–70s, aldrin was available in the USA as a technical-grade product containing 95% minimum active ingredient (equivalent to 90.3% HHDN and 4.7% other insecticidally active related compounds) (Whetstone, 1964; Frear, 1972a). It was formulated into emulsifiable concentrates, wettable powders, dusts, granules, and mixtures with fertilizers (IARC, 1974).

(b) Dieldrin

Some trade names: Alvit; Dieldrix; Octalox; Quintox; Red Shield (ATSDR, 2002)

Impurities:Otherpolychloroepoxyoctahydro-dimethanonaphthalenes and endrin (IPCS, 1989; WHO, 2003)

In the 1960s–70s, dieldrin was available in the USA as a technical-grade product containing 100% active ingredient (equivalent to 85% HEOD and 15% other insecticidally active related compounds) with a chlorine content of 55–56%, free acid (as hydrochloric acid) at < 0.4%, and water at < 0.1% (Whetstone, 1964; Frear, 1972b). It was formulated into emulsifiable concentrates, solutions, wettable powders, dusts, granules, and mixtures with fertilizers (IARC, 1974).

1.2 Production and use

1.2.1. Production process

Aldrin and dieldrin were first synthesized in the laboratory in about 1948 (Whetstone, 1964) (Galley, 1970); commercial production in the USA was first reported in 1950 (US Tariff Commission, 1951).

Aldrin is produced by the Diels–Alder reaction of hexachlorocyclopentadiene with bicycloheptadiene (Whetstone, 1964).

Dieldrin is made commercially by the epoxidation of aldrin with a peracid (e.g. peracetic or perbenzoic acid), but can also be produced by the condensation of hexachlorocyclopentadiene with the epoxide of bicycloheptadiene (Galley, 1970; IARC, 1974).

Table 1.1 Chemical and physical properties of pure aldrin and dieldrin

Property	Aldrin	Dieldrin
Colour	White (pure); tan to brown (technical grade)	White (pure); light brown (technical grade)
Physical state	Crystalline solid	Crystalline solid
Melting point	104–105.5 °C; 49–60 °C (technical grade)	176–177 °C; 95 °C (technical grade)
Boiling point	Decomposes	Decomposes
Density	1.6 g/L at 20 °C	1.75 g/L at 25 °C
Odour	Mild chemical odour	Mild chemical odour
Odour threshold:		
Water	No data	No data
Air	0.017 mg/kg	0.041 mg/kg
Solubility:		
Water at 20 °C	0.011 mg/L	0.110 mg/L
Organic solvents	Very soluble in most organic solvents	Moderately soluble in common organic solvents except aliphatic petroleum solvents and methyl alcohol
Partition coefficients:		
Octanol/water, Log K_{ow}	6.50	6.2
Organic carbon, Log K_{oc}	7.67	6.67
Vapour pressure:		
at 20 °C	7.5×10^{-5} mmHg	3.1×10^{-5} mmHg
at 25 °C	1.2×10^{-4} mmHg	5.89×10^{-6} mmHg
Henry's law constant:		
at 25 °C	4.9×10^{-5} atm-m³/mol	5.2×10^{-6} atm-m³/mol
Flammability limits	Nonflammable	Nonflammable
Conversion factors	1 ppm = 14.96 mg/m³ at 25 °C, 1 atm	1 ppm = 15.61 mg/m³ at 25 °C, 1 atm
Explosive limits	Stable	Stable

ppm, part per million
From ATSDR (2002)

1.2.2. Production volumes

Global production, which was estimated to be 13 000 tonnes per year in 1972, had decreased to less than 2500 tonnes per year in 1984 (IPCS, 1989).

The following European countries were reported to be producing aldrin and/or dieldrin in 1972 or 1973: Belgium (one supplier), Federal Republic of Germany (one), France (two), Italy (two), the Netherlands (one), and the United Kingdom (one) (Ragno, 1972; Chemical Information Services Ltd, 1973). In 1972, Japan was reported to have eight suppliers of aldrin and/or dieldrin and their formulations (Chemical Information Services Ltd, 1973). Imports into Japan were reported to be 143 000 kg for aldrin and 43 000 kg for dieldrin in 1970 (Hayashi, 1971; IARC, 1974).

The production, import, and use of aldrin and dieldrin in the USA were cancelled or at least considerably reduced by the time aldrin was listed as a Toxic Release Inventory (TRI) chemical in 1986 (EPA, 2003). Nonetheless, the industry trade literature revealed that 11 companies in the USA between 1989 and 1999, and 7 companies in the USA in 2016 reported production of aldrin and/or dieldrin (Jorgenson, 2001; Chem Sources, 2016). It is not known whether these chemicals were primarily exported, or whether they were

used as chemical intermediates for other products, or only for scientific research (Jorgenson, 2001).

In 2016, few facilities reported the production of aldrin and/or dieldrin in Europe and in Asia: Germany (one), United Kingdom (one), Belgium (one), Switzerland (one), China (two), Hong Kong Special Administrative Region (one), and Japan (one) (Chem Sources, 2016). In China, small-scale production for research purposes has been reported (Wong et al., 2005). No information was available concerning production in other countries.

1.2.3 Use

Aldrin and dieldrin are synthetic organochlorine insecticides. Originally, they were used as broad-spectrum soil insecticides for the protection of various food crops, as seed dressings, to control infestations of pests such as ants and termites, and to control several insect vectors of disease (EPA, 2003).

The respective quantities of aldrin and dieldrin used in, or sold for, agricultural purposes in 1970 were reported to be as follows (in tonnes): Myanmar (4.2 and not reported, NR); Canada (18.5 and NR); Colombia (198.5 and 27.8); El Salvador (21.9 and 2.6); Ghana (15.5 and 0.5); Iceland (0.1 and NR); Israel (1 and NR); Italy (2.765 and 9.7); Madagascar (3.5 and 0.1); Ryukyu Islands (9.1 and NR); Sudan (NR and 4.5); and Uruguay (9 and 10) (FAO, 1972). Aldrin and dieldrin use in California, a major agricultural state in the USA, was reportedly 22.7 tonnes for aldrin and nearly 32 tonnes for dieldrin in 1971. For aldrin, almost 90% was used for insect control on wooden structures, whereas for dieldrin, 34% was used for insect control on wooden structures, 14% was used on grapes and 13% was used on pears (California Department of Agriculture, 1972, 1973). In 1972, an estimated 80% of the combined production of aldrin and dieldrin in the USA was used on corn crops, and about 10% was used for termite control (IARC, 1974). Minor uses of dieldrin in the USA and in several other countries were for moth-proofing woollen clothes and carpets (Lipson, 1970; IARC, 1974).

An indication of possible uses of aldrin and dieldrin can be derived from the recommended residue limits for aldrin and dieldrin established by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) for the following food products: asparagus, broccoli, Brussels sprouts, cabbages, cauliflowers, cucumbers, aubergines, horse radishes, onions, parsnips, peppers, pimentos, radishes, radish tops, fruits (including citrus), rice, potatoes, carrots, lettuces, milk and milk products, raw cereals, and eggs (FAO/WHO, 1973).

Since the early 1970s, use of aldrin and dieldrin, especially in agriculture, has been severely restricted or banned in many countries all over the world (IPCS, 1995). In 1972, the United States Environmental Protection Agency (EPA) cancelled all except three specific uses of these compounds (subsurface termite control, dipping of non-food plant roots and tops, and completely contained moth-proofing in manufacturing processes), which by 1987 were voluntarily cancelled by the manufacturer (EPA, 2003).

In tropical countries, dieldrin was reported to be used as a residual spray in residential dwellings to control vector-borne diseases such as malaria, and also to control termites (CDC, 2009).

1.3 Analytical methods

The analytical methods available for detecting, measuring, and/or monitoring aldrin and dieldrin, their metabolites, and other biomarkers of exposure to and effects of aldrin and dieldrin have been described in detail elsewhere (ATSDR, 2002).

1.4 Occurrence and exposure

Under most environmental conditions, aldrin is readily converted to dieldrin (ATSDR, 2002). The half-lives of aldrin and dieldrin in air are estimated to range from 1 to 10 hours for aldrin and from 3 to 40.5 hours for dieldrin (Kwok & Atkinson, 1995; Jorgenson, 2001). In surface waters, aldrin has a reported biodegradation half-life of 24 days (Eichelberger & Lichtenberg, 1971). In the soil, aldrin is converted to dieldrin by epoxidation, with an estimated half-life of between 1.5 and 5.4 years, depending on the composition of the soil (Jorgenson, 2001). In contrast, the average half-life of dieldrin in soil ranges between 2.6 and 12.5 years and appears to be a function of its concentration (Jorgenson, 2001). Consequently, aldrin is infrequently measured in occupational and environmental samples. Dieldrin originating from the application or manufacture of aldrin cannot be distinguished from applied dieldrin. Measurements of dieldrin in the air, soil, water, or body may represent exposure to dieldrin, or aldrin, or both. Dieldrin from both sources bioaccumulates in body fat and is typically measured in blood or body tissues. Dieldrin is excreted in the bile, faeces, and breast milk, and can cross the placenta (Jorgenson, 2001; ATSDR, 2002).

1.4.1 Occupational exposure

Occupational exposure may occur in workers involved in the manufacture of dieldrin or aldrin and formulations containing dieldrin or aldrin, applicators who spray or mix dieldrin or aldrin, farm workers engaged in re-entry tasks, and vector-control workers.

(a) Air and skin

In the USA in the 1960s, estimates of potential dermal exposure to dieldrin during orchard spraying ranged from 14.2 to 15.5 mg per hour, and estimates of potential respiratory exposure ranged from 0.03 to 0.25 mg per hour (Wolfe et al., 1963, 1967). Dieldrin was found on the hands of two out of five greenhouse workers (4.9 and 8.4 ng/hand), one out of nine veterinarians (1.9 ng/hand), and none out of seven florists monitored in France in 2002; however, no dieldrin was detected in their breathing air (Bouvier et al., 2006). In a limited number of stationary air samples collected between 1958 and 1960 from a pesticide formulation plant located in the Netherlands, aldrin and dieldrin concentrations were generally less than 0.25 mg/m^3, with concentrations of dieldrin of up to 4 mg/m^3 measured during drum filling (de Jong, 1991).

(b) Biological markers and intake

Dieldrin has been measured in the blood of agricultural workers and pesticide-treatment workers (Table 1.2). Blood concentrations of dieldrin have been steadily declining in agricultural workers since dieldrin and aldrin were banned (Hayes & Curley, 1968; see also Section 1.5). A correlation of 0.6 between concentration of dieldrin in plasma and total hours of exposure was observed in pesticide-manufacturing workers (Hayes & Curley, 1968). The highest blood concentrations of dieldrin were observed in the 1960s in aldrin formulators in the USA (Mick et al., 1972) and in insecticide-plant workers in the Netherlands (de Jong, 1991). In the latter study of 343 insecticide-plant workers between 1963 and 1970, 18% had levels of 100 µg/L or higher and 5% had levels of 200 µg/L and higher (de Jong, 1991). Estimated daily intake of dieldrin was highest in people employed in the formulation plant, with the estimated median daily intake of assistant operators and cleaners decreasing from 2122 µg/day in 1963 to 575 µg/day in 1969, and the estimated median daily intake of operators decreasing from 1546 µg/day in 1963 to 291 µg/day in 1969. Aldrin/dieldrin plant workers had the second highest estimated daily intake of dieldrin: assistant operators' and cleaners' estimated median intake decreased from 1163 µg/day

to 427 µg/day between 1963 and 1969; estimated median intake for maintenance workers varied between 116 µg and 186 µg between 1963 and 1969 (highest levels in 1964 and 1965); and operators' estimated median intake varied from 291 to 826 µg (highest levels in 1964 and 1965) (de Jong, 1991). Dieldrin intake by occupationally exposed workers employed in the manufacture of dieldrin, aldrin, endrin, and other insecticides has been estimated to range from 0.72 to 1.10 mg/person per day (Hayes & Curley, 1968) compared with 0.025 mg/person per day for the general population (Hunter & Robinson, 1967).

Aldrin in the blood of occupationally exposed workers has been infrequently measured (Table 1.2). Mean aldrin concentrations in the blood of pesticide manufacturing workers in the 1960s in the USA were highest in aldrin formulators (29.5 µg/L) and much lower in 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) formulators (0.8 µg/L) (Mick et al., 1972).

1.4.2 Community exposure

The general population can be exposed to dieldrin and aldrin directly or from residues on food or from living near areas where dieldrin or aldrin was sprayed. Exposures may occur during personal use of products containing dieldrin or aldrin, such as during pesticide treatments carried out in and around the home to prevent termites (ATSDR, 2002), and as a result of their persistence in the environment: aldrin and dieldrin are classified as persistent organic pollutants (POPs) (Stockholm Convention, 2001). Aldrin was consistently found less frequently than dieldrin and, when quantified, in smaller quantities (see below).

(a) Water

Dieldrin and aldrin are hydrophobic and do not dissolve easily in water (Mackay & Wolkoff, 1973). Water concentrations are usually < 0.01 µg/L, with higher levels attributed to contamination from industrial effluents and soil erosion during agricultural use (WHO, 2003). Detectable concentrations of dieldrin are regularly reported in samples collected 5–15 years after use of dieldrin and aldrin was discontinued. In water samples collected in the early 1970s, an average dieldrin concentration of 0.3 ng/L was found in drinking-water in Hawaii, USA (Bevenue et al., 1972), and 0.19 µg/L in 50% of cistern-water samples taken in one locality in the Virgin Islands (Lenon et al., 1972). Surface, ground, lake, and marine waters generally contained low concentrations of aldrin and dieldrin.

In the 1980s, dieldrin was detected in surface- and groundwater samples from Canada, from Puerto Rico, and from 48 states of the USA (EPA, 1987). In a similar survey in the USA in1992–2001, dieldrin was found in less than 5% of samples of stream water and ground water, but most frequently and at the highest concentrations in areas where corn crops had been treated extensively with aldrin and dieldrin (USGS, 2006). Dieldrin and aldrin were detected in samples collected from the Sarno River, Italy (Montuori et al., 2014), and rivers in Greece (Golfinopoulos et al., 2003; Konstantinou et al., 2006; Litskas et al., 2012), but not in samples from the Nile River and its estuaries, Egypt (Abbassy et al., 1999).

(b) Sediment and soil

Past use of aldrin and dieldrin has resulted in the presence of residues of these compounds in the soil today. Both compounds bind to soil and are absorbed into the food chain (Jorgenson, 2001). Sunlight and bacteria change aldrin to dieldrin. Dieldrin has low volatility and its half-life in soil has been estimated to range from 2.6 to 12.5 years (Jorgenson, 2001; Beyer & Gale, 2013). Dieldrin has been detected in river-bed sediments in the USA (20–45% of samples) (USGS, 2006), in marine sediments in Portugal (Carvalho et al., 2009), in marine sediments directly exposed

Table 1.2 Concentrations of aldrin and dieldrin in blood samples from occupationally exposed workers

Agent	Country, year	Occupation	Work task or type of worker	No. of workers	Exposure level[a]	Exposure range	Reference
Aldrin	Brazil, 1997	Agricultural workers	Mixing, loading, and/or applying pesticides	26	NR	All < 1.4 µg/L	Paumgartten et al. (1998)
Aldrin	Columbia, 2005–2006	Agricultural workers	Tasks involving pesticide use in the past 2 yr	99	0.0037 µg/L	NR–0.209 µg/L, 15% detects	Varona et al. (2010)
Aldrin	USA	Pesticide manufacturing workers	Aldrin formulators	7	29.5 µg/L		Mick et al. (1972)
Aldrin	USA	Pesticide manufacturing workers	Warehouse	4	7.6 µg/L		Mick et al. (1972)
Aldrin	USA	Pesticide manufacturing workers	Maintenance and miscellaneous	3	2.8 µg/L		Mick et al. (1972)
Aldrin	USA	Pesticide manufacturing workers	2,4-D & 2,4,5-T formulators	6	0.8 µg/L		Mick et al. (1972)
Dieldrin	Brazil, 1997	Agricultural workers	Mixing, loading, and/or applying pesticides	26	NR	< 1.4–3.7 µg/L, 4% detects	Paumgartten et al. (1998)
Dieldrin	Columbia, 2005–2006	Agricultural workers	Tasks involving pesticide use in the past 2 yr	99	0.004 µg/L	< 0.020–0.090 µg/L, 11% detects	Varona et al. (2010)
Dieldrin	USA, NR	Farmer	Pre- and post-pesticide application	12	NR	< 0.23–21 µg/L, 33% detects	Brock et al. (1998)
Dieldrin	USA, NR	Pesticide manufacturing workers	Pipefitters, shippers, and helpers	20	23.3 µg/L	1.2–4.6 µg/L	Hayes & Curley (1968)
Dieldrin	USA, NR	Pesticide manufacturing workers	Operators, foremen	26	18.9 µg/L	3.2–108 µg/L	Hayes & Curley (1968)
Dieldrin	USA, NR	Pesticide manufacturing workers	Painters, carpenters, engineers, inspectors, laboratory workers	17	9.8 µg/L	1.3–21.5 µg/L	Hayes & Curley (1968)
Dieldrin	USA, NR	Pesticide manufacturing workers	Clerical workers	8	5.4 µg/L	< 0.7–25.5 µg/L	Hayes & Curley (1968)
Dieldrin	The Netherlands, NR	Pesticide manufacturing workers	Manufacture of dieldrin, aldrin and endrin	12	26 µg/L	0.5–110 µg/L	Hunter et al. (1972)
Dieldrin	USA, NR	Pesticide manufacturing workers	Aldrin formulators	7	182.5 µg/L	NR– ~300 µg/L	Mick et al. (1972)
Dieldrin	USA, NR	Pesticide manufacturing workers	Warehouse	4	77.5 µg/L	NR– ~150 µg/L	Mick et al. (1972)
Dieldrin	USA, NR	Pesticide manufacturing workers	Maintenance and miscellaneous	3	35.2 µg/L	NR– ~80 µg/L	Mick et al. (1972)

Table 1.2 (continued)

Agent	Country, year	Occupation	Work task or type of worker	No. of workers	Exposure level[a]	Exposure range	Reference
Dieldrin	USA, NR	Pesticide manufacturing workers	2,4-D & 2,4,5-T formulators	6	11.0 µg/L	NR – 20 µg/L	Mick et al. (1972)
Dieldrin	Australia, NR	Pesticide treatment workers	Vehicle and plant maintenance or stores	5	Median, 7 µg/L	0.9–14.5 µg/L	Edwards & Priestly (1994)
Dieldrin	Australia, NR	Pesticide treatment workers	Termiticide applicators	10	Median, 5.3 µg/L	2.5–145 µg/L	Edwards & Priestly (1994)
Dieldrin	Australia, NR	Pesticide treatment workers	Pre-building treatment of building sites and foundations	5	Median, 16 µg/L	2.5–250 µg/L	Edwards & Priestly (1994)
Dieldrin	Australia, NR	Pesticide treatment workers	Office and sales	10	Median, 4.8–5.8 µg/L	0.7–26 µg/L;	Edwards & Priestly (1994)
Dieldrin	Sudan, NR	Pesticide treatment workers	Mixing and spraying insecticides	22	NR	< 10–50 µg/L, 27% detects	El Zorgani & Musa (1976)
Dieldrin	Argentina, NR	Pesticide workers	Sprayers, spray truck drivers, supervisors using hexachlorocyclohexane or DDT	20	Catamarca Province: 1.09 ± 0.60 µg/L; Salta Province: 1.16 ± 0.72 µg/L	NR	Radomski et al. (1971)
Dieldrin	Argentina, NR	Pesticide workers	Former sprayers in malaria control programme	10	Catamarca Province: 2.81 ± 3.59 µg/L	NR	Radomski et al. (1971)
Dieldrin	Argentina, NR	Pesticide workers	Administrative personnel	19	Catamarca Province: 2.09 ± 1.25 µg/L; Salta Province: 0.77 ± 0.74 µg/L	NR	Radomski et al. (1971)
Dieldrin	The Netherlands, 1963–1970	Insecticide manufacturing workers	Various	343	NR	< 10 µg/L to > 200 µg/L	de Jong (1991)

[a] Exposure levels expressed in mean unless indicated otherwise

2,4-D, 2,4-dichlorophenoxyacetic acid ; DDT, dichlorodiphenyltrichloroethane; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; NR, not reported; yr, year(s)

to wastewater in Marseille, France (Syakti et al., 2012), in agricultural soil samples in Shanghai, China (Jiang et al., 2009), and in soil samples from the Czech Republic (Shegunova et al., 2007).

(c) Air

Concentrations of dieldrin in air are generally low; however, exposures may be greater for residents living around sites where aldrin or dieldrin has been used. Atmospheric transport has resulted in detectable concentrations of dieldrin in remote areas of Scandinavia and the Arctic, where it is unlikely that aldrin or dieldrin were ever used (USGS, 2006). Dieldrin was detected at only one out of nine localities in the USA, at a maximum level of 29.7 ng/m^3 (Stanley et al., 1971). In London and its suburbs, England, very small quantities of dieldrin (18–21 g/10^{12} g of air) were detected in air (Abbott et al., 1966). In the Bahamas in the early 1970s, concentrations of dieldrin in air ranged from 0.33 ng/m^3 to 0.86 ng/m^3 (Davies et al., 1975). More recently, air measurements collected between 2001 and 2008 in Mali found dieldrin at concentrations of 0.091–1.8 ng/m^3 (mean, 1.1 ± 0.8 ng/m^3; median, 1.7 ng/m^3) (Garrison et al., 2014). Concentrations were more than twice as high in samples from an urban area of Kati, Mali, than in samples from downwind sites. Alegria et al. (2000) reported mean concentrations of dieldrin of 0.044 ng/m^3 in an inland agricultural area of Belize in 1995–1996. Low dieldrin concentrations (maximum, 0.64 ng/m^3; median, 0.00 ng/m^3) were reported at mid-continental sites in the USA in 1994 (Majewski et al., 1998).

In air samples collected in the USA, aldrin was infrequently detected and, when detected, occurred at low concentrations ranging from 0.1 to 4 ng/m^3 (Tabor, 1966; Stanley et al., 1971).

(d) Residential exposure

Detectable concentrations of dieldrin were found at a range of < 0.1 to 0.3 ng/m^3 in 42% of air samples collected in homes of residents with no occupational exposure, in France (Bouvier et al., 2006). Samples collected from the hands of these residents showed dieldrin at detectable concentrations (30% detects; range, < 0.8–5.5 ng/hand). Dieldrin was detected in house dust in eight out of nine homes in the USA sampled in the early 1990s, with a mean of 0.12 µg/m^2 and maximum of 0.38 µg/m^2 (Lewis et al., 1994). Aldrin was detected in five out of nine homes in the USA sampled in the early 1990s, but in only one to three of the six environmental matrices examined in these homes (Lewis et al., 1994). Concentrations of dieldrin in carpet dust were higher than those in samples from the walkway, entryway, or play-area soil. Detectable concentrations of dieldrin in air were found in four out of eight homes (mean, 0.07 µg/m^3). For children, estimated dieldrin intake ranged from < 0.1 to 0.13 µg/day via air, and from < 0.01 to 0.04 µg/day via dust. Average dieldrin concentrations were higher in samples of interior dust (2.84 ppm) than in samples of exterior soil (0.07 ppm) from homes in the Bahamas (Davies et al., 1975).

(e) Residues in food, and dietary intake

Dieldrin is stored in the adipose tissue, liver, brain, and muscle of mammals, fish, birds, and other organisms in the food chain (WHO, 2003). The half-life in whole fish is estimated to be about 30 years (USGS, 2006). In whole fish collected from streams draining from watersheds with mixed land use in the USA from 1969 to 1999, dieldrin concentrations in fish tissue varied substantially during the early 1970s, then continued to decline slowly through the early 1990s (USGS, 2006). In Australia, the maximum concentration in fish tissue was 0.14 and 1.75 µg/g wet weight in the 1970s and 1980s, respectively, for aldrin, and 0.37, 3.1, and 0.23 µg/g wet weight in the 1970s, 1980s, and 1990s, respectively, for dieldrin (Connell et al., 2002).

In Poland, the mean daily intake of aldrin and dieldrin combined from milk for an adult was 4.1 ng/kg body weight (bw) based on mean

concentrations in cows' milk of 0.5–4.8 ng/g wet weight for aldrin and 0.03–0.2 ng/g wet weight for dieldrin (Witczak et al., 2013).

In a study in which the median concentration of dieldrin in mothers' milk was reported to be 6 µg/L, the estimated intake by breast-fed babies was approximately 1 µg/kg bw per day (IPCS, 1989). In Denmark, the average daily intake of dieldrin in infants was estimated to be 0.045 µg/kg per day based on average dieldrin concentrations in breast milk of 9 ng/g fat (Danish National Board of Health, 1999). Measurements taken in samples of children's meals in the Salinas Valley of California, USA, in 2002 found detectable levels of dieldrin in 10% of toddlers' solid food samples, with a maximum concentration of 6.1 ng/g (Bradman et al., 2007).

The total dietary intake of dieldrin in the late 1960s was found to range between 0.05 and 0.08 µg/kg bw per day in the USA (Duggan & Corneliussen, 1972), 0.07 µg/kg bw per day in Japan (Uyeta et al., 1971), and 0.30 and 0.09 µg/kg bw per day in the United Kingdom for 1965 and 1966–67, respectively (McGill & Robinson, 1968; Abbott et al., 1969).

The average daily intake of aldrin from food ranged from 0.04 to 0.0001 µg/kg bw per day for 1965–1970, with an average intake of 0.01 µg/kg bw per day (Duggan & Lipscomb, 1969). The reduction in use of aldrin since the 1970s has decreased food residues in many countries (IPCS, 1989). Intake in 1980–1982 was estimated to be below 0.2 µg/kg bw per day in several countries (IPCS, 1989).

(f) Biological markers

Dieldrin has been measured in the blood of populations of varying ages and in various geographical locations over the past several decades (Table 1.3). Although serum dieldrin concentrations have generally decreased over time, detectable levels continue to be measured decades after use of dieldrin and aldrin was banned. Serum dieldrin levels at the 95th percentile in samples from the National Health and Nutrition Examination Surveys (NHANES) 2001–2002 and 2003–2004 were approximately 10 times lower than in samples from NHANES 1976–1980 (Stehr-Green, 1989; CDC, 2009). Detection rates for aldrin measured in the blood were generally low. An exception was observed for blood samples collected from people living in an agricultural area of southern Spain. Carreño et al. (2007) found detectable levels of aldrin in 79% of blood samples from young men. Aldrin and dieldrin have also been detected in adipose tissue and breast milk (Table 1.3). For example, dieldrin was detected in 59% of adipose tissue samples collected in a Danish population between 1993 and 1997, with a median concentration of 17 and 19 µg/kg for women and men, respectively (Bräuner et al., 2012). Cerrillo et al. (2006) found detectable levels of aldrin in 30% of adipose tissue samples from women aged 33–75 years. Because aldrin rapidly converts to dieldrin, the high rate of detection of dieldrin may indicate recent exposure to aldrin, despite its ban in the mid-1980s (Cerrillo et al., 2006). [The Working Group noted that the pattern of results across different matrices and for aldrin and dieldrin was difficult to explain by exposure or release from adipose tissue.]

Dieldrin has been detected at a mean concentration of 0.01–11 µg/L in breast milk in Europe and the USA (IPCS, 1989). Dieldrin concentrations in breast milk decreased from an average of 1.33 ng/g milk in 1982 to 0.85 ng/g milk in 1986 (WHO, 2003). However, higher concentrations of up to 35 ng/g were found in the 1980s in breast milk from Australian women whose houses were treated annually with aldrin (Stacey & Tatum, 1985).

Dieldrin has also been measured in breast tissue (Djordjevic et al., 1994; Mathur et al., 2002), and bone marrow (Scheele et al., 1992).

Aldrin and dieldrin were detected in 82% and 75%, respectively, of samples of umbilical cord blood collected in 2013–2014 from 999 pregnant

Table 1.3 Concentrations of aldrin and dieldrin in biological samples from the general population

Agent	Sample matrix	Country, year	Age (years)	No. of samples	Exposure level[a]	Exposure range,% detects	Comments	Reference
Aldrin	Adipose tissue	Spain, NR	33–75, mean 56 ± 10.46	458	10.51 ng/g lipid	NR, 30.3% detects	Women living in agricultural areas of southern Spain that have the largest area of intensive greenhouse agriculture in Europe	Cerrillo et al. (2006)
Aldrin	Adipose tissue	Spain, NR	Mean age, 53	200	25.6 ± ng/g lipid	NR–137 ng/g lipid, 40% detects	Women living in intensive greenhouse agriculture area	Botella et al. (2004)
Aldrin	Blood	India, NR	21–70	50	115 µg/L	NR	Similar levels in rural and urban environments (mean, 168 µg/L vs 101 µg/L)	Mathur et al. (2002)
Aldrin	Serum	Spain, NR	Mean age, 53	200	2.17 µg/L	NR – 14.2 µg/L, 56% detects	Women living in intensive greenhouse agriculture area	Botella et al. (2004)
Aldrin	Serum	Spain, NR	18–23	220 (Males)	3.75 µg/L; Median, 2.62 µg/L	< 3.0–33.76 µg/L, 79% detects	Extensive greenhouse agricultural area	Carreño et al. (2007)
Aldrin	Serum	Nicaragua, 2002	11–15	38	NR	0% detects	LOD estimated, < 10 ng/g lipid; Working and living at municipal waste-disposal site and in nonworking children living both nearby and far from site	Cuadra et al. (2006)
Aldrin	Serum	Brazil, 1999	19–63	33	NR	< 1.4 µg/L, 0% detects	Urban area	Delgado et al. (2002)
Aldrin	Serum	India, NR	≥ 18	50	2.08 µg/L	NR	Controls, no occupational exposure	Tomar et al. (2013)
Aldrin	Serum	Norway, 1973–1991	18–60; mean, 41.2	300	NR	< 0.08–NR ng/g, lipid 1% detects		Ward et al. (2000)
Aldrin	Plasma	France, Germany, Spain, NR	Mean, 56.5 ± 15.7	203	Median of detectable, 19.8 µg/L	6.7–NR µg/L, Spain 9% detects; France and Germany 0% detects		Cocco et al. (2008)
Aldrin	Breast milk	Israel, 2011–2012	23–35; mean, 30	52	NR	NR, 0% detect	Pooled sample	Wasser et al. (2015)

Table 1.3 (continued)

Agent	Sample matrix	Country, year	Age (years)	No. of samples	Exposure level[a]	Exposure range,% detects	Comments	Reference
Aldrin	Breast milk	Turkey, NR	NR	75	36.6 ng/g lipid	< 5–230.6 ng/g, 58.7% detects		Yalçın et al. (2015)
Dieldrin	Adipose tissue	Denmark, 1993–1997	Men, 51–64	126	Mean, 22 µg/kg lipid; median, 19 µg/kg lipid	5–95th percentile: 10–42 µg/kg lipid	59% detects overall (includes women)	Bräuner et al. (2012)
Dieldrin	Adipose tissue	Denmark, 1993–1997	Women, 51–64	119	Mean, 24 µg/kg lipid; median, 17 µg/kg lipid	5–95th percentile: 8–49 µg/kg lipid	59% detects overall (includes men)	Bräuner et al. (2012)
Dieldrin	Adipose tissue	USA, NR	37–66	5		All < 10 µg/kg fatty tissue, 0% detects	Study controls	Djordjevic et al. (1994)
Dieldrin	Adipose tissue	Australia, 1990–1991	NR	31	Median, 40 µg/kg extractable fat	10–1100 µg/kg extractable fat, 100% detects	Nursing mothers	Stevens et al. (1993)
Dieldrin	Adipose tissue	Spain, NR	Mean, 53	200	17 ng/g lipid	NR–84 ng/g lipid, 28.5% detects	Postmenopausal women living in intensive greenhouse agriculture area	Botella et al. (2004)
Dieldrin	Blood	USA, 1999–2004	≥ 20	2341	Median detected, 8.74 ng/g lipid-adjusted	< 10.5 ng/g lipid-adjusted, 22% detects	Nationally representative sample (NHANES)	Everett & Matheson (2010)
Dieldrin	Blood	Israel, 1975–1986	NR	15	2.7 ng/g	NR	Females of reproductive age	Pines et al. (1987)
Dieldrin	Blood	USA, NR	NR	26	1.49 ± 1.00 µg/L	NR	Adults	Radomski et al. (1971)
Dieldrin	Blood	Argentina, NR	NR	20	1.43 ± 1.21 µg/L	NR	Adults	Radomski et al. (1971)
Dieldrin	Blood	Argentina, NR	Children, 5–10	18	0.94 ± 0.92 µg/L	NR		Radomski et al. (1971)
Dieldrin	Blood	Argentina, NR	Children, 1–5	19	0.54 ± 0.29 µg/L	NR		Radomski et al. (1971)
Dieldrin	Blood	Argentina, NR	Newborns	13	0.59 ± 0.42 µg/L	NR	Ratio newborn to mother: 0.44 ± 0.16 µg/L	Radomski et al. (1971)

Table 1.3 (continued)

Agent	Sample matrix	Country, year	Age (years)	No. of samples	Exposure level[a]	Exposure range,% detects	Comments	Reference
Dieldrin	Serum	Spain, NR	Mean, 53	200	1.21 µg/L	NR–6.35 µg/L, 47% detects	Postmenopausal women living in intensive greenhouse agriculture area	Botella et al., (2004)
Dieldrin	Serum	Spain, NR	Men, 18–23	220	Mean, 1.85 µg/L; median, 0.50 µg/L,	< 3–29.42 µg/L, 40.7% detects	Extensive greenhouse agricultural area, association with maternal employment in agriculture	Carreño et al. (2007)
Dieldrin	Serum	Nicaragua, 2002	11–15	38	NR	0% detects	LOD estimated < 10 ng/g lipid; working and living at municipal waste-disposal site and in nonworking children living both nearby and far from site	Cuadra et al. (2006)
Dieldrin	Serum	The Bahamas, 1970–1971	≥ 20; mean, 39	148	Mean, 1.1 µg/L	< 1–9.2 µg/L		Davies et al. (1975)
Dieldrin	Serum	Brazil, 1999	19–63	33	NR	All < 1.4 µg/L	Urban area	Delgado et al. (2002)
Dieldrin	Serum	USA, NR	67.6 ± 14.6	144	Geometric mean, 0.38 µg/L	NR	Study controls; concentrations associated with older age, higher education, higher BMI, a few other factors	Louis et al. (2006)
Dieldrin	Serum	Costa Rica, 2012	≥ 65	53	3.40 µg/L			Steenland et al. (2014)
Dieldrin	Serum	United Republic of Tanzania, NR	NR	47	Females, 0.50 ± 0.07 ng/g; males, 0.55 ± 0.09 ng/g	NR	Adults, reproductive age	Weiss et al. (2006)
Dieldrin	Serum	Germany, NR	NR	42	Women, 0.02 ± 0.01 ng/g; men, 0.08 ± 0.01 ng/g	NR	Adults, reproductive age	Weiss et al. (2006)
Dieldrin	Serum	Norway, 1973–1991	18–60; mean, 41.2	300	Median, 16.1 ng/g lipid	< 0.47 ng/g – NR, 67.9% detects		Ward et al. (2000)

Table 1.3 (continued)

Agent	Sample matrix	Country, year	Age (years)	No. of samples	Exposure level[a]	Exposure range,% detects	Comments	Reference
Dieldrin	Plasma	Spain, Germany, France, NR	54.7	203	Median of detectable, 16.2 µg/L	6.2–NR µg/L, Spain 34% detects; France and Germany 0% detects		Cocco et al. (2008)
Dieldrin	Seminal plasma	United Republic of Tanzania, NR	NR	31	Mean, 0.13 ± 0.05 ng/g	NR	Men, reproductive age	Weiss et al. (2006)
Dieldrin	Seminal plasma	Germany, NR	NR	21	Mean, 0.03 ± 0.01 ng/g	NR	Men, reproductive age	Weiss et al. (2006)
Dieldrin	Follicular fluid	United Republic of Tanzania, NR	NR	31	Mean, 0.17 ± 0.02 ng/g	NR	Women, reproductive age	Weiss et al. (2006)
Dieldrin	Follicular fluid	Germany, NR	NR	21	Mean, 0.03 ± 0.01 ng/g	NR	Women, reproductive age	Weiss et al. (2006)
Dieldrin	Breast milk	Denmark, 1997–2001	NR	36	Median 4.66 ng/g	25th–75th percentiles: 3.06–5.98 ng/g	Women had a narrow age distribution and were mainly from higher social class	Krysiak-Baltyn et al. (2010)
Dieldrin	Breast milk	Finland, 1997–2001	NR	32	Median 2.21 ng/g	25^{th}–75^{th} percentiles: 1.86–3.10 ng/g	Women had a narrow age distribution and were mainly from higher social class	Krysiak-Baltyn et al. (2010)
Dieldrin	Breast milk	USA, NR	NR	1436	Mean 164.2 ± 436.2 ppb fat-adjusted	< 1 – > 500 ppb, 80.8% detects		Savage et al. (1981)
Dieldrin	Breast milk	Israel, 2011–2012	23–35 ; mean, 30	52	2.8 ng/g lipid	NR	Pooled sample	Wasser et al. (2015)
Dieldrin	Breast milk	Turkey, NR	NR	75	NR	All < 5 ng/g lipid, 0% detects		Yalçın et al. (2015)
Dieldrin	Breast milk	Denmark, 1993–94	25–29	36	Median, 8 ng/g fat	3–19 ng/g fat	Women, several days after giving birth	Danish National Board of Health (1999)

[a] Exposure levels expressed as the mean, unless otherwise indicated

BMI, body mass index; LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey; NR, not reported

women in China, with a mean aldrin concentration of 7.29 µg/L and mean dieldrin concentration of 5.27 µg/L (Luo et al., 2016).

1.5. Regulations and guidelines

In the USA, the American Conference of Governmental Industrial Hygienists (ACGIH), the National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA) have all adopted a time-weighted average (TWA) concentration limit of 0.25 mg/m^3 in air for aldrin and dieldrin, also noting dangers from cutaneous absorption (ATSDR, 2002; NIOSH, 2016a). NIOSH has also designated aldrin as a "potential occupational carcinogen," and has determined an Immediately Dangerous to Life or Health (IDLH) concentration of 25 mg/m^3 (NIOSH, 2016a). NIOSH similarly designated dieldrin as a "potential occupational carcinogen," and has determined an IDLH concentration of 50 mg/m^3 (NIOSH, 2016b).

WHO has established a guideline value of 0.03 µg/L for the sum of aldrin and dieldrin concentrations in drinking-water (WHO, 2003). The United States EPA has not established a maximum contaminant level for aldrin in drinking-water, but has published a variety of non-enforceable health advisory levels that depend on duration of exposure and age (EPA, 2012).

Under the European Union harmonized classification and labelling system, both aldrin and dieldrin are suspected of "causing cancer" (Carc. 2) [H 351] and have been determined to be "very toxic to aquatic life" (Aquatic Acute 1) [H 450] and "very toxic to aquatic life with long lasting effects" (Aquatic Chronic 1) [H 410], "toxic if swallowed" (Acute Tox. 3) [H 311], and to "cause damage to organs through prolonged or repeated exposure" (STOT RE 1) [H 372] (ECHA, 2016a, b). In addition, aldrin has been determined to be "toxic in contact with skin" (Acute Tox. 3) [H 301], whereas dieldrin has been determined to be "fatal in contact with skin" (Acute Tox. 1) [H 310] (ECHA, 2016a, b).

In the USA, aldrin and dieldrin uses were restricted to certain non-food applications in 1974, and the sole manufacturer cancelled all remaining uses in 1989 (ATSDR, 2002). In the 1970s, the use of aldrin was banned or severely restricted in a number of additional countries including Germany, Italy, Japan, Norway, the former Soviet Union, and the United Kingdom (IARC, 1974). Use and export of aldrin and dieldrin are banned in the European Union (European Commission, 2004). There are additional restrictions and requirements regarding the presence of aldrin in seeds, effluent, groundwater, water bodies, hazardous waste, and releases to the environment in the USA (ATSDR, 2002).

Aldrin and dieldrin are listed in Annex A of the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2008), under which parties must take steps to eliminate production and use unless they have registered for an exemption.

2. Cancer in Humans

2.1 Aldrin

Aldrin and dieldrin are often discussed together because aldrin readily converts into dieldrin, both in the environment and in the human body (see Sections 1 and 4). The studies in this section may therefore also be discussed or referred to in the section on dieldrin (Section 2.2), when results for both compounds were presented in the same study.

2.1.1 Cohort studies

See Table 2.1.

(a) Occupational cohorts

Two studies have published results related to aldrin exposure in occupational cohorts: a study in workers at an insecticide plant in Pernis-Rotterdam, the Netherlands; and the Agricultural Health Study (AHS) of Iowa and North Carolina, USA, among residents licensed to apply restricted-use pesticides. A study in workers at organochlorine pesticide-manufacturing plants in Colorado, USA, was considered uninformative because the plant had produced many different pesticides and no results specific to aldrin (or dieldrin) were presented (Ditraglia et al., 1981; Brown, 1992; Amoateng-Adjepong et al., 1995).

Several studies have been published on a cohort of 570 male workers at a Dutch plant that produced and formulated aldrin and dieldrin (Ribbens, 1985; de Jong et al., 1997; Sielken et al., 1999; Swaen et al., 2002; van Amelsvoort et al., 2009). The most recent publication included employees who had worked for at least 1 year between 1954 and 1970 inclusive and were followed up until 2006 (van Amelsvoort et al., 2009). Standardized mortality ratios (SMRs) were calculated relative to the national population of the Netherlands. Total intake of dieldrin plus aldrin was calculated using models based on blood monitoring that had been carried out during the 1950s for 343 members of the cohort (de Jong, 1991). Blood monitoring of dieldrin was used as a combined measure of exposure to both aldrin and dieldrin. Workers without samples were allocated the same intake as workers in the same job, workplace, and time.

The standardized mortality ratio for all cancers combined was 0.76 (95% CI, 0.61–0.95) for all workers. When the workers were divided into three groups on the basis of dose, the standardized mortality ratios were 1.00 (95% CI, 0.66–1.46) for the group at the lowest dose (mean intake, 270 mg); 0.75 (95% CI, 0.50–1.09) for the group at the moderate dose (mean intake, 540 mg), and 0.66 (95% CI, 0.44–0.96) for the group at the highest dose (mean intake, 750 mg).

The standardized mortality ratio for cancer of the lung was significantly different from expected (SMR, 0.63; 95% CI, 0.41–0.92; 26 cases) and there was no dose–response pattern. Standardized mortality ratios for cancers of the oesophagus, rectum, liver and biliary tract, and skin were elevated based on small numbers of deaths, but were not statistically significant or systematically related to exposure level. [For the other cancers examined, all had fewer than 10 cases and none had statistically significant results. No internal analyses were performed.]

[The Working Group noted that the strengths of this study were that the plant made only aldrin and dieldrin; the exposure assessment was based on biomonitoring and modelling; and there was a small loss to follow-up. The limitations were that exposure assessment did not separate aldrin and dieldrin; the study reported mortality data, rather than incidence data; there was low power for rare cancers; no adjustment for confounders; and there were no internal analyses.]

In the AHS, more than 57 000 pesticide-user licensees in Iowa and North Carolina, USA, were recruited between 1993 and 1997. At enrolment, participants completed a self-administered questionnaire on whether they had ever mixed or applied 50 specific pesticides (including aldrin and dieldrin), which application methods were used, and the use of personal protective equipment. About half of the cohort also reported the number of years and days per year they had personally mixed aldrin or dieldrin.

Lifetime exposure-days of use for each pesticide were calculated as the product of the number of years a participant had personally mixed or applied each pesticide multiplied by the number of days per year that pesticide was used. In addition, an intensity-weighted lifetime exposure-days score was calculated by multiplying lifetime exposure-days by an exposure intensity

Table 2.1 Cohort studies of cancer and exposure to aldrin

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ward et al. (2000) Norway 1973–1993 Population-based Nested case–control	Cases: 150; random selection from Janus serum bank with samples taken before diagnosis Controls: 150; matched to cases by date of sample and age Exposure assessment method: personal monitoring; gas chromatography	Breast	Aldrin Above LOD	1	0.5 (0.0–6.5)	Age, time of sample collection	
Flower et al. (2004) Iowa and North Carolina, USA Childhood cancers 1975–1998 in Iowa and 1990–1998 in North Carolina Cohort	50 cases; Agricultural Health Study; children of pesticide licensees, born after 1975 Exposure assessment method: questionnaire; parental pesticide use	Childhood cancer	Aldrin, father's use (prenatal)	6	2.66 (1.08–6.59)	Age of child at enrolment	Strengths: large numbers, individual pesticide use Limitations: self-reported data
Engel et al. (2005) Iowa and North Carolina, USA 1993–2000 Cohort	30 454; Agricultural Health Study; wives of pesticide licensees Exposure assessment method: questionnaire	Breast	Aldrin use By wife By husband By husband (premenopausal) By husband (postmenopausal)	 4 52 6 40	 0.9 (0.3–2.5) 1.9 (1.3–2.7) 1.4 (0.6–3.8) 1.7 (1.1–2.6)	Age, state, race	Strengths: large numbers, individual pesticide use Limitations: self-reported data

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2007) Iowa and North Carolina, USA Recruited, 1993–1997, follow-up, 2002 Cohort	51 011; Agricultural Health Study; pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days (take into account factors affecting exposure)	All cancers combined: incidence	Aldrin	680	1.0 (0.9–1.1)	Age, state, sex, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	Strengths: large numbers, individual pesticide use Limitations: self-reported data
		Lung: incidence	Aldrin	53	1.0 (0.7–1.4)		
		Colon: incidence	Aldrin	39	0.7 (0.4–1.0)		
		Rectum: incidence	Aldrin	28	1.4 (0.8–2.4)		
		Malignant melanoma: incidence	Aldrin	23	1.1 (0.7–2.0)		
		Leukaemia: incidence	Aldrin	22	1.4 (0.8–2.7)		
van Amelsvoort et al. (2009) Pernis, the Netherlands 1954–2006 Cohort	570; men employed ≥ 1 year in a pesticide production plant, 1954–1970 Exposure assessment method: modelling; exposure modelled from blood measures in subgroup (n = 343) to produce total dose for each worker; range, 11–7755 mg dieldrin and aldrin combined	All cancers combined	Estimated intake of aldrin+dieldrin			Age, time	Earlier publications from this study are Swaen et al. (2002); Sielken et al. (1999); de Jong et al. (1997); Ribbens (1985) Strengths: biomonitoring data modelled to give quantitative exposure assessment Limitations: no internal comparisons made; unable to separate exposure to dieldrin and Aldrin; small numbers
			All	82	0.76 (0.61–0.95)		
			Low	27	1.00 (0.66–1.46)		
			Moderate	27	0.75 (0.50–1.09)		
			High	28	0.66 (0.44–0.96)		
		All cancers combined: Mortality	SMR			Age, time	
			Assistant operator	28	0.86 (0.58–1.25)		
			Maintenance	11	0.66 (0.33–1.19)		
			Operator	41	0.78 (0.56–1.05)		
			Supervisor	2	0.45 (0.06–1.65)		
		Oesophagus	Estimated intake of aldrin+dieldrin			Age, time	
			All	4	1.59 (0.43–4.08)		
			Low	2	2.87 (0.35–10.35)		
			Moderate	1	1.17 (0.03–6.49)		
			High	1	1.08 (0.03–5.99)		

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
van Amelsvoort et al. (2009) (cont.)		Rectum	Estimated intake of aldrin+dieldrin			Age, time	
			All	6	2.15 (0.79–4.68)		
			Low	3	4.42 (0.91–12.91)		
			Moderate	1	1.10 (0.03–6.11)		
			High	2	1.76 (0.21–6.34)		
		Liver and bile ducts	Estimated intake of aldrin+dieldrin			Age, time	
			All	4	2.16 (0.59–5.54)		
			Low	2	4.26 (0.52–15.41)		
			Moderate	2	3.23 (0.39–11.65)		
			High	0	0.00 (0.00–4.14)		
		Lung	Estimated intake of aldrin+dieldrin			Age, time	
			All	26	0.63 (0.41–0.92)		
			Low	7	0.67 (0.27–1.37)		
			Moderate	12	0.86 (0.44–1.5)		
			High	7	0.43 (0.17–0.89)		
		Skin (non-melanoma)	Estimated intake of aldrin+dieldrin			Age, time	
			All	3	3.02 (0.62–8.84)		
			Low	1	3.57 (0.09–19.9)		
			Moderate	2	6.12 (0.74–22.09)		
			High	0	0.00 (0.00–8.44)		

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2013a) Iowa and North Carolina, USA 1993–2007 Cohort	54 412; Agricultural Health Study; pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days and intensity-weighted exposure days	Prostate (total): incidence	Cumulative lifetime exposure to aldrin			Age, state, smoking status, race, family history of prostate cancer, fruit servings, leisure time physical activity in winter	Strengths: large numbers, individual pesticide use
			Q1	65	1.04 (0.80–1.35)		
			Q2	64	0.94 (0.72–1.22)		
			Q3	64	1.14 (0.88–1.48)		
			Q4	64	1.25 (0.97–1.63)		
			Trend-test *P*-value: 0.07				
		Prostate (aggressive/ advanced): incidence	Cumulative lifetime exposure to aldrin				
			Q1	33	0.97 (0.67–1.41)		
			Q2	33	1.09 (0.75–1.57)		
			Q3	34	1.21 (0.84–1.74)		
			Q4	31	1.49 (1.03–2.18)		
			Trend-test *P*-value: 0.02				
		Prostate: family history of prostate cancer	Cumulative lifetime exposure to aldrin				
			Q1	12	1.29 (0.70–2.4)		
			Q2	20	1.95 (1.17–3.25)		
			Q3	17	1.83 (1.08–3.09)		
			Q4	16	2.13 (1.22–3.72)		
			Trend-test *P*-value: 0.005				

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2013b) Iowa and North Carolina, USA 1993–2004 Nested case–control	Cases: 776; prostate cancer cases in AHS who had provided DNA of good quality Controls: 1444; non-cancer subjects in AHS who had provided DNA of good quality Controls: 1444; non-cancer subjects in AHS who had provided DNA of good quality Exposure assessment method: Questionnaire; lifetime exposure days, and intensity-weighted exposure days	Prostate: TET2 Genotype AA	Aldrin, low Aldrin, high Trend-test *P*-value: 0.006 for interaction	10 13	1.86 (0.73–4.75) 3.67 (1.43–9.41)	Age, state	Strengths: large numbers, individual pesticide use
Alavanja et al. (2014) Iowa and North Carolina, USA Recruited, 1993–1997, follow-up, 2011 Cohort	54 306; AHS; pesticide licensees. Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days (take into account factors affecting exposure)	NHL: incidence MM: incidence	Aldrin Aldrin	116 29	0.9 (0.7–1.1) 1.5 (0.9–2.5)	Age, state, sex, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	Strengths: large numbers, individual pesticide use

Table 2.1 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2016) Iowa and North Carolina, USA Recruited, 1993–1997, follow-up, 2011 Cohort	57 310; AHS, male pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days	Urinary bladder: incidence	Ever exposed	88	1.2 (0.92–1.57)	Age, state, smoking status, race	Men only Strengths: large numbers, individual pesticide use. Limitations: self-reported data
		Urinary bladder: incidence	Cumulative intensity weighted days of use			Age, state, education level, smoking status, race	
			Tertile 1	15	0.88 (0.5–1.53)		
			Tertile 2	18	1.61 (0.96–2.68)		
			Tertile 3	17	1.51 (0.89–2.55)		
			Trend-test *P*-value: 0.08				

AHS, Agricultural Health Study; CI, confidence interval; LOD, limit of detection; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; SMR, standardized mortality ratio

score based on modifying factors such as use of personal protective equipment.

Between 1999 and 2005, participants were asked to report all pesticides used in the year before the interview, as well as frequency of use. This interview was completed by only 63% of participants, and in reports after 2012 multiple imputation with logistic regression and stratified sampling were used to impute missing pesticide-exposure information (Heltshe et al., 2012). A wide range of potential confounders including lifestyle factors, other agricultural factors, and medical history were also collected at baseline.

Pertinent results from this study have been published in several publications focused on different cancers. The most recent results for each cancer are reviewed below.

There were no statistically significant increases in risk of all cancers associated with exposure to aldrin, or risk of cancer of the lung, colon, rectum, or melanoma, or leukaemia (Purdue et al., 2007), or of non-Hodgkin lymphoma (NHL) (relative risk, RR, 0.9; 95% CI, 0.7–1.1), or multiple myeloma (Alavanja et al., 2014). For cancer of the bladder, there was no significant association with ever use of aldrin or high use (RR, 1.51; 95% CI, 0.89–2.55) (Koutros et al., 2016). There was a non-statistically significant increase in risk of all prostate cancer associated with aldrin use (RR, 1.25; 95% CI, 0.97–1.63; *P* for trend, 0.07), which was more marked for the highest quartile (RR, 1.49; 95% CI: 1.03–2.18), with a significant exposure–response trend for aggressive prostate cancer (*P* for trend, 0.02) (Koutros et al., 2013a). Cancer of the prostate was also associated with aldrin use in those with a family history of prostate cancer (*P* for trend, 0.005) (Koutros et al., 2013a); and in further gene–environment analyses, men carrying two AA alleles at rs7679673 were at increased risk of prostate cancer when they had high aldrin use (Koutros et al., 2013b). Cancer of the breast in wives of the pesticide licensees was not increased for self-use of aldrin, but was increased for husband's use (RR, 1.9; 95% CI, 1.3–2.7), and this was more marked in postmenopausal women (RR, 1.7; 95% CI, 1.1–2.6; 40 cases) than in premenopausal women (RR, 1.4; 95% CI, 0.6–3.8; 6 cases) (Engel et al., 2005). Finally, prenatal use of aldrin by fathers was associated with an increase in risk of childhood cancer (RR, 2.66; 95% CI, 1.08–6.59), although this was based on only six cases (Flower et al., 2004). [The Working Group noted that the strengths of this study were that it was large, there was adjustment for other pesticides and potential confounding factors (including major risk factors for cancer of the breast), the exposure assessment was extensive, and the authors were able to separate exposures to aldrin, dieldrin, and other pesticides. The limitations included the small numbers of cases for some analyses, especially in early publications.]

(b) Population cohort study

(i) Cancer of the breast

A case–control study nested within the Janus cohort in Norway used serum samples that had been collected between 1973 and 1991 (Ward et al., 2000). Of 25 431 women who were working outside the home or were resident on farms as of the 1970 or 1980 census and who were followed for cancer incidence until 1993, 272 incident cases of cancer of the breast were reported by 1993. Of these, 150 were randomly chosen, and 150 controls who were alive and cancer-free at time of case diagnosis were matched to cases by date of sample and date of birth. Aldrin, and dieldrin (which may reflect exposure to aldrin and/or dieldrin) were measured in the sera. There were only three samples that contained aldrin at a concentration above the limit of detection (LOD) and the matched odds ratio for aldrin was 0.5 (95% CI, 0–6.5).

[The Working Group noted that the strengths of this study were that exposure was measured before diagnosis, while the limitations were that the exposure assessment was based solely

on serum measurements, given conversion of aldrin to dieldrin, and that only three samples contained aldrin at a level above the LOD.]

2.1.2 Case–control studies

See Table 2.2.

The associations between cancer risk and exposure to organochlorine pesticides, including aldrin and dieldrin, have been investigated in case–control studies in the USA, Canada, and countries in Europe.

Exposure assessment in case–control studies has mainly been performed in two ways. First, questionnaires can be used to obtain self-reports of pesticides used by the participant, and often also some information about methods of application and use of personal protective equipment. Studies using such questionnaires were able to report results for dieldrin and aldrin separately. Second, samples of serum or adipose tissue can be collected and analysed for pesticides. Because of the conversion of aldrin to dieldrin noted above, results for serum dieldrin may represent exposure to both aldrin and dieldrin.

The methods used in studies presenting results for both aldrin and dieldrin are given in the section on aldrin (Section 2.1) and are referred to in the section on dieldrin (Section 2.2). Studies reporting only results related to dieldrin (which may include aldrin in the case of serum measurements) are described in the section on dieldrin.

The Working Group excluded two case–control studies that did not report results specifically for aldrin or dieldrin (Cocco et al., 2008; Tomasallo et al., 2010), and three case–control studies that did not adequately report their methods (Shukla et al., 2001; Mathur et al., 2002, 2008). A study in Gran Canaria, Spain, (Boada et al., 2012) measured aldrin and dieldrin in serum samples from 121 cases of breast cancer and 103 women who had given serum samples in a survey several years earlier. The controls were significantly younger than the cases. [The Working Group noted that the reported prevalence of exposure and serum levels of aldrin (mean, 72.5 ng/g lipid for cases, with 74% above the LOD; and 27.1 ng/g lipid for controls, with 38% above the LOD) and dieldrin (mean, 12.6 ng/g lipid for cases, with 22% above the LOD; and mean, 9.5 ng/g lipid for controls, with 32% above the LOD) was unusually high. The very narrow confidence intervals around odds ratios based on small numbers were also unusual (aldrin odds ratio, 1.027; 95% CI, 0.991–1.065; and dieldrin odds ratio, 1.002; 95% CI, 0.956–1.050) given that, in the same model, the results for lindane (with similar numbers of exposed cases as dieldrin) were 1.097 (95% CI, 0.420–28.412). The Working Group therefore had little confidence in the results of this study and it was also excluded.]

(a) Non-Hodgkin lymphoma

Several case–control studies have investigated the association between NHL and exposure to aldrin. Three of these studies used questionnaires to obtain self-reported data separately on aldrin and dieldrin, and four of these studies used serum or tissue levels of dieldrin to measure combined exposure to dieldrin and aldrin (see Section 2.3).

A population-based case–control study included 622 newly diagnosed cases of NHL among white men aged ≥ 30 years from Iowa and Minnesota, USA (Cantor et al., 1992). The controls were 1245 men without haematopoietic or lymphatic cancer, randomly selected from the general population and frequency-matched to NHL cases by 5-year age group, vital status at interview, and state of residence. In-person structured interviews included detailed questions about farming and pesticide-use history. Adjusted odds ratios indicated non-significantly elevated risk among subjects who had ever personally handled, mixed, or applied aldrin on crops (OR, 1.1; 95% CI, 0.7–1.7). The risks were somewhat higher for those who had handled

Table 2.2 Case–control studies of cancer and exposure to aldrin

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown et al. (1990) Iowa and Minnesota, USA 1980–1984	Cases: 578; tumour registry and hospital records Controls: 1245; matched to case by 5-yr age group, vital status and state via random-digit dialling, Medicare records or state death certificates Exposure assessment method: questionnaire; detailed questions with days per year for each pesticide	Leukaemia: newly diagnosed cases	Ever handled aldrin/days per year			Vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, high-risk exposures	US midwest studies. cases and controls residing in cities with little farming activity (i.e. Minneapolis, St Paul, Duluth, and Rochester) were excluded from the study Strengths: large population-based study in farming areas; in-person interviews; detailed questionnaires including quantification; collection of other potential risk factors; reviewed diagnosis Limitations: multiple comparisons; self-report of pesticide use and limited numbers of participants with aldrin and dieldrin use
			Aldrin, ever handled	33	0.9 (0.6–1.4)		
			Aldrin, 1–4 days/year	11	1 (0.5–2)		
			Aldrin 5–9 days/year	7	0.8 (0.3–2)		
			Aldrin 10+ days/year	4	0.5 (0.2–1.4)		
Cantor et al. (1992) Iowa and Minnesota, USA 1980–1983	Cases: 622; health registry and hospital and pathology records Controls: 1245; matched to cases by age, vital status and state via random-digit dialling, Medicare record or state death certificate files Exposure assessment method: questionnaire; in-person interview	NHL: newly diagnosed cases of four subtypes, follicular, diffuse, small lymphocytic, and "other NHL"	Aldrin exposure: ever handled	47	1.1 (0.7–1.7)	Vital status, age, state, smoking, family history of lympho-haematopoietic cancer, high-risk occupation, high-risk exposures	Data subsequently pooled in De Roos et al. (2003); white men only Strengths: large population-based study in farming areas Limitations: not controlled for exposure to other pesticides
			Handled before 1965	34	1.3 (0.8–2.1)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McDuffie et al. (2001) Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia, Canada 1991–1994	Cases: 517; cancer registries Controls: 1506; health insurance and voting records; frequency-matched on province and ± 2 yr to the age distribution of entire case group Exposure assessment method: questionnaire; self-administered postal questionnaire, followed by telephone interview	NHL	Ever use of aldrin			Age, province of residence, medical variables, mecoprop	Strengths: large study; detailed exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders. Some modelling of multiple pesticide exposures Limitations: potential recall bias; poor response rates; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides
			Model adjusted for age and province of residence	10	3.81 (1.34–10.79)		
			Fully adjusted model	10	4.19 (1.48–11.96)		
Schroeder et al. (2001) Iowa and Minnesota, USA 1980–1983	Cases: 622; state health registry and hospital/ pathology laboratory records Controls: 1245; matched to cases by age, state and vital status via random-digit dialling, Medicare records or state death certificate files Exposure assessment method: questionnaire; in-person structured interviews	NHL: t(14;18)-Positive or t(14;18)-negative cases	Ever use of aldrin			Age, state	Same study population as Cantor et al. (1992); the study looked at NHL subtypes but > 70% of cases had missing subtypes; small numbers of cases with aldrin or dieldrin exposure Strengths: large population-based study in farming areas Limitations: relative small numbers of t(14;18)-positive or -negative NHL
			Aldrin: t(14;18)-positive NHL vs controls	11	1.5 (0.8–2.7)		
			Aldrin: t(14;18)-negative NHL vs controls	10	0.7 (0.4–1.4)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos et al. (2003) Nebraska, Iowa and Minnesota, Kansas, USA 1979–1986	Cases: 650; Nebraska Lymphoma Study Group, hospitals, state health registry, cancer registry Controls: 1933; matched to cases by race, sex, age, region and vital status via random-digital dialling, Medicare records or state mortality files Exposure assessment method: questionnaire; 47 pesticides	NHL: newly diagnosed cases	Aldrin, ever use Aldrin (logistic regression) Aldrin (hierarchical regression)	 47 47	 0.5 (0.3–0.9) 0.6 (0.4–1.0)	Age, study site, all other pesticides	USA midwest studies (pooled) from 3 previous case–control studies (Zahm, Cantor, Hoar); analysis restricted to potentially carcinogenic pesticides
Ibarluzea et al. (2004) Granada and Almeria provinces, Spain April 1996 to June 1998	Cases: 198; breast cancer histologically diagnosed Controls: 260; matched by age (± 3 yr) and hospital; undergoing gall bladder, inguinal hernia, abdominal, varicose vein or other surgery Exposure assessment method: personal monitoring; adipose tissue; aldrin measured with gas chromatography	Breast Breast (premenopausal) Breast (postmenopausal)	Aldrin > LOD > LOD > LOD	 NR 27 40	 1.55 (1–2.4) 1.07 (0.47–2.42) 1.84 (1.06–3.18)	Age, reference hospital, in BMI, number of children, age first pregnancy, family history of breast cancer, alcohol, tobacco	Strengths: medium-sized study; able to adjust for multiple potential confounders Limitations: aldrin measured after diagnosis

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee et al. (2004) Iowa, Minnesota, Nebraska, USA 1980–1986	Cases: 872; state health registry, hospitals and Nebraska Lymphoma Study Group Controls: 2336; matched to case on age, race, and state via random-digit dialling, Medicare records or mortality files Exposure assessment method: questionnaire; telephone or personal interviews with subjects or next-of-kin in Nebraska	NHL	Ever use of aldrin Aldrin among asthmatics Aldrin among non-asthmatics	 10 66	 2.1 (0.9–5.1) 1.0 (0.7–1.5)	Age, vital status, state	Strengths: pooled study so larger numbers Limitations: use of proxy respondents may have led to nondifferential misclassification; no adjustment for co-exposures
Pahwa et al. (2011) Six provinces in Canada 1991–1994	Cases: 357 STS; provincial cancer registries or hospitals Controls: 1506; matched to case by age constraints (± 2 yr) from provincial health insurance records, telephone listings, voters' lists Exposure assessment method: questionnaire; self-administered postal questionnaire and telephone interview	STS	Ever handled aldrin/days per year Aldrin, ever handled	 4	 3.71 (1.00–13.76)	Statistically significant medical variables (history of measles, rheumatoid arthritis, mononucleosis, whooping cough and a positive family history of cancer in a first-degree relative), age group, province of residence	Same controls and data collection methods as McDuffie et al. (2001) Strengths: population-based study; large number of cases; detailed questionnaires on pesticide exposure information; did not use surrogates Limitations: diversity in exposure situations (crops and animals) but no distinction in analysis; self-reported questionnaire; low response from potential controls (48%)

AHS, Agricultural Health Study; CI, confidence interval; LOD, limit of detection; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; SMR, standardized mortality ratio; STS, soft tissue sarcoma; yr, year(s)

these crop insecticides before 1965 (OR, 1.3; 95% CI, 0.8–2.1).

A further analysis (Schroeder et al., 2001) included the same cases and controls as those in the study by Cantor et al. (1992), but investigated subtypes of NHL, t(14;18)-positive or t(14;18)-negative. Because subtype was missing for more than 70% of study cases, an expectation–maximization algorithm was used to impute missing values. Adjusted odds ratios and 95% confidence intervals for various agricultural risk factors and t(14;18)-positive and -negative cases of NHL were estimated based on polytomous logistic regression models. Aldrin use was not significantly associated with t(14;18)-positive NHL or with t(14;18)-negative NHL.

[The Working Group noted that the strengths of this study were that it was a large population-based study with in-person interview on detailed farming and pesticide-use history, there were adequate numbers of exposed cases; and it was possible to differentiate exposures to aldrin and dieldrin. The limitations were the self-reported exposure and the fact that NHL subtypes were missing for more than 70% of the cases.]

A study by De Roos et al. (2003) pooled data from three case–control studies (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) in the midwest USA to examine pesticide exposures in farming as risk factors for NHL in men. Newly diagnosed NHL cases among white men aged ≥ 30 years in Iowa and Minnesota from 1980 to 1983 and aged ≥ 21 years in eastern Nebraska counties from 1983 to 1986, and a random sample of cases among white men aged ≥ 21 years diagnosed between 1979 and 1981 in Kansas were identified. The Minnesota and Iowa portions of this study overlapped with the population studies by Cantor et al. (1992). Population-based controls were randomly selected from the same geographical areas as the cases, frequency-matched to cases by race, sex, age, and vital status at the time of interview via various sources. Interviews were conducted to obtain pesticide uses and other known or suspected risk factors for NHL. Subjects with a missing or "don't know" response for any of the 47 pesticides of interest (about 25% of subjects) were excluded from analyses, resulting in 650 cases and 1933 controls available in the regression analyses. There was a significantly decreased risk of NHL associated with aldrin use (OR, 0.5; 95% CI, 0.3–0.9). Analysis by hierarchical regression gave similar results. [The Working Group noted that this was a large study, which used adjustment for multiple pesticides with hierarchical logistic regression. The limitations were the lack of univariate analyses of single pesticides, and the exclusion of subjects with any missing data. The Working Group noted a difference between the results of this pooled analysis and those of the original analysis by Cantor et al. (1992), which included all subjects and did not adjust for use of other pesticides.]

A further analysis investigated whether asthma modifies the risk of NHL associated with pesticide exposure (Lee et al., 2004). This study included men from Iowa and Minnesota and men and women from Nebraska, and excluded subjects without asthma information (n = 25), leaving 872 cases and 2336 controls for analysis. Odds ratios were adjusted for age, state, and vital status. The risk of NHL was non-significantly elevated with exposure to aldrin (OR, 2.1; 95% CI, 0.9–5.1) in asthmatics compared with non-farmers without asthma. No increase in risk was reported for non-asthmatics. [The Working Group noted the very small numbers of subjects with asthma and aldrin use, resulting in wide confidence intervals.]

The Cross-Canada Study of Pesticides and Health was a population-based case–control study in male residents in six Canadian provinces (McDuffie et al., 2001). Incident cases with first diagnosis of NHL between 1991 and 1994 and randomly selected, age-matched controls were sent postal questionnaires, with follow-up telephone interviews to obtain details of pesticide

use for subjects who reported pesticide exposure of 10 hours per year or more, plus 15% random samples with lower exposure. The results were based on 517 NHL cases (10 exposed to aldrin) and 1506 controls who responded to the postal questionnaires. NHL was significantly associated with reported exposure to aldrin (OR, 3.81; 95% CI, 1.34–10.79) with adjustment for age and province of residence. NHL risk associated with aldrin use increased to 4.19 (95% CI, 1.48–11.96) when statistically significant medical variables were also adjusted. In additional multivariate models with independent predictors, which included histories of measles, previous cancer, first-degree relatives with cancer and allergy desensitization, as well as exposure to mecoprop, aldrin was significantly associated with increased risk of NHL (OR, 3.42; 95% CI, 1.18–9.95). [The Working Group noted that the strengths of the study were the use of postal questionnaire followed by telephone interviews to obtain details of pesticide use, the fact that surrogates were not used, and that many pesticides/chemicals were analysed and many covariates considered. However, there was limited precision for aldrin, and a low response rate from potential controls (48%).]

(b) Leukaemia

One study investigated leukaemia and aldrin exposure (Brown et al., 1990). This population-based case–control interview study included 578 newly diagnosed leukaemia cases among white men and 1245 controls from Iowa and Minnesota, part of the midwest studies by the United States National Cancer Institute (NCI) (Cantor et al., 1992). Additional interviews to obtain number of days of handling pesticides were completed for 86 cases and 203 controls from Iowa who reported agricultural use of pesticides in the initial interview. Odds ratios relative to nonfarmers for 243 cases and 547 controls were adjusted for multiple risk factors. The odds ratio for subjects who had ever personally handled, mixed, or applied aldrin was 0.9 (95% CI, 0.6–1.4). Odds ratios for leukaemia by the number of days per year that aldrin was reportedly handled showed a decreasing dose–response trend. [The Working Group noted that this was a large population-based study with in-person and follow-up phone interviews in farming areas. A limitation was that more surrogates were interviewed for cases (73%) than for controls (28%) in follow-up.]

(c) Soft tissue sarcoma

The association between soft tissue sarcoma (STS) and aldrin exposure was investigated in the previously described Cross-Canada Study of Pesticides and Health (McDuffie et al., 2001). Details of the study methods are given above. The results for STS were based on 357 cases and 1506 controls who responded to the postal questionnaires (Pahwa et al., 2011). STS was associated with reported exposure to aldrin (OR, 3.71; 95% CI, 1.00–13.76; 4 exposed cases) in multivariate models. In additional multivariate models with independent predictors, which included histories of whooping cough and first-degree relatives with cancer as well as exposure to diazinon, the odds ratio for aldrin was 3.35 (95% CI, 0.89–12.56). [The Working Group noted the very small number of exposed cases, resulting in poor precision.]

(d) Cancer of the breast

A hospital-based case–control study recruited residents of two provinces of Spain in 1996–1998 (Ibarluzea et al., 2004). Cases were women undergoing surgery for breast cancer and controls were women undergoing non-cancer-related surgery (gall bladder surgery, 65%). Of 260 eligible cases and 352 controls, 198 (76%) cases and 260 (74%) controls consented and provided adequate adipose tissue samples and interviews. Dieldrin and aldrin were measured using gas chromatography in adipose tissue: more than 40% of subjects had measurable levels of aldrin, while less than 40% had measurable dieldrin. After

adjusting for a range of potential confounders, a positive association was seen between breast cancer and aldrin levels above the LOD (OR, 1.55; 95% CI, 1.0–2.4) and this relationship was stronger in postmenopausal women (OR, 1.84; 95% CI, 1.06–3.18). [The Working Group noted that the strengths of this study were the biomarker assessment of exposure in adipose tissue and adjustment for a range of potential confounders. The Working Group considered that the finding that the concentration of aldrin was higher than that of dieldrin was surprising, given that aldrin should not have been in active use at the time the study was conducted.]

2.2 Dieldrin

2.2.1 Cohort studies

See Table 2.3.

(a) Occupational cohort studies

Workers at an insecticide plant in the Netherlands were exposed to dieldrin and aldrin. The study methods and results are described in Section 2.1.1 because data were reported for both pesticides combined.

Exposure to dieldrin was specifically investigated in the AHS and the methods are presented in Section 2.1.1. For exposure to dieldrin, there were no increases in risk for all cancers, or for cancer of the colon or rectum (Purdue et al., 2007), for total prostate cancer (Koutros et al., 2013a), for NHL (RR, 0.9; 95% CI, 0.6–1.2), or any NHL subtype, including multiple myeloma (Alavanja et al., 2014). Risks were non-significantly increased for leukaemia (RR, 1.7; 95% CI, 0.8–3.6) and melanoma (RR, 1.4; 95% CI, 0.7–2.9) (Purdue et al., 2007), aggressive prostate cancer (RR, 1.39; 95% CI, 0.65–2.94) (Koutros et al., 2013a), and bladder cancer (RR, 1.19; 95% CI, 0.82–1.72) (Koutros et al., 2016). Lifetime days of dieldrin use showed a positive association with incidence of lung cancer in the highest exposure category (hazard ratio, HR, 1.93; 95% CI, 0.70–5.30). The results were very similar for either a 5- or 15-year lag. Additionally, the results using intensity-weighted lifetime days of dieldrin use showed a similar increase of 2-fold in the highest exposure category (HR, 2.06; 95% CI, 0.95–4.43) (Bonner et al., 2017). Risk of cancer of the breast in wives of the pesticide licensees was increased for husband's use of dieldrin (RR, 2.0; 95% CI, 1.1–3.3) (Engel et al., 2005). [The Working Group noted that the strengths of this study were that it was large, and there was adjustment for other pesticides and potential confounding factors, there was an extensive exposure assessment effort, and the study was able to separate exposures to aldrin, and other pesticides. The limitations were the small numbers for some analyses, especially in early publications.]

(b) Population cohort studies

(i) Non-Hodgkin lymphoma

From 25 802 adults in Washington County, Maryland, USA, who enrolled in 1974 in the Campaign Against Cancer and Stroke (CLUE I) study, 74 incident NHL cases with serum samples available and 147 matched controls were included in a nested case–control study (Cantor et al., 2003). The medians of lipid-corrected serum concentrations of dieldrin (which may reflect exposure to aldrin and/or dieldrin) were 129.9 and 116.9 ng/g lipid for cases and controls, respectively (Wilcoxon signed rank test, $P = 0.26$). Odds ratios showed no evidence of an association between NHL risk and quartiles of serum dieldrin (adjusted OR, 0.9; 95% CI, 0.4–2.4 in the highest versus the lowest quartile, P for trend, 0.88). [The Working Group noted that the strengths of the study included the collection of biological samples, and matching and/or adjustment for potential confounders; however, serum aldrin was not considered but may contribute to serum dieldrin.]

Table 2.3 Cohort studies of cancer and exposure to dieldrin

Reference, location enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Høyer et al. (1998) Denmark Enrolled, 1976, follow-up to 1993 Nested case–control	Cases: 240; all women who developed breast cancer, with enough serum sample Controls: 477; random selection of 2 women matched for age, date of examination, vital status and breast cancer status of case Exposure assessment method: biomarker; gas chromatography	Breast	Dieldrin Q2 Q3 Q4 Trend-test *P*-value: 0.01	 57 66 73	 1.58 (0.93–2.67) 1.96 (1.14–3.39) 2.05 (1.17–3.57)	Age, number of full-term pregnancies, weight	Strengths: serum taken before diagnosis; adequate sample size
Ward et al. (2000) Norway Sera collected 1973–1991, follow-up to 1993 Nested case–control	Cases: 150; random selection from 272 incident breast cancer cases where sera was taken 2+ yr before diagnosis Controls: 150; matched to cases by date of sample and date of birth, alive and free of cancer at time of case diagnosis Exposure assessment method: personal monitoring; gas chromatography	Breast	Dieldrin > LOD	 NR	 1.0 (0.4–2.6)	Age, time of sample collection	Strengths: nested case–control so exposure measured before diagnosis Limitations: very few aldrin-exposed subjects; only 22 discordant pairs for dieldrin; not clear if any confounding factors were added to model

Table 2.3 (continued)

Reference, location enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Høyer et al. (2001) Denmark Enrolled, 1976–78, follow-up to 1993 Nested case–control	Cases: 161 Controls: 318 Exposure assessment method: personal monitoring; gas chromatography	Breast: estrogen receptor-positive	Dieldrin in quartiles (ng/mL)			Age, number of full-term pregnancies, weight, HRT	Strengths: serum taken before diagnosis; adequate sample size
			Q2, 12.01–28.30	28	1.3 (0.7–2.2)		
			Q3, 28.30–57.11	33	1.5 (0.8–2.7)		
			Q4, > 57.11	28	1.4 (0.8–2.5)		
			Trend-test *P*-value: > 0.20				
		Breast: estrogen receptor-negative	Dieldrin in quartiles (ng/mL)			Age, number of full-term pregnancies, weight, HRT	
			Q2, 12.01–28.30	5	1.2 (0.3–5.4)		
			Q3, 28.30–57.11	13	4.9 (0.9–28.3)		
			Q4, > 57.11	20	7.6 (1.3–46.1)		
			Trend-test *P*-value: 0.01				
Høyer et al. (2002) Denmark Enrolled, 1976–78, follow-up to 1993 Nested case–control	Cases: 240 Controls: 477 Exposure assessment method: personal monitoring; gas chromatography	Breast: wildtype p53	Dieldrin			Age, number of full-term pregnancies, weight, HRT	Strengths: serum taken before diagnosis; adequate sample size
			Q2	28	1.0 (0.49–2.04)		
			Q3	31	1.15 (0.53–2.47)		
			Q4	35	1.2 (0.56–2.58)		
			Trend-test *P*-value: 0.6				
		Breast: p53 mutation	Dieldrin			Age, number of full-term pregnancies, weight, HRT	
			Q2	7	2.07 (0.48–8.88)		
			Q3	13	4.57 (0.94–22.24)		
			Q4	12	3.53 (0.79–15.79)		
			Trend-test *P*-value: 0.12				

Table 2.3 (continued)

Reference, location enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cantor et al. (2003) Maryland, USA 1974 enrolment in the Campaign Against Cancer and Stroke (CLUE I) and 1989 CLUE II Nested case–control	Cases: 74; Washington County Cancer Registry from CLUE I or II cohort Controls: 147; matched to case on race, sex, date of birth, CLUE I or II or private census between 1963–75, date of blood sample, location of stored serum Exposure assessment method: total lipid corrected serum values	NHL	ng/g lipid 26.6–84.2 85.3–116.7 116.9–153.8 163.0–393.9 Trend-test *P*-value: 0.88	 18 15 17 24	 1.0 1.0 (0.4–2.7) 1.2 (0.4–3) 0.9 (0.4–2.4)	Years of education, ever smoked cigarettes, currently smoking cigarettes, EBV early antigen seropositivity, quartile of PCB concentration	Strengths: most cases confirmed from pathology information; serum collected pre-diagnosis. matched and/or adjusted for potential confounders Limitations: larger than expected levels obtained for some compounds such as PCB and DDT may imply that there was some measurement error. aldrin was not reported
Engel et al. (2005) Iowa and North Carolina, USA 1993–2000 Cohort	30 454; AH; wives of pesticide licensees Exposure assessment method: questionnaire	Breast: incidence in farmers' wives	Dieldrin, husband's use Premenopausal, husband's use Postmenopausal, husband's use	16 NR 12	2.0 (1.1–3.3) – 1.6 (0.9–3)	Age, state, race	Strengths: large numbers, individual pesticide use Limitations: self-reported data
Purdue et al. (2007) Iowa and North Carolina, USA Recruited, 1993–1997, follow-up, 2002 Cohort	51 011; AHS, pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days (take into account factors affecting exposure)	All cancers combined: incidence Lung: incidence Colon: incidence Rectum: incidence Malignant melanoma: incidence Leukaemia: incidence	Dieldrin	257 21 16 11 10 10	1.0 (0.8–1.1) 1.1 (0.6–1.8) 0.7 (0.4–1.3) 1.1 (0.5–2.4) 1.4 (0.7–2.9) 1.7 (0.8–3.6)	Age, state, sex, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	Strengths: large numbers, individual pesticide use Limitations: self-reported data

Table 2.3 (continued)

Reference, location enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2013a) Iowa and North Carolina, USA 1993–2007 Cohort	54 412; AHS; pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days and intensity-weighted exposure days	Prostate (total): incidence	Dieldrin, quartile of exposure			Age, state, smoking status, race, family history of prostate cancer, fruit servings, leisure time physical activity in winter	Strengths: large numbers, individual pesticide use
			Q1	19	0.94 (0.60–1.49)		
			Q2	19	0.86 (0.54–1.36)		
			Q3	18	0.93 (0.58–1.49)		
			Trend-test *P*-value: 0.68				
		Prostate: aggressive (incidence)	Dieldrin, quartile of exposure				
			Unexposed	429	1.00		
			Q1	8	0.83 (0.41–1.68)		
			Q2	7	2.00 (0.94–4.23)		
			Q3	8	0.68 (0.33–1.37)		
			Q4	7	1.39 (0.65–2.94)		
			Trend-test *P*-value: 0.54				
		Prostate: family history of prostate cancer	Dieldrin, tertile of exposure			Age, state, smoking status, race, fruit servings, leisure time physical activity in winter	
			Unexposed	4	1.00		
			T2	5	1.55 (0.63–3.82)		
			T3	5	1.54 (0.62–3.83)		
			Trend-test *P*-value: 0.29				
Alavanja et al. (2014) Iowa and North Carolina, USA Recruited 1993–1997, follow-up 2011 Cohort	54 306; AHS; pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days (take into account factors affecting exposure)	NHL: incidence	Dieldrin	35	0.9 (0.6–1.2)	Age, state, sex, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	Strengths: large numbers; individual pesticide use
		MM: incidence	Dieldrin	10	0.9 (0.5–1.4)		

Table 2.3 (continued)

Reference, location enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2016) Iowa and North Carolina, USA Recruited, 1993–1997, follow-up 2011 Cohort	57 310; AHS; male pesticide licensees Exposure assessment method: questionnaire; lifetime exposure days, and intensity-weighted exposure days	Urinary bladder: incidence	Dieldrin	32	1.19 (0.82–1.72)	Age, state, sex, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	Strengths: large numbers; individual pesticide use Limitations: self-reported data; men only
Bonner et al. (2017) Iowa and North Carolina, USA Enrolment, 1993–1997 and follow-up 31 December 2011 Cohort	57 310; AHS; included 57 310 restricted-use pesticides applicators residing in Iowa and North Carolina between 1993 and 1997 Exposure assessment method: questionnaire; information about lifetime pesticide use was ascertained at enrolment (1993–1997) and updated with a follow-up questionnaire (1999–2005)	Lung	Lifetime days of use (exposure tertile)			Age, smoking status and pack-years, sex, total lifetime pesticide use	Strengths: large population of pesticide applicators; initial and follow-up questionnaire; controlled for smoking and other potential confounders Limitations: about 40% of applicators did not complete the follow-up interview so missing pesticide needed to be imputed/estimated
			Non-exposed	230	1.00		
			Dieldrin T1	6	0.58 (0.26–1.31)		
			Dieldrin T2	6	1.49 (0.66–3.37)		
			Dieldrin T3	4	1.93 (0.70–5.3)		
			Trend-test *P*-value: 0.472				
		Lung	Intensity-weighted lifetime days of use (exposure tertile)			Age, smoking status and pack-years, sex, total lifetime pesticide use	
			Non-exposed	230	1.00		
			Dieldrin T1	5	1.01 (0.42–2.47)		
			Dieldrin T2	4	0.5 (0.18–1.34)		
			Dieldrin T3	7	2.06 (0.95–4.43)		
			Trend-test *P*-value: 0.880				
		Lung	15-year lagged lifetime days pesticide exposure (tertile)			Age, smoking status and pack-years, sex, total lifetime pesticide use	
			Non-exposed	230	1		
			Dieldrin T1	6	0.59 (0.26–1.32)		
			Dieldrin T2	6	1.44 (0.64–3.26)		
			Dieldrin T3	4	2.09 (0.76–5.75)		
			Trend-test *P*-value: 0.468				

AHS, Agricultural Health Study; CI, confidence interval; CLUE, Campaign Against Cancer and Stroke; DDT, dichlorodiphenyltrichloroethane; EBV, Epstein–Barr virus; HRT, hormone-replacement therapy; LOD, limit of detection; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; PCB, polychlorinated biphenyl; SMR, standardized mortality ratio; STS, soft tissue sarcoma

(ii) Cancer of the breast

A case–control study on cancer of the breast was nested within the Copenhagen City Heart Study (Høyer et al., 1998). In 1976, 7712 of 10 317 participating women agreed to provide demographic information and a serum sample. The cohort was matched with the Danish Cancer Registry and 240 incident cases of breast cancer to 1993 with sufficient serum for analysis were included, while 477 controls were matched for age and vital status. There was an increase in risk of cancer of the breast with increasing quartile of dieldrin exposure (*P* for trend, 0.01); the odds ratio for the highest quartile was 2.05 (95% CI, 1.17–3.57). An analysis stratified by estrogen-receptor (ER) status was reported in a later publication (Høyer et al., 2001). Serum dieldrin was associated with ER-negative tumours (OR, 7.6; 95% CI, 1.3–46.1 for the highest quartile of exposure; *P* for trend, 0.01). There was no association with ER-positive tumours. A further analysis by *p53* (*TP53*) status (wildtype vs mutation) and found no statistically significant associations or trend with increasing serum dieldrin, although odds ratios for the three highest quartiles were raised for cases with mutant *p53* (OR for highest quartile, 3.53; 95% CI, 0.79–15.79; *P* for trend, 0.12) (Høyer et al., 2002). [The Working Group noted that the strengths of this study included that serum was taken before diagnosis of breast cancer, and that there were controls for multiple confounders.]

In the case–control study nested in the Norwegian Janus cohort of serum donors described above (Ward et al., 2000), there were 11 discordant case–control pairs with serum dieldrin levels (which may reflect exposure to aldrin and/or dieldrin) above the LOD. The matched odds ratio for dieldrin was 1.0 (95% CI, 0.4–2.6). [The Working Group considered that this was a reasonably high-quality study on dieldrin, with serum taken before diagnosis and control for multiple confounders; however, there were relatively small numbers of exposed cases.]

2.2.2 Case–control studies

See Table 2.4.

Several case–control studies that reported results for dieldrin also presented data for aldrin. The methods for these studies are described in detail in Section 2.1.2 and only the findings for dieldrin are presented here. In some other studies, exposures to dieldrin were assessed, but no risk estimates were reported (Cocco et al., 2008), or data for dieldrin were reported only as part of a broader grouping of pesticides (McDuffie et al., 2001; Pahwa et al., 2011). These studies were considered uninformative for dieldrin and are not considered further in this section. A cross-sectional study based on the United States NHANES survey of associations of self-reported cancer of the breast and prostate with serum dieldrin levels was also considered uninformative (Xu et al., 2010).

(a) Non-Hodgkin lymphoma

In the previously described study of NHL in Iowa and Minnesota, USA, by Cantor et al. (1992), a non-significant elevation in risk was observed among subjects who had ever personally handled, mixed, or applied dieldrin (OR, 1.4; 95% CI, 0.7–2.8). The risks were higher for those who had handled dieldrin for crop use before 1965 (OR, 1.9; 95% CI, 0.8–4.4). Additionally, elevated risk was found for dieldrin (OR, 2.2; 95% CI, 1.0–4.9; 13 cases) when pre-1965 use on either animals or crops was considered. In the subanalysis investigating subtypes of NHL (Schroeder et al., 2001), dieldrin was associated with t(14;18)-positive NHL (OR, 3.7; 95% CI, 1.9–7.0; 7 cases), but not with t(14;18)-negative NHL. [The Working Group noted that this was a large population-based study with in-person interviews on detailed farming and pesticide-use history, but the number of dieldrin uses was

Table 2.4 Case–control studies on cancer and exposure to dieldrin

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown et al. (1990) Iowa and Minnesota, USA 1980–1984	Cases: 578; tumour registry and hospital records Controls: 1245; matched to case by 5-year age group, vital status and state via random-digit dialling, Medicare records or state death certificates Exposure assessment method: questionnaire; detailed questions with days per year for each pesticide	Leukaemia: newly diagnosed cases	Ever handled dieldrin Ever handled dieldrin	8	0.8 (0.4–2.0)	Vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, high-risk exposures	USA midwest studies Cases and controls residing in cities with little farming activity (i.e. Minneapolis, St Paul, Duluth, and Rochester) were excluded from the study Strengths: large population-based study in farming areas; in-person interviews; detailed questionnaires including quantification; collection of other potential risk factors; reviewed diagnosis Limitations: multiple comparisons; self-report of pesticide use and limited numbers of aldrin and dieldrin use
Cantor et al. (1992) Iowa and Minnesota, USA 1980–1983	Cases: 622; health registry and hospital and pathology records Controls: 1245; matched to cases by age, vital status and state via random-digit dialling, Medicare record or state death certificate files Exposure assessment method: questionnaire; in-person interview	NHL: newly diagnosed cases divided into four subtypes: follicular, diffuse, small lymphocytic, and "other NHL"	Dieldrin ever handled Dieldrin ever handled before 1965	17 10	1.4 (0.7–2.8) 1.9 (0.8–4.4)	Vital status, age, state, smoking, family history of lympho-haematopoietic cancer, high-risk occupation, high-risk exposures	Data subsequently pooled in De Roos et al. (2003) Strengths: large population-based study in farming areas Limitations: not controlled for exposure to other pesticides; white men only

Table 2.4 (continued)

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schroeder et al. (2001) Iowa and Minnesota, USA 1980–1983	Cases: 622; state health registry and hospital/ pathology laboratory records Controls: 1245; matched to cases by age, state and vital status via random-digit dialling, Medicare records or state death certificate files Exposure assessment method: questionnaire; in-person structured interviews	NHL: t(14;18)-positive or t(14;18)-negative cases	Ever use of dieldrin Dieldrin: t(14;18)-positive NHL vs controls	7	3.7 (1.9–7)	Age, state	Same study population as Cantor et al. (1992) Strengths: large population-based study in farming areas Limitations: relative small numbers of t(14;18)-positive or -negative NH; the study looked at NHL subtypes but > 70% of the cases had missing subtypes; small numbers of cases with aldrin or dieldrin exposure
Gammon et al. (2002) Long Island, New York, USA, 1996–1997	Cases: 1508; pathologically diagnosed breast cancer Controls: 1556; frequency-matched to cases by 5-year age group; identified by random-digit dialling Exposure assessment method: personal monitoring; dieldrin in serum	Breast	Dieldrin (ng/g lipid) 14.97–20.90 20.91–26.67 26.68–33.45 33.46–179.29	 38 32 22 46	 1.19 (0.59–2.41) 0.91 (0.45–1.84) 0.64 (0.3–1.35) 1.37 (0.69–2.72)	Age, race	Strengths: serum measures, adjusted for multiple potential confounders Limitations: small numbers (10% of original number of participants); includes in situ breast cancer; blood taken after diagnosis

Table 2.4 (continued)

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Clary & Ritz (2003) California, USA 1989–1996	Cases: 950; death tape files within three counties (Fresno, Kern, and Tulare) Controls: 9435; all non-cancer deaths that occurred during the same time period in the same three counties Exposure assessment method: residential pesticide measure from the California Department of Pesticide Regulation PUR database	Pancreas (ICD-9, 157)	Dieldrin (tonnage) Highest vs lower three quartiles Highest vs lower three quartiles, ≥ 20 yr residence	 114 98	 1.38 (0.90–2.11) 1.52 (0.94–2.46)	Sex, age, year of death, years of living in county, urban residence, race, education	Strengths: examined all pesticides individually, simultaneously and in various combinations of pesticide subgroups Limitations: mortality and pesticide data without individual measurement or questionnaire conducted; residence duration before death may not represent exposure duration
De Roos et al. (2003) Nebraska, Iowa and Minnesota, Kansas, USA 1979–1986	Cases: 650; Nebraska Lymphoma Study Group, hospitals, state health registry, cancer registry Controls: 1933; matched to cases by race, sex, age, region and vital status via random-digital dialling, Medicare records or state mortality files Exposure assessment method: questionnaire; 47 pesticides	NHL: newly diagnosed cases	Dieldrin – ever use Dieldrin (logistic regression) Dieldrin (hierarchical regression)	 21 21	 1.8 (0.8–3.9) 1.4 (0.8–2.6)	Age, study site, all other pesticides	USA midwest studies (pooled) from three previous case–control studies (Zahm, Cantor, Hoar) analysis restricted to potentially carcinogenic pesticides

Table 2.4 (continued)

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ritchie et al. (2003) Iowa, USA 2000–2001 Case–control	Cases: 58; two clinics in Iowa Controls: 99; physical examination and annual check-ups from the university hospital; frequency-matched by age in 5-year increments to cases Exposure assessment method: blood samples collected and analysed questionnaire along with chemical checklist; medical history form for all study participants	Prostate: ICD-O 61.9	Dieldrin (μg/g) Non-detectable 0.006–0.024 > 0.024	 41 12 5	 1.00 0.97 (0.40–2.36) 0.28 (0.09–0.88)	Age, BMI, history of prostatitis	Organochlorine levels were analysed using both the unadjusted and lipid-adjusted serum values Strengths: collected blood samples; questionnaire included demographic and risk characteristics; a medical history for all study participants Limitations: small sample size
Lee et al. (2004) Iowa, Minnesota, Nebraska, USA 1980–86	Cases: 872; State Health Registry, hospitals and Nebraska Lymphoma Study Group Controls: 2336; matched to case on age, race and state via random-digit dialling, Medicare records or mortality files Exposure assessment method: questionnaire; telephone or personal interviews with subjects or next of kin in Nebraska	NHL	Ever use of dieldrin Among asthmatics Dieldrin among non-asthmatics	 5 30	 4.2 (0.98–18.2) 1.2 (0.7–1.9)	Age, vital status, state	Strengths: pooled study so larger numbers; same population as De Roos et al. (2003) Limitations: use of proxy respondents may have led to nondifferential misclassification; no adjustment for co-exposures

Table 2.4 (continued)

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Quintana et al. (2004) USA 1969–1983 Case–control	Cases: 175; cases without missing lipid-adjusted pesticide exposure data in EPA NHATS data Controls: 481; controls with a diagnosis of accidental injury (or death) or myocardial infarction, matched on age, sex, geographical region and race Exposure assessment method: human adipose tissue samples collected during surgery and post mortem	NHL	Dieldrin exposure (ppm; µg/g lipid) < 0.09 0.09–0.15 0.15–0.24 > 0.24 Trend-test *P*-value: 0.0002	 37 37 38 63	 1.0 1.24 (0.71–2.17) 1.56 (0.88–2.74) 2.70 (1.58–4.61)	Year of sample collection	Strengths: relatively large study size with biological samples collected; analyses with dieldrin as well as other compounds were conducted Limitations: two sources used for sample collection and mostly post mortem (> 90% from cadavers); control selection also from two groups; no information on lifestyle factors; essentially a cross-sectional study
De Roos et al. (2005) Iowa, Los Angeles County, Detroit and Seattle, USA 1998–2000	Cases: 100; SEER registry Controls: 100; matched to cases by age, sex and race via random-digit dialling and Medicare records Exposure assessment method: personal interview and laboratory measurements of organochlorines including aldrin and dieldrin from blood samples	NHL: untreated, newly diagnosed cases	Dieldrin, quartiles (ng/g lipid) ≤ 8.1 > 8.1–10.9 > 10.9–14.3 > 14.3 Continuous (per 10 ng/g lipid) Trend-test *P*-value: 0.82	 31 14 8 25 78	 1.0 0.5 (0.17–1.5) 0.31 (0.1–0.96) 0.76 (0.31–1.88) 0.98 (0.71–1.37)	Sex, study site, birth date, date of blood draw	Strengths: laboratory measurements included aldrin and dieldrin exposures; multiple imputation approach for values below detection limits; potential confounders were considered in analyses with quartiles and continuous exposure Limitations: small numbers of cases and controls relative to the original study; all measurements for some pesticides including aldrin were below LOD

BMI, body mass index; CI, confidence interval; EPA, Environmental Protection Agency; LOD, limit of detection; NHATS, National Human Adipose Tissue Survey; NHL, non-Hodgkin lymphoma; NR, not reported; PUR, Pesticide Use Reporting Database; SMR, standardized mortality ratio; vs, versus

limited. An attempt was made to investigate NHL subtypes, but subtype was missing for more than 70% of the cases.]

A pooled analysis of studies in the midwest USA (De Roos et al., 2003), including the previous study by Cantor et al. (1992), found an elevated risk of NHL associated with dieldrin use, although the effect estimate was not statistically significant (RR, 1.8; 95% CI, 0.8–3.9, with conventional logistic regression; and RR, 1.4; 95% CI, 0.8–2.6, with hierarchical regression). In a further analysis investigating whether asthma modifies the risk of NHL associated with pesticide exposure, risk was non-significantly elevated with exposure to dieldrin (OR, 4.2; 95% CI, 0.98–18.2; 5 exposed cases) in asthmatics compared with non-farmers without asthma (Lee et al., 2004). [The Working Group noted that this was a pooled analysis, which used hierarchical regressions to control for multiple pesticide exposures. A large number (n = 47) of insecticides and herbicides was included in regression modelling, but there was no analysis for single pesticides. Subjects with any missing exposure data were excluded. There were a very small number of subjects with asthma and dieldrin use, resulting in wide confidence intervals.]

In the population-based case–control study of NHL in four different areas of the USA (the state of Iowa, Los Angeles county, and metropolitan areas of Detroit and Seattle) (De Roos et al., 2005), 100 untreated, newly diagnosed NHL cases aged 20–74 years identified between 1998 and 2000 with adequate plasma volume were randomly selected with 100 controls matched by birth date, date of blood draw, sex, and study site. Concentrations of organochlorines including aldrin and dieldrin were measured in blood samples obtained before treatment, but no sample contained aldrin at above detection limits. Plasma dieldrin was not associated with risk of NHL in analyses of quartiles or continuous exposure (OR, 0.98 per 10 ng/g lipid; 95% CI, 0.71–1.37). [The Working Group noted that study strengths included use of a conditional logistic regression analysis with consideration of potential confounders. Analyses of quartiles and of continuous exposure were conducted, although no significant associations were observed. The main study limitation was that biological samples were obtained after diagnosis. The Working Group further noted that measurements of dieldrin may additionally reflect exposure to aldrin.]

Another study used cases and controls from a data set originally collected in the United States EPA National Human Adipose Tissue Survey (NHATS) to examine the relationship between NHL and exposure to organochlorine pesticides (Quintana et al., 2004). Adipose tissue samples from more than 20 000 people were collected during surgery or post mortem between 1969 and 1983 in selected cities in the USA, and lipid-adjusted pesticide exposures were estimated. Cases (n = 175) were those with a diagnosis of NHL. Controls (n = 481) were subjects with a diagnosis of accidental injury or myocardial infarction matched on age, sex, geographical region, and race. Virtually all samples from cases and controls were obtained from cadavers. Dieldrin levels were significantly associated with increased risk of NHL among cases in the quartile of highest exposure (OR, 2.70; 95% CI, 1.58–4.61, with adjustment for year of sample collection; P for trend, 0.0002). Serum dieldrin levels showed moderate correlation with exposure to other compounds. When heptachlor epoxide was included in the model, the odds ratio for the highest quartile of dieldrin exposure was attenuated, while adjustment for β-hexachlorocyclohexane (β-HCH), and *para*,*para*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE) did not have a notable effect. [The Working Group noted that the strengths of this study were that it was relatively large, and some biological samples were collected when dieldrin was in active use, with most measurements being above the LOD. The limitations were that it was essentially a

cross-sectional study, and two sources were used for sample collection, mostly post mortem.]

(b) Leukaemia

In the previously cited population-based case–control study in Iowa and Minnesota, USA, a lower risk of leukaemia was observed among subjects who had ever personally handled, mixed, or applied dieldrin (OR, 0.8; 95% CI, 0.4–2.0; 8 exposed cases) (Brown et al., 1990). [The Working Group noted that this was a large population-based study in farming areas. A limited number of subjects using dieldrin were included.]

(c) Cancer of the prostate

A pilot study compared serum levels of organochlorines in cases and controls (Ritchie et al., 2003). Cases (n = 58) were pathologically confirmed, newly diagnosed patients with cancer of the prostate from two clinics in Iowa, USA. Controls (n = 99) were men seen for routine examinations at a university hospital, frequency-matched by age to cases. Polychlorinated biphenyls and 18 organochlorine pesticides were measured in serum. Dieldrin (which may reflect exposure to aldrin and/or dieldrin) was detected in serum from 29.3% of cases and 38.4% of controls (P = 0.25). There was no apparent trend in the regression analysis of association between prostate cancer and dieldrin concentrations, and subjects with the highest levels of dieldrin appeared to have a reduced risk of cancer of the prostate compared with those with non-detectable dieldrin levels (P = 0.13). [The Working Group noted that blood samples were collected after diagnosis; the questionnaire included demographic and risk characteristics. The study was hospital-based, with a small sample size.]

(d) Cancer of the pancreas

A case–control study of mortality from cancer of the pancreas and long-term residential exposure to pesticides used computerized death tape files (1989–1996) and pesticide-use reporting records (1972–1989) from three counties in California (Clary & Ritz, 2003). Between 1989 and 1996, 950 deaths from cancer of the pancreas were identified and 9435 non-cancer deaths were randomly selected as controls within the same time period in these counties. Exposure was assigned based on information on duration of residency and pesticide-use reporting data on pesticide use with date and location of application for 18 chlorinated organic compounds, including dieldrin. Odds ratios and 95% confidence intervals for mortality from cancer of the pancreas were estimated for the quartile of highest pesticide usage at the postal code level (≥ 75%) in comparison with all other quartiles (< 75%). A non-significantly elevated risk of pancreatic cancer was observed for potential dieldrin exposure for all cases (OR, 1.38; 95% CI, 0.90–2.11) and after restricting to subjects with ≥ 20 years of residency (OR, 1.52; 95% CI, 0.94–2.46) in analyses mutually adjusted for the 18 measured pesticides. Single pesticide models (not adjusted for multiple pesticides) did not suggest increases in risk associated with exposure to dieldrin. [The Working Group noted that this study examined all pesticides individually, simultaneously, and in various combinations of pesticide subgroups. Death certificate data only were used. The exposure assessment was ecological.]

(e) Cancer of the breast

A case–control study on cancer of the breast recruited residents of Long Island, New York, USA, in 1996 and 1997 (Gammon et al., 2002). Cases were pathologically diagnosed incident cases of cancer of the breast (both invasive and in situ) and controls were selected by random-digit dialling and frequency-matched to the cases by

5-year age group. Blood samples were available for 646 cases and 429 controls; serum samples for 181 cases and 148 controls contained dieldrin measured at the time of recruitment (i.e. after diagnosis for cases). Geometric mean levels of dieldrin were 20.4 ng/g lipid in cases and 21.3 ng/g lipid in controls. There was a non- significant positive association between dieldrin serum level and breast cancer after adjustment for age and race (OR, 1.37; 95% CI, 0.69–2.72, for the highest compared with the lowest quintile of dieldrin concentration). [The Working Group noted that dieldrin measured in serum may reflect exposure to aldrin and/or dieldrin. A study limitation is that serum dieldrin levels were assessed at the time of breast cancer diagnosis.]

2.3 Exposure assessment in epidemiological studies of aldrin and dieldrin

2.3.1 Exposure questionnaires and interviews

Individual exposure to aldrin and dieldrin has been assessed in epidemiological studies using several different methods. The simplest method, commonly used in case–control studies and also used in some cohort studies, used retrospective interviews or questionnaires to ascertain past use of aldrin, dieldrin, and other pesticides (Brown et al., 1990; Cantor et al., 1992; McDuffie et al., 2001; Schroeder et al., 2001; De Roos et al., 2003; Lee et al., 2004; Engel et al., 2005; Purdue et al., 2007; Pahwa et al., 2011; Koutros et al., 2013a, b, 2016; Alavanja et al., 2014). Such studies may also elicit information on the duration, timing, and frequency of use, specific tasks performed with pesticides, or numbers of animals and crops treated (e.g. Brown et al., 1990; Purdue et al., 2007). At least one study asked about use of pesticides in hobbies or home gardening, as well as farming (McDuffie et al., 2001). It has been argued that workers in stable careers can reliably report on past production methods and frequent chemical use (Friesen et al., 2015; IARC, 2017). For example, orchardists in one study showed good consistency in recalling commonly used pesticides and pesticide categories for repeated exposure questionnaires after 21–25 years; however, long-term recall of specific pesticides can be poor (Engel et al., 2001). In retrospective questionnaires, the types and timing of pesticide use are potentially subject to recall bias (differential accuracy of recall for cases versus controls), particularly if cancers have already occurred when study participants or next-of-kin proxies are interviewed (Nam et al., 2005).

The AHS, a prospective cohort study, collected information on use of specific pesticides from participants before follow-up for health outcomes. Exposure questionnaires collecting information on active ingredients, decades of use, application methods, and use of personal protective equipment were administered both at baseline and after 5 years of follow-up of a cohort (Flower et al., 2004; Koutros et al., 2013a, b; Alavanja et al., 2014; Koutros et al., 2016), rather than after cancer cases had been identified. These studies were unlikely to be affected by recall bias. On the basis of participant responses, the intensity of pesticide use was estimated and combined with information reported on frequency and duration of use to obtain cumulative exposure of each participant to each active ingredient (Dosemeci et al., 2002).

2.3.2 Employment records

An alternative approach was used in a study of workers in a pesticide-production plant in the USA, in which work records such as start dates for work areas and production units, payroll classifications, and job titles were used to classify workers into production unit categories such as “operations” or “maintenance” (Amoateng-Adjepong et al., 1995). [The Working Group noted that although not subject to recall bias, these are crude surrogates for exposure to aldrin

and dieldrin, because a wide variety of chemicals were used and manufactured at the plant.]

2.3.3 Exposure biomarkers

In some studies, pesticide-use questionnaires or work records were supplemented or replaced by measurements of dieldrin and aldrin in the blood (Cantor et al., 2003; Ritchie et al., 2003; De Roos et al., 2005; Cocco et al., 2008; van Amelsvoort et al., 2009), or in adipose tissue (Quintana et al., 2004). Most aldrin is rapidly converted to dieldrin in humans (ATSDR, 2002), so measurements of dieldrin in blood and adipose samples may reflect exposure to aldrin and/or dieldrin. It is unclear whether aldrin measurements in the blood and adipose reflect only recent exposures or long-term storage of unmetabolized aldrin. Most dieldrin in the body is associated with lipids, so biomarker concentrations are typically reported as "lipid-adjusted" values (mass of dieldrin per unit mass of lipids). The mean apparent half-life of dieldrin in humans has been reported as 266–369 days (ATSDR, 2002), so dieldrin concentrations in blood may reflect exposure to aldrin and/or dieldrin in recent years, as well as any dieldrin mobilized from longer-term storage in adipose tissue.

In case–control studies, biomarker measurements were obtained after determination of case status and used as surrogates for past exposure. Such temporal misalignment induces some degree of exposure measurement error, with a larger degree of measurement error with shorter biological half-lives, larger exposure variability, or longer exposure durations (Bartell et al., 2004). Biomarkers may be affected by reverse causation if case status is associated with altered storage, metabolism, or excretion of a toxicant. For example, concentrations of organochlorines increase in plasma and adipose after weight loss (Baris et al., 2000; Pelletier et al., 2003), which results in differential exposure measurement error if cases experienced more weight loss than controls (or vice versa). This may be a concern for interpretation of studies in which cases experienced weight loss before sample collection as a result of illness, chemotherapy, or radiation therapy (De Roos et al., 2005).

In the study by De Roos, serum samples were collected from untreated cases of NHL and matched controls in the USA during 1998–2000. Of the dieldrin measurements, 19% were below the LOD, and an additional 22.5% were unreportable due to interference. The median LOD was 6.5 ng/g lipid and the median serum concentration was 10.9 ng/g lipid. Eighteen quality-control pairs were available for which both measurements were above the LOD; these had an average intrabatch coefficient of variation of 6.6% and an intraclass correlation coefficient of 0.98 (De Roos et al., 2005).

Cocco et al. (2008) measured a variety of polychlorinated biphenyls and organochlorine pesticides in serum samples obtained from NHL cases and controls in France, Germany, and Spain. Among these, 54% of dieldrin measurements were below the LOD in Spain, and 100% were below the LOD in France and Germany. Poor intraclass correlation (< 0.5) was reported for duplicate samples, possibly due to low sample volumes (1 mL).

Ritchie et al. (2003) measured 31 toxicants (including dieldrin) in serum samples in a pilot case–control study of cancer of the prostate in the USA. Serum was collected from newly diagnosed cases and controls in 2000–2001; 71% of cases and 62% of controls had serum dieldrin concentrations that were below the LOD.

In a study by Quintana and colleagues, samples of adipose tissue were collected from a nested case–control study of cadavers and surgery patients in the USA National Human Adipose Tissue Survey (NHATS) from 1969 to 1983. About 14% of NHL cases were excluded due to missing lipid-adjusted pesticide concentrations or low lipid content in the adipose samples. Fewer than 2% of the remaining samples contained

dieldrin at less than the LOD. Median adipose dieldrin concentrations were 180 ng/g lipid and 150 ng/g lipid for cases and controls, respectively (Quintana et al., 2004).

Several studies used stored blood samples to conduct cohort-based studies using prediagnostic biomarkers (Ward et al., 2000; Høyer et al., 2001; Gammon et al., 2002; Cantor et al., 2003; van Amelsvoort et al., 2009). The case–cohort study by Cantor and colleagues used a cohort with stored serum samples collected in the USA in 1974, identifying incident NHL cases from 1975–1994. Median serum dieldrin concentrations were 129.9 ng/g lipid for cases and 116.9 ng/g lipid for controls. Intraset and interset coefficients of variation for serum dieldrin were 0.22 and 0.30, respectively. The few values below the LOD were retained (Cantor et al., 2003). [The Working Group noted that although this design also had temporal misalignment of the exposure measurement and disease outcome, the resulting exposure measurement error was most likely to be non-differential due to the use of prediagnostic rather than postdiagnostic serum samples.]

The cohort study by van Amelsvoort and colleagues of workers at plants manufacturing aldrin and dieldrin in the Netherlands also used dieldrin concentration in prediagnostic blood samples (from 1963–1970) to assess exposure, and followed participants for cause-specific mortality until 2006 (van Amelsvoort et al., 2009). Aldrin and dieldrin exposures were substantially decreased for these workers after 1970 due to improved production processes. Blood samples were collected one to four times per year as part of routine biomonitoring at the plant; repeated dieldrin measurements were available for 60% of participants. The study used a one-compartment pharmacokinetic model and a piecewise constant-exposure model to estimate total intake of aldrin and dieldrin for each worker over time, imputing missing values based on measurements in workers with the same job and work dates (de Jong, 1991). [The Working Group considered that this exposure assessment was of relatively high quality because of the use of repeated prediagnostic biomarkers sampled during the years of peak exposure.]

2.3.4 *Pesticide-use reporting and residential locations*

Clary & Ritz (2003) used a different approach to exposure assessment for their epidemiological analysis, relying on geographical information systems and the California pesticide-use reporting database. They sorted 102 zip (postal) codes by relative commercial use of each of 18 organochlorine pesticides (including dieldrin) from 1972 to 1989, matching each study participant to a postal code using residential address at death. Duration of residency in county of residence was also available from death records. In California, reporting for commercial use of pesticides has been mandatory since the 1970s and recent data are highly resolved spatially and temporally, but earlier records were often incomplete and usage was likely underreported due to lack of enforcement (Clary & Ritz, 2003). [The Working Group noted that it was unclear to what extent dieldrin use by zip (postal) code is a reasonable surrogate for personal exposure.]

3. Cancer in Experimental Animals

3.1 Aldrin

See Table 3.1.

3.1.1 *Mouse*

Oral administration

A group of 215 young male and female C3HeB/Fe mice [age, numbers, and sex were not reported; mice were divided approximately equally by sex] were fed diets containing aldrin [purity not reported] at a concentration of

10 ppm for up to 2 years (Davis & Fitzhugh, 1962). The control group consisted of 217 male and female mice. Treated mice died 2 months earlier than controls: the average survival time in treated mice was 51.8 weeks compared with 59.8 weeks for the controls. Survival at 18 months was decreased in treated mice (32/215; 15%) compared with the control group (47/217; 22%). All survivors at 2 years were killed and autopsied. Pneumonia and intestinal parasitism probably contributed to the decreased survival of the mice. It was reported that caging of mice in groups of 5–8 contributed to the spread of disease within groups. Partial re-evaluation by Reuber and others of the available histopathology data from Davis & Fitzhugh (1962) and from Davis (1965) indicated that most tumours initially classified by Davis & Fitzhugh (1962) as "hepatic cell adenoma" were actually hepatocellular carcinomas (Epstein, 1975; Reuber, 1975, 1976a). A statistically significant increase in the incidence of "hepatic cell adenoma" [hepatocellular carcinoma] was noted in treated mice when compared with the control group. On average, treated mice developed "hepatic cell adenomas" [hepatocellular carcinomas] after 80 weeks on study compared with 89 weeks on study for control mice. [The Working Group noted that the limitations of this study included low survival rate, combination of data for both sexes, lack of detailed histopathology, reports of disease, pneumonia, and intestinal parasitism, and the disposal of a large number of animals at autopsy. The Working Group considered that the re-evaluation by Epstein (1975) was accurate, but limited by the number of cases reviewed.]

In a subsequent study, groups of 100 male and 100 female C3H mice were fed diets containing aldrin [purity not reported] at a concentration of 0 or 10 ppm for up to 2 years (Davis, 1965, reported in Epstein, 1975). The number of survivors at 104 weeks was 64 and 31 for control and treated mice, respectively. Whereas the reported number of hepatic carcinomas [hepatocellular carcinomas] was about the same, the incidence (for both sexes combined) of "benign hepatomas" [hepatocellular carcinomas] in the treated group (10 ppm) was significantly elevated, being approximately double that of controls, (Epstein, 1975). An independent partial re-evaluation of the Davis & Fitzhugh (1962) and Davis (1965) by Reuber and others concluded that most of the "benign hepatomas" were hepatocellular carcinomas. This re-evaluation indicated significant increases in the incidence of hepatocellular carcinoma in males and females in the treated group compared with the control groups (Epstein, 1975; Reuber, 1976a). Morphological descriptions of the liver lesions were reported by Reuber (1975) and Reuber (1976a). There were often two hepatocellular carcinomas present at the same time in treated animals, while solitary hepatocellular carcinomas were reported in the control animals (Reuber, 1976a). In addition, transplantation studies were conducted in which hepatocellular carcinomas were transplanted into mice [sex not reported] with a similar genetic background. Nine out of ten tumours from mice fed diets containing aldrin at 10 ppm grew when transplanted and histologically resembled the primary tumours (Reuber, 1976b). [The Working Group noted that the limitations of this study included the combination of data for both sexes, lack of detailed histopathology, and the absence of report on the number of animals evaluated for histopathology. The Working Group considered that the re-evaluation by Epstein (1975) was accurate, but limited by the number of cases reviewed.]

In a study by the NCI, groups of 50 male and 50 female $B6C3F_1$ mice were fed diets containing aldrin (technical grade; purity, 95% [impurities unspecified]) at a concentration of 4 or 8 ppm (time-weighted exposure) for males, and 3 or 6 ppm (time-weighted exposure) for females, for 80 weeks, and then held untreated for an additional 10–13 weeks (NTP, 1978a). The matched-control group consisted of 20 males

Table 3.1 Studies of carcinogenicity in experimental animals exposed to aldrin

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, C3HeB/Fe (M+F combined) NR 2 yr Davis & Fitzhugh (1962)	Oral Aldrin, purity NR Diet 0, 10 ppm, ad libitum 217, 215 47, 32 (at 18 mo)	*Liver* Hepatic cell adenoma [hepatocellular carcinoma]: 9/134 (7%), 35/151* (23%)	*$P < 0.001$, statistical test NR	Principal strengths: adequate duration Principal limitations: low survival rate; data combined for sexes; lack of detailed histopathology; disease, pneumonia and intestinal parasitism reported; large numbers of animals discarded at autopsy Partial re-evaluation of hepatic lesions of the combined studies by Davis & Fitzhugh (1962) and Davis (1965) by Reuber and others reported in Epstein (1975) (Table 3) – most tumours classified as hepatic cell adenomas were re-evaluated as hepatocellular carcinomas. Total hepatocellular carcinomas reported in Reuber (1976a): control male – 22/73, 30%; aldrin male – 75/91 [$P < 0.0001$], 82%; control female – 2/53, 4%; aldrin female – 72/85, 85% [$P < 0.0001$]
Full carcinogenicity Mouse, C3H (M+F combined) NR 2 yr Davis (1965)	Oral Aldrin, NR Diet 0, 10 ppm, ad libitum 200, 200 64, 31 (at 104 wk)	*Liver* Benign hepatoma [hepatocellular carcinoma]: 27/200, 65/200* Hepatic carcinoma [hepatocellular carcinoma]: 4/200, 3/200	*[$P < 0.0001$] [NS]	Principal strengths: adequate duration Principal limitations: data combined for sexes, lack of detailed histopathology, number of animals evaluated for histopathology not reported Davis (1965) is reported in Epstein (1975). Data presented in Table 2 of Epstein (1975). Partial re-evaluation of hepatic lesions of the Davis & Fitzhugh (1962) and Davis (1965) combined studies by Reuber and others reported in Epstein (1975) (Table 3) – most tumours classified as benign hepatomas were re-evaluated as hepatocellular carcinomas. Total hepatocellular carcinomas reported in Reuber (1976a): control male – 22/73, 30%; aldrin male – 75/91, 82% [$P < 0.0001$]; control female – 2/53, 4%; aldrin female – 72/85, 85% [$P < 0.0001$]

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 35 days 90–93 wk NTP (1978a)	Oral Aldrin, 95% (technical grade; impurities, NR) Diet 0, 0, 4 (TWA), 8 (TWA) ppm, ad libitum; treated for 80 wk then control diet for 10–13 wk 20, 92, 50, 50 NR	*Liver* Hepatocellular carcinoma: 3/20* (matched control), 17/92** (pooled control), 16/49***, 25/45***,****	*P = 0.001 by the Cochran-Armitage trend test **P < 0.001 by the Cochran-Armitage trend test ***P = 0.048 by the Fisher exact test vs pooled control group for intermediate-dose group, and P < 0.001 for high-dose group ****P = 0.002 by the Fisher exact test vs matched control group	Principal strengths: adequate duration; studies in M and F; complete histopathology
Full carcinogenicity Mouse, B6C3F$_1$ (F) 35 days 90–91 wk NTP (1978a)	Oral Aldrin, 95% (technical grade; impurities, NR) Diet 0, 0, 3 (TWA), 6 (TWA) ppm, ad libitum; treated for 80 wk then control diet for 10–11 wk 10, 79, 50, 50 NR	*Liver* Hepatocellular carcinoma: 0/10, 3/78, 5/48, 2/43	NS	Principal strengths: adequate duration; studies in M and F; complete histopathology

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Osborne-Mendel (M+F combined) 3 wk 104 wk Fitzhugh et al. (1964)	Oral Aldrin, ≥ 99% Diet 0, 0.5, 2, 10, 50, 100, 150 ppm, ad libitum 24, 24, 24, 24, 24, 24, 24 50%, 50%, 50%, 42%, 25%, 17%[a], 4%[b]	All sites combined 3/17, 10/19*, 7/19, 8/22, 5/18, 5/11, 1/9	*[$P = 0.041$, Fisher's exact test; increase]	Principal limitations: data combined for sexes; only 68% of animals treated with aldrin (or dieldrin) were examined histologically Survival was significantly decreased in M and F (combined) exposed to 100 or 150 ppm at 24 mo ([a]$P \leq 0.01$ or [b]$P \leq 0.05$, respectively). Tumours reported as "pulmonary lymphosarcoma," "fibroadenoma of breast," "carcinoma of breast," "lymphoid except lung," "fibrosarcoma," and "other", were confirmed by independent re-evaluations by Reuber and others (Epstein, 1975). No liver tumours were initially reported, but a partial re-evaluation of the liver histopathology identified a total of 18 liver carcinomas in rats fed diets containing aldrin or dieldrin
Full carcinogenicity Rat, Osborne-Mendel (M) NR (weanling) 25 mo Deichmann et al. (1967)	Oral Aldrin, 95% (technical grade) Diet 0, 5 ppm, ad libitum 30, 30 50%, 66% (at 24 mo)	All tumours: 1/30, 2/30 Total tumours: 1, 2	[NS]	Principal limitation: only one dose group
Full carcinogenicity Rat, Osborne-Mendel (F) NR (weanling) 25 mo Deichmann et al. (1967)	Oral Aldrin, 95% (technical grade) Diet 0, 5 ppm, ad libitum 30, 30 60%, 63% (at 24 mo)	All tumours: 13/30, 13/30 Total tumours: 14, 13	[NS]	Principal limitation: only one dose group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Osborne-Mendel (M) NR (weanling) ≤ 31 mo Deichmann et al. (1970)	Oral Aldrin, 95% (technical grade) Diet 0, 20, 30, 50 ppm, ad libitum 100, 50, 50, 50 NR	All tumours: 19/75, 5/45, 7/46, 4/45 Total tumours: 46, 15, 15, 13	NS	Principal limitations: histopathology limited to examination of the lung, kidney, liver and all macroscopic changes Doses during the first 10 wk (0, 10, 15, 25 ppm) were half the final concentrations. Maximum survival of controls was only 27 mo. Tumour incidence: total number of rats with tumours/number of rats examined histologically
Full carcinogenicity Rat, Osborne-Mendel (F) NR (weanling) > 27–< 31 mo Deichmann et al. (1970)	Oral Aldrin, 95% (technical grade) Diet 0, 20, 30, 50 ppm, ad libitum 100, 50, 50, 50 NR	All tumours 60/88, 20/47, 24/44, 11/31 Total tumours: 104, 26, 28, 16	NS	Principal limitations: histopathology limited to examination of the lung, kidney, liver and all macroscopic changes Doses during the first 10 wk (0, 10, 15, 25 ppm) were half the final concentrations. Maximum survival of controls was only 27 mo. Tumour incidence: total number of rats with tumours/number of rats examined histologically
Full carcinogenicity Rat, Osborne-Mendel (M) 35 days 111–112 wk NTP (1978a)	Oral Aldrin, 95% (technical grade; impurities NR) Diet 0, 0, 30, 60 ppm, ad libitum; treated for 74 wk then control diet for 37–38 wk 10, 58, 50, 50 NR	*Thyroid* Follicular cell adenoma or carcinoma (combined): 3/7 (matched control), 4/48 (pooled control), 14/38*, 8/38 Follicular cell carcinoma: 0/7 (matched control), NR (pooled control), 4/38, 2/38 *Pancreas* Islet cell adenoma or carcinoma (combined): 0/9 (matched control), 1/52 (pooled control), 5/37*, 2/39	*$P = 0.002$ by the Fisher exact test vs pooled control group NS *$P = 0.043$ by the Fisher exact test vs pooled control group	Principal strengths: adequate duration; covered most of the life span; studies in M and F; complete histopathology

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Osborne-Mendel (F) 35 days 112–113 wk NTP (1978a)	Oral Aldrin, 95% (technical grade; impurities NR) Diet 0, 0, 30, 60 ppm, ad libitum; treated for 80 wk then control diet for 32–33 wk 10, 60, 50, 50 NR	*Adrenal gland* Cortical adenoma: 0/10, 0/55, 8/45*, 1/48 *Thyroid* Follicular cell adenoma or carcinoma (combined): 1/9, 3/52, 10/39*, 7/46 Follicular cell carcinoma: 0/9 (matched control), NR (pooled control), 2/39, 4/46	*$P = 0.002$ by the Fisher exact test vs pooled control group *$P = 0.009$ by the Fisher exact test vs pooled control group NS	Principal strengths: adequate duration; covered most of the life span; studies in both M and F; complete histopathology

F, female; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; TWA, time-weighted average; wk, week(s); yr, year(s)

and 10 females, and the study duration was 90–93 weeks. Time-weighted doses were used to assess the results, because the concentration of aldrin was reduced after study start due to toxicity. Because the number of matched-control mice was small, pooled controls were also used for statistical comparisons. The pooled-control groups consisted of the matched controls from the bioassay of aldrin combined with matched controls from contemporary bioassays with dieldrin, chlordane, heptachlor, dichlorvos, and dimethoate, giving groups of 92 male and 79 female mice. There was no significant effect on the survival of male mice. There was a significant ($P = 0.037$) dose-related trend in the mortality of female mice, primarily due to the early deaths in the groups at the higher dose. Mean body weights of males and females were similar to those of the controls.

In comparisons with the matched or pooled controls, the incidence of hepatocellular carcinoma was significantly increased at 4 and 8 ppm in males, with a significant positive trend. The incidence of hepatocellular carcinoma in the treated groups was above the mean for incidence in historical controls (44/285, 16.8%). There were no other significant increases in tumour incidence compared with the matched or pooled controls. There was no significant increase in the incidence of tumours in female mice (NTP, 1978a).

3.1.2 Rat

A study in male and female Carworth rats fed diets containing aldrin (Treon & Cleveland, 1955; Cleveland, 1966; also reported in Epstein, 1975) was judged inadequate for the evaluation by the Working Group because of the lack of histopathological evaluation, difficulties in interpretation of the mortality data, limited reporting, and discrepancies between Treon & Cleveland (1955) and Cleveland (1966).

Groups of 12 male and 12 female Osborne-Mendel rats were fed diets containing aldrin (purity, not less than 99%) at a concentration of 0, 0.5, 2, 10, 50, 100, or 150 ppm for 2 years (Fitzhugh et al., 1964). Survival was significantly decreased in males and females (combined) at 100 or 150 ppm at 24 months. Mean body weights of males and females were similar to those of the controls. Six tumour categories were identified, including "pulmonary lymphosarcoma", "fibroadenoma of breast", "carcinoma of breast", "lymphoid except lung", "fibrosarcoma", and "other". Epstein (1975) reported that independent histopathological re-evaluations by Reuber and others confirmed these multiple site tumours. No benign or malignant liver tumours were initially reported by Fitzhugh et al. (1964), but a partial re-evaluation of the liver histopathology identified a total of 18 hepatocellular carcinomas in rats fed diets containing aldrin or dieldrin (Epstein, 1975). [The Working Group noted that the limitations of this study were that only 68% of the animals treated with aldrin (or dieldrin) were examined histologically, and that the data were combined for both sexes.]

Groups of 30 male and 30 female weanling Osborne-Mendel rats [age not reported] were fed diets containing aldrin (purity, 95%) at a concentration of 0 or 5 ppm for 25 months (Deichmann et al., 1967). Survival at 24 months was 50% and 66% for control and treated male rats, respectively, and 60% and 63% for control and treated female rats, respectively. Mean body weights were similar between control and treated groups. Tumour incidence (all sites) was not significantly increased in male or female rats relative to that in the respective control groups. [As a limitation of the study, the Working Group noted that only one dose concentration was used.]

Groups of 50 male and 50 female weanling Osborne-Mendel rats [age not reported] were fed diets containing aldrin (purity, 95%) at a concentration of 20, 30, or 50 ppm for 31 months (Deichmann et al., 1970). Control groups were

comprised of 100 males and 100 females. Doses during the first 10 weeks were half the final concentrations. Mean survival of the control male and female rats was 19.7 and 19.5 months, respectively. The survival rate was not affected in treated males, but the mean survival of female rats at 50 ppm (13.0 months) was significantly decreased relative to the control group. The maximum survival of control males and control females was 27 months. Mean body-weight gain was similar for treated and control groups. Tumour incidence (all sites) was not significantly increased in male or female rats relative to the respective control groups. No benign or malignant tumours of the liver were found in treated animals. [The Working Group noted that limitations of this study included that not all tissues were examined histologically.]

In a study by the NCI, groups of 50 male and 50 female Osborne-Mendel rats (age, 35 days) were fed diets containing aldrin (purity, 95% [impurities not reported]) at a concentration of 30 or 60 ppm (NTP, 1978a). Male rats were treated 74 weeks followed by 37–38 weeks of observation, and female rats were treated for 80 weeks followed by 32–33 weeks of observation. For matched controls (10 males and 10 females per group) the study duration was 111 weeks for males and 111–112 weeks for females. The pooled-control groups consisted of the matched controls from the bioassay of aldrin combined with matched controls from the contemporary bioassays of dieldrin, chlordane, heptachlor, dichlorvos and dimethoate, giving groups of 58 male and 60 female rats. There was no significant effect on the survival of males or females. Mean body weights of the treated male and female rats were lower than those of the controls during the second year of the study. The incidences of follicular cell adenoma or carcinoma (combined) of the thyroid gland increased in male and female Osborne-Mendel rats (NTP, 1978a). The increases were significant in groups at the lower dose, but not in the groups at the higher dose for males, or for females when compared with the pooled controls, but were not significant when compared with the matched controls. The incidence of follicular cell carcinoma of the thyroid gland was not increased significantly in males or females. A significant increase in the incidence of adenoma or carcinoma (combined) of pancreatic islet cells was observed in males at the lower dose, but not at the higher dose, when compared with the pooled control group. A significant increase in the incidence of cortical adenoma of the adrenal gland was also observed in females at the lower dose, but not at the higher dose when compared with the pooled control group. [The Working Group noted that these increases in tumour incidence were only for the groups at the lower dose, and only when compared with the pooled control group, and thus concluded that they were not treatment-related.]

3.2 Dieldrin

See Table 3.2.

Dieldrin was reviewed in *IARC Monographs* Volume 5 (IARC, 1974) and Supplement 7 (IARC, 1987). The previous *IARC Monographs* Working Group (IARC, 1987) concluded that there was *limited evidence* in experimental animals for the carcinogenicity of dieldrin. This section provides an evaluation of the animal carcinogenesis studies reviewed in previous *Monographs* and Supplement and a review of any studies published since the earlier reviews.

3.2.1 Mouse

(a) Dietary administration

In a study by Davis & Fitzhugh (1962), a group of 218 young [age not reported] male and female C3HeB/Fe mice [numbers and sex were not reported; mice were divided approximately equally by sex] were fed diets containing dieldrin [purity not reported] at a concentration of 10 ppm for up to 2 years. The control group

Table 3.2 Studies of carcinogenicity in experimental animals exposed to dieldrin

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, C3HeB/Fe (M+F combined) NR 2 yr Davis & Fitzhugh (1962)	Oral Dieldrin, NR Diet 0, 10 ppm, ad libitum 217, 218 47, 33 (at 18 mo)	*Liver* Hepatic cell adenoma [hepatocellular carcinoma]: 9/134, 36/148* (24%)	$P < 0.001$, statistical test NR	Principal strength: adequate duration Principal limitations: low survival rate; lack of detailed histopathology; disease, pneumonia and intestinal parasitism reported; large numbers of animals discarded at autopsy; data combined for both sexes Partial re-evaluation of hepatic lesions of the Davis & Fitzhugh (1962) and Davis (1965) combined studies by Reuber and others reported in Epstein (1975) (Table 3) – most tumours classified as hepatic cell adenomas were re-evaluated as hepatocellular carcinomas. Total hepatocellular carcinomas reported in Reuber (1976a): control male – 22/73, 30%; dieldrin male – 62/71 [$P < 0.0001$], 87%; control female – 2/53, 4%; dieldrin female – 62/71, 87% [$P < 0.0001$]
Full carcinogenicity Mouse, C3H (M+F combined) NR 2 yr Davis (1965)	Oral Dieldrin, NR Diet 0, 10 ppm, ad libitum 200, 200 64, 39 (at 104 wk)	*Liver* Benign hepatoma [hepatocellular carcinoma]: 27/200, 69/200* Hepatic carcinoma [hepatocellular carcinoma]: 4/200, 5/200	*[$P < 0.0001$] [NS]	Principal strength: adequate duration Principal limitations: lack of detailed histopathology; number of animals evaluated for histopathology not reported; data combined for both sexes Davis (1965) is reported in Epstein (1975). Data presented in Table 2 of Epstein (1975). Partial re-evaluation of hepatic lesions of the Davis & Fitzhugh (1962) and Davis (1965) combined studies by Reuber and others reported in Epstein (1975) (Table 3) – most tumours classified as benign hepatomas were hepatocellular carcinomas. Total hepatocellular carcinomas reported in Reuber (1976a): control male – 22/73 (30%); dieldrin male – 62/71 (87%) [$P < 0.0001$]; control female – 2/53 (4%); dieldrin female – 62/71 (87%) [$P < 0.0001$]

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J, C3H/He, B6C3F$_1$ (M) NR (weanling) ≤ 132 wk Meierhenry et al. (1983)	Oral Dieldrin, > 99% Diet C57BL/6J : 0, 10 ppm; C3H/He: 0, 10 ppm; B6C3F$_1$: 0, 10 ppm, ad libitum; dieldrin was given for 85 wk 69, 71, 50, 50, 76, 62 NR	*Liver* Benign hepatoma [benign hepatocellular tumour]: 10/69 (14%), 20/71* (28%), 9/50 (18%), 10/50 (20%), 3/76 (4%), 18/62* (29%) Hepatocellular carcinoma: 0/69, 21/71* (30%), 6/50 (12%), 19/50* (38%), 3/76 (4%), 26/62* (42%)	*$P < 0.01$ compared with control using the one-tailed test for the difference of proportions *$P < 0.01$ compared with control using the one-tailed test for the difference of proportions	Principal limitations: no data on survival or body weight
Full carcinogenicity Mouse, C3H/He (M) NR (weanling) 2 yr Ruebner et al. (1984)	Oral Dieldrin, 99% Diet 0, 10 (dieldrin "stopped"), 10 (dieldrin "continued") ppm, ad libitum 21, 12, 11 NR	*Liver* Hepatocellular adenoma: 6/21, 10/12*, 3/11	*[$P < 0.004$ by Fisher's exact test]	Principal limitations: small number of animals; limited exposure duration; number of animals at start not reported "stop-dieldrin" group: dieldrin fed until mice were aged 57 wk; "continue-dieldrin" group: dieldrin fed until mice were aged 67 wk
Full carcinogenicity Mouse, CF-1 (M) 4 weeks 132 wk Walker et al. (1973); Hunt et al. (1975)	Oral Dieldrin, > 99% Diet 0, 0.1, 1.0, 10 ppm, ad libitum 300, 125, 125, 200 NR	*Liver* Hepatocellular adenoma: 16%, 22%, 23%, 37% Hepatocellular carcinoma: 4%, 5%, 9%, 58% Hepatocellular adenoma and carcinoma (combined): 20%, 27%, 32%, 95% *Lung* Adenoma: 32%, 38%, 37%, 18% Carcinoma: 7%, 11%, 13%, 1%	NR	Principal limitations: neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Study 1 (Experiment 1); data reported in this table are from the values given in Hunt et al. (1975). Total liver tumours: type (a) (simple nodular growth of parenchymal cells) + type (b) (areas of papilliform and adenoid growth of tumour cells). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (F) 4 wk 132 wk Walker et al. (1973); Hunt et al. (1975)	Oral Dieldrin, > 99% Diet 0, 0.1, 1.0, 10 ppm, ad libitum 300, 125, 125, 200 NR	*Liver* Hepatocellular adenoma: 12%, 18%, 23%, 37% Hepatocellular carcinoma: 0%, 3%, 5%, 59% Hepatocellular adenoma and carcinoma (combined): 12%, 21%, 28%, 96% *Lung* Adenoma: 17%, 19%, 25%, 11% Carcinoma: 6%, 10%, 10%, 0%	NR	Principal limitations: neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Study 1 (Experiment 1); data reported in this table are from the values given in Hunt et al. (1975). Total liver tumours: type (a) (simple nodular growth of parenchymal cells) + type (b) (areas of papilliform and adenoid growth of tumour cells). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively
Full carcinogenicity Mouse, CF-1 (M) 4 wk 128 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 0, 1.25, 2.5, 5, 10, 20 ppm, ad libitum 78, 30, 30, 30, 30, 30 NR	*Liver* Hepatocellular adenoma: 12%, 13%, 40%, 77%, 36%, 18% Hepatocellular carcinoma: 0%, 7%, 3%, 10%, 9%, 53% Hepatocellular adenoma and carcinoma (combined): 12%, 20%, 43%, 87%, 45%, 71% *Lung* Adenoma: 58%, 57%, 37%, 47%, 18%, 6% Carcinoma: 1%, 3%, 3%, 3%, 0%, 0%	NR	Principal limitations: neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Study 2 – Experiment 2.1; animals received ethylene oxide-sterilized diet (standard procedure at that time). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (F) 4 wk 128 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 0, 1.25, 2.5, 5, 10, 20 ppm, ad libitum 78, 30, 30, 30, 30, 30 NR	*Liver* Hepatocellular adenoma: 10%, 17%, 39%, 43%, 41%, 24% Hepatocellular carcinoma: 0%, 0%, 4%, 17%, 12%, 14% Hepatocellular adenoma and carcinoma (combined): 10%, 17%, 43%, 60%, 53%, 38% *Lung* Adenoma: 31%, 23%, 11%, 10%, 6%, 0% Carcinoma: 10%, 0%, 0%, 3%, 0%, 0% *Ovary* Tumour, NOS: 26%, 40%, 14%, 10%, 6%, 0%	NR	Principal limitations: neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Other comments: Study 2 – Experiment 2.1; animals received ethylene oxide-sterilized diet (standard procedure at that time). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (M) 4 wk Unsterilized diet experiment (104 wk), γ-irradiated diet experiment (128 wk), γ-irradiated diet and bedding experiment (100 wk) Walker et al. (1973)	Oral Dieldrin, > 99% Diet Unsterilized diet: 0, 10 ppm; irradiated diet: 0, 10 ppm; irradiated diet and bedding: 0, 10 ppm, ad libitum 24, 24, 30, 30, 24, 24 NR	*Liver* Hepatocellular adenoma: 30%, 58%, 20%, 40%, 42%, 63% Hepatocellular carcinoma: 4%, 25%, 3%, 20%, 0%, 23% Hepatocellular adenoma and carcinoma (combined): 34%, 83%, 23%, 60%, 42%, 86% *Lung* Adenoma: 30%, 17%, 43%, 10%, 46%, 36% Carcinoma: 0%, 0%, 3%, 0%, 4%, 0% *Lymphoid tissue* Tumour, NOS: 13%, 4%, 37%, 10%, 13%, 5% *Kidney* Tumour, NOS: 4%, 4%, 13%, 0%, 4%, 9%	NR	Principal limitations: few dose groups; neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Study 2 – Experiment 2.2; diet sterilization. Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design **Species, strain (sex)** **Age at start** **Duration** **Reference**	**Route** **Agent tested, purity** **Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**Incidence of tumours**	**Significance**	**Comments**
Full carcinogenicity Mouse, CF-1 (F) 4 wk Unsterilized diet experiment (104 wk), γ-irradiated diet experiment (128 wk), γ-irradiated diet and bedding experiment (100 wk) Walker et al. (1973)	Oral Dieldrin, > 99% Diet Unsterilized diet: 0, 10 ppm; irradiated diet: 0, 10 ppm; irradiated diet and bedding: 0, 10 ppm, ad libitum 24, 24, 30, 30, 24, 24 NR	*Liver* Hepatocellular adenoma: 23%, 36%, 11%, 32%, 17%, 42% Hepatocellular carcinoma: 0%, 23%, 0%, 11%, 0%, 21% Hepatocellular adenoma and carcinoma (combined): 23%, 59%, 11%, 43%, 17%, 63% *Lung* Adenoma: 32%, 9%, 18%, 0%, 17%, 29% Carcinoma: 0%, 0%, 0%, 0%, 13%, 0% *Ovary* Tumour, NOS 23%, 0%, 25%, 5%, 29%, 17% *Lymphoid tissue* Tumour, NOS: 32%, 5%, 32%, 0%, 2%, 21%	NR NR NR NR NR NR NR	Principal limitations: few dose groups; neoplasm incidences not reported; effective number of animals unclear; statistics cannot be calculated Study 2 – Experiment 2.2; diet sterilization. Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Co-carcinogenicity Mouse, CF-1 (M) 4 wk 112 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 0, 50 (DDT), 100 (DDT), 5 +50 (dieldrin+DDT) ppm, ad libitum 48, 32, 32, 32 NR	*Liver* Hepatocellular adenoma: 13%, 28%, 44%, 38% Hepatocellular carcinoma: 0%, 9%, 9%, 50% Hepatocellular adenoma and carcinoma (combined): 13%, 37%, 53%, 88% *Lung* Adenoma: 38%, 41%, 50%, 34% Carcinoma: 0%, 0%, 0%, 3% *Testes* Tumour, NOS: 0%, 0%, 6%, 3%	NR	Principal limitations: few dose groups.; neoplasm incidences not reported.; effective number of animals unclear; statistics cannot be calculated Study 2 – Experiment 2.3; DDT and dieldrin co-carcinogenicity. Animals received ethylene oxide-sterilized diet (standard procedure at that time). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively. As reported in Epstein (1975), Reuber re-evaluated the liver histopathology data and reported the following incidences for hepatocellular adenoma and carcinoma (combined): control, 0%; 50 ppm DDT, 6%; 5 ppm dieldrin+50 ppm DDT, 58%
Co-carcinogenicity Mouse, CF-1 (F) 4 wk 112 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 0, 50 (DDT), 100 (DDT), 5 +50 (dieldrin+DDT) ppm, ad libitum 48, 32, 32, 32 NR	*Liver* Hepatocellular adenoma: 17%, 43%, 63%, 28% Hepatocellular carcinoma 0%, 7%, 13%, 50% Hepatocellular adenoma and carcinoma (combined): 17%, 50%, 76%, 78% *Lung* Adenoma: 40%, 20%, 22%, 28% *Lung* Carcinoma: 6%, 17%, 3%, 6%	NR	Principal limitations: few dose groups; effective number of animals unclear; neoplasm incidences not reported; statistics cannot be calculated Study 2 – Experiment 2.3; DDT and dieldrin co-carcinogenicity. Animals received ethylene oxide-sterilized diet (standard procedure at that time). Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively. As reported in Epstein (1975), Reuber re-evaluated the liver histopathology data and reported the following incidences for hepatocellular adenoma and hepatocellular carcinoma (combined): control, 0%; 50 ppm DDT, 16%; 5 ppm dieldrin+50 ppm DDT, 94%

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (M) 4 wk 104 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 10 ppm fed for 0 wk, 2 wk, 4 wk, 8 wk, 16 wk, 32 wk, 64 wk, ad libitum 29, 29, 29, 29, 29, 29, 29 NR	*Liver* Hepatocellular adenoma: 2/18, 2/13, 0/10, 3/10, 4/11, 4/10, 6/13*	*[$P < 0.043$ by Fisher's exact test]	Study 2 – Experiment 2.4; limited exposure Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively
		Hepatocellular carcinoma: 0/18, 0/13, 1/10, 1/10, 0/11, 0/10, 7/13	[NS]	
		Hepatocellular adenoma and carcinoma (combined): 2/18, 2/13, 1/10, 4/10, 4/11, 4/10, 13/13*	*[$P < 0.0001$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	
		Lung Tumour, NOS: 8/18, 6/13, 3/10, 6/10, 6/11, 5/10, 7/13	[NS]	
		Spleen/lymphatic tissue Tumour, NOS: 0/18, 2/13, 1/10, 3/10, 0/11, 1/10, 2/13	[NS]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (F) 4 wk 104 wk Walker et al. (1973)	Oral Dieldrin, > 99% Diet 10 ppm fed for 0 wk, 2 wk, 4 wk, 8 wk, 16 wk, 32 wk, ad libitum 29, 29, 29, 29, 29, 29, 29 NR	*Liver* Hepatocellular adenoma: 1/16, 2/9, 3/12, 4/12, 3/8, 4/10, 6/9*	*[$P < 0.003$ by Fisher's exact test]	Study 2 – Experiment 2.4; limited exposure Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively
		Hepatocellular carcinoma: 0/16, 0/9, 1/12, 0/12, 0/8, 0/10, 2/9	[NS]	
		Hepatocellular adenoma and carcinoma (combined): 1/16, 2/9, 4/12, 4/12, 3/8, 4/10, 8/9*	*[$P < 0.0001$ by Fisher's exact test]; [$P = 0.004$ by Cochran-Armitage trend-test]	
		Lung Tumour, NOS: 4/16, 7/9*, 2/12, 6/12, 3/8, 4/10, 2/9	*[$P < 0.017$ by Fisher's exact test]	
		Ovary Tumour, NOS: 4/16, 3/9, 3/12, 4/12, 2/8, 3/10, 3/9	[NS]	
		Spleen/lymphatic tissue Tumour, NOS: 2/16, 1/9, 1/12, 2/12, 0/8, 3/10, 1/9	[NS]	
		Other tissues Tumour, NOS: 2/16, 2/9, 1/12, 0/12, 0/8, 0/10, 2/9	[NS]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (M) 4 wk 110 wk Thorpe & Walker (1973)	Oral Dieldrin, > 99% Diet 0, 10 ppm, ad libitum 45, 30 NR	*Liver* Hepatocellular adenoma and carcinoma (combined): 24%, 100%*	*$P < 0.01$ by a 2 × 2 contingency table	Principal limitations: few dose groups; neoplasm incidences not reported; statistics cannot be calculated Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively
Full carcinogenicity Mouse, CF-1 (F) 4 wk 110 wk Thorpe & Walker (1973)	Oral Dieldrin, > 99% Diet 0, 10 ppm, ad libitum 45, 30 NR	*Liver* Hepatocellular adenoma and carcinoma (combined): 23%, 93%*	*$P < 0.01$ by a 2 × 2 contingency table	Principal limitations: few dose groups; neoplasm incidences not reported; statistics cannot be calculated Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively
Full carcinogenicity Mouse, CF-1 (M) NR (weanling) ≤ 65 wk of age Tennekes et al. (1979)	Oral Dieldrin, NR Diet (SSD or CD) SSD/FPB, 0, 10; SSD/SB, 0, 10; CD/FPB, 0, 10; CD/SB, 0, 10 mg/kg diet, ad libitum 15, 15, 15, 12, 15, 16, 15, 16 NR	*Liver* Hepatocellular adenoma: 2/15 (13.3%), 7/15 (46.7%), 1/15 (6.7%), 10/12 (83.3%)*, 0/15, 9/16 (56.3%)**, 0/15, 7/16 (43.7%)*** Hepatocellular carcinoma: 0/15, 4/15 (26.7%), 0/15, 2/12 (16.7%), 0/15, 4/16 (25%), 0/15, 5/16 (31.3%)* Hepatocellular adenoma and carcinoma (combined): 2/15 (13.3%), 11/15 (73.3%)*, 1/15 (6.7%), 12/12**, 0/15, 13/16 (81.3%)**, 0/15, 12/16 (75.0%)**	*[$P < 0.0001$] **[$P \leq 0.0008$] ***[$P < 0.007$] *[$P < 0.05$] *[$P \leq 0.0025$] **[$P < 0.0001$]	Principal limitations: few dose groups; small number of animals per group SSD: semisynthetic diet; FPB: filter paper bedding; SB: sawdust bedding; CD: conventional diet. Liver neoplasms were diagnosed as type (a) and type (b). In current terminology, these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (M) NR (weanling) 110 wk Tennekes et al. (1981)	Oral Dieldrin, > 99% Diet (SSD or CD) SSD/FPB, 0, 10; SSD/SB, 0, 10; CD/FPB, 0, 10; CD/SB, 0, 10 mg/kg diet, ad libitum 55, 31, 47, 19, 68, 51, 82, 38 NR	*Liver* Hepatocellular adenoma: 2/55, 10/31*, 12/47, 13/19**, 4/68, 23/51***, 5/82, 13/38**** Hepatocellular carcinoma: 1/55, 11/31*, 0/47, 3/19**, 0/68, 21/51*, 1/82, 19/38* Hepatocellular adenoma and carcinoma (combined): 3/55 (5.5%), 21/31 (67.7%)*, 12/47 (25.5%), 16/19 (84.2%)*, 4/68 (5.9%), 44/51 (86.3%)*, 6/82 (7.3%), 32/38 (84.2%)*	*[$P = 0.0005$] **[$P \leq 0.0019$] ***[$P < 0.0001$] ****[$P = 0.0002$] *[$P < 0.0001$] **[$P = 0.0212$] *[$P < 0.0001$]	Principal limitation: few dose groups SSD: semi-synthetic diet; FPB: filter paper bedding; SB: sawdust bedding; CD: conventional diet. On average, only 10% of dieldrin-treated mice survived to 100 wk (controls, 40%). Lung metastases were observed in hepatocellular carcinoma-bearing treated mice
Full carcinogenicity Mouse, CF-1 (M) 4 wk ≤ 132 wk Tennekes et al. (1982)	Oral Dieldrin, > 99.9% NR 0, 0.1, 1, 10 ppm, ad libitum 289, 124, 111, 176 NR	*Liver* Hepatocellular carcinoma: 11/289, 6/124, 10/111*, 102/176** Hepatocellular adenoma and carcinoma (combined): 58/289, 33/124, 35/111*, 167/176**	*[$P < 0.046$ by Fisher's exact test]; **[$P < 0.0001$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test] *[$P < 0.018$ by Fisher's exact test]; **[$P < 0.0001$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	Re-evaluation of Walker et al. (1973). Publication includes data from two different long-term feeding studies: Experiment 1: dieldrin at 0, 0.1, 1, and 10 ppm; Experiment 2.1: dieldrin at 0, 2.5, 5, and 20 ppm. At 20 ppm, 5/17 males died from "acute intoxication" within the first 13 wk of treatment

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (F) 4 wk Up to 132 wk Tennekes et al. (1982)	Oral Dieldrin, > 99.9% NR 0, 0.1, 1, 10 ppm, ad libitum 297, 120, 117, 148 NR	*Liver* Hepatocellular carcinoma: 0/297, 4/120*, 6/117**, 88/148**	*[$P < 0.007$ by Fisher's exact test]; **[$P < 0.0001$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	Re-evaluation of Walker et al. (1973). Publication includes data from two different long-term feeding studies: Experiment 1: dieldrin at 0, 0.1, 1 and 10 ppm; Experiment 2.1: dieldrin at 0, 2.5, 5, 10, and 20 ppm. At 20 ppm, 11/21 females died from "acute intoxication" within the first 13 wk of treatment
		Hepatocellular adenoma and carcinoma (combined): 37/297, 25/120*, 33/117**, 142/148**	*[$P < 0.034$ by Fisher's exact test]; **[$P = 0.0002$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	
Full carcinogenicity Mouse, CF-1 (M) 4 wk Up to 132 wk Tennekes et al. (1982)	Oral Dieldrin, > 99.9% NR 0, 2.5, 5, 20 ppm, ad libitum 78, 30, 30, 17 NR	*Liver* Hepatocellular carcinoma: 0/78, 2/30, 3/30*, 9/17**	*[$P < 0.020$ by Fisher's exact test]; **[$P < 0.0001$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	Re-evaluation of Walker et al. (1973). Publication includes data from two different long-term feeding studies: Experiment 1: dieldrin at 0, 0.1, 1, and 10 ppm; Experiment 2.1: dieldrin at 0, 2.5, 5, and 20 ppm. At 20 ppm, 5/17 males died from "acute intoxication" within the first 13 wk of treatment
		Hepatocellular adenoma and carcinoma (combined): 9/78, 14/30*, 26/30*, 12/17*	*[$P < 0.0002$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, CF-1 (F) 4 wk Up to 132 wk Tennekes et al. (1982)	Oral Dieldrin, > 99.9% NR 0, 2.5, 5, 10, 20 ppm, ad libitum 78, 28, 30, 17, 21 NR	Hepatocellular carcinoma: 0/78, 2/28, 5/30*, 2/17**, 3/21***	*[$P < 0.0014$ by Fisher's exact test]; **[$P < 0.031$ by Fisher's exact test]; ***[$P < 0.009$ by Fisher's exact test] [$P = 0.013$ by Cochran-Armitage trend-test]	Re-evaluation of Walker et al. (1973). Publication includes data from two different long-term feeding studies: Experiment 1: dieldrin at 0, 0.1, 1, and 10 ppm; Experiment 2.1: dieldrin at 0, 2.5, 5, 10, and 20 ppm. At 20 ppm, 11/21 females died from "acute intoxication" within the first 13 wk of treatment
		Hepatocellular adenoma and carcinoma (combined): 8/78, 13/28*, 18/30*, 9/17*, 8/21**	*[$P < 0.0001$ by Fisher's exact test]; **[$P = 0.0002$ by Fisher's exact test]; [$P < 0.001$ by Cochran-Armitage trend-test]	
Full carcinogenicity Mouse, B6C3F$_1$ (M) 35 days 91–93 wk NTP (1978a)	Oral Dieldrin (technical grade), > 96% (impurities, NR) Diet 0, 0, 2.5, 5 ppm, ad libitum; mice treated for 80 wk, followed by observation periods of 11–13 wk 20, 92, 50, 50 NR	*Liver* Hepatocellular carcinoma: 3/18 (matched control), 17/92* (pooled control), 12/50, 16/45**	*$P = 0.020$; Cochran-Armitage trend test **$P = 0.025$; Fisher exact test vs pooled control group	Principal strengths: adequate duration; studies in M and F; complete histopathology

Table 3.2 (continued)

Study design **Species, strain (sex)** **Age at start** **Duration** **Reference**	**Route** **Agent tested, purity** **Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**Incidence of tumours**	**Significance**	**Comments**
Full carcinogenicity Mouse, B6C3F$_1$ (F) 35 days 90–93 wk NTP (1978a)	Oral Dieldrin (technical grade), > 96% (impurities NR) Diet 0, 0, 2.5, 5 ppm, ad libitum; mice treated for 80 wk, followed by observation periods of 10–13 wk 20, 79, 50, 50 NR	*Liver* Hepatocellular carcinoma: 0/20 (matched control), 3/78 (pooled control), 6/50, 2/49	 NS	Principal strengths: adequate duration; studies in M and F; complete histopathology
Full carcinogenicity Mouse, Balb/c (M) NR (young) 52 and 75 wk Lipsky et al. (1989)	Oral Dieldrin, NR Diet 0 (52 wk), 10 (52 wk), 0 (75 wk), 10 (75 wk) ppm 10, 10, [unclear], 20 NR	*Liver* Hepatocellular adenoma: Incidence: 0/10, 2/10, 2/36, 16/20* Total tumours: 0, 2, 7, 29 Hepatocellular carcinoma: Incidence: 0/10, 1/10, 1/36, 3/20 Total tumours: 0, 1, 1, 3 Hepatocellular adenoma or carcinoma (combined): 0/10, 2/10 (20%), 3/36 (8%), 16/20 (80%)*	 *[$P < 0.0001$] [NS] *[$P < 0.0001$]	Principal limitation: number of 75-wk control animals at start unclear
Full carcinogenicity Mouse, Swiss-Webster (M) NR (weanling) ≤ 30 mo Epstein (1975)	Oral Dieldrin, technical grade NR 0, 3, 10 ppm, ad libitum 125, 129, 130 NR	*Liver* Nodules: 0/93, 2/81 (2%), 32/91* (35%) Hepatoma: 0/93, 0/81, 0/91	 *[$P < 0.0001$ by Fisher's exact test] [$P < 0.001$ by Cochran-Armitage trend-test] NS	Re-evaluation of some of the histopathology data by Reuber and others concluded that more than half of the re-examined liver lesions from high-dose mice were hepatocellular carcinomas

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss-Webster (F) NR (weanling) ≤ 32 mo Epstein (1975)	Oral Dieldrin, technical grade NR 0, 3, 10 ppm, ad libitum 100, 100, 100 NR	*Liver* Nodules: 0/71, 2/78 (3%), 44/70* (63%) Hepatoma: 2/71, 0/78, 0/70	*[$P < 0.0001$ by Fisher's exact test] [$P < 0.001$ by Cochran-Armitage trend-test] NS	Re-evaluation of some of the histopathology data by Reuber and others concluded that more than half of the re-examined liver lesions from high-dose mice were hepatocellular carcinomas
Full carcinogenicity Mouse (C57BL/6J × C3HeB/FeJ)F_1 (M) 1 or 5 wk Up to 90 wk Vesselinovitch et al. (1979)	Gavage and/or oral Dieldrin, NR NR 0 µg (untreated control); 12.5 µg daily by gavage from age 1 to 5 wk; 10 ppm in the diet from age 5 to 90 wk; 12.5 µg daily by gavage from age 1 to 5 wk, then 10 ppm in the diet from age 5 to 90 wk NR NR	*Liver* Hepatocellular tumours: 1/58 (2%), 3/46 (7%), 7/60 (12%), 21/70 (30%)*	$P < 0.001$	Principal limitations: number of animals at start, NR; lack of vehicle control; no data on survival or body weight; purity, NR; statistical test, NR

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse (transgenic), FVB/N-TgMMTV/neu (offspring) (F) NA Offspring killed at 22 wk Cameron & Foster (2009)	Transplacental/lactation/gavage Dieldrin, NR Corn oil 0, 0.45, 2.25, 4.5 µg/g bw Dams: gavage for 5 days, 2 wk before mating, 1×/week throughout gestation and lactation until weaning (age 3 wk); offspring: 1×/week until age 9 wk NR 84, 79, 81, 19	*Mammary* Total tumours: Tumour multiplicity: 4.62, 4.82, 4.54, 7.58* Thoracic tumours: Tumour multiplicity: 3.81, 4.24, 3.82, 6.37* Inguinal tumours: Tumour multiplicity: 0.75, 1.00, 0.73, 1.21	*$P < 0.05$ as determined by one way ANOVA and appropriate post hoc test *$P < 0.05$ as determined by one way ANOVA and appropriate post hoc test NS	Tumours were primarily adenocarcinoma of the mammary gland. There was also an increased ($P < 0.05$) volume of thoracic mammary tumours: 49.12, 45.55, 18.28, and 77.30* mm^3, respectively. Preliminary evidence of increased incidence of ovarian and liver tumours in groups at the intermediate and highest dose
Full carcinogenicity Rat, Carworth Farm "E" (M) 5 wk 2 yr Walker et al. (1969)	Oral Dieldrin, > 99% Diet 0, 0.1, 1.0, 10 ppm, ad libitum 45, 25, 25, 25 NR	All tumours 12/43, 6/23, 5/23, 8/23 *Pituitary gland* Tumour: 2/43, 2/23, 1/23, 2/23 *Thyroid gland* Tumour: 3/43, 2/23, 2/23, 4/23	NS NS NS	Stevenson et al. (1976) re-evaluated the study and concluded again there was not a treatment-related increase in tumour incidence. Number of males with tumours was 12/43, 9/23, 5/23, and 9/23, respectively
Full carcinogenicity Rat, Carworth Farm "E" (F) 5 wk 2 yr Walker et al. (1969)	Oral Dieldrin, > 99% Diet 0, 0.1, 1.0, 10 ppm, ad libitum 45, 25, 25, 25 NR	*All tumours* 19/43, 15/23, 14/23, 12/23 *Pituitary gland* Tumour: 2/43, 1/23, 1/23, 2/23 *Thyroid gland* Tumour: 3/43, 6/23, 4/23, 3/23	NS NS NS	Stevenson et al. (1976) re-evaluated the study and concluded again there was not a treatment-related increase in tumour incidence. Number of females with tumours was 18/43,18/23,16/23,and 13/23, respectively

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Carworth Farm "E" (F) 5 wk 2 yr Walker et al. (1969) (cont.)		*Mammary gland* Tumour: 13/43, 11/23, 10/23, 8/23 *Other tumours* 3/43, 2/23, 4/23, 0/23	 NS NS	
Full carcinogenicity Rat, Osborne-Mendel (M+F combined) 3 wk 104 wk Fitzhugh et al. (1964)	Oral Dieldrin, 100% Diet 0, 0.5, 2, 10, 50, 100, 150 ppm, ad libitum 24, 24, 24, 24, 24, 24, 24 50%, 42%, 63%, 25%, 21%[a], 13%[b], 4%[a]	*All tumours* 3/17, 8/22, 8/23, 4/18, 4/20, 3/18, 0/11	NS	Principal limitations: data combined for sexes; only 68% of animals treated with dieldrin (or aldrin) were examined histologically Survival was significantly decreased in M and F (combined) at 50, 100 or 150 ppm at 24 mo ([a]$P \leq 0.05$, [b]$P \leq 0.01$). Tumours reported as "pulmonary lymphosarcoma," "fibroadenoma of breast," "carcinoma of breast," "lymphoid except lung," "fibrosarcoma," and "other were confirmed by" independent re-evaluations by Reuber and others (Epstein, 1975). No liver tumours were initially reported, but a partial re-evaluation of the liver histopathology identified a total of 18 liver carcinomas in rats fed diets containing dieldrin or aldrin
Full carcinogenicity Rat, Osborne-Mendel (M) NR (weanling) < 31 mo Deichmann et al. (1970)	Oral Dieldrin, 100% Diet 0, 20, 30, 50 ppm, ad libitum 100, 51, 50, 50 NR	*All tumours* 19/75, 4/48, 7/38, 1/44 Total tumours: 46, 10, 19, 1	NS	Doses during the first 10 wk were half the final concentrations. Maximum survival of controls was only 27 mo. Histopathology was re-evaluated in limited samples of the group receiving dieldrin at 30 ppm: as reported in Epstein (1975), the total number of rats (M and F combined) with malignant tumours was reported as 12.6% (10/79) in Deichmann et al. (1970), whereas a re-evaluation by Reuber reported 34.2% (26/76). Tumour incidence: total number of rats with tumours/number of rats examined histologically

Table 3.2 (continued)

Study design **Species, strain (sex)** **Age at start** **Duration** **Reference**	**Route** **Agent tested, purity** **Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**Incidence of tumours**	**Significance**	**Comments**
Full carcinogenicity Rat, Osborne-Mendel (F) NR (weanling) < 31 mo Deichmann et al. (1970)	Oral Dieldrin, 100% Diet 0, 20, 30, 50 ppm, ad libitum 100, 50, 48, 50 NR	*All tumours* 60/88, 23/48, 16/41, 16/41 Total tumours: 104, 33, 23, 23	NS	Doses during the first 10 wk were half the final concentrations. Maximum survival of controls was only 27 mo. Histopathology was re-evaluated in limited samples of the group receiving dieldrin at 30 ppm: as reported in Epstein (1975), the total number of rats (M and F combined) with malignant tumours was reported as 12.6% (10/79) in Deichmann et al. (1970), whereas a re-evaluation by Reuber reported 34.2% (26/76). Tumour incidence: total number of rats with tumours/number of rats examined histologically
Full carcinogenicity Rat, Osborne- Mendel (M) 35 days 110–111 wk NTP (1978a)	Oral Dieldrin (technical grade), > 96% (impurities NR) Diet Matched controls – 0, pooled controls – 0, 29 (TWA), 65 (TWA) ppm, ad libitum; low-dose rats treated for 80 wk, followed by observation periods of 30 wk; high-dose rats treated for 59 wk followed by observations periods of 52 wk 10, 58, 50, 50 NR	Any tumour type No significant increase	NS	Principal strengths: adequate duration; covered most of the life span; studies in M and F; complete histopathology

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Osborne- Mendel (F) 35 days 110–111 wk NTP (1978a)	Oral Dieldrin (technical grade), > 96% (impurities NR) Diet Matched controls – 0, pooled controls – 0, 29 (TWA), 65 (TWA) ppm, ad libitum; Low-dose rats treated for 80 wk, followed by observation periods of 30–31 wk; high-dose rats treated for 59 wk followed by observation periods of 51–52 wk 10, 60, 50, 50 NR	*Adrenal gland* Cortical adenoma or carcinoma (combined): 0/9, 0/55, 6/45*, 2/40	*P = 0.007 by the Fisher exact test when compared with the pooled-control group	Principal strengths: adequate duration; covered most of the life span; studies in M and F; complete histopathology
Full carcinogenicity Rat, F344 (M) 51–55 days 104–105 wk NTP (1978b)	Oral Dieldrin, technical grade, purified Diet 0, 2, 10, 50 ppm, ad libitum 24, 24, 24, 24 22, 18, 18, 16	No increase in incidence of any tumour type	NS	Principal limitation: small number of animals
Full carcinogenicity Rat, F344 (F) 51–55 days 104–105 wk NTP (1978b)	Oral Dieldrin, technical grade, purified Diet 0, 2, 10, 50 ppm, ad libitum 24, 24, 24, 24 21, 21, 20, 17	No increase in incidence of any tumour type	NS	Principal limitation: small number of animals

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Hamster, Syrian golden (M) NR Lifetime (up to 120 wk of age) Cabral et al. (1979)	Diet Dieldrin, 99% Diet 0, 20, 60, 180 ppm, ad libitum 40, 34, 32, 41 2, 3, 5, 13 (at age 90 wk)	All tumours: 3/40 (7.5%), 5/32 (15.6%), 5/32 (15.6%), 10/40 (25%) Total tumours: 3, 5, 8, 11	NS	Tumour sites were reported as [all], thyroid gland, adrenal gland, liver, and "other"
Full carcinogenicity Hamster, Syrian golden (F) NR Lifetime (up to 120 wk of age) Cabral et al. (1979)	Diet Dieldrin, 99% Diet 0, 20, 60, 180 ppm, ad libitum 40, 33, 34, 38 3, 0, 3, 4 (at age 90 wk)	All tumours: 5/39 (12.8%), 1/32 (3.2%), 5/34 (14.7%), 5/38 (13.2%) Total tumours: 5, 1, 7, 9	NS	Tumour sites were reported as [all], thyroid gland, adrenal gland, liver, and "other"

DDT, dichlorodiphenyl trichloroethane; F, female; M, male; mo, month; NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; TWA, time-weighted average; wk, week; yr, year

consisted of 217 male and female mice. Treated mice died 2 months earlier than controls; the average survival time in the treated mice was 51.4 weeks compared with 59.8 weeks for the controls. Survival at 18 months was decreased in treated mice (33/218, 15%) compared with the control group (47/217, 22%). All survivors at 2 years were killed and autopsied. Pneumonia and intestinal parasitism were observed in the study and probably contributed to the decreased survival of the mice. Caging of mice in groups of 5–8 contributed to the spread of disease within groups (Davis & Fitzhugh, 1962). Partial re-evaluation by Reuber and others of the histopathology data of the Davis & Fitzhugh (1962) and Davis (1965) studies indicated that most tumours initially classified by Davis & Fitzhugh (1962) as "hepatic cell adenomas" were hepatocellular carcinomas (Epstein, 1975; Reuber 1975, 1976a). A statistically significant increase in the incidence of "hepatic cell adenoma" [hepatocellular carcinoma] (36/148, 24%; $P < 0.001$) was noted in treated mice when compared to the control group (9/134, 7%). On average, treated mice developed "hepatic cell adenomas" [hepatocellular carcinomas] after 77 weeks on study compared with 89 weeks on study for control mice. [Limitations of this study included the low survival rate, combination of data for both sexes, lack of detailed histopathology, reports of disease, pneumonia and intestinal parasitism, and the disposal of a large number of animals at autopsy. The Working Group noted that the re-evaluation by Epstein (1975) was accurate, but limited by the number of cases reviewed.]

In a subsequent study, groups of 100 male and 100 female C3H mice were fed diets containing dieldrin [purity not reported] at a concentration of 0 or 10 ppm for up to 2 years (Davis, 1965, reported in Epstein, 1975). The number of survivors at 104 weeks was 64 and 39 for control and treated mice, respectively. The incidence (for both sexes combined) of "benign hepatoma" [hepatocellular carcinoma] in the treated group was significantly increased and approximately double that of controls, whereas the number of hepatic carcinomas [hepatocellular carcinoma] was about the same (Epstein, 1975). An independent partial re-evaluation of the Davis & Fitzhugh (1962) and Davis (1965) combined studies by Reuber and others concluded that most of the "benign hepatomas" were hepatocellular carcinomas. This re-evaluation indicated significant increases in the incidence of hepatocellular carcinoma in the treated compared with the control group in males and females (Epstein, 1975; Reuber 1976a). Morphological descriptions of the liver lesions were reported by Reuber (Reuber, 1975, 1976a). There were often two hepatocellular carcinomas present at the same time in treated animals compared with a solitary hepatocellular carcinoma in the control animals (Reuber, 1976a). In addition, transplantation studies were conducted in which hepatocellular carcinomas were transplanted into mice [sex not reported] with a similar genetic background. Eight out of nine tumours from mice fed dieldrin at 10 ppm grew when transplanted and histologically resembled the primary tumours (Reuber, 1976b). [Limitations of this study included the combination of data for both sexes, lack of detailed histopathology and the absence of report on the number of animals evaluated for histopathology. The Working Group noted that the re-evaluation by Epstein (1975) was accurate but limited by the number of cases reviewed.]

Groups of 71 C57BL/6, 50 C3H/He and 62 B6C3F$_1$ weanling male mice [age not reported] were fed diets containing dieldrin (purity, > 99%) at a concentration of 10 ppm for 85 weeks and observed up to age 132 weeks (Meierhenry et al., 1983). Control groups consisted of 69, 50, and 76 mice per strain, respectively. Hepatic tumours [hepatocellular tumours] developed earlier in mice treated with dieldrin than in controls, particularly in the C3H/He strain, in which the first tumour was observed in dieldrin-treated animals 25 weeks earlier (12 weeks)

than in the controls (37 weeks). There was a statistically significant increase in the incidence of benign hepatocellular neoplasms in C57BL/6J and B6C3F$_1$ mice fed diets containing dieldrin compared with controls. The incidence of hepatocellular carcinoma was significantly increased in all strains of mice treated with dieldrin compared with controls. [The Working Group noted that limitations of this study included the absence of data on survival and body weight.]

A subsequent study investigated the histological progression of hepatocellular adenomas to carcinomas in sequential liver biopsies in two groups of C3H/He weanling male mice [age not reported] that were fed diets containing dieldrin (purity, 99%) at a concentration of 10 ppm until the mice reached either age 57 or 67 weeks (Ruebner et al., 1984). A control group was untreated. The animals were killed at age 2 years. There was a significant increase in the incidence of hepatocellular adenoma in the dieldrin-treated group (for 57 weeks) compared with the control group. More frequent progression of hepatocellular lesions from adenoma to carcinoma was observed in dieldrin-treated mice than in control mice. [The Working Group noted that limitations of this study included that the number of animals at start was not reported, the small number of animals, and the short exposure duration.]

Bauer-Hofmann et al. (1992) evaluated the frequency and pattern of c-Ha-*ras* mutations in hepatocellular lesions induced in 20 male C3H/He mice (age, 4 weeks) fed diets containing dieldrin at a concentration of 10 ppm for 52 weeks. A control group of 40 animals was fed basal diet. There was an increase in the incidence [not significant] and multiplicity of hepatocellular lesions in dieldrin-treated mice relative to controls. [This mechanistic study was not a carcinogenicity study: a distinction between neoplastic and non-neoplastic lesions was not made. Limitations of this study also included the limited number of dose groups and short exposure duration. The Working Group considered this study inadequate for the evaluation.]

Walker et al. (1973) conducted several studies in which male and female CF-1 mice (age, 4 weeks) were fed diets containing dieldrin (purity, > 99%) at concentrations ranging from 0.1 to 20 ppm for up to 132 weeks.

In the first study, male and female CF-1 mice were fed diets containing dieldrin (purity, > 99%) at a concentration of 0, 0.1, 1.0, or 10 ppm for 132 weeks (Walker et al., 1973; Epstein, 1975; Hunt et al., 1975). Groups consisted of 600, 250, 250, and 400 mice, respectively, divided equally by sex. By experimental month 15, half of the males and females fed diet containing dieldrin at 10 ppm had died or been killed, while the controls reached 50% mortality by experimental months 20–24. The increase in incidence of hepatocellular carcinoma [type (b) tumours] was dose-related and markedly increased in groups of males and females at the highest dose compared with their respective controls. The increases in incidence of hepatocellular adenoma [type (a) tumours] and carcinoma [type (b) tumours] (combined) were also dose-related and strongly increased in groups of males and females at the highest dose compared with their respective controls. [The Working Group noted that the limitations of the study included that the incidence of neoplasms was not reported and that the effective number of animals was unclear. Liver neoplasms were diagnosed as type (a) and type (b) tumours; in current terminology (Thoolen et al., 2010), these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively.]

In the first study ["Experiment 2.1"] of a second series of studies ["Experiments 2.1-2.4" on the original publication] by Walker and colleagues (Walker et al., 1973; Epstein, 1975), groups of 30 male and 30 female CF-1 mice were given diet containing dieldrin (purity, > 99%) at a concentration of 1.25, 2.5, 5, 10, or 20 ppm for 128 weeks. Control groups consisted of 78 males and 78 females. The incidences of hepatocellular

adenoma and carcinoma (combined) were increased in all the dose groups relative to controls, with the highest incidence observed in males and females given a diet containing dieldrin at a concentration of 5 ppm. In a second study ["Experiment 2.2"], similar incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma (combined), and hepatocellular carcinoma were observed in groups of male and female CF-1 mice given non-irradiated diet and bedding, gamma-irradiated diet, or gamma-irradiated diets and bedding, with clearly higher incidences in the dieldrin-treated groups; the diet contained dieldrin (purity, > 99%) at concentrations of 0 or 10 ppm. A third (co-carcinogenicity) study ["Experiment 2.3"] investigated the influence of dieldrin (purity, > 99%) exposure (5 ppm) on groups of 32 male and 32 female CF-1 mice given a diet containing 4,4′-dichlorodiphenyltrichloroethane (DDT) at a concentration of 50 ppm for 112 weeks. An untreated control group consisted of 48 male and 48 female mice. The incidence of hepatocellular adenoma or carcinoma (combined) (but also of hepatocellular carcinoma) was increased in male and female mice fed dieldrin at 5 ppm and DDT at 50 ppm compared with controls and in groups fed DDT alone at 50 ppm. Reuber re-evaluated the histopathology data and diagnosed fewer liver neoplasms in the control and DDT groups than Walker et al. did (Walker et al., 1973; Epstein, 1975), resulting in a more pronounced effect of dieldrin on the incidence of hepatocellular neoplasms: males, 0 ppm (control), 0%; 50 ppm DDT, 6%; 5 ppm dieldrin+50 ppm DDT, 58%; and females: 0 ppm (control), 0%; 50 ppm DDT, 16%; 5 ppm dieldrin+50 ppm DDT, 94%. Epstein (1975) indicated that Walker et al. "overestimated the incidence of total liver tumours in the DDT groups, largely by inclusion of hyperplastic and nodular lesions as type (a) tumours." In a fourth study ["Experiment 2.4"], Walker et al. (1973) conducted a time course in which groups of 29 male and 29 female CF-1 mice were given a diet containing dieldrin (purity, > 99%) at a concentration of 10 ppm for varying periods of time from 0 (control) up to 64 weeks, and were maintained until experimental week 104. The incidences of hepatocellular adenoma and carcinoma (combined) were significantly increased in males and in females fed dieldrin at 10 ppm for 64 weeks. There was also a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) in males and females. [The Working Group noted that limitations of some of the studies included that neoplasm incidence was not reported, the effective number of animals was unclear, and the number of dose groups was limited. Liver neoplasms were diagnosed as type (a) and type (b) tumours; in current terminology (Thoolen et al., 2010), these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively.]

Tennekes et al. (1982) re-evaluated the dose–response relationship for the data on tumours of the liver from two long-term studies conducted by Walker et al. (1973) [Experiment 1 and Experiment 2.1]. In both sexes, treatment appeared to result in dose-related increases in the incidence of both hepatocellular adenoma and carcinoma (combined) and hepatocellular carcinoma, up to 10 ppm; the somewhat lower incidence at 20 ppm was hypothesized to result from considerable toxicity and lethality at that concentration. Dieldrin also induced a dose-dependent reduction in tumour latency periods; the lowest doses associated with a significant reduction in median time-to-tumour formation were 0.1 and 1.0 ppm for females and males, respectively.

In another study in CF-1 mice, groups of 30 males and 30 females were fed diets containing dieldrin (purity, > 99%) at a concentration of 10 ppm for 110 weeks (Thorpe & Walker, 1973). The control group consisted of 45 males and 45 females. Mortality increased in male mice fed diets containing dieldrin at 10 ppm after 22

months. A statistically significant increase in the incidence of hepatocellular adenoma and carcinoma (combined) was found in treated males and females compared with controls. The liver tumours appeared much earlier in treated animals than in controls. [The Working Group noted that limitations of the study included the lack of reporting on neoplasm incidence and the small number of dose groups. Liver neoplasms were diagnosed as type (a) and type (b) tumours; in current terminology (Thoolen et al., 2010), these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively.]

In a study by Tennekes et al. (1979), eight groups of 12–16 male weanling CF-1 mice [age not reported] were fed diets containing dieldrin [purity not reported] at a concentration of 0 or 10 ppm until age 65 weeks. The incidence of benign and malignant liver tumours (hepatocellular adenoma and hepatocellular carcinoma, respectively) or their combination was significantly increased in some groups fed dieldrin relative to the respective control groups. The Working Group noted that limitations of the study included the small number of animals per group and the small number of dose groups. Liver neoplasms were diagnosed as type (a) and type (b) tumours; in current terminology (Thoolen et al., 2010), these neoplasms correspond to hepatocellular adenoma and hepatocellular carcinoma, respectively.]

In a subsequent study by Tennekes et al. (1981), eight groups of 19–82 male weanling CF-1 mice [age not reported] were fed diets containing dieldrin (purity, > 99%) at a concentration of 0 or 10 ppm over the duration of their lifespan (for up to 110 weeks). Dieldrin had no effect on the mean body weights of treated mice relative to controls. Survival was significantly reduced in mice fed diets containing dieldrin at 10 ppm. On average, 10% of mice fed dieldrin survived to 100 weeks compared with 40% of control mice. A significant increase in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) was observed in all four groups of mice fed diets containing dieldrin at 10 ppm. Several dieldrin-treated mice with hepatocellular carcinomas had lung metastases.

In a study by the NCI, groups of 50 male and 50 female B6C3F$_1$ mice were fed diets containing dieldrin (technical grade; purity, > 96% [impurities not reported]) at a concentration of 2.5 or 5 ppm for 80 weeks, and then held untreated for an additional 10–13 weeks (NTP, 1978a). The matched-control group consisted of 20 male and 20 female mice, and the study duration was 91–93 weeks. Because the number of matched-control mice was small, pooled controls were used for statistical comparisons. The pooled-control groups consisted of the matched controls from the bioassay of dieldrin combined with matched controls from contemporary bioassays of aldrin, chlordane, heptachlor, dichlorvos, and dimethoate, giving groups of 92 male and 79 female mice. There was no significant effect on the survival or mean body weights of males and females compared with the controls.

In males, a significant positive trend in the incidence of hepatocellular carcinoma was noted when the treated groups were compared with the pooled controls. The incidence of hepatocellular carcinoma was significantly increased in males at 5 ppm (16/45; 36%), and exceeded the incidence for historical controls (48/285, 16.8%). The incidence of other neoplasms was not significantly increased when compared with the matched or the pooled controls. There was no significant increase in the incidence of neoplasms in female mice (NTP, 1978a).

In a study by Lipsky et al. (1989), groups of young [number of control animals at start unclear, and age not further specified] male Balb/c mice were fed diets containing dieldrin [purity not reported] at a concentration of 0 or 10 ppm for 2, 4, 8, 16, 36, 52, or 75 weeks. Hepatocellular adenomas were reported in the groups at 52

and 75 weeks. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in mice fed diets containing dieldrin at 10 ppm for 52 weeks and 75 weeks when compared with controls.

Epstein (1975) reviewed and provided re-evaluation of the slides from a study (see also EPA, 1974) in which groups of 125–130 male and 100 female Swiss-Webster mice were fed diets containing dieldrin (technical grade) [purity not reported] at a concentration of 0, 3, or 10 ppm for up to 32 months. The treated animals were initially given dieldrin at 1.5 or 5 ppm for the first 2 months of the study. Only 71% of the mice were examined histologically. According to the authors, dieldrin was not carcinogenic, but it increased the incidence of various non-neoplastic lesions of the liver (including liver hepatomas and nodules). A re-evaluation of some of the histopathology data by Reuber and others concluded that more than half of the re-examined liver lesions from male and female mice at the highest dose were hepatocellular carcinomas. [The Working Group noted the incomplete histopathological examination and re-evaluation, and the limited reporting in this study.]

(b) Gavage plus dietary administration

Four groups of male (C57BL/6J × C3HeB/FeJ)F_1 mice [number of animals at start, not reported] were treated with dieldrin [purity not reported]. Group I received 12.5 µg of dieldrin daily by gavage from age 1 week to age 4 weeks. Group II was given diet containing dieldrin at a concentration of 10 ppm from age 5 weeks to age 90 weeks. Group III received 12.5 µg of dieldrin daily by gavage from age 1 week to age 4 weeks, and was subsequently given diet containing dieldrin at a concentration of 10 ppm from age 5 weeks to age 90 weeks. Group IV (control) was untreated. The experiment was terminated at age 90 weeks. Histopathological examination was performed on the liver only. Only when both treatment schedules were combined (group III) did dieldrin significantly increase the incidence of liver [hepatocellular] tumours (30% vs 2% in controls; $P < 0.001$) (Vesselinovitch et al., 1979). [The Working Group noted the lack of vehicle controls, and the lack of data on survival and body weight.]

(c) Transplacental exposure, lactation, and gavage

In a study by Cameron & Foster (2009), four groups of 29–30 transgenic FVB/N-TgMMTV-neu female mice were given vehicle (corn oil) or dieldrin [purity not reported] at a dose of 0.45, 2.25, or 4.5 µg/g bw daily by gavage for 5 days 2 weeks before mating, and then once per week throughout gestation and lactation until weaning (age, 3 weeks). At weaning, four groups of female pups [number of animals at start, not reported] began weekly dosing (same doses as their respective groups of dams) by gavage until age 9 weeks and were killed at 22 weeks. Treatment with dieldrin had no effect on litter size, birth weight, or the number of pups surviving to weaning. The highest dose of dieldrin (4.5 µg/g bw) resulted in an increased multiplicity of thoracic mammary tumours [primarily mammary adenocarcinomas] per mouse and in increased volume of incident thoracic tumours. The multiplicity of total mammary tumours was also significantly increased at the highest dose. In contrast, the mean number of inguinal mammary tumours was not significantly increased. Preliminary histopathological assessment of the ovaries revealed an increased incidence of ovarian tumours in groups receiving dieldrin at 2.25 (7.5%) and 4.5 (10.5%) g/g bw compared with controls (2.65%). An increase in the incidence of liver tumours was also found in groups receiving dieldrin at 2.25 (18.8%) and 4.5 (52.6%) µg/g bw compared with controls (11.8%).

3.2.2 Rat

A study in male and female Carworth rats fed diets containing dieldrin (Treon & Cleveland, 1955; Cleveland, 1966; also reported in Epstein, 1975) was judged inadequate by the Working Group because of the lack of histopathological evaluation, difficulties in interpretation of the mortality data, limited reporting, and discrepancies between Treon & Cleveland (1955) and Cleveland (1966).

Three groups of 25 male and 25 female Carworth (Farm "E") rats (age, 5 weeks) were fed diets containing dieldrin (purity, > 99%) at a concentration of 0.1, 1.0, or 10 ppm for 2 years (Walker et al., 1969). Control groups consisted of 45 males and 45 females. Survival and body weight were not affected by feeding with dieldrin for 2 years. The incidence of tumours was not increased in treated groups relative to the controls for any of four tissue sites, including the thyroid, pituitary, and mammary gland, or "other" after 2 years.

Groups of 12 male and 12 female Osborne-Mendel rats were fed diets containing dieldrin (purity, 100%) at a concentration of 0, 0.5, 2, 10, 50, 100, or 150 ppm for 2 years (Fitzhugh et al., 1964). Survival was significantly decreased in males and females (combined) at 50, 100, or 150 ppm at 24 months. Mean body weights of males and females were similar to those of the controls. The incidence of tumours (all six categories listed below) in treated males and females (combined) was not increased compared with the control group. Six tumour categories were identified, including "pulmonary lymphosarcoma", "fibroadenoma of breast", "carcinoma of breast", "lymphoid except lung", "fibrosarcoma", and "other". Epstein (1975) reported that independent histopathological re-evaluations by Reuber and others confirmed these multiple-site tumours. No benign or malignant tumours of the liver were initially reported by Fitzhugh et al. (1964), but a partial re-evaluation of the liver histopathology identified a total of 18 hepatocellular carcinomas in rats fed diets containing dieldrin or aldrin (Epstein, 1975). [The Working Group noted that limitations of this study included that only 68% of animals treated with dieldrin (or aldrin) were examined histologically, and that the data were combined for both sexes.]

Groups of [about] 50 male and 50 female weanling Osborne-Mendel rats [age not reported] were fed diets containing dieldrin (purity, 100%) at a concentration of 20, 30, or 50 ppm for less than 31 months (Deichmann et al., 1970). Control groups were comprised of 100 males and 100 females. Doses during the first 10 weeks were half the final concentrations. The mean survival of the control male and female rats was 19.7 and 19.5 months, respectively. The survival rate was not affected in treated males, but the mean survival of females at 30 ppm (17.4 months) and 50 ppm (16.6 months) was significantly lower than that in the control group. Mean body-weight gain was similar in treated and control groups. The tumour incidence (all sites) was not significantly increased in male or female rats relative to the respective control groups. No benign or malignant tumours of the liver were found in the treated animals. [The Working Group noted that limitations of this study included that not all tissues were examined histologically, and that partial re-evaluation of the histopathology indicated that the authors may have underestimated or underreported the incidence of malignant tumours by approximately 3-fold (Epstein, 1975).]

In a study by the NCI, groups of 50 male and 50 female Osborne-Mendel rats (age, 35 days) were fed diets containing technical-grade dieldrin (purity, > 96% [impurities not reported]) at a concentration of 29 or 65 ppm (time-weighted average) (NTP, 1978a). Rats at the lower dose were treated for 80 weeks, followed by observation periods of 30–31 weeks. Rats at the higher dose were treated for 59 weeks, followed by 51–52 weeks of observation. For matched

controls (10 males and 10 females per group), the study duration was 110 weeks. Time-weighted doses were used to assess the results, because the concentration of dieldrin was reduced after study start due to toxicity with initial exposures. The pooled-control groups consisted of the matched controls from the bioassay of dieldrin combined with matched controls from the contemporary bioassays of aldrin, chlordane, heptachlor, dichlorvos and dimethoate, giving groups of 58 male and 60 female rats. There was no significant effect on the survival of rats at the end of the study because there was decreased survival in male and female rats during the first 90 weeks of the study and in the control groups during the remaining 20 weeks. Mean body weights of the treated male and female rats were lower than those of the controls during the second year of the study.

There was no statistically significant increase in the incidence or positive trend in the incidence of any tumour in treated males or females when compared with the matched controls. There was a significant increase in the incidence of adrenal cortical adenoma or carcinoma (combined) in female rats at the lower dose compared with the pooled controls. [The Working Group noted that the increase in tumour incidence was only for the group at the lower dose, and only when compared with the pooled-control group, and concluded that it was not treatment-related.]

In a second study by the NCI (NTP, 1978b), groups of 24 male and 24 female Fischer 344 rats were fed diets containing dieldrin (technical grade, purified) at a concentration of 0, 2, 10, or 50 ppm for 104–105 weeks. There was no significant effect on the survival or mean body weights of rats of either sex relative to matched controls. There was no treatment-related increase in the incidence of tumours in males or females.

3.2.3 Hamster

Four groups of 32–41 male and four groups of 33–40 female Syrian golden hamsters were fed diets containing dieldrin (purity, 99%) at a concentration of 0, 20, 60, or 180 ppm for their life span (up to age 120 weeks) (Cabral et al., 1979). The survival rate at age 50 weeks was comparable to that of controls (males: 0 ppm (control), 32/40; 20 ppm, 24/34; 60 ppm, 27/32; 180 ppm, 35/41; females: 0 ppm (control), 25/40; 20 ppm, 14/33; 60 ppm, 26/34; 180 ppm, 25/38). Only 0–13 hamsters per group survived to age 90 weeks. Male and female hamsters fed diets containing dieldrin at 20 and 180 ppm showed a marked retardation of growth. Tumour sites were reported as [all], thyroid gland, adrenal gland, liver, and "other". The percentage of tumour-bearing animals did not differ significantly between control and treated groups.

3.2.4 Dog

Groups of five male and five female beagle hounds (age, 4–7 months) were fed gelatin capsules containing dieldrin (purity, > 99%) at a dose of 0, 0.005, or 0.05 mg/kg bw per day for 2 years (Walker et al., 1969). Survival and body weight were not affected by feeding with dieldrin for 2 years. In females, the liver weights and liver:body weight ratios in the group at 0.05 mg/kg bw per day dose were increased. No tumours or other specific lesions attributable to dieldrin were reported. [Limitations of this study included the small number of animals. The Working Group concluded that this study was inadequate for the evaluation.]

3.2.5 Monkey

Epstein (1975) summarized the findings from an unpublished study in five groups of five male rhesus monkeys (age, 4 years) given a diet containing dieldrin at a concentration of 0.1, 0.1, 0.5, 0.1, or 1.75 ppm for 5.5–6 years.

An untreated control group consisted of five males and one female. Monkeys in the group receiving the highest dose received dieldrin at 5.0 ppm for 4 months, then 2.5 ppm for approximately 2.5 months, and finally 1.75 ppm for the remainder of the study; for one monkey in the group, dieldrin concentrations were gradually increased to 5 ppm, and were maintained at this concentration for 5 years. No histological differences were observed in the liver or other tissues when the treated monkeys and the controls were compared. [The Working Group noted that the limitations of this study included the short duration, the small number of animals, and the lack of detailed histopathology data.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

Aldrin is readily converted to epoxide-containing dieldrin in the environment and in living organisms (see Fig. 4.1), thus exposures related to aldrin also involve exposure to dieldrin (Jorgenson, 2001). Overall, there was less information available on the toxicokinetics and disposition of aldrin than of dieldrin. Toxicokinetic data on dieldrin were briefly reviewed by the *IARC Monographs* Working Group more than 40 years before the present meeting (see *IARC Monographs* Volume 5; IARC, 1974). The present Working Group updated its review of the literature encompassing both aldrin and dieldrin, with separate discussions on each where possible.

4.1.2 Absorption

(a) Humans

(i) Aldrin

Several studies indicated that aldrin is absorbed by humans, mainly on the basis of its detection in blood, adipose tissue, and breast milk (Mick et al., 1971; Feldmann & Maibach, 1974; Nair et al., 1992; Stevens et al., 1993; Teixeira et al., 2015). However, it was unclear which route of exposure – oral, dermal, or inhalation – was the most important quantitatively for absorption. Moreover, aldrin was detected less frequently than dieldrin in humans, which is probably due to the ease of conversion of aldrin to dieldrin in the environment and body (IARC, 1974).

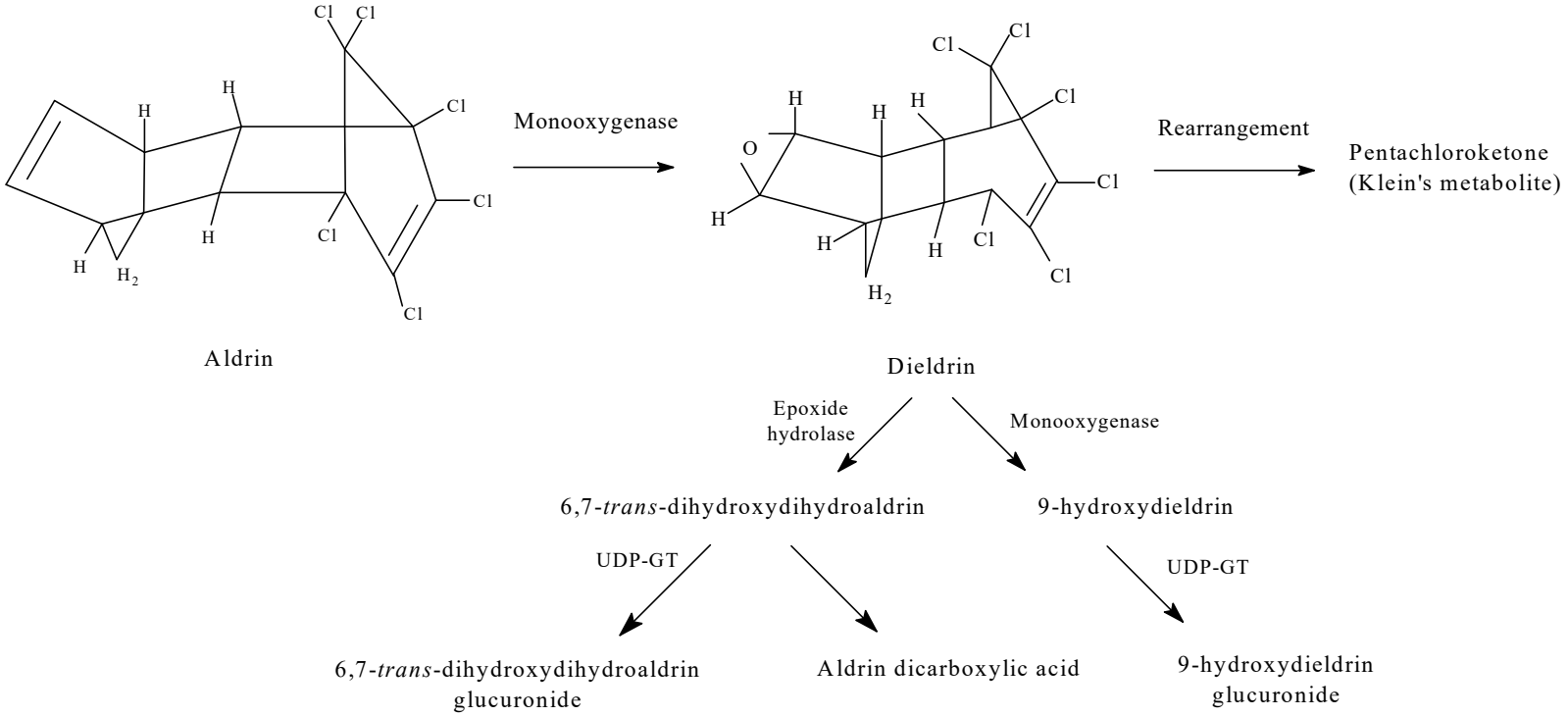
Evidence for the absorption of aldrin in humans comes from occupational as well as non-occupational exposures (see Table 1.2 and Table 1.3). Direct evidence for percutaneous absorption came from studies of deliberate exposure to aldrin (Feldmann & Maibach, 1974). For example, dermal application of [^{14}C]-labelled aldrin (single dose, 0.004 mg/cm^2) to the forearm of six subjects resulted in its rapid absorption, based on the excretion of radiolabel in the urine within 4 hours after administration (Feldmann & Maibach, 1974).

(ii) Dieldrin

On the basis of detection in blood, adipose tissue, and breast milk, dieldrin can be absorbed systemically by humans (Hunter & Robinson, 1967; Hayes & Curley, 1968; Mick et al., 1971; Feldmann & Maibach, 1974; Nair et al., 1992; Stevens et al., 1993; Teixeira et al., 2015). Detection of dieldrin after occupational as well as non-occupational exposures (see Table 1.2 and Table 1.3) indicated that dieldrin was absorbed, or that dieldrin was produced in vivo after exposure to and absorption of aldrin.

When humans were exposed for up to 2 years, concentrations in blood and adipose tissue were strongly correlated with the orally administered

Fig. 4.1 Major biotransformation pathways of aldrin to the epoxide-containing dieldrin metabolite and polar metabolites excreted via faeces and urine in experimental animals



The Chemical Abstracts Service numbering system was used in the designation of aldrin and its downstream metabolites.
UDP-GT, uridine 5′-diphospho-glucuronosyltransferase
Structures of aldrin and dieldrin are from Wikipedia Commons, the remaining metabolites are designated by their names.
Adapted with permission from Matthews et al. (1971). Dieldrin metabolism, excretion, and storage in male and female rats, *Journal of Agricultural and Food Chemistry*, Volume 19, issue 6, pages 1244-1248. Copyright (1971) American Chemical Society; adapted from *Biochemical Pharmacology*, Volume 36, issue 16, Wolff & Guengerich (1987). Rat liver cytochrome P-450 isozymes as catalysts of aldrin epoxidation in reconstituted monooxygenase systems and microsomes, Pages 2581-2588, Copyright (1987), with permission from Elsevier.

dose of dieldrin (Hunter et al., 1967, 1969; Hunter & Robinson, 1967). Direct evidence for percutaneous absorption came from the deliberate exposure of volunteers to dieldrin (Feldmann & Maibach, 1974). Dermal application of [^{14}C]-labelled dieldrin (single dose, 0.004 mg/cm^2) to the forearm of six subjects resulted in its rapid absorption, on the basis of excretion of radiolabel in the urine within 4 hours after administration (Feldmann & Maibach, 1974).

(b) Experimental systems

(i) Aldrin

Multiple studies in experimental animals demonstrated absorption of aldrin via oral and dermal routes; however, no studies were available on exposure to aldrin by inhalation. In dogs, rats, mice, and hens, oral treatment with aldrin resulted in rapid absorption into the systemic circulation (Brown et al., 1964; Korte & Kochen, 1966; Furusawa, 2002). In addition, dose-dependent increases in dieldrin levels in adipose tissue were seen in rats fed diets supplemented with aldrin for several months (Quaife et al., 1967).

In female rats, dermal administration of aldrin at increasing doses (0.1, 1, and 10 mg/kg per bw) resulted in detectable amounts of aldrin and dieldrin in the skin and in the blood stream (Graham et al., 1991). The amount of aldrin absorbed was proportional to the dose administered. This observation was supported by studies in vitro (Macpherson et al., 1991). Aldrin applied onto isolated rat skin was absorbed into the skin. Although percutaneous absorption of aldrin occurs, its major metabolite (dieldrin) is persistent in the skin (Macpherson et al., 1991).

Although the literature on absorption of aldrin in experimental systems was sparser than that for dieldrin, aldrin can penetrate the body after oral and dermal exposures. [Because of its highly lipophilic nature, aldrin is most likely to be absorbed into the body and tissues via processes involving first-order passive diffusion.]

(ii) Dieldrin

Several studies in experimental animals have shown absorption of dieldrin via oral and dermal routes. Mice, rats, rabbits, rhesus monkeys, and chimpanzees all effectively absorbed an oral dose of [^{14}C]-labelled dieldrin (0.5 mg/kg) (Müller et al., 1979). Absorption into the blood stream within 2 hours was noted in rats given a single oral dose of dieldrin (10 mg/kg) (Hayes, 1974). Absorption of dieldrin from the gastrointestinal tract was not complete, because unmetabolized dieldrin was detected in the faeces within 24–48 hours after administration, indicating the presence of unabsorbed compound.

Studies ex vivo examining the kinetics of dieldrin absorption in the mouse intestinal tract have also been conducted (Shah & Guthrie, 1970). The rate constant for penetration of the upper intestinal wall by dieldrin was 0.268×10^{-3} min^{-1}, suggesting that intestinal absorption of dieldrin is slow.

Thus dieldrin is absorbed at variable rates into the body after oral and dermal exposures. [Because of its highly lipophilic nature, dieldrin, like aldrin, is likely to be absorbed into the body via passive diffusion through membranes.]

4.1.3 Distribution

(a) Humans

(i) Aldrin

After its absorption into the body, aldrin is distributed to systemic tissues, with adipose tissues being an important storage depot (Mick et al., 1971; Nair et al., 1992; Botella et al., 2004; Teixeira et al., 2015). While aldrin bioaccumulates in adipose tissues and can be detected in breast milk, no information was available to the Working Group concerning levels in non-adipose tissues (except for blood) in exposed populations or individuals. On the other hand, numerous

studies have reported on the distribution of dieldrin in human tissues, as discussed below.

(ii) Dieldrin

After its absorption into the body, dieldrin is distributed via the circulation to systemic tissues, with adipose, mammary glands, and breast milk being notable storage depots (Mick et al., 1971; Nair et al., 1992; Botella et al., 2004; Teixeira et al., 2015). Dieldrin was detected at levels ranging from 0.13 to 0.36 mg/kg in adipose tissue obtained from autopsy patients (Ahmad et al., 1988; Adeshina & Todd, 1990). In subjects deliberately exposed to dieldrin for up to 24 months (0.1, 0.7, or 3 µg/kg per day), dieldrin concentrations in either blood or adipose tissue correlated strongly with the dose administered (Hunter & Robinson, 1967; Hunter et al., 1967, 1969). In addition, dieldrin concentrations in blood and adipose tissue were also strongly correlated with each other. The average ratio of steady-state dieldrin concentrations in adipose tissue versus blood was 156:1, indicating poor excretability and avid affinity for adipose tissues. When exposure was terminated, the concentration of dieldrin in the blood decreased exponentially, with an average half-life of 369 days (Hunter & Robinson, 1967), indicating slow elimination from the body. [Whereas tissue levels of dieldrin have been reported in the literature, the Working Group noted that quantitative levels of dieldrin-derived polar metabolites (Fig. 4.1) in human tissues were not available. This is mainly due to the metabolic stability of dieldrin (see Section 4.1.4).]

Transport of lipophilic dieldrin in the blood stream involves its avid interaction with albumin, α-globulins, and lipoprotein particles (Moss & Hathway, 1964; Tanaka et al., 1981; Maliwal & Guthrie, 1982). Studies in vitro indicated that dieldrin bound to albumin could be exchanged with human lipoproteins for subsequent transport (Maliwal & Guthrie, 1982). For instance, dieldrin was found to undergo efficient exchange reactions with all lipoprotein types in human plasma within 1 minute. [Similar interactions between aldrin and plasma proteins and lipoproteins in the blood are likely, although no specific data for aldrin were available to the Working Group.]

(b) Experimental systems

(i) Aldrin

Aldrin is widely distributed in the body (Korte & Kochen, 1966; Rumsey & Bond, 1974; Cooke et al., 2001). Intravenous injection of [^{14}C]-labelled aldrin in rats resulted in a broad tissue distribution of radiolabel after about 48 hours, with abdominal fat and subcutaneous fat exhibiting the highest amounts (nearly 15% and 7% of the administered dose, respectively), followed by liver (about 1.5%), and intestines (1%) (Korte & Kochen, 1966).

(ii) Dieldrin

Several studies on dieldrin demonstrated rapid and wide distribution in experimental animals (Bäckström et al., 1965; Robinson et al., 1969; Hayes, 1974; Iatropoulos et al., 1975). In rats given a single oral dose (10 mg/kg), dieldrin was absorbed into the blood stream within 2 hours, and then distributed systemically (Hayes, 1974). Maximum concentrations of dieldrin in muscle, liver, brain, and kidney were reached within 2–4 hours, whereas the maximum concentration in adipose tissue was attained by 24 hours. Over the 10 days after dosing, concentrations of dieldrin slowly declined in all tissues (by day 10: adipose tissue, 5–6% of administered dose; muscle, 0.1% of administered dose). The rank order of dieldrin concentrations (ppm) in tissues was: adipose tissue > > liver > kidney > brain > muscle > plasma, with adipose having by far the highest concentrations of dieldrin over the 10-day period. Thus, after its absorption, dieldrin was distributed rapidly to all systemic tissues, with slow redistribution from non-adipose tissues to adipose tissue for long-term storage (Hayes, 1974).

There were also data indicating that dieldrin can distribute through the body via the lymphatic system (Iatropoulos et al., 1975). The levels of dieldrin in mesenteric lymph nodes in Sprague-Dawley rats gradually increased over a 48-hour period after administration of a single oral dose (150 µg).

4.1.4 Metabolism and modulation of metabolic enzymes

The primary metabolic transformation of aldrin in the body is its rapid conversion to epoxide-containing dieldrin, followed by subsequent slow metabolism of dieldrin to polar metabolites that are excreted (Fig. 4.1).

(a) Humans

(i) Aldrin

No studies were found that examined the metabolism of aldrin in exposed humans; however, epoxidation of aldrin to dieldrin was demonstrated in human liver microsomes and in a human hepatoma cell line (Limbosch, 1983; McManus et al., 1984). The rate of aldrin epoxidation correlated with cytochrome P450 content in liver microsomes and varied by 2.4-fold across samples from 28 individuals (McManus et al., 1984). Comparison of rates of aldrin epoxidation activity with aryl hydrocarbon hydroxylase activity provided context for the rate of conversion of aldrin to dieldrin in human liver. From Wolff & Strecker (1985), it was estimated that aldrin epoxidase activity in human liver microsomes ranged from ~50 to 200 pmol/min per mg protein, whereas the benzo[*a*]pyrene aryl hydrocarbon hydroxylase activity ranged from 0.5 to 1.75 pmol/min per mg protein (an approximately 100-fold difference in rates). Although liver is the most important site of aldrin metabolism (McManus et al., 1984), other tissues probably have minor roles depending on their cytochrome P450 content. [The Working Group noted that the human cytochrome P450 isoforms responsible for aldrin epoxidation have yet to be characterized.]

(ii) Dieldrin

No studies directly examined the metabolism of dieldrin in exposed humans. 9-Hydroxydieldrin, an oxidation product of the metabolism of dieldrin in vivo, was detected in the faeces of occupationally exposed workers (Richardson & Robinson, 1971), indicating that this biotransformation reaction can occur in humans. Hunter et al. (1969) reported on the disposition of dieldrin in subjects given dieldrin for 18–24 months, but did not provide information on metabolites. Most of the information regarding dieldrin metabolism was obtained from experimental animals, as discussed below.

(b) Experimental systems

(i) Aldrin

The primary metabolic transformation of aldrin in rodents and other animal species is its conversion to epoxide-containing dieldrin, particularly in the liver and to a lesser degree in extrahepatic tissues. Nicotinamide adenine dinucleotide phosphate reduced (NADPH)-dependent epoxidation of aldrin was evident in rat liver, lung, and skin microsomes (Wong & Terriere, 1965; Wolff et al., 1979; Lambotte-Vandepaer et al., 1981; Graham et al., 1991), and mouse liver and skin microsomes (Williams & Woodhouse, 1996). Aldrin metabolism in rat liver microsomes was substantially faster than in lung and skin. In addition, the rate of aldrin epoxidation was sex-dependent in adult Sprague-Dawley rats; catalysis of this reaction was more efficient in liver microsomes obtained from males than in those obtained from females (Wolff & Guengerich, 1987). Aldrin epoxidation was sensitive to monooxygenase inhibitors in intact living rats (Clark & Krieger, 1976), and in vitro in rat liver microsomes (Wolff et al., 1979).

Aldrin was also metabolized to dieldrin in the skin. For instance, after dermal administration of

aldrin to rats (0.1, 1.0, or 10mg/kg bw), dieldrin was detectable in the skin at the application site after 1 hour (Graham et al., 1991). Studies in vitro in isolated rat skin preparations (Macpherson et al., 1991) or in rabbit lung (Mehendale & El-Bassiouni, 1975) showed that metabolism of aldrin to dieldrin could occur in these tissues.

In terms of catalysts of aldrin epoxidation in experimental models, cytochrome P450 monooxygenases were identified as the major enzymes responsible for aldrin epoxidation in rat liver (Wolff & Guengerich, 1987). [The Working Group noted that the rodent cytochrome P450 isoforms responsible for aldrin epoxidation have yet to be characterized.]

(ii) Dieldrin

Biotransformation of dieldrin was studied in several animal models and experimental systems. In general, the rate of metabolism of dieldrin is considered to be very slow, but excretion via formation of water-soluble metabolites has been reported (Matthews & Matsumura, 1969).

Despite the relative stability of dieldrin with regard to metabolic transformation, two sites on dieldrin, the epoxide moiety and the non-chlorinated methylene carbon, are susceptible to metabolic attack (Lykken & Casida, 1969). The epoxide can be opened by epoxide hydrolases to yield 6,7-*trans*-dihydroxydihydroaldrin, although this reaction is slow because of steric hindrance (Moody et al., 1991). In addition, the non-chlorinated methylene carbon is susceptible to hydroxylation producing 9-hydroxydieldrin, with the hydroxyl group oriented *syn* to the epoxide moiety. Most species can perform these metabolic transformations, although at variable rates between species or between sexes within a species (Matthews et al., 1971; Müller et al., 1979). Furthermore, the metabolites 6,7-*trans*-dihydroxydihydroaldrin and 9-hydroxydieldrin are often conjugated with glucuronic acid to give terminal products that are excreted (Fig. 4.1) (Matthews & Matsumura, 1969; Matthews et al., 1971).

Compared with epoxide opening, the formation of 9-hydroxydieldrin is the more quantitatively important reaction in rats (Matthews et al., 1971), although all species appear to perform this hydroxylation, including humans (Lykken & Casida, 1969; Richardson & Robinson, 1971). The metabolite 6,7-*trans*-dihydroxydihydroaldrin was also formed in several species, including rats, rabbits, sheep, rhesus monkeys, and chimpanzees (Korte & Arent, 1965; Feil et al., 1970; Müller et al., 1979). In addition to these main reactions, dieldrin can also be oxidatively dechlorinated to give the pentachloro-bridged ketone (pentachloroketone metabolite) (Fig. 4.1; Lykken & Casida, 1969). This product, also termed Klein's metabolite, is most important in male rats; it is detected in relatively high levels in the kidney and urine of males, while females make and excrete little (Matthews et al., 1971).

Studies of metabolism in vitro indicated that rat liver microsomes supplemented with uridine diphosphoglucuronic acid and NADPH could metabolize dieldrin to glucuronides of 9-hydroxydieldrin and 6,7-*trans*-dihydroxydihydroaldrin (Matthews & Matsumura, 1969). In addition, 6,7-*trans*-dihydroxydihydroaldrin can be further oxidized to give aldrin dicarboxylic acid (Baldwin et al., 1972; Hutson, 1976).

Collectively, metabolism of dieldrin to yield polar products does occur and can be modified by competing pathways (Fig. 4.1). The rate of overall biotransformation of dieldrin, however, is deemed to be very slow, accounting for its poor excretion and persistence in the body. Furthermore, on the basis of its half-lives in humans (Hunter & Robinson, 1967) and rodents (Robinson et al., 1969), dieldrin appears to persist longer in humans. [The Working Group noted that this is either because of slower degradation by human enzymes than by rodent enzymes, or slower release from human fat than rodent fat.]

(c) Modulation of metabolic enzymes

Multiple studies indicated that dieldrin can modulate metabolic enzymes. For example, exposure to dieldrin could induce the synthesis of xenobiotic metabolizing enzymes in the liver of many different species, including rats, mice, cattle, fish, and birds (Davison & Sell, 1972; Campbell et al., 1983; Abdelsalam & Ford, 1986; Haake et al., 1987; Barber et al., 2007; Dail et al., 2007). Mixed-function oxidases (i.e. cytochrome P450s) were the most affected by dieldrin (Campbell et al., 1983), and induction of hepatic enzymes by dieldrin could modulate the metabolism of carcinogens and hormones.

Dieldrin is a ligand for the xenoreceptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR), resulting in the increased transcription of cytochrome P450 2B and 3A genes in mice (Zhang et al., 2004). PXR-dependent induction of human CYP3A4 gene expression by dieldrin in cultured cell lines was also reported (Coumoul et al., 2002). Furthermore, a double-null mouse lacking CAR and PXR was completely insensitive to broad-range xenobiotics, such as dieldrin, that activate both types of receptor (Zhang et al., 2004).

[The Working Group considered that, taken together, these data supported the idea that dieldrin (and perhaps aldrin indirectly) can activate xenobiotic receptors that regulate the expression of xenobiotic metabolic enzymes.]

4.1.5 Excretion

(a) Humans

Aldrin and dieldrin

9-Hydroxydieldrin was detectable in the faeces of occupationally exposed workers, suggesting that this is an important route of elimination of aldrin- or dieldrin-derived metabolites in humans (Richardson & Robinson, 1971). Furthermore, aldrin and dieldrin were excreted in the breast milk of nursing mothers (Sant'Ana et al., 1989; Nair et al., 1992), suggesting that this is an important route of excretion in lactating women.

(b) Experimental systems

(i) Aldrin

One study in rats indicated that metabolites of aldrin were predominantly excreted via the faecal route, whereas urinary excretion was a minor route (Korte & Kochen, 1966). For example, in rats given an intravenous injection of [^{14}C]-labelled aldrin, 15% and 5% of the administered dose was excreted in the faeces and urine, respectively, within about 48 hours (Korte & Kochen, 1966). Polar metabolites accounted for the bulk of the radiolabel in the excreta, with only trace amounts of aldrin detected.

(ii) Dieldrin

Because aldrin is converted to dieldrin in vivo, studies on the excretion of dieldrin are also informative for aldrin. Faecal excretion was the major route of elimination of dieldrin and its metabolites, whereas urinary excretion was a minor route (Matthews et al., 1971; Hutson, 1976; Müller et al., 1979). Single oral doses of [^{14}C]-labelled dieldrin (0.5 mg/kg) administered to rats, mice, monkeys, and chimpanzees resulted in the faecal excretion of 10% (average of male and female Sprague-Dawley rats), 36% (average of male and female Swiss white mice), 16% (male rhesus monkeys), and 5% (female chimpanzees) of the administered [^{14}C]-labelled dose within 10 days, whereas urinary excretion accounted for only 0.6–4.4% of the administered dose (Müller et al., 1979). Thus, the bulk of the total radiolabel recovered in the excreta from these different species was present in the faeces (79–95% of excreted radiolabel).

In a separate study, 62% and 7% of a single oral dose of [^{14}C]-labelled dieldrin (3 mg/kg bw) was excreted in the faeces and urine, respectively, within 8 days after administration to male CFE rats (Hutson, 1976). Unchanged dieldrin and

9-hydroxydieldrin and its glucuronide were the major ^{14}C-labelled compounds detected in the faeces, with lesser amounts of 6,7-*trans*-dihydroxydihydroaldrin and aldrin dicarboxylic acid detected.

After an oral dose, the presence of [^{14}C]dieldrin-derived radiolabel in the faeces could indicate incomplete absorption. However, intraperitoneal and intravenous injections of [^{14}C]-labelled dieldrin in male rats also resulted in the excretion of most of the radiolabel by the faecal route (Cole et al., 1970) (Chipman & Walker, 1979). This suggested that biliary excretion of dieldrin and its metabolites is important for its elimination. Indeed, perfusion of isolated rat liver with [^{14}C]-labelled dieldrin resulted in significant biliary excretion of [^{14}C]-labelled dieldrin equivalents (Cole et al., 1970). Using this same approach, endrin, a stereoisomer of dieldrin, was excreted at a significantly higher rate than dieldrin was (Cole et al., 1970). This was attributed to faster metabolism of endrin in the liver when compared with dieldrin, and the subsequent biliary excretion of endrin metabolite 9-hydroxyendrin (Hutson et al., 1975). Elimination via the bile was also measured directly in bile-cannulated rats after an intraperitoneal dose of [^{14}C]-labelled dieldrin (Chipman & Walker, 1979). Interestingly, when dieldrin was compared with another chlorinated cyclodiene analogue (termed HCE), which is metabolically labile, its rate of biliary excretion was substantially slower than that of HCE (3.17 and 204 nmol/min per kg bw for dieldrin and HCE, respectively) (Chipman et al., 1979). [This result further supported the notion that the excretion rates for chlorinated cyclodienes are dependent on their metabolism rates.]

In general, 9-hydroxydieldrin and 6,7-*trans*-dihydroxydihydroaldrin and their glucuronides were the major ^{14}C-labelled compounds detected in rat, mouse, and monkey faeces (via bile excretion), with lesser amounts of unchanged dieldrin detected (Matthews et al., 1971; Hutson, 1976; Müller et al., 1979). On the basis of the profile of metabolites in faecal extracts, male rats excreted greater proportions of 9-hydroxydieldrin and 6,7-*trans*-dihydroxydihydroaldrin than females, whereas female rats excreted more unchanged dieldrin than male rats (Matthews et al., 1971). This result was consistent with a faster rate of dieldrin metabolism in male rats than in female rats. Furthermore, 9-hydroxydieldrin was also detectable in mouse and monkey urine, but not in rat urine (Hutson, 1976; Müller et al., 1979).

A comparison of excretion rates in mice and rats indicated that mice excreted 37–39% of an oral dose of [^{14}C]-labelled dieldrin within 10 days, whereas rats excreted 10–12% of the dose, signifying a species difference in excretion (Müller et al., 1979). In both rodent species, 95% of the radiolabel recovered in the excreta was present in the faeces. Further, substantially more 6,7-*trans*-dihydroxydihydroaldrin was excreted by mice than by rats.

Overall, these studies indicated that faecal excretion of aldrin and dieldrin metabolites via bile is the major route of elimination by rodents, whereas urinary excretion is a minor route. Several polar metabolites and trace amounts of unchanged dieldrin could be detected in the faeces and urine, while aldrin was generally not detectable in excreta (Fig. 4.1). For most species, the overall excretion rate of aldrin and dieldrin was generally slow. Whereas aldrin is rapidly converted to dieldrin in vivo, the slow excretion rate of dieldrin was attributable both to its slow release from fat as well as its inefficient metabolism to water-soluble products.

4.2 Mechanisms of carcinogenesis

This section summarizes in the following order the available evidence for the key characteristics for carcinogens, concerning whether aldrin or dieldrin is genotoxic; modulates receptor-mediated effects; induces inflammation and is immunosuppressive; induces oxidative stress; and alters cell proliferation, cell death, and

nutrient supply. For the other key characteristics of human carcinogens, insufficient data were available for evaluation.

4.2.1 Genetic and related effects

(a) Humans

(i) Exposed humans

See Table 4.1.

Aldrin

DNA damage in lymphocytes was not correlated with levels of aldrin or of other organochlorine pesticides in peripheral blood from mothers, or in umbilical cord blood in a study of mother–infant pairs (Alvarado-Hernandez et al., 2013). Aldrin was detected in maternal blood (median concentration, 412 ng/g lipid) and in umbilical cord blood (median concentration, 906 ng/g lipid); however, no correlation was found between pesticide levels and the frequency of micronucleus formation, chromatin buds, nucleoplasmic bridges, or DNA strand breaks as measured by the comet assay. Edwards & Priestly (1994) did not find increased frequencies of sister-chromatid exchange in the lymphocytes of pest-control workers and pesticide-treatment company employees exposed to aldrin. Aldrin exposures were confirmed by plasma dieldrin detection; median plasma levels ranged from 4.8 ng/mL to 16.0 ng/mL in the lowest and highest exposure groups, respectively. The plasma levels of dieldrin in exposed workers correlated with the duration of employment.

Dieldrin

Chromosomal aberrations were analysed in the lymphocytes of current and former dieldrin-manufacturing plant workers (Dean et al., 1975). The incidence of chromatid and chromosome-like aberrations was not increased in plant workers when compared with matched controls.

(ii) Human cells in vitro

See Table 4.2.

Exposure to aldrin or dieldrin induced unscheduled DNA synthesis, with and without the addition of rat liver S9 microsomal fraction, in SV-40 transformed human fibroblasts (Ahmed et al., 1977a). Aldrin induced chromosome aberrations (gaps, breaks, deletions, and fragments) in human peripheral blood lymphocytes exposed for 22 hours in vitro (Georgian, 1975). [The Working Group noted the lack of methodological detail reported in Georgian (1975).]

Dieldrin induced chromosomal aberrations in cultured human embryonic lung cells at cytotoxic exposures (Majumdar et al., 1976).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

Aldrin

Aldrin induced chromosomal aberrations in bone marrow cells in mice and rats given a single intraperitoneal dose (Georgian, 1975; see comment above). Aldrin exposure via drinking-water for 2 days did not increase the frequency of micronucleus formation in the bone marrow of mice (Usha Rani et al., 1980).

Dieldrin

Bachowski et al. (1998) investigated oxidative damage to DNA and unscheduled DNA synthesis in mice and rats fed diets containing dieldrin for up to 90 days. In the urine of male mice, 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels increased up to day 28. This increase correlated with increased hepatic DNA synthesis, although 8-OHdG levels were not changed in mouse liver (Klaunig et al., 1995). No increases in 8-OHdG formation or unscheduled DNA synthesis were detected in the liver or urine of rats (Klaunig et al., 1995).

Ha-*ras* proto-oncogene codon 61 mutations were not detected in tumours of the liver of dieldrin-exposed CF1 mice (Bauer-Hofmann et al., 1990, 1992).

Table 4.1 Genetic and related effects of aldrin and dieldrin in exposed humans

End-point	Tissue, cell line	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Aldrin					
DNA strand breaks, micronucleus formation	Blood, lymphocytes	50 mother–infant pairs in rural agricultural region of San Luis Potosi, Mexico	–	Multiple pesticides were detected; no exposure information	Alvarado-Hernandez et al. (2013)
	Umbilical cord blood, lymphocytes		–		
Sister-chromatid exchange	Blood, lymphocytes	29 pest-control workers in south Australia Exposure time: range, 3 mo to 20 yr; split into 4 groups based on job duties; 3 matched controls Plasma dieldrin levels were 4.8, 5.8, 7.0, 5.3, and 16.0 ng/mL in groups 1, 1a, 2, 3, and 4, respectively	–		Edwards & Priestly (1994)
		33 pesticide-treatment company employees; Australia	–		
Dieldrin					
Chromosomal aberrations	Blood, lymphocytes	9 former and 12 current dieldrin-plant workers; 17 matched controls	–	No exposure information	Dean et al. (1975)

[a] –, negative; mo, month(s); yr, year

Table 4.2 Genetic and related effects of aldrin and dieldrin in human cells in vitro

End-point	Tissue, cell line	Results[a]		Dose (LED or HID)	Comments	Reference
		With metabolic activation	Without metabolic activation			
Aldrin						
Unscheduled DNA synthesis	Fibroblasts	+	+	NR		Ahmed et al. (1977a)
Chromosomal aberrations	Lymphocytes (primary)	(+)	NT	9.56 µg/mL	Lack of methodological detail	Georgian (1975)
Dieldrin						
Unscheduled DNA synthesis	Fibroblast	+	+	1 µM	No positive controls	Ahmed et al. (1977a)
Chromosomal aberrations	Embryonic lung, WI-38	+	NT	1 µg/mL	No positive controls; cytotoxicity observed	Majumdar et al. (1976)

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

HID, highest ineffective dose; LED, lowest effective dose; NR, not reported; NT, not tested

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

End-point	Species, strain (sex)	Tissue	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aldrin							
Chromosomal aberrations	Mouse, AKR (M) Rat, Wistar (M)	Bone marrow	(+)	9.56 µg/g bw	i.p.; one injection of 9.56, 19.125, 38.25, or 76.50 µg/g bw, 24 h before the harvesting of the bone marrow; controls received an equivalent volume of vehicle	Lack of methodological detail	Georgian (1975)
Micronucleus formation	Mouse, Swiss albino (M)	Bone marrow (PCE)	–	13 mg/kg	Drinking-water; 2 d (1 × 24 h)		Usha Rani et al. (1980)
Dieldrin							
8-OHdG, unscheduled DNA synthesis	Rat, F344 (M)	Liver, urine	–	10 mg/kg diet	0.1, 1.0, or 10 mg/kg diet; 7, 14, 28, and 90 d		Bachowski et al. (1998)
8-OHdG	Mouse, $B6C3F_1$ (M)	Liver	–	10 mg/kg diet	0.1, 1.0, or 10 mg/kg diet; 7, 14, 28, and 90 d		Bachowski et al. (1998)
8-OHdG	Mouse, $B6C3F_1$ (M)	Urine	+	10 mg/kg diet	0.1, 1.0, or 10 mg/kg diet; 7, 14, 28, and 90 d	Increased at d 14 and 28	Bachowski et al. (1998)
Unscheduled DNA synthesis	Mouse, $B6C3F_1$ (M)	Liver	+	1.0 mg/kg diet	0.1, 1.0, or 10 mg/kg diet; 7, 14, 28, and 90 d		Bachowski et al. (1998)
c-Ha-*ras* proto-oncogene codon 61 mutations	Mouse, C3H (M)	Liver tumours	–	10 ppm [10 000 µg/L]	10 ppm in the diet; 52 wk		Bauer-Hofmann et al. (1992)
Ha-*ras* mutations at codons 12 or 61	Mouse, CF1 (M)	Liver tumours	–	10 ppm [10 000 µg/L]	10 ppm in the diet; 96 wk		Bauer-Hofmann et al. (1990)
Dominant lethal test	Mouse, CF1 (M)	Germinal tissue	–	25 mg/kg bw	Oral; HEOD at 12.5, 25, or 50 mg/kg; 8 wk (1×/wk)	Purity, > 99% (HEOD); vehicle, DMSO	Dean et al. (1975)
Chromosomal aberrations	Hamster, Chinese (M, F)	Bone marrow	–	60 mg/kg bw	Oral; HEOD at 30 or 60 mg/kg bw; 8 or 24 h	Purity, > 99% (HEOD); vehicle, DMSO	Dean et al. (1975)
Chromosomal aberrations	Mouse, STS (M)	Bone marrow	+	1 mg/kg bw	i.p.; single dose, 24 h	No positive controls; 4 animals/dose group; mitotic index decreased 40%	Majumdar et al. (1976)

Table 4.3 (continued)

End-point	Species, strain (sex)	Tissue	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, CBA (M)	Bone marrow (PCE)	+	60 mg/kg bw	i.p.; 24 and 48 h	No concurrent cytotoxicity; positive and dose-dependent at lethal and sublethal doses; most micronuclei were kinetochore-negative	Cicchetti et al. (1999)

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

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; HEOD, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-*exo*-1,4-*endo*-5,8-dimethanonapthalene (the major constituent of dieldrin); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male; mo, month; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCE, polychromatic erythrocytes; ppm, parts per million; wk, week

In the dominant-lethal assay in mice exposed orally to dieldrin for 8 weeks, no significant differences in fetal implantation rates or early fetal deaths were detected in the offspring of exposed male mice (Dean et al. (1975). In the same study, chromosomal aberrations were not induced in the bone marrow of Chinese hamsters 8 or 24 hours after a single oral dose of dieldrin . In two studies in male mice exposed intraperitoneally to dieldrin, chromosomal aberrations (Majumdar et al., 1976) and micronucleus formation (Cicchetti et al., 1999) were significantly induced in bone marrow.

(ii) Non-human mammalian cells in vitro

See Table 4.4.

No studies on aldrin were available to the Working Group.

Dieldrin induced 8-OHdG lesions in actively proliferating and in differentiated rat PC12 cells (Stedeford et al., 2001), and in mouse but not rat hepatocytes (Klaunig et al., 1995).

Dieldrin induced forward mutation at the thymidine kinase locus in the mouse lymphoma assay in two out of three replicate experiments (McGregor et al., 1991). The increases in mutant fraction correlated with dose, but the lowest effective concentration reduced the relative total cell growth to 40%.

Ahmed et al. (1977b) reported a significant induction in the frequency of ouabain-resistant mutants in Chinese hamster V79 cells exposed to nontoxic concentrations of dieldrin.

Dieldrin also increased the frequency of micronucleus formation in mouse primary lung fibroblasts (Cicchetti & Argentin, 2003).

In Chinese hamster ovary cells, an increased incidence of sister-chromatid exchange was observed after exposure to dieldrin, both with and without metabolic activation (S9 microsomal fraction), but there was no increase in chromosomal aberrations at up to toxic concentrations (Galloway et al., 1987). [At concentrations that induced sister-chromatid exchange, a precipitate formed.]

(iii) Non-mammalian systems in vivo

See Table 4.5.

Aldrin

Aldrin induced chromosomal aberrations in the plant species *Vicia faba* (Pandey, 2008), but did not increase micronucleus formation in *Tradescantia* clone 4430 (Sandhu et al., 1989).

Dieldrin

Dieldrin was studied in fish, insects, and plants. In fish, significant increases in the frequency of oxidative damage to DNA were seen 7 days after a single intraperitoneal injection of dieldrin in the gilthead seabream *Sparus aurata* (Rodríguez-Ariza et al., 1999).

Dieldrin gave negative results in the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*, and was toxic to larvae at 0.005 mM (Osaba et al., 1999).

In plants, chromosomal damage was significantly increased by dieldrin exposures. Chromosomal aberrations were induced in *Vicia faba* bean (Pandey, 2008), and increased micronucleus formation was reported in *Tradescantia* (Sandhu et al., 1989; Gill & Sandhu, 1992).

(iv) Non-mammalian systems in vitro

See Table 4.6.

Aldrin

Aldrin gave an equivocal response in strain TA100 in the presence of hamster liver S9 (NTP, 2016a), but otherwise gave negative results when tested with and without S9 fractions in *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538) and in *Escherichia coli* (strain WP2 *hcr*) (Moriya et al., 1983; NTP, 2016a). Aldrin did not induce DNA adducts in calf thymus DNA (Decloître et al., 1975), or DNA strand breaks in ColE1 plasmid DNA (Griffin & Hill, 1978).

Table 4.4 Genetic and related effects of dieldrin in non-human mammals in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
8-OHdG	Rat adrenal gland pheochromocytoma, PC12	+	NT	100 µM [38.1 µg/mL]		Stedeford et al. (2001)
8-OHdG	Rat, hepatocytes	–	NT	50 µM		Klaunig et al. (1995)
8-OHdG	Mouse, hepatocytes	+	NT	10 µM		Klaunig et al. (1995)
Tk mutation	Mouse L5178Y lymphoma cells	+	NT	20 µg/mL	Vehicle, DMSO Positive in 2/3 replicate experiments with dose–response relationship; relative total growth, approx. 40% at 20 µg/mL	McGregor et al. (1991)
Mutation	Chinese hamster fibroblasts, V79	+	NT	10 µM [3.8 µg/mL]	No positive controls; 77.8% cell survival	Ahmed et al. (1977b)
Micronucleus formation	Mouse lung fibroblasts (primary)	+	NT	25 µM [9.5 µg/mL]		Cicchetti & Argentin (2003)
Chromosomal aberrations	Chinese hamster ovary, CHO-W-B1	–	–	60 µg/mL (–S9); 800 µg/mL (+S9)		Galloway et al. (1987)
Sister-chromatid exchange	Chinese hamster ovary, CHO-W-B1	+	+	< 40 µg/mL (–S9); < 300 µg/mL (+S9)	Doses induced cell cycle delay and formed precipitate	Galloway et al. (1987)

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

DMSO, dimethyl sulfoxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; S9, 9000 × *g* supernatant

Table 4.5 Genetic and related effects of aldrin and dieldrin in non-mammalian experimental systems in vivo

Test system (species, strain)	End-point	Results	Dose (LED or HID)	Comments	Reference
Aldrin					
Vicia faba (bean)	Chromosomal aberrations	+	50 ppm [50 000 µg/L]	Toxic at ≥ 500 ppm	Pandey (2008)
Tradescantia clone 4430 (spiderworts)	Micronucleus formation	–	36.59 µg/mL	Vehicle, DMSO; precipitated out of solution when water was added. Reported as ppm	Sandhu et al. (1989)
Dieldrin					
Sparus aurata (gilthead seabream)	8-OHdG	+	0.6 mg/kg	Levels significant ($P < 0.05$) in liver and not blood or gills	Rodríguez-Ariza et al. (1999)
Drosophila melanogaster	Somatic mutation and recombination test (SMART)	–	0.005 mM	Highly toxic to larvae	Osaba et al. (1999)
Vicia faba (bean)	Chromosomal aberrations	+	50 ppm [50 µg/mL]	Toxic at ≥ 500 ppm	Pandey (2008)
Tradescantia clone 4430 (spiderwort)	Micronucleus formation	+	3.81 µg/mL	Vehicle, DMSO	Sandhu et al. (1989)
Tradescantia (spiderwort)	Micronucleus formation	+	4 µg/mL	Also positive at 4 mg/kg in soil Vehicle, DMSO	Gill & Sandhu (1992)

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

DMSO, dimethyl sulfoxide; h, hour; HID, highest ineffective dose; LED, lowest effective dose; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ppm, parts per million

Table 4.6 Genetic and related effects of aldrin and dieldrin in non-mammalian experimental systems in vitro

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aldrin						
Salmonella typhimurium TA98, TA1535, TA1537, TA1538	Reverse mutation	–	–	10 000 µg/plate	Vehicle, DMSO Incubations with rat and hamster liver S9 fractions at 10% and 30% were tested; highest two doses had observable precipitate	NTP (2016a)
Salmonella typhimurium TA100	Reverse mutation	–	+/–	10 000 µg/plate	Vehicle, DMSO Two replicate experiments with 30% hamster liver S9 fractions induced equivocal and negative results, respectively, with precipitate observed	NTP (2016a)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	5000 µg/plate	Vehicle, DMSO	Moriya et al. (1983)
Escherichia coli WP2 hcr	Reverse mutation	–	–	5000 µg/plate	Vehicle, DMSO	Moriya et al. (1983)
Calf thymus DNA	DNA adducts	NT	–	26.3 µM		Decloître et al. (1975)
ColE1 plasmid DNA	DNA strand breaks	–	NT	1 mg/mL		Griffin & Hill (1978)
Dieldrin						
Aspergillus nidulans 35 (haploid)	Forward mutation	–	NT	26 mM [9.9 mg/mL] [converted from 26 mM]	Purity, 97% No decrease in survival	Crebelli et al. (1986)
Aspergillus nidulans P1 (diploid)	Aneuploidy	–	NT	26 mM [9.9 mg/mL] [converted from 26 mM]	Purity, 97% 61% survival	Crebelli et al. (1986)
Saccharomyces cerevisiae D4	Gene conversion	–	NT	50 mg/kg bw	Purity, > 99% (HEOD); vehicle, DMSO Host-mediated assay of male CF1 mice dosed orally with dieldrin and injected i.p. with yeast	Dean et al. (1975)
Salmonella typhimurium TA98, TA100, TA1535	DNA strand breaks	+	+	1 µg/mL	No positive control; only group not positive was TA1535 –S9	Majumdar et al. (1977)
Salmonella typhimurium TA1535, TA1536, TA1537, TA1538	Reverse mutation	–	–	1000 µg/plate	Vehicle, DMSO Highest dose +S9 was toxic	Marshall et al. (1976)

Table 4.6 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	1 mg	Vehicle, DMSO	Wade et al. (1979)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	3333 µg/plate	Vehicle, DMSO Incubations with 10% rat and hamster liver S9 fractions were tested; highest three doses had observable precipitate	NTP (2016b)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	NT	3000 µg/plate	Concentrations of ≥ 300 µg/plate had visible precipitate, but were not toxic Results reported from other experiments, all negative: TA1535 –S9, TA1537 –S9, TA98 +S9 ± TCPO, TA100 +S9 (Oesch & Daly, 1972)	Glatt et al. (1983)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	NT	1000 µg/plate	Positive with UVC light exposure	De Flora et al. (1989)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	5000 µg/plate	Vehicle, DMSO	Moriya et al. (1983)
Calf thymus DNA	DNA adducts	NT	–	31.6 µM		Decloître et al. (1975)
ColE1 plasmid DNA	DNA strand breaks	–	NT	0.1 mg/mL		Griffin & Hill (1978)

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

bw, body weight; DMSO, dimethyl sulfoxide; h, hour; HEOD, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-*exo*-1,4-*endo*-5,8-dimethanonapthalene (the major constituent of dieldrin); HIC, highest ineffective concentration; i.p., intraperitoneal; LEC, lowest effective concentration; NT, not tested; S9, 9000 × *g* supernatant; TCPO, hypoxide hydrolase inhibitor; UVC, ultraviolet C

Dieldrin

Dieldrin did not induce forward mutation or aneuploidy in *Aspergillus nidulans* strains 35 and P1 (Crebelli et al., 1986). Dieldrin gave negative results in the host-mediated assay, in which mice orally exposed to dieldrin were injected intraperitoneally with *Saccharomyces cerevisiae* strain D4 (Dean et al., 1975).

Majumdar et al. (1977) reported DNA strand breaks in *S. typhimurium* strains TA98 and TA100 (with or without S9), and in TA1535 (with S9 only).

Dieldrin did not induce reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1536, TA1537, and TA1538, with or without S9 microsomal fraction (Marshall et al., 1976; Wade et al., 1979; Glatt et al., 1983; Moriya et al., 1983; De Flora et al., 1989; NTP, 2016b).

Dieldrin did not induce DNA adducts in calf thymus DNA (Decloître et al., 1975), or DNA strand breaks in ColE1 plasmid DNA (Griffin & Hill, 1978).

4.2.2 Receptor-mediated effects

(a) Humans

(i) Exposed humans

No studies on aldrin or dieldrin in exposed humans were available to the Working Group.

(ii) Human cells in vitro

Aldrin

Aldrin increased aromatase activity and CYP19 mRNA aromatase expression in the human choriocarcinoma JEG3 cell line (Laville et al., 2006). While aldrin bound human estrogen receptor α (ERα), it had greater affinity for the human progesterone receptor (Scippo et al., 2004). However, aldrin did not have estrogenic activity in transcriptional activation assays using human cell lines that either expressed ERα (MCF-7 cells) or that were stably transfected with human ERα receptors (HeLa cells) (Tully et al., 2000; Mumtaz et al., 2002; Kim et al., 2011). Lemaire et al. (2006) demonstrated antagonism of the ER by aldrin (0.1–10 μM), suggesting a potential anti-estrogenic effect, in HELN cells expressing human ER subtypes ERα and ERβ.

Aldrin has been shown to activate human retinoic acid receptors (Lemaire et al., 2005), although other studies were unable to demonstrate similar results (Laville et al., 2006).

Dieldrin

The receptor-mediated effects of dieldrin are summarized in Table 4.7, Table 4.8, and Table 4.9.

Dieldrin bound to the human ER and induced estrogen-dependent cell proliferation in human breast cancer cell lines at concentrations of 1 μM or above (Soto et al., 1994, 1995; Rasmussen & Nielsen, 2002) Activation of ERs in transactivation assays was also reported in several different human breast cell lines (Legler et al., 1999; Andersen et al., 2002; Charles et al., 2002; Buteau-Lozano et al., 2008). Dieldrin did not elicit estrogenic responses in MCF-7 or HeLa cells in other studies (Arcaro et al., 1998; Tully et al., 2000; Mumtaz et al., 2002). Dieldrin treatment induced activation of the pregnane X receptor in MCF-7 and HepG2 cells in culture (Coumoul et al. 2002), but did not appear to activate peroxisome proliferator-activated receptor gamma (PPARγ) (Moreno-Aliaga & Matsumura, 1999).

(b) Experimental systems

(i) Aldrin

Aldrin was weakly estrogenic in an assay in zebra fish (Hodges et al., 2000).

(ii) Dieldrin

Several studies in experimental systems demonstrated estrogenic effects, while others have been unable to document similar findings, either with dieldrin alone or in combination with other estrogenic contaminants (Ratnasabapathy et al., 1997; Wade et al., 1997). Dieldrin did not bind to ERs

Table 4.7 Estrogenic activity of dieldrin

Assay system	Cell line	Agent	Concentration	Results	Reference
Breast cancer cell lines	MCF-7	Dieldrin + 9 pesticides	1.0 pM–10 μM	Induced cell proliferation at 10 μM dose only Mixture of 10 pesticides induced cell proliferation at concentrations that were ineffective on their own	Soto et al. (1994)
	MCF-7	Dieldrin + estrogenic compounds	1–10 μM	Dieldrin treatment induced cell proliferation at 10 μM and pS2 expression at 1–10 μM Cell proliferation was increased by chemical mixture containing dieldrin (1.0 μM) showing additivity	Soto et al. (1995)
	MCF7-BUS	Dieldrin	0.1–100 μM	Significant increase in cell proliferation beginning at 5 mM, reaching a maximum at 25 mM, and EC_{50} of 7.0 mM Effects mediated via ER-mediated transactivation of the reporter gene	Rasmussen & Nielsen (2002)
	MCF-7	Dieldrin	1.0 μM–10 μM	Treatments induced PXR transactivation	Coumoul et al. (2002)
	MCF-7	Dieldrin	1.0 pM–10 nM	Dieldrin failed to induce an estrogenic response	Arcaro et al. (1998)
	MELN	Dieldrin	1.0 pM–10 μM	Dieldrin-induced transactivation of the ER and upregulated VEGF expression at 10 μM concentration	Buteau-Lozano et al. (2008)
	T47D	Dieldrin + others	0.1 nM–10 μM	Lowest concentration of dieldrin to cause estrogen receptor transactivated luciferase induction was 1.0 μM Dieldrin and endosulfan acted additively in the range of 3–6 μM	Legler et al. (1999)
	MCF-7	Ternary mixture including dieldrin	0.1–1.0 μM	Estrogenic effects even at highest concentrations were no more than additive	Charles et al. (2002)
Other mammalian cell lines	HELN ERα & ERβ	PCP, dieldrin & aldrin	0.1–10 μM	Pentachlorophenol, aldrin& dieldrin demonstrated antagonism towards hERα & hERβ Dieldrin (10 μM) treatment also caused significant transactivation of ERα but not ERβ	Lemaire et al. (2006)
	HeLa	4,4-DDT, 4,4-DDD, 4,4-DDE, aldrin, dieldrin, or endrin	1.0 nM–10 μM	No estrogenic effects alone or in binary mixtures	Mumtaz et al. (2002)
	HeLa	Aldrin & dieldrin	1.0 nM–10 μM	No estrogenic effects alone or in binary mixtures	Tully et al. (2000)
	GH3/B6 pituitary cells	Dieldrin	1.0 pM–10 nM	Dieldrin-induced Ca^{++} fluxes and PRL release at all concentrations tested although dose–response relationship for PRL release was not evident Effects thought to be mediated via activation of membrane ERα	Wozniak et al. (2005)
	HepG2 cells	Dieldrin	1.0 μM–10 μM	Treatment (10 μM) caused transactivation of PXR	Coumoul et al. (2002)

Table 4.7 (continued)

Assay system	Cell line	Agent	Concentration	Results	Reference
	Eker rat leiomyoma cells	Dieldrin	10 nM–10 μM	Dieldrin exposure did not induce cell proliferation but did upregulate PR message	Hodges et al. (2000)
Yeast gene reporter		Dieldrin	0.1 nM–10 mM	Estrogenic response at concentrations above 1.0 μM	Graumann et al. (1999)
	rtER	PCP and/or + dieldrin	0.1 pM–100 μM	PCP inhibited estrogen-dependent cell growth, whereas dieldrin was weakly estrogenic in both yeast cells expression a rainbow trout ER	Petit et al. (1997)

Ca^{++}, calcium; DDD, dichlorodiphenyl dichloroethane; DDE, dichlorodiphenyl dichloroethylene; DDT, bis(*p*-chlorophenyl)-2,2,2-trichloroethane; EC_{50}, half maximal effective concentration; ER, estrogen receptor; PCP, pentachlorophenol; PR, progesterone; PRL, prolactin; PXR, pregnane X receptor; VEGF, vascular endothelial growth factor

Table 4.8 Summary of studies quantifying receptor-binding capacity of dieldrin

Model	Concentration	Results	Reference
Recombinant yeast assay	0.1 nM–10 mM	^{3}H-E_2 was displaced by dieldrin concentrations above 1.0 μM	Graumann et al. (1999)
Displacement of ^{3}H-E_2 binding to estrogen receptor fusion proteins from human (ERα), mouse (ERα), chicken, green anole (ERdef), and rainbow trout (rtERdef)	0.1 nM–100 μM	Dieldrin, at a concentration of 100 μM, was considered a weak binder of the ER across all species tested	Matthews et al. (2000)
Displacement of ^{3}H-E_2	2.5, 15, 60 μmol/kg	Alone or in combination with equimolar concentration of toxaphene did not bind with mouse uterine ER	Ramamoorthy et al. (1997)
MCF-7 cells and immature female SD rats. Displacement of ^{3}H-E_2	10 nM–10 μM	Micromolar concentrations inhibited ^{3}H-E_2 binding	Wade et al. (1997)
Displacement of ^{3}H-E_2 from alligator & human ER	630 nM	Dieldrin alone failed to bind appreciably with either cytosolic aER or hER. However, in combination with other chemical contaminants additive to synergistic displacement of ^{3}H-E_2 from the ER was detected	Arnold et al. (1997)
Displacement of ^{3}H-DHT from rat ventral prostate	10 μM	Dieldrin non-competitively inhibited the binding of ^{3}H-DHT to androgen receptors in the rat prostate in vitro	Wakeling & Visek (1973)
Displacement of ^{3}H-E_2 from CN & CGC in vitro	0.06–3 μM	Dieldrin displaced ^{3}H-E_2 in the competitive binding assay with the LOEC of between 1 and 3 μM for CGC and CN cells, respectively	Briz et al. (2011)
Displacement of ^{3}H- E_2 from Atlantic salmon and rainbow trout estrogen receptors	10 μM/L to 1 mM/L	Dieldrin failed to show any evidence of binding with either the salmon or rainbow trout ER	Tollefsen et al. (2002)
Displacement of ^{3}H-E_2 from recombinant catfish ERα and ERβ	6–20 μM	Dieldrin demonstrated little competition for binding with either ER	Gale et al. (2004)
Competitive radioreceptor binding assay in two nematode species	25 nM	Significant inhibition of estrogen binding in nematode homogenates; however, binary mixtures with other chlorinated contaminants failed to reveal any evidence of additive or synergistic effects	Hood et al. (2000)

CGC, cerebellar granule cells; CN, cortical neuron; DHT, dihydrotestosterone; E_2, 17β estradiol; ER, estrogen receptor; LOEC, lowest observed effect concentration

Table 4.9 Summary of dieldrin effects on receptor expression from animal and tissue culture studies

Test system	Concentration	Results	Reference
CN and CGC in vitro	0.03–1.0 μM	ERα expression was downregulated in both CGC and CN but not ERβ	Briz et al. (2011)
FVB-MMTV/*neu* mice	0.45–4.5 μg/g bw	Treatments induced a significant dose-dependent increase in Ntrk2 expression	Cameron & Foster (2009)
Largemouth bass	0.4–0.81 ppm	Dieldrin exposure had no effect on AR and ERα expression but downregulated expression of ERβ in the gonad of both sexes. Exposure upregulated AR in the liver of males only	Garcia-Reyero et al. (2006a)
Largemouth bass	0.4–0.81 μg/g feed	Exposure had no effect on ERα expression in the liver of both sexes but induced decreased ERα expression of ERβ in the liver and gonad of females whereas the highest dose increased its expression in the liver of both sexes. Effects of dietary dieldrin exposure on AR expression varied by dose, sex, and target tissue	Garcia-Reyero et al. (2006b)
Immature female Sprague-Dawley rats	3 mg/kg per day	Treatments had no effect on nuclear or cytosolic ER expression	Wade et al. (1997)
PC12 cells (rat adrenal gland pheochromocytoma)	30 μM	Upregulation of Fgfr1, Ntrk1, and Ntrk3 expression after 72 h in culture	Slotkin et al. (2010)
Differentiating PC12 cells (rat adrenal gland pheochromocytoma)	30 μM	Treatment increased Avpr1b, Cckbr, and Smstr28 expression	Slotkin & Seidler (2010b)
Embryonic d14 brainstem cell cultures	10 μM	Treatments upregulate GABA(β3) expression while expression of GABA(γ2S) and GABA(γ2L) expression was downregulated. GABA1 subunit expression was unaffected	Liu et al. (1997b)
In utero exposure, embryonic d12–17 brainstem cells		Decreased expression of GABA(α1), GABA(β3) and GABA(γ1) but did not affect expression of GABA(γ2S) and GABA(γ2L)	Liu et al. (1998)
CN cultures	60 and 200 nM	200 nM of dieldrin decreased the expression of NR2A	Briz et al. (2012)
Testis explants	1 pM–1 nM	Both concentrations of dieldrin increased LHR expression	Fowler et al. (2007)

AR, androgen receptor; Avpr, arginine vasopressin receptor; bw, body weight; Cckbr, cholecystokinin B receptor; CGC, cerebellar granule cells; CN, cortical neuron; d, day of gestation; ER, estrogen receptor; Fgfr, fibroblast growth factor receptor; GABA, α-aminobutyric acid receptor; h, hour; LHR, luteinizing hormone receptor; NR2A, glutamate (NMDA) receptor subunit 2A; Ntrk, neutrophin receptor kinase; Smstr, somatostatin receptor

of Atlantic salmon and rainbow trout (Tollefsen et al., 2002) and weakly bound ERα and ERβ in catfish (Gale et al., 2004). Dieldrin also inhibited binding of [^{3}H]estradiol (E_2) to the ER in a nematode species (*Panagrellus redivivus*) (Hood et al., 2000).

Androgen uptake by prostate cells in culture was adversely affected by dieldrin treatment (Blend, 1975), and adverse effects of dieldrin on rat thymocytes in culture have also been reported (Hallegue et al., 2002).

Using cultures of the rat ventral prostate, dieldrin inhibited binding of 5α[^{3}H]dihydrotestosterone to the androgen receptor (Wakeling & Visek, 1973; Wakeling et al., 1973). However, other investigators were unable to demonstrate any interference of dieldrin with 5α[^{3}H]dihydrotestosterone binding in the anterior prostate, seminal vesicle, kidney, and liver of mice (Schein et al., 1979).

Dieldrin has been shown to induce non-genomic effects in a rat prolactinoma cell line (GH3/B6/F10) as shown by an increase in calcium influx and prolactin release (Watson et al., 2007a). β-Hexosaminadase release from cultures of a human mast cell line were significantly increased after treatment with dieldrin at concentrations as low as 1.0 pM, an effect that was abolished in ERα knockout mouse primary mast cell cultures (Narita et al., 2007). Similar non-genomic effects have also been documented in rat GH3/BH6 cells, a pituitary tumour cell line (Wozniak et al., 2005).

Regarding other receptors, several studies have demonstrated that dieldrin affects gamma-aminobutyric acid (GABA)- and *N*-methyl-D-aspartate (NMDA)-mediated signalling (Lawrence & Casida, 1984; Briz et al., 2012; Martyniuk et al., 2013). The expression of other neurotrophin receptors, including neurotrophin receptor kinase 1 (Ntrk) and Ntrk 2, Ntrk 3, was significantly affected by dieldrin treatment in PC12 cells after 72 hours in culture (Slotkin et al., 2010). Dieldrin was also a ligand for PXR and CAR receptors (Wei et al., 2002; Zhang et al., 2004).

4.2.3 Inflammation and immunosuppression

(a) Humans

(ii) Exposed humans

Aldrin

A cross-sectional study of agricultural workers suggested an association between pesticide exposure and immune dysfunction (Rosenberg et al., 1999). Aldrin residue in the plasma and adipose tissue of pre- and postmenopausal obese women was infrequently detected in this study population, and thus links with inflammation and cardiometabolic risk could not be established (Teixeira et al., 2015). However, maternal exposure to pesticides including aldrin and dieldrin has been associated with inflammation and dysregulation of coagulation mechanisms in infants (Schaalan et al., 2012).

Dieldrin

In a study on Inuit infants exposed perinatally to organochlorines, dieldrin exposure was associated with an increased relative risk (RR, 1.75; 95% CI, 1.05–2.91) of otitis media only in the age group 4–7 months for the highest tertile versus the lowest (Dewailly et al., 2000). Of note, similar effects were not elicited in infants aged 0–3 or 8–12 months.

(ii) Human cells in vitro

For aldrin, no data were available to the Working Group.

Dieldrin induced reactive oxygen species (ROS) in human neutrophils in culture (Pelletier et al., 2001), and lead to a calcium-dependent induction of arachidonic acid and eicosanoid production by human monocytes in culture (Mangum et al., 2015).

(b) Experimental system

No studies on aldrin and immune function or inflammation were available to the Working Group.

Dieldrin treatment is pro-inflammatory and drives the generation of ROS in rat neutrophils, as well as calcium-dependent induction of arachidonic acid and eicosanoid production (Hewett & Roth, 1988; Tithof et al., 2000; Mangum et al., 2015).

4.2.4 Oxidative stress

(a) Humans

(i) Exposed humans

Significantly higher levels of aldrin, but not dieldrin, were observed in patients with chronic kidney disease than in healthy controls. Plasma levels of malondialdehyde (MDA) and advanced oxidation protein production were positively associated with plasma levels of total organochlorine pesticides, including aldrin and dieldrin, indicating augmentation of oxidative stress with increased accumulation of organochlorine pesticides in patients with chronic kidney disease (Siddharth et al., 2012).

(ii) Human cells in vitro

No data on aldrin were available to the Working Group.

For dieldrin, several studies demonstrated ROS production in various types of human cells in vitro, reporting increased levels of oxidative markers, cell-cycle progression, and apoptosis. In human THP-1 monocyte cultures, dieldrin (10 μM) elevated levels of intracellular ROS, as shown by dichlorofluorescence-derived fluorescence by flow cytometry (Mangum et al., 2015). Dieldrin also induced human neutrophil superoxide dismutase (SOD) production (Pelletier et al., 2001), although dieldrin did not induce P4501A and 1B nor deplete GSH in human HepG2 cells (Dehn et al., 2005). ROS generated by dieldrin activated the ERK pathway in human HaCaT cells (Ledirac et al., 2005), and induced caspase-3 activation leading to apoptosis via alteration of mitochondrial transmembrane permeability in human peripheral blood lymphocytes (Michałowicz et al., 2013).

(b) Experimental systems

(i) Non-human mammals in vivo

No data on aldrin were available to the Working Group.

For dieldrin, the potential to induce oxidative stress in experimental animals has been investigated in rats and mice. Hfaiedh et al. (2012) reported perturbations of oxidative stress in hepatic and renal tissues induced by dieldrin (50 mg/kg bw by gavage for 4 consecutive days), as shown by increased lipid peroxidation levels associated with increased SOD activity and decreases in glutathione peroxidase and catalase activities. Increased urinary MDA was observed in B6C3F$_1$ mice fed diets containing dieldrin at 0.1, 1.0, or 10 mg/kg for 7, 14, 28, or 90 days. In rats, while dieldrin had no effects on urinary MDA levels after 7, 14, or 28 days of treatment, a dose-dependent increase in urinary MDA was observed at 90 days. Only in mice fed dieldrin was there a temporal association of increases in hepatic MDA and hepatic DNA synthesis (Bachowski et al., 1998; see also Section 4.2.1). In short-term studies in mice and rats exposed to dieldrin, hepatic vitamin E was decreased in correlation with dieldrin dose. Because of normally lower levels of vitamin E in the mouse, MDA formation in the liver was found only in this species (not in the rat). Also, dieldrin produced a dose-dependent increase in DNA synthesis only in the mouse (Klaunig et al., 1995). Dieldrin (10 mg/kg) increased the liver focal lesion volume, focal lesion number, and focal lesion labelling index in B6C3F$_1$ mouse liver induced by diethylnitrosamine (DEN). Supplementation with vitamin E at 50 mg/kg

blocked this effect (Kolaja et al., 1998). Vitamin E inhibited hepatic DNA synthesis in B6C3F$_1$ mice fed diet containing dieldrin at 1, 3, and 10 mg/kg for 7 or 28 days, but not liver enlargement, hypertrophy of centrilobular hepatocytes, or induction of hepatic ethoxyresorufin O-deethylase activity (Stevenson et al., 1995).

In studies in the brain, dieldrin caused global oxidative stress as shown by increased levels of lipid peroxidation in all brain regions in the mouse. Dieldrin also elicited increases in SOD activity and oxyguanosine glycosylase activity (Sava et al., 2007) and decreases in total glutathione (Hatcher et al., 2007) in the mouse brain.

(ii) Non-human mammalian cells in vitro

Aldrin did not generate oxygen-free radicals in rat cerebellar granule cells (Rosa et al., 1996).

Dieldrin increased concentrations of 8-OHdG (see Section 4.2.1), ROS, and MDA, and decreased cellular antioxidants in cultured mouse hepatocytes (Klaunig et al., 1995).

Neuronal cells, such as PC12, SN4741, and microglial cells, were used in many studies in vitro. After treatment of PC12 cells with dieldrin, ROS generation (analysed by flow cytometric analysis) was evident within 5 minutes, and lipid peroxidation was increased within 1 hour (Kitazawa et al., 2001). ROS generation was inhibited by SOD (Kitazawa et al., 2001), and lipid peroxidation increase was inhibited by ascorbate or vitamin E (Slotkin & Seidler, 2010a). Dieldrin also increased the frequency of 8-OHdG in PC12 cells (Stedeford et al., 2001), induced haem oxygenase-1 (Kim et al., 2005), and generated ROS (Chun et al., 2001) in SN4741 cells, and increased ROS in microglia cells (Mao et al., 2007).

(c) Non-mammalian experimental systems

The effects of aldrin on amphibian neuronal, hepatic and muscular tissue were reported to be attributable to changes in oxidative enzymes (Joseph & Rao, 1990), and ascorbic acid (vitamin C) was able to prevent aldrin toxicity in an air-breathing fish (Agrawal et al., 1978).

Dieldrin induced thiobarbituric acid reactive substances and 8-OHdG in *Sparus aurata* (Pedrajas et al., 1995, 1998; Rodríguez-Ariza et al., 1999).

4.2.5 Altered cell proliferation, cell death, or nutrient supply

(a) Apoptosis

(i) Humans

No data on aldrin were available to the Working Group.

Schroeder et al. (2001) (described in Section 2.1.2) reported an association of dieldrin with t(14;18)-positive, but not t(14;18)-negative, NHL. The *BCL2* gene is overexpressed in t(14;18), prolonging survival through the inhibition of apoptosis (Schroeder et al., 2001).

Dieldrin increased resistance to anoikis (apoptosis triggered by inappropriate anchorage) in the human breast cancer cell line MDA-MB-231 (Cameron & Foster, 2008). An increase in the expression of tyrosine kinase B (TrkB), a suppressor of anoikis, by dieldrin was also demonstrated.

(ii) Experimental systems

No data on aldrin were available to the Working Group.

Dose-dependent thymic atrophy [an effect associated with apoptosis], apparently mediated by endogenous corticosteroids, was induced in rats after exposure to dieldrin in vivo (Hallegue et al., 2002). However, apoptosis was not decreased in foci by dieldrin at any concentration (0.1, 1.0, or 10.0 mg/kg diet) in rat or mouse liver (Kolaja et al., 1996). Kamendulis et al. (2001) also found no effect of dieldrin (0, 1, 3, or 10 mg/kg diet) on hepatocyte apoptosis in male F344 rats or B6C3F$_1$ mice after 7, 14, 28, or 90 days.

Incubation of rat thymocytes for 6 hours with dieldrin in vitro resulted in a dose-dependent decrease in cell viability comparable to that of dexamethasone (Hallegue et al., 2002). DNA fragmentation was induced by dieldrin, demonstrating apoptosis, whereas higher concentrations stimulated necrosis. Apoptosis, downregulated gap junction intracellular communication, and interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α) expression were induced in mouse CID-9 mammary cells exposed to dieldrin (5 or 25 µM, up to 9 hours) (Tarraf et al., 2003).

Dieldrin treatment promoted apoptosis in a dopaminergic neuronal cell model, inducing caspase-3 activation and apoptosis in PC12 cells by generating oxidative stress (Buchmann et al., 1999; Kitazawa et al., 2001, 2003; Slotkin et al., 2007). Overexpression of human Bcl-2 in PC12 cells completely suppressed dieldrin-induced caspase-3 activation and DNA fragmentation (Kanthasamy et al., 2003; Kitazawa et al., 2004).

(b) Proliferation

(i) Humans

Exposed humans

In a representative sample of the general population of the Canary Islands, Spain, levels of insulin-like growth factor-1 (IGF-I) were significantly lower in women and men with detectable levels of aldrin (Boada et al., 2007).

No data on dieldrin were available to the Working Group.

Human cells in vitro

No data on aldrin were available to the Working Group.

In a study on the effects of dieldrin on mitogen-activated protein kinase (MAPK) cascades in human HaCaT cells, it was reported that dieldrin strongly activates the ERK1/2 pathway (Ledirac et al., 2005).

(ii) Experimental systems

Büsser & Lutz (1987) investigated stimulation of liver DNA synthesis after a single gavage dose of aldrin in rats and mice. Aldrin gave positive results only in male mice, doubling thymidine incorporation at 0.007 mmol/kg, but not in male rats or female mice.

In swine IB-RS-2 cells, aldrin (0.1–100 µg/mL medium for 48 hours) decreased cell growth, and also decreased cellular protein, RNA, and DNA levels (Rodrigues & Puga, 1979).

Bulayeva & Watson (2004) demonstrated that dieldrin (10^{-10} to 10^{-8} M) can rapidly activate the phosphorylation of extracellular signal-regulated kinases (ERKs) in the rat pituitary tumour cell line GH_3/B6/F10.

(c) Cell–cell communication

(i) Humans

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

In human teratocarcinoma cells, dieldrin inhibited gap junctional intercellular communication at non-cytotoxic doses (Lin et al., 1986).

(ii) Experimental systems

Trosko et al. (1987) reported that aldrin inhibits gap junctional communication using Chinese hamster cells. Similarly, aldrin and dieldrin were shown to affect metabolic cooperation in V79 cells (Kurata et al., 1982).

Dieldrin (1–10 µg/mL) inhibited intercellular communication between primary cultured hepatocytes from four different strains of male mice ($B_6C_3F_1$, C3H, C57BL and Balb/c strains), but not from male F344 rats (Klaunig & Ruch, 1987).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays carried out by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) programmes of the government of the USA, see Section 4.3 of the *Monograph* on pentachlorophenol in the present volume.

4.4 Cancer susceptibility

Koutros et al. (2013a) examined single nucleotide polymorphism–environment interactions for prostate cancer susceptibility loci and pesticide exposures. For aldrin, a statistically significant increased risk (corrected for multiple comparisons) of prostate cancer was observed among men carrying two A alleles at rs7679673 in *TET2*. Høyer et al. (2002) examined the interaction between dieldrin exposure and *TP53* status on risk of breast cancer. No statistically significant change in risk was observed on the basis of *TP53* status, but cases with "wild-type" *TP53* had a significant increased risk of dying associated with dieldrin exposure.

4.5 Other adverse effects

Several case reports of liver toxicity and haemolytic anaemia after oral exposure to aldrin or dieldrin have been published (ATSDR, 2002). No additional studies in humans were available to the Working Group.

In experimental systems, liver toxicity was observed in multiple studies on aldrin or dieldrin administered orally in mice, rats, and dogs; effects observed included elevated serum enzyme levels, decreased serum proteins, hyperplasia, bile-duct proliferation, focal degeneration, and necrosis (ATSDR, 2002).

5. Summary of Data Reported

5.1 Exposure data

Aldrin and dieldrin are synthetic organochlorine pesticides that act as effective contact and ingested poisons for insects. They have been used to control infestations of pests such as ants and termites, and to control several insect vectors of disease. Commercial formulations of aldrin contain 90.3% (1R,4S,4aS,5S,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene and 4.7% other insecticidally active related compounds. Commercial formulations of dieldrin contain 85% 1*R*,4*S*,4a*S*,5*R*,6*R*,7*S*,8*S*,8a*R*)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene and 15% other insecticidally active, related compounds.

Both aldrin and dieldrin have been classified as persistent organic pollutants under the Stockholm Convention, which requires parties to take measures to eliminate their production and use. Since the early 1970s, use of these two compounds have been banned or severely restricted in several countries, especially in agriculture. Use for specific purposes, including as a termiticide and for vector control, continued up to the 1980s and 1990s, when many countries implemented complete bans. Some continued use has been reported, primarily for malaria vector control.

Aldrin rapidly converts to dieldrin in the human body and in soil. Measurements of dieldrin in the body and the soil represent exposure to dieldrin, aldrin, or both. Occupational exposure to aldrin and dieldrin has been measured in aldrin- and dieldrin-manufacturing workers, agricultural workers, and pesticide-treatment workers. The highest concentrations of dieldrin were observed in insecticide-plant workers in the USA, with mean serum concentrations in aldrin formulators of aldrin, 29.5 µg/L, and dieldrin, 182.5 µg/L. Pesticide-treatment workers had

median dieldrin serum concentrations ranging from < 1 to16 µg/L in several studies. The general population can be exposed to dieldrin and aldrin directly from residues on food, from living near areas where dieldrin or aldrin was sprayed, or from (past) use of aldrin or dieldrin for insecticide treatments in and around the home. The 95th percentile of dieldrin serum concentrations in the general population in the USA has decreased by 10 times between 1976–1980 and 2001–2004. In measurements from the 1980s until the 2010s in various countries, mean dieldrin concentrations were ~0.5–2 µg/L in blood, 2–5 ng/g lipid in breast milk, and 17–40 ng/g lipid in adipose tissue.

5.2 Human carcinogenicity data

An important consideration in the interpretation of the studies on aldrin and dieldrin that were available to the Working Group was the type of exposure assessment used. In studies that used questionnaires, it was possible to differentiate between dieldrin and aldrin use, while in studies based on measurements in serum or adipose tissue, the dieldrin measurements may reflect exposure to aldrin and/or dieldrin.

5.2.1 Aldrin

Data were available from two cohort studies: the AHS, in which dieldrin use was assessed using questionnaires; and a cohort study of male workers at a Dutch manufacturing plant, in which combined exposure to dieldrin and/or aldrin was assessed. In the most recent update of the Dutch cohort, there was no increase in overall cancer mortality or mortality from cancer of the lung associated with total intake of aldrin and dieldrin. The AHS reported a decrease in risk of non-Hodgkin lymphoma (NHL) associated with aldrin use.

Three population-based case–control studies in the USA and Canada have investigated the association between NHL and exposure to aldrin, and reported conflicting results. The only statistically significant positive finding was based on results for 10 cases who had ever handled aldrin in a study in Canada.

A study of cancer of the breast among wives of pesticide applicators in the AHS showed increased risk associated with use of aldrin by the husband, but not by the wife, although the latter finding was based on results for only four cases. A case–control study on cancer of the breast that was nested within the Janus cohort in Norway found only three serum samples that contained aldrin at above the detection limit, with an odds ratio of 0.5. A case–control study on cancer of the breast and pesticide exposures in Spain reported increased risk associated with adipose tissue levels of aldrin at greater than the limit of detection, but this result was difficult to interpret because of the unexpected finding that aldrin was detected more frequently than dieldrin.

One case–control study investigated the association between soft tissue sarcoma and exposure to aldrin, and another study reported on the association between leukaemia and exposure to aldrin.

Because of the inconsistent results reported in studies on NHL and cancer of the breast, the different study designs used, different countries in which the studies were set, and the small number of studies available for other cancer sites, together with the small number of cases exposed to aldrin in most studies, the Working Group concluded that there were insufficient data to draw a conclusion regarding carcinogenicity associated with exposure to aldrin.

5.2.2 Dieldrin

Data were available from two cohort studies: the AHS, in which dieldrin use was assessed using questionnaires; and a cohort study of male workers at a Dutch manufacturing plant in which

combined exposure to dieldrin and/or aldrin was assessed. There were also several case–control studies nested within large population cohorts, and most of these reported levels of serum dieldrin that had been measured at baseline. Other case–control studies either used questionnaires or measured serum dieldrin concentrations at the time of recruitment (after diagnosis for cases).

Two or more studies considered other cancers including NHL, leukaemia, and cancers of the breast, prostate, or lung; the results of these studies are discussed below.

(a) Cancer of the breast

Two nested case–control studies with very similar methods assessed serum dieldrin concentrations in samples taken at baseline. The Danish study found a doubling in risk of cancer of the breast for the highest quartile of exposure, with a strong dose–response relationship limited to subjects with estrogen-receptor-negative (ER–) tumours. The Norwegian study found no increase in risk (but had fewer cases). The case–control study of cancer of the breast in Long Island, USA, found risk of breast cancer to be increased for the highest quintile of serum dieldrin concentration measured at diagnosis, but this was not statistically significant. In the AHS, risk of breast cancer in wives of pesticide licensees was statistically significantly doubled if the husband had ever used dieldrin. The number of wives who had used dieldrin themselves was too small to provide meaningful results. The Working Group considered that there was evidence for an association between dieldrin and cancer of the breast, but that chance, bias, and confounding could not be ruled out.

(b) Non-Hodgkin lymphoma

In two studies in the USA that measured biomarkers, no increase in risk of NHL was seen with serum dieldrin concentration measured at time of diagnosis in the case–control study, nor at enrolment in the cohort study. In a study in the USA that used stored adipose tissue mainly from cadavers, the highest quartile of dieldrin concentration at time of death was significantly associated with an increased risk of NHL. In questionnaire studies, the AHS cohort study reported that ever use of dieldrin was not associated with an increase in NHL or in any NHL subtype, including multiple myeloma. The case–control study in the midwest USA (De Roos et al., 2003) found an elevated risk of NHL associated with dieldrin use, although the effect estimate was not statistically significant.

(c) Other cancers

For leukaemia, the AHS found a non-statistically significant increase in risk for ever use of dieldrin, while an older case–control study found no increase in risk among subjects who had ever used dieldrin. Dieldrin exposure was not associated with cancer of the prostate in two studies, nor was it associated with cancer of the colorectum in the two cohort studies. Lung cancer risk was increased with dieldrin use in the AHS, but not in the Dutch cohort study. Only one study was available for cancer of the bladder, melanoma, or for cancer of the pancreas, and no associations with dieldrin were reported.

5.3 Animal carcinogenicity data

5.3.1 Aldrin

Three studies in mice fed diets containing aldrin were available to the Working Group: two studies in males and females combined, and one study in males and females considered separately. Aldrin increased the incidence of hepatocellular carcinoma in both studies in males and females combined, and in males only in the study in males and females considered separately.

Five studies in rats fed diets containing aldrin were available to the Working Group: one study in males and females combined, and four studies in males and females considered

separately. In one study in males and females considered separately, there was an increase in the incidence of tumours of the thyroid in males and females, and in the incidence of tumours of the pancreas in males and of the adrenal gland in females. These increases were not considered to be treatment-related, because they were significant only for groups at the lower dose, and only when compared with pooled controls. No significant increase in the incidence of neoplasms was observed in three other studies, and an additional study in males and females separately was judged inadequate for the evaluation.

5.3.2 Dieldrin

Sixteen studies in mice fed diets containing dieldrin were available to the Working Group: two studies in males and females combined, six studies in males only (one was judged inadequate for the evaluation), and eight studies (including one co-carcinogenicity study) in males and females considered separately. Dieldrin increased the incidence of hepatocellular carcinoma and/or hepatocellular adenoma or carcinoma (combined) in males and females in most of these studies. In one additional study in female transgenic offspring mice treated with dieldrin by gavage in addition to transplacental exposure and exposure throughout lactation, there was an increase in the multiplicity of tumours of the thoracic mammary gland (mainly adenocarcinomas). In another study, in male mice exposed to dieldrin by gavage and/or in the diet, there was an increase in the incidence of hepatocellular tumours when mice were exposed to dieldrin by gavage and in the diet.

Six studies in rats fed diets containing dieldrin were available to the Working Group: one study in males and females combined and five studies (one was judged inadequate for the evaluation) in males and females considered separately. In one study in males and females separately, there was an increase in the incidence of tumours of the adrenal gland in females. This increase was not considered to be treatment-related, because it was significant only for the group at the lower dose, and only when compared with pooled controls. No significantly increased incidence of neoplasms was observed in the other studies.

One study in male and female hamsters fed diets containing dieldrin gave negative results. One study in male rhesus monkeys fed diets containing dieldrin gave negative results. One study in dogs fed diets containing dieldrin was judged inadequate for the evaluation.

5.4 Mechanistic and other relevant data

Absorption of aldrin and dieldrin in humans has been documented after occupational and non-occupational exposures. Both compounds are detected in the blood and in adipose tissue biopsies. Gastrointestinal and percutaneous absorption have been reported in studies in human volunteers. In studies in experimental animals, absorption occurs readily via oral and dermal routes. In all species, aldrin and dieldrin are rapidly distributed by blood circulation to systemic tissues, with adipose tissue being an important storage depot. Metabolism of aldrin involves its rapid conversion to the epoxide-containing dieldrin, but there are no data to suggest that dieldrin forms protein or DNA adducts. Subsequently, dieldrin is very slowly metabolized to polar glucuronide metabolites that are excreted in the bile and, to a lesser degree, in the urine. The blood half-life of dieldrin in humans is about 1 year. The slow excretion of dieldrin is attributed to inefficient metabolism and to sequestration in adipose tissue.

With respect to the key characteristics of carcinogens, adequate data were available to evaluate whether dieldrin is genotoxic, modulates receptor-mediated effects, induces inflammation, is immunosuppressive, induces oxidative

stress, and alters cell proliferation, cell death, or nutrient supply. For aldrin, the available data were sparse or inconsistent.

There is *weak* evidence that aldrin is genotoxic. No effect was seen in human lymphocytes from exposed populations, on end-points such as DNA damage and strand breaks. Aldrin also gave negative results in human lymphocytes treated in vitro, and in other experimental systems (animals in vivo, bacteria, and plants).

There is *moderate* evidence that dieldrin is genotoxic. The frequency of chromosomal aberrations was not increased in exposed workers, and two studies in human cell lines were not informative. Chromosomal aberrations (at toxic exposures) and micronucleus formation were induced in the bone marrow of male mice, but not in Chinese hamsters. Levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were elevated in the urine, but not in the liver, of male mice fed diets containing dieldrin for up to 90 days. In vitro, dieldrin increased the formation of 8-OHdG in mouse hepatocytes and PC12 cells, but not in rat hepatocytes. Findings of mutations and micronucleus formation in mammalian cells may have been compromised by excessive toxicity.

There is *weak* evidence that aldrin modulates receptor-mediated effects. Aldrin did not bind the estrogen receptor or activate estrogen receptor-mediated signalling pathways. Results were conflicting in two studies on activation of the human retinoic acid receptor.

There is *moderate* evidence that dieldrin modulates receptor-mediated effects, on the basis of anti-estrogenic effects in complementary assay systems in vitro. Dieldrin is a ligand for the xenobiotic receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR), resulting in increased transcription of cytochrome P450 2B and 3A genes.

There is *weak* evidence that aldrin induces oxidative stress, based on sparse data; the evidence is *moderate* for dieldrin. No studies in exposed humans or human primary cells in vitro were available, but dieldrin induced production of reactive oxygen species in several studies in human cell lines. In rodents fed dieldrin, levels of various markers of oxidative stress were increased. Supplementation with vitamin E blocked mouse liver focal-lesion enhancement by dieldrin after initiation with diethylnitrosamine.

There is *weak* evidence that aldrin induces chronic inflammation and is immunosuppressive; the evidence is *moderate* for dieldrin. Maternal exposure to multiple chlorinated pesticides, including aldrin and dieldrin, was associated with inflammation and dysregulation of coagulation mechanisms in infants. Dieldrin stimulated an oxidative burst in human THP-1 monocytes and in rat neutrophils.

No studies on aldrin were available, and there is *moderate* evidence that dieldrin alters cell proliferation, cell death, or nutrient supply. Although no studies in exposed humans were available, dieldrin increased resistance to anoikis (apoptosis triggered by inappropriate anchorage) in a human breast-cancer cell line, MDA-MB-231. Dieldrin induced dose-dependent thymic atrophy (an effect associated with apoptosis) in rats. Dieldrin strongly activated the ERK1/2 pathway in human HaCaT cells.

In high-throughput testing in the Toxicity Testing in the 21st Century and Toxicity Forecaster research programmes of the USA government, aldrin and dieldrin were active for multiple assay end-points measuring markers of oxidative stress. Aldrin and dieldrin were cytotoxic in cell lines and primary cells, and were also active for many assay end-points related to modulation of receptor-mediated effects that may be related to cytotoxicity.

Few data were available concerning cancer susceptibility. The liver was consistently identified as a target organ of toxicity and carcinogenicity.

6. Evaluation

6.1 Cancer in humans

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

There is *limited evidence* in humans for the carcinogenicity of dieldrin. A positive association has been observed between dieldrin and cancer of the breast.

6.2 Cancer in experimental animals

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

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

6.3 Overall evaluation

Dieldrin, and aldrin metabolized to dieldrin, is *probably carcinogenic to humans* (*Group 2A*).

6.4 Rationale

Because aldrin is rapidly metabolized to dieldrin in humans and experimental animals, exposure to aldrin always leads to internal exposure to dieldrin. Therefore, for the evaluation of aldrin, the evidence on the carcinogenicity of dieldrin was taken into account.

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AhR	aryl hydrocarbon receptor
AHS	Agricultural Health Study
bw	body weight
CHAMACOS	Center for the Health Assessment of Mothers and Children of Salinas
DCFD	dichlorodihydrofluorescein diacetate
DMSO	dimethyl sulfoxide
dw	dry weight
E2	17β-estradiol
EPN	*O*-ethyl *O*-*para*-nitrophenyl phenylphosphonothioate
ER	estrogen receptor
GABA	gamma-aminobutyric acid
GC-MS	gas chromatography-mass spectrometry
GJIC	gap-junctional intercellular communication
gpt	guanine-hypoxanthine phosphoribosyl transferase
GSH	glutathione
GSSG	oxidized glutathione
HCB	hexachlorobenzene
β-HCH	β-hexachlorocyclohexane
HENU	2-hydroxyethylnitrosourea
HPLC-ECD	high-performance liquid chromatography-electrochemical detection
HPLC-UV	high-performance liquid chromatography-ultraviolet
HpCDD	heptachlorodibenzo-*para*-dioxin
HxCDD	hexachlorodibenzo-*para*-dioxin
JEM	job–exposure matrix
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
NADPH	nicotinamide adenine dinucleotide phosphate reduced form
Na-PCP	sodium pentachlorophenate
NCI	National Cancer Institute
NDEA	*N*-nitrosodiethylamine
Nrf2	nuclear factor erythroid 2-related factor
NHANES	National Health and Nutrition Examination Survey

NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NQO1	NAD(P):quinone oxidoreductase 1
NMDA	*N*-methyl-*D*-aspartate
OCDD	octachlorodibenzo-*para*-dioxin
OCDF	octachlorodibenzofuran
8-OHdG	8-hydroxy-2′-deoxyguanosine
OR	odds ratio
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*para*-dioxin
PCDF	polychlorinated dibenzofuran
PeCDD	pentachlorodibenzo-*para*-dioxin
PeCDF	polychlorinated dibenzofuran
ROS	reactive oxygen species
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
STS	soft tissue sarcoma
T3	triiodothyronine
T4	thyroxine
TCAB	3,3′,4,4′-tetrachloroazobenzene
TCAOB	3,4,3′,4′-tetrachloroazoxybenzene
TCBQ	tetrachlorobenzoquinone
TCHQ	tetrachlorohydroquinone
TCDD	2,3,7,8-tetrachlorodibenzo-*para*-dioxin
TDCF	tetrachlorodibenzofuran
TrCP	trichlorophenol
TEF	toxicity equivalence factor
TEQ	toxic equivalency
UDP-glucuronosyltransferase	uridine 5′-diphospho-glucuronosyltransferase
USA	United States of America
vs	versus
WHO	World Health Organization
ww	wet weight

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: http://publications.iarc.fr/574) contains spreadsheets (.xlsx) and a ToxPi data file (.csv) analysed by the Working Group for Volume 117 of the *IARC Monographs*. The spreadsheets list the ToxCast/Tox21 assay end-points, the associated target and/or model system (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10 "key characteristics" of known human carcinogens, and whether each chemical was "active" or "inactive" (EPA, 2015). The ToxPi file integrates the results by "key characteristic" and can be accessed using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

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IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of pentachlorophenol, 2,4,6-trichlorophenol, 3,3′,4,4′-tetrachloroazobenzene, aldrin, and dieldrin.

Pentachlorophenol, aldrin, and dieldrin are classified as persistent organic pollutants under the Stockholm Convention. Pentachlorophenol has been widely used as a wood preservative and insecticide, but its production and use are now restricted. 2,4,6-Trichlorophenol has also been used as a wood preservative and insecticide, and in the synthesis of some fungicides. Aldrin and dieldrin are synthetic organochlorine pesticides used as broad-spectrum soil insecticides for the protection of various food crops, as seed dressings, and to control infestations of pests such as ants and termites. In several countries their use has been banned or severely restricted since the early 1970s. 3,3′,4,4′-Tetrachloroazobenzene is not manufactured commercially but is formed during the production and degradation of chloroanilide herbicides such as propanil, linuron, and diuron.

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

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