IARC MONOGRAPHS

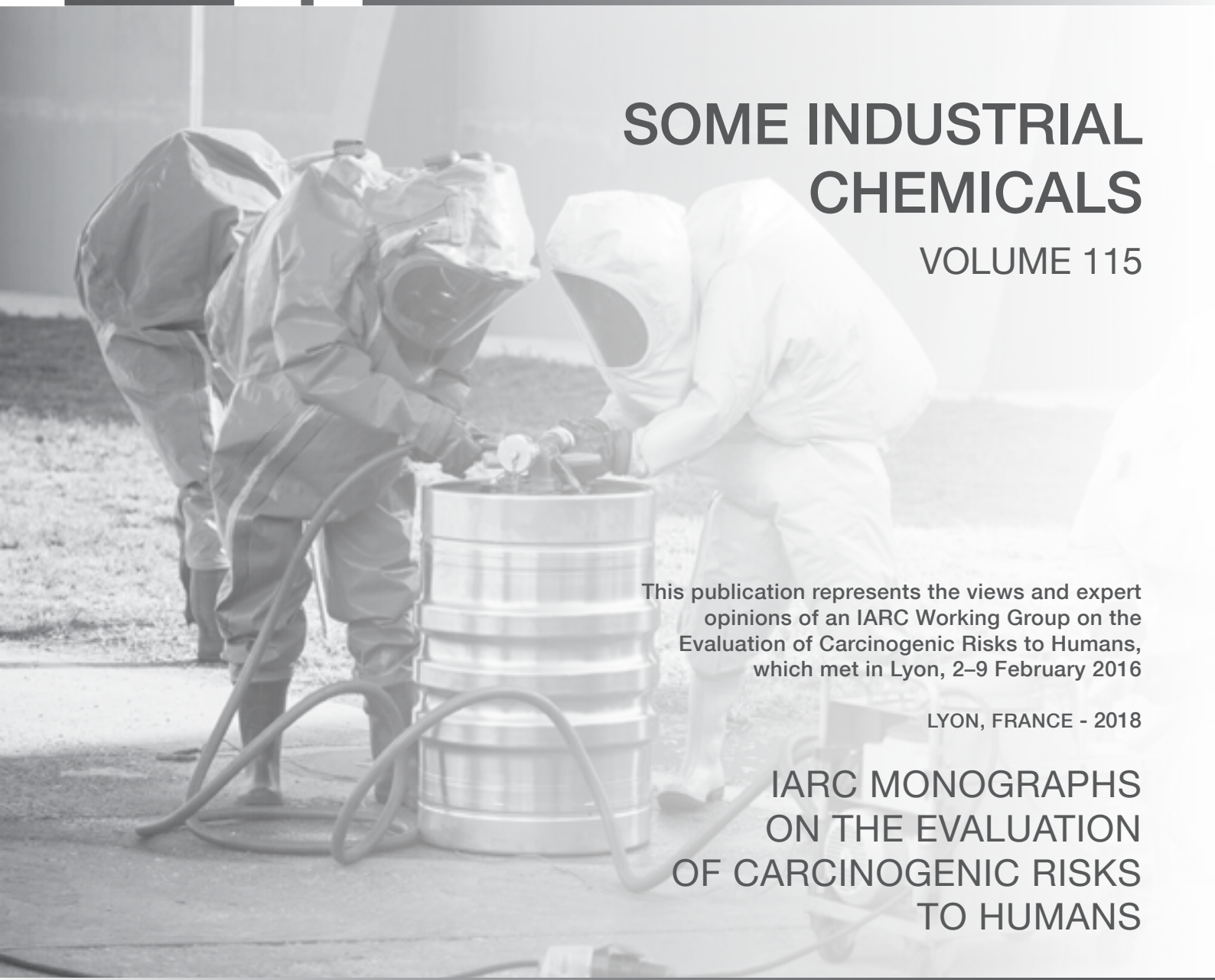
SOME INDUSTRIAL CHEMICALS

VOLUME 115

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS



SOME INDUSTRIAL CHEMICALS

VOLUME 115

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 2–9 February 2016

LYON, FRANCE - 2018

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

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IARC MONOGRAPHS

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

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

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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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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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[4] Deborah Glass receives significant research funding from the Australian Institute of Petroleum Ltd, Australia for the Health Watch cohort.

[5] Tom Sorahan received significant research funding for a study of European rubber workers from the European Tyre and Rubber Manufacturers Association (ETRMA) and paid via the International Prevention Research Institute (IPRI), France; and for a study of oil refinery and petroleum distribution workers from the UK Energy Institute, a trade association.

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[6] Michele Bisson attended as a Representative of the National Competence Centre for Industrial Safety and Environmental Protection (INERIS), France.
[7] Each Observer agreed to respect the Guidelines for Observers at IARC Monographs meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a Monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[8] Susan Borghoff attended as an Observer for the American Chemistry Council's North American Flame Retardant Alliance. She is employed by ToxStrategies, a consulting firm that has provided research services related to tetrabromobisphenol A.
[9] Wolfgang Dekant attended as an Observer for the Bromine Science and Environmental Forum. He has received significant honoraria from the Bromine Science Environmental Forum and the American Chemistry Council for preparation of reviews on tetrabromobisphenol A.
[10] Pinar Erkekoglu attended as an Observer for Hacettepe University, Turkey
[11] Sylvia Jacobi attended as an Observer for Albemarle Europe SPRL. She is employed by and holds stock in Albermarle Europe, which manufactures 1-bromopropane and tetrabromobisphenol A.
[12] Orit Manor attended as an Observer for ICL-Industrial Products. She is employed by ICL-Industrial Products, a company producing tetrabromobisphenol A.
[13] Valentin Thomas attended as an Observer for the University of Paris Dauphine, France.

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IARC would like to dedicate this Monograph to Jane Mitchell (1952–2017), who was the Technical Editor for the present volume of the *IARC Monographs*. Jane joined IARC as a clerk-stenographer in 1976 and remained until 2007, when she was a technical assistant performing editing for the *IARC Monographs* programme, and subsequently continued her relationship with IARC as a freelance technical editor until 2017.

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-fifteenth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of seven industrial chemicals (*N,N*-dimethylformamide, 2-mercaptobenzothiazole, hydrazine, tetrabromobisphenol A, 1-bromopropane, 3-chloro-2-methylpropene, and *N,N*-dimethyl-*p*-toluidine) to which workers or the general population are or can be potentially exposed. All seven agents were accorded high or medium priority for evaluation in the *IARC Monographs* programme by an Advisory Group that met in 2014 (Straif et al., 2014). 3-Chloro-2-methylpropene was evaluated previously in Volume 63 of the *IARC Monographs* (IARC, 1995) as *not classifiable as to its carcinogenicity to humans* (Group 3). Hydrazine was evaluated previously as *possibly carcinogenic to humans* (Group 2B) and *N,N*-dimethylformamide as *not classifiable as to its carcinogenicity to humans* (Group 3) in Volume 71 of the *IARC Monographs* (IARC, 1999). Since the previous evaluations, new data have become available. A summary of the findings of this volume appears in *The Lancet Oncology* (Grosse et al., 2016).

High production volume chemicals and exposure measurement data

Four "high production volume" chemicals (*N,N*-dimethyl-*p*-toluidine, 2-mercaptobenzothiazole, tetrabromobisphenol A, and *N,N*-dimethylformamide) were evaluated. The Working Group noted that for two of these (*N,N*-dimethyl-*p*-toluidine and 2-mercaptobenzothiazole) no data on exposure measurement were available in occupational settings or the general population.

Data on exposure measurement were also lacking for two other chemicals evaluated in the present volume (3-chloro-2-methylpropene and hydrazine).

Isoniazid

Hydrazine is a metabolite of isonicotinic acid hydrazide (isoniazid), which was evaluated in Supplement 7 of the *IARC Monographs* as *not classifiable as to its carcinogenicity to humans* (Group 3) (IARC, 1987). Data on hydrazine and its metabolites from studies on isoniazid and isoniazid metabolites are included in Section 4 of the monograph on hydrazine, but isoniazid was not re-evaluated.

Disruption of the thyroid hormone pathway

The regulation of thyroid hormone action is perhaps more complex than the regulation of action of other hormones that act on nuclear receptors. This is because the predominant hormone in the blood, thyroxine (T_4), must be actively transported across membranes. In the central nervous system, T_4 must be taken up by glial cells, converted to T_3, and then be transported to neurons (Bernal et al., 2015). The conversion of T_4 to T_3 itself represents another regulated step in the control of thyroid hormone action. This complexity is difficult to account for when considering the impact of environmental chemicals such as tetrabromobisphenol A on thyroid hormone action. In addition, the effect of chemicals on the thyroid hormone receptor itself probably depends on the receptor isoform (TRα or TRβ), as well as the DNA motif to which it is bound (Gilbert et al., 2012). This is important because the measures of thyroid "disruption" in humans are a measure of hormone levels in the blood, which may not faithfully capture the impact of chemical exposures on thyroid hormone action (Bansal et al., 2014). Moreover, while the concentration of thyroid hormone in the blood is maintained within a relatively narrow range under normal conditions, there is significant variation among individuals in the set-point around which hormone levels are maintained (Andersen et al., 2002, 2003). Thus it is to be expected that there will be variation between studies – in humans and in vitro – employing different cell lines, different receptor isoforms, and different strategies for evaluating the impact of a particular chemical on the thyroid system. Finally, and because disruption of pathways involving the thyroid hormone receptor is closely associated with the development of specific cancers (Rosen & Privalsky, 2011), the effects of chemical exposures on thyroid hormone receptor should not be ignored.

Evaluation of data on the mechanisms of cancer

In its evaluation of data on mechanisms of carcinogenesis, the Working Group used the procedures first introduced in Volume 112 of the *IARC Monographs* for assessing the strength of evidence with respect to 10 key characteristics of carcinogens (Smith et al., 2016) and for reviewing data from large-scale toxicity testing programmes (IARC, 2017).

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1-BROMOPROPANE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 106-94-5

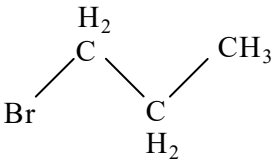
Chem. Abstr. Serv. Name: Propane, 1-bromo

IUPAC Systematic Name: 1-Bromopropane

Synonyms: 1-Propyl bromide; *n*-propyl bromide

Acronyms: 1BP; nPB.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C_3H_7Br

Relative molecular mass: 122.99

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless to pale yellow liquid with a strong, characteristic sweet odour (Merck index, 2013)

Boiling point: 71 °C at 760 mm Hg (Merck index, 2013)

Melting point: –110 °C (Merck index, 2013)

Density: 1.353 at 20 °C (Merck index, 2013)

Vapour density: 4.25 (air = 1) (HSDB, 2016)

Solubility: Poorly soluble in water (2.45 mg/L at 20 °C) (HSDB, 2016); soluble in acetone, ethanol, ether, benzene, chloroform and carbon tetrachloride (HSDB, 2016)

Volatility: Vapour pressure, 110.8 mm Hg at 20 °C (HSDB, 2016)

Stability: Will not polymerize (HSDB, 2016)

Reactivity: Incompatible with strong oxidizing agents, acids and alkalis, alkali metals and finely powdered aluminium (GESTIS, 2015)

Flammability: Neat (pure) solvent, highly flammable liquid and vapour; hazardous when heated or exposed to flame or oxidizers (HSDB, 2016)

Flash point: –10 °C (GESTIS, 2015)

Auto-ignition temperature: 490 °C (HSDB, 2016)

Decomposition: Combustion by-product emits hydrogen bromide (HSDB, 2016)

Octanol/water partition coefficient: log K_{ow}, 2.10 (HSDB, 2016)

Conversion factor (at 25 °C, 760 mm Hg): 1 ppm = 5.03 mg/m^3.

1.1.4 Impurities

Production of commercial-grade 1-bromopropane produces low levels of a contaminant (0.1–0.2%), 2-bromopropane (i.e. isopropyl bromide; Chem. Abstr. Serv. Reg. No. 75-26-3). Contemporary manufacturing processes, however, result in < 0.1% 2-bromopropane contamination in neat 1-bromopropane solvents (Boekelheide et al., 2004; HSDB, 2016).

1.2 Production and use

1.2.1 Production

(a) Manufacturing process

1-Bromopropane is produced by treating *n*-propanol with bromide in the presence of sulfuric acid; once the propanol is unstable, hydrobromic acid is added and *n*-propyl bromide is flashed from the hot mixture. The resultant product is condensed, neutralized and fractionated. The procedure can be modified by using bromine (gas) together with a reducing agent such as sulfur, sulfur dioxide, phosphorus, or sodium borohydride (Kirk-Othmer, 1978).

(b) Production volume

In 2001, the United Nations Environment Programme (UNEP) projected that manufacture and use of 1-bromopropane would expand because it was being marketed to replace ozone-depleting solvents with high production volume for a range of applications in Asia (China, Japan, and the Republic of Korea), Europe (France, Germany, and the United Kingdom) and the USA (UNEP, 2001). Global production was estimated to be 20 000–30 000 tonnes in 2007 (UNEP, 2010), but specific volumes produced in each country were not available. The United States Environmental Protection Agency (EPA) reported in 2006 that national aggregate production was > 1–10 million pounds [> 450–4500 tonnes] (EPA, 2010). In 2011, the EPA listed the national production volume as > 15.3 million pounds [~7000 tonnes] (EPA, 2012).

1.2.2 Use

1-Bromopropane is a solvent for fats, waxes and resins and is primarily used as a chemical intermediate in the production of pesticides, quaternary ammonium compounds, flavours and fragrances, and pharmaceuticals in closed processes (NTP, 2013). In the mid-to-late 1990s, 1-bromopropane was introduced as a non-toxic, fast-drying solvent that does not leave surface residue for cleaning metals, plastics, and optical, electrical and electronic components (NTP, 2011; NIOSH, 2013). It was marketed as a substitute solvent for ozone-depleting and other solvents such as trichloroethylene, tetrachloroethylene (perchloroethylene) and methylene chloride. 1-Bromopropane is used for vapour degreasing and immersion cleaning, liquid and spray adhesive applications, fabric dry cleaning, and aerosol spray products (Blando et al., 2010; NTP, 2013).

1.3 Measurement and analysis

1.3.1 Detection and quantification

(a) Air monitoring

A few methods that are available for analysing 1-bromopropane in air are described in Table 1.1. In 2003, the United States National Institute for Occupational Safety and Health (NIOSH) issued a validated analytical method for 1-bromopropane in air (NIOSH, 2003a), which prescribes adsorption on activated charcoal, desorption with carbon disulfide (CS_2) and analysis by gas chromatography with flame ionization detection; the limit of detection for NIOSH method 1025 is 1 µg using a 12 L air sample. Occupational Safety and Health Administration (OSHA) method 1017 collects 1-bromopropane on activated charcoal but uses CS_2 (99%) and *N*,*N*-dimethylformamide for desorption and gas chromatography with electron capture detection; it is fully validated with a quantitative limit of 5.9 µg/m^3 for a 12 L air sample (OSHA, 2014).

Table 1.1 Selected methods of analysis for 1-bromopropane and biomarkers of exposure

Sample method	Sample preparation	Assay method	Limit of detection	Reference
1-Bromopropane in air				
NIOSH 1025	Active collection on activated charcoal; flow rate, 0.01–0.2 L/min (12 L); CS_2 desorption	GC/FID	1 µg	NIOSH (2003a)
OSHA 1017	Active collection on activated charcoal; flow rate, 0.05 L/min (12 L); CS_2/DMF, 99:1 (v/v) desorption	GC/ECD	5.9 µg/m^3	OSHA (2014)
OSHA PV2061	Active collection on activated charcoal; flow rate, 0.1 L/min (12 L); CS_2 desorption	GC/FID	37 µg/m^3	OSHA (1999)
IRSST 333-1	Active collection on activated charcoal; flow rate, 0.2 L/min (5 L); desorption NR	GC/FID	54 µg	IRSST (2015)
NR	Diffusive sampler; carbon cloth KF-1500; CS_2 desorption	GC	0.1 ppm	Kawai et al. (2001)
NR	Diffusive sampler; CS_2 desorption	GC/EID	0.13 ppm	Ichihara et al. (2004a)
1-Bromopropane in urine				
NR	Headspace collection; 5 mL urine into 20 mL vial; heated at 60 °C for 60 min	GC	2 µg/L	Kawai et al. (2001)
NR	Headspace collection; 5 mL urine into 20 mL vial; heated at 42 °C for 15 min; Tenax GC trap	GC/EID	0.5 ng/L	Ichihara et al. (2004a)
Bromide ion in urine				
NR	48 h, 7 interval composite specimens; nitric acid rinsed bottles; stored below −60 °C	ICP/MS	100 µg/L	Allain et al. (1990), Hanley et al. (2006, 2010)
AcPrCys in urine				
NR	Solid phase extraction (C18) column; methanol/water (40:60) wash; acetone elution	LC/ESI-MS	0.01 µg/L	Hanley et al. (2009) Cheever et al. (2009)
NR	1 mL urine in 1 mL ammonium formate buffer; pH adjusted to 2.4–2.6 with formic acid	LC/MS-MS	2 µg/L	Eckert & Göen (2014)
NR	Urine dissolved in NaOH; mixed in ethanol; acidified to pH 3 with H_3PO_4; ethyl acetate extraction; column chromatography with 2% methanol in ethyl acetate	LC/MS-MS	NR	Valentine et al. (2007)
GSPrCys adducts				
NR	Urine dissolved in NaOH; stirred with 1-bromopropane in ethanol; pH adjusted to 3 with HCl; washed with ice water and ethanol	LC/MS-MS	2.5 pmol	Valentine et al. (2007)

AcPrCys, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine; CS_2, carbon disulfide; DMF, *N*,*N*-dimethylformamide; ECD, electron capture detection; EID, electron ionization detector; ESI, electrospray ionization; FID, flame ionization detector; GC, gas chromatography; GSPrCys, globin-*S*-propylcysteine; HCl, hydrochloric acid; H_3PO_4, phosphoric acid; ICP/MS, inductively coupled plasma/mass spectrometry; LC, liquid chromatography; LC/MS-MS, liquid chromatography-tandem mass spectrometry; MS, mass spectrometry; NaOH, sodium hydroxide; NR, not reported

(b) Biomarkers

Table 1.1 also contains details for laboratory methods of analysis of 1-bromopropane and biomarkers of exposure that include 1-bromopropane in urine, bromide ion in urine, and 1-bromopropane metabolites: *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) and *S*-propylcysteine adducts on globin in urine.

Using high-performance liquid chromatography with mass spectrometry, Cheever et al. (2009) isolated several mercapturic acid conjugates from highly exposed workers who used 1-bromopropane spray adhesives, the most abundant of which was propyl mercapturic acid (i.e. AcPrCys). AcPrCys was also associated with lower exposure to 1-bromoproopane among workers exposed to vapour degreasing solvents (Hanley et al., 2010). AcPrCys is more sensitive and specific than bromide because non-occupational exposure to bromide could occur from diet and medications (Hanley et al., 2009). Valentine et al. (2007) studied the utility of globin-*S*-propylcysteine (GSPrCys) and AcPrCys as biomarkers of exposure to 1-bromopropane in the urine of workers exposed at a factory producing 1-bromopropane. A significant increase in GSPrCys was found in workers exposed to 1-bromopropane compared with controls, and urinary AcPrCys levels increased with increased levels of exposure to 1-bromopropane in the air, supporting the hypothesis that these biomarkers can potentially be used to evaluate the exposure of humans to 1-bromopropane.

1.4 Occurrence and exposure

1.4.1 Natural occurrence

1-Bromopropane was detected qualitatively in six species of marine algae that produce it naturally (HSDB, 2016).

1.4.2 Environmental occurrence

Fugitive emissions of 1-bromopropane from industrial and commercial settings are possible from manufacturer, formulator and user sites and from aerosol products used by public consumers. No quantitative data for 1-bromopropane measured in the environment were available to the Working Group.

1.4.3 Occupational exposures

Several studies of occupational exposure were available in the literature that describes data on human exposure to 1-bromopropane reported from industrial and commercial facilities (Table 1.2). Occupational exposure may occur by inhalation and dermal absorption (NIOSH, 2009; Frasch et al., 2011a; NTP, 2013), particularly if the skin is occluded (Frasch et al., 2011b). Dermal exposure can be a significant source of 1-bromopropane absorption and most common glove and chemical protective materials do not provide adequate skin protection (Hanley et al., 2009, 2010; NIOSH, 2013). Facilities reported in the literature include: (a) chemical manufacturing; (b) spray adhesives in foam cushion fabricators; (c) vapour and immersion cleaning activities; (d) aerosol products; and (e) commercial dry cleaning.

(a) Chemical manufacturing

Using passive (diffusion) monitoring, exposure to 1-bromopropane ranging from non-detectable to 170 ppm were reported in chemical manufacturing facilities in China (Ichihara et al., 2004a, b, 2006).

In another chemical manufacturer in China, the exposures determined by passive air samplers (activated carbon cloth) ranged from 0.07 to 106 ppm for women, and from 0.06 to 115 ppm for men (Li et al., 2010).

In an adhesives manufacturing facility, Hanley et al. (2010) reported on urinary bromide and AcPrCys concentrations (mg/g creatinine)

Table 1.2 Occupational exposure to 1-bromopropane in industrial and commercial facilities

Industry, country, year	Job/process	Concentration, mean (range)			Comments[a]	Reference
		Air[a] (ppm)	Blood (mEq/L)[b]	Urine (mg/g creatinine)[b]		
Chemical synthesis						
China, NR	NR, women	NR (0.9–170.5)	NR	NR	*n* = 24; 1BP in air, BZ, passive (diffusion) monitoring	Ichihara et al. (2004a)
China, NR	NR, men	NR (ND–43.3)	NR	NR	*n* = 13; 1BP in air, BZ, passive (diffusion) monitoring	Ichihara et al. (2004a)
China, NR	NR, women	2.92 (0.34–49.19)	NR	NR	*n* = 23; 1BP in air, BZ, passive (diffusion) monitoring	Ichihara et al. (2004b)
China, NR	NR, women	NR (0.07–106.3)	NR	NR	*n* = 60; 1BP in air, BZ, passive (diffusion) monitoring	Li et al. (2010)
China, NR	NR, men	NR (0.06–114.8)	NR	NR	*n* = 26; 1BP in air, BZ, passive (diffusion) monitoring, 8–12-h TWA	Li et al. (2010)
China, NR	NR	15.3 (0.65–73.7)	NR	NR	*n* = 40; 1BP in air, BZ, passive (diffusion) monitoring, 8–12-h TWA	Ichihara et al. (2006)
Adhesive manufacture						
USA, 2004	Direct user	3.8 (0.26–19)	NR	Br: 4.51 (2.37) AcPrCys: 0.485 (2.30)	Air: *n* = 6, worker–days; urine: *n* = 3; 48-h urine specimens, GM (GSD)	Hanley et al. (2010)
	Non-user	0.33 (0.07–1.6)	NR	Br: 2.01 (1.42) AcPrCys: 0.128 (4.51)	Air: *n* = 16, worker–days; urine: *n* = 8; 48-h urine specimens; GM (GSD)	
Spray adhesives						
China, 2002	NR	133 (60–261)	NR	NR	*n* = 11; AM	Ichihara et al. (2002)
USA, 1998	Sprayers	193 (115.3–250.7)	NR	NR	*n* = 15; initial; AM	NIOSH (2002a)
USA, 1998	Saw	117.1 (85.1–159.2)	NR	NR	*n* = 12; initial; AM	
USA, 1998	Covers	197.0 (117.3–381.2)	NR	NR	*n* = 21; initial; AM	
USA, 1998	Assembly	169.8 (60.0–250.7)	NR	NR	*n* = 36; initial; AM	
USA, 1998	All departments	168.9 (60.0–381.2)	NR	NR	*n* = 69; initial; AM	
USA, 2000	Saw	1.8 (1.6–2.0)	NR	NR	*n* = 6; follow-up; AM	
USA, 2000	Covers	29.2 (2.8–58.0)	NR	NR	*n* = 12; follow-up; AM	
USA, 2000	Assembly	18.8 (6.1–32.0)	NR	NR	*n* = 11; follow-up; AM	
USA, 2000	All departments	19.0 (1.2–58)	NR	NR	*n* = 30; follow-up; AM	
USA, 2000	Sewing	1.2	NR	NR	*n* = 1; follow-up; AM	
USA, 2000	Sprayers	NR (12.3–95.8)	NR	NR	*n* = 12; follow-up; 15-min STEL	

Table 1.2 (continued)

Industry, country, year	Job/process	Concentration, mean (range)			Comments[a]	Reference
		Air[a] (ppm)	Blood (mEq/L)[b]	Urine (mg/g creatinine)[b]		
USA, 2000	Sprayers	65.9 (41.3–143.0)	NR	NR	*n* = 12; TWA; initial; GM	NIOSH (2002b)
USA, 2000	Sprayers	NR (33.7–173.9)	NR	NR	*n* = 9; 15-min STEL; initial; GM	
USA, 2000	Non-sprayers	NR (6.3–14.1)	NR	NR	*n* = 2; TWA; initial	
USA, 2001	Sprayers	16.6 (8.8–31.9)	NR	Br: 7.7 (2.5–38.0)	*n* = 12; TWA; follow-up; urine (mg/L); GM	
USA, 2001	Non-sprayers	NR (1.1–5.8)	NR	NR	*n* = 10; TWA; follow-up; GM	
USA, 1999	All exposed	81.2 (18.1–253.9)	NR	NR	*n* = 16; TWA; initial; GM	NIOSH (2003b)
USA, 1999	Sprayers	107.6 (57.7–253.9)	NR	NR	*n* = 12; TWA; initial; GM	
USA, 1999	Sprayers	101.4 (38.0–280.5)	NR	NR	*n* = 8; TWA; follow-up; GM	
USA, 2003 USA, 2007	Sprayers	130 (91–176) 108 (92–127)	Br: 107 (AM) (44–170) Reference (Br) = 0–40	NR	Air: TWA-actual; t = 7 h; blood: serum Br (mg/dL), *n* = 6 Air: 8-h TWA	Majersik et al. (2007)
USA, 2001	Sprayers	63.45 (4.3–271.4)	Br: 13.6 (0.62–43.5)	Br: 153 (ND–595.4)	*n* = 19; blood: serum Br (mg/dL); urinary Br (mg/dL); end-of-week specimens; AM	Toraason et al. (2006)
	Unexposed	1.7 (ND–10.4)	Br: 1.48 (ND–4.6)	Br: 16.6 (0.27–42.2)	*n* = 45; blood: serum Br (mg/dL); urinary Br (mg/dL); end-of-week specimens; AM	
USA, 2007	Sprayers All jobs	107 (58–254) 81 (18–254)	7.1 (AM) (3.0–12.5)	NR	Air: 9 months after hospital cases by NIOSH, *n* = 12 (sprayers), *n* = 16 (all jobs); blood: *n* = 4 patients	Raymond & Ford (2007)
USA, 2006	Sprayers	92 (45–200)	NR	Br: 195 (119–250) AcPrCys: 41.1 (22–127)	Air: *n* = 26, worker–days; urine: *n* = 13; 48-h urine specimens: GM	Hanley et al. (2006, 2009)
	Non-sprayers	11 (0.6–60)	NR	Br: 42.9 (5.5–149) AcPrCys: 10.2 (1.23–81.5)	Air: *n* = 34, worker–days; urine: *n* = 17; 48-h urine specimens	
	Controls	NR	NR	Br: 3.8 (2.6–5.9) AcPrCys: 0.024 (ND–0.318)	Air: *n* = 0; urine: *n* = 7 (Br), *n* = 21 (AcPrCys); single "spot" urine specimens	

Table 1.2 (continued)

Industry, country, year	Job/process	Concentration, mean (range)			Comments[a]	Reference
		Air[a] (ppm)	Blood (mEq/L)[b]	Urine (mg/g creatinine)[b]		
Vapour degreasing and immersion cleaning						
Painting workshop, Japan, NR	NR	1.42 NR (ND–27.8)	NR	NR	Air: *n* = 33	Kawai et al. (2001)
Electronic plant, Pennsylvania, USA, 2007	Direct user	178	Br: 48 mg/dL	NR	Air: *n* = 1 case hospitalized for symptoms of neurotoxic effects	CDC (2008)
USA, 2004	Near degreaser	2.6 (0.08–21)	NR	Br: 8.94 (1.69–115) AcPrCys: 1.7 (0.02–32.1)	Air: *n* = 44, worker–days; urine: *n* = 22; 48-h urine specimens: GM	Hanley et al. (2010)
	Away from degreasers	0.31 (0.08–1.7)	NR	Br: 3.74 (1.69–15.6) AcPrCys: 0.13 (0.007–1.88)	Air: *n* = 18, worker–days; urine: *n* = 9; 48-h urine specimens; GM	
	Controls	NR	NR	Br: 2.01 (0.90–3.55) AcPrCys: 0.024 (ND–0.318)	Urine: *n* = 21; single "spot" urine specimens; GM	
Golf club cleaning, Taiwan, China, 2013	NR	128.8 (97.3–188.6)	NR	AcPrCys: NR (0.171–2.71)	Air: *n* = 3, area samples – above wash tank, passive (diffusion) sampling; urine AcPrCys measured between 1–26 days after exposure, *n* = 6	Wang et al. (2015)
Unspecified metal cleaning, Japan, NR	NR	553 (353–663)	Br: 58 and 20 µg/mL	NR	1 case of severe neurotoxicity; air: *n* = NR; blood: serum Br measured 2 and 4 months after exposure, normal level, < 5 µg/mL, *n* = 1	Samukawa et al. (2012)
Aerosol products						
Textile manufacture, Canada, 2000	Cleaners	NR (8.5–23.5)	NR	NR	*n* = 4	Mirza et al. (2000)
Use, locations and dates NR	NR	NR (5.0–30.2)	NR	NR	*n* = 8; 8-h TWA; most sampling by passive (diffusion) badges	EPA (2002)
	NR	NR (45.1–254.0)	NR	NR	15-min STEL	

Table 1.2 (continued)

Industry, country, year	Job/process	Concentration, mean (range)			Comments[a]	Reference
		Air[a] (ppm)	Blood (mEq/L)[b]	Urine (mg/g creatinine)[b]		
Dry-cleaning fabrics						
USA, 2006	Facility 1, operator	40 (23–56)	NR	NR	*n* = 2	NIOSH (2008)
	Facility 1, cashier	17 (10–24)	NR	NR	*n* = 2	
	Facility 2, operator	7.2	NR	NR	*n* = 1; ~4-h TWA; t = 209 min	
	Facility 2, cashier	1.5	NR	NR	*n* = 1; ~4-h TWA; t = 212 min	
	Facility 3, operator	11	NR	NR	*n* = 1; ~4-h TWA; t = 163 min	
	Facility 4, operator	160	NR	NR	*n* = 1; ~4-h TWA; t = 241 min	
	Facility 4, cashier	2.4	NR	NR	*n* = 1; ~4-h TWA; t = 246 min	
USA, 2006	Shop A, operator	NR (12.7–54.55)	NR	NR	*n* = 4; 8-h TWA	Blando et al. (2010)
	Shop A, clerk	NR (8.31–21.85)	NR	NR	*n* = 4; 8-h TWA	
	Shop B, operator	41.65	NR	NR	*n* = 1; 8-h TWA	
	Shop B, clerk	0.65	NR	NR	*n* = 1; 8-h TWA	
	Shop C, operator	0.24 (ND–0.35)	NR	NR	*n* = 3; 8-h TWA	

[a] Reported as breathing zone, full-shift time-weighted average, and geometric mean unless otherwise noted.
[b] Unless indicated otherwise
1BP, 1-bromopropane; AcPrCys, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine; AM, arithmetic mean; Br, bromide ion; BZ, breathing zone; GM, geometric mean; GSD, geometric mean standard deviation; ND, not detected; NR, not reported; STEL, short-term exposure level; t, sampling time; TWA, full-shift time-weighted average

among workers who directly used 1-bromopropane or were indirectly exposed by working in adjacent vicinities. Worker exposures ranged from 0.26 to 19 ppm for those employees directly using 1-bromopropane and from 0.07 to 1.6 ppm for those that did not use 1-bromopropane. The geometric mean (GM) for two consecutive days of air monitoring was 3.8 ppm and 0.33 ppm for users and non-users of 1-bromopropane, respectively.

(b) Spray adhesives in foam cushions

Several publications were available for 1-bromopropane spray adhesives used in foam-fabricating plants where seat cushions were assembled. Adhesive is applied to foam and cloth using compressed air spray guns. Once the foam parts are glued, the workers used bare hands to align and press the pieces together and shape edges; hence skin contact contributed to the absorbed dose for sprayers.

At a foam cushion plant where 1-bromopropane spray adhesives were used, 1-bromopropane exposure concentrations ranged from 60 to 261 ppm, as estimated by passive sampling; the mean exposure was 133 ppm (Ichihara et al., 2002).

The NIOSH conducted health hazard evaluation (HHE) site investigations at three independent foam fabricating facilities (NIOSH, 2002a, b, 2003b) which included initial and follow-up site surveys. At the first plant (NIOSH, 2003b), the initial mean concentration of 1-bromopropane was 81 ppm for sprayers and other workers in spraying areas (range, 18–254 ppm); the mean for sprayers was 108 ppm. Some minor maintenance was performed on the general ventilation system before the return survey. Although the GM exposure to 1-bromopropane was lowered for all exposed jobs combined (GM, 46 ppm; 7.2–281 ppm), the exposures of sprayers remained essentially the same (mean, 101 ppm; range, 38–280 ppm) because no local exhaust ventilation was installed at their work stations.

The initial exposure levels to 1-bromopropane at the second plant (NIOSH, 2002a) were 170 ppm (GM) and ranged from 60 to 381 ppm. Differences in mean exposure were found between departments (i.e. covers, 197 ppm; assembly, 170 ppm; saw, 117 ppm). After implementing engineering controls (i.e. spray booth enclosures, local and general ventilation improvements), the follow-up evaluation showed a substantial reduction in the exposures of workers to 1-bromopropane with a mean of 19 ppm (range, 1.2–58 ppm).

The initial exposures of sprayers to 1-bromopropane at the third foam cushion factory (NIOSH, 2002b) ranged from 41 to 143 ppm, with a GM of 66 ppm. This facility also made modifications to local and general exhaust ventilation and exposures to 1-bromopropane were substantially reduced at the follow-up survey (mean, 16.6 ppm; range, 8.8–32 ppm).

Start-of- and end-of-work week blood and urine samples were collected from 41 and 22 workers at two facilities where 1-bromopropane spray adhesives were used for foam cushion fabrication (Toraason et al., 2006). Bromide was measured in the blood and urine as a biomarker of exposure. Overall, the air concentrations of 1-bromopropane ranged from 0.2 to 271 ppm at facility A and from 4 to 27 ppm at facility B, and the highest exposures were measured in workers classified as sprayers. 1-Bromopropane concentrations were statistically significantly correlated with individual blood and urine bromide concentrations. Serum and urine bromide levels of up to 43.5 and 595.4 mg/dL, respectively, were measured for sprayers.

Majersik et al. (2007) published a case study of six patients who worked at a foam cushion factory where glue containing 1-bromopropane was sprayed. The mean exposure to 1-bromopropane at this plant was 130 ppm (range, 91–176 ppm) collected over a 7-hour sampling period. Serum bromide levels were elevated

(107 mg/dL), ranging from 44 to 170 mg/dL (reference, 0–40 mg/dL).

Hanley et al. (2006, 2009) conducted an exposure assessment study using air sampling and measurement of urinary metabolites at two facilities using 1-bromopropane adhesives to construct polyurethane foam seat cushions. Complete 48-hour urine specimens were obtained from 30 workers on two consecutive days and were collected into composite samples representing three time intervals: at work, after work, but before bedtime, and upon awakening. GM breathing zone concentrations of 1-bromopropane were 92 ppm for adhesive sprayers and 11 ppm for other jobs. For sprayers, urinary bromide concentrations ranged from 77 to 542 mg/g creatinine at work, from 58 to 308 mg/g creatinine after work and from 46 to 672 mg/g creatinine in wake-up samples. Pre-week urinary bromide concentrations for sprayers were substantially higher than those for the non-sprayers and controls, with GMs of 102, 31, and 3.8 mg mg/g creatinine, respectively. Correlation of 48-hour urinary bromide with exposure to 1-bromopropane was statistically significant ($r^2 = 0.89$) for all jobs combined. The GM AcPrCys concentrations were 41 mg/g creatinine ($P < 0.05$) for sprayers, 10 mg/g creatinine ($P < 0.01$) for non-sprayers and 0.024 mg/g creatinine for controls.

(c) Vapour degreasing and immersion cleaning

In a Japanese painting workshop that used 1-bromopropane to clean parts, Kawai et al. (2001) evaluated the concentrations of 1-bromopropane in the air using passive samplers. The GM was 1.42 ppm with a maximum concentration of 27.8 ppm.

Hanley et al. (2010) measured the levels of 1-bromopropane in the air and those of bromide and AcPrCys in the urine and found GM breathing zone concentrations of 2.6 and 0.31 ppm for workers near degreasers and those furthest from degreasers, respectively. Urinary metabolites (mg/g creatinine) showed the same trend, with higher levels observed in workers near degreasers (48-hour GM bromide, 8.9 versus 3.7; 48-hour GM AcPrCys, 1.7 versus 0.13, respectively).

In a metal-cleaning facility, 1-bromopropane concentrations ranged from 353 to 663 ppm, with a mean of 553 ppm, and an elevated level of 58 µg/mL serum bromide was found in one worker (normal level, < 5 µg/mL) (Samukawa et al., 2012).

The mean 1-bromopropane concentration above a degreasing tank used for cleaning golf clubs was 129 ppm (range, 97–189 ppm). Levels of AcPrCys, collected 1–26 days after exposure were reported to range from 0.17 to 2.7 mg/g when normalized with creatinine (Wang et al., 2015).

(d) Aerosol products

Aerosol spray products are typically used intermittently for short periods and spray emissions often are not regulated by engineering controls (NTP, 2013). In a study to assess exposures from aerosol cleaning, 1-bromopropane concentrations ranged from 8.5 to 23.5 ppm (Mirza et al., 2000).

In aerosol samples from eight exposed workers, the full-shift concentration of 1-bromopropane ranged from 5 to 30 ppm (EPA, 2002).

(e) Dry cleaning

NIOSH (2008) conducted an evaluation at four dry-cleaning shops in the USA. At one facility, exposure to 1-bromopropane was 40 ppm for the machine operator and 17 ppm for the cashier clerk. At the other dry-cleaning shops, partial shift exposures to 1-bromopropane were 7.2 (209 minutes), 11 (163 minutes) and 160 (241 minutes) ppm for the operators typically working in the laundry room, and 1.5 (212 minutes) and 2.4 (246 minutes) ppm for cashiers working in the front of the store.

Blando et al. (2010) conducted follow-up investigations at the same four dry-cleaning laundries. The concentrations of 1-bromopropane were in the range of non-detectable to 55 ppm for operators in the laundries and 0.6–22 ppm for cashier clerks.

1.4.4 Exposure of the general population

No data were available to the Working Group.

1.5 Regulations and guidelines

The State of California Department of Industrial Relations has promulgated an 8-hour time-weighted average (TWA) permissible exposure limit for 1-bromopropane of 5 ppm [25 mg/m^3] with a skin notation (CA DIR, 2009). The state of California has also listed 1-bromopropane as a developmental hazard under proposition 65 of the California Clean Water Act (CA EPA, 2008). The Canadian Ministry of Health issued an 8-hour TWA occupational exposure limit for 1-bromopropane of 10 ppm [~50 mg/m^3] (GESTIS, 2015). In 2012, the Japan Society for Occupational Health proposed to set an occupational exposure limit for 1-bromopropane of 0.5 ppm (2.5 mg/m^3) (JSOH, 2015). In 2013, the American Conference of Governmental Industrial Hygienists adopted a TWA threshold limit value of 0.1 ppm short-term exposure limit with an A3 note "known animal carcinogen with unknown relevance to humans" (HSDB, 2016). In the 13th Report on Carcinogens, the United States National Toxicology Program (NTP) classified 1-bromopropane as "reasonably anticipated to be a human carcinogen" (NTP, 2013).

In November 2012, in accordance with Article 57 and 59 of the European Committee regulation 1907/2006, the European Chemical Agency identified 1-bromopropane as a substance of very high concern due to the risk of reproductive toxicity (ECHA, 2012). The European Chemical Bureau has labelled 1-bromopropane as H360FD "may damage fertility or the unborn child", H319 "causes serious eye irritation", H315 "causes skin irritation", H373 "may cause damage to organs from prolonged and repeated exposures", H335 "may cause irritation" and H336 "may cause drowsiness or dizziness" (ECHA, 2016).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Inhalation

One well-conducted good laboratory practice study evaluated the carcinogenicity of 1-bromopropane (NTP, 2011). Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were exposed by inhalation to 1-bromopropane (purity, > 99.9%) at a dose of 0, 62.5, 125, or 250 ppm for 6 hours and 10 minutes per day on 5 days per week for 105 weeks. No effect on survival or body weights was observed.

In females, treatment with 1-bromopropane resulted in a significant increase in the incidence of lung alveolar/bronchiolar adenoma or carcinoma (combined) at all doses: 1/50 controls, 9/50 at 62.5 ppm ($P = 0.010$ by the pairwise Poly-3 test), 8/50 at 125 ppm ($P = 0.016$ by the pairwise Poly-3 test), and 14/50 at 250 ppm ($P < 0.001$ by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend in the incidence of lung alveolar/bronchiolar adenoma or carcinoma (combined) using the Poly-3 test ($P < 0.001$). Treatment with 1-bromopropane at a dose of 250 ppm also significantly increased the incidence of lung alveolar/bronchiolar adenoma:

Table 3.1 Studies of carcinogenicity with 1-bromopropane in experimental animals exposed by inhalation

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals/group No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (F) 5–6 wks 105 wks NTP (2011)	Purity, > 99.9% 0, 62.5, 125 or 250 ppm, 6 h and 10 min/day, 5 days/wk 50/group 36, 40, 37, 42	*Lung, alveolar/bronchiolar* Adenoma: 1/50, 6/50, 4/50, 10/50 Carcinoma: 0/50, 7/50, 5/50, 4/50 Adenoma or carcinoma (combined): 1/50, 9/50, 8/50, 14/50	 Trend test: $P = 0.007$ (Poly-3 test) Pairwise: $P = 0.006$ for 250 ppm (one-sided Poly-3 test) Pairwise: $P = 0.009$ and $P = 0.031$ for 62.5 and 125 ppm, respectively (one-sided Poly-3 test) Trend test: $P < 0.001$ (Poly-3 test) Pairwise: $P = 0.010$, $P = 0.016$, $P < 0.001$ for 62.5, 125, 250 ppm, respectively (one-sided Poly-3 test)	Principal strengths of the study: covered most of the lifespan, multiple dose study; large numbers of animals per group and GLP
Mouse, $B6C3F_1$ (M) 5–6 wks 105 wks NTP (2011)	Purity, > 99.9% 0, 62.5, 125 or 250 ppm, 6 h and 10 min/day, 5 days/wk 50/group 37, 33, 32, 36	*All organs, all sites* None	 NS	Principal strengths of the study: covered most of the lifespan, multiple dose study; large numbers of animals per group and GLP No neoplastic effects
Rat, F344 (M) 5–6 wks 105 wks NTP (2011)	Purity, > 99.9% 0, 125, 250, or 500 ppm, 6 h and 10 min/day, 5 days/wk 50/group 23, 26, 18, 13	*Large intestine (rectum or colon)* Adenoma: 0/50, 0/50, 2/50, 1/50 *Skin* Keratoacanthoma: 0/50, 3/50, 6/50, 6/50 Keratoacanthoma or squamous cell carcinoma (combined): 1/50, 4/50, 6/50, 8/50 Keratoacanthoma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma (combined): 1/50, 7/50, 9/50, 10/50	 NS Trend test: $P = 0.008$ (Poly-3 test) Pairwise: $P = 0.012$, $P = 0.010$ for 250, 500 ppm, respectively (one-sided Poly-3 test) Trend test: $P = 0.006$ (Poly-3 test) Pairwise: $P = 0.044$, $P = 0.009$ for 250, 500 ppm, respectively (one-sided Poly-3 test) Trend test: $P = 0.003$ (Poly-3 test) Pairwise: $P = 0.028$, $P = 0.006$, $P = 0.002$ for 125, 250, 500 ppm, respectively (one-sided Poly-3 test)	Principal strengths of the study: covered most of the lifespan, multiple dose study; large number of animals per group, and GLP Incidence of adenoma of the large intestine (rectum or colon) in historical controls: 0/349 (inhalation); 2/1398 (all routes)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals/group No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 5–6 wks 105 wks NTP (2011) (cont.)		*Multiple organs* Malignant mesothelioma: 0/50, 2/50, 2/50, 4/50	Trend test: $P = 0.031$ (Poly-3 test) Pairwise: $P = 0.046$ for 500 ppm (one-sided Poly-3 test)	Malignant mesothelioma was found in the epididymis in all affected animals, with other tissues variably affected; rate in historical controls: 5/349 (inhalation); 35/1398 (all routes)
		Pancreas Islet cell adenoma: 0/50, 5/50, 4/50, 5/50	Trend test: $P = 0.043$ (Poly-3 test) Pairwise: $P = 0.029$, $P = 0.050$, $P = 0.019$ for 125, 250, 500 ppm, respectively (one-sided Poly-3 test)	
		Islet cell carcinoma: 3/50, 7/50, 5/50, 3/50	NS	
		Islet cell adenoma or carcinoma (combined): 3/50, 10/50, 9/50, 8/50	Pairwise: $P = 0.031$, $P = 0.043$ for 125, 250 ppm, respectively (one-sided Poly-3 test)	
Rat, F344/N (F) 5–6 wks 105 wks NTP (2011)	Purity, > 99% 0, 125, 250, or 500 ppm, 6 h and 10 min/day, 5 days/wk 50/group 34, 33, 30, 24	*Large intestine (rectum or colon)* Adenoma: 0/50, 1/50, 2/50, 5/50	Trend test: $P = 0.004$ (Poly-3 test) Pairwise: $P = 0.018$ for 500 ppm (one-sided Poly-3 test)	Principal strengths of the study: covered most of the lifespan, multiple dose study; large number of animals per group, and GLP Incidence of large intestine (rectum or colon) adenoma in historical controls: 2/350 (inhalation); 16/1350 (all routes)
		Skin Squamous cell papilloma, keratocacanthoma, basal cell adenoma or basal cell carcinoma (combined): 1/50, 1/50, 1/50, 4/50	Trend test: $P = 0.050$ (Poly-3 test)	
		Basal cell carcinoma: 0/50, 0/50, 0/50, 1/50	NS	

F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week

1/50 controls, 6/50 at 62.5 ppm, 4/50 at 125 ppm and 10/50 at 250 ppm (P = 0.006 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test (P = 0.007). At doses of 62.5 and 125 ppm, 1-bromopropane significantly increased the incidence of lung alveolar/bronchiolar carcinoma: 0/50 controls, 7/50 at 62.5 ppm (P = 0.009 by the pairwise Poly-3 test), 5/50 at 125 ppm (P = 0.031 by the pairwise Poly-3 test) and 4/50 at 250 ppm.

Treatment with 1-bromopropane did not result in significant increases in tumour incidence, unusual tumours, or early-onset tumours in males. [The strengths of this study included the large numbers of animals, compliance with good laboratory practice, the evaluation of multiple dose levels and a duration of exposure that involved most of the lifespan.]

3.2 Rat

Inhalation

One well-conducted good laboratory practice study evaluated the carcinogenicity of 1-bromopropane (NTP, 2011). Groups of 50 male and 50 female Fischer 344/N rats (age, 5–6 weeks) were exposed by inhalation to 1-bromopropane (purity, > 99.9%) at a dose of 0, 125, 250, or 500 ppm for 6 hours and 10 minutes per day on 5 days per week for 105 weeks. Survival was significantly decreased in male rats exposed to 500 ppm. No effect on body weights was observed.

In males, treatment with 1-bromopropane significantly increased the incidence of skin tumours (keratoacanthoma, basal cell adenoma, basal cell carcinoma and squamous cell carcinoma combined) at all doses: 1/50 controls, 7/50 at 125 ppm (P = 0.028 by the pairwise Poly-3 test), 9/50 at 250 ppm (P = 0.006 by the pairwise Poly-3 test), and 10/50 at 500 ppm (P = 0.002 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test (P = 0.003). The incidence of this combination of skin tumours in historical controls was 19/349 (range, 0–10%) in inhalation studies and 97/1398 (range, 0–20%) in all studies. At doses of 250 and 500 ppm, 1-bromopropane significantly increased the incidence of skin keratoacanthoma: 0/50 controls, 3/50 at 125 ppm, 6/50 at 250 ppm (P = 0.012 by the pairwise Poly-3 test), and 6/50 at 500 ppm (P = 0.010 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test (P = 0.008). The incidence of skin keratoacanthoma in historical controls was 10/349 (range, 0–8%) in inhalation studies and 66/1398 (range, 0–16%) in all studies. At doses of 250 and 500 ppm, 1-bromopropane significantly increased the incidence of skin keratoacanthoma or squamous cell carcinoma (combined): 1/50 controls, 4/50 at 125 ppm, 6/50 at 250 ppm (P = 0.044 by the pairwise Poly-3 test) and 8/50 at 500 ppm (P = 0.009 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test (P = 0.006). The incidence of this combination of skin tumours in historical controls was 11/349 (range, 0–8%) in inhalation studies and 74/1398 (range, 0–16%) in all studies. The incidence of basal cell carcinoma was 0/50 controls, 2/50 at 125 ppm, 1/50 at 250 ppm and 2/50 at 500 ppm; that of squamous cell carcinoma was 1/50 controls, 1/50 at 125 ppm, 0/50 at 250 ppm, and 2/50 at 500 ppm. 1-Bromopropane also induced a non-significant increase in the incidence of large intestine (rectum or colon) adenoma: 0/50 controls, 0/50 at 125 ppm, 2/50 at 250 ppm, and 1/50 at 500 ppm. Although not statistically significant, this increase equalled or exceeded that observed in male historical controls (0/349 in inhalation studies and 2/1398 in all studies; range, 0–2%). Statistical analysis did not detect a positive dose-related trend. A significant increase in the incidence of malignant mesothelioma of the epididymis was also observed: 0/50 controls, 2/50 at 125 ppm, 2/50 at 250 ppm, and 4/50 at 500 ppm (P = 0.046 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test

(P = 0.031). The incidence of malignant mesothelioma in historical controls was 5/349 (range, 0–6%) in inhalation studies, and 35/1398 (range, 0–6%) in all studies. The incidence of pancreatic islet cell adenoma or carcinoma (combined) was also significantly increased: 3/50 controls, 10/50 at 125 ppm (P = 0.031 by the pairwise Poly-3 test), 9/50 at 250 ppm (P = 0.043 by the pairwise Poly-3 test), and 8/50 at 500 ppm. Statistical analysis did not detect a positive dose-related trend. The incidence of this combination of pancreatic islet cell tumours in historical controls was 37/349 (range, 6–18%) in inhalation studies, and 119/1394 (range, 0–18%) in all studies. A significant increase in the incidence of pancreatic islet cell adenoma was also observed at all doses: 0/50 controls, 5/50 at 125 ppm (P = 0.029 by the pairwise Poly-3 test), 4/50 at 250 ppm (P = 0.050 by the pairwise Poly-3 test), and 5/50 at 500 ppm (P = 0.019 by the pairwise Poly-3 test). Statistical analysis detected a positive dose-related trend using the Poly-3 test (P = 0.043). The incidence of pancreatic islet cell adenoma in historical controls was 20/349 (range, 0–12%) in inhalation studies, and 90/1394 (range, 0–14%) in all studies.

In females, treatment with 1-bromopropane resulted in an increase in the incidence of large intestine (rectum or colon) adenoma at all doses: 0/50 controls, 1/50 at 125 ppm, 2/50 at 250 ppm, and 5/50 at 500 ppm (P = 0.018 by the pairwise Poly-3 test). Although statistically significant only at 500 ppm, this increase equalled or exceeded that observed in female historical controls (0/350 in inhalation studies; 3/1350 (range, 0–2%) in all studies). Statistical analysis detected a positive dose-related trend in the incidence of large intestine (rectum or colon) adenoma using the Poly-3 test (P = 0.004). 1-Bromopropane also resulted in a non-significant positive trend (P = 0.050) in the incidence of skin tumours (squamous cell papilloma, keratoacanthoma, basal cell adenoma and basal cell carcinoma combined): 1/50 controls, 1/50 at 125 ppm, 1/50 at 250 ppm, and 4/50 at 500 ppm. Although not statistically significant, the incidence in the high-dose group exceeded that observed in historical controls (2/350 (range, 0–2%) in inhalation studies; 16/1350 (range, 0–6%) in all studies). The incidence of basal cell carcinoma was 0/50 controls, 0/50 at 125 ppm, 0/50 at 250 ppm, and 1/50 at 500 ppm. [The strengths of this study included the large numbers of animals, compliance with good laboratory practice, the evaluation of multiple dose levels and a duration of exposure that involved most of the lifespan.]

4. Mechanistic and Other Relevant Data

Data on the toxicokinetics and genotoxicity of 1-bromopropane in humans and experimental animals have been reviewed (NTP, 2013). Sections 4.1 and 4.2 present a summary of the most relevant information.

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

(a) Absorption, distribution, and excretion

Exposure to 1-bromopropane in humans is liable to occur by inhalation or dermal contact, and it has been demonstrated that 1-bromopropane undergoes absorption by both routes in humans (Hanley et al., 2006; Cheever et al., 2009).

In a study addressing the absorption characteristics of 1-bromopropane, human epidermal membranes (collected from Caucasian female donors undergoing elective surgical procedures) were subjected in vitro to different exposure scenarios using neat 1-bromopropane or a saturated aqueous solution. The compound was readily absorbed, although the absorption potential depended upon the type and duration

of exposure. Losses due to evaporation were approximately two orders of magnitude greater than dermal absorption (Frasch et al., 2011a).

No data on the distribution of 1-bromopropane in humans were available to the Working Group.

Limited information was available regarding the excretion of 1-bromopropane in humans. Studies of exposed workers have reported the presence of non-metabolized 1-bromopropane in the urine (Kawai et al., 2001; Ichihara et al., 2004a). These studies found a significant correlation between the levels of 1-bromopropane in the urine and the levels of exposure to 1-bromopropane in the air. Bromide ion was also excreted but the high background levels from dietary and pharmaceutical sources represent a confounding factor.

(b) Metabolism

1-Bromopropane metabolites have been detected in the urine of occupationally exposed humans. The major metabolite is AcPrCys, the concentration of which increases with increasing ambient exposure levels (Hanley et al., 2009, 2010). Several other mercapturate conjugates have been identified, including *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, and *N*-acetyl-*S*-(3-hydroxy-*n*-propyl)-L-cysteine (Cheever et al., 2009; Hanley et al., 2009). A proposed phase I metabolite is 3-bromopropanoic acid (B'Hymer & Cheever, 2004; Mathias et al., 2012).

Conjugates of oxidative metabolites identified in experimental animals (see Section 4.1.2b) do not appear to have been reported in humans.

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

Studies in rats and mice have demonstrated that 1-bromopropane is well absorbed after inhalation (Ishidao et al., 2002; Garner et al., 2006), intraperitoneal administration (Jones & Walsh, 1979) or oral exposure (Lee et al., 2010). When male Wistar rats were exposed to 1-bromopropane vapour at doses of either 700 ppm (for 6 hours per day for 1 day or 4 or 12 weeks) or 1500 ppm (for 6 hours per day on 5 days per week for 3 or 4 weeks), the blood concentration of the compound decreased linearly in a time-dependent manner and was below the limit of detection 0.7 hours after the end of the exposure (Ishidao et al., 2002).

One study reported the distribution of [^{14}C]1-bromopropane in rats and mice in exhaled air, urine and faeces that were collected at various time points following intravenous administration. The radioactivity recovered totalled 83–103%, with the largest fractions accounting for volatile organic compounds (25–71%), carbon dioxide (10–31%) and urine (13–23%). The radioactivity recovered from the total carcass (2–6%) and the faeces (< 1–4%) was comparatively negligible. Data for recovery from individual tissues were not presented, with the exception of some limited information for the liver. The liver/blood radioactivity ratios (approximately 3) were similar in both species, regardless of the dose (Garner et al., 2006).

After intraperitoneal administration of a single dose of 200 mg/kg bw of [^{14}C]1-bromopropane in rats, 60% was exhaled unchanged within 4 hours, and only trace amounts were detected in the exhaled air after that time-point. Exhaled carbon dioxide accounted for only 1.4% of the total dose and approximately 45% of the metabolized dose was excreted in the urine after 100 hours (Jones & Walsh, 1979).

In contrast to humans, the urinary excretion of non-metabolized 1-bromopropane does not appear to have been reported in rodents.

(b) Metabolism

The metabolism of 1-bromopropane has been investigated in several studies in experimental animals.

In-vivo studies have been conducted in rats and mice exposed by inhalation and oral, subcutaneous or intraperitoneal administration (Jones & Walsh, 1979; Garner et al., 2006, 2007; Valentine et al., 2007). The four urinary mercapturates identified in exposed humans were also found in experimental animals and additional urinary metabolites were detected in animals, although differences in the metabolite profile were noted. This may result from differences in the routes of administration, species specificities or detection methodologies. Figure 4.1 presents an overview of the metabolite structures that have been identified following inhalation and oral administration and include brominated and debrominated phase I metabolites, and phase II conjugates.

Some of the reactive species that have been identified in vivo or have been postulated, including 2,3-epoxy-1-propanol (glycidol), α-bromohydrin, and 1,2-epoxypropane (propylene oxide), are genotoxic (see Section 4.2.3).

Several debrominated metabolites of 1-bromopropane were identified in studies in vitro with rat liver microsomes, but were not detected in vivo (reviewed in NTP, 2013).

Most of the 1-bromopropane metabolites that have been identified are formed from oxidation reactions and glutathione (GSH) conjugation. Evidence that cytochrome P450 (CYP) 2E1 contributes significantly to the metabolism of 1-bromopropane was presented in a study in which *Cyp2e1*$^{-/-}$ (knockout) and wildtype mice were exposed to the compound by inhalation for 6 hours (Garner et al., 2007). Compared with their wildtype counterparts, the elimination half-life was much longer in the knockout mice (3.2 versus 1.3 hours), the ratio of GSH conjugation to 2-hydroxylation increased fivefold and the urinary concentration of *N*-acetyl-*S*-(2-hydroxypropyl)cysteine was reduced by approximately 50%. A study in which rats were exposed to 1-bromopropane by inhalation and intravenous injection confirmed that the clearance of 1-bromopropane is saturable and that elimination is not only highly dependent on CYP but also on GSH-dependent metabolism (Garner & Yu, 2014).

4.2 Mechanisms of carcinogenesis

The evidence on the "key characteristics" of carcinogens (Smith et al., 2016) – concerning whether 1-bromopropane induces oxidative stress and chronic inflammation, is immunosuppressive, is genotoxic, and modulates receptor-mediated effects – is summarized below.

4.2.1 Oxidative stress

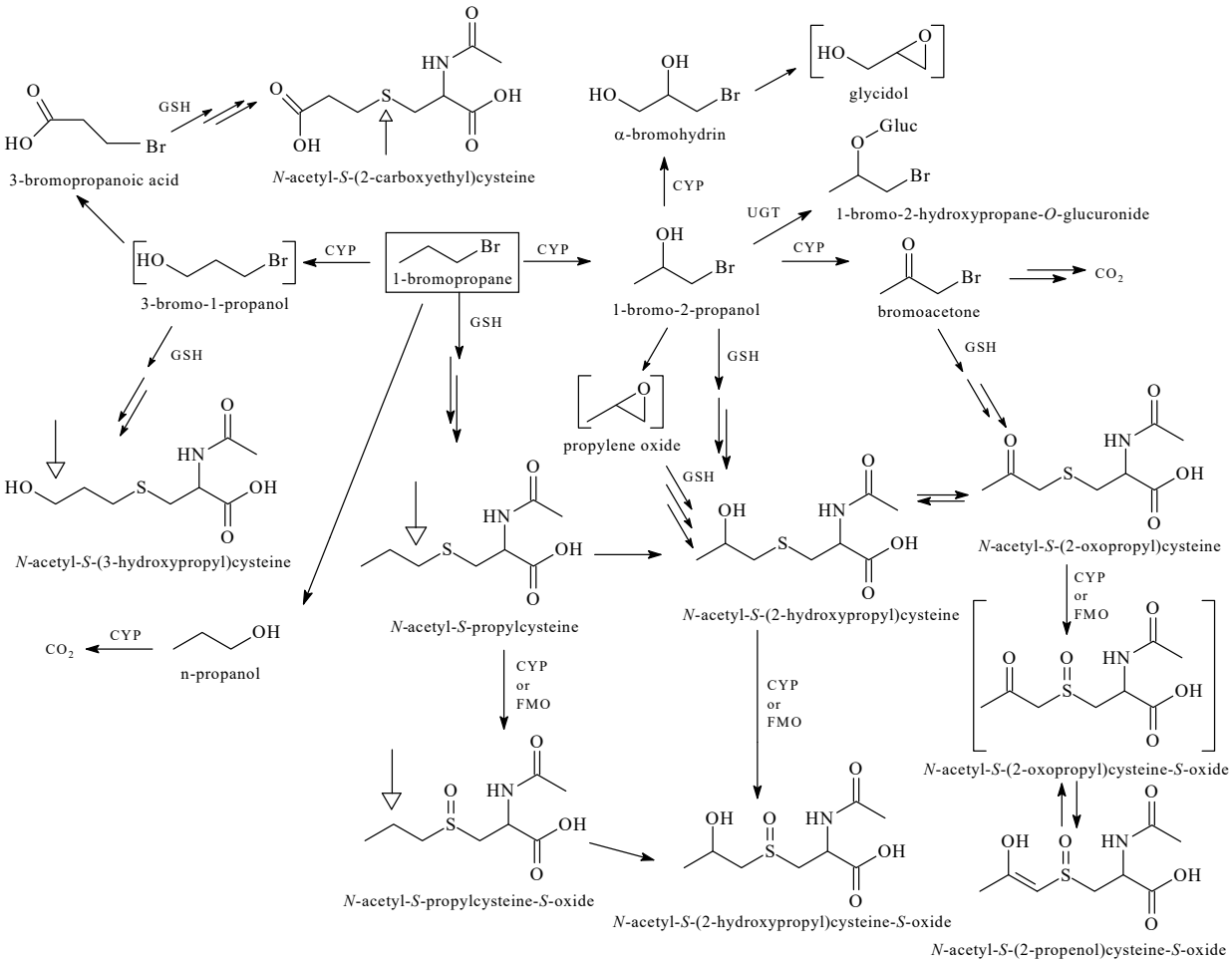
(a) Humans

The detection of urinary *N*-acetyl-*S*-propylcysteine indicated that 1-bromopropane induced GSH conjugation (Valentine et al., 2007; Hanley et al., 2009, 2010; Mathias et al., 2012), but did not directly signify the induction of oxidative stress.

(b) Experimental systems

Studies of urinary metabolites in experimental animals have revealed mercapturates derived from 1-bromopropane conjugated with GSH (Grenby & Young, 1959, 1960; Barnsley, 1966; Barnsley et al., 1966; Jones & Walsh 1979; Garner et al., 2006). An in-vitro study showed that 1-bromopropane is oxidized by liver microsomes, resulting in the production of metabolites including propene, 1,2-epoxypropane, and 1,2-propanediol (Tachizawa et al., 1982). The addition of GSH to the incubation mixture yielded *S*-propyl GSH, and *S*-hydroxyl propyl GSH. [The Working Group noted that the above studies showed that 1-bromopropane or its oxidized metabolites can be conjugated with GSH, which might result in an increase in oxidative stress through the depletion of GSH.]

Fig. 4.1 Metabolism of 1-bromopropane in rodents and humans



Structures in square brackets represent postulated intermediates. The white arrows indicate the urinary metabolites that have been identified in humans

CYP, cytochrome P450; FMO, flavin-containing monooxygenase; Gluc, glucuronide; GSH, glutathione; UGT, uridine 5′-diphospho (UDP)-glucuronosyltransferase

Compiled by the Working Group using data from Jones & Walsh (1979) and Garner et al. (2006, 2007)

A decrease in GSH was observed in the liver of mice 12 hours after a single oral dose of 200–1000 mg/kg body weight (bw) of 1-bromopropane (Lee et al., 2005, 2007), in the spleen of the mice after a single oral dose of 1000 mg/kg bw of 1-bromopropane (Lee et al., 2007), in the cerebral cortex of rats after daily oral administration of 200–800 mg/kg bw of 1-bromopropane for 12 days (Zhong et al., 2013; Guo et al., 2015), and in the cerebrum, cerebellum, and brainstem of rats after inhalation exposure to 800 ppm of 1-bromopropane for 7 days or 12 weeks (Wang et al., 2002, 2003). In contrast, an increase in GSH was observed in the spinal cord of rats after inhalation exposure to 200–800 ppm of 1-bromopropane for 7 days or 12 weeks (Wang et al., 2002, 2003).

The ratio of GSH to oxidized GSH was decreased in the liver after inhalation exposure for 28 days of C57BL/6J or BALB/cA mice to 50 ppm of 1-bromopropane and BALB/cA mice to 110 ppm of 1-bromopropane (Liu et al., 2009), and in the cerebral cortex of rats following oral administration of 100–800 mg/kg bw of

1-bromopropane for 12 days (Zhong et al., 2013; Guo et al., 2015). The level of oxidized GSH did not change in the cerebrum, cerebellum or spinal cord of rats exposed by inhalation to 200–800 ppm of 1-bromopropane for 7 days (Wang et al., 2002), but was increased in the cerebrum of rats after 12 weeks of inhalation exposure to 800 ppm of 1-bromopropane (Wang et al., 2003).

Several studies have indicated lipid peroxidation, protein affected by lipid peroxidation and protein carbonylation induced by exposure to 1-bromopropane. Malondialdehyde (MDA) levels were significantly increased in the liver of mice after a single oral administration of 1-bromopropane at a dose of 500 and 1000 mg/kg bw (Lee et al., 2005), or after inhalation exposure to 300 ppm of 1-bromopropane for 28 days in *Nrf2*-null mice (Liu et al., 2010). Thiobarbituric acid-reactive substances were significantly increased in the liver of mice after a single oral dose of 1-bromopropane at 1000 mg/kg bw, and this increase was enhanced significantly by pretreatment with phenobarbital (Lee et al., 2010). The level of lipid peroxide in the liver microsomes was significantly increased after 8 weeks of exposure by inhalation to 1-bromopropane at doses of 300 and 1800 ppm in female rats, and at 1800 ppm in male rats (Kim et al., 1999a). Exposure by inhalation for 4 weeks to 1-bromopropane increased thiobarbituric acid-reactive substances at doses of 400–1000 ppm, and protein carbonyl and reactive oxygens species at doses of 800–1000 ppm in the cerebellum of rats (Subramanian et al., 2012). The levels of MDA (at a dose of 800 mg/kg bw), MDA-modified proteins (at a dose of 800 mg/kg bw) and 4-hydroxy-2-nonenal-modified proteins (at doses of 200–800 mg/kg bw) (Zhong et al., 2013) and those of *N*-epsilon-hexanoyl-lysin-modified proteins (at doses of 200–800 mg/kg bw), and 4-hydroxy-2-nonenal-modified proteins (at doses of 100–800 mg/kg bw) (Guo et al., 2015) were increased in the cerebral cortex of rats after oral administration of 1-bromopropane for 12 days. Male Fischer 344 rats were exposed by inhalation to 1-bromopropane at doses of 0, 400, or 1000 ppm for 8 hours per day, on 7 days per week for 4 weeks (Huang et al., 2012). Hippocampal reactive oxygen species and protein carbonyl were increased significantly. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry identified 10 individual proteins with increased carbonyl modification.

Two studies have investigated the role of GSH depletion and lipid peroxidation in the mechanism of hepatotoxicity. Male ICR mice that received a single oral dose of 200, 500, or 1000 mg/kg bw of 1-bromopropane had increased serum alanine amino transferase and aspartate amino transferase activities, decreased GSH content and increased levels of *S*-propyl GSH conjugate in the liver 12 hours after the treatment. The GSH conjugate was maximally increased 6 hours after treatment with 1000 mg/kg bw of 1-bromopropane in parallel with the decrease in GSH content. MDA increased dose-dependently 12 hours after the treatment (Lee et al., 2005). Groups of 24 male *Nrf2*-null mice and 24 wildtype mice were exposed by inhalation to 1-bromopropane at doses of 0, 100, or 300 ppm for 8 hours per day for 28 days (Liu et al., 2010). *Nrf2*-null mice had higher levels of MDA, a higher ratio of oxidized GSH to the reduced form of GSH and lower total GSH content than wildtype mice. The constitutive level and the increase in the ratio per exposure level of GSH *S*-transferase (GST) activity were lower in the liver of *Nrf2*-null mice than that of wildtype mice. *Nrf2*-null mice showed greater areas of necrosis in the liver compared with wildtype mice. The level of MDA in the liver was only increased by the dose of 300 ppm in *Nrf2*-null mice, but did not change in wildtype mice at any exposure level. [The Working Group noted that these two studies suggested that treatment with 1-bromopropane induced hepatotoxicity and lipid peroxidation through GSH depletion due to the formation of GSH conjugates in the liver of mice.]

A proteomic study suggested a change in the expression levels of proteins related to immunity in the brain hippocampus of rats. Male Fischer 344 rats were exposed by inhalation to 1-bromopropane at doses of 0, 400, or 1000 ppm for 8 hours per day for 1 or 4 weeks (Huang et al., 2011). Changes in the expression level of proteins related to immunity and the response to stress, including the upregulation of glucose-regulated protein 78, heatshock protein 60, GSTA3 and GSTP1 and the downregulation of protein DJ-1 (also known as Parkinson disease protein 7), were observed. [The Working Group interpreted the results to be consistent with effects on oxidative stress.]

4.2.2 Chronic inflammation and immunosuppression

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) In vivo

Male and female Fischer 344/N rats exposed by inhalation to 1-bromopropane (at doses of 125, 250, or 500 ppm) for 2 years demonstrated chronic inflammation in the nose and larynx at several doses (Morgan et al., 2011; NTP, 2011). Chronic inflammation of the lung was also observed in females exposed to 500 ppm. In the trachea, an increased incidence of chronic active inflammation was observed in all exposed groups of females, and in males exposed to 500 ppm. Inflammatory lesions with Splendore-Hoeppli material were present primarily in the nose and skin of male and female rats, indicating that 1-bromopropane caused immunosuppression.

Significant decreases in the spleen immunoglobulin M response to sheep erythrocytes were observed in mice (at doses of 125–500 ppm) and rats (at a dose of 1000 ppm) after exposure to 1-bromopropane for 10 weeks. Total spleen cells and T cells were significantly decreased after 4 weeks of exposure in mice and rats (Anderson et al., 2010).

A single oral administration of 1-bromopropane to female mice suppressed the immune response, including the number of antibody-forming cells per spleen (at doses of 200–1000 mg/kg bw), antibody-forming cells per spleen cell (at doses of 500–1000 mg/kg bw), and $CD4^+$ $IL\text{-}2^+$ cells per spleen (at doses of 200–1000 mg/kg bw) in response to concanavalin A (Lee et al., 2007).

(ii) In vitro

Serial studies have shown that exposure to 1-bromopropane at doses of 1–10 μM produced inducible nitric oxide synthases, interleukin-1β, interleukin-6 and tumour necrosis factor-α (Han et al., 2008) and upregulated cyclooxygenase-2 (Han et al., 2012) through nuclear factor-κB in a murine macrophage cell line. In contrast, exposure to 1-bromopropane inhibited the DNA-binding activity of nuclear factor-κB (Yoshida et al., 2007) and decreased brain-derived neurotrophic factor mRNA expression in murine astrocytes (Yoshida et al., 2009) [but these data on astrocytes should be interpreted carefully because the concentration of 1-bromopropane was very high (0.1 or 1 mM)].

4.2.3 Genetic and related effects

See Table 4.1 and Table 4.2

(a) Humans

S-Propylcysteine haemoglobin adducts were measured in 26 Chinese female factory workers exposed to 1-bromopropane by inhalation and possibly by skin contact. The controls were age-matched workers (n = 32) from a Chinese beer factory. Exposure levels ranged from 0.34 to 49.2 ppm for the workers who gave blood samples and from 0 to 170.54 ppm for the workers who gave urine samples. A significant increase in

Table 4.1 Genetic and related effects of 1-bromopropane in humans

Tissue, cell type	End-point	Test	Results without metabolic activation	Dose (LED or HID)	Comments	Reference
Venous blood, leukocytes; 64 workers occupationally exposed at two facilities in the USA (18 men and 46 women); one group with low exposure and one group with high exposure (sprayers); no controls	DNA damage	DNA strand breaks (comet assay)	± (not significant)	Up to 5 ± 1 ppm in the low-exposure group; up to 83 ± 85 ppm in the high-exposure group	Higher tail moments (non-sprayers) and dispersion coefficients (sprayers) at the end of the week in the same individuals ($P < 0.05$)	Toraason et al. (2006)
Venous blood, leukocytes (in vitro)	DNA damage	DNA strand breaks (comet assay)	+	1 mM	Increased DNA damage (tail moments) at ≥ 4 h	Toraason et al. (2006)

+, positive; ±, equivocal; h, hour; HID, highest ineffective dose; LED, lowest effective dose; wk, week

Table 4.2 Genetic and related effects of 1-bromopropane in experimental systems

Species, strain, sex	End-point	Test	Results		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Mouse, B6C3F$_1$, M/F (peripheral blood erythrocytes)	Chromosomal damage	Micronuclei	–	–	Inhalation, 500 ppm, 3 h/day on 5 days/wk for 3 mo		NTP (2011)
Rat, Sprague-Dawley, M	Mutation	Dominant lethal test	–	NA	Gastric intubation, 400 mg/kg bw once daily for 5 days		Saito-Suzuki et al. (1982)
Mouse, ICR, M	Mutation	Dominant lethal test	–	NA	Gavage, 600 mg/kg bw once daily for 10 days		Yu et al. (2008)
Salmonella typhimurium TA98	Mutation	Reverse mutation	–	–	20.3 µmol/plate [2497 µg/plate]	Closed system incubation	Barber et al. (1981)
Salmonella typhimurium TA100, TA1535	Mutation	Reverse mutation	+	+	4.9 µmol/plate [603 µg/plate]	Closed system incubation	Barber et al. (1981)
Salmonella typhimurium TA97, TA98, TA100, TA1535	Mutation	Reverse mutation	–	–	10 000 µg/plate	Mutagenicity at ≥ 3333 µg/plate, not reliable due to high toxicity	NTP (2011)
Salmonella typhimurium TA98, TA100	Mutation	Reverse mutation	–	–	5000 (10 000 with metabolic activation) µg/plate		NTP (2011)
Escherichia coli WP2uvrA/pKM101	Mutation	Reverse mutation	–	–	5000 (10 000 with metabolic activation) µg/plate		NTP (2011)

+, positive; –, negative; bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month, NA, not applicable; wk, week

S-propylcysteine adducts was observed in the haemoglobin of exposed workers (1.52 pmol/mg globin) compared with the controls (0.11 pmol/mg globin). In addition, the level of urinary AcPrCys increased with increasing exposure concentrations (Valentine et al., 2007).

The comet assay was used to assess DNA damage (strand breaks) in the peripheral blood leukocytes from 64 workers (18 men and 46 women) exposed occupationally to 1-bromopropane at two facilities in the USA that used spray adhesives containing 1-bromopropane. Because no unexposed population was available, the workers were divided into groups with higher exposure (sprayer) and lower exposure (non-sprayers). Overall, the exposures ranged from 0.2 to 271 ppm in one facility and from 4 to 27 ppm in the other facility, with workers classified as sprayers having the highest exposures. The TWA concentrations of 1-bromopropane were significantly correlated with blood and urine bromide concentrations. No significant differences in DNA damage in leukocytes were observed between the workers with high exposure (sprayers) and low exposure (no-sprayers). At the facility with the higher exposures, a paired analysis between the end- and start-of-the-week values indicated that non-sprayers had significantly increased comet tail moments and sprayers had significantly increased comet tail moment dispersion coefficients. Although not statistically significant, all of the other associations between the 1-bromopropane exposure indices and DNA damage were positive, with the exception of the end-of-work-week urinary bromide. A marginal correlation was found between DNA damage and *GSTM1*-positive genotypes (Toraason et al., 2006). [The Working Group noted the small number of subjects and the lack of controls. This study provided some evidence that exposure to 1-bromopropane induces DNA damage in humans.]

When assessed in vitro, DNA damage (comet assay) was significantly increased in human leukocytes exposed to 1 mM 1-bromopropane for 8 hours. Under the same conditions, apoptosis was significantly induced at 10-fold lower concentrations of 1-bromopropane (Toraason et al., 2006).

(b) Experimental systems

In rodents exposed in vivo, 1-bromopropane did not increase micronucleus formation in peripheral blood erythrocytes (NTP, 2011) or cause dominant lethal mutations (Saito-Suzuki et al., 1982; Yu et al., 2008). [However, the dominant lethal mutation assay is generally regarded as relatively insensitive for the detection of mutagenic agents.]

Although 1-bromopropane did not induce mutations in bacteria under standard assay conditions (NTP, 2011), it did induce mutations in bacteria in both the presence and absence of exogenous mammalian metabolic activation in the only reported study, the design of which was appropriate for testing a highly volatile chemical (Barber et al., 1981).

Reactive metabolites cause genotoxic effects in vitro, including DNA adduct formation, mutations and DNA or chromosome damage. Glycidol and propylene oxide cause cytogenetic effects in vivo and are carcinogenic in experimental animals (Stolzenberg & Hine, 1979, 1980; IARC, 1994, 2000).

4.2.4 Receptor-mediated effects

(a) Humans

One epidemiological study suggested that exposure to 1-bromopropane affected thyroid-stimulating hormone or follicular-stimulating hormone in female workers. A significant increasing trend in thyroid-stimulating hormone with exposure levels from 0.07 to 106.4 ppm and a significant increasing trend in thyroid-stimulating hormone and follicular-stimulating hormone with cumulative exposure levels from 2 to 3618 ppm × months were shown in 60 female

workers exposed to 1-bromopropane (Li et al., 2010).

(b) Experimental systems

Two studies investigated the reproductive toxicity and endocrine effects of 1-bromopropane in male and female rats (Ichihara et al., 2000a; Yamada et al., 2003). Exposure to 1-bromopropane (200, 400 and 800 ppm for 12 weeks) significantly decreased the weight of seminal vesicles, which is known to reflect blood testosterone levels, in male rats. The weight of the pituitary glands was decreased by 400 and 800 ppm and failure of spermiation, shown by an increase in retained sperm at post-spermiation stages, also suggested endocrine effects (Ichihara et al., 2000a). In female rats, 1-bromopropane increased the disruption of estrous cycles and also decreased antral and growing follicles in the ovary but not primordial follicles. The levels of luteinizing hormone and follicle-stimulating hormone were not altered in females exposed to 1-bromopropane at a dose of 800 ppm for 7 weeks or at doses of 200 and 400 ppm for 12 weeks (Yamada et al., 2003).

4.2.5 Altered cell proliferation or death

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling-positive granular cells (Subramanian et al., 2012) and pyknotic shrinkage in the cytoplasm of Purkinje cells (Ohnishi et al., 1999; Mohideen et al., 2013) and nuclei of granular cells (Mohideen et al., 2013) in the cerebellum have been reported in rats exposed to 1-bromopropane by inhalation at high doses of 1000–1500 ppm. Exposure of rats to 1-bromopropane at doses of 800–1000 ppm also suppressed neurogenesis in the dentate gyrus (Zhang et al., 2013). Exposure of rats to 1-bromopropane by inhalation increased phosphorylation of 14-3-3-θ protein in the hippocampus (at a dose of 1000 ppm for 4 weeks), which is related to apoptosis signalling, increased mitochondrial Bax (at a dose of 1000 ppm for 1 week or doses of 400–1000 ppm for 4 weeks), decreased cytosolic Bax (at doses of 400–800 ppm for 4 weeks), decreased mitochondrial cytochrome c and increased cytosolic cytochrome c (at a dose of 1000 ppm for 1 week or doses of 400–1000 ppm for 4 weeks) (Huang et al., 2015).

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 seven chemicals reviewed in the present volume of *IARC Monographs* (1-bromopropane, 2-mercaptobenzothiazole, 3-chloro-2-methylpropene, *N*,*N*-di-methylformamide, *N*,*N*-dimethyl-*p*-toluidine, hydrazine and tetrabromobisphenol A). The EPA has systematically analysed concentration–response sample-assay pairs from ToxCast and Tox21. The resulting concentration–response models and bioactivity determination have been publicly released via the interactive Chemical Safety for Sustainability ToxCast Dashboard (EPA, 2015a, b). Summary matrix files, the ToxCast data analysis pipeline R package and a connected database (invitrodb_v1) are also available (EPA, 2015c). The ToxCast data analysis pipeline R package and the associated database enable access to all of the underlying concentration–response data, the analysis decision logic and methods, concentration–response model outputs, bioactivity determination, and bioactivity 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 seven chemicals in the current Volume, no testing data were available for two, one was tested only in Tox21 assay components and the remaining four 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 National Institutes of Health, comprise 821 assay end-points derived from 558 assay components (i.e. readouts) and 342 assays (i.e. experiments). The 342 assays were sourced from seven vendors or collaborators spanning diverse technological and biological space, including over 300 gene targets. Approximately half of the final assay end-points were analysed from biochemical (cell-free) assay formats and the remainder from cell-based or 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. Chemical effects requiring biotransformation to active metabolites may be missed in some or all of the in-vitro assays. Relatively uniform testing concentration ranges were used, from low nanomolar up to approximately 100–200 µM. Compounds with a 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 in-vitro screening at the concentrations used in ToxCast and Tox21 may be insufficient to detect molecular receptor-type interactions. These compounds with a very low relative molecular mass may also have high vapour pressure, which could lead to loss of the sample during testing and thus a failure to reach effective active concentrations.

The Tox21 and ToxCast in-vitro assays were selected to cover a broad range of potential toxicity mechanisms and are not specifically focused on carcinogenesis. Therefore, the Working Group of the *IARC Monographs* Volume 112 mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens (IARC, 2014). The consensus assignments resulted in 263 assay end-points mapped to seven of 10 key characteristics (IARC, 2017). Additional assay end-points have been included in Tox21 and ToxCast data since that determination. These were reviewed and 18 further assay end-points were added to the mapped key characteristics resulting in a total of 281. The assay end-points used, the bioactivity determination, and the mapping to key characteristics is included as supplementary material to *IARC Monographs* Volume 115 (IARC, 2018). The key characteristics listing and the number of assays included are briefly described below.

1. *Is electrophilic or can be metabolically activated* – 81 assay end-points consisting of CYP biochemical activity assays including aromatase;
2. *Is genotoxic* – 14 assay end-points consisting of cellular TP53 induction and DNA repair-sensitive cellular assays;
3. *Alters DNA repair or causes genomic instability* – 0 assay end-points;
4. *Induces epigenetic alterations* – 18 assay end-points including biochemical assays targeting histone deacetylases and other enzymes that modify chromatin as well as cellular transcription factor assays involved in epigenetic regulation;
5. *Induces oxidative stress* – 34 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 reactive oxygen species;

6. *Induces chronic inflammation* – 48 assay end-points measuring protein expression levels in primary human cells in complex environments;
7. *Is immunosuppressive* – 0 assay end-points;
8. *Modulates receptor-mediated effects* – 143 assay end-points targeting nuclear receptors (including aromatic hydrocarbon receptor, AhR) 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* – 157 assay end-points measuring cytotoxicity by a wide variety of assay formats in cell lines, primary human cells and developing zebrafish larvae.

The assay end-point groupings were not intended to serve as definitive linkages to carcinogenic outcomes but to provide insight into the bioactivity profile of a chemical highlighting its potential to interact with or disrupt targets biologically associated with cancer. The specific assays tested, the mapping to the key characteristics of carcinogens and the bioactivity determination can be found in the Supplementary Material (IARC, 2018). A summary of potentially significant outcomes for 1-bromopropane and other chemicals evaluated in the present volume are outlined below.

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

(a) 3-Chloro-2-methylpropene

This compound was not tested.

(b) N,N-Dimethyl-p-toluidine

This compound was not tested.

(c) 1-Bromopropane

This compound was tested as part of the Tox21 programme, but not in ToxCast assays, and was inactive in all 179 bioassays with the exception of a single testing in an assay for *TP53* activation. However, a parallel cell viability test was positive, confounding the results, and four other tests of the *TP53* activation assay gave negative results for 1-bromopropane. No chemical quality control information was available for the tested sample due to the current unavailability of appropriate detection methods for this structure. [The Working Group noted that the chemical has a fairly low relative molecular mass (122.99). In addition, 1-bromopropane has a vapour pressure of 13.3 kPa at 18 °C (ILO, 2004), which may be sufficiently high to allow vaporization during testing and loss of the sample. Thus, the ability to detect bioactivity in the Tox21 assays may have been limited.]

(d) 2-Mercaptobenzothiazole

Analysis of 2-mercaptobenzothiazole included bioactivity data from high-throughput screening assays from Tox21 and ToxCast. It was tested across the full assay suite of ToxCast and Tox21 with data for 887 assay end-points. The analytical quality control of the tested sample showed the intended structure was present and purity was > 90%.

1. Is electrophilic or can be metabolically activated: 2-mercaptobenzothiazole was tested in 11 assay end-points and found active in six, all of which were CYP inhibition assays. This activity does not necessarily demonstrate that the chemical generates electrophilic products upon CYP-mediated metabolism, but does support that 2-mercaptobenzothiazole can be a promiscuous CYP substrate and/or inhibitor.

2. Is genotoxic: 2-mercaptobenzothiazole was tested in nine assay end-points related to genotoxicity and was found to be active in none.

4. *Induces epigenetic alterations*: 2-mercaptobenzothiazole was active in two of

10 assay end-points, both of which were transcription factor assays (DNA binding) that lacked high confidence in epigenetic activity due to lack of validity with reference chemicals.

5. *Induces oxidative stress*: 2-mercaptobenzothiazole was active in three of 16 assay end-points mapped to oxidative stress, two assays for NRF2/ARE activation and one for nuclear respiratory factor 1.

6. *Induces chronic inflammation*: 2-mercaptobenzothiazole was tested in 45 assay end-points mapped to chronic inflammation and was active in none.

8. *Modulates receptor-mediated effects*: 2-mercaptobenzothiazole was active in 13 of 93 assay end-points mapped to receptor-mediated effects. It had the most pronounced activity towards the peroxisome proliferator-activated receptors (PPARs), in particular PPARγ, and AhR.

10. *Alters cell proliferation, cell death or nutrient supply*: 2-mercaptobenzothiazole was active in 7 of 67 assay end-points.

Overall, 2-mercaptobenzothiazole was very weakly cytotoxic, did not show evidence of genotoxicity but did inhibit CYP activities and show signs of inducing oxidative stress. It was notably active as a modulator of nuclear receptor activity, in particular AhR and PPARs, and was active in 47 of 636 assays not mapped to cancer end-points. Notably, it induced developmental abnormalities in zebrafish larvae in two different assays including morphological effects linked to AhR activity, jaw and snout malformations (Prasch et al., 2003). It also modulated steroid biosynthesis pathways in H295R human adrenocortical carcinoma cells.

(e) N,N-*Dimethylformamide*

N,*N*-Dimethylformamide was tested in 36 Tox21 and 865 ToxCast assay end-points and had no bioactivity. Although one sample showed bioactivity for four ToxCast end-points (retinoid X receptor α, retinoid X receptor β, nuclear receptor-related factor-1, and peroxisome proliferation response element γ), a second sample of *N*,*N*-dimethylformamide showed no bioactivity in these assays. No analytical quality control of the tested sample for ToxCast and Tox21 was presented because no structure detection method was available at the time of analysis. [The Working Group noted that the low relative molecular mass of *N*,*N*-dimethylformamide (73.1) as well as the limited biotransformation capacity of the assays may have made the detection of activity in the ToxCast and Tox21 assays unlikely.]

(f) Hydrazine sulfate

Hydrazine sulfate was tested in 182 Tox21 bioassays and the multiplexed transcription factor activation assays in ToxCast (135 end-points). It was marginally active in the Tox21 AHR reporter gene assay (concentration at half-maximal activity, AC_{50}, 48 μM), but was inactive in the ToxCast AhR end-point in the Attagene transcription factor assay platform. [The Working Group interpreted this as only very weak evidence of possible AhR activity.] The only other ToxCast active call was a very marginal activity (AC_{50}, 58 μM) in downregulation of NRF2, an assay end-point of undetermined significance in the downregulation direction. No analytical quality control of the tested sample for ToxCast and Tox21 was presented because no structure detection method was available at the time. [The Working Group noted that the molecular weight of hydrazine is low (32 g/mol) and that minimal biotransformation was present in the assays, probably limiting the ability to detect any potential activity of hydrazine.]

(g) Tetrabromobisphenol A

Tetrabromobisphenol A was tested across the full assay suite of ToxCast and Tox21 with data available for 836 assay end-points. The analytical quality control of the tested sample showed the intended structure was present and purity was greater than 90%.

Specific effects across seven of the 10 key characteristics based on data from high-throughput screening in vitro were:

1. *Is electrophilic or can undergo metabolic activation*: tetrabromobisphenol A was tested in 31 assay end-points and was found to be active in three – the inhibition of CYP2C9, CYP2C19 and CYP19 (aromatase), the latter of which regulates the conversion of androgens to estrogens. This activity is consistent with the inhibition of enzyme activity, possibly by acting as a substrate of the CYPs, but is not necessarily indicative of activation.

2. *Is genotoxic*: tetrabromobisphenol A was tested in nine assay end-points related to genotoxicity and was found to be active in six (five were repeated testing over time of the same assay end-point, the other was from a different assay technology), all of which were related to the activation of *TP53* in human hepatoma HepG2 cells. [The Working Group noted that *TP53* activation can occur in response to a variety of cell stress in addition to DNA damage.]

4. *Induces epigenetic alterations*: tetrabromobisphenol A was active in four of 11 assay end-points, all of which were transcription factor activation end-points mapped to the DNA-binding subcategory. [The Working Group noted that these end-points have not been validated extensively with reference compounds for epigenetic alterations.]

5. *Induces oxidative stress*: tetrabromobisphenol A was active in eight of 18 assay end-points mapped to oxidative stress. The eight active assay end-points were all stress-related genes, in particular the NRF2/ARE pathway together with the heatshock response factor, metal response factor and the endoplasmic reticulum stress response.

6. *Induces chronic inflammation*: tetrabromobisphenol A was tested in 45 assay end-points mapped to chronic inflammation and was active in none. Most of these assays used primary human cell lines with clear evidence of cytotoxicity, potentially confounding the results for inflammatory responses.

8. *Modulates receptor-mediated effects*: tetrabromobisphenol A was active in 24 of 93 assay end-points mapped to receptor-mediated effects. Strong evidence of activity was found for the PPARγ receptor as well as activity for the androgen receptor, glucocorticoid receptor, farnesyl X receptor and the xenobiotic receptor PXR. Tetrabromobisphenol A was determined to be inactive as an estrogen receptor agonist or antagonist using a model combining results from 18 estrogen receptor pathway assay end-points (Judson et al., 2015).

10. *Alters cell proliferation, cell death or nutrient supply*: tetrabromobisphenol A was active in 37 of 73 assay end-points mapped to this category and was the highest-ranked chemical among all chemicals evaluated in the *IARC Monographs* with ToxCast data (195 total). The majority of the active end-points were for cytotoxicity in cell lines and primary human cells. It was also active in several end-points mapped to cell cycle as well as those mapped to mitochondrial toxicity by loss of mitochondria membrane potential.

Overall, tetrabromobisphenol A demonstrated strong cytotoxic effects that may have confounded the results from other end-points. It activated several stress pathways, in particular the oxidative stress pathway. It was also a promiscuous nuclear receptor modulator with higher potency towards PPARγ than other receptors, but also active for steroid hormone receptors and the xenobiotic receptor PXR. In assay end-points not currently mapped to the key characteristics of carcinogens, tetrabromobisphenol A disrupted steroidogenesis in H295R human adrenal corticocarcinoma cells through the upregulation of progesterone and hydroxyprogesterone.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Neurotoxicity

(a) Humans

The major adverse effect of 1-bromopropane identified in humans is neurotoxicity, which affects the peripheral nerves and the central nervous system. After repetitive exposure to 1-bromopropane at levels of up to several hundred parts per million, workers showed a disability in walking and reported paresthesia or anaesthesia mainly in the lower limbs. Sensory and motor conduction velocity decreased and elongation of distal latency in lower extremities was also reported. Intoxicated cases also showed aggressive behaviour during exposure to 1-bromopropane but cognitive dysfunction and depressive mood after exposure (Sclar, 1999; Ichihara et al., 2002; Majersik et al., 2007; Wang et al., 2015). Epidemiological studies of workers producing 1-bromopropane showed dose-dependent changes including an increase in tibial motor distal latency, a decrease in sural nerve conduction velocity, a decrease in scores in the Benton cognitive test and an increase in the threshold for vibration sense in the toes (Li et al., 2010).

(b) Experimental systems

Numerous animal studies have demonstrated the neurotoxicity of 1-bromopropane (e.g. Ichihara et al., 2000b; Mohideen et al., 2011; Subramanian et al., 2012). Wistar rats exposed to 1-bromopropane at doses of 200, 400, or 800 ppm for 8 hours per day on 7 days per week for 12 weeks showed degeneration of the myelin sheath in the posterior tibial nerve or tibial nerve and decreased cerebrum weight (Ichihara et al., 2000b). Exposure to 1-bromopropane induced DNA fragmentation (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling-positive cells) (Subramanian et al., 2012) and decreased myelin basic protein (Mohideen et al., 2013) in the cerebellum.

4.5.2 Other types of toxicity

(a) Humans

No clinical case reports have indicated the haematotoxicity of 1-bromopropane. Epidemiological studies showed lower leukocyte counts compared with age-matched controls (Ichihara et al., 2004b) and a significant trend in the decrease in erythrocytes or haematocrit with exposure level or cumulative exposure level (Li et al., 2010).

There are no clinical case reports showing liver injury after exposure to 1-bromopropane (Sclar, 1999; Samukawa et al., 2012). An epidemiological study showed a positive trend in the increase in lactate dehydrogenase with exposure to 1-bromopropane in female workers, but did not show any trend in the change in alanine aminotransferase or aspartate aminotransferase. The same study showed significant trend in the increase in blood urea nitrogen with exposure in male workers (Li et al., 2010).

(b) Experimental systems

In mice, necrotic changes were observed in the liver at a low exposure level of 50 ppm (Liu et al., 2009) compared with the subtle changes in the liver of rats exposed to 1-bromopropane at a higher level of 800 ppm (Ichihara et al., 2000b). Oxidation of 1-bromopropane by CYP, GSH depletion and oxidative stress may be involved in 1-bromopropane-induced hepatotoxicity (Lee et al., 2005, 2007, 2010; Liu et al., 2010). Female rats exposed to 1-bromopropane at concentrations of 800 or 1590 ppm showed increased absolute kidney weight (Yamada et al., 2003; Ichihara, 2005). Another study identified tubular casts in the kidney after exposure to 1800 ppm (Kim et al., 1999b).

Exposure to 1-bromopropane reduced the motility of epididymal sperm in rats (Ichihara et al., 2000a; Banu et al., 2007) and mice (Liu et al., 2009). The effects of 1-bromopropane on sperm motility were less marked in

CYP2E1-null mice, suggesting a contribution of CYP2E1 to the reduction in sperm motility (Garner et al., 2007).

5. Summary of Data Reported

5.1 Exposure data

1-Bromopropane is a solvent that is used in spray adhesives to fabricate polyurethane foam cushions, as a vapour degreasing agent and as a cleaning solvent for metals, plastics, optical and electronic components, and for dry cleaning fabrics. 1-Bromopropane is also used as a chemical intermediate in the manufacture of pesticides, flavours and fragrances, and pharmaceuticals. Occupational exposures to 1-bromopropane through inhalation and dermal uptake have been reported in the production of chemicals, the manufacture of adhesives, in the production and use of spray adhesives, vapour degreasing and in dry cleaning fabrics. 1-Bromopropane has been measured in the air, urine and serum of workers, but exposures of the general population have not been reported.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1-Bromopropane was tested for carcinogenicity by inhalation in one good laboratory practice (GLP) study in male and female mice, and in one GLP study in male and female rats.

In the study in mice, inhalation of 1-bromopropane was associated with a significantly increased incidence of alveolar/bronchiolar carcinoma of the lung, and a significantly increased incidence (with a significant positive trend) of alveolar/bronchiolar adenoma or carcinoma (combined) and alveolar/bronchiolar adenoma of the lung in females, but no significant increase in tumour incidence in males.

In the study in male rats, inhalation of 1-bromopropane caused a significantly increased incidence (with a significant positive trend) of tumours of the skin (keratoacanthoma, basal cell adenoma, basal cell carcinoma and squamous cell carcinoma combined), and of keratoacanthoma and keratoacanthoma or squamous cell carcinoma (combined). It also caused a significant increase in the incidence of malignant mesothelioma of the epididymis (with a significant positive trend). It was associated with a significantly increased incidence of pancreatic islet cell adenoma (with a significant positive trend) and of pancreatic islet cell adenoma or carcinoma (combined), and was associated with a non-significant increase in the incidence of adenoma of the large intestine (colon or rectum), a tumour never observed in historical controls for inhalation studies.

In the study in female rats, inhalation of 1-bromopropane caused a significantly increased incidence (with a significant positive trend) of adenoma of the large intestine (colon or rectum) and was associated with a non-significant positive trend in the incidence of tumours of the skin (squamous cell papilloma, keratoacanthoma, basal cell adenoma and basal cell carcinoma combined).

5.4 Mechanistic and other relevant data

In humans, metabolites of 1-bromopropane have been detected in the urine of workers after occupational exposure. The concentration of the major metabolite identified, *N*-acetyl-*S*-propylcysteine, increased with increasing levels of ambient exposure. 3-Bromopropanoic acid identified in rats exposed to 1-bromopropane has been postulated as a phase I metabolite.

With respect to the key characteristics of human carcinogens, there is *strong* evidence that 1-bromopropane is electrophilic or can be metabolically activated. No reports in humans were available on the conjugates of oxidative metabolites identified in experimental animals. *N*-Acetyl-*S*-propylcysteine was detected in the urine of exposed workers and *S*-propylcysteine haemoglobin adducts were measured in the blood of Chinese factory workers exposed to 1-bromopropane.

There is *strong* evidence that 1-bromoproane induces oxidative stress, induces chronic inflammation and is immunosuppressive, based on studies in rodents exposed in vivo. Inflammation was seen in the nose, larynx, trachea and lung in exposed rodents. 1-Bromopropane blocked the activation of nuclear factor-κB in murine astrocytes in vitro and suppressed neurogenesis in rats.

There is *moderate* evidence that 1-bromoproane modulates receptor-mediated effects and is genotoxic. In exposed humans, some evidence of DNA damage in leukocytes was available from a small study of workers exposed to 1-bromopropane. In rodents in vivo, 1-bromopropane did not induce micronucleus formation in peripheral blood erythrocytes or cause dominant lethal mutations.

There were few data on other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, causes immortalization, or alters cell proliferation, cell death, or nutrient supply).

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1-bromopropane.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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Yu WJ, Kim JC, Chung MK (2008). Lack of dominant lethality in mice following 1-bromopropane treatment. *Mutat Res*, 652(1):81–7. doi:10.1016/j.mrgentox.2008.01.001 PMID:18291709

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Zhong Z, Zeng T, Xie K, Zhang C, Chen J, Bi Y et al. (2013). Elevation of 4-hydroxynonenal and malondialdehyde modified protein levels in cerebral cortex with cognitive dysfunction in rats exposed to 1-bromopropane. *Toxicology*, 306:16–23. doi:10.1016/j.tox.2013.01.022 PMID:23395825

2-MERCAPTOBENZOTHIAZOLE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 149-30-4

Chem. Abstr. Serv. Name:

2-Mercaptobenzothiazole

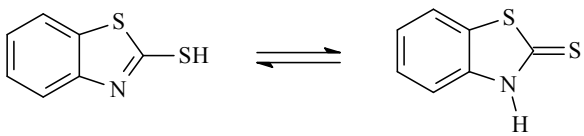
IUPAC Systematic Name:

3*H*-1,3-Benzothiazole-2-thione

Synonyms: Benzothiazole-2-thiol; 1,3-benzothiazole-2-thiol; 2-benzothiazolethiol; 3*H*-benzothiazole-2-thione; 2-sulfanylbenzothiazole

Acronyms: MBT; 2-MBT.

1.1.2 Structure and molecular formula, and relative molecular mass



(tautomers in crystals and aqueous solutions)

Molecular formula: $C_7H_5NS_2$

Relative molecular mass: 167.25

1.1.3 Physical and chemical properties of the pure substance

Description: Yellowish crystals or powder with a characteristic sulfurous odour (IFA, 2015)

Melting point: 180–182 °C (HSDB, 2015)

Density (at 20 °C): 1.42 g/cm^3 (HSDB, 2015)

Octanol/water partition coefficient: log K_{ow}, 2.41 (HSDB, 2015)

Water solubility: Moderately soluble (100–1000 mg/L) (ECHA, 2015)

Dissociation constant: pK_a 7.0 at 20 °C (HSDB, 2015)

Volatility: Vapour pressure, 2.25 × 10^{-8} mm Hg at 20 °C (HSDB, 2015)

Flash point: 243 °C (GESTIS, 2015)

Conversion factor: 1 ppm = 6.83 mg/m^3 (HSDB, 2015).

1.2 Production and use

1.2.1 Production

(a) Production process

2-Mercaptobenzothiazole is produced by reacting aniline, carbon disulfide, and sulfur at high temperature and pressure; the product is then purified by dissolution in a base to remove the dissolved organics. Re-precipitation is achieved by the addition of acid (Kirk-Othmer, 1982; NTP, 1988).

(b) Production volume

2-Mercaptobenzothiazole is listed as a chemical with high production volume by the Organisation for Economic Co-operation and Development (OECD, 2004) and in the USA (Federal Register, 2000; HSDB, 2015).

In 2006, the inventory-aggregated national production volume for 2-mercaptobenzothiazole in the USA was between 10 and < 50 million pounds [~4536 to < 22 680 tonnes]. The United States Environmental Protection Agency (EPA) noted that 500 000–1 000 000 pounds/year [~227–454 tonnes/year] of 2-mercaptobenzothiazole were produced, imported, and used in the USA in 2012 (EPA, 2012). In 2012, three facilities in three states were listed as manufacturing 2-mercaptobenzothiazole in the USA (HSDB, 2015).

2-Mercaptobenzothiazole is registered with the European Chemicals Agency, and production was stated to be 1000–10 000 tonnes per year from three manufacturers in three countries, one each in Belgium, Spain, and the United Kingdom (ECHA, 2012).

A commercial website identified a larger number of suppliers: 225 in China, 13 in the USA, five in India, two in Hong Kong Special Administrative Region, and one each in Canada, France, Germany, Japan, the Russian Federation, and Turkey (GuideChem, 2015).

1.2.2 Use

2-Mercaptobenzothiazole is principally used as a reactant in the manufacture of rubber products, but is also used as a corrosion inhibitor in oils, greases and cooling fluids. It is added to polyether polymers as a stabilizer to resist damage by air and ozone, and is a component approved in the USA in some skin medications for dogs (HSDB, 2015).

2-Mercaptobenzothiazole is also used as an intermediate in the production of pesticides such as 2-(thiocyanomethylthio)benzothiazole (Azam & Suresh, 2012), and sodium and zinc salts of 2-mercaptobenzothiazole are approved for use as pesticides by the EPA (1994).

1.3 Measurement and analysis

Several analytical methods are available for the determination of 2-mercaptobenzothiazole in environmental samples (e.g. air, water, and food), in rubber products (e.g. disposable medical gloves), in products that come into contact with rubber materials (e.g. medical drug solutions or industrial coolant solutions), and in the urine of exposed persons (Table 1.1). Generally, the use of stable isotope-labelled surrogate standards is recommended for the specific analysis of 2-mercaptobenzothiazole (Wick et al., 2010). A single method has been described for the determination of 2-mercaptobenzothiazole in dietary products (Barnes et al., 2003).

1.4 Occurrence and exposure

Due to its use as an accelerant in rubber vulcanization, 2-mercaptobenzothiazole can be found as a contaminant in rubber products. Sensitization to 2-mercaptobenzothiazole is common in occupational and non-occupational settings and can be used as an indicator of exposure (HSDB, 2015).

1.4.1 Natural occurrence

2-Mercaptobenzothiazole is not known to occur in nature.

1.4.2 Environmental occurrence

(a) Air

Urban particulate matter was sampled in a street in Stockholm, Sweden, using a device made in-house; average concentrations of 2-mercaptobenzothiazole were 64 pg/m^3 in airborne particulate matter, and 591 pg/m^3 in total suspended particulate matter, and were thought to derive from tyre wear (Avagyan et al., 2014).

Table 1.1 Selected methods of analysis for 2-mercaptobenzothiazole

Sample matrix	Sample preparation	Assay method	Limit of detection	Reference
Air				
Workplace air	Collection on quartz fibre filters; stabilization by covering with double-distilled water; desorption using an ultrasonic bath; filtration of the sample Sampling recommendation: 2 h at 1 L/min (120 L)	CE/DAD	0.2 mg/m^3 [a]	Breuer et al. (2002)
Water				
Municipal and industrial wastewater	Extraction using methylene chloride at pH 6–8; drying and concentrating the sample; change solvent to methanol; if necessary: silica gel column clean-up	HPLC/UV	1.7 µg/L	EPA (1993)
Treated and raw wastewater	Direct injection of the (diluted) effluent samples	HPLC/ESI-MS/MS	0.2 µg/L	Reemtsma (2000)
Treated wastewater and raw municipal wastewater	SPE using a co-polymeric sorbent (divinylbenzene, *N*-vinylpyrrolidone); elution with methanol/acetone; addition of the internal standard; concentration of the eluent	HPLC/ESI-MS	0.05 µg/L 0.12 µg/L	Kloepfer et al. (2004a)
Cooling water (spiked)	Direct analysis of the undiluted samples at pH 8 (buffered)	SWV	0.8 mg/L	Parham et al. (2008)
Cooling water (spiked)	SPE using a cartridge loaded with copper oxide nanoparticles at pH 5–8; washing the sorbent with 0.5 M sodium thiosulfate; desorption of the cartridge using methanol	HPLC/UV	1.9 µg/L	Parham & Khoshnam (2013)
Cooling water and drinking-water (spiked)	Addition of gold nanoparticle solution and citrate buffer (pH 6)	RRS, SFP	1.0 µg/L	Parham et al. (2015)
Food				
Dietary products	Milk, yoghurt, infant formula: addition of an internal standard; protein precipitation by the addition of acetonitrile; sample filtration All other foodstuffs: addition of an internal standard, acetic acid and acetonitrile; sonication of the sample followed by centrifugation and filtering	HPLC/APCI-MS	8–43 µg/kg [b]	Barnes et al. (2003)
Products				
Coolant formulations	Direct analysis of the undiluted samples	HPLC/UV	0.02%	Schmitt & Muzher (1981)
Injectable solutions	Mixing with 1 M hydrochloric acid; extraction with chloroform; evaporation to dryness and reconstitution in acetonitrile	HPLC/UV	NA	Reepmeyer & Juhl (1983)
Protective gloves	Extraction using acetone; evaporation to dryness and reconstitution in acetonitrile; filtration of the sample	HPLC/DAD	1 mg/L	Bergendorff et al. (2006)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Limit of detection	Reference
Urine				
Experimental animals	Acidic hydrolysis using 5 M sulfuric acid; incubation at room temperature; SPE using C_{18} cartridges; elution with ethyl acetate; evaporation to dryness and reconstitution in ethanol	GC/MS	20 µg/L	Manninen et al. (1996)
Exposed workers and non-exposed controls	Homogenization and addition of ammonium acetate buffer (pH 6.5), deuterated internal standard (MBT-d_4) and β-glucuronidase/arylsulfatase; homogenization of the samples, incubation overnight at 37 °C and centrifugation	HPLC/ESI-MS/MS	0.4 µg/L	Gries et al. (2015)

[a] The analysis is considered to be semi-quantitative. The coefficient of variation of the calibration is 1.8%, therefore the analytical procedure itself is precise; however, due to the instability of 2-mercaptobenzothiazole on the filter, large overall variation can occur (up to 16% depending on the concentration)

[b] Depending on the food

APCI, atmospheric pressure chemical ionization; CE, capillary electrophoresis; DAD, diode array detection; ESI, electrospray ionization; GC, gas chromatography; HPLC, high-performance liquid chromatography; MBT, 2-mercaptobenzothiazole; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NA, not available; RRS, resonance Raleigh scattering; SFP, spectrofluorophotometry; SPE, solid-phase extraction; SWV, square wave voltammetry; UV, ultraviolet detection

(b) Water

Effluent from a waste dump was analysed for 2-mercaptobenzothiazole by liquid chromatography in a study aimed at developing analytical methods; the concentration of 2-mercaptobenzothiazole was estimated at 30 µg/L in the sample tested (Cox, 1976).

A study of municipal wastewater in Germany found 2-mercaptobenzothiazole at a concentration of up to 0.19 µg/L (Kloepfer et al., 2004b). In a comprehensive survey of wastewater from 4000 industrial and publicly owned treatment works sponsored by the Effluent Guidelines Division of the EPA, 2-mercaptobenzothiazole was identified in one discharge each from a rubber-processing and pesticide-manufacturing industry at a concentration of 1.27 ppm [8.67 µg/L] and 0.86 ppm [5.87 µg/L], respectively (Shackelford et al., 1983). The mean concentration of 2-mercaptobenzothiazole in the effluent from a paper mill was 0.025 ppm [0.17 µg/L] (Keith, 1976), and a concentration of 30 µg/L was found in the wastewater from a tyre-manufacturing plant (Jungclaus et al., 1976). 2-Mercaptobenzothiazole has been detected in tannery wastewater at concentrations ranging from 420 to 840 µg/L (Rodríguez et al., 2004), and in trace amounts in natural surface-water samples as a degradation product of the wood preservative 2-(thiocyanomethylthio)benzothiazole, which leaked from an upstream sawmill (Khoroshko et al., 2005).

(c) Products

(i) Medical devices

An investigation of adverse reactions to an excretory urography contrast agent detected 2-mercaptobenzothiazole at concentrations of up to 3.3 µg/mL. At this concentration, a dose of the contrast agent of 100 mL would contain 0.33 mg of 2-mercaptobenzothiazole (Hamilton, 1987).

Leaching of 2-mercaptobenzothiazole into drug preparations of several constituents of elastomeric closures was assessed. In syringe cartridges, concentrations of 2-mercaptobenzothiazole ranged from 8.3 to 13.8 µg/mL (Airaudo et al., 1990).

An investigation of instability of a therapeutic protein for infusion revealed that 2-mercaptobenzothiazole and its zinc salt leached from the stopper used for the infusion bags (100 mL) (Chang et al., 2010).

(ii) Consumer products

A survey of 19 rubber gloves found that two contained 2-mercaptobenzothiazole (5–8 µg/g) (Bergendorff et al., 2006).

In 2001, a retail market survey of 19 samples of baby bottle teats and soothers was performed in the Netherlands. The migration of 2-mercaptobenzothiazole was detected in only one natural rubber sample, and was considerably lower than the limit of 0.3 mg/teat (Bouma et al., 2003).

In the United Kingdom, in 2000, 2-mercaptobenzothiazole was not detected in 236 retail samples of food that may have been in contact with rubber material during their fabrication, transport, or storage (Barnes et al., 2003).

1.4.3 Occupational exposure

2-Mercaptobenzothiazole has been detected in the urine samples of four workers employed at a plant producing 2-mercaptobenzothiazole, one worker from the administration department of the plant, and only one out of forty persons not knowingly exposed to 2-mercaptobenzothiazole (Gries et al., 2015). Mean concentrations of 2-mercaptobenzothiazole in the four exposed workers were 3958 µg/L after enzymatic hydrolysis of the urine sample during sample preparation; without hydrolysis, the concentration determined was only 69 µg/L. The results showed that most 2-mercaptobenzothiazole in the urine was excreted in conjugated forms (e.g.

2-mercaptobenzothiazole glucuronide) rather than in its unchanged form. Using this method (with hydrolysis), 2-mercaptobenzothiazole was detected in only one (11 µg/L) out of the 40 samples of urine collected from non-exposed individuals. The person from the administration department excreted 2-mercaptobenzothiazole at 2.5 µg/L and was thus within the range for non-exposed individuals.

The data from all employed patients (age range, 16–68 years; *n* = 14 234) patch-tested between 2003 and 2013 in the German Information Network of Departments of Dermatology, and diagnosed as having occupationally acquired contact dermatitis were analysed. The control group comprised all other patients (*n* = 31 706) within the same time frame who tested negative for occupationally acquired allergic contact dermatitis. The prevalence ratio (indicating risk) was significantly increased for 2-mercaptobenzothiazole (prevalence ratio, 3.88; 95% confidence interval [CI], 3.09–4.89) (Bauer et al., 2015).

A series of 23 patients with allergic contact dermatitis (some with disseminated dermatitis) due to rubber accelerators in rubber gloves treated during a 2-year period was described. Sixteen were health-care workers from a single institution whose dermatitis was temporally related to the switch to latex-safe gloves. Each had positive patch-test reactions to one or more rubber accelerators, including 2-mercaptobenzothiazole. Chemical analysis identified 2-mercaptobenzothiazole in four out of six glove samples (Cao et al., 2010).

A retrospective analysis of data from the Information Network of Departments of Dermatology in 2002–10 found that, of 93 615 patients who were patch tested, 3448 had occupational dermatitis and were tested because of a suspected glove allergy. Of all the occupational dermatitis patients, 3% were sensitized to 2-mercaptobenzothiazole and/or its derivatives (Geier et al., 2012).

Standard patch test results of employed persons with an initial report of an occupational skin disease were analysed within 24 occupational groups. Among the occupationally relevant sensitizers, mercapto-mix/mercaptobenzothiazole contributed to 35% of the positive results (Dickel et al., 2002).

1.4.4 Exposure of the general population

Case reports that included a patch test confirmed that contact with 2-mercaptobenzothiazole was from a foley catheter (Ancona et al., 1985), a rubber earplug (Deguchi & Tagami, 1996), safety shoes (Foussereau et al., 1986), rubber gloves (Geier et al., 2012), a condom catheter (Harmon et al., 1995), and a bikini with rubberized elastic (Jung et al., 2006).

A total of 155 cases with footwear dermatitis were evaluated from July 2005 to June 2006 from detailed histories, clinical examinations, and patch testing. Contributory allergens included 2-mercaptobenzothiazole (12.9%; *n* = 20) (Chowdhuri & Ghosh, 2007).

In a study of dermatitis among athletes, 43 young adults (31 men and 12 women) with eczematous skin lesions suggesting allergic contact dermatitis were patch-tested; 21% tested positive for 2-mercaptobenzothiazole (Ventura et al., 2001).

Investigators in Spain used gas chromatography–mass spectrometry to analyse the 2-mercaptobenzothiazole content of samples of crumb rubber from urban playgrounds and from rubber pavers. Ten of the 21 samples from playgrounds contained quantifiable 2-mercaptobenzothiazole (mean, 195 µg/g; median, 185 µg/g; maximum, 398 µg/g). No 2-mercaptobenzothiazole was detected in the nine samples from rubber pavers analysed (Llompart et al., 2013).

1.4.5 Exposure assessment in epidemiological studies

Strauss et al. (1993) studied a production facility in West Virginia, USA, where 2-mercaptobenzothiazole had been produced since 1934. A former plant industrial hygienist developed annual airborne exposure estimates throughout the study period for all hourly production jobs, using sampling data available from 1977 to 1989, historical company documents, and interviews with knowledgeable retirees for the period before 1977. Jobs with potential exposure to 2-mercaptobenzothiazole were assigned to four exposure categories: very low (> 0–0.5 mg/m^3), low (> 0.5–2.0 mg/m^3), medium (> 2.0–5.0 mg/m^3) and high (> 5.0–20.0 mg/m^3). Jobs in these categories were not named. A cumulative exposure index for each job was calculated by multiplying the midpoint of each exposure category by duration in a 2-mercaptobenzothiazole-exposed job. Three categories of cumulative exposure to 2-mercaptobenzothiazole were calculated: < 2 mg/m^3–year (46% of the cohort), 2–7 mg/m3–year (32% of the cohort), and 8–129 mg/m^3–year (22% of the cohort). No description of the processes was included in the report, therefore no information on exposure characteristics by process could be derived. Collins et al. (1999) extended the follow-up of this cohort and noted that, in 1943–54, average exposures generally exceeded 2 mg/m^3. Again, no information on the processes was provided.

A mortality study was conducted among employees at a plant in Wales, United Kingdom, that had manufactured 2-mercaptobenzothiazole since 1932 and other chemicals, including *ortho*-toluidine, aniline, phenyl-β-naphthylamine, and polymerized 2,2,4-trimethyl-1,2-dihydroquinoline. A former occupational hygienist from the plant provided assessments of 8-hour time-weighted average (TWA) exposures to both 2-mercaptobenzothiazole and its derivatives over a range of years and for each job and department title. Various jobs entailed either zero exposure, very low (0–1 mg/m^3), low (1–2.5 mg/m^3), medium (2.5–6 mg/m^3) or high (6–20 mg/m^3) exposure. Estimates were made on the basis of monitoring data from 1977 onwards, a review of process manuals and other company records for earlier years, and discussions with long-serving employees. Annual exposure estimate were adjusted by a "year fraction" factor per job, to take into account the duration of exposure for a working year. Cumulative exposure strata were reported as none, 0.01–21.24 mg/m^3–year, 21.25–63.74 mg/m^3–year, and ≥ 63.75 mg/m^3–year. No description of the processes was supplied and no exposures by process or distribution of workers by exposure strata were reported (Sorahan & Pope, 1993). A second report on this cohort provided further details of the matrix of exposure levels by job title and year. The highest exposures were 11.7 mg/m^3 for day pack and pellet operators, and 8.5 mg/m^3 for bag flake operators and daymen. The same cumulative exposure strata were reported (Sorahan et al., 2000). A third update (Sorahan, 2008) and a fourth update (Sorahan, 2009) of this cohort used the same exposure assessment. [The Working Group noted that exposure to 2-mercaptobenzothiazole dust would be associated with jobs identified within the compounding area or described as batch preparation. Downstream, skin contact with expressed 2-mercaptobenzothiazole and absorption would be higher in jobs before curing (green rubber), such as milling, calendaring, and tyre building, although there may still be skin absorption from cured rubber.]

1.5 Regulations and guidelines

The legal occupational exposure in Germany for 2-mercaptobenzothiazole is 4 mg/m^3 inhalable dust. No other permissible limits were found (GESTIS, 2015).

The Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

designation of 2-mercaptobenzothiazole is H317, "may cause an allergic skin reaction" and H410 "very toxic to aquatic life with long-lasting effects" (ECHA, 2015).

2. Cancer in Humans

See Table 2.1

A series of studies of workers at a chemical production plant in north Wales, United Kingdom, provided most of the pertinent evidence pertaining to associations between occupational exposure to 2-mercaptobenzothiazole and cancer (Sorahan & Pope, 1993; Sorahan et al., 2000; Sorahan, 2008, 2009). Some workers in the plant were also exposed to other chemicals (aniline, phenyl-β-naphthylamine, *ortho*-toluidine, and polymerized 2,2,4-trimethyl-1,2-dihydroquinoline) (Sorahan et al., 2000).

Within a cohort of 2160 male production workers employed for at least 6 months and with some employment during 1955–84, 363 were exposed to 2-mercaptobenzothiazole, 94 were exposed to phenyl-β-naphthylamine, 53 were exposed to *ortho*-toluidine, and 442 were exposed to aniline, but overlaps among the exposed groups were not specified (Sorahan, 2008). Sorahan (2008) focused on morbidity and mortality from cancer of the urinary bladder in the cohort exposed to 2-mercaptobenzothiazole and found an excess in mortality (8 deaths; standardized mortality ratio [SMR], 3.74; 95% CI, 1.62–7.37) and incidence (12 cases; standardized relative risk [SRR], 2.53; 95% CI, 1.31–4.41) compared with national rates (population of England and Wales). In an internal multivariate analysis of incident bladder cancer using the full cohort and adjusting for age, calendar period, and duration of employment with exposure to *ortho*-toluidine, aniline, and phenyl-β-naphthylamine, a positive non-significant trend ($P = 0.16$) was found with cumulative exposure to 2-mercaptobenzothiazole (0.01–21.24 mg/m^3–year: 6 cases; relative risk [RR], 0.97; 95% CI, 0.38–2.43; 21.25–63.74 mg/m^3–year: 6 cases; RR, 1.70; 95% CI, 0.65–4.41; ≥ 63.75 mg/m^3–year: 3 cases; RR, 2.12; 95% CI, 0.64–7.06). The 15 cases in the three exposed groups included three with benign or in-situ tumours.

A subsequent report that presented data on the mortality from and incidence of cancers other than those of the urinary bladder among male workers exposed to 2-mercaptobenzothiazole relative to unexposed workers found excesses for cancers of the large intestine, lung and multiple myeloma (Sorahan, 2009). In the subcohort exposed to 2-mercaptobenzothiazole, there were eight deaths (SMR, 2.32; 95% CI, 1.00–4.57) from and nine diagnoses (SRR, 1.81; 95% CI, 0.83–3.44) of cancer of the large intestine, 27 deaths (SMR, 1.38; 95% CI, 0.91–2.01) from and 26 diagnoses (SRR, 1.52; 95% CI, 0.99–2.23) of lung cancer, and three deaths (SMR, 4.40; 95% CI, 0.91–12.87) from and four diagnoses (SRR, 4.65; 95% CI, 1.27–11.91) of multiple myeloma. In internal multivariate analyses of incidence in the full cohort that adjusted for the duration of employment with exposure to *ortho*-toluidine, aniline and phenyl-β-naphthylamine, significant trends of increased incidence with increased cumulative exposure to 2-mercaptobenzothiazole remained for cancer of the large intestine ($P < 0.001$) and multiple myeloma ($P = 0.019$). [The trend analysis for multiple myeloma was based on small numbers (4 exposed cases).]

Collins et al. (1999) studied a cohort of 1059 male chemical-production workers in Nitro, West Virginia, USA, exposed to 2-mercaptobenzothiazole and 4-aminobiphenyl (classified in IARC Group 1 as a cause of cancer of the urinary bladder) in an update of a report by Strauss et al. (1993). Among 511 2-mercaptobenzothiazole workers with no documented exposure to 4-aminobiphenyl, five deaths from urinary bladder cancer (SMR, 4.3; 95% CI, 1.4–10.0)

Table 2.1 Cohort studies of cancer and exposure to 2-mercaptobenzothiazole

Reference, location, enrolment/ follow-up period	Population size, description; method of exposure assessment	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (1999) Nitro, WV, USA 1955–77	1059 (600 with MBT exposure), white male production workers active for ≥ 1 day; JEM Social Security Administration, National Death Index, motor vehicle bureau, credit records	All cancers combined	Total MBT cohort	63	1.0 (0.8–1.3)	NR	Strengths: extended follow-up Limitations: sampling data from 1977–89 only; no data on cigarette smoking
			Job involving MBT with 4-ABP	23	2.0 (1.3–3.0)		
			Job involving MBT without 4-ABP	40	0.8 (0.5–1.0)		
		Lung	Total MBT cohort	27	1.0 (0.7–1.5)	NR	
			Job involving MBT with 4-ABP	11	2.4 (1.2–4.3)		
			Job involving MBT without 4-ABP	16	0.7 (0.4–1.2)		
		Prostate	Total MBT cohort	4	0.9 (0.2–2.3)	NR	
			Job involving MBT without 4-ABP	4	1.1 (0.3–2.9)		
		Urinary bladder	Total MBT cohort	13	8.9 (4.7–15.2)	NR	
			Job involving MBT with 4-ABP	8	27.1 (11.7–53.4)		
			Job involving MBT without 4-ABP	5	4.3 (1.4–10.0)		
			Unexposed to MBT or 4-ABP	1	1.2 (0.0–6.5)		
			Cumulative exposure, < 2 mg/m^3-year, no 4-ABP	0	0.0 (0.0–13.8)		
			Cumulative exposure, 2–7.9 mg/m^3-year, no 4-ABP	1	3.5 (0.1–19.5)		
			Cumulative exposure, 8–129 mg/m^3-year, no 4-ABP	4	6.5 (1.8–16.6)		
			Trend test *P*-value: 0.04				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description; method of exposure assessment	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2008) North Wales, UK 1955–2005	2160 men, hourly staff working for ≥ 6 mo; 8-h TWA to MBT, MBT derivatives, by job and department: zero, very low (0–1 mg/m³), low (1–2.5 mg/m³), medium (2.5–6 mg/m³), high (6–20 mg/m³) 363 men exposed to MBT, 94 men exposed to PBN, 442 men exposed to aniline, 53 men exposed to *ortho*-toluidine) Office of National Statistics	Urinary bladder	0	41	1.00	Duration of employment in departments with exposure to PBN, aniline and *ortho*-toluidine	Trend analysis included benign or in-situ tumours. Strengths: long follow-up, little loss to follow-up Limitations: levels estimated based on monitoring data ≥ 1977, 266 workers in > 1 subcohort, 8 workers in all 4 subcohorts, cancer morbidity data only from 1971 to 2005
			0.01–21.24 mg/m³-year (RR)	6	0.97 (0.38–2.43)		
			21.25–63.74 mg/m³-year (RR)	6	1.70 (0.65–4.41)		
			≥ 63.75 mg/m³-year (RR)	3	2.12 (0.64–7.06)		
			Trend test *P*-value: 0.16				
			SMR	8	3.74 (1.62–7.37)	Age, sex, and period	
			SRR	12	2.53 (1.31–4.41)	Age, sex, and period	
Sorahan (2009) North Wales, UK 1955–2005	2160 men (363 in MBT subcohort), hourly staff working ≥ 6 mo and some employment 1955–84; 8-h TWA to MBT, MBT derivatives, by job and department: zero, very low (0.1–1 mg/m³), low (1–2.5 mg/m³), medium (2.5–6 mg/m³) and high (6–20 mg/m³) Office of National Statistics	Colon: large intestine	SMR	8	2.32 (1.00–4.57)	Duration of employment in departments with exposure to PBN, aniline, and *ortho*-toluidine	Cases represent incidence and include death certificate notifications. Report focused on MBT and adjusted for other chemical exposures Strengths: long follow-up, little loss to follow-up Limitations: levels estimated based on monitoring data ≥ 1977, most workers exposed to > 1 chemical
			SRR	9	1.81 (0.83–3.44)		
			Cumulative exposure, 0 (RR)	27	1.00		
			Cumulative exposure, 0.01–21.24 mg/m³-year (RR)	3	1.24 (0.36–4.26)		
			Cumulative exposure, 21.25–63.74 mg/m³-year (RR)	6	4.76 (1.82–12.43)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description; method of exposure assessment	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2009) North Wales, UK 1955–2005 (cont.)			Cumulative exposure, > 63.75 mg/m³-year (RR)	3	4.69 (1.38–15.90)		
			Trend test *P*-value: < 0.001				
		Lung	SMR	27	1.38 (0.91–2.01)	Duration of employment in departments with exposure to PBN, aniline and *ortho*-toluidine	
			SRR	26	1.52 (0.99–2.23)		
			Cumulative exposure, 0 (RR)	107	1.00		
			Cumulative exposure, 0.01–21.24 mg/m³-year (RR)	21	2.17 (1.30–3.61)		
			Cumulative exposure, 21.25–63.74 mg/m³-year (RR)	4	0.65 (0.23–1.81)		
			Cumulative exposure, ≥ 63.75 mg/m³-year (RR)	4	1.44 (0.53–3.96)		
			Trend test *P*-value: 0.38				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description; method of exposure assessment	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2009) North Wales, UK 1955–2005 (cont.)		Multiple myeloma	SMR	3	4.40 (0.91–12.87)	Duration of employment in departments with exposure to PBN, aniline and *ortho*-toluidine	
			SRR	4	4.65 (1.27–11.91)		
			Cumulative exposure, 0 (RR)	2	1.00		
			Cumulative exposure, 0.01–21.24 mg/m^3-year (RR)	2	10.21 (1.27–81.7)		
			Cumulative exposure, 21.25–63.74 mg/m^3-year (RR)	2	20.57 (2.58–164.00)		
			Trend test *P*-value: 0.019				
		All cancers combined	SMR	76	1.41 (1.11–1.77)	NR	
			SRR	97	1.48 (1.20–1.81)		

4-ABP, 4-aminobiphenyl; CI, confidence interval; JEM, job–exposure matrix; MBT, 2-mercaptobenzothiazole; mo, month; NR, not reported; PBN, phenyl-β-naphthylamine; RR, relative risk; SMR, standardized mortality ratio; SRR, standardized relative risk; TWA, time-weighted average

occurred. The standardized mortality ratios for bladder cancer in this cohort also showed a statistically significant increasing trend with increasing cumulative exposure to 2-mercaptobenzothiazole (*P* for trend = 0.04). No excess or trend was reported for lung cancer overall, or among workers exposed only to 2-mercaptobenzothiazole; however, the SMR for workers also exposed to 4-aminobiphenyl was elevated (SMR, 2.4; 95% CI, 1.2–4.3). No findings were presented for multiple myeloma or colon cancer in the cohort exposed to 2-mercaptobenzothiazole.

The use of 2-mercaptobenzothiazole was mentioned in studies conducted at another rubber chemical plant by the United States National Institute for Occupational Safety and Health (NIOSH), but results for urinary bladder cancer were only given for exposure to *ortho*-toluidine (NIOSH, 1989, 1992; Carreón et al., 2014).

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

Groups of 18 male and 18 female B6C3F$_1$ and C57BL/6 × AKR mice (age, 7 days) received 2-mercaptobenzothiazole [purity not reported; "commercial product" Captax] at a dose of 0 (control) or 100 mg/kg body weight (bw) in 0.5% gelatin daily by gavage for 3 weeks, followed immediately by continuous treatment with diets containing 2-mercaptobenzothiazole at a concentration of 0 (control) or 323 ppm for up to 18 months. [These dose rates were considered to be the maximal tolerated dose based on short-term studies of dose-related mortality.] Control mice were housed in the same room. At 18 months, mice were subjected to a post-mortem evaluation of the major thoracic and abdominal organs and the thyroid, but the cranium was not dissected. Results were characterized as the incidence of hepatoma, pulmonary tumours, and lymphoma, and the total number of mice with tumours. Statistical analyses compared treated mice by sex and strain combination with five pooled negative-control groups using the Mantel-Haenszel procedure for the combined relative risk, using the weighted geometric mean with the ½ correction. Under the conditions of this study, administration of 2-mercaptobenzothiazole did not cause a significant increase in the incidence of tumours (Innes et al., 1969). [The limitations of this single-dose study were the small number of animals per group and the inadequate reporting of results, including the lack of information on survival, and the limited macroscopic and microscopic post-mortem evaluation. The Working Group considered this study to be inadequate for an evaluation of the carcinogenicity of 2-mercaptobenzothiazole.]

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given 2-mercaptobenzothiazole (purity, 96.3%) at a dose of 0, 375, or 750 mg/kg bw by gavage in corn oil on 5 days per week for 103 weeks. Survival was significantly decreased in female mice at 750 mg/kg bw. In male mice, mean body weights were 6–14% lower in those at 750 mg/kg bw, and 4–8% lower in those at 375 mg/kg bw, compared with controls. In female mice, mean body weights in the group at 750 mg/kg bw were within 6% of the level in vehicle controls, while those of the group at 375 mg/kg bw were generally greater than the level in vehicle controls.

In female mice, treatment with 2-mercaptobenzothiazole resulted in an increase in the incidence of hepatocellular adenoma or carcinoma (combined) only at 375 mg/kg bw (4/50 [adjusted rate, 10.8%] controls; 12/49 [adjusted rate, 29.8%] at 375 mg/kg bw (P = 0.035 by the pairwise life-table test, P = 0.028 by pairwise incidental-tumour test) and 4/50 [adjusted rate, 18.2%] at 750 mg/kg bw). The historical incidence of

Table 3.1 Studies of carcinogenicity in experimental animals given 2-mercaptobenzothiazole by oral administration

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M+F, combined) 7 days 18 mo Innes et al. (1969)[a]	Commercial product, "Captax" 0.5% gelatin (gavage) 0, 323 ppm Daily on days 7–35 (100 mg/kg bw), in the diet (0 or 323 ppm) for remainder 36/group 32, NR	Hepatomas, pulmonary tumours and lymphoma: NR	NS	Groups of 18 male and 18 female animals at start of study; the authors reported no significant increase in the incidence of hepatomas, pulmonary tumours, lymphoma or total tumours in treated mice
Mouse, C57BL/6 × AKR (M+F, combined) 7 days 18 mo Innes et al. (1969)[a]	Commercial product, "Captax" 0.5% gelatin (gavage) 0, 323 ppm Daily gavage on days 7–35 (100 mg/kg bw), in the diet (0 or 323 ppm) for remainder 36/group 33, NR	Hepatomas, pulmonary tumours and lymphoma: NR	NS	Groups of 18 male and 18 female animals at start of study; the authors reported no significant increase in the incidence of hepatomas, pulmonary tumours, lymphoma or total tumours in treated mice
Mouse, $B6C3F_1$ (M) 8 wks 103 wks NTP (1988)[b]	Purity, 96.3% Corn oil 0, 375, 750 mg/kg bw by gavage 5 days/wk 50/group 38, 33, 30	Any tumour type	NS	No evidence of carcinogenicity
Mouse, $B6C3F_1$ (F) 8 wks 103 wks NTP (1988)[b]	Purity, 96.3% Corn oil 0, 375, 750 mg/kg bw 5 days/wk 50/group 37, 39, 22	*Liver* Hepatocellular adenoma: 3/50, 7/49, 4/50 Hepatocellular carcinoma: 1/50, 5/49, 0/50 Hepatocellular adenoma or carcinoma (combined): 4/50, 12/49, 4/50	NS NS 375 mg/kg: pairwise $P = 0.035$ (life-table test), $P = 0.028$ (incidental-tumour test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 6–7 wks 103 wks NTP (1988)[b]	Purity, 96.3% Corn oil 0, 375, 750 mg/kg bw by gavage 5 days/wk 50/group 42, 22, 20	*Adrenal gland*		
		Pheochromocytoma, benign or malignant: 18/50, 27/50, 24/49	Trend, $P < 0.001$ (life-table test, $P = 0.038$ (incidental-tumour test); 375 mg/kg: pairwise $P < 0.001$ (life-table test), $P = 0.021$ (incidental-tumour test); 750 mg/kg: pairwise $P < 0.001$ (life-table test, $P = 0.034$ (incidental-tumour test).	
		Pheochromocytoma, benign: 18/50, 25/50, 22/49	Trend, $P = 0.002$ (life-table test); 375 mg/kg: pairwise $P < 0.001$ (life-table test); 750 mg/kg: pairwise $P = 0.002$ (life-table test)	
		Pheochromocytoma, malignant: 0/50, 2/50, 2/49	NS	
		Haematopoietic system		
		Mononuclear cell leukaemia: 7/50, 16/50, 3/50	375 mg/kg: pairwise $P = 0.002$ (life-table test)	
		Pancreas		
		Acinar cell adenoma: 2/50, 13/50, 6/49	Trend, $P = 0.017$ (life-table test); 375 mg/kg: pairwise $P < 0.001$ (life-table test), $P < 0.001$ (incidental-tumour test); 750 mg/kg: pairwise $P = 0.030$ (life-table test)	
		Mesothelium		
		Mesothelioma (multiple organs): 0/50, 2/50, 3/50	Trend, $P = 0.039$ (life-table test), $P = 0.041$ (incidental-tumour test); historical corn oil vehicle controls NTP studies: 55/1450 (4% ± 3%); range, 0–6/50	
		Preputial gland		
		Adenoma or carcinoma (combined): 1/50, 6/50, 5/50	Trend, $P = 0.027$ (life-table test); 375 mg/kg: pairwise $P = 0.021$ (life-table test); 750 mg/kg: pairwise $P = 0.030$ (life-table test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 6–7 wks 103 wks NTP (1988)[b] (cont.)		Adenoma: 0/50, 4/50, 4/50	Trend, $P = 0.016$ (life-table test), $P = 0.042$ (incidental-tumour test); 375 mg/kg: pairwise $P = 0.019$ (life-table test); 750 mg/kg: pairwise $P = 0.021$ (life-table test)	
		Carcinoma: 1/50, 2/50, 1/50	NS	
		Pituitary gland		
		Adenoma: 14/50, 21/50, 12/48	375 mg/kg: pairwise $P = 0.003$ (life-table test)	
		Skin, subcutaneous		
		Fibroma, neurofibroma, sarcoma, or fibrosarcoma (combined): 3/50, 6/50, 7/50	Trend, $P = 0.031$ (life-table test); 750 mg/kg: pairwise $P = 0.037$ (life-table test); historical controls, corn oil vehicle, NTP studies: 126/1450 (9% ± 4%)	
Rat, F344 (F) 6–7 wks 103 wks NTP (1988)[b]	Purity, 96.3% Corn oil 0, 188, 375 mg/kg bw by gavage 5 days/wk 50/group 28, 31, 25	*Adrenal gland*		
		Pheochromocytoma (benign): 1/50, 5/50, 6/50	Trend, $P = 0.030$ (life-table test), $P = 0.038$ (incidental-tumour test); 750 mg/kg: pairwise $P = 0.041$ (life-table test)	
		Pituitary gland		
		Adenoma: 15/49, 24/50, 25/50	Trend, $P = 0.014$ (life-table test), $P = 0.015$ (incidental-tumour test); 375 mg/kg: pairwise $P = 0.021$ (life-table test), $P = 0.027$ (incidental-tumour test)	
		Adenoma or adenocarcinoma (combined):16/49, 24/50, 25/50	Trend, $P = 0.024$ (life-table test,) $P = 0.028$ (incidental-tumour test); 375 mg/kg: pairwise $P = 0.036$ (life-table test); 1 (control) animal had a pituitary gland adenocarcinoma	

[a] Principal limitations of the study: inadequate reporting of results, limited macroscopic and microscopic evaluation, purity not reported and single dose tested

[b] Principal strengths of the study: good laboratory practice, covers most of the lifespan, multiple doses tested and large number of animals per group

bw, body weight; F, female; M, male; mo, month; NR, not reported; NS, not significant; NTP, United States National Toxicology Program; wk, week

hepatocellular adenoma or carcinoma (combined) among female control mice in studies conducted by the National Toxicology Program (NTP) cited in the report was 116/1489 (8% ± 6%). The incidence of hepatocellular adenoma and hepatocellular carcinoma was 3/50 and 1/50 in the control group, 7/49 and 5/49 in the group at 375 mg/kg bw, and 4/50 and 0/50 at 750 mg/kg bw, respectively. In male mice, treatment with 2-mercaptobenzothiazole did not result in significant increases in tumour incidence, unusual tumours or early-onset tumours (NTP, 1988). [The strengths of this study included the large numbers of animals, compliance with good laboratory practice, the evaluation of multiple dose levels, and the duration of exposure that involved most of the lifespan.]

3.2 Rat

Oral administration

Groups of 50 male and 50 female Fischer 344/N rats (age, 6–7 weeks) were given 2-mercaptobenzothiazole (purity, 96.3%) at a dose of 0, 188 (females only), 375, or 750 (males only) mg/kg bw daily by gavage in corn oil on 5 days per week for 103 weeks. Survival was significantly decreased in male rats at 375 or 750 mg/kg bw. The mean body weights of treated males were similar to or greater than those of control males, and those of treated females were greater than those of control females.

In male rats, treatment with 2-mercaptobenzothiazole resulted in an increase in the incidence of pheochromocytoma of the adrenal gland (benign or malignant, combined) in the groups at 375 and 750 mg/kg bw: 18/50 controls, 27/50 at 375 mg/kg bw ($P < 0.001$ by the pairwise life-table test, $P = 0.021$ by the pairwise incidental-tumour test), and 24/49 at 750 mg/kg bw ($P < 0.001$ by the pairwise life-table test, $P = 0.034$ by the pairwise incidental-tumour test). Statistical analyses detected a positive dose-related trend using the life-table ($P < 0.001$) and incidental-tumour ($P = 0.038$) tests. The reported historical incidence of benign or malignant pheochromocytoma (combined) in male control rats was 347/1442 (24% ± 9%). In addition, an increase in the incidence of benign pheochromocytoma was observed in treated males: 18/50 controls, 25/50 at 375 mg/kg bw ($P < 0.001$ by the pairwise life-table test), and 22/49 at 750 mg/kg bw ($P = 0.002$ by the pairwise life-table test). Statistical analyses detected a positive dose-related trend using the life-table test ($P = 0.002$). The incidence of malignant pheochromocytoma was 0/50 controls, 2/50 at 375 mg/kg bw, and 2/49 at 750 mg/kg bw. An increase in the incidence of mononuclear cell leukaemia of the haematopoietic system was also observed in the group at 375 mg/kg bw only (7/50 controls, 16/50 at 375 mg/kg bw ($P = 0.002$ by the pairwise life-table test) and 3/50 at 750 mg/kg bw). The reported historical incidence of mononuclear cell leukaemia in male control rats was 202/1450 (14% ± 8%). The incidence of pancreatic acinar cell adenoma was increased in treated males (2/50 controls, 13/50 at 375 mg/kg bw ($P < 0.001$ by the pairwise life-table test, $P < 0.001$ by the pairwise incidental-tumour test), and 6/49 at 750 mg/kg bw ($P = 0.030$ by the pairwise life-table test)). Statistical analyses detected a positive dose-related trend using the life-table test ($P = 0.017$), and the reported historical incidence of pancreatic acinar cell neoplasms in male control rats was 80/1381 (6% ± 8%). The incidence of preputial gland adenoma or carcinoma (combined) was also increased in treated males (1/50 controls, 6/50 at 375 mg/kg bw ($P = 0.021$ by the pairwise life-table test), and 5/50 at 750 mg/kg bw ($P = 0.030$ by the pairwise life-table test)). Statistical analyses detected a positive dose-related trend using the life-table test ($P = 0.027$), and the reported historical incidence of preputial gland adenoma or carcinoma (combined) in male control rats was 65/1450 (4% ± 4%). In addition, the treatment resulted in an increase in the incidence of preputial gland adenoma in males: 0/50

controls, 4/50 at 375 mg/kg bw (P = 0.019 by the pairwise life-table test), and 4/50 at 750 mg/kg bw (P = 0.021 by the pairwise life-table test). Statistical analyses detected a positive dose-related trend using the life-table (P = 0.016) and incidental tumour (P = 0.042) tests, but did not detect a positive dose-related trend or increases in the incidence of preputial gland carcinoma in individual treatment groups (1/50 controls, 2/50 at 375 mg/kg bw, and 1/50 at 750 mg/kg bw) using either the life-table or incidental-tumour tests. The reported historical incidence of carcinoma of the preputial gland in male control rats was 35/1450 (2% ± 3%). A significant positive trend (P = 0.041 by the incidental-tumour test, P = 0.039 by the life-table test) in the incidence of mesothelioma (multiple organs) was observed (0/50 controls, 2/50 at 375 mg/kg bw, and 3/50 at 750 mg/kg bw). This increase was not statistically significant by pairwise comparison for either of the treated groups, and the incidence did not exceed that of historical corn-oil vehicle controls (55/1450, 4% ± 3%; reported range, 0–6/50). Also, an increase in the incidence of tumours of the skin (fibroma, neurofibroma, sarcoma, or fibrosarcoma combined) was observed in treated males (3/50 controls, 6/50 at 375 mg/kg bw, and 7/50 at 750 mg/kg bw (P = 0.037 by the pairwise life-table test)), and was associated with a significant positive trend (P = 0.031 by the life-table test). The incidence of adenoma of the pituitary gland was increased only at a dose of 375 mg/kg bw (14/50 controls, 21/50 at 375 mg/kg bw (P = 0.003 by the pairwise life-table test), and 12/48 at 750 mg/kg bw) with a reported historical incidence of 344/1411 (24% ± 8%).

In female rats, treatment with 2-mercaptobenzothiazole resulted in an increase in the incidence of benign pheochromocytoma of the adrenal gland only at a dose of 375 mg/kg bw (1/50 controls, 5/50 at 188 mg/kg bw, and 6/50 at 375 mg/kg bw (P = 0.041 by the pairwise life-table test)). Statistical analyses detected a positive dose-related trend using the life-table test (P = 0.030) and the incidental-tumour test (P = 0.038), and the reported historical incidence of benign pheochromocytoma in female controls was 82/1443 (6% ± 4%). An increase in the incidence of adenoma of the pituitary gland was also observed in females at 375 mg/kg bw (15/49 controls, 24/50 at 188 mg/kg bw, and 25/50 at 375 mg/kg bw (P = 0.021 by the pairwise life-table test, P = 0.027 by the pairwise incidental-tumour test)). Statistical analyses detected a positive dose-related trend using the life-table (P = 0.014) and incidental-tumour (P = 0.015) tests. One rat in the control group had a pituitary adenocarcinoma. The reported historical incidence of adenoma, carcinoma, or adenocarcinoma (combined) of the pituitary gland in female control rats was 561/1407 (40% ± 8%) (NTP, 1988). [The strengths of this study included the large numbers of animals, compliance with good laboratory practice, the evaluation of multiple dose levels, and the duration of exposure that involved most of the lifespan.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

2-Mercaptobenzothiazole has been detected in the urine samples of four workers employed at a plant producing 2-mercaptobenzothiazole, one worker from the administration department of the plant, and one out of forty persons not knowingly exposed to 2-mercaptobenzothiazole. Most 2-mercaptobenzothiazole in the urine was excreted in conjugated forms (e.g. 2-mercaptobenzothiazole glucuronide) rather than in its unchanged form (Gries et al., 2015).

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

An early study of pharmacokinetics after subcutaneous administration of radiolabelled 2-mercaptobenzothiazole to guinea-pigs demonstrated that the compound was well absorbed, with abrasion increasing the rate of absorption. Distribution occurred primarily in the kidney, liver, and thyroid gland. The thyroid gland contained the highest concentration of the compound 48 hours after injection. Ninety percent of the compound was conjugated with glucuronides and sulfates (cited in NTP, 1988).

When administered by gavage, the half-life of 2-mercaptobenzothiazole in Fischer 344 rats was less than 8 hours, possibly as short as 4–6 hours. Absorption was rapid and was not affected by doses up to 55 mg/kg bw. The major products of metabolism were polar metabolites, consistent with the findings of earlier studies in rats and guinea-pigs (cited in NTP, 1988).

After administration in Fischer 344 rats treated by gavage, 2-mercaptobenzothiazole-derived radioactivity in the blood decreased very little between 24 and 96 hours, suggesting that residual 2-mercaptobenzothiazole-derived material accumulated in the blood. After intravenous administration in Fischer 344 rats, whole blood, plasma, urine, and faeces were analysed for radioactivity at multiple time-points, from 5 minutes up to 72 hours. Most of the radiolabel (91–96%) was excreted in the urine and 4–15% in the faeces by 72 hours. A small amount (1.5–2%) remained in the erythrocytes. The metabolites found in the urine samples were the same as those found after gavage (el Dareer et al., 1989).

(b) Metabolism

In rats treated by intraperitoneal injection, the urinary metabolites of [^{35}S-mercapto]-2-mercaptobenzothiazole comprised glutathione and glucuronic acid conjugates, and inorganic sulfate (Colucci & Buyske, 1965).

4.2 Mechanisms of carcinogenesis

The evidence on the key characteristics of carcinogens (Smith et al., 2016), concerning whether 2-mercaptobenzothiazole modulates receptor-mediated effects and is genotoxic, is summarized below. Studies relevant to whether 2-mercaptobenzothiazole induces chronic inflammation are presented in Section 4.5.

4.2.1 Receptor-mediated effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

The ability of leachates from rubber tyre materials to stimulate aryl hydrocarbon receptor (AhR)–DNA binding and AhR-dependent gene expression was recently demonstrated using recombinant mouse hepatoma (Hepa1c1c7)-based clonal cell lines containing a stably integrated AhR/dioxin-responsive element and a luciferase reporter gene with different intracellular locations and stabilities. Among the individual components of the leachates that might be involved in receptor-mediated effects, 2-mercaptobenzothiazole was identified as an AhR agonist (He et al., 2011).

2-Mercaptobenzothiazole has also been shown to inhibit rat and porcine thyroid peroxidase activity in vitro (Paul et al., 2013) and to interfere with the thyroid hormone pathway in a standard in-vivo protocol in larvae of the amphibian *Xenopus laevis* (Tietge et al., 2013). These findings were corroborated in a subsequent study that measured the inhibition of thyroid peroxidase derived from pig thyroid glands, and the inhibition of thyroxine release in a *X. laevis* thyroid gland explant culture assay and in a 7-day in-vivo assay in *X. laevis* (Hornung et al., 2015).

2-Mercaptobenzothiazole appears to accumulate in the rat thyroid after gavage (el Dareer et al., 1989; see Section 4.1.2).

4.2.2 Genetic and related effects

See Table 4.1 and Table 4.2

(a) Humans

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

In one study in vitro, 2-mercaptobenzothiazole did not induce micronucleus formation in human gastric and lung carcinoma cell lines (MGC-803 and A549, respectively) (Ye et al., 2014).

(b) Experimental systems

Zinc mercaptobenzothiazole, the zinc salt of 2-mercaptobenzothiazole, did not induce chromosomal aberrations in the bone marrow of Swiss albino mice 36 hours after administration of a single intraperitoneal dose of the compound. Groups of four mice received three different doses (480, 960, and 1920 µg/20 g) [24, 48 and 96 mg/kg bw] (Mohanan et al., 2000).

In male and female Fischer 344 rats, 2-mercaptobenzothiazole (375 mg/kg bw by gavage) did not bind to DNA in any of the tissues examined (liver, adrenal glands, pituitary gland, pancreas, and bone marrow) (Brewster et al., 1989).

2-Mercaptobenzothiazole induced chromosomal aberrations and sister-chromatid exchange in Chinese hamster ovary (CHO) cells in the presence of a metabolic activation system, as well as mutations at the $Tk^{+/-}$ locus in mouse L5178Y lymphoma cells (NTP, 1988). It also induced polyploidy in Chinese hamster lung (CHL) cells in the presence and absence of a metabolic activation system (Matsuoka et al., 2005). However, it gave negative results for the induction of 6-thioguanine-resistant mutants (*Hgprt* gene mutation) in Chinese hamster V79 cells (Donner et al., 1983).

2-Mercaptobenzothiazole was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, in the presence or absence of metabolic activation (NTP, 1988). A previous study also reported negative results in *S. typhimurium*, although the purity of the compound and the dose were not specified (Donner et al., 1983). Similarly, 2-mercaptobenzothiazole was reported to give negative results in an early study in *Escherichia coli* SD-4-73, but the purity and doses tested were not indicated (Szybalski, 1958). More recently, 2-mercaptobenzothiazole also gave negative results when tested in *S. typhimurium* TA1535/psK1002 (Ye et al., 2014).

4.3 Data relevant to comparisons across agents and end-points

For all compounds evaluated in the present volume of the *IARC Monographs*, including 2-mercaptobenzothiazole, analyses of high-throughput screening data generated by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) are presented in the *Monograph* on 1-bromopropane, in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

The major effect of 2-mercaptobenzothiazole identified in humans is allergic contact dermatitis, which has been reported in numerous case studies after contact with synthetic rubber gloves (Cao et al., 2010; Tomc et al., 2012), rubber ear plugs (Deguchi & Tagami, 1996), or condom catheters (Harmon et al., 1995), after working in the production of photographic films (Rudzki et al., 1981), or in a mining industry in which xanthate, carbamate and 2-mercaptobenzothiazole were used (Sasseville et al., 2003), after being issued

Table 4.1 Genetic and related effects of 2-mercaptobenzothiazole in human and other mammalian systems

Species, strain, sex	Test system	End-point	Test	Results		Dose (LED or HID)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Human	Gastric MGC-803 and lung A549 cell lines	Chromosomal damage	Micronuclei	–	–	100 µg/mL (not cytotoxic)		Ye et al. (2014)
Mouse, Swiss albino, NR	Bone marrow	Chromosomal damage	Chromosomal aberrations	–	NA	96 mg/kg bw i.p.; single dose (zinc salt of MBT)		Mohanan et al. (2000)
Rat, Fischer 344, M/F	Liver, adrenal and pituitary glands, pancreas, bone marrow	DNA damage	DNA binding	–	NA	375 mg/kg bw by gavage		Brewster et al. (1989)
Mouse	L5178 lymphoma cells	Mutation	*Tk*$^{+/-}$	–	+	100 µg/mL without and 5 µg/mL with metabolic activation		NTP (1988)
Chinese hamster	Lung (V79)	Mutation	*Hgprt*	–	NT	300 µg/mL		Donner et al. (1983)
Chinese hamster	Lung (CHL)	Chromosomal damage	Chromosomal aberrations	+	+	200 µg/mL without and 400 µg/mL with metabolic activation	Toxicity at 400 µg/mL without, and 600 µg/mL with, metabolic activation	Matsuoka et al. (2005)
Chinese hamster	Ovary (CHO)	Chromosomal damage	Chromosomal aberrations	–	+	30 µg/mL without and 352 µg/mL with metabolic activation		NTP (1988)
Chinese hamster	Ovary (CHO)	Chromosomal damage	Sister-chromatid exchange	–	+	20 µg/mL without and 352 µg/mL with metabolic activation		NTP (1988)

+, positive; –, negative; bw, body weight; F, female; HID, highest ineffective dose; Hgprt, hypoxanthine-guanine phosphoribosyltransferase; i.p., intraperitoneal; LED, lowest effective dose; M, male; MBT, 2-mercaptobenzothiazole; NA, not applicable; NR, not reported; NT, not tested; Tk, thymidine kinase locus

Table 4.2 Genetic and related effects of 2-mercaptobenzothiazole in non-mammalian systems

Species, strain	End-point	Test	Results		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Salmonella typhimurium, strain NR	Mutation	Reverse mutation	(–)	(–)	NR	Purity and dose NR; response stated to be statistically non-significant	Donner et al. (1983)
Salmonella typhimurium, TA100, TA1535, TA1537	Mutation	Reverse mutation	–	–	0–1000 µg/plate		NTP (1988)
Salmonella typhimurium, TA98	Mutation	Reverse mutation	–	±	0–1000 µg/plate		NTP (1988)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537	Mutation	Reverse mutation	–	–	0–10 000 µg/plate		NTP (1988)
Salmonella typhimurium, TA1535/psK1002	DNA damage	SOS/*umu* test	–	–	19 µg/mL without or 20 µg/mL with metabolic activation	50% lethal concentration: 19 µg/mL without metabolic activation and 20 µg/ml with metabolic activation	Ye et al. (2014)
Escherichia coli, SD-4-73	Mutation	Reverse mutation	(–)	NT	NR	Purity and dose NR	Szybalski (1958)

+, positive; –, negative; (–), negative in a study of limited quality; ±, equivocal (variable response in several experiments within an adequate study); HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested

with hand-based splints made of neoprene that is known to contain 2-mercaptobenzothiazole and its derivatives and formaldehyde (Stern et al., 1998), or after contact with an oil containing 2-mercaptobenzothiazole when making moulds (Wilkinson et al., 1990). The number of actual cases of allergic contact dermatitis attributed to mercapto mix and 2-mercaptobenzothiazole reported to EPIDERM in the United Kingdom from 1996 to 2012 was 177 (Warburton et al., 2015). An analysis of 803 female cleaners who were evaluated for contact dermatitis and 64 736 female controls without occupational dermatitis showed that the cleaners were significantly more frequently sensitized to 2-mercaptobenzothiazole (Liskowsky et al., 2011). In a positive patch-test analysis of 75 patients (38 men and 37 women) with contact allergic dermatitis, 6.66% (4 men and 1 woman) of the subjects tested showed reactivity to 2-mercaptobenzothiazole (Singhal & Reddy, 2000). Among 25 patients who showed a positive reaction to xanthates or other rubber additives, four patients showed reactivity to carbamate mix, thiuram mix, and 2-mercaptobenzothiazole, suggesting cross-reactivity of these chemicals (Sasseville et al., 2007). In 43 young people (31 men and 12 women) with eczematous skin lesions, two of the most frequent substances that showed a positive result in a patch test were thiurams (23.3%) and 2-mercaptobenazothiazole (20.9%) (Ventura et al., 2001).

In-vitro studies showed dose-dependent increases in cell-associated interleukin-18 in human skin cells (Corsini et al., 2009, 2013). In the human monocytic cell line THP-1, increased surface markers of CD54 and CD86 (An et al., 2009) and secretion of macrophage inflammatory protein-1β was detected (Hirota & Moro, 2006).

4.5.2 Experimental systems

The major effects of 2-mercaptobenzothiazole other than carcinogenicity identified in a 2-year bioassay in rats were nephropathy characterized by tubular hyperplasia in exposed males, and ulcers and inflammation of the forestomach in exposed males and females (NTP, 1988). Cell proliferation in the draining lymph nodes was increased by exposure of mice to sensitizers, including 2-mercaptobenzothiazole (Ikarashi et al., 1993; Ahuja et al., 2009).

One study showed that haptenation occurs through the formation of mixed disulfides between the thiol group on 2-mercaptobenzothiazole and a protein sulfhydryl group (Chipinda et al., 2007). 2-Mercaptobenzothiazole can conjugate with lysine or cysteine (Wang & Tabor, 1988b). [The Working Group noted the possibility that the thiol group of 2-mercaptobenzothiazole may react either via oxidation and/or thioester formation with carboxylate groups of amino acids.]

5. Summary of Data Reported

5.1 Exposure data

2-Mercaptobenzothiazole is a chemical of high production volume that is mainly used as an accelerator in the manufacture of rubber products and as an inhibitor of corrosion. Workers are exposed during the production of 2-mercaptobenzothiazole, and can be exposed during the manufacture of tyres and rubber products and the production of pesticides. The general population is exposed to 2-mercaptobenzothiazole through dermal contact with consumer goods containing rubber. 2-Mercaptobenzothiazole has also been detected in samples of urban air, presumably originating from tyre abrasion, and in samples of effluent water from rubber manufacturers and tanneries.

5.2 Human carcinogenicity data

Studies on the carcinogenicity of 2-mercaptobenzothiazole were available for a plant that manufactured chemicals for the rubber industry in north Wales, United Kingdom, and for a general chemical manufacturing plant in West Virginia, USA.

The study on the plant in Wales was described in a series of reports. The Working Group considered that this study was the more informative of those available. Comparisons of exposed workers with the national population of England and Wales showed a significant excess of incident cases of cancer of the urinary bladder. Internal comparisons that controlled for other occupational exposures showed a non-significant trend in increasing incidence of cancer of the urinary bladder with increasing cumulative exposure to 2-mercaptobenzothiazole. A non-significant twofold excess risk was observed in the group with highest exposure.

The study in the plant in the USA reported a statistically significant fourfold excess of mortality from cancer of the urinary bladder based on a small number of deaths in a subgroup of workers exposed to 2-mercaptobenzothiazole, but with no documented exposure to 4-aminobiphenyl (classified in IARC Group 1 as a cause of cancer of the urinary bladder). A statistically significant trend in mortality from cancer of the urinary bladder with increasing cumulative exposure to 2-mercaptobenzothiazole was also observed.

The lack of available data on tobacco smoking was a limitation of both studies; however, confounding by smoking is unlikely to explain the exposure–response patterns observed in these studies.

5.3 Animal carcinogenicity data

2-Mercaptobenzothiazole was tested for carcinogenicity by oral administration in two studies in male and female mice (one of these studies was considered to be inadequate for an evaluation) and in one study in male and female rats.

In one study in male and female mice, oral administration (by gavage) of 2-mercaptobenzothiazole induced a significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; in male mice, 2-mercaptobenzothiazole did not result in any significant increase in tumour incidence.

In the study in male and female rats, oral administration (by gavage) of 2-mercaptobenzothiazole to male rats induced a significantly increased incidence of benign pheochromocytoma and of pheochromocytoma (benign or malignant, combined) of the adrenal gland, with a significant positive trend, and was also associated with a significantly increased incidence of mononuclear cell leukaemia of the haematopoietic system, pancreatic acinar cell adenoma, preputial gland adenoma, preputial gland adenoma or carcinoma (combined), pituitary gland adenoma and tumours of the skin (fibroma, neurofibroma, sarcoma or fibrosarcoma, combined). A significant positive trend in the incidence of mesothelioma (multiple organs) was also observed. In female rats, oral administration (by gavage) of 2-mercaptobenzothiazole resulted in a significantly increased incidence of benign pheochromocytoma of the adrenal gland and of adenoma of the pituitary gland, with a significant positive trend.

5.4 Mechanistic and other relevant data

A study of workers reported urinary excretion of 2-mercaptobenzothiazole glucuronide. In rodents, the compound is well absorbed, and

distribution primarily to the kidney, liver, and thyroid gland occurs after subcutaneous administration. Urinary excretion accounts for > 90% after intravenous administration to rats. In rats, the major urinary metabolites are glutathione and glucuronic acid conjugates.

With respect to the key characteristics of human carcinogens, there is *moderate* evidence that 2-mercaptobenzothiazole modulates receptor-mediated effects. 2-Mercaptobenzothiazole acted as an agonist of the aryl hydrocarbon receptor in an assay in mouse hepatoma cells. 2-Mercaptobenzothiazole also inhibited rat and pig thyroid peroxidase in vitro, and inhibited thyroxine release from *Xenopus laevis* thyroid gland ex vivo and in vivo. ToxCast data supported activation of the aryl hydrocarbon receptor pathway by 2-mercaptobenzothiazole in human cells and in assays for development in zebrafish.

There is *weak* evidence that 2-mercaptobenzothiazole is genotoxic. 2-Mercaptobenzothiazole induced chromosomal aberrations and sister-chromatid exchange in Chinese hamster ovary cells in the presence of a metabolic activation system, and caused mutations at the *Tk* locus in mouse L5178Y lymphoma cells. However, it was not mutagenic in test systems in bacteria or in human gastric and lung carcinoma cell lines, and did not bind to rat DNA in vivo.

The evidence that 2-mercaptobenzothiazole induces chronic inflammation is *weak*. 2-Mercaptobenzothiazole is a contact allergen in humans. It increased the levels of interleukin-18 in human skin cells, and caused inflammation of the forestomach in male and female rats in a 2-year bioassay.

There were few data on the other key characteristics of human carcinogens (is electrophilic or can be metabolically activated, alters DNA repair or causes genomic instability, induces epigenetic alterations, induces oxidative stress, is immunosuppressive, causes immortalization, or alters cell proliferation, cell death, or nutrient supply).

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of 2-mercaptobenzothiazole. A positive association has been observed between exposure to 2-mercaptobenzothiazole and cancer of the urinary bladder.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-mercaptobenzothiazole.

6.3 Overall evaluation

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

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3-CHLORO-2-METHYLPROPENE

1. Exposure Data

3-Chloro-2-methylpropene was considered by the *IARC Monographs* Working Group in 1995 (IARC, 1995). Since that time, new data have become available and these have been taken into consideration in the present evaluation.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 563-47-3

Chem. Abstr. Serv. Name:

3-Chloro-2-methylpropene

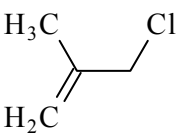
IUPAC Systematic Name:

3-Chloro-2-methylprop-1-ene

Synonyms: 3-Chloroisobutene; γ-chloroisobutylene; 3-chloro-2-methyl-1-propene; 3-chloro-2-methylprop-1-ene; methallyl chloride; 2-methylallyl chloride

Acronym: CMP

1.1.2 Structure and molecular formula, and relative molecular mass



Molecular formula: C_4H_7Cl

Relative molecular mass: 90.55 (IFA, 2015)

1.1.3 Physical and chemical properties of the pure substance

Description: Colourless, highly flammable liquid and vapour with a pungent odour; the substance may decompose when heated, explode when in contact with oxidizing agents, can polymerize and react dangerously with acids (IFA, 2015)

Impurities: Light and/or heat may form low concentrations of dimeric methallyl chloride (HSDB, 2014)

Density (at 20 °C): 0.93 g/cm³ (IFA, 2015)

Melting point: < −80 °C (IFA, 2015)

Boiling point: 72.2 °C (IFA, 2015)

Octanol/water partition coefficient (P): log K_{ow}, 2.48 (NTP, 2014)

Vapour pressure (at 20 °C): 13.5 kPa [101.7 mm Hg] (HSDB, 2014)

Vapour density: 3.1 (air = 1) (IFA, 2015)

Solubility: Moderately soluble in water (1.4 g/L at 25 °C) (NTP, 2014)

Stability: Lower explosion limit, ~2.3 vol. %; upper explosion limit, ~8.1 vol. % (IFA, 2015)

Flash point: −12 °C (IFA, 2015)

Conversion factor (at 101 kPa, 20 °C): 1 ppm = 3.76 mg/m³ (IFA, 2015)

1.1.4 Technical grade and impurities

3-Chloro-2-methylpropene is available commercially at purities ranging from 95% (technical grade) to 98%, with dimethyl-vinyl-chloride (1-chloro-2-methylpropene) as an impurity (IARC, 1995).

1.2 Production and use

1.2.1 Production

3-Chloro-2-methylpropene is produced by the chlorination of 2-methyl-1-propene (isobutylene) (HSDB, 2014).

Production of 3-chloro-2-methylpropene in the USA was estimated to be 12–24 million pounds [~5400–11 000 tonnes] in 1984 (NTP, 2014). Data filed between 1986 and 2006 under the Toxic Substances Control Act Inventory Update Rule of the United States Environmental Protection Agency (EPA) indicated a total annual production plus import of 3-chloro-2-methylpropene between 10 million and 50 million pounds [~4500–23 000 tonnes] in the USA (NTP, 2014). In 2009, 3-chloro-2-methylpropene was produced by one manufacturer in Asia and was available from 18 suppliers (NTP, 2014). No information on European production or import of 3-chloro-2-methylpropene was available to the Working Group.

1.2.2 Use

3-Chloro-2-methylpropene is an important intermediate in the production of pesticides such as carbofuran, ethalfluralin, and fenbutatin oxide (HSDB, 2014). However, it can also be used as a fumigant for seeds of several vegetables, including cucumbers, tomatoes, onions and beetroot. In addition, 3-chloro-2-methylpropene is an intermediate in the manufacture of plastics and organic chemicals, in particular in the production of 2-methylepichlorohydrin (NTP, 2014).

1.3 Measurement and analysis

3-Chloro-2-methylpropene was analysed semi-quantitatively, among other volatile and semivolatile compounds, in air samples using gas chromatography-mass spectrometry after their sorption on TENAX®. The limit of detection of the method is 62 ng/m^3 ± 30% for 30 L of air sampled (Krost et al., 1982). No other specific methods of detection have been described for 3-chloro-2-methylpropene in the literature.

1.4. Occurrence and exposure

1.4.1 Natural occurrence

3-Chloro-2-methylpropene is not known to occur as a natural product.

1.4.2 Environmental occurrence

3-Chloro-2-methylpropene was degraded by hydroxyl radicals and ozone in air, and its estimated half-lives were 10 and 27 hours by hydroxyl radicals and ozone, respectively (HSDB, 2014).

Environmental exposure to 3-chloro-2-methylpropene was monitored at concentrations of 110–400 µg/m^3 in ambient air in an industrial area near Curtis Bay, MD, USA. The source of the emission was not reported, but four halogenated hydrocarbons, including 3-chloro-2-methylpropene, analysed in this study were speculated to come from the same source (Pellizzari, 1982).

1.4.3 Occupational exposure

No data on occupational exposure to 3-chloro-2-methylpropene were available to the Working Group.

1.4.4 Exposure of the general population

No data on exposure to 3-chloro-2-methylpropene were available to the Working Group.

1.5 Regulations and guidelines

No occupational exposure limits have been reported for 3-chloro-2-methylpropene (IFA, 2015). According to the risk phrases of the Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations, 3-chloro-2-methylpropene is harmful if swallowed (H302) and if inhaled (H332), causes severe skin burns and eye damage (H314), may cause an allergic skin reaction (H317) and is toxic to aquatic life with long-lasting effects (H411) (ECHA, 2015).

The United States National Toxicology Program (NTP) states that 3-chloro-2-methylpropene "is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals" (NTP, 2014). The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK) has designated 3-chloro-2-methylpropene as a substance for which there is some evidence of carcinogenicity (Category 3B) (MAK, 2015).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3-Chloro-2-methylpropene was evaluated previously by an IARC Monographs Working Group (IARC, 1995) which concluded that there was *limited evidence* in experimental animals for its carcinogenicity.

See Table 3.1

3.1 Mouse

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given technical-grade 3-chloro-2-methylpropene (two different lots; purity, 93% and > 95%, respectively, containing 5% and 3.6% dimethylvinylchloride [1-chloro-2-methylpropene, an IARC Group 2B carcinogen with *sufficient evidence* in experimental animals (IARC, 1995)], respectively) in corn oil by gavage at doses of 0, 100, or 200 mg/kg body weight (bw) on 5 days per week for 103 weeks (Chan et al., 1986; NTP, 1986). Mean body weights of the males given 200 mg/kg bw and of all treated females were significantly lower (by less than 10%) than those of vehicle controls throughout most of the study. Survival in the treated groups was not significantly lower than that in vehicle controls; the numbers of survivors at the end of the experiment (105 weeks) were: 26 male controls, 37 males at 100 mg/kg bw and 32 males at 200 mg/kg bw; and 37 female controls, 43 females at 100 mg/kg bw and 27 females at 200 mg/kg bw.

In males, the incidence of forestomach squamous cell papilloma was: 3/49 controls, 19/49 at 100 mg/kg bw ($P < 0.001$ by the Fisher's exact test); and 30/49 at 200 mg/kg bw ($P < 0.001$ by the Fisher's exact test) (dose-related increase at $P < 0.001$ by the Cochran-Armitage trend test); that of squamous cell carcinoma was: 0/49 controls, 5/49 at 100 mg/kg bw ($P = 0.028$ by the Fisher's exact test) and 7/49 at 200 mg/kg bw ($P = 0.006$ by the Fisher's exact test) (dose-related increase at $P = 0.008$ by the Cochran-Armitage trend test); and that of forestomach squamous cell papilloma or carcinoma (combined) was: 3/49 controls, 24/49 at 100 mg/kg bw ($P < 0.001$ by the Fisher's exact test) and 36/49 at 200 mg/kg bw ($P < 0.001$ by the Fisher's exact test) (dose-related increase at $P < 0.001$ by the Cochran-Armitage trend test). The historical incidence of forestomach squamous cell papilloma or carcinoma (combined) in male mice at the study laboratory

Table 3.1 Studies of carcinogenicity with 3-chloro-2-methylpropene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M) 8 wk 105 wk NTP (1986)	Gavage Purity, ≥ 93% Corn oil 0, 100, 200 mg/kg bw 5 days/wk for 103 wk 50/group 26, 37, 32	*Forestomach* Squamous cell papilloma: 3/49, 19/49, 30/49 Squamous cell carcinoma: 0/49, 5/49, 7/49 Squamous cell papilloma or carcinoma (combined): 3/49, 24/49, 36/49	Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P < 0.001$ at 100 and 200 mg/kg bw (Fisher's exact test) Trend test: $P = 0.008$ (Cochran-Armitage test) Incidental tumour test: $P = 0.028$ and $P = 0.006$ at 100 and 200 mg/kg bw, respectively (Fisher's exact test) Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P < 0.001$ at 100 and 200 mg/kg bw (Fisher's exact test)	Principal strengths: GLP, large number of animals per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 3.6–5%
Mouse, $B6C3F_1$ (F) 8 wk 105 wk NTP (1986)	Gavage Purity, ≥ 93% Corn oil 0, 100, 200 mg/kg bw 5 days/wk for 103 wk 50/group 37, 43, 27	*Forestomach* Squamous cell papilloma: 0/50, 15/48, 29/44 Squamous cell carcinoma: 0/50, 1/48, 2/44 Squamous cell papilloma or carcinoma (combined): 0/50, 16/48, 31/44	Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P < 0.001$ at 100 and 200 mg/kg bw (Fisher's exact test) NS Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P < 0.001$ at 100 and 200 mg/kg bw (Fisher's exact test)	Principal strengths: GLP, large number of animals per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 3.6–5% Incidence of forestomach squamous cell papilloma or carcinoma (combined) in historical controls: study laboratory, 0/145; NTP studies, 4/1027

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, BDF_1 (M) 6 wk 104 wk Katagiri et al. (2000)	Whole-body inhalation Purity, 95% vapour 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50/group 40, 44, 43, 46	*Forestomach* Squamous cell papilloma: 1/50, 0/49, 3/50, 4/50 Squamous cell carcinoma: 0/50, 0/49, 1/50, 0/50 *Harderian gland* Adenoma: 3/50, 7/49, 9/50, 5/50	 Trend test: $P = 0.0392$ (Peto's test) NS NS	Principal strengths of the study: GLP, large number of animals per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 1.4% Incidence of Harderian gland adenoma in historical controls: 30/598 (5.0%), 2.0–10.0% (Ohnishi et al., 2013)
Mouse, BDF_1 (F) 6 wk 104 wk Katagiri et al. (2000)	Whole-body inhalation Purity, 95% vapour 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50/group 29, 29, 31, 33	*Forestomach* Squamous cell papilloma: 1/50, 0/48, 5/50, 4/49 Squamous cell carcinoma: 0/50, 0/48, 0/50, 0/49 *Harderian gland* Adenoma: 0/50, 4/48, 7/50, 8/49	 Trend test: $P = 0.0437$ (Peto's test) NS Trend test: $P = 0.0056$ (Peto's test) and $P = 0.0061$ (Cochran-Armitage test) Incidence: $P = 0.0101$ and $P = 0.0051$ at 100 and 200 ppm, respectively (Fisher's exact test)	Principal strengths of the study: GLP, large number of mice per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 1.4% Incidence of Harderian gland adenoma in historical controls: 29/849 (3.4%), 0–12.0%

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/N (M) 8 wk 105 wk NTP (1986)	Gavage Purity, ≥ 93% Corn oil 0, 75, 150 mg/kg bw 5 days/wk for 103 wk 50/group 30, 25, 17	*Forestomach* Squamous cell papilloma: 1/50, 5/50, 30/48 Squamous cell carcinoma: 0/50, 0/50, 2/48 *Kidney* Renal tubular cell adenoma: 0/50, 1/50, 0/49 Renal tubular cell adenocarcinoma: 0/50, 1/50, 1/49 Renal transitional cell carcinoma: 0/50, 0/50, 1/49 *Urinary bladder* Transitional cell papilloma: 0/48, 0/49, 1/46 *Testis* Interstitial cell tumours: 36/50, 43/50, 43/48	Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P < 0.001$ at 150 mg/kg bw (Fisher's exact test) NS NS NS NS Trend test: $P < 0.001$ (life-table test), $P = 0.003$ (incidental tumour test) and $P = 0.015$ (Cochran-Armitage test) Incidence: $P = 0.009$ (life-table test at 75 mg/kg bw), $P < 0.001$ (life-table test at 150 mg/kg bw), $P = 0.012$ (incidental tumour test at 150 mg/kg bw) and $P = 0.025$ (Fisher's exact test at 150 mg/kg bw)	1-Chloro-2-methylpropene, 3.6–5% Incidence of renal tubular cell tumours, renal transitional cell tumours and urinary bladder tumours in historical corn oil-vehicle controls at the study laboratory: 1/150, 0/150 and 0/150, respectively Incidence of testicular (interstitial cell) tumours in historical controls: 92.0 ± 6.9%, study laboratory; 90.4 ± 5.7%, all NTP studies

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/N (F) 8 wk 105 wk NTP (1986)	Gavage Purity, ≥ 93% Corn oil 0, 75, 150 mg/kg bw 5 days/wk for 103 wk 50/group 31, 32, 26	*Forestomach* Squamous cell papilloma: 1/50, 1/50, 10/50 *Subcutis* Fibroma: 0/50, 2/50, 4/50	Trend test: $P < 0.001$ (Cochran-Armitage test) Incidental tumour test: $P = 0.004$ at 150 mg/kg bw (Fisher's exact test) NS	Principal strengths of the study: GLP, large number of rats per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 3.6–5% Incidence of subcutaneous fibroma in historical controls at study laboratory: 2/150
Rat, F344/DuCrj (M) 6 wk 104 wk MHLW (1998b)	Whole-body inhalation Purity, 95% Vapour 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 39, 35, 33, 30	*Thyroid gland* Follicular cell adenoma: 2/50, 0/50, 2/50, 6/50 Follicular cell adenoma or adenocarcinoma (combined): 4/50, 4/50, 3/50, 10/50	Trend test: $P = 0.00213$ (Cochran-Armitage test) Trend test: $P = 0.0388$ (Cochran-Armitage test)	Principal strengths of the study: GLP, large number of animals per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 1.4%
Rat, F344/DuCrj (F) 6 wk 104 wk MHLW (1998b)	Whole-body inhalation Purity, 95% Vapour 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 40, 45, 44	*Any tumour type* No significant increase	NS	Principal strengths of the study: GLP, large number of animals per group, multiple doses tested, and covered most of the lifespan 1-Chloro-2-methylpropene, 1.4%

bw, body weight; F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week

and that in NTP studies was 2/147 and 7/1005, respectively. Evidence of metastasis or invasion of other organs was observed in two males at 100 mg/kg bw and three males at 200 mg/kg bw. The incidence of forestomach epithelial hyperplasia was significantly increased in all treated males.

In females, the incidence of forestomach squamous cell papilloma was: 0/50 controls, 15/48 at 100 mg/kg bw ($P < 0.001$ by the Fisher's exact test) and 29/44 at 200 mg/kg bw ($P < 0.001$ by the Fisher's exact test) (dose-related increase at $P < 0.001$ by the Cochran-Armitage trend test); that of forestomach squamous cell carcinoma was: 0/50 controls, 1/48 at 100 mg/kg bw and 2/44 at 200 mg/kg bw; and that of forestomach squamous cell papilloma or carcinoma (combined) was: 0/50 controls, 16/48 at 100 mg/kg bw ($P < 0.001$ by the Fisher's exact test) and 31/44 at 200 mg/kg bw ($P < 0.001$ by the Fisher's exact test) (dose-related increase at $P < 0.001$ by the Cochran-Armitage trend test). The historical incidence of forestomach squamous cell papilloma or carcinoma (combined) in female mice at the study laboratory and in NTP studies was 0/145 and 4/1027, respectively. Evidence of metastasis or invasion of other organs was observed in one female mouse at 200 mg/kg bw. The incidence of forestomach epithelial hyperplasia was significantly increased in females at 200 mg/kg bw.

[The strengths of this study included compliance with good laboratory practice, large numbers of animals, the evaluation of multiple dose levels and a duration that involved most of the lifespan.]

3.1.2 Inhalation

Groups of 50 male and 50 female BDF_1 (C57BL/6NCrj × DBA/2NCrj) mice (age, 6 weeks) were exposed to technical-grade 3-chloro-2-methylpropene (purity, 95%, containing 1.4% 1-chloro-2-methylpropene) by whole-body inhalation at a concentration of 50, 100 or 200 ppm for 6 hours per day on 5 days per week for 104 weeks (MHLW, 1998a; Katagiri et al., 2000). The control mice were handled in the same manner and exposed to clean air in similar chambers. A significant decrease in body weight was observed in all exposed groups. Survival in the treated groups was slightly higher than that in the controls; the numbers of survivors at the end of the experiment were: 40/50 male controls, 44/50 males at 50 ppm, 43/50 males at 100 ppm, and 46/50 males at 200 ppm; and 29/50 female controls, 29/48 females at 50 ppm, 31/50 females at 100 ppm, and 33/49 females at 200 ppm.

Histopathological evaluation revealed a significant positive trend in the incidence of forestomach squamous cell papilloma in males and females ($P = 0.0392$ and $P = 0.0437$ by Peto's trend test, respectively). The incidence of squamous cell papilloma was 1/50 controls, 0/49 at 50 ppm, 3/50 at 100 ppm, and 4/50 at 200 ppm in males, and 1/50 controls, 0/48 at 50 ppm, 5/50 at 100 ppm, and 4/49 at 200 ppm in females. A forestomach squamous cell carcinoma was also observed in one male mouse at 100 ppm. The incidence of forestomach epithelial hyperplasia was also significantly increased ($P \leq 0.01$) in males and females at 200 ppm. In addition, a significant positive trend was found in the incidence of Harderian gland adenoma in female mice ($P = 0.0056$ by Peto's trend test, $P = 0.0061$ by the Cochran-Armitage trend test) with an incidence of 0/50 controls, 4/48 at 50 ppm, 7/50 at 100 ppm ($P = 0.0101$ by the Fisher's exact test) and 8/49 at 200 ppm ($P = 0.0051$ by the Fisher's exact test). The incidence in the groups exposed to 100 and 200 ppm was higher than the maximum incidence (29/849, 3.4%; 0–12.0%) in historical controls at the institute performing this experiment using female BDF_1 mice. The incidence of Harderian gland adenoma in males was: 3/50 controls, 7/49 at 50 ppm, 9/50 at 100 ppm, and 5/50 at 200 ppm (historical control incidence in male BDF_1 mice: 30/598 (5.0%); range, 2.0–10.0%; Ohnishi et al., 2013).

[The strengths of this study included compliance with good laboratory practice, large numbers of animals, the evaluation of multiple dose levels and a duration that involved most of the lifespan.]

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female Fischer 344/N rats (age, 8 weeks) were given technical-grade 3-chloro-2-methylpropene (two different lots; purity, 93% and > 95%, respectively, containing 5% and 3.6% dimethylvinylchloride [1-chloro-2-methylpropene, an IARC group 2B carcinogen with *sufficient evidence* in experimental animals (IARC, 1995)], respectively) in corn oil by gavage at doses of 0, 75, or 150 mg/kg bw on 5 days per week for 103 weeks (Chan et al., 1986; NTP, 1986). The mean body weight of the males given 150 mg/kg bw was significantly lower than that of the vehicle controls, beginning at week 10 of the study. The mean body weights of males given 75 mg/kg bw and of all treated females were comparable with those of the vehicle controls throughout the study. Survival was marginally reduced among males receiving 150 mg/kg bw; the numbers of survivors at the end of the experiment (105 weeks) were: 30 male controls, 25 males at 75 mg/kg bw and 17 males at 150 mg/kg bw; and 31 female controls, 32 females at 75 mg/kg bw, and 26 females at 150 mg/kg bw.

A dose-related increase in the incidence of squamous cell papilloma of the forestomach was observed in both males and females ($P < 0.001$ by the Cochran-Armitage trend test): 1/50 male controls, 5/50 males at 75 mg/kg bw, 30/48 males at 150 mg/kg bw ($P < 0.001$ by the Fisher's exact test), 1/50 female controls, 1/50 females at 75 mg/kg bw, and 10/50 females at 150 mg/kg bw ($P = 0.004$ by the Fisher's exact test). Forestomach squamous cell carcinomas were observed in 2 of the male rats bearing forestomach squamous cell papillomas at 150 mg/kg bw but not in controls or animals given 75 mg/kg bw. Consequently, the incidence was significantly increased (with a significant positive trend) for papilloma and carcinoma (combined) in males at 150 mg/kg bw. The historical incidence of forestomach squamous cell papilloma at the study laboratory and in NTP studies was 0/147 and 5/1062 in male rats, and 1/150 and 5/1073 in female rats, respectively. The incidence of basal cell or epithelial hyperplasia (combined) of the forestomach in groups of treated males and that of basal cell hyperplasia in groups of treated females was also increased. In addition to forestomach tumours in males, a significant increase and positive trend in the incidence ($P < 0.001$ by the life-table trend test, $P = 0003$ by the incidental tumour trend test, $P = 0.015$ by the Cochran-Armitage trend test) of testicular interstitial cell tumours was observed: 36/50 controls, 43/50 at 75 mg/kg bw ($P = 0.009$ by the life-table test), and 43/48 at 150 mg/kg bw ($P < 0.001$ by the life-table test, $P = 0.012$ by the incidental tumour test, $P = 0.025$ by the Fisher's exact test), which was within the historical control range. The historical incidence for this tumour at the study laboratory was 92.0% ± 6.9% (138/150), and that in all NTP studies was 90.4% ± 5.7% (985/1090). [The Working Group concluded that it was uncertain that these testicular tumours were related to treatment.]

A non-significant increase in tumours was observed in the kidney and urinary bladder of male rats and in the subcutaneous tissue of female rats. One renal tubular cell adenoma was observed in one male given 75 mg/kg bw and renal tubular cell adenocarcinomas were observed in another male given 75 mg/kg bw and in one male given 150 mg/kg bw. At 150 mg/kg bw, a renal transitional cell carcinoma was observed in one male and a transitional cell papilloma was observed in the urinary bladder of another. No renal tubule tumours, renal transitional cell tumours or urinary bladder tumours were observed in male or female controls. The historical incidence

of renal tubular cell tumours and renal transitional cell tumours in corn oil vehicle-control male rats at the study laboratory was 1/150 and 0/150, respectively, and that in NTP studies was 4/1091 and 1/1091 (1 transitional cell papilloma), respectively; that of urinary bladder tumours in male rats treated with corn oil vehicle was 0/150 at the study laboratory and 0/1040 in NTP studies. [The Working Group concluded that it was uncertain that these kidney and urinary bladder tumours were related to treatment.] Subcutaneous fibromas were observed in 2/50 females at 75 mg/kg bw and in 4/50 females at 150 mg/kg bw. No such subcutaneous tumour was seen in control female rats. The historical incidence of subcutaneous fibroma in control female rats at the study laboratory and in NTP studies was 2/150 and 13/1095, respectively [the Working Group concluded that these subcutaneous tumours may have been related to treatment] (NTP, 1986).

[The strengths of this study included compliance with good laboratory practice, large numbers of animals, the evaluation of multiple dose levels and a duration that involved most of the lifespan.]

3.2.2 Inhalation

Groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were exposed to technical-grade 3-chloro-2-methylpropene (purity, 95%, containing 1.4% 1-chloro-2-methylpropene) by whole-body inhalation at a concentration of 50, 100 or 200 ppm for 6 hours per day on 5 days per week for 104 weeks. The control rats were handled in the same manner and exposed to clean air in similar chambers. Growth rates were slightly suppressed in males treated with 100 or 200 ppm and in females treated with 200 ppm compared with the respective controls. Survival in the treated males was slightly lower than that in controls; the numbers of male survivors at the end of the experiment were: 39 controls, 35 at 50 ppm, 33 at 100 ppm and 30 at 200 ppm. Survival in the treated females tended to be higher than that in controls; the numbers of female survivors at the end of the experiment were: 38 controls, 40 at 50 ppm, 45 at 100 ppm and 44 at 200 ppm. Histopathological evaluation revealed a slightly increased incidence of thyroid follicular cell adenoma and of thyroid follicular cell adenoma or adenocarcinoma (combined) in treated males. The incidence of thyroid follicular cell adenoma in males was 2/50 controls, 0/50 at 50 ppm, 2/50 at 100 ppm, and 6/50 at 200 ppm (significant positive trend at $P = 0.00213$ by the Cochran-Armitage trend test). The incidence of thyroid follicular cell adenoma or adenocarcinoma (combined) in males was 4/50 controls, 4/50 at 50 ppm, 3/50 at 100 ppm and 10/50 at 200 ppm (significant positive trend at $P = 0.0388$ by the Cochran-Armitage trend test). The historical control incidence of thyroid follicular cell adenoma in male Fischer 344 rats at the study laboratory was 8/899 (0.9%; range, 0–4%). No significant increase in the incidence of tumours was observed in treated female Fischer 344/DuCrj rats (MHLW, 1998b). [The strengths of this study included compliance with good laboratory practice, large numbers of animals, the evaluation of multiple dose levels and a duration that involved most of the lifespan.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

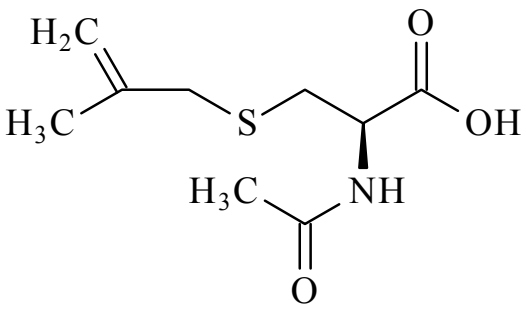
No data were available to the Working Group.

4.1.2 Experimental systems

The toxicokinetics of 3-chloro-2-methylpropene in experimental systems was reviewed in *IARC Monographs* Volume 63 (IARC, 1995). At that time, only one study was available (Ghanayem & Burka, 1987). The reported data indicated extensive absorption and rapid excretion in rats after a single dose or up to four daily doses of 150 mg/kg bw of 3-chloro-2-methylpropene (stated purity, 93%; containing ~5% 1-chloro-2-methylpropene [dimethylvinyl chloride] by oral gavage. Distribution to the tissues was rapid and the highest concentrations were found in the forestomach, liver and kidney. Excretion was primarily via the urine but large amounts were exhaled, partly as carbon dioxide.

The major 3-chloro-2-methylpropene metabolite in rat urine was characterized as *N*-acetyl-*S*-(2-methylpropenyl)cysteine, presumably formed by direct conjugation of glutathione with the allylic carbon of 3-chloro-2-methylpropene (see Fig. 4.1), followed by catabolism to the mercapturate (Ghanayem & Burka, 1987).

Fig. 4.1 Structure of *N*-acetyl-*S*-(2-methylpropenyl)cysteine, the major metabolite of 3-chloro-2-methylpropene in rat urine



4.2 Mechanisms of carcinogenesis

The evidence on the key characteristics of carcinogens (Smith et al., 2016) concerning whether 3-chloro-2-methylpropene is genotoxic, induces chronic inflammation and alters cell proliferation, cell death or nutrient supply is summarized below.

4.2.1 Genetic and related effects

See Table 4.1

(a) Humans

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

(b) Experimental systems

3-Chloro-2-methylpropene is considered to be an electrophilic agent. The genotoxicity of 3-chloro-2-methylpropene was reviewed in *IARC Monographs* Volume 63 (IARC, 1995). Most of the studies reported were conducted with compounds that were 90.7% pure (the remaining material being primarily 1-chloro-2-methylpropene). The test preparations were mutagenic to *Salmonella typhimurium* TA100 in the presence and absence of metabolic activation and to strain TA1537 in the presence of an exogenous metabolic system. Single studies also reported the induction of somatic recombination in *Drosophila melanogaster*, mutation at the *Tk* locus in mouse L5178Y lymphoma cells, and sister-chromatid exchange and chromosomal aberrations in Chinese hamster cells, but not micronucleus formation in the bone marrow of mice treated in vivo.

An earlier study reported the induction of unscheduled DNA synthesis in HeLa cells by 3-chloro-2-methylpropene (purity, 100%; reported in Eder et al., 1982) at a minimum dose of 10^{-3} mol/L [90.55 µg/mL] (Schiffmann et al., 1983). [The Working Group noted that no statistical analysis was presented.]

Table 4.1 Genetic and related effects of 3-chloro-2-methylpropene

Species	End-point	Test	Results		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Human, HeLa cell line	DNA damage	Unscheduled DNA synthesis	(+)	NT	10^{-3} mol/L [90.55 µg/mL]	Stated purity, 100% (Eder et al., 1982); no statistical analysis presented; the lowest dose was one to two orders of magnitude higher than that for other structurally related compounds; lethal dose NR	Schiffmann et al. (1983)
Drosophila melanogaster	Mutation	Sex-linked recessive lethal mutations	+	NA	4500 ppm		Foureman et al. (1994)
Drosophila melanogaster	Mutation	Heritable translocation test	–	NA	5000 ppm		Foureman et al. (1994)
Drosophila melanogaster	Mutation	Somatic mutation and recombination test	+	NA	2.75 µg/L (50% lethal concentration)		Chroust et al. (2007)

+, positive; –, negative; (+), weakly positive; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NT, not tested

An additional study in *Drosophila melanogaster* (analysed purity, 93.3%) reported a positive result for the induction of sex-linked recessive lethal mutations in post-meiotic and meiotic germ cells of adult males fed 3-chloro-2-methylpropene. The same test sample gave negative results for the induction of reciprocal translocations (Foureman et al., 1994). 3-Chloro-2-methylpropene [purity not specified] also gave positive results in the wing spot test in *Drosophila melanogaster* when administered by inhalation at the 50% lethal concentration (Chroust et al., 2007). A quantitative structure–activity relationship multivariate analysis of a series of structurally similar halogenated aliphatic compounds, including 3-chloro-2-methylpropene, indicated that nucleophilic superdelocalizability of the halogen atom (calculated by quantum mechanics) was a good structural parameter to predict the toxicity and genotoxicity of these compounds, consistent with the direct reactivity or bioactivation at the halogenated carbon (Chroust et al., 2007).

4.2.2 Other mechanisms

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 13-week study on the toxicity of 3-chloro-2-methylpropene (same material as that used in the 2-year bioassay), inflammation and necrosis of the liver were observed in treated rats and mice, and necrosis of cortical tubules was observed in the kidney of mice. In the 2-year study, a dose-dependent increase in the incidence of forestomach inflammation was

observed in treated male and female mice. The incidence of inflammation in the nasal cavity and of nephropathy/nephrosis was higher in exposed groups than in the controls in male and female mice and rats (NTP, 1986).

The incidence and severity of epithelial cell proliferation of the forestomach was increased in Fischer 344/N rats given 3-chloro-2-methylpropene at 150 mg/kg bw, but not at 75 mg/kg bw, by gavage on 5 days per week for 2 weeks (Ghanayem et al., 1986). A strong association between early forestomach mucosal cell proliferation and forestomach neoplasia was observed in treated rats and mice in the 2-year carcinogenicity bioassay conducted by the NTP, in which 3-chloro-2-methylpropene (technical grade, containing 5% dimethylvinyl chloride) was administered by gavage (Chan et al., 1986; NTP, 1986).

In the nasal cavity, eosinophilic exudate associated with atrophy of the olfactory epithelia, respiratory metaplasia of the olfactory epithelia and olfactory gland, and eosinophilic changes in the respiratory and olfactory epithelia were observed in male and female BDF_1 mice exposed to 3-chloro-2-methylpropene (stated purity, 95%) at 0, 50, 100, or 200 ppm on 5 days per week for 104 weeks (Katagiri et al., 2000).

4.3 Data relevant to comparisons across agents and end-points

3-Chloro-2-methyl propene was not tested by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013). Analyses of other compounds evaluated in this volume with high-throughput screening results are presented in the *Monograph* on 1-bromopropane in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

No additional data were available to the Working Group.

5. Summary of Data Reported

5.1 Exposure data

3-Chloro-2-methylpropene is available commercially at purities ranging from 95% (technical grade) to 98%, with dimethylvinyl chloride (1-chloro-2-methylpropene) as an impurity. 3-Chloro-2-methylpropene is an important intermediate in the production of pesticides, is used as a fumigant for seeds and is also an intermediate in the manufacture of plastics and other organic chemicals. Apart from a single study of environmental exposure published in 1982, no data on environmental or occupational exposure were available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Technical-grade 3-chloro-2-methylpropene was tested for carcinogenicity by oral administration (gavage) in one study in male and female mice and in one study in male and female rats, and by whole-body inhalation in one study in male and female mice and in one study in male and female rats.

In the study in mice treated by gavage, technical-grade 3-chloro-2-methylpropene caused a significantly increased incidence (with a significant positive trend) of forestomach squamous cell papilloma, forestomach squamous cell carcinoma, and forestomach squamous cell papilloma or carcinoma (combined) in males and that of forestomach squamous cell papilloma and

forestomach squamous cell papilloma or carcinoma (combined) in females.

In the study in mice treated by inhalation, administration of technical-grade 3-chloro-2-methylpropene resulted in a significantly increased positive trend in the incidence of forestomach squamous cell papilloma in males and females. It also caused a significantly increased incidence (with a significant positive trend) of adenoma of the Harderian gland in females.

In the study in rats treated by gavage, technical-grade 3-chloro-2-methylpropene caused a significantly increased incidence (with a significant positive trend) of forestomach squamous cell papilloma and forestomach squamous cell papilloma or carcinoma (combined) in males and that of forestomach squamous cell papilloma in females. Treated female rats also developed subcutaneous fibromas that may have been related to treatment.

In the study in rats treated by inhalation, exposure to technical-grade 3-chloro-2-methylpropene resulted in a significant positive trend in the incidence of follicular cell adenoma and of follicular cell adenoma or adenocarcinoma (combined) of the thyroid gland in males. No significant increase in the incidence of tumours was observed in female rats.

5.4 Mechanistic and other relevant data

3-Chloro-2-methylpropene is considered to be an electrophilic agent. The main urinary metabolite is a mercapturate conjugate. No data were available on the toxicokinetics of 3-chloro-2-methylpropene in humans. In rats given 3-chloro-2-methylpropene by oral administration, the compound is rapidly absorbed, distributed (primarily to the forestomach, liver and kidney) and excreted. Urinary excretion predominates, although large amounts are exhaled, partly as carbon dioxide.

With respect to the key characteristics of human carcinogens, there is *strong* evidence that 3-chloro-2-methylpropene is genotoxic, and these effects can operate in humans. 3-Chloro-2-methylpropene induced gene mutation in bacterial and mammalian cells, chromosomal aberrations and sister-chromatid exchange in mammalian cells, and genetic crossing-over (or recombination) and sex-linked recessive lethal mutation in post-meiotic and meiotic germ cells in *Drosophila melanogaster*. Only one study of DNA damage in human (HeLa) cells in vitro was available, but the results were positive.

There is *moderate* evidence that 3-chloro-2-methylpropene induces chronic inflammation. 3-Chloro-2-methylpropene induced inflammation in the liver and nasal cavity in rats and mice, and inflammation in the forestomach in male and female mice.

There is *weak* evidence that 3-chloro-2-methylpropene alters cell proliferation. Proliferation of the epithelial cells of the forestomach was increased in rats exposed to 3-chloro-2-methylpropene.

There were few data on the other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, induces oxidative stress, is immunosuppressive, modulates receptor-mediated effects or causes immortalization).

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 3-chloro-2-methylpropene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of technical-grade 3-chloro-2-methylpropene.

6.3 Overall evaluation

Technical-grade 3-chloro-2-methylpropene is *possibly carcinogenic to humans* (*Group 2B*).

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N,N-DIMETHYLFORMAMIDE

N,N-Dimethylformamide was considered by the *IARC Monographs* Working Group in 1989 and 1998 (IARC, 1989, 1999). New data have become available and have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 68-12-2

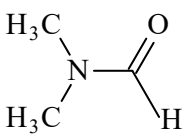
Chem. Abstr. Serv. Name: *N,N*-Dimethylformamide

IUPAC Systematic Name: *N,N*-Dimethylformamide

Synonyms: *N,N*-Dimethylmethane amide, formic acid dimethyl amide, *N*-formyldimethylamine

Acronym: DMF

1.1.2 Structure and molecular formula, and relative molecular mass



Molecular formula: C_3H_7NO

Relative molecular mass: 73.09

1.1.3 Physical and chemical properties of the pure substance

Description: Colourless to slightly yellow liquid with a faint amine-like odour (HSDB, 2015)

Degradation: Dimethylamine can be released from *N,N*-dimethylformamide and, in the presence of nitrogen oxides, carcinogenic nitrosamines can be formed. Other potential degradation products are ammonia, carbon monoxide, carbon dioxide, amines and formaldehyde.

Density (at 20 °C): 0.95 g/cm^3 (IFA, 2015)

Octanol/water partition coefficient: log K_{ow}, –1.01 (HSDB, 2015)

Melting point: –61 °C (IFA, 2015)

Boiling point: 153 °C (IFA, 2015)

Vapour pressure (at 20 °C): 0.377 kPa (2.83 mm Hg) (IFA, 2015)

Vapour density: 2.51 (air = 1) (HSDB, 2015)

Solubility: Entirely soluble in water at 20 °C (IFA, 2015)

Flammable limits: Lower explosion limit: 2.2 vol. %; upper explosion limit: 16 vol. % (IFA, 2015)

Flash point: 58 °C (IFA, 2015)

Ignition temperature: 440 °C (IFA, 2015)

Conversion factor (101 kPa, 20 °C): 1 ppm = 3.04 mg/m^3 (IFA, 2015).

1.2 Production and use

1.2.1 Production

N,*N*-Dimethylformamide is predominantly produced in a single-step reaction between dimethylamine and carbon monoxide under pressure at high temperatures and in the presence of basic catalysts such as sodium methoxide. The crude product contains methanol and *N*,*N*-dimethylformamide with increased purity (up to 99.9%) is obtained by multiple distillations (HSDB, 2015). Alternatively, it can be produced by a two-step process in which methyl formate is prepared separately and, in a second step, reacts with dimethylamine under similar conditions as those described for the single-step reaction. No catalysts are involved in the process (HSDB, 2015).

N,*N*-Dimethylformamide is listed as a high production volume chemical by the Organisation for Economic Co-operation and Development (OECD), indicating that this chemical is produced or imported at levels greater than 1000 tonnes per year in at least one member country or region (OECD, 2004, 2009). The OECD 2007 list of high production volume chemicals was compiled on the basis of submissions from eight member countries (including Australia, Canada, Japan, and the USA) in addition to the list provided by the European Union (OECD, 2009).

N,*N*-Dimethylformamide is also listed as a high production volume chemical in the USA by the Environmental Protection Agency (EPA) indicating that > 1 million pounds [~450 tonnes] were produced in or imported into the USA in 1990 and/or 1994 (HSDB, 2015).

The annual production volume of *N*,*N*-dimethylformamide (excluding imports) in the USA remained constant between 1986 and 2002, and was reported to be between 50 and 100 million pounds [approximately 20 000 and 45 000 tonnes] (HSDB, 2015). The annual production and import volume of nine USA companies was about 50 million pounds [~25 000 tonnes] (HSDB, 2015) and the total annual production volume in the European Union ranged from 50 000 to 100 000 tonnes in 2000 (SCOEL, 2006). No production volumes could be traced for Asia, but more than 300 suppliers of *N*,*N*-dimethylformamide could be identified globally, including > 200 in Asia, > 60 in the USA, and > 30 in the European Union (ChemBook, 2015).

1.2.2 Use

N,*N*-Dimethylformamide is used predominantly as an aprotic solvent in the manufacture of polyacrylonitrile fibres, and trends in its production parallel those of the polyacrylic fibre industry (HSDB, 2015). It is also used in the manufacture of high quality polyurethane and polyamide coatings (e.g. for leather or artificial leather fabrics), which are otherwise difficult to solubilize, and where a solvent with a slow rate of evaporation is needed. *N*,*N*-Dimethylformamide is commonly used as a solvent in the electronics industry, in pesticides, in industrial paint-stripping applications, and as a reaction and crystallizing solvent in the pharmaceutical industry. It has limited use as a selective solvent for the separation of aliphatic hydrocarbons such as the extraction of acetylene or butadiene from hydrocarbon streams (HSDB, 2015).

1.3 Measurement and analysis

Multiple methods exist for the analysis of *N*,*N*-dimethylformamide and its metabolites in air, water, urine, and blood. The methods are largely based on gas chromatography (GC) and high-performance liquid chromatography (HPLC) with various detection systems such as

flame ionization, nitrogen-sensitive, or mass-sensitive detection.

N,*N*-Dimethylformamide is most commonly measured in air by adsorbing on a silica gel and analysis by GC/flame ionization detection. The level of detection is approximately 0.05 mg per sample in a 15-L air sample or higher at a flow rate of 0.01–1 L/min (NIOSH, 1994).

Several diffusive sampling devices have been described for exposure assessment of *N*,*N*-dimethylformamide in the air of the workplace (Tanaka et al., 2002; Baglioni et al., 2007).

Because of the potential for dermal uptake, exposure to *N*,*N*-dimethylformamide should be measured by biomonitoring. Major metabolites in urine are *N*-hydroxymethyl-*N*-methylformamide (HMMF), *N*-methylformamide and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) (Gescher, 1993). HMMF degrades at injection temperatures > 250 °C, which allows the determination of total *N*-methylformamide in biological samples (Kawai et al., 1992).

An HPLC method also has been described that has a pre-heat phase to convert HMMF to *N*-methylformamide. The reported detection limit was 0.5 mg/L (Tranfo et al., 1999). As an alternative, HMMF can be converted chemically to *N*-methylformamide in the presence of potassium carbonate during sample preparation (Mráz & Turecek, 1987). The limits of detection are usually approximately 1 mg/L for total *N*-methylformamide and sufficient for exposure assessment in *N*,*N*-dimethylformamide-exposed workers (Mráz et al., 1987).

Recently, a method for simultaneously analysing total *N*-methylformamide and AMCC by capillary GC and nitrogen-selective detector with an injector temperature > 250 °C has been described (Käfferlein & Angerer, 2005). Under these conditions, the limits of detection were 1 mg/L of urine for total *N*-methylformamide and 0.5 mg/L of urine for AMCC.

The measurement of AMCC in urine can also form part of a multi-analysis of various mercapturic acids of organic compounds rather than measuring AMCC alone (Schettgen et al., 2008; Schettgen, 2010; Alwis et al., 2012). [The Working Group noted that AMCC can also be detected in the urine of the general population as a result of its endogenous production in the body (Käfferlein & Angerer, 1999).]

1.4 Occurrence and exposure

1.4.1 Natural occurrence

N,*N*-Dimethylformamide is not known to occur as a natural product.

1.4.2 Environmental occurrence

Industrial releases of *N*,*N*-dimethylformamide into the air appear to be considerably larger than those into other environmental media. *N*,*N*-Dimethylformamide is expected to exist almost entirely in the vapour phase in ambient air. When emitted into air, most of the *N*,*N*-dimethylformamide released remains in that compartment where it is degraded by chemical reactions with hydroxyl radicals; its half-life in the air is estimated to be in the range of few days. Some atmospheric *N*,*N*-dimethylformamide can reach the aquatic and terrestrial environment, presumably during periods of rain. Releases into the water or soil are expected to be followed by relatively rapid biodegradation (half-life, 18–36 hours) (IPCS, 2001; HSDB, 2015).

Measurements of *N*,*N*-dimethylformamide in air and water are presented in Table 1.1.

(a) Air

In Lowell, MA, USA, *N*,*N*-dimethylformamide was detected in the air over an abandoned chemical waste reclamation plant (mean, 7 µg/m^3), a neighbouring industry (> 150 µg/m^3), and a residential area (24 µg/m^3) (Amster et al., 1983; cited in IPCS, 2001). In ambient air samples collected in the north-eastern USA in 1983, levels

Table 1.1 Environmental exposure to *N,N*-dimethylformamide

Country	Duration	Sites/situation		DMF exposure											Reference
				DMF in air (μg/m³)				NMF in urine (mg/g creatinine)			DMF in surface water (μg/L)				
				No.	Mean	SD	Range	No.	Mean	SD	No.	Mean	SD	Range	
China	1-year follow-up	"Unpolluted" area in Longwan		366[a]	98.3	109.7	4.4–678.3								Wang et al.(2014)
	Cross-sectional	Near synthetic leather factories in Longwan	A	25	297.5	95		25	7.7	6.2					
			B	39	430	122.5		39	6.7	2.7					
			C	22	180	0		22	1.5	1.7					
			D	23	565	516.2		23	23.4	24.9					
			E	24	270	84.9		24	1.8	0.8					
Japan	Cross-sectional	All over Japan		105[b]	0.092[c]		0.016–0.49				47[d]	0.27[c]		ND–0.53	Ministry of the Environment Japan (2012)
USA		North-eastern					ND–14								Kelly et al. (1994)
USA		Massachusetts/ polluted area					7– > 150								Amster et al. (1983) cited in IPCS (2001)

[a] Monitoring daily throughout 2008
[b] Three samples were measured from 35 sites in Japan. *N,N*-Dimethylformamide was detected in all samples
[c] Geometric mean
[d] One sample was measured from 47 sites in Japan. *N,N*-Dimethylformamide was below the detection limit (0.019 μg/L) in 10 samples out of 47
DMF, *N,N*-dimethylformamide; ND, not detected; NMF, *N*-methylformamide; SD, standard deviation

of *N*,*N*-dimethylformamide ranged from not detected to 14 µg/m³ (Kelly et al., 1994).

Airborne concentrations of *N*,*N*-dimethylformamide, measured near synthetic leather factories in China where it is used, ranged from 180 to 565 µg/m³. In the same region, but away from the factories, mean concentration in 366 samples was 98.3 µg/m³ (Wang et al., 2014). Airborne concentrations of *N*,*N*-dimethylformamide measured at 35 sites all over Japan in 2008 ranged from 0.016 to 0.49 µg/m³ (Ministry of the Environment Japan, 2012).

(b) Water

Concentrations of *N*,*N*-dimethylformamide in water from rivers, lakes, and bays were measured throughout Japan and, in a total of 47 samples from 47 sites, ranged from not detected to 0.53 µg/L (Ministry of the Environment Japan, 2012).

1.4.3 Exposure of the general population

A study on biological monitoring in the general population living in areas near synthetic leather factories in China investigated the consistency between the concentration of *N*,*N*-dimethylformamide in outdoor air and levels of urinary *N*-methylformamide (Wang et al., 2014). The mean concentration of urinary *N*-methylformamide ranged from 1.5 to 23.4 mg/g creatinine. Under the most intensive exposure (> 450 µg/m³ of *N*,*N*-dimethylformamide in the air), the maximum value of urinary *N*-methylformamide was 41.03 mg/g creatinine. The correlation between urinary *N*-methylformamide and air sampling was strong ($P < 0.01$) with a coefficient of 0.80 (Wang et al., 2014; Table 1.1).

1.4.4 Occupational exposure

According to the 1981–83 United States National Occupational Exposure Survey, as many as 125 000 workers in the USA were potentially exposed to *N*,*N*-dimethylformamide (NOES, 1997).

Exposures to *N*,*N*-dimethylformamide were assessed in workplaces by both air monitoring and biomonitoring (urinary *N*-methylformamide) beginning in the 1970s (Table 1.2).

1.4.5 Exposure assessment in epidemiological studies

In a retrospective cohort study (Chen et al., 1988a, b), at an acrylic-fibre production plant in the USA, exposure to *N*,*N*-dimethylformamide was assessed semi-quantitatively by an "exposure classification committee" comprising 15 employees. They classified all jobs into three categories: "low" exposure (no direct contact with liquids, workplace concentrations of *N*,*N*-dimethylformamide in air consistently below 10 ppm and no odour of *N*,*N*-dimethylformamide), "moderate" exposure (intermittent contact with liquids containing more than 5% *N*,*N*-dimethylformamide and workplace concentrations of *N*,*N*-dimethylformamide in air occasionally (more than once a week) above 10 ppm) and "high" exposure (frequent contact with liquids containing more than 5% *N*,*N*-dimethylformamide, workplace concentrations of *N*,*N*-dimethylformamide in air often above 10 ppm and breathing protection often required for 15–60 min). Monitoring data on *N*,*N*-dimethylformamide were not available for the period; exposure was estimated for 1950–70 and exposure intensity for each job was assumed to be constant during this time period.

In a nested case–control study in four plants (Walrath et al., 1989), a much more detailed exposure assessment was performed. Data on air measurements of *N*,*N*-dimethylformamide

Table 1.2 Occupational exposure to *N,N*-dimethylformamide

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Personal air sampling										
China	7	Leather production		43	27.7					Cai et al. (1992)
		Polyurethane production		65	11.9					
		Shoe sole production		17	2.1					
		Laboratory A		23	1.2					
		Laboratory B		59	0.6					
		Leather printing		52	7.6					
		Resin production		59	1.8					
Germany	1	Polyacrylic fibre		63	5.3[b]	ND–485.7	92	13.1	ND–108.7	Käfferlein et al. (2000)
Germany	1	Polyacrylic fibre	Total	118	12.5	ND–115.2	125	4.7[b,c]	0.4–62.3[c]	Wrbitzky & Angerer (1998)
			Wet-spinning	30	22.2	0.9–115.2	30	8.9[b,c]	0.4–54.0[c]	
			Dry-spinning	25	19.5	2.4–112.2	28	6.7[b,c]	0.9–62.3[c]	
			Finishing	51	4.3	ND–41.6	55	3.0[b,c]	0.6–19.9[c]	
			Dyeing	12	7.6	0.3–29.8	12	5.5[b,c]	0.8–17.2[c]	
Italy	2	Synthetic leather		100	22.0	8.0–58.0				Cirla et al. (1984)
Italy	1	Synthetic leather		125	13.5	0.4–75.2	125	17.1[d]	1.5–114.2	Imbriani et al. (2002)
Japan	1	Synthetic resin	Blending	3	7.0[d,e]	6.1–9.1				Sakai et al. (1995)[f]
			Kneading	3	14.6[d,e]	6.1–24.3				
			Extruding	3	14.9[d,e]	12.2–18				
			Ageing	3	4.3[d,e]	3.0–9.1				
			Total				10	24.7[c]		
Japan	1	Synthetic resin	Summer	128	5.2[d]		128	4.1[d]		Miyauchi et al. (2014)
			Winter	142	3.0[d]		142	1.4		

Table 1.2 (continued)

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Republic of Korea	15	Fibre coating		57	14.9	0.3–48.9	57	17.5[d]	0.4–97.9	Yang et al. (2000)
		Synthetic leather		108	25.8[d]	4.0–168.7	108	32.8[d]	0.8–235.8	
		Paint		13	3.5[d]	0.3–22.5	13	4.2[d]	1.2–12.7	
		Synthetic fibre		116	2.4[d]	0.3–32.2	116	8.3[d]	1.2–91.4	
		Synthetic leather		4	2.1[d]	0.6–11.6	4	7.8[d]	0.4–54.9	
		Synthetic fibre		2	0.5[d]	0.3–1.0	2	3.7[d]		
		Fibre coating		11	3.7[d]	0.5–21.0	11	4.4[d]	0.4–19.5	
		Synthetic leather		19	13.0[d]	2.1–49.2	19	15.8[d]	4.7–65.4	
		Paint		5	1.2[d]	0.3–8.1	5	3.2[d]	0.4–10.8	
Republic of Korea	9	Synthetic leather		116	26.8[d]		143	47.5[d]		Kim et al. (2004)
Taiwan, China	1	Synthetic leather and resin		176	35.3	0.3–263.3				Luo et al. (2001)
Taiwan, China	1	Synthetic leather		12	34.7		12	17.9		Chang et al. (2004a)
Taiwan, China	4	Various	Total	75	4.6[d]	0.1–58.9	75	0.47[d]	0.03–104.4	Chang et al. (2004b)
		Synthetic acrylic fibre factory		23	2.1[d]	0.1–17.5	23	0.09[d]	0.03–3.20	
		Synthetic leather factory A		8	19.9[d]	10.5–58.9	8	4.95[d]	1.32–21.01	
		Synthetic acrylic leather factory B		24	13.3[d]	2.4–50.7	24	14.3[d]	4.9–104.4	
		Circuit board factory		20	1.7[d]	0.3–29.9	20	0.05[d]	0.03–1.20	
Taiwan, China	4	Synthetic leather		59	12.4[d]	1.6–155.3		20.8[d]	1.35–178.6	Wang et al. (2004)
Taiwan, China	1	Synthetic leather		65	34.9	2.2–104.8				Chang et al. (2005b)
Taiwan, China	1	Synthetic leather		13	32.2[b]	20.2–104.8	13	13.8[b]	7.5–73.7	Shieh et al. (2007)

Table 1.2 (continued)

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Air sampling in the workplace										
Belgium	1	Synthetic acrylic fibre	Mixer	54	32.7					Lauwerys et al. (1980)
			Spinning	54	13.4					
China	2	Synthetic resin	Resin line	12	15.0[d]	5.4–30.3				He et al. (2010)
			Control line	4	2.9[d]	2.1–4.7				
		Synthetic leather	Mixing	3	46.7[d]	42.5–51.2				
			Wet process	9	49.1[d]	13.1–199.8				
			Dry process	6	22.3[d]	14.9–72.4				
			Laboratory	7	3.3[d]	1.1–7.7				
			Product inspection	3	4.3[d]	1.3–17.2				
			Administration	4	1.5[d]	ND–2.2				
Italy	1	Synthetic leather	Washing	10	21.5[d]	5–40				Fiorito et al. (1997)
			Production	22	18.7[d]	2–35				

[a] Post-shift urine samples
[b] Median
[c] mg/g creatinine
[d] Geometric mean
[e] Stationary sampling
[f] Temperature of the injector port < 250 °C for biomonitoring/method unclear
DMF, *N*,*N*-dimethylformamide; ND, not detected; NMF, *N*-methylformamide

and urinary concentrations of *N*-methylformamide were used whenever possible. For plant A, 167 personal *N*,*N*-dimethylformamide samples (1979–86), 107 urinary *N*-methylformamide samples (1975–79) and 14 *N*,*N*-dimethylformamide area samples (1975–80) were available. For plants B and C, respectively, 2916 and 2718 personal or area air samples of *N*,*N*-dimethylformamide (1974–86) were available; ~61 000 urinary (1980–86) and ~12 000 air *N*-methylformamide measurements (1975–86) were used for the exposure assessment. For plant D, 2361 *N*,*N*-dimethylformamide samples (1974–86) and 2037 *N*-methylformamide samples (1978–1986) were available. Two estimates of 8-hour time-weighted average (TWA) exposure to *N*,*N*-dimethylformamide (average and peak exposure) were assigned to each job title based on the measurements; however, the final exposure classification was similar to the classification for the cohort study described in Chen et al. (1988a). Jobs were ranked as having "low" (1.0–< 2.0 ppm of *N*,*N*-dimethylformamide in air), "moderate" (2.0–< 10.0 ppm) and "high" (≥ 10.0 ppm) exposure. Controls were ranked as "none" or "present" if not exposed or exposed to < 1 ppm of *N*,*N*-dimethylformamide in air, respectively. Contrary to the retrospective cohort study, a job could be ranked differently over time because of the use of time-resolved monitoring data.

1.5 Regulations and guidelines

Depending on the country, 8-hour TWA occupational exposure limits have been set at 15 mg/m³ (5 ppm) or 30 mg/m³ (10 ppm) for *N*,*N*-dimethylformamide in the air (GESTIS, 2015). For example, the permissible exposure limit issued in the USA by the Occupational Safety and Health Administration is 30 mg/m³, whereas the threshold limit value recommended by the Scientific Committee for Occupational Exposure Limits of the European Union is 15 mg/m³ (SCOEL, 2006; GESTIS, 2015).

The European Union and the Occupational Safety and Health Administration label *N*,*N*-dimethylformamide with a skin notation (SCOEL, 2006; OSHA, 2016). As a consequence, biological limit values, in terms of measuring metabolites of *N*,*N*-dimethylformamide in urine, have been recommended to take into account all routes of exposure (inhalation, dermal, oral). The Scientific Committee for Occupational Exposure Limits recommends a biological limit value of 15 mg *N*-methylformamide per litre urine in end-of-shift samples, based on the association between exposure to 5 ppm airborne *N*,*N*-dimethylformamide and excretion of *N*-methylformamide in urine at the end of shift (SCOEL, 2006). Several countries of the European Union list *N*,*N*-dimethylformamide as a reproductive toxicant. For example, in Germany, *N*,*N*-dimethylformamide is categorized in group B of reproductive toxicants because embryonic or fetal damage cannot be excluded if women are exposed to the compound during pregnancy, even when the respective threshold limit values are observed (DFG, 2015).

According to the risk phrases of the Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations, *N*,*N*-dimethylformamide is harmful if inhaled (H332) and if skin contact occurs (H312). It also can cause serious eye irritation (H319) and may damage fertility or the unborn child (H360D) (ECHA, 2016).

N,*N*-Dimethylformamide has recently been categorized as a group 4 carcinogen by the Commission for the Investigation of Health Hazards at the Workplace in Germany (DFG, 2015).

EPA (1990) promulgated a reference concentration for *N*,*N*-dimethylformamide in the air of 0.03 mg/m³.

2. Cancer in Humans

2.1 Aircraft repair

See Table 2.1

The human carcinogenicity of *N*,*N*-dimethylformamide was questioned by Ducatman et al. (1986) beginning with an investigation of a cluster of three cases of testicular germ cell cancer that occurred between 1981 and 1983. The three testicular cancers (< 1 expected) occurred among 153 white civilian repairmen who were exposed to *N*,*N*-dimethylformamide while repairing the exterior surfaces and exterior electrical components of the F4 Phantom jet aircraft at a single United States naval air rework facility in North Carolina, USA. The finding led to an evaluation of testicular cancer in two groups of civilian aircraft repair workers, which was reported in the same paper. The two groups of workers were at a United States naval air rework site in California. Similar to the North Carolina workers, the first of these groups also repaired the F4 Phantoms among other naval aircraft and were exposed to *N*,*N*-dimethylformamide, while workers in the second group performed re-work on a variety of naval aircraft, but not the F4 Phantoms, and were not exposed to *N*,*N*-dimethylformamide. At the California facility, four of the 680 men with a history of working on F4 phantoms were diagnosed with testicular germ cell cancers. The men in this groups of exposure workers had an estimated 19 040 person–years of experience from 1970 to 1983, and that finding represented an apparent increase of fourfold [4.21; 95% confidence interval (CI), 1.15–10.78] in incidence compared with national incidence rates ($P < 0.02$). [It was not clear whether the expected numbers reported in the paper were age-adjusted.] In contrast, none of the 446 men in the unexposed population (who did not repair the F4 Phantom) had a diagnosis of testicular germ cell cancer. Within the North Carolina and California populations involved in F4 Phantom repairs, the men with a diagnosed cancer (five seminomas and two embryonal cell cancers) had long histories of aircraft repair before their diagnosis.

Naval air rework facilities (dedicated to military aircraft repair) presented opportunities for repair workers to be exposed to surface coatings and associated emulsifiers and surfactants including "Teflon" paints and dyes, solvents and metals (Ducatman et al., 1986). A relatively uncontrolled process that was unique to F4 Phantom repair at the two facilities involved open air spraying of an 80% *N*,*N*-dimethylformamide solution onto in-situ electrical cables in quantities sufficient to dissolve the elastomeric surface coatings of the cables, with the goal of performing needed maintenance on the bare cables in place. The process was open and no specific ventilation was employed: it was reported to involve substantial inhalation and dermal exposure. At the time of the investigation, the process had been out of use for more than a decade. A formal exposure model was not created. Among the seven cases at both facilities (age range, 30–46 years), three reported direct responsibility for the setting up and use of this process and the others recalled that they were exposed in proximity. [The Working Group was aware that this group of military aircraft mechanics had respiratory and dermal exposure to *N*,*N*-dimethylformamide. Limitations of the study included the lack of quantitative exposure measurements and the fact that follow-up for cancer incidence included only the years when the workers were employed.]

The above findings in civilian repairmen working for the United States Navy motivated an evaluation of testicular cancer hospitalizations of United States active duty military personnel from 1974 to 1979 (Garland et al., 1988). This study found 143 incident cases of testicular cancer in currently active duty personnel. No excess risk was observed overall but, among aviation support technicians, the standardized incidence ratio was 6.2 (95% CI, 1.9–13.0; 5 cases). [The Working

Table 2.1 Cohort studies of cancer and exposure to *N,N*-dimethylformamide

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
Ducatman et al. (1986) Naval air rework facility North Island (the 2nd facility investigated, which did not contain the original cluster), CA, USA 1970–1983	680 exposed, 446 not exposed; male civilian employees at a US naval air rework facility Exposure assessment method: direct observation; onsite survey, linkage to local cancer registry, outreach to local physicians and hospitals, and review of medical records	Testis	All exposed workers	4	[4.21 (1.15–10.78)]	NR	Strengths: the investigation was prompted by a specific hypothesis related to a cluster investigation at a previous facility; there was a nested case–control comparison to men who had similar jobs but never used the same process Limitations: statistical comparison was to national SEER data; only active workers included; the implicated exposure process had stopped before the investigation, and no exposure model was created
CDC (1989) Fulton County, NY, USA 1 January 1975 to 31 December 1987	80 male workers; males employed in the finishing line of a leather tannery from 1975–1987 Exposure assessment method: environmental monitoring; medical record confirmed cases and linkage to the New York State cancer registry	Testis	All workers in the cluster plant	3	40.5 (8.1–118.4)	Age, sex, period, location	Exposure monitoring was performed after the relevant exposure had ceased; a historical survey attempted to assess the previous history of high exposure by interrogating the presence of alcohol intolerance during the period of the DMF operation Strengths: additional follow-up of Fulton County testicular cancer cluster Limitations: statistical calculations include the initial cancer cluster; the exposure monitoring did not include the process of interest

Table 2.1 (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
Chen et al. (1988a) Fibre-producing industrial facility, USA 1 January 1956 to 31 December 1984	3859 total: 2530 DMF only; 1329 to DMF and acrylonitrile; and 1130 with exposures to neither. Data are tabulated for the cohort with any exposure to DMF; male workers with potential exposure to DMF between 1950 and 1970 Exposure assessment method: assessment by employee committee; corporate cancer registry, begun in 1956	Upper aerodigestive tract: buccal cavity & pharynx	All DMF cohort	11	[1.67 (0.83–2.98)]	Age, sex, period	SRRs were generally elevated, but not statistically significant for the DMF-only cohort Strengths: cancer incidence study; exposure matrix Limitations: exposure measurements only after 1970
		All cancers combined	All DMF cohort	88	[0.92 (0.73–1.13)]		
		Prostate	All DMF cohort	10	[1.92 (0.92–3.54)]		
		Malignant melanoma	All DMF cohort	7	[1.55 (0.56–3.21)]		
		Lung	All DMF cohort	21	[0.97 (0.60–1.48)]		
		Testis	All DMF	1	[0.59 (0.01–3.28)]		
		Upper aerodigestive tract: buccal cavity & pharynx	Low/ moderate DMF	5	[4.17 (1.35–9.72)]		
			High DMF	6	[3.0 (1.10–6.5)]		
			< 5 yrs DMF exposure	4	[3.64 (0.99–9.31)]		
			≥ 5 yrs DMF exposure	7	[3.18 (1.28–6.56)]		

Table 2.1 (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
Chen et al. (1988b) Fibre-producing industrial facility, USA 1 January 1950 to 31 December 1982	See incidence study by Chen et al. (1988a) Male workers with potential exposure to DMF between 1950 and 1970 Exposure assessment method: assessment by employee committee; corporate mortality data	All cancers combined	All DMF cohort	75	[0.98 (0.77–1.23)]	Age, sex, period, location	Same population and methods as Chen et al. (1988a) Strengths: local worker comparison group Limitations: mortality only; no testicular cancer deaths were reported (mortality is not the optimal end-point for investigating this cancer)
			DMF only cohort	38	[0.95 (0.67–1.30)]		
		Upper aerodigestive tract: buccal cavity & pharynx	All DMF cohort	3	[1.88 (0.39–4.48)]		
			DMF only cohort	2	[2.5 (0.30–9.0)]		
		Lung	All DMF cohort	33	[1.24 (0.85–1.74)]		
		Lung	DMF-only cohort	19	[1.40 (0.85–2.20)]		

CI, confidence interval; DMF, *N*,*N*-dimethylformamide; NR, not reported; SEER, Surveillance, Epidemiology and End Results; SRR, standardized relative risk; US, United States; yr, year

Group considered it unlikely that active duty aviation support technicians in general would have had significant exposure to the unique process involving *N*,*N*-dimethylformamide.]

2.2 Leather workers

See Table 2.1 and Table 2.2

Levin et al. (1987) reported a cluster of three cases of testicular germ cell cancer in workers who worked on a spray line in a leather tannery (Fulton County, NY, USA) where *N*,*N*-dimethylformamide was used. They were aged 25–36 years at the time of diagnosis, which occurred between 1982 and 1984, and had worked for 8–14 years on the spray line before diagnosis. Other than *N*,*N*-dimethylformamide and "dyes", this report did not discuss other potential exposures.

In a further investigation, a team including personnel from the United States National Institute of Occupational Safety and Health performed air sampling of the spray process for partially tanned hides at the same facility (CDC, 1989). At the time of the investigation, the facility had ceased to use *N*,*N*-dimethylformamide in the tanning process and therefore none was detected in air sampling. This investigation established that *N*,*N*-dimethylformamide had been used historically in the spraying process. The exposures identified included dyes, pigments, surface coatings and solvents, including *N*,*N*-dimethylformamide. Sampling after the *N*,*N*-dimethylformamide process had stopped detected several glycol ethers, including 2-butoxyethanol, at up to 10.9 ppm. A cohort study of testicular cancer in 80 workers in the index tannery found a standardized incidence ratio of 40.5 (95% CI, 8.1–118.4) compared with state Surveillance, Epidemiology and End Results data. In addition, this report featured a case–control study, performed at the county level, of testicular cancer cases in male residents aged 20–54 years from January 1974 to March 1987. The three men reported in the Levin et al. (1987) report were included, as well as an additional seven cases. A group of 129 male controls of similar age who had other cancer diagnoses from the same registry was used. Five of the 10 cases had histories of leather-related employment, resulting in an odds ratio of 5.8 (95% CI, 1.5–22.0) (CDC, 1989). [The Working Group noted that the inclusion a posteriori of an initially detected disease cluster within a small population calculation can contribute to an expected outcome of an elevated standardized incidence ratio.]

Workers from the index tannery workplace were also enrolled in a prospective testicular cancer screening programme. The National Institute of Occupational Safety and Health investigators reported that 51 out of 83 workers participated and, as of 1990, no additional cases of testicular cancer had been found (Calvert et al., 1990).

2.3 Chemical manufacturing

2.3.1 Cohort study

See Table 2.1

A major chemical manufacturer conducted retrospective cohort cancer incidence (Chen et al., 1988a) and cancer mortality (Chen et al., 1988b) studies of workers at an acrylic-fibre production plant in the USA whose employment provided exposure to *N*,*N*-dimethylformamide alone (2530 workers), acrylonitrile alone (16 workers), neither (1130 workers) or both (1329 workers). The two groups with any exposure to *N*,*N*-dimethylformamide were then combined into a single historical cohort (3859 workers).

Monitoring data were reportedly unavailable for most of the historical period (1950–1970) considered in these reports, and exposures were classified as "low", "moderate" or "high" by a committee of workers. Diagnostic information on cancer was obtained from the manufacturer's internal cancer registry and was therefore

Table 2.2 Case–control studies of cancer and exposure to *N,N*-dimethylformamide

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate	Covariates controlled	Comments
CDC (1989) Fulton County, NY, USA Testicular cancer cases from county-level analysis of New York State cancer registry data	Cases: 10; New York State cancer registry Controls: 129; New York State cancer registry Exposure assessment method: based on recorded usual occupation at diagnosis	Testis	Leather tannery workers	5	5.8 (95% CI, 1.5–22.0)	Date, year of diagnosis	Strengths: added information (two additional cases) to earlier cluster investigation Limitations: the original cluster was included in the case numerator
Walrath et al. (1989) Four industrial facilities of one employer 1956–1985	Cases: 39 buccal cavity & pharynx, 6 liver, 43 prostate, 11 testis, 39 skin; employer cancer registry Controls: 276; other workers from cohort Exposure assessment method: expert assessment; existing monitoring data were characterized by job title, for mean and peak DMF; a facility history was developed for each of four facilities	Liver/ hepatocellular carcinoma	Minimum latency 10 yrs	NR	6.10 (90% CI, 0.38–72.00)	Age, sex, plant, pay class	Strengths: improved description of JEM for this employer Limitations: exposure monitoring data not available for the earlier years: extrapolations were made
		Liver/ hepatocellular carcinoma	> 10 yr duration	NR	2.74 (90% CI, 0.16–47.3)	Age, study site, pay class, year of diagnosis, age at diagnosis	
		Upper aerodigestive tract: buccal cavity & pharynx	All categories	15	0.89 (90% CI, 0.35–2.29)	Age, sex, plant, pay class	
		Prostate	All categories	17	1.47 (90% CI, 0.66–3.30)	Age, sex, plant, pay class	
		Malignant melanoma	All categories	16	1.70 (90% CI, 0.52–5.51)	Age, sex, plant, pay class	
		Testis	All categories of exposure	3	0.99 (90% CI, 0.22–4.44)	Age, sex, plant, pay class	
		Testis	Moderate exposure only	2	11.60 (90% CI, 0.47–286)	Age, study site, pay class, year of diagnosis, age at diagnosis	

CI, confidence interval; DMF, *N,N*-dimethylformamide; JEM, job–exposure matrix; NR, not reported; SEER, Surveillance, Epidemiology and End Results; US, United States; yr, year

limited to the time of employment. Person–years were calculated from first exposure or first employment from 1956 to 1984. Comparisons of corporate registry data were then made to national Surveillance, Epidemiology and End Results cancer registry data for 1973–1977. Thirty-four incident cases of all types of cancer were recorded in the cohort of wage workers exposed to *N*,*N*-dimethylformamide only (Chen et al., 1988a). Only one case of testicular cancer was found compared with 1.7 expected [standardized relative risk, 0.59; 95% CI, 0.01–3.28] in the cohort exposed to *N*,*N*-dimethylformamide only. Buccal cavity and pharyngeal cancers (combined) were significantly increased (8 observed, 1 expected [standardized relative risk, 8.00; 95% CI, 3.45–15.76]) in the cohort exposed to *N*,*N*-dimethylformamide only, but not in the cohort exposed to both *N*,*N*-dimethylformamide and acrylonitrile. No dose–response relationship was observed related to the classified exposure level.

Chen et al. (1988b) used the same methods to study mortality from all causes and cancer in the same population. Compared with internal company rates, non-statistically significant excesses of buccal cavity and pharyngeal cancers (combined) and lung cancer were reported (Chen et al., 1988a). [The exposure characterization for this *N*,*N*-dimethylformamide-manufacturing cohort was more complete than that in the previously cited studies in aircraft repair and leather work and provides an impression that exposures were better controlled than those for aircraft and leather workers. The *N*,*N*-dimethylformamide solution at the manufacturing sites is reported to have generally been up to 5% in concentration and intermittently > 5% – less concentrated than the 80% solutions used by aircraft repairmen and leather workers – and the observation that respirators were supposed to have been used if atmospheric concentrations were > 10 ppm of *N*,*N*-dimethylformamide does not appear to pertain to the aircraft repair and leather operations previously described.]

2.3.2 Case–control study

See Table 2.2

The same chemical manufacturer subsequently performed a broader case–control study on exposure to *N*,*N*-dimethylformamide among male active employees at four of its manufacturing facilities including the one studied by Chen et al. (1988a, 1988b), from 1956 to 1985, based on cancer cases reported to the company cancer registry (Walrath et al., 1989). The study population included only cases identified among currently employed workers. For each case, two controls from the entire cohort were matched to the case worker based on year of employment, year of birth, sex, plant, and payroll/salary class. The study provided a more detailed exposure assessment than that used in the preceding cancer incidence and mortality studies (Chen et al., 1988a, 1988b), including measurements of *N*,*N*-dimethylformamide in the air and its urinary metabolites. Average and peak exposures were considered. When data were not available, an extrapolation was made using exposure data from later years. The exposure classifications were more comprehensive but ultimately similar to those used in the cohort study (Chen et al., 1988a): "low", "medium", "high" and none. The geometric mean concentration of *N*,*N*-dimethylformamide in the air ranged from 1 to 2 ppm [3–6 mg/m^3] for jobs in the low exposure group to > 10 ppm [30 mg/m^3] for jobs in the high exposure group. Air concentrations > 50 ppm [149 mg/m^3] were possible in the high exposure category. Mantel-Haenszel and multiple logistic regression methods were used to calculate odds ratios. The odds ratio for testicular cancer for ever being exposed was 0.99 (90% CI, 0.22–4.44; 3 exposed cases). Odds ratios for testicular cancer by level of exposure were 0.86 (90% CI, 0.09–8.56) for low

and 11.6 (90% CI, 0.47–286; 2 exposed cases, 2 exposed controls) for moderate exposure. No cases occurred in the high exposure category. The same data set was also considered with regard to different durations of exposure. The odds ratio for testicular cancer was 1.28 (90% CI, 0.14–7.12) for those with < 10 years exposure and no cases occurred among those with ≥ 10 years exposure. Buccal cavity and pharyngeal cancer were not found to be significantly increased when all facilities were combined, nor were any other studied cancer sites. [The Working Group noted that interpretation of these studies was limited by the small numbers of testicular cancer cases and by the restriction to cases occurring among active workers.]

3. Cancer in Experimental Animals

N,*N*-Dimethylformamide was first reviewed by the *IARC Monographs* Working Group (IARC, 1989) when it had been tested for carcinogenicity by oral administration and by subcutaneous injection in one rat strain (Druckrey et al., 1967) and by intraperitoneal injection in another strain (Kommineni, 1972). All of these studies were judged to be inadequate for an evaluation and were still considered to be inadequate by the current Working Group because of the small number of animals, the short duration of treatment and the incomplete reporting of the results. *N*,*N*-Dimethylformamide was subsequently reviewed by a second Working Group (IARC, 1999) when it had also been tested for carcinogenicity by inhalation in one study in mice and one study in rats, with no significant increase in tumour incidence (Malley et al., 1994).

Studies that were judged to be adequate for an evaluation (including studies released since the previous *IARC Monographs*) are summarized below.

3.1 Mouse

See Table 3.1

Inhalation

Groups of 78 male and 78 female Crl:CD-1 (ICR) BR mice (age, 55 days) were exposed to *N*,*N*-dimethylformamide (purity, 99.9%) by whole-body inhalation at concentrations of 0, 25, 100, and 400 ppm for 6 hours per day on 5 days per week for 18 months. Five male and five females per group were killed at 2 weeks, 3 months and 12 months to evaluate cell proliferation in the liver. Survival in all exposure groups was similar to that of controls. Higher body weights and body-weight gain were observed in the male and female mice exposed to 100 and 400 ppm compared with controls and were considered to be compound-related. The males exposed to 100 and 400 ppm and females exposed to 400 ppm had higher liver weights relative to body weight at necropsy. No significant increase in tumour incidence was observed in any dose group. All treated males had significant increases in centrilobular hepatocellular hypertrophy and hepatic single cell necrosis. Centrilobular hepatocellular hypertrophy was also significantly increased in the females exposed to 100 and 400 ppm and hepatic single cell necrosis in all treated females (Malley et al., 1994). [The Working Group noted the short duration of the study.]

In a well-conducted study that complied with good laboratory practice, groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were exposed to *N*,*N*-dimethylformamide (purity, > 99.8%) by whole-body inhalation at concentrations of 0, 200, 400, and 800 ppm for 6 hours per day on 5 days per week for 104 weeks. No significant difference in survival was observed between the exposed groups and the controls. The survival rate of females exposed to 800 pm decreased marginally (but not significantly) after the 78th week because of the development of liver tumours.

Table 3.1 Studies of carcinogenicity with *N,N*-dimethylformamide in mice and rats

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Crl:CD-1 (ICR) BR (M) 55 days 18 mo Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 78, 78, 78, 78 56%, 68%, 60%, 59%	*Liver or testis* No increase in tumour incidence	NS	Principal limitations: short duration of the study 5 males per group were killed at 2 wks, 3 mo, and 12 mo, for evaluation of cell proliferation in the liver
Full carcinogenicity Mouse, Crl:CD-1 (ICR) BR (F) 55 days 18 mo Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 78, 78, 78, 78 68%, 57%, 62%, 76%	*Liver or mammary gland* No increase in tumour incidence	NS	Principal limitations: short duration of the study 5 males and 5 females per group were killed at 2 wks, 3 mo, and 12 mo, for evaluation of cell proliferation in the liver
Full carcinogenicity Mouse, Crj:BDF1 (M) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 37, 33, 37, 40	*Liver* Hepatocellular adenoma: 6/50, 36/50*, 41/49*, 41/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appears to have complied with GLP
		Hepatocellular carcinoma: 2/50, 12/50*, 16/49*, 16/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma: 0/50, 13/50*, 7/49*, 4/50	*$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 8/50, 42/50*, 46/49*, 44/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF1 (F) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 49, 50, 50, 49 29, 30, 21, 22	*Liver* Hepatocellular adenoma: 1/49, 42/50*, 47/50*, 48/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appears to have complied with GLP
		Hepatocellular carcinoma: 3/49, 25/50*, 32/50*, 35/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma: 0/49, 0/50, 4/50, 0/49	NS	
		Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 3/49, 45/50*, 49/50*, 49/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
Full carcinogenicity Rat, Crl:CD BR (M) 47 days 2 yrs Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 87, 87, 87, 87 27%, 34%, 40%, 44%	*Liver, testis or mammary gland* No increase in tumour incidence	NS	Principal limitations: survival for all dose groups and controls was < 45% 5 males per group were killed at 2 wks, 3 mo, and 12 mo for evaluation of cell proliferation in the liver; 10 males per group were killed for interim pathological evaluation at 12 mo
Full carcinogenicity Rat, Crl:CD BR (F) 47 days 2 yr Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 87, 87, 87, 87 35%, 23%, 19%, 39%	*Liver or mammary gland* No increase in tumour incidence	NS	Principal limitations: survival for all dose groups and controls was less than 40% Five females per group were killed at 2 wks, 3 mo, and 12 mo for evaluation of cell proliferation in the liver; 10 females per group were killed for interim pathological evaluation at 12 mo

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (M) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 42, 38, 40, 37	*Liver* Hepatocellular adenoma: 1/50, 3/50, 13/50*, 20/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well conducted study that appeared to comply with GLP The authors stated that multiple occurrences of hepatocellular tumours were found in the liver of DMF-exposed rats, in contrast to the occurrence of a single tumour in the liver of the animals of the control group
		Hepatocellular carcinoma: 0/50, 1/50, 0/50, 24/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined): 1/49, 4/50, 13/50*, 33/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
Full carcinogenicity Rat, F344/DuCrj (F) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 42, 38, 38, 30	*Liver* Hepatocellular adenoma: 1/49, 1/50, 6/50, 16/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appeared to comply with GLP The authors stated that multiple occurrences of hepatocellular tumours were found in the liver of DMF-exposed rats, in contrast to the occurrence of a single tumour in the liver of the animals of the control group
		Hepatocellular carcinoma: 0/49, 0/50, 0/50, 5/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.05$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined): 1/49, 1/50, 6/50, 19/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	

d, day; DMF, *N*,*N*-dimethylformamide; F, female; GLP, good laboratory practice; M, male; mo, month; NA, not applicable; NS, not significant; ppm, parts per million; wk, week; yr, year

Body weights of all treated males and of females exposed to 800 ppm were decreased by more than 10% compared with controls. The incidence of hepatocellular adenoma (males: 6/50 controls, 36/50 at 200 ppm, 41/49 at 400 ppm, and 41/50 at 800 ppm; females: 1/49 controls, 42/50 at 200 ppm, 47/50 at 400 ppm, and 48/49 at 800 ppm), hepatocellular carcinoma (males: 2/50 controls, 12/50 at 200 ppm, 16/49 at 400 ppm, and 16/50 at 800 ppm; females: 3/49 controls, 25/50 at 200 ppm, 32/50 at 400 ppm, and 35/49 at 800 ppm) and hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in all groups of treated males and females ($P < 0.01$, Fisher's exact test) in a dose-related manner ($P < 0.01$, Peto's trend test). There was also a significant increase in the incidence of hepatoblastoma in males exposed to 200 and 400 ppm (0/50 controls, 13/50* at 200 ppm, 7/49* at 400 ppm and 4/50 at 800 ppm; * $P < 0.01$, Fisher's exact test). The incidence of hepatoblastoma in females was 0/49 controls, 0/50 at 200 ppm, 4/50 at 400 ppm and 0/49 at 800 ppm. Multiple hepatocellular adenomas and carcinomas were found in the livers of treated mice, and the tumour tissues often occupied almost all areas of the entire liver. No *N*,*N*-dimethylformamide-related neoplastic or non-neoplastic lesions were found in any other organ except the liver (Senoh et al., 2004). [The strengths of this study included the use of multiple doses, a large number of animals per group and the testing of two sexes.]

3.2 Rat

3.2.1 Inhalation

See Table 3.1

Groups of 87 male and 87 female Crl:CD BR rats (age, 47 days) were exposed to *N*,*N*-dimethylformamide (purity, 99.9%) by whole-body inhalation at concentrations of 0, 25, 100, and 400 ppm for 6 hours per day on 5 days per week for 2 years. Five male and five females per group were killed at 2 weeks, 3 months, and 12 months to evaluate cell proliferation in the liver, and 10 males and 10 females per group were killed for interim pathological evaluation at 12 months. Exposure to the highest concentration reduced body-weight gain in both sexes. Survival in all treated groups was similar to that of controls; however, survival in all treated groups and controls was less than 45%. The males treated with 100 and 400 ppm and females treated with 400 ppm had higher liver weights relative to body weight at necropsy. No significant increase in tumour incidence was observed in any treated group. The incidence of minimal to mild centrilobular hepatocellular hypertrophy was increased in males and females treated with 100 and 400 ppm (Malley et al., 1994). [The Working Group noted the poor survival of all groups, including controls.]

In a well-conducted study that complied with good laboratory practice, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were exposed to *N*,*N*-dimethylformamide (purity, > 99.8%) by whole-body inhalation at concentrations of 0, 200, 400, and 800 ppm for 6 hours per day on 5 days per week for 104 weeks. Survival rates of treated males did not differ significantly from those of controls. Survival of the females treated with 800 ppm was significantly decreased compared with that of controls after the 9th week. Body weights of the males and females treated with 400 and 800 ppm were decreased by more than 10% compared with their respective controls. Exposure to *N*,*N*-dimethylformamide caused a dose-related increase ($P < 0.01$, Peto's trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in males and females. The incidence of hepatocellular adenoma was significantly increased in male rats exposed to 400 and 800 ppm (1/50 controls, 3/50 at 200 ppm, 13/50* at 400 ppm

Table 3.2 Incidence of hepatocellular tumours in male F344/DuCrlCrj rats exposed to *N,N*-dimethylformamide by inhalation and/or in drinking-water

Parameter	Inhalation (ppm)								
	0			200			400		
	Drinking-water (ppm)								
	0	800	1600	0	800	1600	0	800	1600
Number of rats examined	50	50	50	50	50	50	50	50	50
Surviving animals	41	34	40	36	36	41	37	43	38
Adenoma[a]	1	6*	8*	15*	28**	45**	26*	43**	46**
Carcinoma[a]	0	0	4*	1	6**	14**	2	12**	14**
Adenoma or carcinoma (combined)[a]	1	6*	12*	16*	30**	46**	26*	45**	47**

[a] Number of tumour-bearing animals
* Significantly different from untreated control group, at $P < 0.05$ by chi-square test
** Significantly different from untreated control group, each drinking-water-alone group and each inhalation-alone group with matching concentrations, respectively, at $P < 0.05$ by chi-square test
Adapted from Ohbayashi et al. (2009), *J Toxicol Sci*, 34(1):53–63.

and 20/50* at 800 ppm; *$P < 0.01$, Fisher's exact test) and in females exposed to 800 ppm (1/49 controls, 1/50 at 200 ppm, 6/50 at 400 ppm and 16/50* at 800 ppm; *$P < 0.01$, Fisher's exact test). The incidence of hepatocellular carcinoma was significantly increased in both sexes exposed to 800 ppm (males: 0/50 controls, 1/50 at 200 ppm, 0/50 at 400 ppm, and 24/50* at 800 ppm; females: 0/49 controls, 0/50 at 200 ppm, 0/50 at 400 ppm and 5/50** at 800 ppm; *$P < 0.01$, **$P < 0.05$, Fisher's exact test). Multiple occurrences of hepatocellular tumours were found in the liver of exposed rats and no multiplicity of hepatocellular tumours was observed in the control groups. No *N,N*-dimethylformamide-related neoplastic or non-neoplastic lesions were found in any other organ except the liver (Senoh et al., 2004). [The strengths of this study included the use of multiple doses, a large number of animals per group, and testing in males and females.]

3.2.2 Inhalation and drinking-water (combined)

See Table 3.2

In a well-conducted study that complied with good laboratory practice, groups of 50 male Fischer 344/DuCrlCrj rats (age, 6 weeks) were exposed to *N,N*-dimethylformamide (purity, > 99.5%) by whole-body inhalation at concentrations of 0, 200, and 400 ppm for 6 hours per day on 5 days per week, and given *N,N*-dimethylformamide-formulated drinking-water at 0, 800, or 1600 ppm (w/w) ad libitum for 24 hours per day on 7 days per week for 104 weeks. No significant difference in survival was found between the untreated control group and the two inhalation-only groups, the two oral administration-only groups or the four combined exposure groups. The terminal body weight was significantly decreased in the inhalation-only group, both oral administration-only groups and all four combined exposure groups compared with controls. Exposure to *N,N*-dimethylformamide caused a significant increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in the four combined exposure groups, the two oral administration-only groups and the two inhalation-only groups compared with the untreated controls, a

significant increase in the incidence of hepatocellular carcinoma in the four combined exposure groups and in the oral administration-only group exposed to 1600 ppm compared with the untreated controls, and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in the four combined exposure groups compared with each of the oral administration-only and inhalation-only groups with matching concentrations (see Table 3.2 for tumour incidences and statistics). The incidence of hepatocellular tumours induced by the combined exposures tended to exceed the dose–response relationship that would be expected under the assumption that the incidence of hepatocellular tumours induced by the single-route exposures through inhalation and ingestion are additive [the authors also stated that the combined exposures were found to produce multiple occurrences of hepatocellular adenomas compared with the single-route exposures but did not show these data] (Ohbayashi et al., 2009). [The strengths of this study included the use of multiple doses and a large number of animals per group. The Working Group noted that only one sex was used and that no mention or discussion was made of any observations in any tissue or organs other than the liver.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

(a) Absorption, distribution, and excretion

N,*N*-Dimethylformamide is readily absorbed after inhalation, dermal and oral exposure. The absorption and elimination of *N*,*N*-dimethylformamide was studied after inhalation of vapour (Mráz & Nohová, 1992a), and after percutaneous absorption of both vapour and liquid (Mráz & Nohová, 1992b). In the inhalation study (Mráz & Nohová, 1992a), retention of *N*,*N*-dimethylformamide in the lungs was 90% in 10 volunteers (5 men and 5 women) exposed for 8 hours to concentrations of 10, 30, or 60 mg/m^3. After a single exposure, for the metabolites that accounted for 49% of the dose, the half-lives of urinary excretion and urinary recoveries were: *N*-methylformamide, 4 hours (22%); formamide, 7 hours (13%); and AMCC, 23 hours (13%). A small amount (0.3% of the dose) of unchanged *N*,*N*-dimethylformamide was also detected with a half-life of excretion of 2 hours. With repeated exposure to 30 mg/m^3 for 8 hours per day on 5 consecutive days, a significant accumulation of AMCC was observed with an equilibrium being reached on day 4. [The Working Group noted that *N*-methylformamide and formamide, which were determined by gas chromatography, were at least in part formed from corresponding hydroxymethyl derivatives by thermal decomposure (formaldehyde release) in the hot sample chamber of the gas chromatograph.]

After a 4-hour exposure to *N*,*N*-dimethylformamide vapour (50 mg/m^3), 13–36% of the total dose (depending on air humidity and temperature) was absorbed through the skin in 8 volunteers (3 men and 5 women). Absorption

of liquid *N*,*N*-dimethylformamide was studied (i) by dipping one hand up to the wrist for 2, 10, 15, and 20 minutes; and (ii) by a patch experiment, in which *N*,*N*-dimethylformamide was placed on a patch of teflon foil, which was then attached to the forearm of the volunteers for 8 hours. The rate of percutaneous absorption in the dipping experiment was 9.4 ± 4 mg/cm^2 per hour (mean ± standard deviation (SD); $n = 4$). In the patch experiment, the urinary excretion of metabolites was not delayed compared with the short-term dipping experiments, but the metabolites amounted to only half of those excreted after inhalation at the same absorbed dose levels (Mráz & Nohová, 1992b). A similar value of percutaneous absorption rate (11 mg/cm^2 per hour), based on in-vitro experiments on excised human skin, was reported (Bortsevich, 1984). The same study showed that aqueous *N*,*N*-dimethylformamide is poorly absorbed; < 1% in 4 hours was absorbed from 15% aqueous *N*,*N*-dimethylformamide, whereas absorption of pure liquid *N*,*N*-dimethylformamide was 51% in 4 hours (Bortsevich, 1984). Absorption of *N*,*N*-dimethylformamide vapour through the skin was further evaluated in an experiment on 13 male volunteers exposed for 4 hours through whole-body exposure (but inhaling clean air via a respirator) as well as lung-only inhalation (at intervals of 96 hours or more) to *N*,*N*-dimethylformamide at concentrations below 10 ppm. Based on the comparison of excreted amounts of *N*-methylformamide, it was estimated that the skin and the lung absorption amounted to 40.4% and 59.6%, respectively. The biological half-life of urinary *N*-methylformamide after dermal exposure (4.75 ± 1.63 hours) was significantly longer than that after respiratory exposure (2.42 ± 0.63 hours) (Nomiyama et al., 2001a).

An even higher contribution of skin absorption amounting to 71% of the total dose of *N*,*N*-dimethylformamide was found under conditions of actual occupational exposure by Wang et al. (2007) at airborne concentrations below 10 ppm.

Internal exposure determined by urinary *N*-methylformamide was monitored in two groups of occupationally exposed workers across a working week to determine the total body burden. Twenty-five workers in a synthetic leather factory and 20 workers in a copper laminate circuit board factory were recruited (Chang et al., 2005a). The average airborne concentration of *N*,*N*-dimethylformamide was similar for both groups (about 4 ppm) but dermal exposure to *N*,*N*-dimethylformamide of the synthetic-leather workers was significantly higher. A significant pattern of linear accumulation was found across a 5-day work cycle for synthetic leather workers but not for copper laminate circuit board workers. [The Working Group noted that dermal exposure to *N*,*N*-dimethylformamide over 5 consecutive days can result in its accumulation in the body.]

In a recent study of the percutaneous absorption of *N*,*N*-dimethylformamide in 193 occupationally exposed workers, the mean breathing zone concentrations in both summer and winter were below the occupational exposure limit of 10 ppm. However, the urinary levels of *N*-methylformamide and AMCC were 2.6 and 1.6 times higher, respectively, in the summer (at 34.1 ± 2.6 °C; mean ± SD) than in the winter (at 17.1 ± 4.8 °C; mean ± SD) (Tsuda et al., 2014).

(b) Metabolism

In an early GC study by Kimmerle & Eben (1975a), *N*-methylformamide and formamide were reported to be major urinary metabolites. However, further studies showed that these metabolites were actually HMMF and *N*-hydroxymethylformamide (HMF), respectively, which released formaldehyde under the conditions of GC (in the sample chamber) (Brindley et al., 1983; Scailteur et al., 1984; Kestell et al., 1986). AMCC was identified for the first time in the urine of a volunteer who inhaled

an unspecified dose of *N*,*N*-dimethylformamide (Mráz & Tureček, 1987).

[The Working Group noted that the above mentioned data indicated the formation of electrophilic metabolites, namely methyl isocyanate and/or another *N*-methylcarbamoylating species and formaldehyde.]

More recently, a liquid chromatography-tandem mass spectrometry method was developed for simultaneous determination of HMMF, *N*-methylformamide, and AMCC and was applied to the analysis of 13 urine samples taken from workers occupationally exposed to *N*,*N*-dimethylformamide. The molar excretion ratio of HMMF:*N*-methylformamide:AMCC was approximately 4:1:1. HMMF was confirmed as the most abundant urinary metabolite in humans (Sohn et al., 2005).

Interactions of *N*,*N*-dimethylformamide with ethanol were also studied. In an early study, four volunteers were exposed to *N*,*N*-dimethylformamide at concentrations of 50–80 ppm [152–243 mg/m^3] for 2 hours with or without preceding oral administration of ethanol. Persons who received ethanol excreted slightly elevated urinary concentrations of *N*,*N*-dimethylformamide during the first 4 hours and lower blood levels of *N*-methylformamide (Eben & Kimmerle, 1976).

A haemoglobin adduct of methyl isocyanate, a proposed metabolic intermediate of *N*,*N*-dimethylformamide and *N*-methylformamide, with *N*-terminal valine (*N*-(*N*-methylcarbamoyl)valine) was identified in the blood of 35 workers in the polyacrylic fibre industry exposed to *N*,*N*-dimethylformamide. The exposure was assessed by measuring urinary *N*-methylformamide which was between 1.3 and 46.5 mg/L (mean, 17.0 mg/L; median, 9.8 mg/L). The methyl isocyanate adduct was released by modified Edman degradation and converted to 3-methyl-5-isoprolylhydantoin, the concentrations of which in globin samples ranged from 26.1 to 412.0 nmol/g of globin (Käfferlein & Angerer, 2001). More recently, a new lysine adduct, N_ε-(*N*-methylcarbamoyl)lysine, was identified in globin samples of humans occupationally exposed to *N*,*N*-dimethylformamide (Mráz et al., 2006).

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

The toxicokinetics of *N*,*N*-dimethylformamide in rats, mice and cynomolgus monkeys was extensively studied by Hundley et al. (1993a, b). Male and female cynomolgus monkeys were exposed by whole-body inhalation to *N*,*N*-dimethylformamide at concentrations of 30, 100, or 500 ppm [90, 300, or 1500 mg/m^3] for 6 hours per day on 5 days per week for 13 consecutive weeks. Disproportionate increases were observed in *N*,*N*-dimethylformamide plasma area under the concentration curve (AUC) values of 19–37-fold in male monkeys and 35–54-fold in females as the atmospheric concentrations increased fivefold from 100 to 500 ppm. Plasma half-lives of *N*,*N*-dimethylformamide were 1–2 hours and those of *N*-methylformamide (actually HMMF + *N*-methylformamide) were significantly longer (4–15 hours). Plasma *N*-methylformamide concentrations exceeded those of *N*,*N*-dimethylformamide 0.5 hour after the beginning of each exposure. HMMF formed 56–95% of the urinary metabolites, regardless of exposure level and duration of the study (Hundley et al., 1993b). The AUC values and peak plasma levels for *N*,*N*-dimethylformamide in rats and mice following a single exposure to 500 ppm (Hundley et al., 1993a) were substantially greater than the respective values in monkeys after a similar exposure. In contrast, the enhancement of metabolism by repeated exposure to *N*,*N*-dimethylformamide at 500 ppm, which was observed in rats and mice, was not clearly demonstrated in monkeys (Hundley et al., 1993b).

In another study, cynomolgus monkeys were exposed to *N*,*N*-dimethylformamide at 500 ppm

[1500 mg/m^3] for 6 hours per day on 5 days per week for 2 weeks either by head-only or whole-body inhalation (one monkey per exposure route), plasma samples taken 0.5–18 hours after the first exposure showed *N*,*N*-dimethylformamide AUC values that were three times higher in the monkey exposed by whole-body inhalation, indicating considerable absorption by non-inhalation route(s). The same comparison of plasma samples taken after the final (10th) exposure revealed an *N*,*N*-dimethylformamide AUC value that was sixfold for the monkey exposed by whole-body inhalation (Hurtt et al., 1991).

Rapid absorption was observed in pregnant Sprague-Dawley rats treated on gestation days 12 and 18 with a single oral dose of 100 mg/kg bw [^{14}C]-*N*,*N*-dimethylformamide. The radioactivity in plasma peaked within 1 hour after treatment. A major part of the dose (60–70%) was excreted in the urine and 3–4% in the faeces. Levels of radioactivity in embryonic and fetal tissues were nearly equal to those in maternal plasma up to 8 and 24 hours, respectively, but were higher thereafter. In lactating rats treated with a single oral dose of 100 mg/kg bw [^{14}C]-*N*,*N*-dimethylformamide on lactation day 14, *N*,*N*-dimethylformamide, HMMF, and *N*-methylformamide were found in the milk at concentrations equal to those in the plasma (Saillenfait et al., 1997).

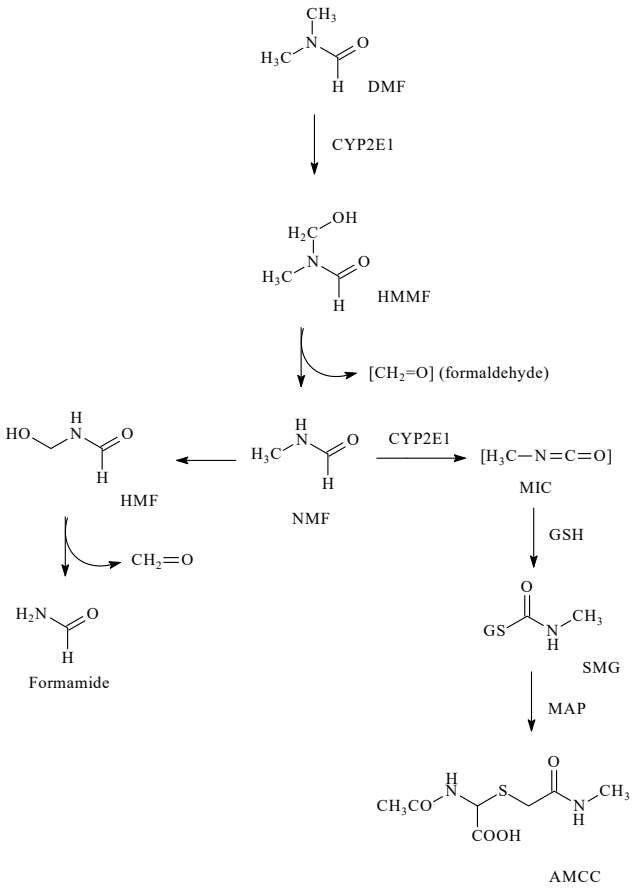
A single ethanol drinking episode significantly enhanced the dermal absorption of *N*,*N*-dimethylformamide in a study using the skin of ethanol-dosed and control Wistar rats to test the penetration of *N*,*N*-dimethylformamide in vitro (Brand et al., 2006).

(b) Metabolism

The metabolism of *N*,*N*-dimethylformamide has been extensively reviewed by Gescher (1993). In both humans and animals, *N*,*N*-dimethylformamide is primarily oxidized by cytochrome P450 (CYP) 2E1 to HMMF. Although relatively stable in neutral or mildly acidic solutions, HMMF may release formaldehyde under physiological conditions to produce *N*-methylformamide. In early studies, *N*-methylformamide was identified by GC as a major metabolite in rats and dogs (Kimmerle & Eben, 1975b). Further metabolic oxidation of *N*-methylformamide leads to HMF, which has been identified as a minor urinary metabolite in rats (Tulip et al., 1989). Unequivocal evidence exists that HMF is formed after the metabolism of *N*-methylformamide in rats and mice (Tulip et al., 1986; Threadgill et al., 1987; Cross et al., 1990). In a close analogy with HMMF, HMF undergoes thermal decomposition to formamide when analysed by GC; therefore, GC data on formamide actually usually include HMF and formamide (Mráz & Tureček, 1987; Mráz et al., 1987; Mráz & Nohová, 1992a).

The mercapturic acid AMCC is formed by a toxicologically relevant pathway via glutathione (GSH) conjugation with a presumed electrophilic *N*-methylcarbamoylating intermediate, probably methyl isocyanate or its chemical equivalent formed by oxidation of *N*-methylformamide and/or HMMF. The GSH conjugate, *S*-(*N*-methylcarbamoyl)glutathione undergoes mercapturic acid pathway to yield AMCC, which is then excreted in the urine. AMCC was found in the urine of rats, mice, Syrian hamsters (Mráz et al., 1989) as well as in human urine (Mráz & Tureček, 1987; Mráz & Nohová, 1992a, b). The pattern of *N*,*N*-dimethylformamide metabolites is qualitatively the same in various rodent species and in humans (Fig. 4.1). Experiments with liver microsomes from rats and mice treated with inducers of CYP2E1 and with CYP2E1 purified from rat and mouse liver microsomes indicated the pivotal role of CYP2E1 in the oxidation of *N*,*N*-dimethylformamide to HMMF as well as in the formation of the key reactive intermediate, which was proposed to be methyl isocyanate (Gescher, 1993; Mráz et al., 1993; Chieli et al., 1995). The key role of CYP2E1 was later confirmed in an in-vitro study on *N*,*N*-dimethylformamide dealkylation. Among several forms of human

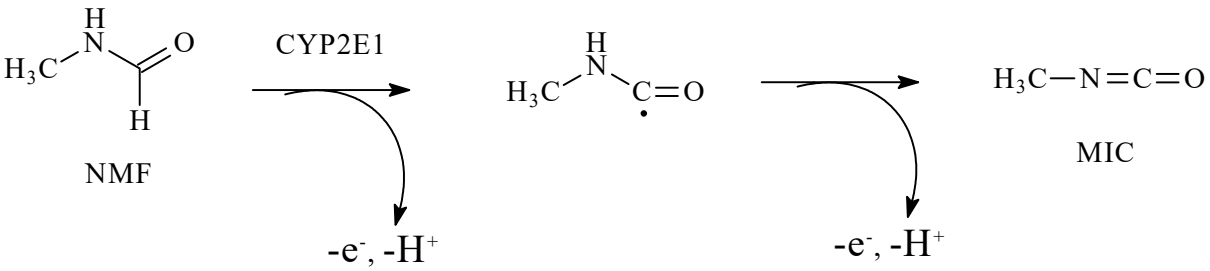
Fig. 4.1 Metabolism of *N*,*N*-dimethylformamide



AMCC, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine; CYP2E1, cytochrome P450 2E1; DMF, *N*,*N*-dimethylformamide; GSH, glutathione; HMF, *N*-hydroxymethylformamide; HMMF, *N*-hydroxymethyl-*N*-methylformamide; MAP, mercapturic acid pathway; MIC, methyl isocyanate; NMF, *N*-methylformamide; SMG, *S*-(*N*-methylcarbamoyl)glutathione
Adapted with permission from Gescher (1993) and Mráz et al. (1993)

recombinant CYPs, namely, CYP1A1, 1A2, 2B6, 2C10, 3A4 and 2E1, only CYP2E1 oxidized *N*,*N*-dimethylformamide (Amato et al., 2001). Methyl isocyanate or its chemically equivalent metabolite is formed via *N*-methylformamide as an intermediate, although HMMF is also a substrate of CYP2E1. However, the amount of *S*-(*N*-methylcarbamoyl)glutathione generated from HMMF in vitro was much smaller than that formed from *N*-methylformamide under identical conditions (Mráz et al., 1993). The mechanism by which the key reactive intermediate is formed was studied by kinetic isotope effect measurements in experiments in mice. The conversion of *N*-methylformamide to urinary AMCC and biliary *S*-(*N*-methylcarbamoyl)glutathione was found to be subject to large primary kinetic isotope effects when hydrogen was replaced by deuterium in the formyl group (k_H/k_D , 4.5 ± 1.0 and 7 ± 2, respectively) indicating the cleavage of the formyl C–H bond is the rate limiting step (Threadgill et al., 1987). The proposed mechanism of the key step in the metabolic activation of *N*,*N*-dimethylformamide through the oxidation of *N*-methylformamide is shown in Figure 4.2. Methyl isocyanate was also reported to be a main

Fig. 4.2 Proposed mechanism of generation of the key electrophilic carbamoylating species, methyl isocyanate



MIC, methyl isocyanate; NMF, *N*-methylformamide
Adapted from Gescher (1993)

product of *N*-methylformamide photo-oxidation with hydroxyl radicals under aerobic conditions and the carbonyl centred radical was proposed as a precursor to methyl isocyanate based on quantum chemical calculations (Bunkan et al., 2015). This observation represents indirect support for the above-mentioned mechanism (Fig. 4.2).

Adducts with globin at *N*-terminal valine and N_{ε}-lysine, *N*-(*N*-methylcarbamoyl)valine and N_{ε}-(*N*-methylcarbamoyl)lysine, respectively, were identified in rats exposed to high doses of *N*,*N*-dimethylformamide (1000 mg/kg bw) (Mráz et al., 2004) as well as in humans exposed to *N*,*N*-dimethylformamide (Mráz et al., 2002, 2006). As cysteine sulfhydryl groups are much stronger nucleophiles than amino acid amine groups, *N*-methylcarbamoylation is likely to occur also at the sulfhydryl groups of cysteine to yield reactive thiocarbamate species capable of transferring the *N*-methylcarbamoyl moiety to other nucleophilic sites (transcarbamoylation). [The Working Group noted that, due to the reactivity of the thiocarbamate moiety, *N*-methylcarbamoylated cysteine residues may have easily escaped detection in the analyses of adducted globin. The *N*-methylcarbamoylating species, either methyl isocyanate or a *N*-methylcarbamoylated cysteine residue, may carbamoylate nucleophilic sites in proteins and in the DNA.]

The effects of the co-administration of ethanol and *N*,*N*-dimethylformamide on the metabolism of *N*,*N*-dimethylformamide, *N*-methylformamide and ethanol were investigated in several early studies. Increases in the concentrations of *N*,*N*-dimethylformamide, *N*-methylformamide, ethanol or acetaldehyde in blood were observed after co-exposure. These results were attributed to the inhibition by *N*,*N*-dimethylformamide of the activity of alcohol dehydrogenase observed both in vitro and in vivo (Eben & Kimmerle, 1976; Hanasono et al., 1977; Sharkawi, 1979) and of aldehyde dehydrogenase observed in vivo (Elovaara et al., 1983). The effect of the competitive inhibition of CYP2E1, which seems to be very probable in the light of more recent observations (Mráz et al., 1993; Chieli et al., 1995), was not considered at the time of these early studies.

In conclusion, three metabolic events appear to play key roles in *N*,*N*-dimethylformamide toxicity: (i) *N*-methylcarbamoylation mediated by methyl isocyanate and/or its chemical equivalents; (ii) free radical damage caused by carbamoyl radicals; and (iii) the probable formation of formaldehyde by oxidative demethylation (Fig. 4.1 and Fig. 4.2). The main enzyme responsible for metabolic activation is CYP2E1.

4.2 Mechanisms of carcinogenesis

The evidence on the key characteristics of carcinogens (Smith et al., 2016), concerning whether *N*,*N*-dimethylformamide induces oxidative stress; alters cell proliferation, cell death, or nutrient supply; is genotoxic; or modulates receptor-mediated effects – is summarized below.

4.2.1 Oxidative stress

(a) Humans

(i) Exposed humans

N,*N*-Dimethylformamide-exposed Chinese workers (n = 104) and 101 controls were studied for oxidative and antioxidative status. The *N*,*N*-dimethylformamide concentration in workplace air was within the range of 3.3–12.4 mg/m^3. The *N*-methyl-carbamoylated haemoglobin adduct (NMHb) in blood was chosen as a biomarker measured as the Edman degradation product, 3-methyl-5-isopropylhydantoin (MVH). The MVH level in exposed workers was 19.69 ± 12.52 mg/kg, and MVH was not detected in the control group. The activity of superoxide dismutase (SOD) in exposed workers (125.30 ± 21.23 U/mL) was significantly higher than the control group (118.35 ± 18.48 U/mL). However, the activity of SOD showed different trends with increasing MVH levels. When MVH ≤ 24 mg/kg, the SOD activity increased with the increasing of MVH level. When MVH > 24 mg/kg, SOD activity decreased with increasing MVH level. No significant differences were observed in glutathione-*S*-transferase, malondialdehyde or 3-nitrotyrosine levels among the two groups. It was concluded that *N*,*N*-dimethylformamide exposure did not cause obvious lipid and/or protein peroxidative damage (Cheng et al., 2014).

(ii) Human cells in vitro

HL-7702 normal human liver cells were used to study *N*,*N*-dimethylformamide-induced oxidative stress. Reactive oxygen species (ROS)-induced fluorescence was determined by flow cytometry using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) and propidium iodide to identify viable cells. ROS levels increased with *N*,*N*-dimethylformamide dose (0–40 mM) to a maximum of ~1.5-fold in cells treated for 24 hours. The levels of 8-hydroxy-2′-deoxyguanosine measured by enzyme-linked immunosorbent assay (ELISA) increased to ~1.5-fold after incubation with *N*,*N*-dimethylformamide at 40 and 100 mM for 24 hours (Wang et al., 2015).

N,*N*-Dimethylformamide (60 mM) induced differentiation of human HL-60 promyelocytic leukaemia cells, and these cells acquired polymorphonuclear leukocyte functions and the ability to generate ROS when stimulated (Speier & Newburger, 1986). HL60 cells are known to contain CYP2E1 (Nagai et al., 2002), which can metabolize *N*,*N*-dimethylformamide. Treatment of HL-60 cells with *N*,*N*-dimethylformamide resulted in a decline in total SOD, while catalase activity declined slightly. Oxidized glutathione (GSSG) reductase activity and reduced glutathione (GSH) and GSSG levels declined slightly over time. Concomitantly with the decrease in antioxidant enzymes during the course of *N*,*N*-dimethylformamide-induced differentiation of human HL-60 cells, treatment with phorbol myristate acetate (PMA, an ROS stimulating agent) increased the ability of the cells to generate hydrogen peroxide (Speier & Newburger, 1986). At a higher dose, *N*,*N*-dimethylformamide (80 mM) induced granulocytic cell differentiation of human HL-60 promyelocytic leukaemia and PLB-985 human myeloid leukaemia cells. *N*,*N*-Dimethylformamide (80 mM) treatment increased glutathione peroxidase enzymatic activity, gene expression (mRNA) and protein levels in HL60 and PLB-985 cells (Shen et al., 1994).

N,*N*-Dimethylformamide (0.5%) induced granulocytic differentiation in PLB-985 human myelomonoblastic leukaemia cells after 6 days of treatment. Further treatment with PMA (1 μM) and formyl-methionyl-leucine phenylalanine (800 nM), ROS stimulating agents, induced ROS detected by nitroblue tetrazolium staining in the cells (Katschinski et al., 1999).

Both *N*,*N*-dimethylformamide and its metabolite *N*-methylformamide reduced total GSH levels in a concentration–response manner (0–200 mM) after incubation in DLD-1 Clone A human colon carcinoma cells (Cordeiro & Savarese, 1984). In a follow-up study using the same experimental conditions, both reduced GSH and GSSG were measured separately. In concentration–response studies with both *N*,*N*-dimethylformamide and *N*-methylformamide, it was found that that the levels of GSSG remained relatively constant over 0–200 mM, while the levels of reduced GSH decreased with increasing concentrations of *N*,*N*-dimethylformamide or *N*-methylformamide (Cordeiro & Savarese, 1986).

(b) Non-human mammalian systems

(i) In vivo

N,*N*-Dimethylformamide treatment of Sprague-Dawley rats after intraperitoneal injection (500 mg/kg bw per day for three consecutive days) had no effect on the hepatic levels of thiobarbituric acid reactive substances (TBARS). *N*,*N*-Dimethylformamide decreased the hepatic GSH content to 61% of control values (Kim et al., 2010).

Malondialdehyde (MDA) levels (measured as TBARS) increased four- to ninefold in the liver, and four- to sevenfold in the kidney of male Wistar rats, 24 and 48 hours after intraperitoneal injection with a single dose of *N*,*N*-dimethylformamide (1.5 g/kg bw). The activities of the hepatic antioxidant enzymes decreased at both time-points: SOD (~50%), catalase (~50%), and glutathione peroxidase (~58%). The hepatic levels of reduced GSH and vitamin C also decreased 55% and ~62%, respectively. The levels of SOD, catalase, glutathione peroxidase, reduced GSH, and vitamin C each decreased 40–60% in the kidney (Jyothi et al., 2012). In a spectroscopic and modelling study, Kalyani et al. (2014) reported a destabilization of SOD, which might explain the decreases in activity reported by Jyothi et al. (2012).

ICR mice (both sexes) treated with *N*,*N*-dimethylformamide daily by gavage for 90 days had significant increases in MDA levels measured as TBARS in heart homogenates and in liver homogenates (at the 0.32, 0.63, and 1.26 g/kg bw dose levels) compared with controls. SOD activity levels decreased significantly in heart homogenates and liver homogenates (at the 0.63 and 1.26 g/kg bw dose levels) compared with controls. GSH levels decreased only in liver homogenates at 0.32, 0.63, and 1.26 g/kg bw (Rui et al., 2011).

Male Sprague-Dawley rats were dosed with *N*-methylformamide or *N*,*N*-dimethylformamide by intraperitoneal injection (1 mL/kg bw). Only *N*-methylformamide depleted the hepatic GSH level by about 30% 1 hour after injection. Twenty-four hours after the treatment, the GSH level returned to the control value (Scailteur & Lauwerys, 1984).

GSH levels were measured in liver, kidney, heart, lung and spleen tissues from male CBA/CA mice dosed with single doses of *N*-methylformamide (400 mg/kg bw), *N*-ethylformamide (495 mg/kg bw) or formamide (dose not reported) after 1 hour. *N*-Methylformamide, but not *N*-ethylformamide or formamide, decreased hepatic GSH levels by 59.8%. Of the other tissues investigated only the kidneys exhibited GSH depletion (21.6%). In a dose–response study, hepatic GSH levels in male BALB/c mice decreased with increasing dosage of *N*-methylformamide (0–400 mg/kg bw) 1 hour after intra-

peritoneal injection of *N*-methylformamide (Gescher et al., 1982).

N-Methylformamide (200 mg/kg bw) injected intraperitoneally into BALB/c mice depleted total hepatic GSH to 21% of control levels 2 hours after administration and induced hepatotoxicity. In CBA/CA and BDF1 mice, the same dose of *N*-methylformamide depleted total hepatic GSH levels to 53% of control levels and did not cause hepatotoxicity (Pearson et al., 1987).

N-Methylformamide treatment of male BALB/c mice (single intraperitoneal injection of 50–200 mg/kg bw) induced a dose-dependent decrease in hepatic non-protein sulfhydryls (reduced GSH is the major non-protein sulfhydryl in cells). Higher doses (up to 800 mg/kg bw) did not induce a further decline. A time-course study revealed that 4 hours after a dose of 300 mg/kg bw, non-protein sulfhydryls were reduced to 25% of control values. *N*-Methylformamide did not significantly decrease the levels of hepatic non-protein sulfhydryls in male Sprague-Dawley rats 4 hours after an intraperitoneal injection of 1000 mg/kg bw (Tulip & Timbrell, 1988).

Changes in gene expression were examined in the livers of male BALB/c mice dosed with a single intraperitoneal injection (300 mg/kg bw) of *N*-methylformamide. Comparison of the gene expression patterns of *N*-methylformamide versus saline control identified a series of significantly altered genes associated with oxidative stress (e.g. upregulation of aldehyde oxidase 1 *Aox1,* heat shock protein *Hspb1, Hspa8,* and *Hsp105*) (Mutlib et al., 2006).

N,*N*-dimethylformamide induced CYP2E1 protein levels in liver microsomes from male Sprague Dawley rats after intraperitoneal injection of *N*,*N*-dimethylformamide at 0, 450, 900 or 1800 mg/kg bw once per day for 3 days. The activities of *p*-nitrophenylhydroxylase (a measure of CYP2E1 enzymatic activity) in the *N*,*N*-dimethylformamide-treated groups were significantly higher than that of the control group after 3 days of treatment. Under the same experimental conditions there were no significant increases in CYP1A1 or CYP2B1/2 proteins or increases in their associated enzymes ethoxyresorufin-*O*-deethylase (EROD) or pentoxyresorufin-*O*-dealkylase (PROD) activities, respectively (Koh et al., 1999). The effects of *N*,*N*-dimethylformamide on the induction of CYP2E1 protein levels, *p*-nitrophenylhydroxylase, glutathione *S*-transferase and glutathione peroxidase activities were studied in the livers of male Sprague-Dawley rats after intraperitoneal injection of *N*,*N*-dimethylformamide at 0, 450, 900, or 1800 mg/kg bw once per day for 3 days. The levels of CYP2E1 analysed by Western immunoblot increased and *p*-nitrophenylhydroxylase activity increased ~3.7-fold after 3 days of treatment. Glutathione *S*-transferase levels increased slightly with *N*,*N*-dimethylformamide treatment while *N*,*N*-dimethylformamide had no effect on glutathione peroxidase levels (Kim & Chung, 2013). In another report, CYP2E1 protein was induced ~1.5-fold in liver homogenates from rats treated with *N*,*N*-dimethylformamide (500 mg/kg bw daily for 3 days) (Kim & Kim, 2011). It has been reported that CYP2E1 induction can lead to the over production of ROS and oxidative stress (Caro & Cederbaum, 2004; Gonzalez, 2005). [A possible explanation for the ability of *N*,*N*-dimethylformamide to induce hepatotoxicity is the production of ROS by *N*,*N*-dimethylformamide through CYP2E1 induction and uncoupling of the catalytic cycle (release or escape of the superoxide anion radical during the course of the P450 catalytic cycle) (Gonzalez, 2005) combined with *N*,*N*-dimethylformamide and the depletion of GSH by *N*-methylformamide.]

(ii) In vitro

N-Methylformamide decreased total intracellular GSH levels in murine TLX5 lymphoma cells in a concentration-related fashion up to 160 mM after a 48-hour incubation (Bill et al., 1988)

(c) Non-mammalian systems

N,*N*-Dimethylformamide induced oxidative stress in the proteobacteria, *Paracoccus* sp. SKG. Glutathione *S*-transferase, catalase, and SOD activities increased in a concentration–response manner. Cellular levels of methionine as well as protein levels of the enzyme methionine sulfoxide reductase, which catalyses the reduction of both free and protein-bound methionine sulfoxide residues back to methionine (Ezraty et al., 2005) were increased (Kirankumar et al., 2014).

[The Working Group noted that both *N*,*N*-dimethylformamide and *N*-methylformamide have been shown to alter indices of oxidative stress. *N*,*N*-Dimethylformamide induced ROS in human liver cells and increased malondialdehyde levels in the livers of mice and rats. *N*,*N*-dimethylformamide altered the levels of glutathione peroxidase and SOD in human tumour cells and lowered the levels of hepatic GSH in the livers of mice and rats. *N*-Methylformamide reduced the levels of GSH in tumour cells and in the livers of mice and rats and increased the expression of genes associated with oxidative stress in the livers of treated mice.]

4.2.2 Altered cell proliferation or death

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human tumour cells implanted into experimental animals

N,*N*-Dimethylformamide and *N*-methylformamide inhibited the growth of two human colon cancer cell lines xenografted into female nude mice. Mice received subcutaneous transplants of HCT-15 or DLD-2 human colon cancer cells and were treated intraperitoneally with *N*,*N*-dimethylformamide or *N*-methylformamide, 2219 and 374 mg/kg bw given daily for 21 days, respectively. HCT-15 tumour growth was inhibited 65% by *N*,*N*-dimethylformamide compared with controls. In two independent experiments with *N*,*N*-dimethylformamide, DLD-2 tumour growth was inhibited by 45 and 67%. *N*-Methylformamide treatment produced 43 and 75% growth inhibition of HCT-15 and DLD-2 tumours, respectively (Dexter et al., 1982). Nude mice (15 mice per group) were subcutaneously injected with SHG-44 human glioma cells grown in the absence or presence of *N*,*N*-dimethylformamide. Pretreatment of SHG-44 cells with *N*,*N*-dimethylformamide reduced the growth rate of the xenografts (Li et al., 1997).

CD-1 male nude mice (eight mice per group) were injected subcutaneously with human colon carcinoma HT-29 cells or HT-29 cells grown in medium containing 1% (170 mM) *N*-methylformamide (designated HT29-NMF cells) inducing cell differentiation. HT29-NMF cells had an increased latency period in the appearance of palpable tumours compared with HT-29 cells and tumour weights were lower in mice carrying the HT29-NMF cell xenographs compared with mice carrying the HT29 cell xenographs, indicating slower growth induced by *N*-methylformamide (D'Agnano et al., 1992).

(iii) Human cells in vitro

N,*N*-Dimethylformamide has been shown to inhibit the growth of human tumour cells in culture in many studies.

The growth of RKO colon carcinoma cells (Brattain et al., 1984) treated with *N*,*N*-dimethylformamide (0.7%) resulted in an 88% reduction in cell number compared with untreated control cells (Zipfel et al., 1993). Similarly, cell proliferation of MOSER human colon carcinoma cells, transforming growth factor-β (TGF-β)-resistant MOSER human colon carcinoma cells and HCT 116 human colon carcinoma cells was reduced by 1%

N,*N*-dimethylformamide; there was essentially minimal cell growth for each cell line (Mulder et al., 1988).

DLD-1 and HCT-15 human colon carcinoma cell lines and several subclones of DLD-1 cells (Clone A, Clone D) were treated with *N*,*N*-dimethylformamide (0.8%) which induced morphological changes in the cells and alterations in their growth properties. *N*,*N*-Dimethylformamide also induced differentiation and maturation to a less malignant phenotype (Dexter et al., 1979; Dexter & Hager, 1980). Growth in the presence of *N*,*N*-dimethylformamide also caused a marked reduction in the tumorigenicity of the cells in vivo. While 20 of 20 nude mice injected with DLD-1 cells developed tumours in 10–14 days, only one nude mouse out of ten inoculated with *N*,*N*-dimethylformamide-treated DLD-1 cells developed a tumour in the third month after the injection (Dexter et al., 1979; Dexter & Hager, 1980).

N-Methylformamide increased the doubling times of DLD-1 Clone A cells in culture in a concentration–response fashion (Dibner et al., 1985). *N*-Methylformamide (1%, 170 mM) treatment of human HT-29 colon carcinoma cells induced cell differentiation and reduced cell proliferation. *N*-Methylformamide treatment induced a G_0/G_1 phase accumulation, with a higher percentage of treated cells in G_0/G_1 phase compared with controls (D'Agnano et al., 1992). *N*,*N*-Dimethylformamide (0.25, 0.5, 0.75, and 1%) produced a concentration-dependent inhibitory effect on cell proliferation in monolayers of human glioma SHG-44 cells Li et al. (1997). The percentage of DNA fragmentation and apoptotic nuclei increased in cultures of human colonic carcinoma HT-29 cells after treatment with *N*,*N*-dimethylformamide (1%) for 72 hours (Heerdt et al., 1996).

The effects of *N*,*N*-dimethylformamide on hyperthermia-induced apoptosis, DNA fragmentation and cell cycle effects were studied in PLB-985 human myelomonoblastic leukaemia cells. Hyperthermia (42 or 43 °C, 1 hour) induced apoptosis and DNA fragmentation in PLB-985 cells and led to a decrease in the number of G_0/G_1 cells as determined by flow cytometry. *N*,*N*-Dimethylformamide (0.5%) treatment of these cells caused differentiation along the granulocytic pathway and prevented the induction of apoptotic death (Katschinski et al., 1999).

In the normal human liver cell line, HL-7702, *N*,*N*-dimethylformamide (0, 50, 100, 150, and 200 mM) for 12 hours increased the apoptotic rate of hepatocytes in a concentration-related manner as measured by flow cytometry. *N*,*N*-Dimethylformamide induced a steady decrease of the expression of the anti-apoptotic Bcl-2 protein shown by Western blotting and its level was lower than the control group. The expression of the pro-apoptotic Bax protein showed no significant differences between the different *N*,*N*-dimethylformamide concentration groups. Increasing the *N*,*N*-dimethylformamide concentration decreased the ratio of Bcl-2/Bax; at 200 mM this ratio was significantly lower than that of the controls. Procaspase-3 protein was observed at 150 and 200 mM, which demonstrated that there was active caspase-3 (Lu et al., 2008).

(b) Non-human mammalian systems

(i) In vivo

Dose related increases in cell proliferation were seen in male F344/DuCrlCrj rats (SPF) exposed to *N*,*N*-dimethylformamide by both inhalation (0, 200, or 400 ppm for 6 hours per day, 5 days per week, for 4 weeks) and in drinking-water (0, 800, 1600, or 3200 ppm for 24 hours per day, 7 days per week, for 4 weeks). Maximal induction up to 9.7-fold was reported at the 200 ppm inhalation/3200 ppm oral dose level. Rats treated with *N*,*N*-dimethylformamide only by inhalation or only by oral dosing also showed

modest dose-related increases in cell proliferation (Ohbayashi et al., 2008).

The effects of *N*,*N*-dimethylformamide on several hepatic enzymes associated with apoptosis were investigated in Sprague-Dawley rats after intraperitoneal injections of 500 mg/kg bw per day for 3 consecutive days. *N*,*N*-Dimethylformamide induced a marginal increase in the ratio of the protein levels of Bax to Bcl-xL (an apoptosis inhibitor), and had no effects on poly (ADP-ribose) polymerase (PARP) cleavage or caspase-3 activity (Kim et al., 2010).

Fourteen of 17 CE/J mice receiving intraperitoneal injections of murine rhabdomyosarcoma cells (pre-cultured in media containing *N*,*N*-dimethylformamide (1%) for 10 days) did not develop tumours after 6 months. This was compared with 21 mice receiving intraperitoneal injections of untreated rhabdomyosarcoma cells, which died of tumour between 11 and 31 days (Dexter, 1977).

The growth of murine M5076 ovarian sarcoma cells injected intramuscularly into female BDF_1 mice was inhibited by daily intraperitoneal injections of *N*-methylformamide (25, 50, 100, or 200 mg/kg bw) for 17 days. In similar types of studies, formamide (200 mg/kg bw) or *N*-methylformamide (200 mg/kg bw) administered to female BDF_1 mice daily during the course of 17 days markedly reduced the tumour volume of M5076 tumours. Daily dosing with *N*-methylformamide (300 mg/kg bw) or formamide (300 mg/kg bw) for 9 days by intraperitoneal injections reduced murine sarcoma 180 tumour volume in female BDF_1 mice injected with this tumour (Gescher et al., 1982).

N-Methylformamide (300 mg/kg bw) administered intraperitoneally daily for 6 days slowed the growth of FSA fibrosarcoma, and HCA-I hepatocarcinoma tumour xenographs and totally inhibited the growth of the MCA-K mammary carcinoma tumour xenographs in CH_3Hf/Kam mice. However, the effects were transient; tumours resumed their pretreatment growth rate upon cessation of the treatment (Iwakawa et al., 1987b). In a similar study, CH_3Hf/Kam mice were injected intramuscularly with a single-cell suspension of the FSA fibrosarcoma. Treatment with *N*-methylformamide (300 mg/kg bw) only slightly inhibited the tumour growth when administered for 8 days (Iwakawa et al., 1987a).

Changes in gene expression associated with cell proliferation were reported in the livers of male BALB/c mice dosed with a single intraperitoneal injection (300 mg/kg bw) of *N*-methylformamide (Mutlib et al., 2006).

(ii) In vitro

N,*N*-Dimethylformamide (1%, 170 mM) decreased the growth of both murine rhabdomyosarcoma cells (Dexter, 1977), AKR-2B mouse embryo fibroblast cells and their methylcholanthrene-transformed counterpart AKR-MCA cells in culture (Hoosein et al., 1988).

N-Methylformamide (43 to 170 mM) decreased the growth of TLX5 murine lymphoma cells and changed the distribution of these cells in the cell cycle determined by flow cytofluorimetry, increasing the proportion of G_1 cells and reducing the proportion of cells in the S and G_2/M phases. *N*-Methylformamide (0–160 mM) also inhibited the ability of TLX5 cells to grow in soft agar (Bill et al., 1988).

N-Methylformamide (0.5 or 1%) reduced the clonogenicity of MCA-K (mammary carcinoma), but not FSA (fibrosarcoma) or HCA-I cells (hepatocarcinoma) in vitro (Iwakawa et al., 1987b).

One study reported that *N*,*N*-dimethylformamide increased apoptosis. Treatment of immortalized Rat-1 fibroblasts in monolayer with *N*,*N*-dimethylformamide at 50–175 mM for 24 hours induced cell death by apoptosis with early apoptotic changes observed in the cells, cell shrinkage with nuclear condensation, and the formation of membrane and DNA fragmentation (Boyle & Hickman, 1997).

[The Working Group noted that *N*,*N*-dimethylformamide and *N*-methylformamide have been used in preclinical cancer chemotherapeutic trials. Experimental studies showed that *N*,*N*-dimethylformamide and *N*-methylformamide inhibited the growth of cancer cells in culture, and cancer cell lines implanted into mice. In these studies, cancer cells were cultured in the presence of *N*,*N*-dimethylformamide or *N*-methylformamide and injected into mice, or mice carrying cancer xenographs were dosed with *N*,*N*-dimethylformamide or *N*-methylformamide. *N*,*N*-Dimethylformamide also altered the malignant phenotype of cancer cells and reduced cell growth in vitro. One study in rats showed that *N*,*N*-dimethylformamide increased hepatic cell proliferation. *N*,*N*-Dimethylformamide increased apoptosis in tumour cells and in a normal human liver cell line.]

4.2.3 Genetic and related effects

(a) Humans

(i) Exposed humans

See Table 4.1

In the analytical study of Hennebrüder & Angerer (2005), urine samples taken from male workers exposed to *N*,*N*-dimethylformamide vapours (exposure level not given) were analysed by a sensitive LC/MS method for DNA adducts. N^4-(*N*-methylcarbamoyl)cytosine (N4-NMCC), a DNA adduct, which was already known to be formed from MIC (Segal et al., 1989), a probable metabolic intermediate of *N*,*N*-dimethylformamide, was detected. In 10/32 urine samples collected from occupationally exposed subjects N4-NMCC was detected in concentrations of 31–172 ng/L (detection limit was 8 ng/L). No N4-NMCC was detected in urine samples taken from 24 subjects with no record of *N*,*N*-dimethylformamide exposure (Hennebrüder & Angerer, 2005).

Significant differences in the comet extent, tail extent, Olive tail moment, and tail DNA/total DNA (%) were reported by Chen (2004) in workers who were occupationally exposed to *N*,*N*-dimethylformamide (6–7 years of exposure) as compared with controls ($P < 0.05$).

Changes in mitochondrial DNA in blood leukocytes, namely, a common deletion $\Delta mtDNA^{4977}$ and mtDNA copy numbers, were reported in a study on 13 male synthetic-leather factory workers. Exposure to *N*,*N*-dimethylformamide was followed by air sampling (median, 10.59 ppm; range, 6.65–34.38 ppm), as well as by urine analysis for the biomarkers "NMF" (actually HMMF+NMF; median, 13.77 mg/L; range, 7.47–73.64 mg/L) and AMCC (median, 40.70 mg/L; range, 6.76–442.24 mg/L). The values found in subjects exposed to *N*,*N*-dimethylformamide were significantly higher than those in controls, which were matched by age, seniority, smoking, and alcohol drinking habits. Moreover, both parameters appeared to be exposure-dependent when subjects exposed to *N*,*N*-dimethylformamide at concentrations higher and lower than 10 ppm (permissible exposure limit) were compared. The frequencies of $\Delta mtDNA^{4977}$ were significantly elevated also in subjects with high urinary levels of AMCC (≥ 40 mg/L). On the other hand elevated mtDNA copy number was found in subjects with relatively high urinary "NMF" concentrations (≥ 15 mg/L) considered as a short-term marker of exposure to *N*,*N*-dimethylformamide (Shieh et al., 2007).

Rates of sister-chromatid exchange were determined in peripheral blood lymphocytes of 22 non-smoking women exposed to *N*,*N*-dimethylformamide working in a synthetic-leather manufacturing plant for 1.1–9.9 years. The exposed group was divided into three subgroups according to the exposure level, which was 0.3, 0.7, and 5.8 ppm (time-weighted average, TWA) in the low, intermediate, and high exposure group, respectively. A co-exposure by low

Table 4.1 Genetic and related effects of *N,N*-dimethylformamide in exposed humans

Biological sample	Description of exposed and controls	End-point	Test	Results	Agent, dose (LED or HID)	Comments	Reference
Urine	32 male workers in acrylic fibre industry and 24 control from general population	DNA damage	N^4-(*N*-methylcarbamoyl) cytosine by LC-ESI-MS/MS	+	DMF		Hennebrüder & Angerer (2005)
Peripheral blood cells	Workers with 6–7 yrs of exposure to DMF	DNA damage	DNA strand breaks (comet assay)	+ ($P < 0.05$)	DMF		Chen (2004)
Leukocytes	13 male synthetic leather factory workers; 13 control subjects from the administrative department of the same factory	DNA damage	End-point deletion ΔmtDNA^{4977}, mitochondrial mtDNA copy number	+	DMF	Smoking taken into account	Shieh et al. (2007)
Lymphocytes	22 non-smoking women; synthetic leather production, 3 exposure groups; 22 sex-, age- and residence-matched controls	Chromosomal damage	Sister-chromatid exchange	+ ($P < 0.01$)	DMF + toluene, 0.7 ppm (TWA)	Exposure-related effect; co-exposure with toluene in the intermediate exposure group	Seiji et al. (1992)
Lymphocytes	26 male viscose rayon plant workers; 26 matched controls and 6 industrial controls (administrative workers)	DNA and chromosomal damage	UDS, chromosomal aberrations, aneuploidy, sister-chromatid exchange	(+) ($P < 0.01$)	DMF + acetonitrile	Effect of smoking separated; effects of DMF and acrylonitrile not separated [synergism is likely]	Major et al. (1998)
Lymphocytes	40 occupationally exposed workers, 31 unexposed persons	Chromosomal damage	Chromosomal aberrations	(+) $P < 0.05$	DMF, 150 mg/m^3 (TWA)	Smoking not reported	Koudela & Spazier (1981)
Lymphocytes	56 workers in printed circuit-board manufacture; no unexposed controls	Chromosomal damage	Sister-chromatid exchange	(–)	DMF + epichlorohydrin, 33.6 ± 3.9 ppm, long-term	No association with DMF exposure but strong association with exposure to epichlorohydrin	Cheng et al. (1999)
Lymphocytes	29 workers synthetic leather manufacture; no unexposed controls	Chromosomal damage	Sister-chromatid exchange	(–)	DMF, 34.6 ± 6.3 ppm, long-term	Smoking and GST genotypes taken into account	Cheng et al. (1999)

+, positive; (+) or (–), positive or negative in a study with limited quality; DMF, *N,N*-dimethylformamide; GST, glutathione *S*-transferases; HID, highest ineffective dose; LC-ESI-MS/MS, liquid chromatography and electrospray ionization mass spectrometry; LED, lowest effective dose; NMF, *N*-methylformamide; TWA, time-weighted average; UDS, unscheduled DNA synthesis; yr, year

concentrations of toluene (0.9 ppm, TWA) was reported for the intermediate exposure group but not for other groups. Age-, sex-, and residence-matched controls from the same factory (administrative staff) were used. A dose-dependent increase in sister-chromatid exchanges was found in the exposed group (rates of 8.26, 7.23, and 5.67 for the high, intermediate, and low exposure subgroup, respectively) and the mean of exposed group was significantly higher than that of controls ($P < 0.01$). However, the mean sister-chromatid exchange rate value in the low exposure group appeared to be lower than that in controls ($P < 0.05$) (Seiji et al., 1992).

Two other field studies in humans exposed to *N*,*N*-dimethylformamide in vivo found increases in chromosomal aberrations (Major et al., 1998; Koudela & Spazier, 1981), aneuploidy, sister-chromatid exchange, and unscheduled DNA synthesis (Major et al., 1998). [The Working Group noted that the genotoxic effects observed may not be related to *N*,*N*-dimethylformamide exposure because of co-exposure with high concentrations of acrylonitrile (Major et al. 1998), and because no data were available on smoking and co-exposure with numerous other chemicals occurring at the workplace (Koudela & Spazier, 1981).]

Cheng et al. (1999) studied sister-chromatid exchange in peripheral blood lymphocytes of 85 plant workers occupationally exposed to *N*,*N*-dimethylformamide and/or epichlorohydrine. Airborne *N*,*N*-dimethylformamide concentrations during the work-shifts ranged from 1 to 83 ppm (TWA). An association between sister-chromatid exchange frequencies and epichlorohydrin exposure levels, but not *N*,*N*-dimethylformamide exposure levels, was found. No unexposed control group was used and the exposure history of the subjects studied was not reported.

(ii) Human cells in vitro

See Table 4.2

In vitro, *N*,*N*-dimethylformamide gave negative results in a human hepatocyte primary culture DNA repair assay based on autoradiographic detection of unscheduled DNA synthesis at concentrations up to 10 mM (McQueen et al., 1988).

Positive results were reported when phosphorylated histone H2AX (γH2AX) was used as a biomarker of double-strand breaks in human liver cell DNA treated with *N*,*N*-dimethylformamide (1.5–100 mM) (Xuan et al., 2008; Wang et al., 2015).

The levels of 8-hydroxy-2′-deoxyguanosine were increased 1.5-fold in human liver cells HL-7702 exposed to *N*,*N*-dimethylformamide at 40 and 100 mM (Wang et al., 2015).

Negative results for *N*,*N*-dimethylformamide (up to 10 mM, 731 μg/mL) were obtained in a study of genotoxic insult evaluated by GreenScreen HC *GADD45α-GFP* genotoxicity assay (Hastwell et al., 2006).

No increase in the frequency of sister-chromatid exchange was observed in human lymphocytes incubated in vitro with *N*,*N*-dimethylformamide at concentrations up to 1.1 M for 24, 48, or 72 hours (Antoine et al., 1983).

(b) Experimental systems

See Table 4.3

Studies on genetic and related effects in various experimental systems in vivo (e.g. rats, mice, and Syrian hamsters) and in vitro (including human lymphocytes, rat and mouse hepatocytes, mouse lymphoma cells, Chinese hamster ovary cells, yeast, *Salmonella typhimurium*, and *Escherichia coli*) were comprehensively reviewed in a previous *IARC Monograph*, when the data on *N*,*N*-dimethylformamide were evaluated by the Working Group in 1999. At the time, the results were mostly negative or inconclusive, and did not provide evidence supporting a genotoxic mechanism (IARC, 1999). For instance, *N*,*N*-dimethylformamide gave negative results

Table 4.2 Genetic and related effects of *N,N*-dimethylformamide in human cells in vitro

Species	Cell type	End-point	Test	Results	Dose (LED or HID)	Reference
Human	Hepatocytes	DNA damage	UDS	–	10 mM [731 µg/mL]	McQueen et al. (1988)
Human	Hepatocytes	DNA damage	γH2AX	+	1.56 mM [114 µg/mL]	Xuan et al. (2008)
Human	Liver HL-7702 cells	DNA damage	γH2AX	+	6.4 mM [468 µg/mL]	Wang et al. (2015)
Human	Liver HL-7702 cells	DNA damage	8-OHdG (ELISA)	+	40 mM [2924 µg/mL]	Wang et al. (2015)
Human	Lymphoblastoid TK6 cell line	DNA damage	*GADD45α-GFP* assay	–	10 mM [731 µg/mL]	Hastwell et al. (2006)
Human	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	–	1.1 M [80 400 µg/mL]	Antoine et al. (1983)

+, positive; –, negative; ELISA, enzyme-linked immunosorbent assay; *GADD45α*, growth arrest and DNA damage-inducible 45α gene; GFP, green fluorescent protein; HID, highest ineffective dose; LED, lowest effective dose; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; UDS, unscheduled DNA synthesis

for micronuclei in mice (Antoine et al., 1983) and in the white/white$^+$ eye mosaic test in Drosophila (Vogel & Nivard, 1993).

Recent studies of genotoxicity in vitro have shown predominantly negative results. *N,N*-Dimethylformamide administered by gavage at the dose levels of 50, 100, and 200 mg/kg bw once per day for 14 consecutive days to rats significantly increased lymphocyte comet tail, average tail length, and tail moment (Xing et al., 2014). However, negative results for *N,N*-dimethylformamide at concentrations up to 257 mM were obtained in an *RAD54-GFP* assay on yeast (*Saccharomyces cerevisiae*). *RAD54* is a DNA repair gene induced by a variety of DNA lesions above the constitutive level (Knight et al., 2007). *N,N*-Dimethylformamide (4% v/v) was also not mutagenic in the *umu*-test on *Salmonella typhimurium* TA1535 (Degirmenci et al., 2000).

In a study on the combined action of *N,N*-dimethylformamide and hydrogen peroxide in the presence of Cu(II), *N,N*-dimethylformamide (0.5–4%) markedly enhanced DNA strand breaks and 8-hydroxy-2′-deoxyguanosine induced by 50 µM H_2O_2 and 20 µM Cu(II) (Midorikawa et al., 2000).

Formaldehyde, a necessary product of *N,N*-dimethylformamide demethylation, and MIC, a likely metabolic intermediate of *N,N*-dimethylformamide, can also cause chromosomal damage (Goswami, 1986; Tice et al., 1987; IARC, 2006).

4.2.4 Receptor-mediated effects

(a) Humans

Sperm motility was reduced in 12 workers exposed to *N,N*-dimethylformamide in a synthetic-leather factory compared with 8 non-exposed sociodemographically matched controls (Chang et al., 2004a). Sperm motility was related to urinary *N*-methylformamide level, but not airborne *N,N*-dimethylformamide.

In occupationally exposed workers, effects of *N,N*-dimethylformamide on coagulation were probably related to change on the membrane receptor of platelets and to a modification in phospholipid components. A follow-up experiment in vitro with platelets indicated that effects on aggregation were most likely induced by epinephrine and adenosine diphosphate (Imbriani et al., 1986).

Table 4.3 Genetic and related effects of *N*,*N*-dimethylformamide in experimental systems

Species, strain (sex), tissue	End-point	Test	Results	Dose (LED or HID)	Reference
Rat, Wistar (F), lymphocytes	DNA damage	DNA strand breaks (comet)	+	50 mg/kg bw gavage; 1 ×/day, 14 days	Xing et al. (2014)
Mouse Balb/C (M), bone marrow	Chromosomal damage	Micronuclei	–	2000 mg/kg bw i.p.; dosing 30 h after treatment	Antoine et al. (1983)
Drosophila, Cross C-1, strains y and w	Mutation	Somatic mutation and recombination test (SMART)	–	10 mM [731 µg/mL]	Vogel & Nivard (1993)
Saccharomyces cerevisiae GenT01/GenC01 (control strain)	DNA damage	*RAD54-GFP* assay	–	257 mM [18 800 µg/mL]	Knight et al. (2007)
Salmonella typhimurium TA1535	DNA damage	*umu*-test	NT	4% (v/v), tested with (but not without) metabolic activation	Degirmenci et al. (2000)

+, positive; –, negative; bw, body weight; GFP, green fluorescent protein; F, female; HID, highest ineffective dose; i.p., intraperitoneal; M, male; NT, not tested; *RAD54*, gene coding for a DNA repair/recombination protein; v/v, volume per volume

In human colon carcinoma and other cell lines, *N*,*N*-dimethylformamide stimulated mitogenesis via the epidermal growth factor (EGF) receptor and increased TGF-β receptors, with results variable across cell type (Levine et al., 1985a, b; Levine et al., 1989; Levine & Chakrabarty, 1992). *N*,*N*-dimethylformamide also significantly induced the number of urokinase receptors in a human colon carcinoma cell line (Boyd et al., 1988).

(b) Experimental systems

A 13-week toxicity study in female and male cynomolgus monkeys treated with *N*,*N*-dimethylformamide at 30–500 ppm by inhalation showed a slight trend towards increasing estrous cycle (Hurtt et al., 1992).

Some experimental studies did report receptor perturbations at relatively high (in the range of 7 to 15%) dose levels. While using *N*,*N*-dimethylformamide to increase the solubility of estrogen receptor (ER) agonists, it was observed that *N*,*N*-dimethylformamide was able to interfere with ER-binding kinetics and could cause receptor inactivation on the binding site directly or the ER-estradiol complex (Sasson & Notides, 1988). In addition, *N*,*N*-dimethylformamide was also able to act as a weak ER agonist in fish (Ren et al., 1996). *N*,*N*-Dimethylformamide also influenced the sex ratio and the gonadosomatic index in rainbow trout (van den Hurk & Slof, 1981).

4.2.5 Other mechanisms

In the polyclonal human ovarian adenocarcinoma cell line HOC-7, 0.5% *N*,*N*-dimethylformamide prolonged the cell doubling time, downregulated c-Myc protein expression and caused development of the HOC-7 cells to a less malignant cell phenotype (Somay et al., 1992). In addition, *N*,*N*-dimethyl-formamide has also been found to induce the EGF receptor, but the overall inhibitory effect on c-Myc expression by *N*,*N*-dimethylformamide has been suggested to dominate its eventual anti-proliferative effect in these HOC-7 cells (Grunt et al., 1993).

4.3 Data relevant to comparisons across agents and end-points

For all compounds evaluated in the present volume, including *N*,*N*-dimethylformamide, analyses of high-throughput screening data generated by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) are presented in the *Monograph* on 1-bromopropane in the present volume.

4.4 Susceptibility to cancer

No studies with cancer as an outcome that investigated susceptibility were available to the Working Group.

There were susceptibility studies concerning other end-points. A study showed that workers with *GSTT1* null genotype are more susceptible to *N*,*N*-dimethylformamide exposure-associated abnormal liver function (Luo et al., 2005). Two other studies showed no associations between polymorphism of *CYP2E1* and the levels (Nomiyama et al., 2001b) or half-lives (Nomiyama et al., 2001c) of urinary *N*-methylformamide. A total of 66 male workers exposed to *N*,*N*-dimethylformamide were investigated (Luo et al., 2005). Compared with the *GSTT1*-positive genotype workers, the adjusted odds ratio and 95% confidence intervals for abnormal liver function test were 4.41 (95% CI, 1.15–16.9) for the *GSTT1* null genotype workers. Compared with the *N*,*N*-dimethylformamide low-exposure group with *GSTT1*-positive genotype workers, the odds ratio (adjusted for hepatitis B virus (HBV) status) of abnormal liver function test was 12.38 (95% CI, 1.04–146.9) for the *N*,*N*-dimethylformamide high-exposure group with *GSTT1*-null genotype workers. Multiplicative interactions between *N*,*N*-dimethylformamide exposure and *GSTT1* genotype were demonstrated.

There was no significant difference between slopes of single linear regression model for *N*,*N*-dimethylformamide and *N*-methylformaldehyde in *1C homozygotes and *1D-hetero- and -homozygotes, in a study of the *1C/*1D CYP2E1 polymorphism in 20 male and 24 female workers (Nomiyama et al., 2001b).

A total of 123 workers exposed to *N*,*N*-dimethylformamide were investigated for genotypes of *CYP2E1* and half-lives of urinary *N*-methylformamide (Nomiyama et al., 2001c). The workers comprised 77 c1 homozygotes, 45 c2 heterozygotes, and 1 c2 homozygotes. The half-lives of c1 homozygotes were not significantly different from those of the c2 heterozygotes and there were no differences between *N*-methylformamide half-lives for subjects with or without the c2 allele.

4.5 Other adverse effects

Occupational exposure to *N*,*N*-dimethylformamide induced liver injury in exposed workers (Wrbitzky, 1999; Wang et al., 2014). Exposure to *N*,*N*-dimethylformamide induced various symptoms, such as abdominal pain, nausea, vomiting or diarrhoea, as well as jaundice and disulfiram-type reaction (Potter, 1973; Fiorito et al., 1997). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (ALT > AST generally) and γ-glutamyl transpeptidase were increased (Potter, 1973; Nomiyama et al., 2001d). HBV carrier status and increased body mass index had synergistic effects with *N*,*N*-dimethylformamide exposure in causing liver abnormalities (Luo et al., 2001). A patient who was exposed to *N*,*N*-dimethylformamide by a suicide attempt showed elevation in AST, ALT, and total bilirubin and decrease in prothrombin time (Buylaert et al., 1996).

In experimental systems, there were many studies in rats (e.g. Tanaka, 1971; Kim & Chung, 2013), mice (e.g. Craig et al., 1984; Rui et al., 2011), hamsters (Ungar et al., 1976), cats or rabbits (Massmann, 1956) exposed to

N,*N*-dimethylformamide by inhalation (Craig et al., 1984; Senoh et al., 2003), inhalation and in drinking-water (Ohbayashi et al., 2008), gavage (Rui et al., 2011), and intraperitoneal injection (Ungar et al., 1976; Kim & Chung, 2013), which showed hepatotoxicity (Malley et al., 1994; Senoh et al., 2003). The main histopathological changes were centrilobular hepatocellular necrosis (Ungar et al., 1976; Kim & Chung, 2013), centrilobular hepatocellular hypertrophy (Tanaka, 1971; Rui et al., 2011), massive necrosis associated with centrilobular fibrosis (Senoh et al., 2003), focal necrosis (Senoh et al., 2003), centrilobular single cell necrosis (Malley et al., 1994; Senoh et al., 2003), and lipofuscin/hemosiderin accumulation in Kupffer cells (Malley et al., 1994). Serum sorbitol dehydrogenase (Malley et al., 1994; Lynch et al., 2003), ALT (Tanaka, 1971; Rui et al., 2011), AST (Tanaka, 1971; Rui et al., 2011), lactate dehydrogenase (Tanaka, 1971; Rui et al., 2011), cholesterol (Lynch et al., 2003; Senoh et al., 2003), phospholipid (Senoh et al., 2003), and total bile acids (Lynch et al., 2003) were increased.

In rodents, exposure to *N*,*N*-dimethylformamide by inhalation (Kimmerle & Machemer, 1975), gavage (Saillenfait et al., 1997), in drinking-water (Fail et al., 1998), or by epicutaneous administration (Hansen & Meyer, 1990) reduced fertility (Hansen & Meyer, 1990), and the number of fetuses (Hansen & Meyer, 1990), decreased maternal weight (Hansen & Meyer, 1990; Saillenfait et al., 1997) and fetal weight (Hansen & Meyer, 1990; Saillenfait et al., 1997) as well as inducing skeletal variations (Saillenfait et al., 1997) or malformations (Fail et al., 1998).

5. Summary of Data Reported

5.1 Exposure data

N,*N*-Dimethylformamide is a chemical with a high production volume that is mainly used in the manufacture of acrylic fibres, and in the synthetic-leather industry. *N*,*N*-Dimethylformamide is also used as a solvent in the electronics industry, in pesticide formulations, and as a component of paint strippers. Exposures of the general population have been reported in China among residents living near synthetic-leather factories. *N*,*N*-Dimethylformamide has been detected in the air in the vicinity of a waste site in the USA, and in environmental samples of air and water in Japan. Occupational exposures to *N*,*N*-dimethylformamide have been measured among workers employed in acrylic-fibre and synthetic-leather industries.

5.2 Human carcinogenicity data

Evidence on the carcinogenicity of *N*,*N*-dimethylformamide in humans was available from studies of aircraft-repair workers, leather-tannery workers, and chemical manufacturing workers.

One study described a cluster of three cases of cancer of the testes among 153 workers repairing F4 Phantom jet aircraft in the USA. These workers participated in or worked in proximity to a single process that involved uncontrolled exposure to *N*,*N*-dimethylformamide. The finding of the cluster investigation motivated a further study at a different aircraft-repair facility, where one unit repaired the F4 Phantom aircraft (also with exposure to *N*,*N*-dimethylformamide) and one unit repaired other aircraft (without exposure to *N*,*N*-dimethylformamide). In the exposed group, four cases of cancer of the testes were observed, compared with none in the unexposed group.

Shortly thereafter, a cluster of three cases of cancer of the testes was observed in workers who used *N*,*N*-dimethylformamide at a leather tannery in New York state, USA. A subsequent case–control study of cancer of the testis in the county where the tannery was located, which included the 3 original cases and 7 others, supported the existence of a cluster among leather workers.

A cohort study in one plant producing acrylic fibres in the USA, and a subsequent case–control study including that plant and three others, all of which used *N*,*N*-dimethylformamide and other chemicals, found 11 cases of cancer of the testes, of which three had been exposed to *N*,*N*-dimethylformamide, with no observed excess. It is likely that the exposures to *N*,*N*-dimethylformamide in these manufacturing plants were lower than those of leather-tanning and aircraft-repair workers.

The data from studies of aircraft-repair workers and leather workers provided evidence for a positive association between exposure to *N*,*N*-dimethylformamide and cancer of the testes; however, chance and confounding by other occupational exposures could not be ruled out.

5.3 Animal carcinogenicity data

N,*N*-dimethylformamide was tested for carcinogenicity in two studies by whole-body inhalation in male and female mice, two studies by whole-body inhalation in male and female rats, and one study by whole-body inhalation plus oral (drinking-water) administration (combined) in male rats. The Working Group determined that one study by oral administration, one study by subcutaneous injection, and one study by intraperitoneal injection in rats were inadequate for the evaluation.

In a 2-year study that complied with good laboratory practice (GLP), treatment of mice with *N*,*N*-dimethylformamide by inhalation caused a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined), in males and females in all dose groups, with a significant positive trend. There was also a significant increase in the incidence of hepatoblastoma in males. Multiple hepatocellular adenomas and carcinomas were found in the liver of exposed mice. In the second study by inhalation in mice, no significant increase in tumour incidence was reported in any dose group.

In a 2-year GLP study in rats treated by inhalation, exposure to *N*,*N*-dimethylformamide caused a significant positive trend in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), in males and females; the incidences of hepatocellular adenoma and hepatocellular carcinoma were significantly increased in males and females at the highest dose. Multiple occurrences of hepatocellular tumours were found in the livers of exposed rats. In the second study by inhalation in rats, no significant increase in tumour incidence was reported in any dose group.

In the GLP study of inhalation plus oral administration (combined) in male rats, *N*,*N*-dimethylformamide caused a significant increase in the incidences of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in the groups treated by inhalation plus oral administration, oral administration only, and inhalation only. It also caused a significant increase in the incidence of hepatocellular carcinoma in one group treated by oral administration only and in all groups treated by inhalation plus oral administration.

5.4 Mechanistic and other relevant data

With respect to the key characteristics of human carcinogens, there is *strong* evidence that *N*,*N*-dimethylformamide is metabolically activated, including in exposed humans. *N*,*N*-Dimethylformamide is readily absorbed after inhalation, dermal, and oral exposure. Several urinary metabolites, including the mercapturic acid, have been identified in humans. Carbamoylated adducts formed from methyl isocyanate and/or another carbamoylating metabolic intermediate of *N*,*N*-dimethylformamide have been identified in globin (both lysine and *N*-terminal valine adducts). A carbamoylated cytosine adduct has also been detected in vivo in the urine of occupationally exposed humans. Additionally, formaldehyde is a probable metabolite from oxidative demethylation of *N*,*N*-dimethylformamide.

There is *strong* evidence that *N*,*N*-dimethylformamide induces oxidative stress. A study of occupationally-exposed workers showed a distinct increase in the activity of superoxide dismutase in blood compared with the controls. Experimental studies in vivo and in vitro confirmed that *N*,*N*-dimethylformamide is capable of increasing oxidative stress in rodents, as well as in human liver, leukaemia and colon carcinoma cells.

There is *strong* evidence that *N*,*N*-dimethylformamide alters cell proliferation. No data were available in exposed humans. Experimental studies in vitro have shown an anti-proliferative and differentiating effect in human cancer cells, while it induced cell proliferation in normal human and rodent liver cells.

There is *moderate* evidence that *N*,*N*-dimethylformamide is genotoxic. Chromosomal and DNA damage has been observed in several studies in occupationally exposed humans, but results were equivocal. The results of studies of genotoxicity in various experimental systems in vivo and in vitro were mostly negative or inconclusive.

There is *weak* evidence that *N*,*N*-dimethylformamide modulates receptor-mediated effects. So far there is little mechanistic evidence that *N*,*N*-dimethylformamide exerts any toxic effects through a specific receptor in humans or in experimental systems. Some experimental studies did report perturbations of the receptors for estrogen, transforming growth factor β, and epidermal growth factor.

There were few data on other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, induces chronic inflammation, is immunosuppressive, or causes immortalization).

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of *N*,*N*-dimethylformamide. A positive association has been observed between exposure to *N*,*N*-dimethylformamide and cancer of the testes.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*,*N*-dimethylformamide.

6.3 Overall evaluation

N,*N*-Dimethylformamide is *probably carcinogenic to humans (Group 2A).*

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N,N-DIMETHYL-*P*-TOLUIDINE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 99-97-8

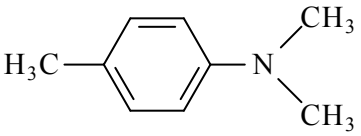
Chen. Abstr. Serv. Name: *N,N*-Dimethyl-*para*-toluidine

IUPAC Systematic Name: *N,N*,4-Trimethyl-aniline

Synonyms: 4-Dimethylaminotoluene, *N,N*-dimethyl-4-methylaniline, *p,N,N*-trimethylaniline, *N,N*,4-trimethylbenzen-amine

Acronyms: DMPT

1.1.2 Structure and molecular formula, and relative molecular mass



Molecular formula: $C_9H_{13}N$
Relative molecular mass: 135.21

1.1.3 Physical and chemical properties

Description: Light yellow to brown, oily fluid with a characteristic amine-like odour (IFA, 2015)

Density (at 20 °C): 0.94 g/cm³ (IFA, 2015)

Octanol/water partition coefficient (P): log K_{ow}, 2.81 (HSDB, 2015)

Melting point: –15 °C (IFA, 2015)

Boiling point: 211 °C (HSDB, 2015)

Volatility: Vapour pressure, 0.01 kPa [0.178 mm Hg], at 20 °C (IFA, 2015)

Vapour density: 4.66 (air = 1) at 20 °C (IFA, 2015)

Solubility: Insoluble to moderately soluble in water (650 mg/L at 37 °C) (ECHA, 2015)

Stability: Lower explosion limit, 1.2 vol. %; upper explosion limit, 7.0 vol. % (IFA, 2015)

Flash point: 83 °C (IFA, 2015)

Ignition temperature: 425 °C (IFA, 2015)

Conversion factor (at 101 kPa, 20 °C): 1 ppm = 5.62 mg/m³ (IFA, 2015)

1.2 Production and use

1.2.1 Production

(a) Production methods

Multiple methods exist for the manufacture of *N*-alkyltoluidines, including *N,N*-dimethyl-*p*-toluidine, such as acid-catalysed alkylation of unalkylated toluidines with lower unbranched alcohols or ether. Another method involves reductive alkylation with lower aldehydes or ketones using metal catalysts under hydrogen pressure (Bowers, 1996).

(b) Production volume

N,*N*-Dimethyl-*p*-toluidine was listed as a high production volume chemical in the USA, indicating that > 1 million pounds [approximately 454 tonnes] were produced or imported between 1990 and 1994 annually (HSDB, 2015). Annual United States production volumes of *N*,*N*-dimethyl-*p*-toluidine from 1986 to 2014 are given in Table 1.1. With annual production reported to be 1–10 million pounds [approximately 454–4536 tonnes] in 2014, it remains a high production volume chemical in the USA (HSDB, 2015). The total tonnage band per annum is estimated to be between 10 and 100 tonnes in the European Union (ECHA, 2015). Globally, more than 130 suppliers of *N*,*N*-dimethyl-*p*-toluidine could be identified in 2016 with about two thirds (> 80) located in Asia (ChemicalBook Inc., 2016).

Table 1.1 Annual production volume of *N*,*N*-dimethyl-*p*-toluidine in the USA, 1986–2014

Year	Production range (pounds)
1986	10 000–500 000 [~4.5–227 tonnes]
1990	10 000–500 000 [~4.5–227 tonnes]
1994	> 1 million–10 million [~ > 454–4536 tonnes]
1998	> 1 million–10 million [~ > 454–4536 tonnes]
2002	> 500 000–1 million [~ > 227–454 tonnes]
2006	> 1 million- 10 million [~ > 454–4536 tonnes]
2014	> 1 million–10 million [~ > 454–4536 tonnes]

Compiled by the Working Group from non-confidential Chemical Data Reporting information (EPA, 2011, 2015; HSDB, 2015)

1.2.2 Use

N,*N*-Dimethyl-*p*-toluidine is used as an accelerator in the preparation of dental materials and bone cements to activate the polymerization reaction or when curing methyl methacrylate monomers. The concentrations in these preparations are usually between 0.5% and 3% (NTP, 2012). It has also been used for the preparation of acrylic denture material for the past 50 years (HSDB, 2015). In addition, *N*,*N*-dimethyl-*p*-toluidine is an important ingredient of industrial glues and artificial fingernail preparations, when short setting times are needed, and is also used as an intermediate in the manufacture of dyes and pesticides (NTP, 2012).

1.3 Measurement and analysis

N,*N*-Dimethyl-*p*-toluidine in dental material was analysed by high-performance liquid chromatography with ultraviolet detection (Shintani et al. 1993; Stea et al. 1997). Sample preparation was carried out by solid-phase extraction.

Air monitoring of *N*,*N*-dimethyl-*p*-toluidine has been described as part of NIOSH Method 2002 (aromatic amines) using gas chromatography and flame ionization detection (NIOSH, 1994). Sample preparation includes sorption on silica gel at a sampling rate of 1 L/minute or less, and the estimated limit of detection is 0.01 mg per sample.

No analytical procedures were found for the determination of *N*,*N*-dimethyl-*p*-toluidine specifically in the environment (air, water), food, urine or blood, with the exception of the determination of its *N*-oxides in enzyme reaction mixtures (i.e. liver microsomes) using high-performance liquid chromatography with ultraviolet detection (Seto & Guengerich, 1993). *N*,*N*-Dimethyl-*p*-toluidine may be determined by current methods for the determination of monocyclic aromatic amines in blood or urine (Richter & Branner, 2002; Weiss & Angerer, 2002; Lamani et al., 2015).

1.4 Occurrence and exposure

1.4.1 Natural occurrence

N,*N*-Dimethyl-*p*-toluidine does not occur naturally.

1.4.2 Environmental occurrence

In the environment, *N*,*N*-dimethyl-*p*-toluidine is broken down rapidly in air. It travels through soil and may volatilize from moist soil and water surfaces (HSDB, 2015).

1.4.3 Occupational exposure

Potential widespread human exposure to *N*,*N*-dimethyl-*p*-toluidine can occur in occupational settings related to its use in bone cements, dental prostheses, industrial glues, and artificial fingernails. Surgeons, surgical staff, dentists, dental technicians, nail salon operators, and users of industrial glues may receive significant exposure to *N*,*N*-dimethyl-*p*-toluidine by inhalation or through the skin (NTP, 1999).

The National Occupational Exposure Survey, which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 62 720 workers (among whom 27 118 were women) were potentially exposed to *N*,*N*-dimethyl-*p*-toluidine in the workplace (NIOSH, 1990). In 2014, three companies in the USA manufactured *N*,*N*-dimethyl-*p*-toluidine (HSDB, 2015).

A NIOSH investigation of air sampling from a nail salon measured only trace amounts of *N*,*N*-dimethyl-*p*-toluidine (Kronoveter, 1977). No other measurements of occupational exposure were identified.

1.4.4 Exposure of the general population

Sniffing glues, some of which contain 1–7% of the compound (Misiak & Scheffler, 2003; 3M Company, 2004), could possibly result in exposure to *N*,*N*-dimethyl-*p*-toluidine (Neumark et al., 1998; Wu et al., 2008; Marsolek et al., 2010).

Due to its use as polymerization accelerator for the manufacture of bone cement and dental materials, *N*,*N*-dimethyl-*p*-toluidine has been identified in a variety of commonly used bone cements at concentrations of about 10–30 g/kg (~1–3%) (Haddad et al., 1996; Stea et al., 1997). Exposure from bone cement is thought to have been responsible for sensitization in some patients (Haddad et al., 1996).

Two cases of the accidental ingestion of artificial fingernail solutions containing unspecified concentrations of *N*,*N*-dimethyl-*p*-toluidine have been reported. In the first case, a child aged 16 months drank 15 mL of solution (estimated to contain about 60 mg of *N*,*N*-dimethyl-*p*-toluidine or 6 mg/kg body weight [bw]) and on admission to hospital, methaemoglobin was 43% (normal value, < 2%) (Potter et al., 1988). In the second case, a baby aged 5 months ingested 30 mL of solution and after 1 hour, methaemoglobin was 11%. The children recovered after the administration of methylene blue and oxygen (Kao et al., 1997).

1.4.5 Exposure assessment and biological markers

No studies to assess exposure in humans by measuring *N*,*N*-dimethyl-*p*-toluidine or its metabolites in the blood or urine were available to the Working Group.

1.5 Regulations and guidelines

No specific occupational exposure limit has been reported for *N*,*N*-dimethyl-*p*-toluidine, but this chemical can cause methaemoglobinaemia. The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area established guidance values for methaemoglobin-forming amino and nitro compounds in terms of an umbrella regulation. Methaemoglobin values > 1.5% indicate that workers are exposed to aromatic amino and nitro compounds, and the Commission has recommended a level of 5% as a "ceiling" (Leng & Bolt, 2006). [The magnitude of exposure to *N*,*N*-dimethyl-*p*-toluidine leading to this level has not yet been specified.]

According to the risk phrases of the Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations, *N*,*N*-dimethyl-*p*-toluidine is harmful if is swallowed (H301), is in contact with skin (H311), or is inhaled (H331). *N*,*N*-Dimethyl-*p*-toluidine may also cause damage to organs through prolonged and repeated exposure (reproductive organs after oral exposure) (H373) and is harmful to aquatic life, with long-lasting effects (H412) (ECHA, 2015).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were given *N*,*N*-dimethyl-*p*-toluidine (purity, > 99%) by gavage in corn oil at doses of 0 (control), 6, 20, or 60 mg/kg bw on 5 days per week for 105 weeks. Survival of females at 60 mg/kg bw was significantly lower than that of the group receiving the vehicle control. The mean body weights of males and females at 60 mg/kg bw were > 10% lower than those of the vehicle controls after week 89 and week 65, respectively. The decreased survival of females at 60 mg/kg bw, and the decrease in body-weight gain in males and females at 60 mg/kg bw were attributed to the development of treatment-related tumours (NTP, 2012; Dunnick et al., 2014).

In males, treatment-related increases in hepatocellular tumours were observed in males, including increases in the incidence of hepatocellular adenoma (multiple): 17/50 controls, 19/50 at 6 mg/kg bw, 27/50 at 20 mg/kg bw ($P \leq 0.05$) and 26/50 at 60 mg/kg bw ($P \leq 0.05$); hepatocellular carcinoma (multiple): 7/50 controls, 7/50 at 6 mg/kg bw, 16/50 at 20 mg/kg bw ($P \leq 0.05$) and 22/50 at 60 mg/kg bw ($P \leq 0.01$); hepatocellular carcinoma (including multiple): 22/50 controls (*P* for trend, 0.002), 25/50 at 6 mg/kg bw, 30/50 at 20 g/kg bw and 36/50 at 60 mg/kg bw ($P = 0.005$); hepatocellular adenoma or carcinoma (combined): 38/50 controls (*P* for trend, 0.005), 44/50 at 6 mg/kg bw, 47/50 at 20 mg/kg bw ($P = 0.010$), and 48/50 at 60 mg/kg bw ($P = 0.006$); and hepatoblastoma: 1/50 controls, 5/50 at 6 mg/kg bw, 10/50 at 20 mg/kg bw ($P = 0.005$) and 8/50 at 60 mg/kg bw ($P = 0.021$). The incidence of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) was 38/50 controls (*P* for trend, 0.006), 45/50 at 6 mg/kg bw, 48/50 at 20 mg/kg bw ($P = 0.004$), and 48/50 at 60 mg/kg bw ($P = 0.006$). The historical incidence for hepatoblastoma in studies by gavage in corn oil was 14/350 (4.0% ± 2.8%; range, 0–8%) and for all routes was 61/1149 (5.3% ± 7.1%; range, 0–34%) and that for hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) in studies by gavage in corn oil was 242/350 (69.1% ± 8.0%; range, 58–78%) and for all routes was 852/1149 (74.2% ± 11.5%; range, 52–92%).

In females, treatment-related increases in the incidence of hepatocellular tumours were observed, including that of hepatocellular adenoma (multiple): 2/50 controls, 6/50 at 6 mg/kg bw, 29/50 at 20 mg/kg bw ($P \leq 0.01$) and 35/50 at 60 mg/kg bw ($P \leq 0.01$); hepatocellular adenoma (including multiple): 17/50 controls (*P* for trend, < 0.001), 19/50 at 6 mg/kg bw, 37/50 at 20 mg/kg bw ($P < 0.001$), and 44/50 at 60 mg/kg bw ($P < 0.001$); hepatocellular carcinoma (multiple): 1/50 controls, 3/50 at 6 mg/kg bw, 5/50 at 20 mg/kg bw, and 19/50 at 60 mg/kg bw ($P \leq 0.01$); hepatocellular carcinoma (including multiple): 6/50 controls (*P* for trend, < 0.001), 13/50 at 6 mg/kg bw ($P = 0.049$), 18/50 at 20 mg/kg bw ($P = 0.002$),

Table 3.1 Studies of carcinogenicity in experimental animals treated with *N,N*-dimethyl-*p*-toluidine by gavage in corn oil

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$/N (M) 5–6 wks 105 wks NTP (2012)	Purity, > 99% 0, 6, 20, 60 mg/kg bw 5 days/wk for 105 wks 50/group 34, 36, 31, 36	*Liver*		GLP study
		Hepatocellular adenoma (multiple): 17/50, 19/50, 27/50*, 26/50*	Trend: NR *$P \leq 0.05$	
		Hepatocellular adenoma (including multiple): 29/50, 34/50, 37/50, 36/50	NS	
		Hepatocellular carcinoma (multiple): 7/50, 7/50, 16/50*, 22/50**	Trend: NR *$P \leq 0.05$ **$P \leq 0.01$	
		Hepatocellular carcinoma (including multiple): 22/50, 25/50, 30/50, 36/50*	Trend: $P = 0.002$ *$P = 0.005$	
		Hepatocellular adenoma or carcinoma (combined): 38/50, 44/50, 47/50*, 48/50**	Trend: $P = 0.005$ *$P = 0.010$ **$P = 0.006$	
		Hepatoblastoma: 1/50, 5/50, 10/50*, 8/50**	*$P = 0.005$ **$P = 0.021$	
		Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 38/50, 45/50, 48/50*, 48/50**	Trend: $P = 0.006$ *$P = 0.004$ **$P = 0.006$	
Mouse, B6C3F$_1$/N (F) 5–6 wks 105 wks NTP (2012)	Purity, > 99% 0, 6, 20, 60 mg/kg bw 5 days/wk for 105 wks 50/group 43, 40, 39, 32	*Liver*		GLP study
		Hepatocellular adenoma (multiple): 2/50, 6/50, 29/50*, 35/50*	Trend: NR *$P \leq 0.01$	
		Hepatocellular adenoma (including multiple): 17/50, 19/50, 37/50*, 44/50*	Trend: $P < 0.001$ *$P < 0.001$	
		Hepatocellular carcinoma (multiple): 1/50, 3/50, 5/50, 19/50*	Trend: NR *$P \leq 0.01$	
		Hepatocellular carcinoma (including multiple): 6/50, 13/50*, 18/50**, 31/50***	Trend: $P < 0.001$ *$P = 0.049$ **$P = 0.002$ ***$P < 0.001$	
		Hepatocellular adenoma or carcinoma (combined): 20/50, 25/50, 42/50*, 45/50*	Trend: $P < 0.001$ *$P < 0.001$	
		Hepatoblastoma: 0/50, 1/50, 0/50, 4/50*	Trend: $P = 0.007$ *$P = 0.044$	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$/N (F) 5–6 wks 105 wks NTP (2012) (cont.)		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined): 20/50, 26/50, 42/50*, 45/50*	Trend: $P < 0.001$ *$P < 0.001$	
		Lung		
		Alveolar/bronchiolar adenoma: 2/50, 4/50, 8/50*, 12/50**	Trend: $P < 0.001$ *$P = 0.039$ **$P < 0.001$	
		Alveolar/bronchiolar carcinoma: 0/50, 1/50, 2/50, 1/50	NS	
		Alveolar/bronchiolar adenoma or carcinoma (combined): 2/50, 5/50, 9/50*, 13/50**	Trend: $P < 0.001$ *$P = 0.021$ **$P < 0.001$	
		Forestomach		
		Squamous cell papilloma: 1/50, 5/50, 6/50*, 7/50**	Trend: $P = 0.037$ *$P = 0.049$ **$P = 0.017$	
		Squamous cell carcinoma: 0/50, 1/50, 0/50, 0/50	NS	
		Squamous cell papilloma or carcinoma (combined): 1/50, 6/50, 6/50*, 7/50**	*$P = 0.049$ **$P = 0.017$	
Rat, F344 (M) 6–7 wks 104 wks NTP (2012)	Purity, > 99% 0, 6, 20, 60 mg/kg bw 5 days/wk for 104 wks 50/group 37, 37, 31, 21	*Liver*		GLP study The incidence of thyroid follicular cell adenoma or carcinoma (combined) at 60 mg/kg bw was outside that of the range for this tumour in historical controls (all routes, 0–6%)
		Hepatocellular adenoma: 0/50, 0/50, 1/50, 1/50	NS	
		Hepatocellular carcinoma: 0/50, 0/50, 1/50, 6/50*	Trend: $P < 0.001$ *$P = 0.009$	
		Hepatocellular adenoma or carcinoma (combined): 0/50, 0/50, 2/50, 6/50*	Trend: $P < 0.001$ *$P = 0.009$	
		Nasal cavity		
		Transitional epithelium adenoma: 0/50, 3/49, 2/50, 11/49*	Trend: $P < 0.001$ *$P < 0.001$	
		Transitional epithelium carcinoma: 0/50, 0/49, 0/50, 2/49	Trend: $P = 0.033$	
		Transitional epithelium adenoma or carcinoma (combined): 0/50, 3/49, 2/50, 13/49*	Trend: $P < 0.001$ *$P < 0.001$	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 6–7 wks 104 wks NTP (2012) (cont.)		*Thyroid* Follicular cell adenoma: 1/50, 0/49, 1/50, 3/49 Follicular cell carcinoma: 0/50, 2/49, 1/50, 2/49 Follicular cell adenoma or carcinoma (combined): 1/50, 2/49, 2/50, 4/49	 NS NS NS	
Rat, F344 (F) 6–7 wks 105 wks NTP (2012)	Purity, > 99% 0, 6, 20, 60 mg/kg bw 5 days/wk for 105 wks 50/group 33, 42, 33, 23	*Liver* Hepatocellular adenoma: 0/50, 1/50, 1/50, 3/49 Hepatocellular carcinoma (multiple): 0/50, 0/50, 0/50, 1/49 Hepatocellular carcinoma (including multiple): 0/50, 0/50, 0/50, 4/49* Hepatocellular adenoma or carcinoma (combined): 0/50, 1/50, 1/50, 7/49* *Nasal cavity* Transitional epithelium adenoma: 0/50, 1/49, 0/50, 2/49	 Trend: $P = 0.044$ NS Trend: $P < 0.001$ *$P = 0.041$ Trend: $P < 0.001$ *$P = 0.003$ NS	GLP study The incidence of transitional epithelium adenoma of the nasal cavity at 60 mg/kg bw was outside that of the range for this tumour in historical controls (corn oil gavage studies, 0%; all routes, 0–2%)

bw, body weight; F, female; GLP, good laboratory practice; M, male; NR, not reported; NS, not significant; wk, week

and 31/50 at 60 mg/kg bw ($P < 0.001$); hepatocellular adenoma or carcinoma (combined): 20/50 controls (P for trend, < 0.001), 25/50 at 6 mg/kg bw, 42/50 at 20 mg/kg bw ($P < 0.001$), and 45/50 at 60 mg/kg bw ($P < 0.001$); and hepatoblastoma: 0/50 controls (P for trend, 0.007), 1/50 at 6 mg/kg bw, 0/50 at 20 mg/kg bw, and 4/50 at 60 mg/kg bw ($P = 0.044$). The incidence of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) was 20/50 controls (P for trend, < 0.001), 26/50 at 6 mg/kg bw, 42/50 ($P < 0.001$) at 20 mg/kg bw, and 45/50 at 60 mg/kg bw ($P < 0.001$). The historical incidence for hepatoblastoma in studies on gavage in corn oil was 1/347 (0.3% ± 0.8%; range, 0–2%) and for all routes was 4/1195 (0.3% ± 0.8%; range, 0–2%) and that for hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) in studies on gavage in corn oil was 91/347 (26.2% ± 12.7%; range, 8–40%) and for all routes was 444/1195 (37.2% ± 22.9%; range, 6–82%). In addition, an increase was observed in the incidence of lung alveolar/bronchiolar adenoma: 2/50 controls (P for trend, < 0.001), 4/50 at 6 mg/kg bw, 8/50 at 20 mg/kg bw ($P = 0.039$), and 12/50 at 60 mg/kg bw ($P < 0.001$); and alveolar/bronchiolar adenoma or carcinoma (combined): 2/50 controls (P for trend, < 0.001), 5/50 at 6 mg/kg bw, 9/50 at 20 mg/kg bw ($P = 0.021$), and 13/50 at 60 mg/kg bw ($P < 0.001$). The historical incidence for alveolar/bronchiolar adenoma or carcinoma (combined) in studies on gavage in corn oil was 23/346 (6.7% ± 3.2%; range, 2–12%) and for all routes was 100/1196 (8.4% ± 4.3%; range, 2–22%). A few alveolar/bronchiolar carcinomas were found in treated groups (0/50 controls, 1/50 at 6 mg/kg bw, 2/50 at 20 mg/kg bw and 1/50 at 60 mg/kg bw). The incidence for this tumour was not significant and was within the historical control range, which was 7/346 (2.0% ± 2.0%; range, 0–4%) in studies on gavage in corn oil and 44/1196 (3.7% ± 3.3%; range, 0–14%) for all studies. Significant increases were also observed in the incidence of forestomach squamous cell papilloma: 1/50 controls (P for trend, 0.037), 5/50 at 6 mg/kg bw, 6/50 at 20 mg/kg ($P = 0.049$), and 7/50 at 60 mg/kg bw ($P = 0.017$); and forestomach squamous cell papilloma or carcinoma (combined): 1/50 controls, 6/50 at 6 mg/kg bw, 6/50 at 20 mg/kg bw ($P = 0.049$) and 7/50 at 60 mg/kg bw ($P = 0.017$). The historical incidence of forestomach squamous cell papilloma in studies of gavage in corn oil was 12/348 (3.5% ± 1.5%; range, 2–6%) and for all routes was 22/1198 (1.8% ± 1.7%; range, 0–6%) and that of forestomach squamous cell papilloma or carcinoma (combined) in studies of gavage in corn oil was 12/348 (3.5% ± 1.5%; range, 2–6%) and for all routes was 23/1198 (1.9% ± 1.6%; range, 0–6%). One female mouse given 6 mg/kg bw had a forestomach squamous cell carcinoma (0/50 controls, 1/50 at 6 mg/kg bw, 0/50 at 20 mg/kg bw, and 0/50 at 60 mg/kg bw). The historical incidence for forestomach squamous cell carcinoma in studies on gavage in corn oil was 0/348 and that for all routes was 1/1198 (0.1% ± 0.4%; range, 0–2%).

Treatment-related non-neoplastic lesions were seen in the liver, nose, lung, olfactory lobe and spleen in males and females. In females, increases in non-neoplastic lesions of the forestomach, bone marrow and mesenteric lymph node were also observed (NTP, 2012). [The strength of this study was that it was a 2-year cancer bioassay in males and females with a large number of animals per group, multiple doses, and in compliance with good laboratory practice.]

3.2 Rat

Groups of 50 male and 50 female Fischer 344/N rats (age, 6–7 weeks) were given *N*,*N*-dimethyl-*p*-toluidine (purity, > 99%) by gavage in corn oil at doses of 0 (control), 6, 20, or 60 mg/kg bw on 5 days per week for 104 weeks (males) or 105 weeks (females). At termination after 2 years, survival in male rats at 60 mg/kg bw was significantly lower than that in controls, which was attributed to the development of

treatment-related tumours. Mean body weights of males and females at 60 mg/kg bw were > 10% lower than those in the vehicle-control group after weeks 61 and 33, respectively (NTP, 2012; Dunnick et al., 2014).

In males, treatment-related increases in the incidence of hepatocellular tumours included those of hepatocellular carcinoma: 0/50 controls (*P* for trend, < 0.001), 0/50 at 6 mg/kg bw, 1/50 at 20 mg/kg bw, and 6/50 at 60 mg/kg bw (*P* = 0.009); and hepatocellular adenoma or carcinoma (combined): 0/50 controls (*P* for trend, < 0.001), 0/50 at 6 mg/kg bw, 2/50 at 20 mg/kg bw, and 6/50 at 60 mg/kg bw (*P* = 0.009). The incidence of hepatocellular adenoma was 0/50 controls, 0/50 at 6 mg/kg bw, 1/50 at 20 mg/kg bw, and 1/50 at 60 mg/kg bw. The historical incidence of hepatocellular carcinoma was 0/299 in studies of gavage in corn oil and 5/1249 (0.4% ± 1.0%; range, 0–4%) for all routes. Increases were also observed in the incidence of nasal cavity transitional epithelium adenoma: 0/50 controls (*P* for trend, < 0.001), 3/49 at 6 mg/kg bw, 2/50 at 20 mg/kg bw, and 11/49 at 60 mg/kg bw (*P* < 0.001); and nasal cavity transitional epithelium adenoma or carcinoma (combined): 0/50 controls (*P* for trend, < 0.001), 3/49 at 6 mg/kg bw, 2/50 at 20 mg/kg bw, and 13/49 at 60 mg//kg bw (*P* < 0.001). The historical incidence for nasal cavity transitional epithelium adenoma indicated that they have not previously been observed in historical controls (studies of gavage in corn oil: 0/299; all routes: 0/1248). The incidence of nasal cavity transitional epithelium carcinoma was not significantly increased by pairwise comparison, although 2 males given 60 mg/kg bw had these tumours (0/50 controls, 0/49 at 6 mg/kg bw, 0/50 at 20 mg/kg bw, and 2/49 at 60 mg/kg bw; *P* for trend, 0.033); no historical control data were available for this tumour type. There was a non-significant increase in thyroid gland follicular cell adenoma or carcinoma (combined) (1/50 controls, 2/49 at 6 mg/kg bw, 2/50 at 20 mg/kg bw, and 4/49 at 60 mg/kg bw), although the incidence at 60 mg/kg bw was outside of the incidence range for this tumour in historical controls. The incidence for thyroid gland follicular cell adenoma or carcinoma (combined) in the historical controls was 9/299 (3.0% ± 2.1%; range, 0–6%) in studies on gavage in corn oil and 23/1239 (1.9% ± 2.2%; range, 0–6%) for all routes. A non-significant increase in the incidence of thyroid gland follicular cell carcinoma was observed (0/50 controls, 2/49 at 6 mg/kg bw, 1/50 at 20 mg/kg bw, and 2/49 at 60 mg/kg bw), for which the historical control incidence was 3/299 (1.0% ± 1.7%; range, 0–4%) in studies on gavage in corn oil and 10/1239 (0.8% ± 1.5%; range, 0–4%) for all routes.

In females, the incidence of hepatocellular adenoma was significantly increased according to trend statistics (*P* = 0.044): 0/50 controls, 1/50 at 6 mg/kg bw, 1/50 at 20 mg/kg bw, and 3/49 at 60 mg/kg bw; and that of hepatocellular carcinoma was significantly increased at the highest dose: 0/50 controls, 0/50 at 6 mg/kg bw, 0/50 at 20 mg/kg bw, and 4/49 at 60 mg/kg bw (*P* = 0.041). A significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) was also observed: 0/50 controls (*P* for trend, < 0.001), 1/50 at 6 mg/kg bw, 1/50 at 20 mg/kg bw, and 7/49 at 60 mg/kg bw (*P* = 0.003). The historical incidence of these tumours was 1/300 (0.3% ± 0.8%; range, 0–2%) in studies with administration by gavage in corn oil, and 12/1200 (1.0% ± 1.6%; range, 0–4%) for all routes. A non-significant increase in the incidence of nasal cavity transitional epithelium adenoma was also observed (0/50 controls, 1/49 at 6 mg/kg bw, 0/50 at 20 mg/kg bw, and 2/49 at 60 mg/kg bw), although the incidence in females given the highest dose was outside the historical range for this tumour, which was 0/299 in studies on gavage in corn oil, and 1/1196 (0.1% ± 0.4%; range, 0–2%) for all routes.

Treatment-related non-neoplastic lesions were seen in the liver and nose of males and females; non-neoplastic lesions also occurred in the spleen, kidney, and bone marrow of males

and females. In addition, significant increases in forestomach and mesenteric lymph node non-neoplastic lesions were found in male rats (NTP, 2012). [The strength of this study was that it was a 2-year cancer bioassay in males and females, with a large number of animals per group, multiple doses, and in compliance with good laboratory practice.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Absorption, distribution, and excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

[U-^{14}C]-Labelled *N*,*N*-dimethyl-*p*-toluidine was rapidly absorbed after a single oral administration of 2.5, 25 or 250 mg/kg bw to male and female Fischer 344 rats and B6C3F$_1$ mice (four animals/treatment group) (Dix et al., 2007). Absorption of the dose was nearly complete based on the data on excretion and their similarity to those obtained from groups of rats and mice injected intravenously. Cumulative excretion amounted to about 90% in the urine and 4% in the faeces 24 hours after gavage. *N*,*N*-Dimethyl-*p*-toluidine-derived radioactivity appeared to have been widely distributed to the tissues. At the terminal 24-hour time-point, the assayed tissues, including blood, liver, kidney, skin, muscle and adipose tissue, contained 2–5% of the total dose. In rats, the highest concentration of radioactivity was detected in the liver, followed by the kidney and urinary bladder. The lung and liver contained the highest concentrations in mice. The tissue-to-blood ratio of *N*,*N*-dimethyl-*p*-toluidine-derived radioactivity at these sites was > 1. Male rats excreted about 60% of the total dose in urine within 6 hours of receiving a dose of 2.5 mg/kg bw, whereas male and female rats receiving 25 mg/kg bw excreted about half as much (i.e. about 30%). However, cumulative excretion in the urine was similar for both doses at the 24-hour time-point. These results indicated initial saturation of absorption and/or elimination of the higher dose. The data on urinary excretion for 2.5 and 25 mg/kg bw were similar over time for male mice. Female mice appeared to excrete less of the higher dose in the urine than males; however, this difference was probably due to poor recovery of radiolabel in the urine of one or more individuals. All of the other data on disposition in female mice were similar to those of males. [The Working Group noted that the data from groups of male rats and mice that received 250 mg/kg bw by gavage were compromised by acute toxicity.] Cumulative excretion of the dose in the high-dose rats amounted to only ~70%, possibly as a result of the initial acute toxicity. Most of the unexcreted radiolabel was present in the gastrointestinal tract at 24 hours. In a supplemental experiment reported to the National Toxicology Program, the absorption and excretion of a high dose were nearly complete at 72 hours after treatment, with about 2% of the total dose detected in tissues and the gastrointestinal tract (NTP, 2012). The data for male mice receiving 250 mg/kg bw in this study were not reported here due to death or unresolved morbidity associated with all individuals in the treatment group.

4.1.2 Metabolism

(a) Humans

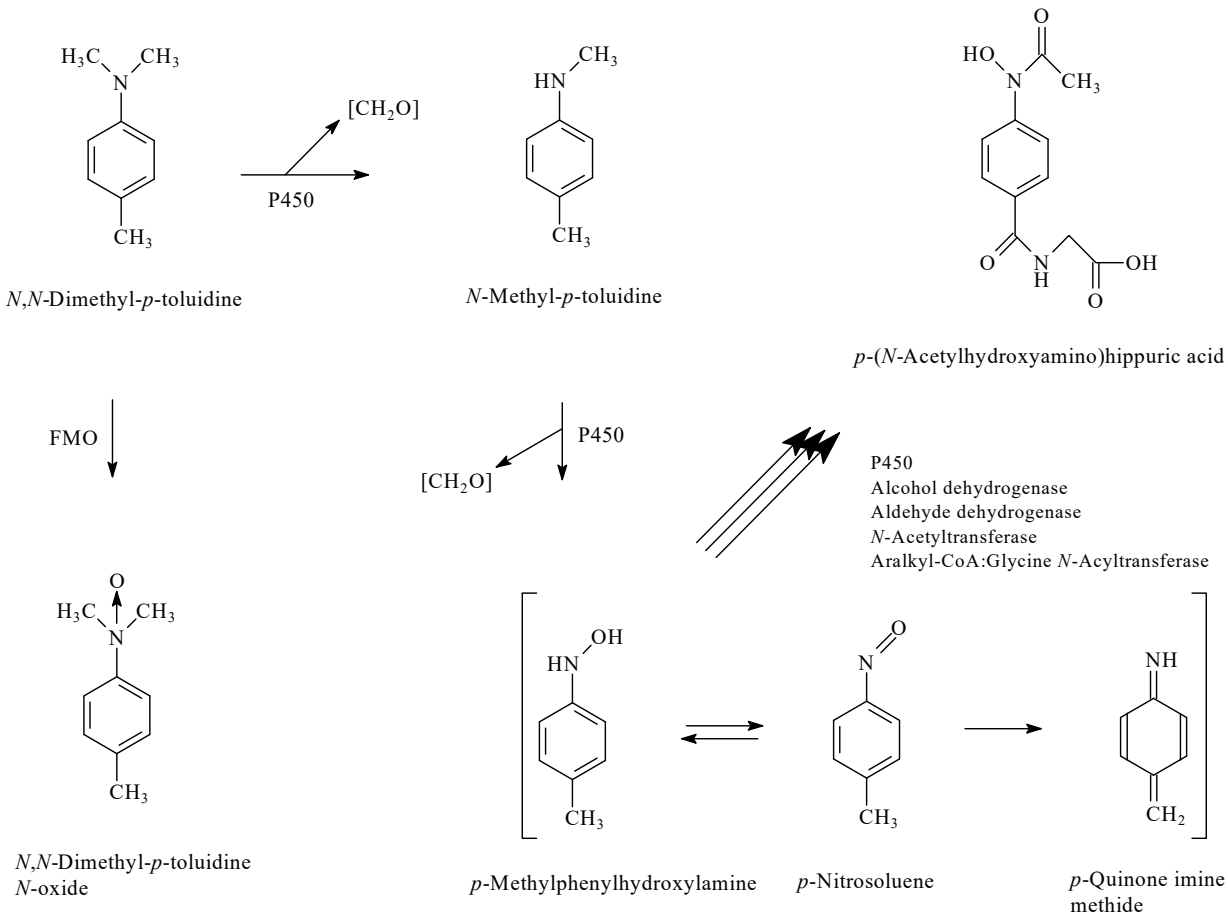
No data were available to the Working Group.

(b) Experimental systems

See Figure 4.1

As reported by Kim et al. (2007), the metabolism of *N*,*N*-dimethyl-*p*-toluidine was rapid and

Fig. 4.1 Known and postulated metabolites of *N,N*-dimethyl-*p*-toluidine in rats



Adapted from Kim et al. (2007) and Dunnick et al. (2014), with permission of the publisher, SAGE Publications, Inc
FMO, flavin-containing monooxygenase; P450, cytochrome P450; coA, coenzyme A

extensive in rats in a study conducted by Dix et al. (2007). The major radiolabelled metabolite in rat urine was identified as *p*-(*N*-acetylhydroxyamino)hippuric acid that was catalysed by cytochrome P450, alcohol and aldehyde dehydrogenases, *N*-acetyl transferase and aralkyl-coenzyme A:glycine-*N*-acyltransferase. The urine also contained *N*-methyl-*p*-toluidine, an oxidative product of *N,N*-dimethyl-*p*-toluidine, and a precursor of the glycine conjugate.

Intermediates in this pathway are postulated to include *p*-methylphenylhydroxylamine and an electrophilic *p*-quinone imine methide (4-methylene-2,5-cyclohexadiene-1-imine) (Kim et al., 2007; Dunnick et al., 2014). The formation of *p*-methylphenylhydroxylamine may lead to the oxidation of haemoglobin (Potter et al., 1988) or DNA binding (Marques et al., 1997). *N,N*-Dimethyl-*p*-toluidine *N*-oxide, considered to be catalysed primarily by flavin-containing monooxygenases, was also excreted in urine, as was a small amount of parent *N,N*-dimethyl-*p*-toluidine (Kim et al., 2007).

Table 4.1 Genetic and related effects of *N,N*-dimethyl-*p*-toluidine in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point/test	Results	Dose (LED/HID)	Reference
Rat, Sprague-Dawley (M)	Liver	DNA damage/ DNA strand breaks	±	4 mg/kg bw, gavage and i.p.	Taningher et al. (1993)
Rat, Sprague-Dawley (M)	Liver	DNA damage/ DNA strand breaks	+	60 mg/kg bw, gavage, 4 days	NTP (2012)
Mouse, Balb/C (M)	Liver	DNA damage/ DNA strand breaks	±	1 mg/kg bw, gavage and i.p.	Taningher et al. (1993)
Mouse, B6C3F$_1$ (M)	Blood leukocytes, liver	DNA damage/ DNA strand breaks	–	75 mg/kg bw × 4 days, gavage	NTP (2012)
Mouse, B6C3F$_1$ (M & F)	Peripheral blood	Chromosomal damage/ micronucleus formation	–	125 mg/kg bw per day for 3 mo; gavage	NTP (2012)
Mouse, B6C3F$_1$ (M)	Peripheral blood	Chromosomal damage/ micronucleus formation	–	75 mg/kg bw × 4 days, gavage	NTP (2012)

+, positive; –, negative; ±, equivocal (variable responses in several experiments within an adequate study); bw, body weight; F, female; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male; mo, month

4.2 Mechanisms of carcinogenesis

The evidence on the key characteristics of carcinogens (Smith et al., 2016), concerning whether *N,N*-dimethyl-*p*-toluidine is genotoxic and alters cell proliferation, cell death, and nutrient supply, is summarized below.

4.2.1 Genetic and related effects

(a) Humans

No data in exposed humans were available to the Working Group. In cultured human lymphocytes, the bone cement polymethyl methacrylate, containing *N,N*-dimethyl-*p*-toluidine (2.6% w/w) and hydroquinone (75 ± 15 ppm), significantly increased the frequency of micronucleus formation. However, the components of the mixture were not tested individually (Bigatti et al., 1994).

(b) Experimental systems

See Table 4.1, Table 4.2, and Table 4.3

(i) In vivo

In the livers of male BALB/c mice and male Sprague-Dawley rats given *N,N*-dimethyl-*p*-toluidine by gavage or intraperitoneal injection, DNA fragmentation was modestly increased by the alkaline elution test (Taningher et al., 1993).

The alkaline comet assay gave negative results in liver cells or blood leukocytes of male B6C3F$_1$ mice treated by gavage once daily for 4 days at 30–75 mg/kg bw. In contrast, male Sprague-Dawley rats given daily doses of 60 mg/kg bw for 4 days had a small but statistically significant (P = 0.024) increased percentage of tail DNA in the liver cells compared with the group administered the corn-oil vehicle only (NTP, 2012).

Neither the frequencies of micronucleated erythrocytes nor the percentage of circulating reticulocytes were altered in the peripheral blood of male or female B6C3F$_1$/N mice treated by gavage with *N,N*-dimethyl-*p*-toluidine, suggesting that neither chromosomal damage nor bone marrow toxicity were induced in mice within the dose range and time periods tested (NTP, 2012).

(ii) In vitro

N,N-Dimethyl-*p*-toluidine showed both aneugenic and clastogenic activity in a concentration–related manner in the absence of metabolic activation in Chinese hamster V79 cells (Taningher et al., 1993).

Table 4.2 Genetic and related effects of *N*,*N*-dimethyl-*p*-toluidine in non-human mammalian cells in vitro

Test system, tissue	End-point/ test	Results	Agent, concentration (LEC/HIC)	Comments	Reference
Chinese hamster, CHO-K1 ovary cell line	Chromosomal damage/ micronucleus formation	+	Camphorquinone/DMPT (equimolar), 0–1 mM	DMPT alone was not tested; antioxidants had preventive effects	Li et al. (2007)
Chinese hamster, CHO-K1 ovary cell line	Chromosomal damage/ micronucleus formation	+	9-Fluorenone/DMPT (equimolar), 0–0.5 mM	DMPT alone was not tested; antioxidants had preventive effects	Li et al. (2008)
Chinese hamster lung, V79 cells	Chromosomal damage/ micronucleus formation	+	DMPT, 0.3 mM	Dose-dependent response (0.3–1.2 mM)	Taningher et al. (1993)

+, positive; DMPT, *N*,*N*-dimethyl-*p*-toluidine; HIC, highest ineffective concentration; LEC, lowest effective concentration

A concentration-related increase in micronuclei was observed in the CHO-K1 ovary cell line exposed to camphorquinone and *N*,*N*-dimethyl-*p*-toluidine or 9-fluorenone and *N*,*N*-dimethyl-*p*-toluidine ($P < 0.05$), with or without visible light irradiation (Li et al., 2007, 2008) [*N*,*N*-dimethyl-*p*-toluidine was not tested alone].

N,*N*-Dimethyl-*p*-toluidine was marginally positive in *Salmonella typhimurium* strain TA100 in the presence and absence of metabolic activation, and in strain TA104 in the presence of metabolic activation, but not in TA98 with or without metabolic activation. The compound was not mutagenic in plate incorporation tests (Miller et al., 1986).

Negative results were reported with or without metabolic activation in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 and in *Escherichia coli* WP2 *uvrA*/pKM101 (Taningher et al., 1993; NTP, 2012).

When tested in a bioluminescent test for bacterial genotoxicity using dark mutants of a marine luminous bacterium (*Vibrio fischeri* M169) and a 24-hour exposure period, *N*,*N*-dimethyl-*p*-toluidine showed considerable genotoxic activity at 4 mM [the half maximal inhibitory concentration value was also approximately 4 mM] (Nomura et al., 2006).

Exposure to camphorquinone in association with *N*,*N*-dimethyl-*p*-toluidine induced DNA strand breaks in PhiX-174 RF double-stranded plasmid DNA (Pagoria et al., 2005; Winter et al., 2005; Lee et al., 2007) [*N*,*N*-dimethyl-*p*-toluidine was not tested alone].

4.2.2 Altered cell proliferation or death

(a) Humans

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

N,*N*-Dimethyl-*p*-toluidine did not induce cytotoxicity in cultured human oral keratinocytes (OKF6/TERT 2), and simultaneous treatment with camphorquinone and *N*,*N*-dimethyl-*p*-toluidine did not affect apoptosis (Volk et al., 2014).

When assessed in human gingival fibroblasts in vitro, *N*,*N*-dimethyl-*p*-toluidine (500 µM) induced a significant and sustained accumulation of cells in the G0/G1 stage of the cell cycle compared with controls at 24 hours, implying that the growth inhibitory effect resulted from the arrest of DNA replication. The pattern of cell

Table 4.3 Genetic and related effects of *N,N*-dimethyl-*p*-toluidine in bacterial mutation tests

Species	Strain	End-point/ test	Results		Concentration (LEC/HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Salmonella typhimurium	TA98 TA100 TA104	Mutation/ reverse mutation (spot test)	– ± –	– ± ±	10 µL (of 300 µg/µL)/ plate	Negative in plate incorporation tests	Miller et al. (1986)
Salmonella typhimurium	TA97, TA98, TA100	Mutation/ reverse mutation	–	–	70 µg/plate		Taningher et al. (1993)
Salmonella typhimurium	TA97, TA98, TA100, TA1535	Mutation/ reverse mutation	–	–	1000 µg/plate		NTP (2012)
Salmonella typhimurium	TA98, TA100	Mutation/ reverse mutation	–	–	1500 µg/plate		NTP (2012)
Escherichia coli	WP2 *uvrA*/ pKM101	Mutation/ reverse mutation	–	–	1500 µg/plate		NTP (2012)
Vibrio fischeri	M169	Mutation/ other	+	NT	4 mM	Bioluminescence assay	Nomura et al. (2006)

+, positive; –, negative; ±, equivocal (variable responses in several experiments within an adequate study); DMPT, *N,N*-dimethyl-*p*-toluidine; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

death was consistent with necrosis (Masuki et al., 2007).

In human osteoblastic cells exposed to different bone-cement extracts, a significant correlation was found between the concentration of *N,N*-dimethyl-*p*-toluidine present in the extracts (up to 1 µg/100 mL) and a delay in cell-replication cycle (Stea et al., 1997). [The Working Group noted that the extracts did not contain *N,N*-dimethyl-*p*-toluidine only.]

(b) Experimental systems

In rat polymorphonuclear leukocytes, *N,N*-dimethyl-*p*-toluidine increased cytotoxicity in a concentration-dependent manner (Liso et al., 1997).

4.3 Data relevant to comparisons across agents and end-points

N,N-Dimethyl-*p*-toluidine was not tested by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013). Analyses of other compounds evaluated in this volume with high-throughput screening data are presented in the *Monograph* on 1-bromopropane in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5. Other adverse effects

4.5.1 Humans

Methaemoglobinaemia, which is consistent with metabolism of *N*,*N*-dimethyl-*p*-toluidine to *p*-methylphenylhydroxylamine, was described in two poisoning cases of children who had ingested artificial fingernail solutions containing acetone, ethyl methacrylate, and *N*,*N*-dimethyl-*p*-toluidine (Potter et al., 1988) or acrylic ester monomers and *N*,*N*-dimethyl-*p*-toluidine (Kao et al., 1997).

Allergic responses to dental materials containing *N*,*N*-dimethyl-*p*-toluidine has been reported (Tosti et al., 1990; Verschueren & Bruynzeel, 1991). Positive reactions to *N*,*N*-dimethyl-*p*-toluidine were found in seven cases, all of whom showed rapid onset of aseptic loosening of total hip replacements, out of 70 patients (Haddad et al., 1996).

4.5.2 Experimental systems

In a chronic study, *N*,*N*-dimethyl-*p*-toluidine caused methaemoglobinaemia in F344/N rats after administration by gavage (NTP, 2012; Dunnick et al., 2014). Metabolic activation to *p*-methylphenylhydroxylamine was inferred from the presence of *p*-(*N*-acetylhydroxyamino) hippuric acid in the urine of rats given the compound (Kim et al., 2007; see Section 4.1.2). Macrocytic regenerative anaemia, methaemoglobinaemia, and increased Heinz body production was seen in rats (and to a lesser extent in mice) in a subchronic gavage study. Bone marrow hyperplasia occurred in exposed rats, but not in mice (NTP, 2012; Dunnick et al., 2014).

5. Summary of Data Reported

5.1 Exposure data

N,*N*-Dimethyl-*p*-toluidine is a chemical with a high production volume that is used worldwide as an accelerator in the preparation of dental materials, bone cements, industrial glues and artificial-fingernail preparations, and also as an intermediate in the production of dyes and pesticides. Chemical-production workers, surgeons, surgical staff, dentists, dental technicians, nail-salon operators, and users of industrial glues may be exposed to *N*,*N*-dimethyl-*p*-toluidine by inhalation and through the skin. Exposure among the general population may occur from dental material, bone cement, sniffing glue and fingernail solutions. No quantitative data on exposure or occupational exposure limits were identified.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

N,*N*-Dimethyl-*p*-toluidine was tested for carcinogenicity by oral administration (gavage) in one good laboratory practice (GLP) study in male and female mice and one GLP study in male and female rats.

In mice, *N*,*N*-dimethyl-*p*-toluidine caused a significantly increased incidence (with a significant positive trend, except for hepatoblastoma in males) of hepatocellular carcinoma, hepatoblastoma, hepatocellular adenoma or carcinoma (combined), and hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) in males, and hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, hepatocellular adenoma or carcinoma (combined), and hepatocellular adenoma,

hepatocellular carcinoma or hepatoblastoma (combined) in females. It also caused a significantly increased incidence of alveolar/bronchiolar adenoma of the lung (with a significant positive trend), alveolar/bronchiolar adenoma or carcinoma (combined) of the lung (with a significant positive trend), forestomach squamous cell papilloma (with a significant positive trend) and forestomach squamous cell papilloma or carcinoma (combined) in female mice.

In rats, *N*,*N*-dimethyl-*p*-toluidine caused a significantly increased incidence (with a significant positive trend) of hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), adenoma of the transitional epithelium of the nasal cavity and adenoma or carcinoma (combined) of the transitional epithelium of the nasal cavity in males. A significant positive trend was observed in the incidence of carcinoma of the transitional epithelium of the nasal cavity in males. Increases in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid gland in males were not significant by the pairwise or trend tests, although the incidence of these tumours at the highest dose exceeded the range for historical controls for these tumours. A significant positive trend was observed in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in females. The incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in females given the highest dose were significantly increased compared with controls. The incidence of adenoma of the transitional epithelium of the nasal cavity in females given the highest dose exceeded the range for historical controls for this tumour.

5.4 Mechanistic and other relevant data

In rats and mice, *N*,*N*-dimethyl-*p*-toluidine is rapidly absorbed after oral administration. *N*,*N*-Dimethyl-*p*-toluidine and/or its metabolites are distributed widely to the tissues and the highest concentrations are detected in the liver and kidney of rats and lung and liver of mice. *N*,*N*-Dimethyl-*p*-toluidine is rapidly metabolized and excreted primarily in the urine. The major metabolic pathway leading to the formation of *p*-(*N*-acetylhydroxyamino)hippuric acid in rats includes putative reactive intermediates. The fate of *N*,*N*-dimethyl-*p*-toluidine has not been determined in humans.

With respect to the key characteristics of human carcinogens, there is *moderate* evidence that *N*,*N*-dimethyl-*p*-toluidine is electrophilic or can be metabolically activated, is genotoxic, or alters cell proliferation, cell death or nutrient supply.

A putative reactive intermediate of *N*,*N*-dimethyl-*p*-toluidine, *p*-methylphenylhydroxylamine, was implicated in the formation of methaemoglobinaemia in a case of unintentional (human) oral exposure to *N*,*N*-dimethyl-*p*-toluidine. *N*-Hydroxylated arylamines are capable of forming DNA adducts. The formation of an electrophilic *p*-quinone imine methide as a result of the metabolism of *N*,*N*-dimethyl-*p*-toluidine is plausible.

A significant increase in the frequency of micronucleus formation was observed in cultured human lymphocytes treated with the bone cement polymethyl methacrylate containing *N*,*N*-dimethyl-*p*-toluidine. *N*,*N*-Dimethyl-*p*-toluidine exhibited aneugenic and clastogenic activity in Chinese hamster V79 cells. *N*,*N*-Dimethyl-*p*-toluidine gave negative or marginally positive results in other assays, including mutation in *Salmonella* and DNA strand breaks in rats and mice.

The cell-replication cycle was delayed in human osteoblastic cells exposed to bone-cement extracts containing *N*,*N*-dimethyl-*p*-toluidine. *N*,*N*-Dimethyl-*p*-toluidine had a growth-inhibitory effect in human gingival fibroblasts in vitro and caused cytotoxicity in rat polymorphonuclear leukocytes, but not in cultured human oral keratinocytes.

There were few other data on other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, induces oxidative stress, induces chronic inflammation, is immunosuppressive, modulates receptor-mediated effects, or causes immortalization).

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *N*,*N*-dimethyl-*p*-toluidine.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*,*N*-dimethyl-*p*-toluidine.

6.3 Overall evaluation

N,*N*-Dimethyl-*p*-toluidine is *possibly carcinogenic to humans (Group 2B)*.

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HYDRAZINE

Hydrazine was considered by the *IARC Monographs* Working Group in 1973, 1987, and 1998 (IARC, 1974, 1987, 1999). New data have since become available and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 302-01-2

Chem. Abstr. Serv. Name: Hydrazine

IUPAC Systematic Name: Hydrazine

Synonyms: Levoxine, nitrogen hydride, diamide, diamine, anhydrous hydrazine, hydrazine base.

1.1.2 Structural and molecular formulae, and relative molecular mass

$H_2N–NH_2$

Molecular formula: H_4N_2

Relative molecular mass: 32.05

1.1.3 Chemical and physical properties of the pure substance

From IPCS (1987a), ECHA (2011), NIOSH (2014), HSDB (2010)

Description: Colourless, fuming, oily liquid with a penetrating ammonia-like odour; odour threshold, 3–9 mg/m^3

Note: Anhydrous hydrazine contains less than 37% water by mass; hydrazine hydrate [CAS No.: 7803-57-8] corresponds to a 64% or less aqueous solution of hydrazine (HSDB, 2010)

Boiling point: 113.5 °C

Melting point: 2 °C

Vapour density: 1.1 (air = 1)

Solubility: 282.0 g/100 g in water at 25 °C; miscible with methyl, ethyl, propyl, and isobutyl alcohols; insoluble in chloroform and diethyl ether

Volatility: Vapour pressure, 14.4 mm Hg at 25 °C

Octanol/water partition coefficient (P): log K_{ow}, –2.07 at 25 °C

Flash point: 52 °C

Explosive limits: Upper, 100%; lower, 1.8% by volume in air

Stability: Hydrazine is stable when stored, but may cause hazardous reactions when in contact with strong oxidizing agents or acids. Contact with cellulose or cotton textiles, especially at elevated temperatures, may

result in ignition. Vapours may form explosive mixtures with air when the substance is heated above its flash point.

Decomposition: Forms nitrogen oxides, ammonia, and hydrogen

Conversion factor: 1 ppm = 1.31 mg/m^3 at 25 °C

1.2 Production and use

1.2.1 Production

(a) Production process

Most hydrazine is produced by a variation of the Raschig process, where ammonia is oxidized using alkaline hypochlorite with or without the presence of a ketone such as acetone or butanone. It can also be made by the oxidation of ammonia by hydrogen peroxide in the presence of butanone and an oxygen-transfer agent (Schiessl, 2003).

Anhydrous hydrazine is made by breaking the hydrazine–water azeotrope with aniline. The aniline–water vapour condenses and phase separates. The water layer, contaminated with a small amount of aniline and hydrazine, flows to a biological treatment pond. The aniline and anhydrous hydrazine are separated in a final column (Schiessl, 1995).

(b) Production volume

(i) Anhydrous hydrazine

In 2009, hydrazine was produced by three manufacturers worldwide (SRI, 2009; as cited in NTP, 2014). Hydrazine was then available from 27 suppliers (ChemSources, 2009; as cited in NTP, 2014). USA imports of "hydrazine and hydroxylamine and their salts" generally increased between 1989 and 2008, to a maximum of 235 000 tonnes in 1999 (USITC, 2009 as cited in NTP, 2014).

Anhydrous hydrazine was not manufactured in the European Union in 2011, and less than 10 tonnes per year was imported to the European Union (ECHA, 2011). The following suppliers of hydrazine in 2016 were identified: 27 suppliers in China, 26 in the USA, 5 in the United Kingdom, 3 in Germany, 2 in Japan, and 1 in each of 5 other countries; production volumes were not reported (ChemicalBook, 2016).

(ii) Hydrazine hydrate

Production capacity estimates for hydrazine hydrate in 1988 were: USA, 25 000 tonnes; France, 10 000 tonnes; Germany, 10 000 tonnes; Japan, 5000 tonnes; and United Kingdom, 3000 tonnes (Schirmann, 1989).

By 2011, hydrazine hydrate was manufactured in China, France, Germany, Japan, and the Republic of Korea, and possibly in the Russian Federation and the USA. The worldwide market demand for hydrazine hydrate was then about 80 000–90 000 tonnes per year (ECHA, 2011).

The following suppliers of hydrazine hydrate in 2016 were identified: 178 suppliers in China, 27 in the USA, 11 in the United Kingdom, 6 in Japan, 4 in Germany, and 1 in each of 5 other countries; production volumes were not reported (ChemicalBook, 2016).

1.2.2 Use

In its hydrated form, hydrazine (solutions with concentrations ranging from 0.01% to 100%) is used in multiple applications. It is used to synthesize pharmaceuticals, agrochemicals, and chemical blowing agents; as a stabilizing agent of aromatic amines for manufacture of paints, inks, and organic dyes; and as a reagent for the treatment of nuclear reactor waste. Hydrazine is used as a monomer in polymerizations, mostly for polyurethane coatings and adhesives; as a corrosion inhibitor in water treatment, mainly for the removal of dissolved oxygen; for pH adjustment in the feed water of boilers; and for the removal of solids from steam generators, notably in nuclear and thermal power-generation plants. Oxygen scavenging of steam by hydrazine may

also be carried out in paper mills, steel manufacture, and chemical production. It is also used as a reducing agent in the deposition of metals (e.g. nickel, chromium, tin, and precious metals); in plastics and glass manufacture; for recovery of precious and basic metals from metallic salt solutions; and as effluent sand in the purification of chemical reagents. Hydrazine is also a laboratory chemical reagent (ECHA, 2011).

The anhydrous form (at high purity levels, > 90%) is used as a propellant for aerospace vehicles (e.g. satellite propulsion and upper stages of satellite launchers); as a fuel in military power units for the F-16 fighter jet aircraft; and in gas generators for submarine rescue systems (ECHA, 2011).

1.3 Measurement and analysis

Hydrazine compounds are highly soluble in water and can be measured by spectrophotometry (HSDB, 2010).

Sampling and analysis methods for hydrazine in a variety of matrices are listed in Table 1.1. Other hydrazines, such as 1,1- and 1,2-dimethylhydrazine, may interfere with these methods.

In addition, direct-reading papers or indicating tubes, based on colorimetric methods, are available commercially, with reported limits of detection of 65 µg/m³ for tapes and 330 µg/m³ for tubes (IPCS, 1987a).

More recently, an optical probe for hydrazine in air has been described. It uses colorimetric, fluorescent, and chemiluminescent outputs, and has a limit of detection of 3.2 ppb (0.1 µM) (Cui et al., 2014).

1.4 Occurrence and exposure

1.4.1 Natural occurrence

Hydrazine is naturally produced by the algae *Azotobacter agile* as a result of nitrogen fixation (ATSDR, 1997), and in tobacco plants (Choudhary & Hansen, 1998).

1.4.2 Environmental occurrence

Production and use of hydrazine may result in its release to the environment. It has been detected at low levels in wastewater samples (HSDB, 2010).

1.4.3 Occupational exposure

The National Occupational Exposure Survey estimated that 60 490 workers (including 2841 women) in the USA were potentially exposed to hydrazine between 1981 and 1983 (NIOSH, 1997). National estimates on exposure were not available from other countries.

Hydrazine is usually handled in closed systems, but fugitive emissions may occur. Table 1.2 presents data on occupational exposures for hydrazine.

Data suggested that open manual transfer of hydrazine hydrate results in exposure to hydrazine of < 0.02 mg/m³, and that exposure is lower when closed transfer is used (HSE, 2006). Exposures of 0.014–0.35 mg/m³ (maximum, 1.18 mg/m³) have been measured in hydrazine hydrate production, and 0.29–2.59 mg/m³ in a rocket propellant facility where hydrazine was handled (Cook et al., 1979; IPCS, 1987a). Refilling F-16 aircraft with hydrazine resulted in exposures as high as 10.5 mg/m³, but they were usually < 0.13 mg/m³ (Christensen, 1978; IPCS, 1987a). Exposure during a spillage of hydrazine was measured as 800 mg/m³ (Suggs et al., 1980).

Hydrazine hydrate and acetylhydrazine were separately measured, as a result combined at 0.8660 µmol/g creatinine (range, not detected to 14.20 µmol/g creatinine) in the urine of production workers in five factories who had been exposed to hydrazine at 0.014 mg/m³ (range, not detected to 0.262 mg/m³) (Nomiyama et al., 1998a).

1.4.4 Exposure of the general population

No data were available to the Working Group.

Table 1.1 Selected methods of analysis of hydrazine in various matrices

Sample matrix	Sample preparation	Assay method	Limit of detection	Reference
Air	Collect in bubbler with hydrochloric acid; neutralize with sodium hydroxide; derivatize with dimethyl-aminobenzaldehyde; dilute with glacial acetic acid [NIOSH method 3503]	SP	0.9 µg/sample	NIOSH (1984)[a]
Air	Adsorb on sulfuric acid-coated silica gel; elute with water; derivatize with 2-furaldehyde; extract with ethyl acetate [NIOSH method 248]	GC/FID	0.002 mg/m^3	NIOSH (1977a)[a]
Air	Collect in bubbler with hydrochloric acid; derivatize with phosphomolybdic acid [NIOSH method S143]	SP	0.02 mg/m^3	NIOSH (1977b)[a]
Air	Collect in micro-impinger containing acetone and glacial acetic acid	GC/NSD	5 µg/m^3	Holtzclaw et al. (1984)[a]
Air	Collect on sulfuric acid coated Gas Chrom R; desorption with water [Method ORG-20]	HPLC	1.6 µg/m^3	OSHA (1980a)
Air	Collect on sulfuric acid-treated glass fibre filters; extract with buffered EDTA disodium solution; derivatize with benzaldehyde [Method ORG-108]	LC/UV	0.076 µg/m^3	OSHA (1980b)
Air	*Method 1* (filter sampling; recommended for personal sampling up to 2 hours): collect on filters treated with phosphoric acid in acetonitrile solution; extract in sulfuric acid; derivatize in benzaldehyde in methanol; add sodium tetraborate buffer solution	HPLC/UV	0.002 ppm	HSE (2014)
Air	*Method 2* (impinge sampling; recommended for long-term static sampling): collect in midget impinger containing dilute sulfuric acid; derivatize in benzaldehyde in methanol; add sodium tetraborate buffer solution	HPLC/UV	0.002 ppm	HSE (2014)
Water	Acidify with hydrochloric acid; derivatize with *p*-dimethyl-aminobenzaldehyde	SP	5 µg/L	ASTM (1991)[a]
Water	Derivatize with vanillin in ethanol; acidify with sulfuric acid	SP	0.065 ppm	Amlathe & Gupta (1988)[a]
Water	Derivatize with 5-chlorosalicylaldehyde in acidified aqueous solution (ethanol/water/acetic acid = 30/66/4)	Fl at 570 nm	0.08 µM	Chen et al. (2008)
Water	Derivatize with acetone; extract with dichloromethane; dry with anhydrous sodium sulfate; concentrate by evaporation	GC/MS	0.70 ng/L	Davis & Li (2008)
Water	Derivatize with propyl chloroformate and pyridine; solid-phase microextraction	GC/MS	4.4 ng/L	Gionfriddo et al. (2014)
Water	Derivatize with *ortho*-phthalaldehyde, extract with methylene chloride	GC/MS	Surface water, 0.002 µg/L; drinking-water, 0.007 µg/L	Oh et al. (2013)
Soil	Extract with sulfuric acid; derivatize with 2,4-pentanedione	GC/TID	0.1 ppm	Leasure & Miller (1988)[a]
Food	Extract with L-ascorbic acid; derivatize with 2-nitrobenzaldehyde; clean-up on alumina column	GC/ECD	10 ppb	Wright (1987)[a]

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Limit of detection	Reference
Food	Derivatize with pentafluorobenzoyl chloride; extract with methylene chloride	GC/MS	0.01 ppm	Rutschmann & Buser (1991)[a]
Tobacco & tobacco smoke	Derivatize with pentafluorobenzaldehyde; enrich with thin-layer chromatography; extract with ether	GC/ECD	0.1 ng/cigarette	Liu et al. (1974)[a]
Smokeless tobacco	Derivatize with pentafluorobenzaldehyde; extract with hexane	GC/MS	10 ng/g smokeless tobacco	McAdam et al. (2015)
Urine	Precipitate protein with hydrochloric acid and ammonium sulfate, extract lipids with methylene chloride, derivatize aqueous fraction with pentafluorobenzaldehyde, extract with ethyl acetate	GC/NPD	8 µmol	Preece et al. (1992b)[a]
Urine	Extract with methylene chloride, discard extract; derivatize aqueous fraction with *p*-chlorobenzaldehyde; extract with methylene chloride; dry and dissolve in ethyl acetate	GC/NPD	0.05 µg/mL	Timbrell & Harland (1979)[a]
Urine	Deproteinate with trichloroacetic acid; derivatize with vanillin in ethanol; acidify with sulfuric acid	SP	0.065 µg/mL	Amlathe & Gupta (1988)[a]
Plasma/Urine	None/Dilute with deionized water	Ion-exchange HPLC/ECD	8 ng/sample	Fiala & Kulakus (1981)[a]
Plasma, liver tissue	Precipitate residual protein with hydrochloric acid and ammonium sulfate, extract lipids with methylene chloride, derivatize aqueous fraction with pentafluorobenzaldehyde, extract with chloroform	GC/MS	~20 nmol/mL	Preece et al. (1992b)[a]
Serum, liver/ brain tissue	Acidify; derivatize with *p*-dimethylaminobenzaldehyde in ethanol	SP	0.025 µg/sample	Alvarez de Laviada et al. (1987)[a]
Serum	Treat with trichloroacetic acid; centrifuge; derivatize supernatant with *p*-dimethylaminobenzaldehyde in ethanol	SP	0.05 µg/mL	Reynolds & Thomas (1965)[a]
Living cells	Probe was synthesized from *n*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride added to resorufin and 4-bromobutyric acid in dichloromethane; evaporated; purified and added to buffered cells	NMR/MS	~2 µM	Qian et al. (2014)

[a] As cited in ATSDR (1997)

ECD, electrochemical detection; EDTA, ethylenediaminetetraacetic acid; FID, flame ionization detector; Fl, fluorescence; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; NPD, nitrogen-phosphorus detector; NSD, nitrogen selective detection; SP, spectrophotometry; TID, thermionic ionization detector; UV, ultraviolet

Table 1.2 Occupational exposures to hydrazine

Country	Job/process	Numbers of people exposed	Air concentration (mg/m³)		Reference
			Typical	Exceptional	
USA	F-16 fighter jet refilling including nitrogen depressurization, catalyst purge, poppet valve replacement	NR	< 0.13	6.6–10.5	Christensen (1978)
USA	F-16 fighter jet applications	32		0.04–0.05	IPCS (1987a)
USA	Production	100–800	< 0.13	0.13–0.26[a]	IPCS (1987a)
USA	Production	≤ 1100	< 0.35	< 1.18	IPCS (1987a)
USA	Derivative manufacture	< 25	< 0.13	~0.13	IPCS (1987a)
NR	Evaporation from liquid spill	NR	4		IPCS (1987b)
NR	At site of leakage	NR		800	Suggs et al. (1980)
USA	Rocket propellant-handling facility	NR	0.29–2.59 0.013 (inside RPE)		Cook et al. (1979)
USA	Rocket testing	10–100	0.01–0.02	0.14[b]	IPCS (1987a)
NR	Process stream sampling	NR	0.05–0.35	1.19	ATSDR (1997)
Japan[c]	Production in 5 factories	NR	0.014	ND–0.262	Nomiyama et al. (1998b)
United Kingdom[c]	Manual processing and distributing hydrazine hydrate solutions, open transfer (45–60 min)	NR NR		0.0045 0.0096	HSE (2006)
	Manual processing and distributing hydrazine hydrate solutions, open transfer (41 min)	NR		0.013	
	Manual processing and distributing hydrazine hydrate solutions, closed transfer (15 min)	NR NR		0.002 0.003	

[a] Short-term samples during specific operations
[b] Level measured during aeration of the wastewater holding period
[c] The data from Japan and the United Kingdom are for hydrazine hydrate, the others for hydrazine; note that exposures are all measured as hydrazine, but the processes involved handling of hydrazine hydrate
ND, not detected; NR, not reported; RPE, respiratory protection equipment
Compiled by the Working Group with data reported by ECHA (2011)

1.4.5 Exposure assessment in epidemiological studies

Workers were commonly also exposed to other carcinogens, which made it difficult to attribute identified risks specifically to hydrazine (Cammarano et al., 1984; Wald et al., 1984; Zhao et al., 2005; Krishnadasan et al., 2007). In a nested case–control study of 6107 aerospace workers and 4607 workers enrolled in a radiation monitoring programme, 87% of those exposed to hydrazine were also exposed to trichloroethylene, while levels of radiation were not reported (Krishnadasan et al., 2007).

In a retrospective cohort study in aerospace workers in southern California, USA, which used interviews, historical facility reports, and walkthrough surveys, job titles were discussed and classified into a time-dependent semi-quantitative job–exposure matrix (JEM) with respect to hydrazine compounds (e.g. hydrazine, 1-methylhydrazine, and 1,1-dimethylhydrazine) (Morgenstern & Ritz, 2001; Zhao et al., 2005; Ritz et al., 2006). The JEM was used to classify jobs held for at least 6 or 12 months (depending on the job) into low, medium, or high exposure. Morgenstern & Ritz (2001) stated that the categories reflect the probability, not the amount,

of hydrazine exposure, but this was not further clarified (Morgenstern & Ritz, 2001); however, subsequent reports referred to the classification in terms of intensity of exposure (Zhao et al., 2005; Ritz et al., 2006); any employee involved in working hands-on with rocket engines or in fuel production and testing was assumed to have been exposed to hydrazine. For example, rocket test-stand mechanics were assigned to the highest intensities of exposure because they probably had the greatest contact with rocket engines (Ritz et al., 2006). Each job title was assigned to one of four categories of presumptive exposure (high, medium, low, or unexposed) for each chemical reflecting the relative intensity of that exposure in each of three periods: the 1950–1960s, the 1970s, and the 1980–1990s. A cumulative exposure score for each worker in the cohort was calculated by summing across all employment periods. Thus, each job received an intensity score (0 to 3, from unexposed to highly exposed) that was multiplied by the number of years in the job and then summed. In separate analyses, to take account of the fact that more hydrazine was used in the 1960s, scores of 1 for low, 4 for medium, and 9 for high intensities were used for that decade, retaining the original scores for all other decades (Ritz et al., 2006). In addition to hydrazine, there was exposure to other known carcinogens at the facility, including trichloroethylene, polyaromatic hydrocarbons, mineral oils, and benzene. In Ritz et al. (2006), a new JEM was derived assessing these exposures, and this information was used to adjust the hydrazine effect estimates for potential confounding by co-exposure to other chemicals (Ritz et al., 2006).

In a subsequent nested case–control study of cancer of the prostate identified through cancer registries, five controls per case were randomly selected, matched on age at start date of employment, and cohort (radiation or aerospace). For cases and controls, 69% had no identified exposure to hydrazine; 21% of cases and 24% of controls were classified as having had low or moderate exposure; and 11% of cases and 7% of controls were classified as having had high exposure (Krishnadasan et al., 2007).

A separate and subsequent study at the same aerospace facility used company records to identify test-stand mechanics, including those monitored for radiation. These workers were paid hourly, unlike most of the test-stand workers (instrument mechanics, inspectors and engineers) who were salaried. The mechanics who were paid hourly were thought to be most likely to have been "hands-on" and hence to have had a higher probability of exposure to hydrazine and trichloroethylene. Hydrazine was present in rocket fuel at some but not all rocket-engine test stands. The electronic personnel files did not identify on which rocket stands the mechanics had worked, so the authors used company telephone numbers to identify a minority of the test-stand mechanics who were thought to have worked on stands that used hydrazine. The assignments were validated by "information gathered from walkthrough surveys at operating and closed test stands with knowledgeable personnel who were involved with engine tests over the years, discussions with over 100 long-term employees (both retired and active), and review of medical records of workers, which often identified the test stands and the chemicals used" (Boice et al., 2006).

Ritz et al. (1999) had been unable to link workers to specific test stands using company records, but former employees stated that many workers frequently changed work locations. In addition, leakage of fuel was thought to be a common source of exposure (Ritz et al., 1999).

In a separate study of a cohort of workers from a site in the east Midlands, United Kingdom, that manufactured hydrazine in open tanks and vessels, the exposure assessment was weaker. No measurements of the concentration of hydrazine in air had been made, exposure estimates were derived by the simulation of spillages and calculations using data on the saturated

vapour pressure of hydrazine. This suggested that maximum levels of 100 ppm were possible (Wald et al., 1984). Exposures were estimated to have been 1–10 ppm in the general plant area, and up to 100 ppm close to the hydrazine storage vessels (Wald et al., 1984; Morris et al., 1995). Those directly involved, for at least 6 months, in the manufacture of hydrazine or its derivatives, or its use as a raw material, were classified as having had high exposure. Moderate exposure was assigned to those with only an incidental presence in the hydrazine-manufacturing area for most of their career, e.g. fitters and engineers. Their exposure was estimated to have probably been "< 0.5 or 1 ppm for most of their employment". The remainder of the cohort were thought to have had "little or no exposure". In the risk analysis, the years at each exposure level were calculated on an individual basis (Morris et al., 1995).

1.5 Regulations and guidelines

Hydrazine is classified as a carcinogen in accordance with Article 57 (a) of the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations (EC-1907/2006; ECHA, 2011). Hydrazine is included in the list of harmonized classification and labelling of hazardous substances as carcinogen category 1B (ECHA, 2011).

According to the European Chemicals Agency (ECHA), the following Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements apply: H301 "toxic if swallowed"; H311 "toxic in contact with skin"; H314 "causes severe skin burns and eye damage"; H317 "may cause an allergic skin reaction"; H331 "toxic if inhaled"; H350 "may cause cancer"; H400 "very toxic to aquatic life"; H410 "very toxic to aquatic life with long lasting effects"; and H226 "flammable liquid and vapour" (ECHA, 2016).

Table 1.3 International limit values for occupational exposure to hydrazine

Country	Limit value, 8 hours		Limit value, short-term	
	ppm	mg/m³	ppm	mg/m³
Australia	0.01	0.013		
Austria	0.10	0.130	0.40	0.52
Belgium	0.01	0.013		
Canada, Ontario	0.01			
Canada, Quebec (Province)	0.10	0.130		
Denmark	0.01	0.013	0.02	0.026
Finland	0.01	0.013	0.05[a]	0.07[a]
France	0.10	0.100		
Germany (AGS)	0.017[b] 0.0017[c]	0.022[b] 0.0022[c]	0.034[a,b]	0.044[a,b]
Hungary				0.13
Ireland	0.01	0.01		
Latvia		0.10		
New Zealand	0.01	0.013		
People's Republic of China		0.06		0.13[a]
Poland		0.05		0.10
Republic of Korea	0.05	0.06		
Singapore	0.10	0.13		
Spain[d]	0.10	0.13		
Switzerland	0.10	0.13		
USA, NIOSH			0.03[e]	0.04[e]
USA, OSHA	1.00	1.30		
United Kingdom	0.02	0.03	0.10	0.13

[a] 15 minutes average value
[b] Workplace exposure concentration corresponding to the proposed tolerable cancer risk
[c] Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk
[d] Skin, sensitivity
[e] Ceiling limit value
AGS, German Committee on Hazardous Substances (Ausschuss für Gefahrstoffe); NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration
From the GESTIS Substance Database (IFA, 2015)

The United States Environmental Protection Agency (EPA) Integrated Risk Information System classifies hydrazine as a B2 carcinogen, a probable human carcinogen based on sufficient evidence of carcinogenicity in animals. The inhalation unit risk rate was stated as 4.9×10^{-3} per µg/m³ based on a linearized multistage procedure

"extra risk model." The oral cancer slope factor was stated as 3.0 (mg/kg per day)$^{-1}$ [these values are applicable to lifetime exposure] (EPA, 2016).

Hydrazine was listed as a chemical that may cause cancer in 1988, under California's proposition 95, The Safe Drinking-water and Toxic Enforcement Act 1986 (OEHHA, 2014).

The American Conference of Governmental Industrial Hygienists (ACGIH) recommended an 8-hour time-weighted average (TWA) of 0.013 mg/m^3 (0.01 ppm) as the threshold limit value for occupational exposures to hydrazine in workplace air, and have categorized it as an A2 "suspected human carcinogen" (ACGIH, 1997).

Other 8-hour TWA workplace limits vary from the proposed preliminary acceptable cancer risk value of 0.0017 ppm [0.0022 mg/m^3] in Germany, to the Occupational Safety and Health Administration (OSHA) value of 1.3 mg/m^3 (IFA, 2015) in the USA. Several countries have also set short-term exposure limits (STELs) varying between 0.02 and 0.4 ppm [0.026–0.52 mg/m^3] (IFA, 2015; Table 1.3).

The Agency for Toxic Substances and Disease Registry of the Centers for Disease Control and Prevention (CDC) in the USA has set a minimal risk level (MRL) of 0.004 ppm [0.00524 mg/m^3] for inhalation exposures of intermediate duration (ATSDR, 2016).

2. Cancer in Humans

See Table 2.1

Wald et al. (1984) studied 427 men in the east Midlands, United Kingdom, who were potentially exposed to hydrazine at a hydrazine-production plant between 1945 and 1971 (exposure at this plant ended in 1971), with at least 6 months of employment during this period. Workers were divided into presumed high, moderate, and low exposure groups. High exposure was estimated to be 1–10 ppm, moderate was < 1 ppm, and low exposure was little or no exposure. Follow-up went to 1982 in this original report and was extended in subsequent reports by Morris et al. (1995, 2015). The most recent follow-up until 2012 identified 205 deaths (Morris et al., 2015). Mean length of exposure was 6.8 years and mean follow-up was 32 years. There was no statistically significant excess risk for any cancer. The relative risk (RR) of mortality for cancer of the lung was 0.72 (95% confidence interval, CI, 0.42–1.15; 17 deaths), and no significant or close to significant excess of lung cancer was seen at any exposure level or duration (RR for high exposure group, 1.22; 95% CI, 0.45–2.67). There was no marked excess of cancer of the digestive system for all men combined (RR, 1.02; 95% CI, 0.65–1.53; 23 deaths) nor for any exposure level or duration group (RR for high exposure, 0.44; 95% CI, 0.05–1.58). Few men were in the high exposure category, and there were only six lung cancers and two digestive cancers in this group. There was an overall deficit of all-cause mortality (standardized mortality ratio, SMR, 0.77), and the authors noted that these workers were not allowed to smoke at work due to the explosive nature of hydrazine.

[The Working Group noted that this cohort was of limited value for the evaluation of hydrazine since there were few deaths among men judged to have high exposure, while other exposure groups were exposed only incidentally or were judged not to have been exposed.]

Ritz et al. (1999) studied a cohort of 6107 male workers who worked at the Santa Susana Field Laboratory rocket testing facility (Rocketdyne) near Los Angeles, California, USA. Workers had to have been employed before 1980 and worked at least 2 years, and not to have been exposed to radiation (i.e. no evidence of monitoring). Mortality follow-up went until 1994; no data on cancer incidence were reported. The average length of follow-up was 29 years. Exposure assessment was based on job title, and a JEM was developed to estimate exposure depending on job title

Table 2.1 Cohort studies of cancer and exposure to hydrazine

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Morris et al. (2015) United Kingdom, east Midlands 1947–2012	427 male hydrazine-production workers employed for ≥ 6 mo Exposure assessment method: JEM; exposure divided into high (regular, 1–10 ppm), moderate (incidental, < 0.5–1 ppm), and little or no exposure; linkage to national mortality database	Lung	All	17	0.72 (0.42–1.15)	Age, sex, period	Mean employment, 6.8 yrs; mean follow-up, 32 yrs; English referent rates Strengths: well-defined cohort Limitations: limited number of men with high exposure
			High	6	1.22 (0.45–2.67)		
		Digestive system	All	23	1.02 (0.65–1.53)		
			High	2	0.57 (0.07–2.08)		
Ritz et al. (2006) USA, California 1960–2001	6044 for mortality; 5049 for incidence; employed for ≥ 2 yrs in 1960–1980 in a rocket-testing facility Exposure assessment method: JEM; 56% had no/low exposure, 26–28% had moderate exposure, 17% had high exposure; semi-quantitative ranking based on estimated intensity of exposure; high exposure defined as likely direct contact with hydrazine; linkage to state cancer registries and National Death Index	Lung, mortality	Moderate	37	1.24 (0.78–1.96)	Pay type (categorical), time since first employment (continuous), other possible carcinogens (TCE, PAHs, mineral oils)	Results presented for 20-yr lag; some overlap with Boice et al. (2006), more rocket testing mechanics assumed exposed than in Boice et al., workers worked in earlier years than in Boice et al., during a time of likely higher exposure due to more rocket testing Strengths: long follow-up, semi-quantitative exposure assessment, cancer incidence data (California and eight adjoining states) as well as mortality Limitations: lack of direct measurement of hydrazine levels; some lack of cancer ascertainment expected
			High	36	1.67 (0.99–2.83)		
			Trend-test *P*-value: 0.031				
		Colorectum, mortality	Moderate	12	0.83 (0.35–1.95)		
			High	10	1.55 (0.61–3.90)		
			Trend-test *P*-value: 0.481				
		Lung, incidence	Moderate	22	1.18 (0.62–2.24)		
			High	26	2.49 (1.28–4.86)		
			Trend-test *P*-value: 0.003				
		Colorectum, incidence	Moderate	28	1.75 (0.93–3.30)		
			High	19	2.16 (1.02–4.59)		
			Trend-test *P*-value: 0.041				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	No. of exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Boice et al. (2006) USA, California 1948–1999	8372; worked ≥ 6 mo at rocket-testing facility in 1948–1999 Exposure assessment method: JEM; analyses of subset of 1651 test-stand mechanics of whom 315 were judged to have had likely hydrazine exposure, with another 205 judged to have had possible but unlikely hydrazine exposure; linkage to state death registries and the national death index	Lung	Mortality:			Age, sex, period	Overlap with Ritz et al. (2006), but many fewer rocket-testing mechanics classified as exposed; includes more recent workers than Ritz et al.; recent workers likely to have lower exposures Strengths: long follow-up; extensive exposure characterization based on assignment to categories Limitations: no cancer incidence data
			All workers	215	0.89 (0.78–1.02)		
			Male test-stand mechanics	63	1.07 (0.82–1.37)		
			Male test-stand mechanics with likely hydrazine exposure	15	1.45 (0.81–2.39)		
		Colorectum	Mortality:				
			All workers	70	0.97 (0.75–1.22)		
			Male test-stand mechanics	19	1.10 (0.66–1.71)		
			Male test-stand mechanics with likely hydrazine exposure	5	1.67 (0.54–3.89)		

CI, confidence interval; JEM, job–exposure matrix; PAHs, polycyclic aromatic hydrocarbons; TCE, trichloroethylene; TWA, time-weighted average

and year of employment. Work history records were available from the company. Inventories of hydrazine over time were also available for 1955–1994. Workers were assigned by expert judgment to categories of no, low, medium, and high probability of exposure to hydrazine, without reference to intensity of exposure; the low exposure group was small and results for this group were not reported. Hydrazine used in rocket fuel can exist as three variants: hydrazine itself, 1-methylhydrazine, and 1,1-dimethylhydrazine, but these could not be separated and exposure was analysed generically as "hydrazine" irrespective of the particular variant. Two sets of analyses were conducted, based on minimum length of duration of exposure (6 months or 24 months: HYD-6 or HYD-24, respectively). In some analyses, the exposure variable was high exposure by decade, as it was thought that intensity changed over time. No quantitative estimates of hydrazine exposure intensity were given. Exposure was analysed as a time-dependent variable, using highest exposure at any time, and retaining that highest exposure category if subsequent exposure was lower. Cancer of the lung was of primary interest in the analysis, but data for cancers of the lymphoid and haematopoietic tissues (ICD-9, 200–208), bladder and kidney, upper aerodigestive tract (ICD-9, 140–141 and 161) and pancreas were also reported. Covariates included in internal analyses were age, time since hire, and pay grade, and lags of 0, 10, and 15 years were used. Results shown were similar for both duration of exposure groups (HYD-6, n = 1053; HYD-24, n = 827). Relative risks for cancer of the lung for high exposure with a 15-year lag were 1.93 (95% CI, 1.27–2.93) or 2.10 (95% CI, 1.36–3.25) for high exposure within the HYD-6 and HYD-24 groups, respectively. No elevation in risk of cancer of the lung was seen for medium exposure. Analyses of high exposure (using the HYD-6 group) by decade showed that excesses of cancers of the lung, and lymphoid and haematopoietic tissues were greatest in 1960–1969. In the high-exposure group, mortality from cancer of the lymphoid and haematopoietic tissues (RR for HYD-6, 2.83; 95% CI, 1.22–6.56; and RR for HYD-24, 1.42; 95% CI, 0.54–3.72; 15-year lag), and bladder and kidney cancer (RR for HYD-6, 1.65; 95% CI, 0.59–4.56; and RR for HYD-24, 1.80; 95% CI, 0.63–5.12) was also increased. Limited data on smoking for 295 workers showed no association between smoking and estimated occupational exposure to hydrazine. Furthermore, relative risks for a group of smoking related-cancers other than lung showed no positive trends with exposure.

Ritz et al. (2006) updated the earlier study (Ritz et al., 1999) on this cohort for mortality until 2001. A few workers from the previous cohort were found not to have been eligible, resulting in a cohort size of 6044. Cancer incidence between 1988 and 2000 was also ascertained in California and eight other states of the USA for 5049 workers who were alive as of the beginning of 1988. Death certificate information indicated that cancer incidence would have approximately 89% complete coverage using only these states during the follow-up. Exposure assessment was updated via walkthrough surveys, interviews with company personnel, and review of records. Each job title was assigned to one of four categories (no exposure, low, moderate, high) in three periods (1950–1969, 1970–1979, and 1980–1999). Employees with direct exposure to rocket engines or fuel production and testing were presumed to have been exposed to hydrazine. Exposure intensity was scored annually 0–3 for each job (3 being highest exposure), based on the four categories above, and then summed across employment years to derive a cumulative exposure score. Further details of exposure assessment are available in Zhao et al. (2005). Cox regression was conducted with calendar time as the time variable. Pay grade, age, time since hire, and exposures to trichloroethylene or mineral oils were included in all models. Cumulative exposure

scores were divided into three semiquantitative categories, low (≤ 3), medium (3 to ≤ 12), and high (> 12) for analyses; 56% (3401) of workers were judged to have had no/low exposure, 26% (1593) moderate exposure, and 17% (1050) high exposure.

During follow-up, 2117 workers had died (35%) and 691 incident cancers were found. Mean duration of employment was 16 years. Rate ratios for cancer of the lung with no lag for mortality (194 deaths) were 1.00, 1.46 (95% CI, 0.96–2.22), and 1.49 (95% CI, 0.94–2.35) for no/low, moderate, and high exposures, respectively (*P* for trend, 0.065), and for incidence (92 cases) the RRs were 1.00, 1.15 (95% CI, 0.60–2.20), and 2.31 (95% CI, 1.21–4.43) for the same exposure categories (*P* for trend, 0.007). With 20 years lag, these were respectively 1.00, 1.24 (95% CI, 0.78–1.96), and 1.67 (95% CI, 0.99–2.83) (*P* for trend, 0.031) for mortality, and 1.00, 1.18, (95% CI, 0.62–2.24), and 2.49 (95% CI, 1.28–4.86) (*P* for trend, 0.003) for incidence. For cancer of the colorectum, the relative risks for incidence (90 cases) were 1.00, 1.64 (95% CI, 0.86–3.11), and 2.09 (95% CI, 1.02–4.31) (*P* for trend, 0.043) for no lag, and 1.00, 1.75 (95% CI, 0.93–3.30), and 2.16 (95% CI, 1.02–4.59) (*P* for trend, 0.041) for 20 years lag. There was evidence for positive trends for cancer of the kidney (17 deaths, 16 incident cases) and pancreas (39 deaths, 21 cases), but these did not attain statistical significance. No trends were seen with "NHL and leukaemia" for either mortality or incidence (0 and 20-year lags), in contrast to the earlier mortality follow-up. Limited data on smoking for 200 workers showed no association between smoking and estimated occupational exposure to hydrazine. Furthermore, relative risks for all smoking related-cancers other than lung showed no positive trends with exposure. [The Working Group noted that this cohort was particularly informative because it is relatively large, with semi-quantitative exposure assessment, and included cancer incidence as well as mortality.]

Krishnadasan et al. (2007) conducted a nested case–control study of 362 cases of cancer of the prostate and 1805 controls, matched on date of first employment and age at diagnosis. The study was nested within two cohorts, 4607 radiation-exposed Rocketdyne workers and the same 6107 rocket-testing Rocketdyne workers studied by Ritz et al. (2006). Exposures to hydrazine, trichloroethylene, polycyclic aromatic hydrocarbons, benzene, and mineral oil were evaluated. Incident cases of prostate cancer were ascertained in California and eight other states of the USA, as in Ritz et al. (2006). Based on a JEM, odds ratios (20-year lag) for those with high and low exposure to hydrazine, versus no exposure, were 0.84 (95% CI, 0.48–1.5) and 0.75 (95% CI, 0.50–1.1), respectively (*P* for trend, 0.30). [This study was largely uninformative, given the earlier data from Ritz et al. (2006), where no trends for prostate cancer were observed for hydrazine exposure among the rocket-testing workers.]

Boice et al. (2006) studied a group of 8372 Rocketdyne workers who worked for 6 months or more at the Santa Susana Field Laboratory rocket-testing facility from 1948 onward, and had potential exposure to hydrazine. Women were included among the rocket-testing facility workers, representing 15% of the cohort. Also included were 182 test-stand mechanics who were exposed to radiation. Analyses were limited to mortality, with no incidence data. Mortality comparisons were made using California referent rates, as well as referent rates based on 32 979 other Rocketdyne workers who did not do rocket testing and had no potential exposure to hydrazine. Internal comparisons were also conducted. Follow-up of test facility workers was conducted until 1999 and identified 2251 deaths (27% of the cohort). Among the 8372 rocket-testing workers, 1651 (20%) were test-stand mechanics, judged to have potential exposure to hydrazine. Of these however, Boice et al. (2006) estimated that only

315 (30%) were likely to have been exposed to hydrazine, while 205 others were judged to have possible but unlikely exposure to hydrazine, and the remainder were judged to have had no exposure to hydrazine. These definitions were based on job title and other company records. Some test-stand mechanics were also classified as being exposed to trichloroethylene, with 121 of these thought to have been exposed to both hydrazine and trichloroethylene.

Cancer mortality analyses of all rocket-testing workers compared with California population rates were unremarkable and not significantly different from the null for any specific type of cancer analysed, including lung (SMR, 0.89; 95% CI, 0.78–1.02). All-cause mortality was also decreased (SMR, 0.83; 95% CI, 0.80–0.86). Analyses by duration of employment also showed no significant excesses for any type of cancer for workers with longest duration of employment. Analyses of male test-stand mechanics paid hourly also did not show significant excesses for any cancer type, nor were there any significant excesses for those with > 5 years employment. The standardized mortality ratio for lung cancer was 1.07 (95% CI, 0.82–1.37) based on California referent data; the standardized mortality ratio for lung cancer for > 5 years employment was 1.06 (95% CI, 0.66–1.60). For the 315 test-stand workers estimated to have likely hydrazine exposure, the standardized mortality ratio for all cancers was 1.09 (95% CI, 0.75–1.52). The standardized mortality ratio for lung cancer in this group was 1.45 (95% CI, 0.81–2.39). Findings for deaths for other specific cancers were unremarkable and based on small numbers. Internal analyses of likely exposed, possibly exposed, versus not exposed to hydrazine for test-stand workers showed no evidence of a positive trend with increasing likelihood of exposure (analyses controlled for pay grade, hourly vs salaried). Analyses of test-stand mechanics by decade of exposure, comparing those with 3 or more years employment with those with less than 3 years employment, showed the highest lung cancer mortality risk (RR, 1.40; 95% CI, 0.80–2.47) in the group exposed 1960–1969 (n = 1454), with an excess (RR, 1.29; 95% CI, 0.70–2.40) also seen in those exposed before 1960 (n = 984). Some internal analyses using Cox regression were also conducted among test-stand mechanics by length of employment and likelihood of hydrazine exposure (likely, possible, none). These analyses, in which follow-up time was the time variable, and which controlled for year of birth, year of hire, pay grade (hourly or salaried), and exposure to trichloroethylene, did not show any significant trends for either lung cancer or colon cancer. A smoking survey carried out among about 75 hourly-paid and 75 salaried test-facility workers, found that about 60% of hourly workers had ever smoked versus 40% salaried workers, suggesting the importance of controlling for pay grade. [The Working Group noted that Boice et al. (2006) is an informative study with relatively large number of deaths and extensive exposure characterization by assigned categories. The Working Group further noted that while the two cohorts of Rocketdyne workers (Boice et al., 2006; Ritz et al., 2006) had important overlap, they were different populations. As noted above, the cohort studied by Boice et al. (2006) had a shorter minimum employment period and included women and workers hired in both earlier and later years. Furthermore, Ritz et al. (2006) considered all test-stand mechanics to have been exposed, while Boice et al. (2006) considered only a minority to have had likely or possible exposures based on presumed test-stand assignment. However, as pointed out in Zhao et al. (2005), test-stand mechanics frequently rotated among different test stands, making the classification used by Boice et al. (2006) imprecise. Furthermore, Ritz et al. (2006) included incidence data, while Boice et al. (2006) included only mortality data. Hence, few results in the two studies are directly comparable. It is of note that test-stand mechanics exposed before 1960

(RR for lung cancer, 1.29) and during the 1960s (RR, 1.40), with ≥ 3 years employment versus < 3 years employment in Boice et al. (2006) may be roughly comparable to the workers with high and moderate exposure with a 20-year lag in Ritz et al. (2006) (lung cancer mortality for those with moderate and high exposure, lung cancer RR, 1.24 and 1.67, respectively). These groups, across the two studies, showed a moderate excess of lung cancer. It should also be noted that in Boice et al. (2006), the group with likely or possible exposure had a lung cancer rate ratio of 1.45 versus the non-exposed. Finally, there was a notable increase in incidence of lung cancer in the Ritz et al. (2006) study in the group with high exposure and a 20-year lag (RR, 2.49).]

3. Cancer in Experimental Animals

Hydrazine or hydrazine salts were previously reviewed by the *IARC Monographs* Working Group (Volume 4, IARC, 1974; Supplement 7, IARC, 1987; and Volume 71, IARC, 1999). The Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of hydrazine (IARC, 1999). In the present *Monograph*, the Working Group evaluated the studies of carcinogenesis in experimental animals that had been reviewed previously, and also reviewed any new studies published since the earlier review.

Hydrazine was found to cause tumours of the lung in mice with an incidence of 100% (Biancifiori & Ribacchi, 1962), and was then subsequently used in the laboratory to study formation of tumours of the lung in mice, rats, and hamsters. Most of the studies in mice in the present *Monograph* were single-dose studies designed to investigate possible mitigating agents for hydrazine, or mechanisms of lung-tumour induction by hydrazine.

See Table 3.1

3.1 Mouse

3.1.1 Gavage

A group of 22 female BALB/c mice (age, 8 weeks) was given hydrazine sulfate in water as 251 daily gavage doses at 1.13 mg per mouse during 46 weeks. By the end of the experiment (46 weeks), the mice had 100% incidence [$P < 0.0001$] of pulmonary adenoma [the majority] or carcinoma. A group of 216 untreated control female mice allowed to live their full lifespan (up to 121 weeks) had no pulmonary tumours (Biancifiori & Ribacchi, 1962). [While a high incidence of pulmonary tumours was reported in treated mice versus controls, the study lacked experimental details and had a poor experimental design. For example, the initial number of treated mice was not given and no vehicle controls were included.]

In a follow-up study, Biancifiori et al. (1963) treated a group of 84 female BALB/c mice (age, ~50 days) with hydrazine sulfate at 1.13 mg per day in water by gavage for up to 310 days. About three mice were killed every 10th day. The incidence of pulmonary tumours for the groups killed between day 200 and 240 was 15/15 (100%), between day 250 and 290 was 13/15 (87%), between day 300 and 340 was 14/14 (100%), and between day 350 and 380 was 8/8 (100%). [No information was provided if controls were used. While high incidences of pulmonary tumours were reported in treated mice, this study lacked experimental details and had a limited experimental design. The study was inadequate for the evaluation.]

Groups of 21 male and 21 female CBA/Cb/Se mice (age, 8 weeks) were treated daily with hydrazine sulfate at a total dose of 283 mg in water by gavage for 36 weeks, and then maintained for their lifetime (Biancifiori et al., 1964). Groups of 37 male and 47 female CBA/Cb/Se mice served as untreated controls. Groups of 10 male and 10 female BALB/c mice (age, 8 weeks) were treated daily with hydrazine sulfate at a total

Table 3.1 Studies of carcinogenicity with hydrazine in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, BALB/c (F) 8 wks 46 wks Biancifiori & Ribacchi (1962)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 1.13 mg/dose 251 treatments over 46 wks 216, NR 166, 22	*Lung* Adenoma or carcinoma (combined): 0/166, 22/22*	*[$P < 0.0001$]	Control animals were held for their lifetime While a high incidence (100%) of pulmonary tumours [mainly adenoma] was reported in treated mice vs "controls" (0%), the lack of experimental detail and poor experimental design (e.g. failure to report number of mice in treated group, single dose used, no discussion of pathology) made the study difficult to evaluate
Full carcinogenicity Mouse, CBA/Cb/Se (M) 8 wks Lifetime Biancifiori et al. (1964)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 283 mg of total dose "Daily" gavage for 36 wks 37, 21 NA	*Lung* Pulmonary adenoma or carcinoma (combined): Incidence: 1/37, 16/21* Multiplicity: 1, 3 *Liver* Hepatoma [hepatocellular tumours]: Incidence: 4/37, 13/21*	*[$P < 0.0001$] *[$P < 0.0001$]	Principal limitations: poor description of experimental details; single dose used; small number of animals in exposure group; exposed for only 36 wks; no discussion of clinical signs
Full carcinogenicity Mouse, CBA/Cb/Se (F) 8 wks Lifetime Biancifiori et al. (1964)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 283 mg of total dose "Daily" gavage for 36 wks 47, 21 NA	*Lung* Pulmonary adenoma or carcinoma (combined): Incidence: 4/47, 19/21* Multiplicity: 1, 6 *Liver* Hepatoma [hepatocellular tumours]: 2/47, 15/21*	 *[$P < 0.0001$] *[$P < 0.0001$]	Principal limitations: poor description of experimental details; single dose used; small number of animals in exposure group; exposed for only 36 wks; no discussion of clinical signs
Full carcinogenicity Mouse, BALB/c (M) 8 wks Lifetime Biancifiori et al. (1964)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 32 mg – total dose "Daily" gavage for 4 wks 22, 10 NA	*Lung* Pulmonary adenoma: Incidence: 6/22, 7/8* Multiplicity: 1.0, 2.4	 *[$P < 0.006$]	Principal limitations: poor description of experimental details; single dose used for very short time; small number of animals in exposure and control groups; exposed for only 4 wks; no discussion of clinical signs

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, BALB/c (F) 8 wks Lifetime Biancifiori et al. (1964)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 32 mg – total dose "Daily" gavage for 4 wks 23, 10 NA	*Lung* Pulmonary adenoma: Incidence: 5/23, 8/10* Multiplicity: 1.0, 2.9	*[$P < 0.003$]	Principal limitations: poor description of experimental details; single dose used for very short time; small number of animals in exposure and control groups; exposed for only 4 wks; no discussion of clinical signs
Full carcinogenicity Mouse, CBA/Cb/Se (M) 8 wks Lifetime Severi & Biancifiori (1968)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 1.13 mg/dose 1 × /day for 36 wks 37, 21 NA	*Liver* Hepatocellular adenoma or carcinoma (combined): 4/37, 13/21* *Lung* Adenoma or carcinoma (combined): 1/37, 16/21*	*[$P < 0.0001$] *[$P < 0.0001$]	Principal limitations: use of single dose; short duration of exposure Same data reported in Biancifiori et al. (1964)
Full carcinogenicity Mouse, CBA/Cb/Se (F) 8 wks Lifetime Severi & Biancifiori (1968)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 1.13 mg/dose 1 × /day for 36 wks 47, 21 NA	*Lung* Adenoma or carcinoma (combined): 4/47, 19/21* *Liver* Hepatocellular adenoma or carcinoma (combined): 2/47, 15/21*	*[$P < 0.0001$] *[$P < 0.0001$]	Principal limitations: use of single dose; short duration of exposure Same data reported in Biancifiori et al. (1964)
Full carcinogenicity Mouse, BALB/c/Cb/Se (M+F, combined) Newborn Up to 425 days Milia et al. (1965)	Gavage Hydrazine sulfate, "pure" Water buffered with sodium bicarbonate 0, 17 mg – total dose 1 × /day for 60 days 20, 20 NR, NR	*Lung* Adenoma or carcinoma (combined): Incidence: 3/20, 20/20* Multiplicity: 1, 10 Total tumours: 3, 200	*[$P < 0.0001$]	Principal limitations: poor description of experimental design; small number of animals; no discussion of clinical signs, survival or body weights

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (F) Age NR Up to 40 wks Roe et al. (1967)	Gavage Hydrazine sulfate (purity, NR) Distilled water 0 (untreated), 0.25 mg/dose 1 × /day, 5 days/wk for 40 wks 85, 25 42, 4	*Lung* Adenoma or carcinoma (combined): 8/79, 6/13*	*$P < 0.001$	Principal limitations: experimental details poorly described; single sex; short duration of exposure; poor survival in treated group
Full carcinogenicity Mouse, CBA/Cb/Se (M) Age NR Lifetime Biancifiori (1969)	Gavage Hydrazine sulfate (purity, NR) Water 0.14 (intact virgin), 0.14 (gonadectomized), 0.28 (intact virgin), 0.28 (gonadectomized), 0.56 (intact virgin), 0.56 (gonadectomized) mg 1 × /day for a total of 150 doses (over 25 wks) 26, 25, 25, 26, 25, 23 NA	*Lung* Pulmonary tumours: 2/26, 3/25, 4/25, 3/26, 5/25, 5/23	NA	Principal limitations: poor description of experimental details; no discussion of pathology or clinical observations and no concurrent controls Study to investigate a possible hormonal effect on the formation of tumours by hydrazine sulfate
Full carcinogenicity Mouse, CBA/Cb/Se (F) Age NR Lifetime Biancifiori (1969)	Gavage Hydrazine sulfate (purity, NR) Water 0.14 (intact virgin), 0.14 (gonadectomized), 0.28 (intact virgin), 0.28 (gonadectomized), 0.56 (intact virgin), 0.56 (gonadectomized) mg 1 × /day for a total of 150 doses (over 25 wks) 25, 25, 25, 25, 24, 25 NA	*Lung* Pulmonary tumours: 10/25, 2/25, 16/25, 6/25, 21/24, 7/25	NA	Principal limitations: poor description of experimental details; no discussion of pathology or clinical observations, and no concurrent controls Study to investigate a possible hormonal effect on the formation of tumours by hydrazine sulfate

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, (BALB/c × DBA/2)F_1 (CDF_1) (F) 7–8 wks 28–33 wks Kelly et al. (1969)	Gavage Hydrazine sulfate (purity, NR) 2% aqueous sodium bicarbonate 0, 41.6 mg total dose 1 × /wk for 8 wks 10, 28 10, 26	*Lung* Pulmonary tumours: 1/10, 13/28*	*[$P < 0.05$]	Principal limitations: experimental details poorly described; inadequate number of controls; no discussion of clinical observations or pathology
Full carcinogenicity Mouse, CBA/Cb/Se M 8 wks Lifetime Biancifiori (1970a)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 0.14, 0.28, 0.56, 1.13 mg/day 150 daily doses over 25 wks 30, 26, 25, 25, 25 NA	*Liver* Hepatoma [hepatocellular tumours]: 3/30, 1/26, 7/25, 12/25*, 15/25**	*[$P < 0.003$] **[$P < 0.0001$]	Principal limitations: short duration of exposure; lack of vehicle controls Hepatomas are mainly hepatocellular carcinomas
Full carcinogenicity Mouse, CBA/Cb/Se (F) 8 wks Lifetime Biancifiori (1970a)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 0.14, 0.28, 0.56, 1.13 mg/day 150 daily doses over 25 wks 29, 25, 25, 24, 24 NA	*Liver* Hepatoma [hepatocellular tumours]: 1/29, 0/25, 2/25, 16/24*, 15/24*	*[$P < 0.0001$]	Principal limitations: short duration of exposure; lack of vehicle controls Hepatomas are mainly hepatocellular carcinomas
Full carcinogenicity Mouse, BALB/c/Cb/Se (M) 8 wks Lifetime Biancifiori (1970b)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 0.14 (21 mg total), 0.28 (42 mg total), 0.56 (84 mg total), 1.13 (170 mg total), 1.13 (32 mg total) mg/dose 28 or 150 doses over 4 or 25 wks 25, 24, 24, 26, 25, 20 NA	*Lung* Pulmonary adenoma or carcinoma (combined): 6/25, 13/24*, 15/24**, 17/26***, 20/22****, 17/20****	*[$P < 0.05$] **[$P < 0.008$] ***[$P < 0.004$] ****[$P < 0.0001$]	Principal limitations: no discussion of body-weight gain, or survival; lack of vehicle controls

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, BALB/c/Cb/Se (F) 8 wks Lifetime Biancifiori (1970b)	Gavage Hydrazine sulfate (purity, NR) Water 0 (untreated), 0.14 (21 mg total), 0.28 (42 mg total), 0.56 (84 mg total), 1.13 (170 mg total), 1.13 (32 mg total) mg/dose 28 or 150 doses over 4 or 25 wks 25, 25, 19, 25, 22, 20 NA	*Lung* Pulmonary adenoma or carcinoma (combined): 1/25, 8/25*, 17/19**, 19/25**, 20/22**, 15/20**	*[$P < 0.02$] **[$P < 0.0001$]	Principal limitations: no discussion of clinical signs, body-weight gain, or survival; lack of vehicle controls
Full carcinogenicity Mouse, BALB/c/Cb/Se (F) 8 wks Lifetime Biancifiori (1970c)	Gavage Hydrazine sulfate (purity, NR) Water 0 mg (intact virgins), 1.13 mg (intact virgins), 0 mg (breeders), 1.13 mg (breeders), 0 mg (gonadectomized), 1.13 mg (gonadectomized) 150 daily doses of 1.13 mg/dose 25, 22, 25, 25, 26, 25 NA	*Lung* Adenoma or carcinoma (combined) Incidence: 1/25, 20/22*, 2/25, 25/25*, 7/26, 15/25** Multiplicity: 1, 3, 1, 14, 1, 5 Total tumours: 1, 60, 1, 201, 1, 50	*[$P < 0.0001$] **[$P < 0.05$]	Principal limitations: poor description of experimental details including duration of actual exposure; single dose used; no discussion of body-weight gain, or survival; lack of vehicle controls Study to test the effect of ovarian stimulation on pulmonary tumours induced by hydrazine sulfate in BALB/c/Cb/Se (BALB/c) mice
Full carcinogenicity Mouse, C3Hb/Cb/Se (M) 8 wks Lifetime Biancifiori (1971)	Gavage Hydrazine sulfate (purity, NR) Water 0 mg (virgin), 1.13 mg (virgin), 0 mg (gonadectomized), 1.13 mg (gonadectomized) – total dose 150 daily doses of 1.13 mg/dose (170 mg total dose) 25, 27, 24, 25 NA	*Lung* Adenoma or carcinoma (combined): 0/25, 7/27*, 1/24, 1/25	*[$P < 0.01$]	Principal limitations: poor description of experimental details including duration of actual exposure; single dose used; no discussion of body-weight gain, or survival; lack of vehicle controls Tumours were mainly lung adenomas

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C3Hb/Cb/Se (F) 8 wks Lifetime Biancifiori (1971)	Gavage Hydrazine sulfate (purity, NR) Water 0 mg (virgin), 1.13 mg (virgin), 0 mg (gonadectomized), 1.13 mg (gonadectomized), 0 mg (breeders), 1.13 mg (breeders) – total dose 150 daily doses of 1.13 mg/dose (170 mg total dose) 25, 27, 25, 26, 23, 31 NA	*Lung* Adenoma or carcinoma (combined): 1/25, 13/27*, 3/25, 5/26, 3/23, 12/31**	*[$P < 0.0004$] **[$P < 0.01$]	Principal limitations: poor description of experimental details including duration of actual exposure; single dose used; no discussion of body-weight gain, or survival; lack of vehicle controls Tumours were mainly lung adenomas
Carcinogenicity with other modifying factors Mouse, Swiss (M+F, combined) 10 wks Up to 15 mo Maru & Bhide (1982)	Gavage Hydrazine sulfate, "analytical grade" Water 0, 1.1 mg; 1.1 mg+1.1 mg L-arginine; 1.1 mg+1.1 mg pyridoxine hydrochloride; 1.1 mg+1.1 mg folic acid, 1.1 mg+1.1 mg L-arginine+L-sodium glutamate; 1.1 mg+1.1 mg L-sodium glutamate+pyridoxine hydrochloride mg/mouse/day 1 × /day, 5 days/wk for up to 15 mo 60, 60, 60, 60, 60, 60, 60 47, 29, 18, 33, 31, 18, 21	*Lung* Incidence: 1/47, 22/29*, 11/18*, 26/33*, 17/31*, 13/18*, 16/21*	*[$P < 0.0001$]	Principal limitations: no histological description of tumours; used only one dose; reported combined tumour incidence for males and females; no clinical signs, body weights or mortality information reported Study on the effect of antioxidants on the formation of lung tumours in mice by hydrazine sulfate
Full carcinogenicity Mouse, Swiss (M) 6 wks Lifetime Toth (1969)	Drinking-water Hydrazine sulfate, Fisher certified ACS Water 0 mg, 0.74 mg average daily consumption Daily/ad libitum in drinking-water 110, 50 NA	*Lung* Adenoma or adenocarcinoma (combined): 11/110, 25/50*	*[$P < 0.0001$]	Principal limitations: use of a single dose

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (F) 6 wks Lifetime Toth (1969)	Drinking-water Hydrazine sulfate, Fisher certified ACS Water 0 mg, 0.65 mg average daily consumption Daily/ad libitum in drinking-water 110, 50 NA	*Lung* Adenoma or adenocarcinoma (combined): 14/110, 24/50*	*[$P < 0.0001$]	Principal limitations: use of a single dose
Full carcinogenicity Mouse, A/J (M) 6 wks Up to 48 wks Yamamoto & Weisburger (1970)	Drinking-water Hydrazine sulfate (purity, NR) Water 0 mg/L, 0 mg/L + 1% L-arginine-L-glutamate (in the diet), 325 mg/L, 325 mg/L + 1% L-arginine-L-glutamate (in the diet), ad libitum in drinking-water 20, 20, 38, 37 NR	*Lung* Adenoma or adenocarcinoma (combined): 12/20, 11/20, 38/38*, 34/37**	*[$P < 0.0001$] **[$P < 0.005$]	Principal limitations: use of a single dose
Full carcinogenicity Mouse, NMRI (M) 5–6 wks 2 yrs Steinhoff et al. (1990)	Drinking-water Hydrazine hydrate (purity, 99.3%) Water 0, 2, 10, 50 ppm ad libitum 50, 50, 50, 50 NR	*Haematopoietic and lymphoid tissues* Malignant lymphoma: 4/50, 13/50, 6/50, 4/50	NS	Principal limitations: limited description of histopathology
Full carcinogenicity Mouse, NMRI (F) 5–6 wks 2 yrs Steinhoff et al. (1990)	Drinking-water Hydrazine hydrate, 99.3% Water 0, 2, 10, 50 ppm ad libitum 50, 50, 50, 50 NR	*Lung* Lung tumours (benign): 6/50, 6/50, 9/50, 15/50* *Haematopoietic and lymphoid tissues* Malignant lymphoma: 19/50, 31/50, 20/50, 19/50	*$P < 0.05$ NS	Principal limitations: limited description of histopathology

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF$_1$ (M) 6 wks 2 yrs Matsumoto et al. (2016)	Drinking-water Hydrazine monohydrate (purity, 100%) Deionized water 0, 20, 40, 80 ppm ad libitum, 7 days/wk 50, 50, 50, 50 31, 30, 36, 40	*Liver* Haemangioma or haemangiosarcoma (combined): 3/50, 1/50, 6/50, 0/50 Hepatocellular adenoma or carcinoma (combined): 34/50, 24/50, 15/50, 10/50 Hepatocellular adenoma: 17/50, 12/50, 8/50, 6/50 Hepatocellular carcinoma: 21/50, 14/50, 9/50, 4/50	 NS NS (increase) NS (increase) NS (increase)	Principal strengths: GLP study
Full carcinogenicity Mouse, Crj:BDF$_1$ (F) 6 wks 2 yrs Matsumoto et al. (2016)	Drinking-water Hydrazine monohydrate (purity, 100%) Deionized water 0, 40, 80, 160 ppm ad libitum, 7 days/wk 50, 50, 50, 50 26, 37, 29, 23	*Liver* Haemangioma or haemangiosarcoma (combined): 1/50, 2/50, 1/50, 4/50 Haemangioma: 0/50, 0/50, 1/50, 3/50 Haemangiosarcoma: 1/50, 2/50, 0/50, 1/50 Hepatocellular adenoma or carcinoma (combined): 7/50, 8/50, 3/50, 17/50* Hepatocellular adenoma: 5/50, 6/50, 2/50, 14/50* Hepatocellular carcinoma: 2/50, 2/50, 1/50, 4/50	 $P < 0.05$ by Peto trend test $P < 0.01$ by Peto trend test NS $P < 0.01$ by Peto trend test, *$P < 0.05$ by Fisher's exact test $P < 0.01$ by Peto trend test, *$P < 0.05$ by Fisher's exact test $P < 0.01$ by Peto trend test	Principal strengths: GLP study Authors reported that hepatic haemangiomas in female mice were observed in three animals in the laboratory historical control data, which consisted of 899 female Crj:BDF$_1$ mice (3/899, 0.3%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, BALB/c/Cb/Se (M+F, combined) Newborn Up to 270 days Milia et al. (1965)	Intraperitoneally Hydrazine sulfate ("pure") Water 0, 19 mg – total dose 1 × /day for 90 days 20, 20 NR, NR	*Lung* Adenoma or carcinoma (combined): Incidence: 2/20, 20/20* Multiplicity: 1, 5 Total tumours: 2, 100	*[$P < 0.0001$]	Principal limitations: poor description of experimental design; small number of animals per group, use of a single dose; no discussion of clinical signs, survival or body weights
Full carcinogenicity Mouse, $(BALB/c \times DBA/2)F_1$ (CDF_1) (M) 7–8 wks 28–33 wks Kelly et al. (1969)	Intraperitoneally Hydrazine sulfate (purity, NR) 2% aqueous sodium bicarbonate 0, 20.8 mg total dose 1 × /wk for 8 wks 9, 30 8, 30	*Lung* Pulmonary tumours: 1/9, 6/30	NS	Principal limitations: experimental details poorly described, inadequate number of controls, no discussion of clinical observations or pathology
Full carcinogenicity Mouse, strain NR (M+F, combined) Age NR 313 days Juhász et al. (1966)	Intraperitoneally Hydrazine (purity, NR) Physiological saline 0, 400 mg/kg bw 16 injections over 46 days 60, 60 NR, 34	*Haematopoietic and lymphoid tissues* Reticular-cell sarcoma or myeloid leukaemia: 0/60, 13/34*	*[$P < 0.0001$]	Principal limitations: experimental details poorly described, use of a single dose, short exposure time, no discussion of clinical observations or pathology, poor description and discussion of tumour incidences
Carcinogenicity with other modifying factor Mouse, C57BL/6 (M+F, combined) 6–8 wk Up to 62 wks Mirvish et al. (1969)	Intraperitoneally Hydrazine sulfate (purity, NR) Water 0 (no irradiation), 0 (irradiated), 95 (irradiated), 95 (no irradiation) mg/kg bw Single 400 R total-body irradiation followed by 10 weekly injections ≥ 75, 29, 40, 36 NR, NR, NR, NR	*Lung* Adenoma: 9/75, NR, NR, 5/18 *Haematopoietic and lymphoid tissues* Leukaemia: NR, 2/25, 4/29, 0/33	[NS] [NS]	Principal limitations: experimental details poorly described; no discussion of clinical observations or pathology

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 7 wks 27 mo Vernot et al. (1985)	Inhalation Hydrazine (purity, 99.8%) Air 0, 0.05, 0.25, 1.0 ppm 6 h/day, 5 days/wk for 1 yr 400, 400, 400, 400 NR, NR, NR, NR	*Lung* Adenoma: 4/378, NR, NR, 12/379*	*[$P < 0.05$]	Principal limitations: single sex and low doses used; tumour incidence for some exposure groups not reported
Full carcinogenicity Rat, Cb/Se (M) 8 wks Lifetime Severi & Biancifiori (1968)	Gavage Hydrazine sulfate (purity, NR) Water 0, 18 mg/dose 1 × /day for 68 wks 28, 14 NA	*Lung* Pulmonary adenoma or carcinoma (combined): 0/28, 3/14* *Liver* Malignant tumours: 0/28, 4/13*	*[$P < 0.05$] *[$P < 0.01$]	Principal limitations: use of single dose, short duration of exposures See also Biancifiori et al. (1966)
Full carcinogenicity Rat, Cb/Se (F) 8 wks Lifetime Severi & Biancifiori (1968)	Gavage Hydrazine sulfate (purity, NR) Water 0, 12 mg/dose 1 × /day for 68 wks 22, 18 NA	*Lung* Pulmonary adenoma or carcinoma (combined): 0/22, 5/18*	*[$P < 0.02$]	Principal limitations: use of a single dose, short duration of exposures See also Biancifiori et al. (1966)
Full carcinogenicity Rat, Wistar (M) 6 wks Lifetime Steinhoff & Mohr (1988)	Drinking-water Hydrazine hydrate (purity, 99.3%) Water 0, 2, 10, 50 ppm ad libitum in the drinking-water 50, 50, 50, 50 NA	*Liver* Hepatocellular adenoma: 0/50, 1/49, 1/50, 4/49* Hepatocellular carcinoma: 0/50, 0/49, 1/50, 0/49	*$P < 0.01$ NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (F) 6 wks Lifetime Steinhoff & Mohr (1988)	Drinking-water Hydrazine hydrate (purity, 99.3%) Water 0, 2, 10, 50 ppm ad libitum in the drinking-water 50, 50, 50, 50 NA	*Liver* Hepatocellular adenoma: 0/50, 0/50, 0/50, 4/47* Hepatocellular carcinoma: 0/50, 0/50, 0/50, 3/47*	 *$P < 0.01$ *$P < 0.01$	One of the hepatocellular carcinoma may not have been a primary tumour
Full carcinogenicity Rat, F344/DuCrj (M) 6 wks 2 yrs Matsumoto et al. (2016)	Drinking-water Hydrazine monohydrate (purity, 100%) Deionized water 0, 20, 40, 80 ppm ad libitum, 7 days/wk 50, 50, 50, 50 37, 39, 44, 39	*Liver* Hepatocellular adenoma or carcinoma (combined): 0/50, 0/50, 0/50, 4/50 Hepatocellular adenoma 0/50, 0/50, 0/50, 3/50 Hepatocellular carcinoma 0/50, 0/50, 0/50, 1/50 *Testis* Interstitial cell tumours: 37/50, 45/50*, 43/50, 44/50	 $P < 0.01$ by Peto trend test $P < 0.01$ by Peto trend test NS $P < 0.05$ by Peto trend test, *$P < 0.05$ by Fisher's exact test	Principal strengths: GLP study
Full carcinogenicity Rat, F344/DuCrj (F) 6 wks 2 yrs Matsumoto et al. (2016)	Drinking-water Hydrazine monohydrate (purity, 100%) Deionized water 0, 20, 40, 80 ppm ad libitum, 7 days/wk 50, 50, 50, 50 40, 39, 44, 29	*Liver* Hepatocellular adenoma or carcinoma (combined): 1/50, 0/50, 3/50, 6/50 Hepatocellular adenoma: 1/50, 0/50, 3/50, 4/50 Hepatocellular carcinoma: 0/50, 0/50, 0/50, 4/50	 $P < 0.01$ by Peto trend test $P < 0.05$ by Peto trend test $P < 0.01$ by Peto trend test	Principal strengths: GLP study Authors reported that hepatocellular carcinoma was observed in only one animal in the laboratory historical control data, which consisted of 898 female F344/DuCrj rats (1/898, 0.1%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 7 wks 30 mo Vernot et al. (1985)	Inhalation Hydrazine (purity, 99.8%) Air 0, 0.05, 0.25, 1.0, 5.0 ppm 6 h/day, 5 days/wk for 1 yr 150, 100, 100, 100, 100 NR, NR, NR, NR, NR	*Nose* Adenoma (adenomatous polyp): 0/146, 2/96, 1/94, 9/97*, 58/98* *Thyroid* Carcinoma: 7/146, 6/96, 5/94, 9/97, 13/98**	*$P \leq 0.01$ **$P \leq 0.05$	
Full carcinogenicity Rat, F344 (F) 7 wks 30 mo Vernot et al. (1985)	Inhalation Hydrazine (purity, 99.8%) Air 0, 0.05, 0.25, 1.0, 5.0 ppm 6 h/day, 5 days/wk for 1 yr 150, 100, 100, 100, 100 NR, NR, NR, NR, NR	*Nose* Adenoma (adenomatous polyp): 0/145, 2/97, 0/98, 2/94, 28/95*	*$P \leq 0.01$	
Full carcinogenicity Hamster, Syrian golden (M) Age NR 2 yrs Bosan et al. (1987)	Drinking-water Hydrazine sulfate (purity, > 99%) Water 0, 170, 340, 510 mg/L ad libitum 40, 40, 40, 40 NR, NR, NR, NR	*Liver* Hepatocellular carcinoma: 0/31, 0/31, 4/34, 11/34*	*[$P < 0.0004$]	Principal limitations: study examined the liver only Study designed to investigate liver DNA methylation over a 2-yr hydrazine exposure period to test the relationship between DNA methylation and carcinogenesis

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested (purity) Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Hamster, Syrian golden (M) Age NR 21 mo FitzGerald & Shank (1996)	Drinking-water Hydrazine sulfate (purity, > 99%; ACS grade) Water 0, 170, 340, 510 mg/L ad libitum 25, 30, 43, 40 NR, NR, NR, NR	*Liver* Hepatocellular adenoma: 0/25, 1/30, 4/43, 10/40* Hepatocellular carcinoma: 0/25, 0/30, 1/43, 3/40	*[$P < 0.005$] [Consequently, the incidence of hepatocellular adenoma or carcinoma (combined) is significantly increased ($P < 0.005$) in high-dose animals]	Principal limitations: study examined the liver only Study designed to investigate methylation status of DNA cytosine during the course of induction by hydrazine of liver cancer in hamsters
Full carcinogenicity Hamster, Syrian golden (M) 7 wks 24 mo Vernot et al. (1985)	Inhalation Hydrazine (purity, 99.8%) Air 0, 0.25, 1.0, 5.0 ppm 6 h/day, 5 days/wk for 1 yr 200, 200, 200, 200 NR, NR, NR, NR	*Nose* Adenoma (adenomatous polyp): 1/181, 0/154, 1/148, 16/160* *Thyroid* Parafollicular cell adenoma: 0/145, 0/117, 0/127, 4/137 *Colon* Adenocarcinoma: 0/158, 0/146, 2/129, 3/139	*$P \leq 0.01$ NS NS	

bw, body weight; F, female; GLP, good laboratory practice; M, male; mo, month; NA, not applicable; NR, not reported; NS, not significant; ppm, parts per million; wk, week; yr, year

dose of 32 mg in water by gavage for 4 weeks and held for their lifetime. Groups of 22 male and 23 female BALB/c mice served as untreated controls. Exposure of male and female CBA/Cb/Se mice to hydrazine sulfate caused a significant increase in the incidence of pulmonary adenoma or carcinoma (combined) [mainly adenoma] (males: 16/21 (76%) [$P < 0.0001$] versus 1/37 (3%) controls; females: 19/21 (90%) [$P < 0.0001$] versus 4/47 (9%) controls) and hepatoma [hepatocellular tumours] (males: 13/21 (62%) [$P < 0.0001$] versus 4/37 (11%) controls; females: 15/21 (71%) [$P < 0.0001$] versus 2/47 (4%) controls) in exposed mice. Exposure of male and female BALB/c mice to hydrazine sulfate caused a significant increase in the incidence of pulmonary adenoma or carcinoma (combined) [mainly adenoma] (males: 7/8 (88%) [$P < 0.006$] versus 6/22 (27%) controls; females: 8/10 (80%) [$P < 0.003$] versus 5/23 (22%) controls). [The data for the CBA/Cb/Se mice experiment were also reported in Severi & Biancifiori (1968). While high incidences of pulmonary and liver tumours were reported in exposed CBA/Cb/Se mice, and a high incidence of pulmonary tumours was reported in exposed BALB/c mice, the Working Group noted the poor experimental design, lack of experimental details and vehicle controls, use of a single dose, and small number of mice in treated groups (especially for the BALB/c groups).]

A group of 20 male and female (combined) newborn BALB/c mice was given hydrazine sulfate in sodium bicarbonate (total dose of 17 mg) daily by gavage for 60 days and were then held for up to age 425 days. A group of 20 male and female (combined) mice served as vehicle controls. There was a 100% (20/20) [$P < 0.0001$] incidence of lung adenoma or carcinoma (combined) (carcinoma, 16%) in the treated group compared with a 15% incidence (3/20, adenoma only) in controls (Milia et al., 1965). [While a high incidence of pulmonary tumours was reported in treated mice versus controls, the Working Group noted the lack of experimental details, limited experimental design, and use of a single dose level.]

A group of 25 female Swiss mice [age at start not reported] was given hydrazine sulfate at a dose of 0.25 mg per day by gavage in water on 5 days per week for up to 40 weeks. A group of 85 untreated female Swiss mice served as controls. The incidence of bronchiolo-alveolar adenoma or carcinoma (combined) was 6/13 (46%) ($P < 0.001$) in treated mice that survived for up to 60 weeks, versus 8/79 (10%) controls (Roe et al., 1967). [The Working Group noted that experimental details were poorly described, only one sex was used, the duration of exposure was short, and there was poor survival in the treated group.]

In a study to investigate a possible hormonal effect on the formation of tumours by hydrazine sulfate, Biancifiori (1969) treated groups of 23 to 26 intact virgin or gonadectomized male and female CBA/Cb/Se mice (age, 8 weeks) with hydrazine sulfate as 150 daily doses of 0.14, 0.28, or 0.56 mg (total doses of 21, 42, or 84 mg) by gavage over about 25 weeks. The mice were then held for their lifetime. No concurrent controls were reported. In the groups receiving the highest dose, the incidence of pulmonary tumours [histopathology not further specified] was 87% (21/24) in intact virgin females and 20% (5/25) in intact virgin males. In the groups receiving the intermediate dose, the incidence of pulmonary tumours was 56% (16/25) and 16% (4/25), respectively. In groups receiving the lowest dose, the incidence of pulmonary tumour was 40% (10/25) and 7% (2/26), respectively. In gonadectomized mice, the incidence of pulmonary tumours [mainly adenoma] was 28% (7/25) in females and 21% (5/23) in males at the highest dose, 24% (6/25) and 11% (3/26) at the intermediate dose, and 8% (2/25) and 12% (3/25) at the lowest dose. [The Working Group noted that the study had limited description of experimental details (including histopathology), and lacked concurrent controls.]

A group of 30 female (BALB/c × DBA/2) F_1 (CDF_1) mice (age, 7–8 weeks) was given hydrazine sulfate (total dose, 41.6 mg) in aqueous 2% sodium bicarbonate by gavage once per week for 8 weeks, and then held for up to 33 weeks. A group of 10 female mice served as vehicle controls. Pulmonary tumours [not further specified] were observed in 13/28 (46%) [$P < 0.05$] treated mice compared with 1/10 (10%) controls (Kelly et al., 1969). [While a high incidence of pulmonary tumours was reported in treated mice versus controls, the Working Group noted the lack of experimental details, limited description of the histopathology, and the limited experimental design including the use of one sex, only 10 control animals, and a single dose.]

Groups of 24–26 male and female CBA/Cb/Se mice (age, 8 weeks) were treated with hydrazine sulfate at a dose of 0.14, 0.28, 0.56, or 1.13 mg per day (total dose: 21, 42, 84, or 170 mg) by gavage in water 150 times over 25 weeks, and then held for their lifetime (Biancifiori, 1970a). Groups of 30 males and 29 females served as untreated controls. Exposure of male and female mice to hydrazine sulfate caused a dose-related significant increase in the incidence of hepatoma [mainly hepatocellular carcinoma] (males: 3/30 (10%), 1/26 (4%), 7/25 (28%), 12/25 (48%) [$P < 0.003$], 15/25 (60%) [$P < 0.0001$]; females: 1/29 (3%), 0/25, 2/25 (8%), 16/24 (66%) [$P < 0.0001$], 15/24 (62.5%) [$P < 0.0001$]; for untreated controls, and the groups at 21, 42, 84, and 170 mg, respectively]). Multiple pulmonary tumours were reported to be present in many treated animals, but data on tumour incidence were not provided. [The Working Group noted the lack of vehicle controls.]

Groups of 20–26 male and 19–25 female BALB/c mice (age, 8 weeks) were treated with hydrazine sulfate at a dose of 0.14, 0.28, 0.56, or 1.13 mg per day (total dose, 21, 42, 84, or 170 mg) by gavage in water for 150 daily doses over 25 weeks, or at a dose of 1.13 mg per day (total dose, 32 mg) for 28 daily doses over 4 weeks, and then held for their lifetime (Biancifiori, 1970b). Groups of 25 males and 25 females served as untreated controls. Hydrazine sulfate significantly increased the incidence of pulmonary adenoma or carcinoma (combined) [mainly adenoma] in all dosed groups compared with controls. Tumour incidence for males was: 6/25 (24%), 13/24 (54%) [$P < 0.05$], 15/24 (62.5%) [$P < 0.008$], 17/26 (65%) [$P < 0.004$], 17/20 (85%) [$P < 0.0001$], 20/22 (91%) [$P < 0.0001$]; and for females was: 1/25 (4%), 8/25 (32%) [$P < 0.02$], 17/19 (89%) [$P < 0.0001$], 19/25 (76%) [$P < 0.0001$], 15/20 (75%) [$P < 0.0001$] and 20/22 (91%) [$P < 0.0001$]; for untreated controls, and the groups treated with 21, 42, 84, 32, and 170 mg, respectively. [The Working Group noted the limited description of experimental details, including body-weight gain or survival, and the lack of vehicle controls.]

Groups of 22–25 intact virgin, breeders, or gonadectomized female BALB/c mice (age, 8 weeks) were treated with hydrazine sulfate at a dose of 1.13 mg (total dose, 170 mg) by gavage in water as 150 daily doses, and then held for their lifetime (Biancifiori, 1970c). Three groups of 25–26 intact virgin, breeders, or gonadectomized mice served as untreated controls. Hydrazine sulfate increased the incidence of pulmonary tumours to 90% (20/22) [$P < 0.0001$, mainly adenoma] in intact virgins versus 4% (1/25) in controls, 100% (25/25) [$P < 0.0001$; adenoma:carcinoma, about 1:1] in breeders versus 8% (2/25) in controls, and 60% (15/25) [$P < 0.05$, mainly adenoma] in gonadectomized mice versus 27% (7/26) in controls. [The Working Group noted the poor description of experimental details, including duration of actual exposure, body-weight gain or survival, and the lack of vehicle controls and use of a single dose.]

Biancifiori (1971) gave five groups of 25–31 8-week-old male and female intact virgin, gonadectomized, or breeder (female only) C3H/Cb/Se mice, 150 daily doses of 1.13 mg/dose (170 mg total dose) of hydrazine sulfate in water by gavage over 25 weeks. The animals were then held for

their lifetime. Five groups of 23–25 intact virgin, gonadectomized or breeder animals served as untreated controls. Hydrazine sulfate increased the incidence of pulmonary tumours to 26% (7/27) [$P < 0.01$, mainly adenoma] in intact virgin males versus 0% (0/25) in controls, and to 48% (13/27) [$P < 0.0004$, mainly adenoma] in intact virgin females versus 4% (1/25) in controls. The incidence of pulmonary tumours was 4% (1/25) [adenoma] in gonadectomized males versus 4% (1/24) in controls, and 19% (5/26) in gonadectomized females versus 12% (3/25) in controls. Hydrazine sulfate increased the incidence of pulmonary tumours to 39% (12/31) [$P < 0.01$; adenoma:carcinoma, about 2:1] in female breeders versus 13% (3/23) in controls. [The Working Group noted the limited description of experimental details, including body-weight gain or survival, and the lack of vehicle controls, and use of a single dose.]

In a study to investigate the effect of antioxidants on the formation of tumours of the lung in mice by hydrazine sulfate, groups of 30 male and 30 female Swiss mice (age, 10 weeks) were treated with hydrazine sulfate at daily doses of 0 (control) or 1.1 mg by gavage in water for 5 days per week for up to 15 months. Five groups of treated mice were also simultaneously treated with 1.1 mg per day of the following antioxidants: L-arginine, pyridoxine hydrochloride, folic acid, L-arginine + L-sodium glutamate, or L-sodium glutamate + pyridoxine hydrochloride. The experiment was terminated at 15 months, except for the control group, which was maintained for lifetime. In all of the six groups treated with hydrazine sulfate, the incidence of lung tumours [not further specified] in males and females (combined) was significantly increased and ranged from 17/31 (55%) to 26/33 (79%) [$P < 0.0001$, for all treated groups versus the control group], while the incidence in the control group was 1/47 (2%) (Maru & Bhide, 1982). [The Working Group noted that this study used a single dose, reported combined tumour incidence for males and females, and reported no clinical signs, body weights, mortality information, or histological description of tumours.]

In a study of perinatal exposure in Swiss mice in which hydrazine sulfate was used to induce lung tumours, exposed groups of male and female Swiss mice (parental generation) and groups of 6 (unexposed F_1) or 22 males and females (combined) of their F_1 generation were treated with hydrazine sulfate (in distilled water) at 0 (unexposed F_1) or 1.1 mg per day, 5 days per week, by gavage, at age 10–11 weeks, for their lifetime, respectively (Menon & Bhide, 1983). Two groups of 10 (unexposed F_2) or 35 males and females (combined) of the F_2 generation, were also exposed or not (unexposed F_2) to hydrazine sulfate transplacentally and through lactation. A group of 20 males and females served as untreated controls. To raise the F_1 progeny of hydrazine-treated females, dams received hydrazine (1.1 mg per day) from day 1 of gestation, and continued to receive hydrazine treatment through lactation until death. To raise the exposed F_2 progeny, F_1 males and females (combined) were treated with hydrazine from the age of 11 weeks for a period of 4 weeks, and were then kept for mating; pregnant F_1 females continued to receive hydrazine from day 1 of gestation through lactation until death. To raise the unexposed F_2 progeny, F_1 parents were of the untreated F_1 generation.

The incidence of adenocarcinoma of the lung in the parental generation exposed to hydrazine sulfate was 72% (21/29; $P < 0.05$, X^2 test, versus 1/20 untreated controls) in females and 88% (30/34; $P < 0.05$, X^2 test, versus 1/20 untreated controls) in males. F_1 mice receiving hydrazine sulfate only during gestation and lactation, and subsequently given distilled water from a young adult age showed a 50% (3/6; $P < 0.05$, X^2 test, versus 1/20 untreated controls) incidence of adenocarcinoma of the lung, while F_1 mice raised from the same stock of parents and receiving hydrazine sulfate at 1.1 mg per day showed a 90% (20/22; $P < 0.05$, X^2 test, versus 1/20 untreated controls)

incidence of adenocarcinoma of the lung. The F_2 generation of mice, whose parents received distilled water during gestation and lactation, and then distilled water by gavage from a young adult age (unexposed F_2 generation controls), had a 10% (1/10) incidence of adenocarcinoma of the lung, while the F_2 generation of mice, whose parents received hydrazine sulfate by gavage for 4 weeks and which were themselves exposed to hydrazine sulfate during gestation and lactation, showed a 45% (16/35; $P < 0.05$, X^2 test, versus 1/10 unexposed F_2 generation controls) incidence of adenocarcinoma of the lung. [The Working Group noted that the study used only a single dose, reported combined tumour incidences for males and females, and did not report on clinical signs, body weights or mortality.]

3.1.2 Drinking-water

Toth (1969) reported on a study in three strains of mice treated with hydrazine sulfate. Groups of 110 (control) or 50 male and female Swiss mice (age, 6 weeks) were given drinking-water containing 0% or 0.012% [120 mg/L] hydrazine sulfate (average daily dose, 0.74 mg and 0.65 mg for males and females, respectively) for their lifetime. Exposure of male and female Swiss mice to hydrazine sulfate caused a significant increase in the incidence of adenoma or carcinoma (combined) of the lung: males, 25/50 (50%) [$P < 0.0001$] versus 11/110 (10%) controls; females, 24/50 (48%) [$P < 0.0001$] versus 14/110 (13%) controls. Additionally, groups of 30 (control) or 40 male and female AKR mice (age, 6 weeks) were given drinking-water containing 0% or 0.012% hydrazine sulfate (average daily dose, 0.63 mg for males and females) for their lifetime, and groups of 30 (control) or 41 male and 30 (control) or 40 female C3H mice (age, 6 weeks) were given drinking-water containing 0% or 0.012% hydrazine sulfate (average daily dose, 0.98 mg and 0.84 mg for males and females, respectively) for their lifetime. No significant increase in tumour incidence was observed in male and female AKR or C3H mice exposed to hydrazine sulfate. [The Working Group noted the use of a single dose.]

In a study to see whether the formation of lung tumours by hydrazine could be inhibited by arginine glutamate, groups of 20 or 37–38 male A/J mice (age, 6 weeks) were given drinking-water containing hydrazine sulfate at a concentration of 0 or 325 mg/L and feed containing 0% or 1% arginine glutamate for up to 48 weeks (Yamamoto & Weisburger, 1970). Hydrazine sulfate caused a significant increase [$P < 0.0001$] in the incidence of lung adenoma or carcinoma (combined) (38/38; 100%) in exposed mice compared with control mice (12/20; 60%). Upon addition of arginine glutamate, the incidence of pulmonary tumours in hydrazine-treated mice was not significantly affected, and arginine glutamate did not change the incidence of pulmonary tumours in control mice. [The Working Group noted the use of a single dose.]

Groups of 50 male and 50 female NMRI mice (age, 5–6 weeks) were given drinking-water containing hydrazine hydrate at a concentration of 0, 2, 10, or 50 ppm ad libitum for up to 2 years. A significant reduction in body weight was observed in groups at the highest dose. There was no difference in survival in any of the treated groups or controls. There was a significant increase ($P < 0.05$) in the incidence of benign lung tumours in females at the highest dose (15/50) compared with controls (6/50). There was no other significant increase in tumour incidence in any of the treatment groups compared with controls (Steinhoff et al., 1990). [The Working Group noted the limited description of the pathology of the lesions observed in treated and control mice.]

Matsumoto et al. (2016) reported the results of a study that complied with good laboratory practice (GLP) in which groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were given drinking-water containing hydrazine

monohydrate at a concentration of 0, 20, 40, or 80 ppm (males), and 0, 40, 80, or 160 ppm (females) ad libitum for 2 years. Body weights of males at the intermediate and highest dose and of all groups of treated females were significantly decreased in a dose-related manner compared with controls. There was no significant difference in survival in any of the treated groups compared with controls, except in females at the lowest dose, in which survival was better than in controls. In females at the highest dose, hydrazine monohydrate caused a significant increase in the incidence of hepatocellular adenoma (14/50, 28%; $P < 0.05$) compared with controls (5/50, 10%), and a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) (17/50, 34%; $P < 0.05$) compared with controls (7/50, 14%). There was a significant, dose-dependent positive trend ($P < 0.01$, Peto trend test) in the incidences of haemangioma of the liver, hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in treated female mice. There was also a significant, dose-dependent positive trend ($P < 0.05$, Peto trend test) for the incidence of haemangioma or haemangiosarcoma (combined) of the liver in females. There was no significant increase in the incidence of any tumours in males. [The strengths of this GLP study included the use of multiple doses, a high number of animals per group, and two sexes.]

3.1.3 Intraperitoneal injection

Two groups of 20 newborn male and female (combined) BALB/c/Cb/Se mice were treated by intraperitoneal injection of hydrazine sulfate (in water) once a day for 90 days (0 or 19 mg total dose/mouse) and then held for up to 270 days (Milia et al., 1965). There was a 100% incidence (20/20) [$P < 0.0001$] of lung adenoma or carcinoma (combined) (carcinomas, 27%) in the treated group compared with a 10% incidence (2/20, adenomas only) in controls. [The Working Group noted the limited description of experimental design, small number of animals per group, use of only one dose, and absence of discussion of clinical signs, survival or body weights.]

Groups of 9 (control) or 30 male CDF_1 mice (age 7–8 weeks) were treated by intraperitoneal injection of saline (control) or hydrazine sulfate in 2% aqueous sodium bicarbonate once a week for 8 weeks (0 or 20.8 mg total dose/mouse) and then held for up to 33 weeks (Kelly et al., 1969). There was a non-significant increase in the incidence of pulmonary tumours [not further specified] in 6/30 treated animals (20%) versus 1/9 (10%) in controls. [The Working Group noted the poor description of experimental details, the use of a single dose, the small number of controls, the short exposure time, and absence of discussion of clinical observations or pathology.]

Groups of 30 male and 30 female (combined) white mice (strain and age not reported) [weight, ~20 g] were treated with hydrazine at a dose of 0 (control) or 400 mg/kg body weight (bw) in physiological saline in 16 intraperitoneal injections administered over 46 days, and were then held for up to 313 days (Juhász et al., 1966). It was reported that 13/34 (38%) mice surviving the treatment developed tumours of the haematopoietic and lymphoid tissues (reticulum cell sarcoma or myeloid leukaemia) [$P < 0.0001$] between days 100 and 313 of the experiment. No reticulum cell sarcoma or myeloid leukaemia was reported in the 60 controls after 1 year. [The Working Group noted the poor description of experimental details, the use of a single dose, the high mortality in treated animals, the reported combined tumour incidences, the short exposure time, the absence of discussion of clinical observations or pathology, and the poor description and discussion of tumour incidences.]

In a study of hydrazine and a modifying factor (irradiation), groups of 29 or 40 male and female C57BL/6 mice (equal numbers of each sex per group) (age, 6–8 weeks) were exposed once to

400 R total-body irradiation and, starting 1 week after irradiation, given intraperitoneal injections of aqueous hydrazine sulfate at a dose of 0 or 95 mg/kg bw once per week for 10 weeks, and then held for up to 62 weeks. A group of 36 mice received no irradiation and weekly injections of hydrazine sulfate, and were also held for up to 62 weeks. A fourth group of at least 75 animals served as untreated controls. In the non-irradiated groups, lung adenomas were observed in 5/18 (28%) [not significant] mice treated with hydrazine sulfate compared with 9/75 (12%) untreated controls. The incidence of leukaemia was not increased in the irradiated/hydrazine treated group (4/29) compared with irradiated-only controls (2/25) (Mirvish et al., 1969). [The Working Group noted the use of a single dose, the limited description of experimental details, the short exposure time, the lack of reporting of clinical signs, body weights, and mortality information or histological description of tumours.]

3.1.4 Inhalation

Groups of 400 female C57BL/6 mice (age, 7 weeks) were exposed to 0 (concurrent control), 0.05, 0.25, or 1.0 ppm [0, 0.04, 0.2, and 0.8 mg/m^3] anhydrous hydrazine (purity, 99.8%) by inhalation for 6 hours per day, 5 days per week, for 1 year, and then held for an additional 15 months. Deaths during exposure were below 10% in all groups, and exposed mice demonstrated significantly higher mortality than controls at the end of the experiment, but without any dose–response relationship (controls, 72–79%; exposed, 84–87%). An increase in the incidence of lung adenoma was reported in mice at the highest dose (12/379, 3%) [$P < 0.05$] versus controls (4/378, 1%). No information on lung tumours was given for the other exposure groups (Vernot et al., 1985). [The Working Group noted the low doses used and the lack of tumour data for the groups receiving the lowest and intermediate doses.]

3.2 Rat

3.2.1 Gavage

Groups of 14–28 male and female CB/Se rats were given "pure" hydrazine sulfate as a daily gavage dose in water at 0, 12 (females only), or 18 (males only) mg/day for 68 weeks, and then held for their lifetime. Pulmonary adenoma or carcinoma was observed in 3/14 (21%) [$P < 0.05$] treated males and 5/18 (28%) [$P < 0.02$] treated females. Malignant liver tumours were observed in 4/13 (30%) [$P < 0.01$] treated males. No pulmonary or liver tumours were observed in control males (0/28) or females (0/22) (Biancifiori et al., 1966; Severi & Biancifiori 1968). [The Working Group noted the short duration of exposure, the small number of rats per exposure group, the use of a single dose, and the limited description of experimental design and results.]

3.2.2 Drinking-water

Groups of 50 male and 50 female Wistar rats were given drinking-water containing hydrazine hydrate (purity, 99.3%) at a concentration of 0, 2, 10, or 50 ppm ad libitum for their lifetime. A significant reduction in body weight was observed in males and females at the highest dose. There was no significant difference in survival in any of the treated groups or controls. There was a significant increase ($P < 0.01$) in the incidences of hepatocellular adenoma in males at the highest dose (4/49) and females at the highest dose (4/47), and of hepatocellular carcinoma in females at the highest dose (3/47; one of the tumours may not have been a primary tumour). The incidence of hepatocellular adenoma or carcinoma (combined) in females at the highest dose (7/47) was significantly increased [$P < 0.01$]. No hepatocellular tumours were observed in the 50 male and 50 female controls (Steinhoff & Mohr, 1988).

In a GLP study, groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were given drinking-water containing hydrazine

monohydrate at a concentration of 0, 20, 40, or 80 ppm ad libitum for 2 years (Matsumoto et al., 2016). Body weights of males and females at the intermediate and highest doses were significantly decreased in a dose-related manner compared with controls. Survival in females at the highest dose was significantly reduced compared with controls, while survival was similar to controls for all other groups of treated females and for all groups of treated males. Hydrazine monohydrate in male rats caused a significant positive trend in the incidence of hepatocellular adenoma (0/50, 0/50, 0/50, 3/50; $P < 0.01$ by Peto trend test) and hepatocellular adenoma or carcinoma (combined) (0/50, 0/50, 0/50, 4/50; $P < 0.01$ by Peto trend test). In female rats, hydrazine monohydrate caused a significant positive trend in the incidence of hepatocellular adenoma (1/50, 0/50, 3/50, 4/50; $P < 0.05$ by Peto trend test), hepatocellular carcinoma (0/50, 0/50, 0/50, 4/50; $P < 0.01$ by Peto trend test), and hepatocellular adenoma or carcinoma (combined) (1/50, 0/50, 3/50, 6/50; $P < 0.01$ by Peto trend test). There was also a significant increase and positive trend in the incidence of interstitial cell tumours of the testis (37/50, 45/50*, 43/50, 44/50; *$P < 0.05$; $P < 0.05$ by Peto trend test). [The strengths of this GLP study included the use of multiple doses, a high number of rats per group, and two sexes.]

3.2.3 Inhalation

Groups of 150 (controls) or 100 male and female F344 rats (age, 7 weeks) were exposed to anhydrous hydrazine (purity, 99%) at a concentration of 0 (control), 0.05, 0.25, 1.0, or 5.0 ppm [0, 0.04, 0.2, 0.8, or 4.0 mg/m^3] by inhalation for 6 hours per day, 5 days per week for 1 year, and then held for an additional 18 months. Deaths during exposure were low in all groups (below 10%), and mortality was similar in all groups at the end of the study. A significant increase in the incidence of adenomatous polyps [adenoma] of the nose was reported in males exposed to the higher two doses (0/146, 2/96, 1/94, 9/97*, 58/98*; *$P \leq 0.01$) and in females at the highest dose (0/145, 2/97, 0/98, 2/94, 28/95*; *$P \leq 0.01$). A significant increase in the incidence of thyroid carcinoma was also reported in males at the highest dose (7/146, 6/96, 5/94, 9/97, 13/98**; **$P \leq 0.05$) (Vernot et al., 1985).

Groups of 100 male and 100 female F344 rats (age, 9–11 weeks) were exposed to anhydrous hydrazine (purity, 98.8%) at a concentration of 0, 75, or 750 ppm [0, 98 and 980 mg/m^3] by inhalation for 1 hour per day, 1 day per week, for 10 weeks, and then held for 24–30 months. Polypoid adenomas of the nose were found in 4/99 (4%, $P < 0.05$) males at the highest dose, and 6/95 (6%, $P < 0.05$) females at the highest dose. In addition, one nasal squamous cell carcinoma was reported in males. No nasal tumours were observed in 98 male and 98 female controls (Latendresse et al., 1995). [The Working Group noted that this study was inadequate for the evaluation because of inadequate exposure for a study of full carcinogenicity.]

3.3 Hamster

3.3.1 Gavage

Groups of 23–56 male and female (sex distribution not given) Syrian golden hamsters (age, 8 weeks) were treated with hydrazine sulfate (purity not reported) at a total dose of 0, 180, or 280 mg by gavage in water for 15 or 20 weeks, and then held for life (Biancifiori, 1970a). There was a significant decrease in survival in treated hamsters compared with controls. No information on body weight was provided. No significant increase in the incidence of tumours was reported. A significant increase in cirrhosis of the liver was reported in both treatment groups, but not in the controls. [The Working Group noted that the study used a short duration of exposure, and was considered inadequate for the evaluation because of inadequate reporting of results in controls.]

3.3.2 Drinking-water

Groups of 50 male and 50 female Syrian golden hamsters (age, 9 weeks) were given drinking-water containing hydrazine sulfate (purity not reported) at a concentration of 0.012% [120 mg/L] for life. The average daily intake of hydrazine sulfate was 2.3 mg for both sexes. There was a slight decrease in body weight, but no effect on survival in treated animals compared with controls. No significant increases in the number of tumours produced were reported in treated hamsters. A non-significant increase in the incidence of polypoid adenoma of the caecum was reported in treated males (3/49, 6%) and females (4/45, 9%) compared with controls, but statistics and tumour incidence in controls were not provided (Toth, 1972). [The Working Group considered that the study was inadequate for the evaluation because of the use of a single dose and because of the inadequate reporting of results in controls.]

Groups of 40 male Syrian golden hamsters (age at start not given; weight, 50–60 g at 1 week before exposure) were given drinking-water containing hydrazine sulfate (purity, > 99%) at a concentration of 0 (control), 170, 340, or 510 mg/L for 2 years (average doses, 0, 4.6, 8.3, and 10.3 mg/kg bw per day) (Bosan et al., 1987). There was a significant decrease in survival in all treated groups. Hepatocellular carcinomas were observed in hamsters treated with the two higher doses of hydrazine sulfate after 78 weeks of exposure. The incidences of hepatocellular carcinoma were 0/31 (controls), 0/31, 4/34 (12%), and 11/34 (32%; [$P < 0.0004$]).

Groups of 25–40 male Syrian golden hamsters (age at start not given; weight, 50–70 g at 1 week before exposure) were given drinking-water containing hydrazine sulfate (purity, > 99%) at a concentration of 0 (control), 170, 340, or 510 mg/L for up to 21 months (average doses calculated from consumption data: 0, 4.2, 6.7, and 9.8 mg/kg bw per day) (FitzGerald & Shank, 1996). There was a significant decrease in survival in all treated groups. The incidence of hepatocellular adenoma was 0/25 (control), 1/30 (3%), 4/43 (9%), and 10/40 (25%) [$P < 0.005$], and the incidence of hepatocellular carcinoma was 0/25 (control), 0/30, 1/43 (2%) and 3/40 (8%), respectively. [The Working Group noted that the incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased [$P < 0.005$] in the group at the highest dose compared with controls.]

3.3.3 Inhalation

Groups of 200 male Syrian golden hamsters (age, 7 weeks) were exposed to anhydrous hydrazine (purity, 98.8%) at a concentration of 0 (control), 0.25, 1.0, or 5.0 ppm [0, 0.2, 0.8 or 4.0 mg/m^3] by inhalation for 6 hours per day, 5 days per week, for 1 year, and then held for an additional 1 year (Vernot et al., 1985). Exposure to hydrazine had no effect on body weights of hamsters in any treated group compared with controls. Deaths during exposure were high in all exposure groups (32–33% versus 19% in controls), but mortality was similar in all groups at the end of the study (2 years). A significant increase in the incidence of nose adenomatous polyps [adenoma] was reported in the group at the highest dose: 1/181 (1%) controls; 0/154; 1/148 (1%); 16/160* (10%), *$P \leq 0.01$. A non-significant increase in the incidence of parafollicular cell adenoma of the thyroid gland was also reported in the group at the highest dose (4/137, 3%, versus 0/145 in controls), as was a non-significant increase in the incidence of adenocarcinoma of the colon in the groups at the intermediate and highest dose (2/129, 2% and 3/139, 2%, respectively, versus 0/158 in controls) (Vernot et al., 1985).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Absorption, distribution, excretion

(a) Humans

Organ toxicity after accidental or intentional exposure to hydrazine demonstrated absorption into the systemic circulation and distribution to target tissues (Nagappan & Riddell, 2000; Kao et al., 2007). After occupational exposure, hydrazine was absorbed and excreted in the urine (Nomiyama et al., 1998a). Exposure to the therapeutic drug isoniazid, containing a hydrazine group, resulted in excretion of hydrazine and its metabolites in the urine (Timbrell et al., 1977; Blair et al., 1985; Donald et al., 1994; Preziosi, 2007).

(b) Experimental systems

Absorption of hydrazine was rapid after either oral or dermal administration in experimental animals. Hydrazine was detected in femoral blood within 30 seconds after application of a dose of 3–15 mmol/kg bw to an area of shaved chest skin of anaesthetized dogs. The serum concentration of hydrazine peaked within the first hour for most doses, followed by a slow decline over a 6-hour holding period. Unchanged hydrazine was excreted in the urine. Mortality was high across the dosing range (Smith & Clark, 1972). When hydrazine hydrate (corresponding to hydrazine free base at 3–81 mg/kg bw) was given orally (gavage) to rats, hydrazine was at its greatest concentration in the plasma and liver within 30 minutes after dosing, with the exception of a peak in plasma concentration 90 minutes after administration of the highest dose (Preece et al., 1992a). The liver to plasma ratio of hydrazine decreased with increasing dose, suggesting saturation of uptake by the liver. An effect on dose elimination was also observed, with about 40% of the lowest dose but less than 20% of the highest dose being excreted in the urine within 24 hours after administration. Dambrauskas & Cornish (1964) investigated the fate of hydrazine in rats given hydrazine hydrate at a dose of 60 mg/kg bw by subcutaneous injection. The dose was well distributed, with hydrazine detected in adipose, blood, brain, kidney, liver, lung, muscle, skin, and other tissues within 2 hours after dosing. About 13% of the total administered dose was recovered in the assayed tissues as unchanged hydrazine, with the highest concentration present in the kidney (41–56 µg/g). Hydrazine (8% of the total administered dose) was excreted in the urine. Matsuyama et al. (1983) provided additional evidence that hydrazine crosses the blood–brain barrier in rats. After intravenous injection, hydrazine was detected in the brain accompanied by an increase in gamma-aminobutyric acid (GABA) over a period of 10 hours. Hydrazine was rapidly absorbed and distributed to tissues when administered as hydrazine sulfate at a dose of 0.31 mmol/kg bw by subcutaneous injection in rats (Kaneo et al., 1984). Springer et al. (1981) recovered up to 75% of [^{15}N]-labelled hydrazine from single doses of 1 mmol/kg bw administered by various injection routes (intraperitoneal, subcutaneous, intravenous) in rats. Up to 25% of the total administered dose was recovered as nitrogen (N_2) in expired air within 48 hours. An additional 50% was excreted in the urine as unchanged hydrazine and acid-labile metabolite(s). The disappearance of intravenously administered hydrazine from blood was described as biphasic, with calculated half-lives of 0.74 and 27 hours. In mice, intraperitoneal administration of [^{15}N]-labelled hydrazine sulfate at 1 mmol/kg bw resulted in rapid distribution to tissues including blood, brain, liver, kidney, and lung (Nelson & Gordon, 1981). Clearance from tissues was extensive by 24 hours. About 30% of the administered dose was recovered as N_2 and

40% was recovered in the urine as hydrazine and metabolites within 48 hours after injection (Nelson & Gordon, 1981).

Mice excreted about 50% of an administered dose of either 40 or 60 mg/kg bw as unchanged hydrazine in the urine within 48 hours after a subcutaneous injection. Less than 1.5% of the administered hydrazine remained in each carcass at this time-point. The unrecovered dose was assumed to be metabolites (Dambrauskas & Cornish, 1964).

4.1.2 Metabolism

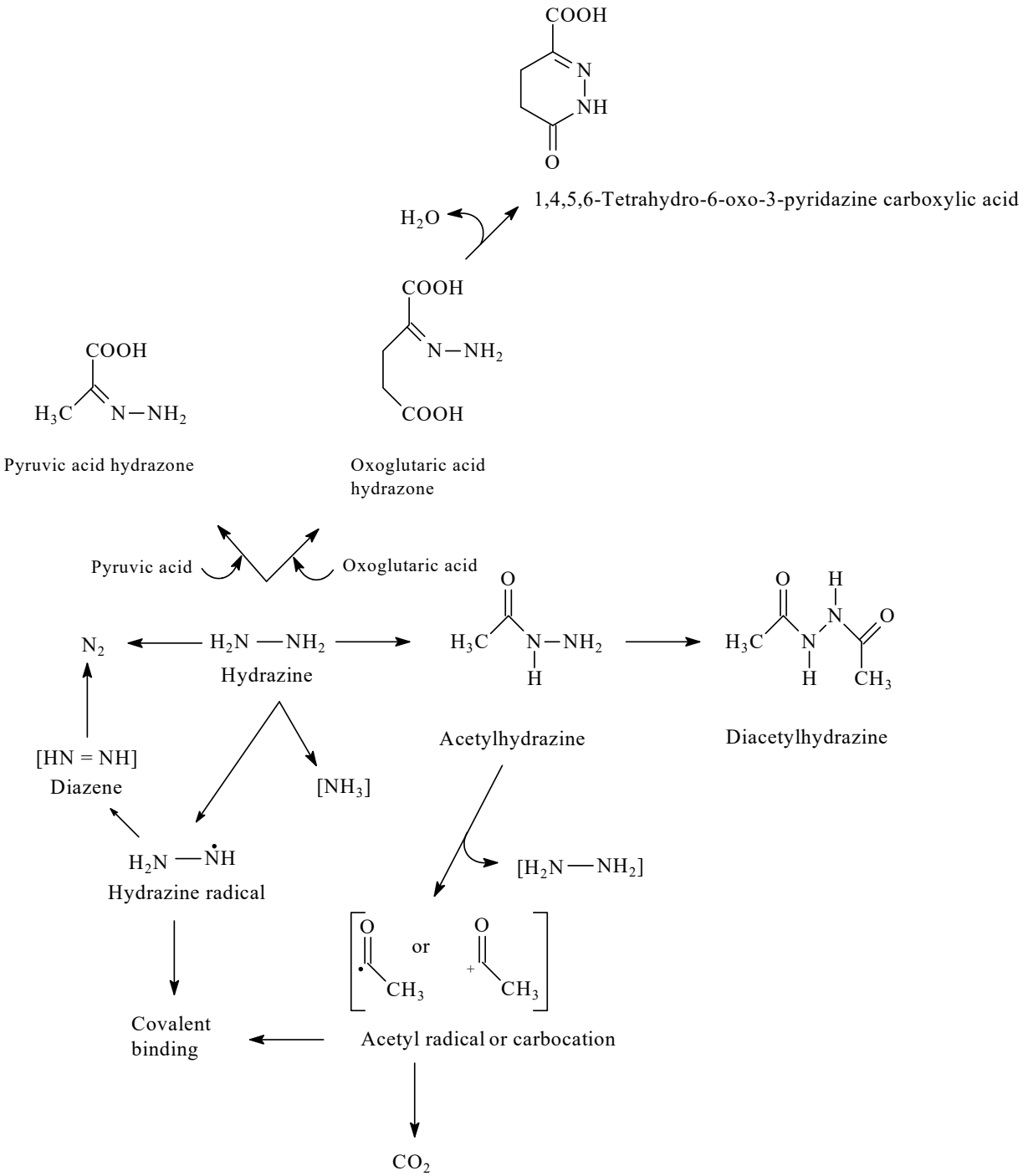
(a) Humans

Hydrazine was metabolized to acetylhydrazine (monoacetylhydrazine) in exposed workers (Nomiyama et al., 1998a). In male Japanese workers, the rate of acetylation was dependent on polymorphisms in *N*-acetyltransferase (NAT2), with half-lives of about 2 and 4 hours in rapid and slow acetylators, respectively (Koizumi et al., 1998). Acetylhydrazine and diacetylhydrazine have been detected in the urine of subjects receiving isoniazid (Ellard & Gammon, 1976; Timbrell et al., 1977). Oxidative metabolism of hydrazine was demonstrated in human microsomes based on disappearance of the chemical over time from the incubation mixture; however, the rate of metabolism was slower than in rat microsomes (Jenner & Timbrell, 1995). A review of isoniazid metabolism indicated that pathways of hydrazine biotransformation are similar in humans and other mammals (Preziosi, 2007). The metabolism of hydrazine is discussed in greater detail below.

(b) Experimental systems

Pathways of hydrazine metabolism in experimental animals include oxidation and acetylation (Colvin, 1969; Fig. 4.1). Acetylhydrazine and/or diacetylhydrazine have been observed in the urine of animals treated with hydrazine. The presence and/or relative abundance of these acetylated metabolites are species-dependent. Bollard et al. (2005) detected diacetylhydrazine in the urine of rats and mice treated orally with hydrazine hydrochloride at a dose of 100 or 250 mg/kg bw. In contrast, acetylhydrazine was detected in the urine in rats, but not in mice. This result was attributed to higher activity of *N*-acetyl transferase in the mouse. Dogs, unlike rats and mice, have little to no capacity to acetylate hydrazine (McKennis et al., 1959). This limitation may have contributed to the prolonged elimination of hydrazine in dogs as reported by Smith & Clark (1972). Rabbits treated with hydrazine excreted diacetylhydrazine in the urine (McKennis et al., 1959). Kaneo et al. (1984) demonstrated that metabolism of hydrazine to acetylhydrazine in rats was reversible. Acetylhydrazine may give rise to a carbon-centred acetyl radical or carbocation, capable of binding to macromolecules or oxidation to carbon dioxide (CO_2) (Sinha, 1987; Mörike et al., 1996; Preziosi, 2007). Hydrazine may also give rise to hydrazones (Preziosi, 2007). The metabolite 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid, a derivative of oxoglutarate hydrazone, was found in the urine of rats and mice treated with hydrazine (Nelson & Gordon, 1981; Delaney & Timbrell, 1995; Bollard et al., 2005). Hydrazine is oxidized to N_2 in rats and mice (Nelson & Gordon, 1981; Springer et al., 1981). Degradation of hydrazine to ammonia (NH_3) may be possible, especially in dogs (McKennis et al., 1959; Colvin, 1969; Preziosi, 2007). Oxidative metabolism of hydrazine was catalysed primarily by cytochrome P450s (CYPs) in rat microsomes (Noda et al., 1987; Jenner & Timbrell, 1995). Mixed function oxidases may also play a role (Jenner & Timbrell, 1995). Results from incubation in rat hepatocytes suggested the involvement of CYP2E1, CYP2B1, and CYP1A1/2 in the metabolism of hydrazine (Delaney & Timbrell, 1995). CYP activity was demonstrated in the metabolism of $[^{15}N_2]$-$[^{14}C]$-labelled acetylhydrazine to N_2 and CO_2 in the rat (Mörike et al., 1996). A hydrazine radical,

Fig. 4.1 Proposed metabolic scheme for hydrazine in mammals



Adapted from Nelson & Gordon (1981), Noda et al. (1985), Delaney & Timbrell (1995), and Mörike et al. (1996)

potentially giving rise to a diimide (diazene), was identified in microsomal incubations of hydrazine using a spin-trapping method (Noda et al., 1985).

4.2 Mechanisms of carcinogenesis

The evidence on the "key characteristics" of carcinogens (Smith et al., 2016) – concerning whether hydrazine is genotoxic, induces oxidative stress, alters cell proliferation, cell death or nutrient supply, and modulates receptor-mediated mechanisms – is summarized below.

4.2.1 Genetic and related effects

See Table 4.1 and Table 4.2

(a) Humans

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

One study reported the induction of single-strand breaks and alkali-labile sites (comet assay) in human lung cells in vitro (Robbiano et al., 2006).

(b) Experimental systems

Considerable information was previously reviewed by the *IARC Monographs* Working Group regarding whether hydrazine is genotoxic in experimental systems (IARC, 1999). Multiple studies identified *N*7-methylguanine and O^6-methylguanine in the livers of mice, rats (including neonates) and hamsters treated with hydrazine in vivo. The available data suggested that the DNA methylation mechanism involved reaction of hydrazine with endogenous formaldehyde, followed by metabolism of the resulting hydrazone to a methylating agent, most likely diazomethane. Other reports concerned the formation of DNA adducts (not characterized) in M13mp18 viral DNA in vitro.

One study found that hydrazine induced organ-specific genotoxicity in mice, and that the target organs for DNA damage (alkaline comet assay) depended on the route of administration. DNA damage was found in the stomach, liver, and lungs of mice given hydrazine as a single intraperitoneal dose at 100 mg/kg bw. When the same dose was administered orally, DNA damage was also found in the colon and brain (Sasaki et al., 1998). More recently, another study reported the induction of single-strand breaks and alkali-labile sites (comet assay) in primary lung cells from male rats as well as in the lungs of rats given a single oral dose of hydrazine (Robbiano et al., 2006). Lack of induction of sister-chromatid exchanges in bone marrow or liver of mice, and conflicting results on induction of micronuclei in mouse bone-marrow cells (in one study out of three), were observed (IARC, 1999).

Hydrazine induced DNA strand breaks in rat hepatocytes and unscheduled DNA synthesis in mouse hepatocytes. There were conflicting results for the induction of gene mutations in mouse lymphoma L5178Y cells (one positive result and two negative, all in the absence of exogenous metabolic activation). Hydrazine induced sister-chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, but gave negative results for the induction of chromosomal aberrations in rat liver RL1 cells (IARC, 1999).

Hydrazine was mutagenic in yeast and bacteria, induced DNA damage in bacteria, and caused somatic mutations in *Drosophila* (IARC, 1999).

4.2.2 Oxidative stress

(a) Humans

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

In human hepatoma HepG2 cells, hydrazine (0.25–2.0 mM) depleted reduced glutathione in a concentration-dependent manner, whereas reactive oxygen species (ROS) were decreased, as assessed using the dye 2′,7′-dichlorodihydrofluorescein diacetate (Olthof et al., 2009).

Table 4.1 Genetic and related effects of hydrazine in human and rodent cells in vitro

Species, strain	Tissue, cell line	End-point	Test	Results	Concentration (LEC or HIC)	Comments	Reference
Human	Lung	DNA damage	DNA strand breaks (comet assay)	+	Hydrazine, 0.5–4 mM	Cells from four donors, analysed independently; all had dose-dependent increases in single-strand breaks and alkali-labile sites	Robbiano et al. (2006)
Rat, Sprague-Dawley	Lung	DNA damage	DNA strand breaks (comet assay)	+	Hydrazine, 0.5–4 mM	Dose-dependent increases in single-strand breaks and alkali-labile sites	Robbiano et al. (2006)

+, positive; HIC, highest ineffective concentration; LEC, lowest effective concentration

Table 4.2 Genetic and related effects of hydrazine in experimental animals in vivo

Species, strain, sex	Tissue	End-point	Test	Results	Dose (LED/HID)	Comments	Reference
Rat, Sprague-Dawley, M	Lung, liver, and kidney	DNA damage	DNA strand breaks (alkaline comet assay)	+	Hydrazine, 30 mg/kg bw; i.p.; single dose	Purity, 98%; positive in all tissues tested	Robbiano et al. (2006)
Mouse, CD-1, M	Stomach, liver, lung	DNA damage	DNA strand breaks (alkaline comet assay)	+	Hydrazine, 100 mg/kg bw; i.p.; single dose	DNA damage was not increased in colon, kidney, bladder, brain, or bone marrow	Sasaki et al. (1998)
Mouse, CD-1, M	Stomach, colon, liver, lung, brain	DNA damage	DNA strand breaks (alkaline comet assay)	+	Hydrazine, 100 mg/kg bw; i.g.; single dose	DNA damage was not increased in kidney, bladder, or bone marrow	Sasaki et al. (1998)

+, positive; bw, body weight; HID, highest ineffective dose; i.g., gavage; i.p., intraperitoneal; LED, lowest effective dose; M, male

(b) Experimental systems

(i) Non-human mammals in vivo

In Wistar rats fed diets containing 0.5% hydrazine dichloride for 7 days, there were significant increases in lipid-soluble fluorophores (lipofuscin) in the liver, heart, muscle, and spleen (Antosiewicz et al., 2002). This index of oxidative stress induction by hydrazine was diminished in the heart and skeletal muscle by the antioxidant α-tocopherol diacetate.

In Wistar rats, hydrazine (intraperitoneal dose of 80 mg/kg bw) decreased hepatic glutathione levels, increased levels of malondialdehyde (a measure of lipid peroxidation), and increased levels of 8-hydroxy-2′-deoxyguanosine DNA adducts (8-OHdG, a measure of oxidative DNA damage). These changes were prevented by tea melanin (Hung et al., 2003).

In Sprague-Dawley rats, hydrazine dihydrochloride (0, 120, or 240 mg/kg bw by gavage) increased levels of the precursor amino acids of glutathione biosynthesis in the urine and/or plasma and increased plasma 5-oxoproline (a product of glutathione metabolism) (Bando et al., 2011).

Several studies have reported suppression by antioxidants of the induction of megamitochondria (enlarged and abnormally shaped mitochondria that are thought to arise by membrane fusion) by hydrazine. For instance, in Wistar rat liver, hydrazine-induced formation of megamitochondria, and accompanying increases in lipid peroxidation were suppressed by co-treatment with coenzyme Q_{10} (CoQ_{10}). CoQ_{10} did not prevent the decrease in reduced glutathione that was observed in rats treated with hydrazine (Adachi et al., 1995). The formation of megamitochondria in the liver of Wistar rats fed diets containing 1.0% hydrazine for 7 days was also suppressed by α-tocopherol (intraperitoneal dose of 700 mg/kg bw). A marked increase in hepatic level of lipid-soluble fluorophores, an indicator of oxidative stress, was also observed in hydrazine-treated rats; however, these increases were not prevented by α-tocopherol (Antosiewicz et al., 1994). The formation of megamitochondria in the livers of Wistar rats fed diets containing 0.5% hydrazine for 7 days was suppressed by various free radical scavengers including CoQ_{10}, α-tocopherol, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-OH-TEMPO), and by allopurinol, a xanthine oxidase inhibitor (Wakabayashi et al., 1997). In addition, 4-OH-TEMPO lowered hepatic lipid peroxidation, assessed by measuring levels of thiobarbituric acid reactive substances (TBARS) and of lipid-soluble fluorophores. Allopurinol was less effective than 4-OH-TEMPO in preventing loss of mitochondrial phosphorylating ability and in preventing lipid peroxidation in liver (Matsuhashi et al., 1997; Wakabayashi et al., 1997). An increase in the rate of generation of hydrogen peroxide was also observed in isolated liver mitochondria obtained from Wistar rats that had been fed diets containing 1% hydrazine for 3 days (Karbowski et al., 1999). [The Working Group noted that induction of megamitochondria by hydrazine is probably due to free radicals generated by exposure to this agent, and that the hepatotoxicity of hydrazine is probably due to induction of oxidative stress.]

Perfusion of Sprague-Dawley rat livers with hydrazine, acetylhydrazine, or isoniazid at 5 mM in the presence of the spin-trapping agent α-phenyl-*tert*-butylnitrone produced the carbon-centred radical that was shown to be the same acetyl radical (Sinha, 1987).

(ii) Non-human mammalian cells in vitro

During CYP-mediated oxidative metabolism of hydrazine by rat liver microsomes, the formation of a free radical intermediate was detected by electron spin resonance spectroscopy using α-phenyl-*tert*-butylnitrone as the spin-trapping agent; the radical species trapped with α-phenyl-*tert*-butylnitrone was identified as a hydrazine-derived metabolite by mass spectrometry (Noda et al., 1985). Inhibitors of CYP, and the

antioxidant ascorbic acid decreased the generation of hydrazine radical by rat liver microsomes (Matsuki et al., 1991).

Lipid peroxidation (TBARS) was increased significantly in rat liver slices incubated with hydrazine at 15 mM for 10 hours. This increase was associated with extensive hepatic necrosis (Walubo et al., 1998).

Exposure of primary rat hepatocytes (isolated from Fischer 344 rats) to hydrazine (at 25 mM and above) reduced catalase activity, depleted reduced glutathione, and increased oxidized glutathione and lipid peroxidation (TBARS), while ROS was increased at hydrazine concentrations of 100 mM and above (Hussain & Frazier, 2002).

Hydrazine (8 mM) increased the formation of hydrogen peroxide and ROS, and protein carbonylation in hepatocytes isolated from Sprague-Dawley rats. ROS formation and protein carbonylation were decreased by a ROS scavenger (4-OH-TEMPO) and by a CYP inhibitor (1-aminobenzotriazole) (Tafazoli et al., 2008).

Hung et al. (2003) showed that melanin derived from tea reduced hydrazine-induced free radical formation in rat hepatocytes isolated from Wistar rats, as assessed by measuring chemiluminescence intensity.

In hepatocytes isolated from Sprague-Dawley rats, the effects of hydrazine and isoniazid on lipid peroxidation and mitochondrial depolarization were significantly reduced by pre-treatment with *N*-acetylcysteine. Hydrazine (8 mM) significantly increased ROS whether or not glutathione was depleted, and also increased lipid peroxidation and mitochondrial membrane depolarization (Heidari et al., 2013).

Incubation of primary Wistar rat hepatocytes with hydrazine (2 mM) induced the formation of megamitochondria, decreased the mitochondrial membrane potential, and increased intracellular levels of ROS (assessed using 2′,7′-dichlorodihydrofluorescein diacetate) (Teranishi et al., 1999). These changes were suppressed by co-treatment of hepatocytes with the free radical scavenger CoQ_{10} (1 μM).

In primary cultures of Wistar rat hepatocytes, mitochondria were enlarged by hydrazine, and mitochondria were substantially larger in hepatocytes isolated from rats pre-treated with phenobarbital and then incubated with hydrazine. The effect of phenobarbital was attributed to the induction of CYP, which had been reported to metabolize hydrazine and generate free radicals (Noda et al., 1987). In addition, compared with controls, levels of malondialdehyde in homogenates of hepatocyte cultures treated with hydrazine at 2 mM were elevated (155%) after incubation for 4 hours and significantly increased (240%) after incubation for 22 hours (Karbowski et al., 1997).

(iii) Acellular systems

Hydrazine (0.5 mM) produced fragmentation of calf thymus DNA in a cell-free system containing manganese (Mn) or copper (Cu) ions. DNA damage induced by hydrazine plus Mn(II) or Mn(III) was inhibited by hydroxyl radical scavengers or superoxide dismutase, but not by catalase; while DNA damage caused by hydrazine plus Cu(II) was inhibited by catalase, but not by hydroxyl radical scavengers or superoxide dismutase. Electron spin trapping using 5,5-dimethyl-1-pyrroline *N*-oxide confirmed that hydrazine plus Mn(III) produced hydroxyl free radical via superoxide and not via hydrogen peroxide. Thus, ROS may also be produced by nonenzymatic activation of hydrazine (Yamamoto & Kawanishi, 1991).

4.2.3 Altered cell proliferation, death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) In vivo

In Syrian golden hamsters given drinking-water containing hydrazine sulfate (170, 340, or 510 mg/L for up to 21 months), increases in megalocytosis, intranuclear inclusions, bile duct hyperplasia, and foci of cellular alteration were observed after 18 months in the groups receiving the intermediate and highest doses. However, incorporation into liver DNA of ^{14}C-thymidine, administered before termination as an index of cell replication, was not different in any of the exposure groups compared with controls at any interim evaluation (FitzGerald & Shank, 1996).

No studies were identified that showed suppression of apoptosis by hydrazine. In an inhalation study, increases in the incidence of apoptosis based on histological criteria were reported in the nose of Fischer 344 rats exposed to hydrazine (750 ppm) for 1 or 10 hours. Increased incidences of proliferative nasal lesions, including epithelial hyperplasia and adenoma, were also observed in these rats held for up to 28 months after exposure (Latendresse et al., 1995).

Hepatic steatosis and hyperlipidaemia were induced in Wistar rats given hydrazine as a single intraperitoneal injection at 50 mg/kg bw. Compared with controls, exposed rats had increased levels of triglycerides, cholesterol, free fatty acids, and total lipids in plasma and liver tissue. Hydrazine also caused a decrease in levels of triglycerides and total lipids in adipose tissue (Vivekanandan et al., 2007). [The Working Group noted that increased mobilization of triglycerides from adipose tissue to the liver by hydrazine may contribute to the development of hepatic steatosis by this chemical.]

In C57Bl/6 mice, significant increases (more than twofold) in the hepatic expression of several genes involved in triglyceride and cholesterol synthesis, lipid transport, and fatty acid oxidation were detected 24 hours after administration of a single oral dose of hydrazine sulfate (100 mg/kg bw). The gene expression profiles resulting from hydrazine exposure were consistent with production and intracellular transport of hepatic lipids being favoured over removal of fatty acids (Richards et al., 2004).

(ii) In vitro

In hepatocytes isolated from Wistar rats, pre-labelled with [^{14}C]palmitate, and incubated with 2–12 mM hydrazine, the percentage of radiolabelled triglycerides appearing in the medium decreased with increasing concentrations of hydrazine [these results indicated that reduced secretion of triglycerides from liver cells might also be a factor in hydrazine-induced hepatic steatosis] (Waterfield et al., 1997). Waterfield et al. (1997) also reported the following hepatic or hepatocellular effects: lactate dehydrogenase leakage, adenosine triphosphate (ATP) and glutathione *S*-transferase depletion, increase in citrulline level, inhibition of protein synthesis, taurine leakage, and triglyceride accumulation.

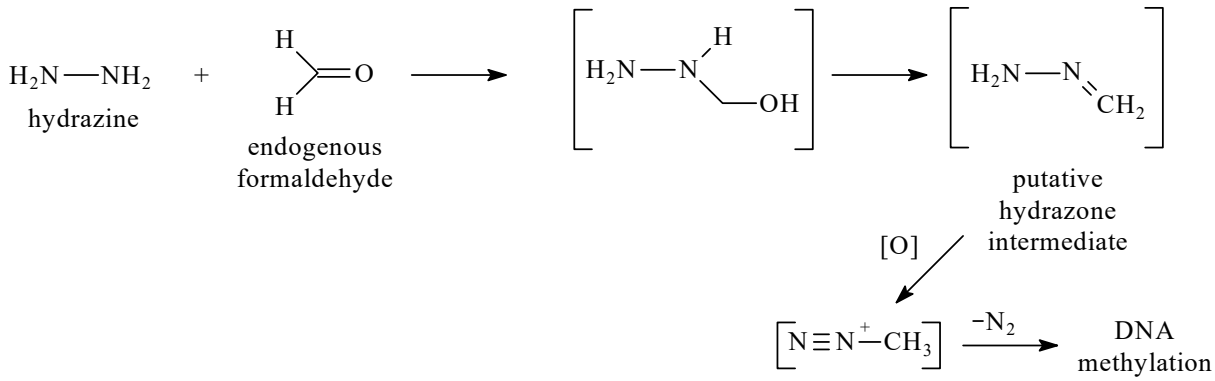
Dilworth et al. (2000), using metabolically competent rat liver spheroids, also showed ATP depletion after hydrazine treatment that required even higher concentrations than hepatocytes in primary culture. Garrod et al. (2005) observed an increase in triglycerides and β-alanine, combined with a decrease in hepatic glycogen, glucose, choline, taurine, and trimethylamine-*N*-oxide (TMAO) in the rat liver 24 hours after a dose of hydrazine of 90 mg/kg bw. In the renal cortex, 2-aminoadipate, and β–alanine increased, which concurred with a decrease in TMAO, *myo*-inositol, choline, taurine, glutamate, and lysine.

4.2.4 Receptor-mediated effects

(a) Humans

No data were available to the Working Group.

Fig. 4.2 Proposed pathway for the metabolic generation of a methylating electrophile from formaldehyde hydrazone, a condensation product of hydrazine and formaldehyde



Postulated metabolites are represented in square brackets
Adapted from Lambert & Shank (1988)

(b) Experimental systems

In C57Bl/6 mice, a single dose of hydrazine sulfate at 100 or 300 mg/kg bw increased hepatic gene expression by peroxisome proliferation-activated receptor (PPAR) and sterol regulatory element-binding protein transcription factors after 24 hours (Richards et al., 2004)

To detect possible toxicity pathways with hydrazine in Sprague-Dawley rat liver, a combination of genomics, proteomics, and metabolomics was used to detect changes in mRNA, proteins, and endogenous metabolites after a single oral dose (30 or 90 mg/kg bw). The results of these combined techniques suggested that hydrazine can affect hepatic oxidative stress, Ca^{2+} concentration, and thyroid hormone homeostasis, glucose and lipid metabolism; several mechanistic pathways for toxicity in the rat liver were described (Klenø et al., 2004a, b).

In addition to hepatotoxicity, effects on the central nervous system in rodents have been associated with changes in GABA levels. Specifically, these changes were caused by depletion of pyridoxal phosphate, which requires gamma-aminobutyrate aminotransferase and glutamate decarboxylase (IPCS, 1987a).

4.2.5 Other mechanisms

No additional mechanistic data in exposed humans or human cells were available to the Working Group.

Concerning epigenetic effects, hydrazine increased the labelling of methylguanines upon co-administration of L-[methyl-^{14}C]methionine or [^{14}C]formate in rodents (Lambert & Shank, 1988). In Syrian golden hamsters given drinking-water containing hydrazine sulfate (170–510 mg/L) for up to 21 months, hypomethylation of cytosines occurred at the highest exposure level (FitzGerald & Shank, 1996). In this 21-month study, Zheng & Shank (1996) reported hypomethylation in the *p53* tumour suppressor gene and in the *c-jun* proto-oncogene, and hypermethylation in the c-Ha-*ras* proto-oncogene and in the DNA methyltransferase gene. No changes were detected for *c-fos, c-myc*, and γ-glutamyltranspeptidase. Lambert & Shank (1988) proposed a pathway for the generation of a methylating elctrophile from formaldehyde hydrazine, a condensation product of hydrazine and formaldehyde (Fig. 4.2).

Inflammatory infiltrates were only of minimal severity in animals killed after a single exposure or 10 exposures of 1 hour to hydrazine at 750 ppm, or at least 2 years after exposure (Latendresse et al., 1995).

4.3 Data relevant to comparisons across agents and end-points

For all compounds evaluated in the present volume of the *IARC Monographs*, including hydrazine, analyses of high-throughput screening data generated by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) are presented in the *Monograph* on 1-bromopropane, in the present volume.

4.4 Cancer susceptibility

No data were available to Working Group.

4.5 Other adverse effects

4.5.1 Humans

Hydrazine is hepatotoxic, nephrotoxic, immunotoxic, and neurotoxic in humans. Hydrazine produces strong irritation of the skin, eye, and mucous membranes, and can also cause skin sensitization. After ingestion, reported systemic effects are vomiting, muscle tremor, convulsions, seizures, paresthesia, anorexia, weight loss, kidney damage, and centrolobular fatty changes of the liver (SCOEL, 2010). No data concerning reproductive toxicity in humans were available to the Working Group (HSDB, 2010).

4.5.2 Experimental systems

The toxicological effects of hydrazine in experimental animals were comparable to those seen in humans, with pronounced effects on the liver, kidneys and lungs. In the rat, liver accumulation of lipids, swelling of mitochondria, and formation of microbodies in the hepatocytes were observed. In general, similar hepatic lesions were observed in rat, mice, hamsters, dogs, and monkeys, but the intensity of the effects was species-dependent. In addition, effects in experimental animals have also been observed on the proximal tubular kidney cells, lungs, central nervous system, haematological system, and ocular regions, while behavioural effects like lethargy and depression were also reported (HSDB, 2010).

Hydrazine and mono-acetyl hydrazine are formed as reactive metabolites of isoniazid, and may play a role in its toxicity (reviewed by Hassan et al., 2015). In the metabolism of isoniazid, NAT2 is responsible for the formation of acetyl hydrazine, which is further oxidized by CYP2E1 to *N*-hydroxy acetyl hydrazine that is eventually converted to acetyl diazine. The latter compound may be the toxic metabolite itself, or break down further to the reactive acetyl onium ion, acetyl radical, and ketene, which in turn can bind covalently to hepatic macromolecules, resulting in liver toxicity.

5. Summary of Data Reported

5.1 Exposure data

Hydrazine exists as anhydrous hydrazine (containing < 37% water by mass), or as hydrazine hydrate (an aqueous solution containing ≤ 64% hydrazine). Hydrazine is used in the manufacture of pharmaceuticals, agrochemicals, chemical blowing agents, paints, inks and organic dyes, polyurethane coatings, and adhesives. In addition, hydrazine has several direct applications as an oxygen scavenger, a corrosion inhibitor, a reducing agent, and a propellant. Exposure predominantly occurs in the workplace, with highest exposures in facilities where hydrazine is handled as rocket propellant and in the refilling of fighter aircrafts. No exposure of the general population has been identified.

5.2 Human carcinogenicity data

Associations between cancer and exposure to hydrazine have been studied in workers manufacturing hydrazine in the United Kingdom, and in workers testing rocket engines at a field laboratory in California, USA.

The manufacturing cohort study used a crude measure of exposure and had a small number of cancer deaths. This study was judged to be uninformative for the purposes of evaluation.

Two investigations featuring overlapping cohorts of rocket-testing workers at the same facility in California, USA, were considered to be more informative. One study was restricted to mortality with wider criteria for eligibility, including workers employed for shorter duration. This study also had a narrower definition of exposure, classifying a smaller proportion of workers as highly exposed. The other study at the California rocket-testing facility included incidence data and had a broader definition of exposure. Nevertheless, some subgroups in both studies were roughly comparable and these showed an excess of cancer of the lung. The study of incidence also showed a statistically significant positive exposure–response relationship for cancer of the lung. Taken together, the findings of these studies were suggestive of excesses of cancer of the lung attributable to exposure to hydrazine for workers with higher exposure and earlier exposure periods. Although analyses of these studies were not adjusted for tobacco smoking, internal analyses of exposure–response are unlikely to be confounded by smoking. Furthermore, in the study of incidence, smoking data for a subset of workers suggested that the proportion of smokers was similar in workers with or without exposure to hydrazine, and no excess of other smoking-related cancers was observed.

5.3 Animal carcinogenicity data

Hydrazine (usually administered as hydrazine sulfate) was tested in studies of carcinogenicity in mice treated by gavage, in the drinking-water, by intraperitoneal injection, or by inhalation. Hydrazine was tested in studies in rats and hamsters treated by gavage, in the drinking-water, or by inhalation.

In mice, hydrazine caused a significant increase in the incidence of adenoma and/or carcinoma of the lung in four different strains of males and/or females treated by gavage in 11 studies. Most of these studies were single-dose studies; tumour incidences usually ranged from 75% to 100%, and tumour multiplicity often ranged from 3 to 10 in the treated groups. A significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) was also observed in males and females in two of these gavage studies. In addition, hydrazine caused a significant increase in the incidence of adenoma or carcinoma of the lung in three different strains of male and/or female mice when administered in the drinking-water in three studies; a significant increase in the incidence of lung adenoma or carcinoma (combined) in males and females (combined) when administered by intraperitoneal injection in one study; and a significant increase in the incidence of tumours of the haematopoietic and lymphoid tissues (reticulum cell sarcoma or myeloid leukaemia) in males and females (combined) when administered by intraperitoneal injection in one study. In one study in female mice given drinking-water containing hydrazine, there was a significant increase (with a significant positive trend) in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined); there was also a significant positive trend in the incidence of hepatocellular carcinoma, liver haemangioma, and liver haemangioma or haemangiosarcoma (combined). Hydrazine also caused a significant

increase in the incidence of lung adenoma in one study in female mice treated by inhalation.

In one study in rats, hydrazine caused a significant increase in the incidence of lung adenoma or carcinoma (combined) in males and females treated by gavage; a significant increase in the incidence of malignant liver tumours was also reported in males. Hydrazine caused a significant increase in the incidence of hepatocellular adenoma in two strains of male and female rats given drinking-water containing hydrazine in two studies, a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) and of hepatocellular carcinoma in female rats in these two studies, and a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) and of interstitial cell tumours of the testes in male rats in one of these studies. Hydrazine caused a significant increase in the incidence of nasal adenoma in male and female rats, and of thyroid carcinoma in male rats, treated by inhalation in one study.

In male hamsters given drinking-water containing hydrazine, there was a significant dose-related increase in the incidence of hepatocellular adenoma or carcinoma (combined) in one study, and a significant increase in the incidence of hepatocellular carcinoma in another study. Hydrazine also caused a significant dose-related increase in the incidence of nasal adenoma in male hamsters treated by inhalation in a third study.

5.4 Mechanistic and other relevant data

In humans and other mammals, hydrazine is absorbed rapidly.

With respect to the key characteristics of human carcinogens, there is *strong* evidence that hydrazine is electrophilic or can be metabolically activated. Acetylated metabolites as well as unchanged hydrazine have been detected in the urine of exposed humans. Hydrazine is acetylated in rodents and rabbits. Oxidative metabolism of hydrazine via formation of nitrogen and carbon dioxide has been demonstrated in mammals. Reactive intermediates include nitrogen- and carbon-centred–radicals, as well as a postulated carbocation.

There is *strong* evidence that hydrazine is genotoxic, primarily from experimental systems. DNA single-strand breaks were observed in a single study of human lung cells exposed to hydrazine. The frequency of single-strand breaks was also increased in the lungs of exposed rats and in rat lung cell cultures. In mice, hydrazine induced formation of DNA strand breaks in the liver, lung, and stomach. DNA adducts from hydrazine, *N*7-methylguanine and O^6-methylguanine, were detected in the liver of mice, rats, and hamsters exposed to hydrazine in vivo. Hydrazine was mutagenic in yeast and bacteria, and induced DNA damage in bacteria and somatic mutations in *Drosophila*.

There is *strong* evidence that hydrazine induces oxidative stress in experimental systems. Numerous studies have shown that exposure to hydrazine induces oxidative stress in mammalian systems, both in vivo and in vitro. In hepatocytes isolated from rats, hydrazine decreased levels of reduced glutathione, and increased various indicators of the formation of reactive oxygen species. In addition, most of these changes were prevented by co-treatment with antioxidants. The production of free-radical intermediates during cytochrome P450 mediated metabolism of hydrazine was demonstrated by electron spin resonance spectroscopy using a spin-trapping agent.

There is *strong* evidence that hydrazine alters cell proliferation, cell death, and nutrient supply in experimental systems. Hepatic steatosis was induced in rats, and reduced secretion of triglycerides was seen in isolated rat hepatocytes treated with hydrazine. In a study of exposure by

inhalation, apoptosis was increased in the nasal epithelium of rats.

There is *weak* evidence that hydrazine modulates receptor-mediated effects. Gene interactions with the peroxisome proliferator-activated receptors and sterol regulatory-element binding protein have been observed in mice. Hydrazine was considered marginally active in the Tox21 aryl hydrocarbon receptor reporter-gene assay.

There were few data on other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, induces chronic inflammation, is immunosuppressive, or causes immortalization).

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of hydrazine. A positive association has been observed between exposure to hydrazine and cancer of the lung.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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TETRABROMOBISPHENOL A

1. Exposure Data

1.1. Identification of the agent

From NTP (2014), ECHA (2006)

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 79-94-7

Chem. Abstr. Serv. Name: Tetrabromobisphenol A

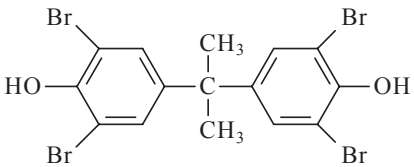
EINECS No.: 201-236-9

IUPAC Name: 2,2′,6,6′-Tetrabromo-4,4′-isopropylidenediphenol

Synonyms: 2,2-Bis(3,5-dibromo-4-hydroxyphenyl) propane; phenol, 4,4′–iso-propylidenebis, (dibromo-); 4,4′-isopropylidene-bis(2,6-dibromophenol); phenol, 4,4′-(1-methylethylidene)bis(2,6-dibromo-); 3,3′,5,5′-tetrabromobisphenol-A; tetrabromodihydroxy diphenylpropane

Acronyms: TBBP-A; TBBP; TBBPA

1.1.2 Structure and molecular formula, and relative molecular mass



Molecular formula: $C_{15}H_{12}Br_4O_2$

Relative molecular weight: 543.88

1.1.3 Physical and chemical properties of the pure substance

Description: White crystalline powder at 20 °C containing 58.4% bromine

Boiling point: ~316 °C (decomposes at 200–300 °C)

Melting point: 181–182 °C

Density: 2.12 g/cm^3

Volatility: Vapour pressure, 6.24 × 10^{-9} kPa at 25 °C

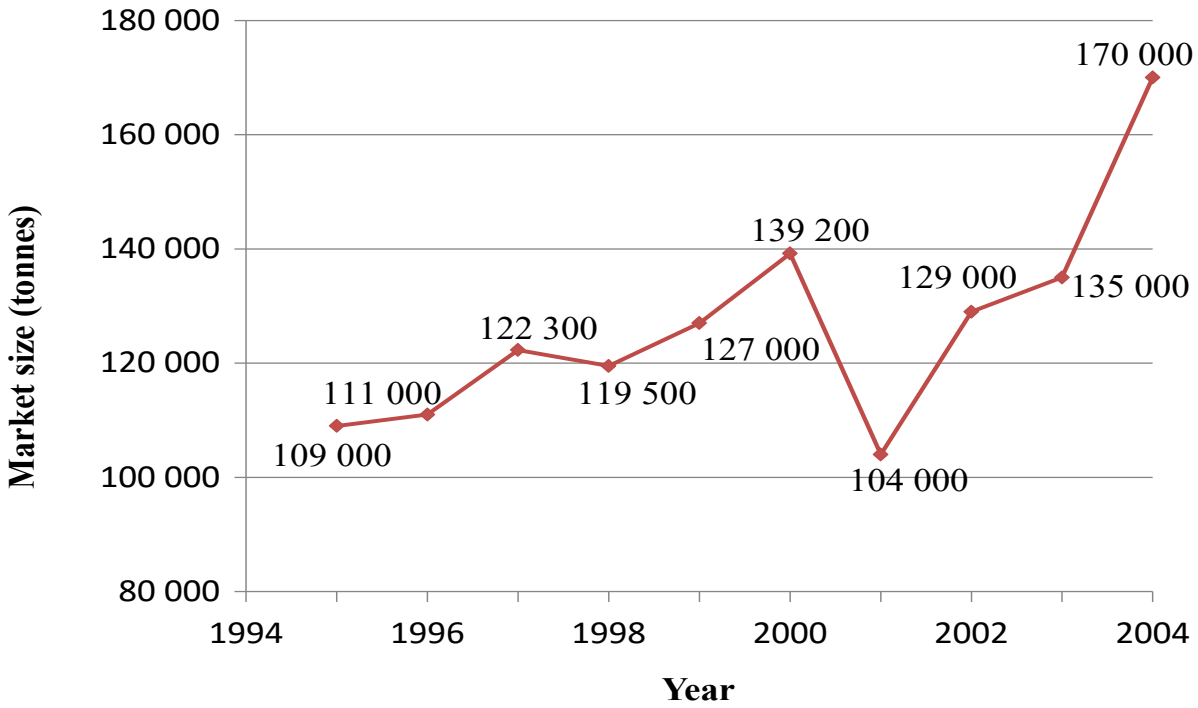
Water solubility: 1.26 mg/L at 25 °C

Octanol/water partition coefficient: log K_{ow}, 5.9

Decomposition: When heated to decomposition, emits bromine vapours

Conversion factor: 1 ppm = 22.6 mg/m^3 at 20 °C.

Fig. 1.1 Global market demand for tetrabromobisphenol A, 1995–2004



From Covaci et al. (2009); numbers were calculated from data published in ECHA (2008)

1.2 Production and use

1.2.1 Production

(a) Production methods

The production process for tetrabromobisphenol A involves the bromination of bisphenol A in the presence of a solvent, such as methanol, a halocarbon alone, or a halocarbon with water, or 50% hydrobromic acid, or aqueous alkyl monoethers (ECHA, 2006). Due to the nature of the process and the by-products (e.g. hydrobromic acid and methyl bromide) that can be formed, the production process is largely conducted in closed systems (Covaci et al., 2009).

(b) Production volume

Tetrabromobisphenol A is a compound with a high production volume that is currently produced in China, Israel, Japan, Jordan, and the USA, but no longer in the European Union. The total global production volume of tetrabromobisphenol A is estimated at > 100 000 tonnes per year (ECHA, 2008). Except for a minor reduction in production between 2000 and 2002, an overall increasing trend was observed in the estimated global market demand for tetrabromobisphenol A from 109 000 tonnes per year in 1975 to 170 000 tonnes per year in 2004 (Fig. 1.1).

1.2.2 Use

Approximately 58% of tetrabromobisphenol A is used as a reactive brominated flame retardant in epoxy, polycarbonate and phenolic resins in printed circuit boards, 18% is used for the production of tetrabromobisphenol A derivatives and oligomers, while 18% is used as additive flame retardant in the manufacture of acrylonitrile–butadiene–styrene resins or high impact polystyrene (Covaci et al., 2009).

(a) Reactive applications

Tetrabromobisphenol A is used primarily as an intermediate in the manufacture of polycarbonate unsaturated polyester and epoxy resins, in which it becomes covalently bound in the polymer. Polycarbonates are used in communication and electronic equipment, electronic appliances, transportation devices, sports and recreational equipment, and lighting fixtures and signs. Unsaturated polyesters are used in the manufacture of simulated marble floor tiles, bowling balls, furniture, coupling compounds for sewer pipes, buttons, and automotive patching compounds. Flame-retardant epoxy resins may be used mainly for the manufacture of printed circuit boards (Lassen et al., 1999). Moreover, epoxy resins containing tetrabromobisphenol A are used to encapsulate certain electronic components (e.g. plastic/paper capacitors, microprocessors, bipolar power transistors, "integrated gate bipolar transistor power modules" and "application specific integrated circuits" on printed circuit boards) (ECHA, 2008).

(b) Additive applications

Tetrabromobisphenol A is generally used with antimony oxide for optimum performance as an additive fire retardant (IPCS, 1995) that is applied in acrylonitrile–butadiene–styrene resins that are used in automotive parts, pipes and fittings, refrigerators, business machines and telephones (ECHA, 2008), and can also be applied to high-impact polystyrene resins used in casings of electrical and electronic equipment, furniture, building and construction materials (IPCS, 1995). The largest additive use of tetrabromobisphenol A is in television casings for which approximately 450 tonnes are used per year. Other uses include: personal computer monitor casings, components in printers, fax machines and photocopiers, vacuum cleaners, coffee machines and plugs/sockets (ECHA, 2008). As additive flame retardant, tetrabromobisphenol A does not react chemically with the other components of the polymer, and may therefore leach out of the polymer matrix after incorporation (Covaci et al., 2009).

1.3 Measurement and analysis

Several studies have reported on different methods for extraction of tetra-bromobisphenol A from different environmental and biological matrices (Table 1.1; Covaci et al., 2009). Solid-phase extraction on pre-packed C_{18} cartridges was the most commonly reported method for the extraction/clean-up of tetrabromobisphenol A from liquid samples, including water (Wang et al., 2015a), plasma (Chu & Letcher, 2013) and milk (Nakao et al., 2015). More aggressive extraction techniques such as Soxhlet extraction, pressurized liquid extraction, ultrasound-assisted extraction and microwave-assisted extraction were required for the efficient extraction of tetrabromobisphenol A from solid samples, including dust (Abdallah et al., 2008), soil (Tang et al., 2014), sediment (Labadie et al., 2010), sewage sludge (Guerra et al., 2010), polymers (Vilaplana et al., 2009), and frozen animal tissues (Tang et al., 2015). The inclusion of a relatively polar organic solvent (e.g. dichloromethane or acetone) was found to be necessary for the efficient extraction of tetrabromobisphenol A (Covaci et al., 2009). Hyphenated chromatographic methods coupled to mass spectrometric detection were commonly applied for the quantitative determination of tetrabromobisphenol A in various media (Table 1.1; Covaci et al., 2009). Other methods of analysis including enzyme-linked immunosorbent assay (Bu et al., 2014) and capillary electrophoresis (Blanco et al., 2005) were also reported for the determination of tetrabromobisphenol A in environmental samples.

Table 1.1 Overview of typical analytical procedures used for the determination of tetrabromobisphenol A in selected matrices

Matrix	Pretreatment	Extraction procedure (solvent)	Extract purification	Instrumental analysis	Recovery (%)	Limit of detection	References
River water, tap water, waste water	Filtration	HLB-SPE cartridge (2% ammonia in methanol)	SPE cartridge (2% formic acid in methanol)	LC-ESI-MS/MS	78–91	0.003 ng/mL	Yang et al. (2014)
Surface water	Filtration	SPE-cartridge (ethyl acetate)	–	UHPLC-UV	76	0.081 ng/mL	Kowalski & Mazur (2014)
Air samples	125-mm glass fibre filters and PUF disks	Soxhlet (DCM, 8 h)	SPE cartridge containing acidified silica (44% concentrated sulfuric acid)	LC-ESI-MS/MS	89	0.027 ng/m^3	Abdallah & Harrad (2010)
Dust	Sieving (250 µm)	Dispersive liquid–liquid microextraction	–	GC-MS	89	250 ng/g	Barrett et al. (2015)
Dust	Sieving (63 µm)	Ultrasonication (methanol:AcN:isopropanol (1:1:2), 60 °C, 30 min)	Filtration and online clean-up	LC-APCI-MS/MS	88	0.6 ng/g	Kopp et al. (2012)
Soil	Freeze-dried and sieved	PLE (DCM)	Activated silica gel column	LC-APCI-MS/MS	82–96	0.025 ng/g	Tang et al. (2014)
Soil	Mixed with sodium sulfate	Soxhlet (hexane:acetone (1:1), 24 h)	SPE cartridge	GC-MS	84–122	0.19 ng/g	Han et al. (2013)
Sediment	Sieved (230 mesh sieve)	PLE (hexane:DCM (1:3), activated copper)	Silica gel column	LC-ESI-MS/MS	70–110	0.05 ng/g	Lee et al. (2015)
Sediment	Mixed with sodium sulfate	Ultrasonication (hexane:acetone (1:1), 20 min)	Hydrochloric acid-activated copper strings and SPE cartridge	GC-NCI-MS	93	0.05 ng/g	Labadie et al. (2010)
Sewage sludge	–	Shaking with 5 mL methanol at 350 rpm, 60 min (3 cycles)	SPE cartridge	LC-ESI-MS/MS	106	–	Song et al. (2014)
Polymer fractions of WEEE	Powdered/granulated	MAE (isopropanol:hexane (1:1), 130 °C, 60 min)	0.45 mm Teflon filter	HPLC-UV	74–96	1630 ng/g	Vilaplana et al. (2009)
Human serum, human milk, dietary homogenate	Formic acid	LLE (hexane, ethanol then diethyl ether)	GPC with silica gel column	GC-NCI-MS	87–99	0.2 ng/g	Fujii et al. (2014b)

Table 1.1 (continued)

Matrix	Pretreatment	Extraction procedure (solvent)	Extract purification	Instrumental analysis	Recovery (%)	Limit of detection	References
Tissues of humans, dolphins and sharks	Mixed with sodium sulfate	Soxhlet (DCM:hexane (3:1), 16 h)	GPC and LLE with sulfuric acid and filtration	LC-ESI-MS/MS	93	0.0003 ng/g	Johnson-Restrepo et al. (2008)
Fish tissues	Freeze-dried, powdered	Soxhlet (hexane:acetone (1:1), 48 h)	Sulfuric acid and silica gel column	LC-ESI-MS/MS	84–99	0.025 ng/g	Tang et al. (2015)
Scallops, gills and digestive glands	Homogenization sodium sulfate	Soxhlet (hexane:DCM (4:1), 12 h)	SPE column	LC-ESI-MS/MS	79	5 ng/g	Hu et al. (2015b)
Egg	Homogenization sodium sulfate	Column extraction (acetone:cyclohexane (1:3), 1 h)	GPC and SPE column and derivatization	LC-TOF-MS GC-LRMS GC-HRMS	79 57	0.02 ng/g 0.01 ng/g 0.001 ng/g	Berger et al. (2004)
Birds muscle	Mixed with sodium sulfate, and ground	Soxhlet (hexane:acetone (1:1), 48 h)	GPC with silica gel column	LC-ESI-MS/MS	75	0.27 ng/g	He et al. (2010)

AcN, acetonitrile; DCM, dichloromethane; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; GPC, gel permeation chromatography; HLB, hydrophilic lypophilic balanced; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; LC-APCI-MS-MS/MS, liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry; LC-ESI-MS/MS, liquid chromatography-electrospray tandem mass spectrometry; LLE, liquid-liquid extraction; LRMS, low-resolution mass spectrometry; MAE, microwave-assisted extraction; MS, mass spectrometry; NCI, negative chemical ionization; PLE, pressurized liquid extraction system; PUF, polyurethane foam; rpm, revolutions per min; SPE, solid-phase extraction; TOF, time-of-flight; UHPLC, reversed phase ultra high-performance liquid chromatography; UV, ultraviolet detection; WEEE, waste electrical and electronic equipment
Solvent mixtures: proportions as volume per volume (v/v)

1.4 Occurrence and exposure

1.4.1 Natural occurrence

Tetrabromobisphenol A does not occur naturally (ECHA, 2006).

1.4.2 Environmental occurrence

Tetrabromobisphenol A was first detected in the environment in 1983 at a level of 20 ng/g in sediment from the Neya River in Japan (Watanabe et al., 1983). Several studies have detected tetrabromobisphenol A in various biotic and abiotic matrices from different parts of the world over the past few years (Table 1.2 and Table 1.3). This chemical was detected in air, dust, water, soil, sediment, and sewage sludge from various areas across the globe (Covaci et al., 2009), including the Arctic, which indicates its ability to undergo long-range transport (Xie et al., 2007; de Wit et al., 2010). The frequent detection of tetrabromobisphenol A and the ubiquitous nature of this contaminant indicates that it is continuously released into the environment due to its reported half-life in the soil ($t_{0.5}$ = 48–84 days) (NTP, 2014). Moreover, tetrabromobisphenol A is frequently detected in biotic samples, including fish, birds, and human tissue (Covaci et al., 2009; Table 1.3).

A recent review reported that China was the region most affected by pollution with tetrabromobisphenol A. The most serious cases of tetrabromobisphenol A pollution were found in Guiyu, Guangdong (a primitive e-waste dismantling site), with concentrations reaching 66 010–95 040 pg/m^3 in the air (mean, 82 850 pg/m^3), in Shouguang, Shandong (a tetrabromobisphenol A-manufacturing site) with concentrations ranging from 1.64 to 7758 ng/g dry weight in the soil (mean, 672 ng/g) and in Chaohu Lake, Anhui (industrial concentration site), with concentrations reaching 850–4870 ng/L in water (Liu et al., 2016).

1.4.3 Occupational exposure

Occupational exposures to tetrabromobisphenol A have been measured in facilities manufacturing electronic products and, at higher concentrations, in recycling facilities.

Mean concentrations of tetrabromobisphenol A in the air were reported to be 30 ng/m^3 in the dismantling hall and 140 ng/m^3 in the shredder at an electronic products recycling plant, and to be several orders of magnitude higher than those found in the other indoor microenvironments investigated (e.g. 0.036 ng/m^3 in the offices) (Sjödin et al., 2001).

A low concentration of tetrabromobisphenol A (0.011 ng/m^3) was measured in the particulate matter collected from a medical equipment-manufacturing building (Batterman et al., 2010).

Occupational exposure of workers to tetrabromobisphenol A at a Chinese printed circuit-board plant via ingestion, dermal absorption, and inhalation of dust varied widely by process, with the greatest estimated exposures being 1930, 431, and 96.5 pg/kg body weight (bw) per day, respectively. Raw-material warehouse workers were the most highly exposed, with an average overall exposure of 2413 pg/kg bw per day. Dust ingestion was the predominant pathway of exposure (Zhou et al., 2014).

Low levels (< 0.09–63 ng/cm^2) of tetrabromobisphenol A were detected in patch samples attached to clothing of workers at an electronics dismantling facility and a circuit board factory in Finland. However, tetrabromobisphenol A was below the limit of quantification (< 2 ng/hand) in handwash samples (Mäkinen et al., 2009).

Tetrabromobisphenol A was also detected in the serum of workers at levels that are presented in Table 1.3.

Table 1.2 Concentrations of tetrabromobisphenol A in abiotic matrices

Matrix (number of samples)	Location	Concentration Mean[a] (range) or range	Reference
Air			
Particulate matter in office air (56)	China	949 [GM] (30–59 140) ng/g	Ni & Zeng (2013)
Homes (5)	United Kingdom	16 (9–22) pg/m³	Abdallah et al. (2008)
Offices (5)		16 (4–33) pg/m³	
Public microenvironments (4)		26 (17–32) pg/m³	
Outdoor air (5)		0.8 (0.7–0.9) pg/m³	
Cars (20)	United Kingdom	3 (0.2–5) pg/m³	Abdallah & Harrad (2010)
Homes (2)	Japan	8–20 pg/m³	Takigami et al. (2009)
Outdoor air (2)		7.0–9.5 pg/m³	
Indoor air microenvironments (4)	Japan	200 (< 100–600) pg/m³	Inoue et al. (2006)
Indoor air at an electronics recycling plant and other work environments	Sweden	200 (110–370) pg/m³	Sjödin et al. (2001)
Outdoor air rural site	Germany	< 0.04–0.85 pg/m³	Xie et al. (2007)
Outdoor air	Wadden Sea	0.31–0.69 pg/m³	
Outdoor air	North-eastern Atlantic	< 0.04–0.17 pg/m³	
E-waste recycling site	China	82 850 (66 010–95 040) pg/m³	Reported in Liu et al. (2016)
Dust			
House dust (34)	China	250 (< 1–2300) mg/g	Wang et al. (2015b)
House dust (42)	Colombia	21 (< 1–280) mg/g	
House dust (28)	Greece	36 (< 1–630) mg/g	
House dust (35)	India	45 (< 1–640) mg/g	
House dust (14)	Japan	360 (12–1400) mg/g	
House dust (16)	Republic of Korea	130 (43–370) mg/g	
House dust (17)	Kuwait	12 (< 1–36) mg/g	
House dust (22)	Pakistan	50 (< 1–800) mg/g	
House dust (23)	Romania	28 (< 1–380) mg/g	
House dust (19)	Saudi Arabia	61 (< 1–360) mg/g	
House dust (22)	USA	91 (< 1–650) mg/g	
House dust (12)	Viet Nam	99 (< 1–670) mg/g	
Houses (35)	United Kingdom	87 (0.5–382) mg/g	Abdallah et al. (2008)
Offices (28)		49 (0.5–140) mg/g	
Cars (20)		6.0 (0.5–25) mg/g	
Public microenvironments (4)		220 (52–350) mg/g	
Primary schools and daycare centres (43)	United Kingdom	200 (17–1400) mg/g	Harrad et al. (2010)
House dust (45)	Belgium	11.7 [median] mg/g	D'Hollander et al. (2010)
Office dust (10)		70.4 [median] mg/g	
House dust (20)	Germany	44 (3–233) mg/g	Fromme et al. (2014)
Gym dust (4)	USA	680 (200–900) mg/g	La Guardia & Hale (2015)
Water			
Lake water (9)	United Kingdom	0.14–3.20 mg/L	Harrad et al. (2009)
River and lake water (9)	Poland	260–490 mg/L	Kowalski & Mazur (2014)
River water (5)	France	< 0.035–0.064 mg/L	Labadie et al. (2010)
Surface water (14)	China	230 (ND–920) mg/L	Xiong et al. (2015)

Table 1.2 (continued)

Matrix (number of samples)	Location	Concentration Mean[a] (range) or range	Reference
Surface water in industry site	China	850–4870 mg/L	Reported in Liu et al. (2016)
Soil			
Soil from e-waste recycling site (5)	China	5–17 mg/g	Han et al. (2013)
Land-use soils (6)	China	< 0.3–144 mg/g	Huang et al. (2014)
Agricultural and industrial soils (11)	Spain	Agricultural, 0.3 mg/g; industrial, 3.4–32.2 mg/g	Sánchez-Brunete et al. (2009)
Agricultural soils (38)	China	107 [GM] (1.6–7758) mg/g	Zhu et al. (2014)
Soil from tetrabromobisphenol A manufacturing site	China	672 (1.64–7758) mg/g	Reported in Liu et al. (2016)
Sediment and sewage sludge			
Sediment (7) and sludge (7)	Spain	Sludge, < 10–1329 mg/g; sediment, < 9–15 mg/g	Guerra et al. (2010)
Lake sediment (9)	England	0.3–3.8 mg/g	Harrad et al. (2009)
Sediment (rivers and ponds) (31)	Czech Republic	3.8–17.7 mg/g	Hloušková et al. (2014)
River bed sediments (5)	France	0.07–0.3 mg/g	Labadie et al. (2010)
Nakdong river sediment	Republic of Korea	0.5–150 mg/g	Lee et al. (2015)
Dongjiang river sediment (17)	China	3.8–230 mg/g	Zhang et al. (2009)
Scheldt basin sediment (20)	Netherlands	5.4 (< 0.1–67) mg/g	Morris et al. (2004)
Skerne river sediment (22)	England	451 (< 2.4–9750) mg/g	Morris et al. (2004)
Lake Mjøsa sediment (3)	Norway	0.04–0.13 mg/g	Schlabach et al. (2004)
Marine sediment	Japan	5.5 mg/g	Suzuki & Hasegawa (2006)
Marine sediment	Singapore	0.05–0.06 mg/g	Zhang et al. (2015a)
Sewage sludge (Montreal water waste treatment plant)	Canada	300 mg/g	Saint-Louis & Pelletier (2004)
Municipal sewage sludge (52)	China	20.5 [GM] (1–259) mg/g	Song et al. (2014)
Municipal sewage sludge (57)	Sweden	2 [median] (< 0.3–220) mg/g	Oberg et al. (2002)
Municipal (4) and industrial (7) sludge	Republic of Korea	4–618 mg/g	Hwang et al. (2012)
Sewage sludge (17)	Spain	104 (< 10–472) mg/g	Gorga et al. (2013)
Sewage sludge (4)	Canada	2–28 mg/g	Chu et al. (2005)

[a] Arithmetic mean unless indicated otherwise in square brackets
GM, geometric mean; ND, not detected

Table 1.3 Concentrations of tetrabromobisphenol A reported in biological matrices or tissues

Species (No. of samples)	Matrix or tissue	Location	Concentration Mean (range) or range (ng/g lipid weight)[a]	Reference
Humans				
Occupational exposure:				
Electronics dismantling (4)	Serum	Sweden	< 1.1–4.0	Hagmar et al. (2000a)
Computer technicians (19)	Serum	Sweden	0.54–1.85	Jakobsson et al. (2002)
Electronics dismantling (5)	Serum	Norway	1.3 (0.64–1.8)	Thomsen et al. (2001)
Circuit board producers (5)	Serum		0.54 (< 0.1–0.80)	
Laboratory personnel (5)	Serum		0.34 (< 0.1–0.52)	
General population:				
General population (21)	Serum	Belgium	80 ng/L	Dirtu et al. (2008)
General population (93)	Serum	Norway	0.31–0.71	Thomsen et al. (2002)
General population (24)	Serum	Japan	0.001 (ND–3.7)	Nagayama et al. (2000)
General population (20)	Adipose tissue	New York, USA	0.048 (< 0.003–0.464)	Johnson-Restrepo et al. (2008)
Men (60)	Serum	Japan	0.05–0.95	Fujii et al. (2014a)
Women (91)	Milk Serum Cord serum	France	4.11 (0.06–37.3) mg/g fresh weight 19.8 (0.23–93.2) mg/g fresh weight 103 (2–649) mg/g fresh weight	Cariou et al. (2008)
Mothers (30)	Milk	Czech Republic	< 2–688	Lankova et al. (2013)
Mothers (19)	Milk	Japan	1.9 (< 0.02–8.7)	Nakao et al. (2015)
Mothers (110)	Milk	Ireland	0.33	Pratt et al. (2013)
Mothers (34)	Milk	England	0.06 (0.04–0.65)	Abdallah & Harrad (2011)
Mothers (43)	Milk	Boston, USA	0.03–0.55	Carignan et al. (2012)
Mothers (103)	Milk	China	0.41 (< LOD–12.5)	Shi et al. (2013)
Mothers and infants (78)	Serum	Republic of Korea	Mothers, 10.7 (< 0.05–74); infants, 83 (< 0.05–713)	Kim & Oh (2014)
Other species				
Common whelk (3)	Whole	North Sea	45 (5.0–96)	Morris et al. (2004)
Sea star (1)	Whole	Tees estuary, UK	205	
Hermit crab (9)	Whole	North Sea	11 (< 1–35)	
Mysid (2)	Whole	Scheldt estuary	0.8–0.9	Verslycke et al. (2005)
Snakehead fish (5)	Whole	China	0.04–1.3	Tang et al. (2015)
Mud carp (5)	Whole	China	0.03–2.85	Tang et al. (2015)
Fish (45)	Whole	Japan	0.01–0.11	Ashizuka et al. (2008)
Fresh water fish (30)	Muscle	England	< 0.3–1.7	Harrad et al. (2009)
Fish (59)	Muscle	Czech Republic	60.8 (5–203)	Svihlikova et al. (2015)
Whiting (3)	Muscle	North Sea	136 (< 97–245)	Morris et al. (2004)
Cod (2)	Liver	North Sea	< 0.3–0.8	
Hake (1)	Liver	Atlantic	< 0.2	
Eel (19)	Muscle	Scheldt estuary	1.6 (< 0.1–13)	
Eel (11)	Muscle	Dutch rivers	0.3 (< 0.1–1.3)	
Yellow eel (4)	Muscle	Scheldt basin	< 0.1–2.1	
Yellow eel (5)	Muscle	Dutch rivers	< 0.1–1.0	
Perch, pike, smelt, vendace, trout (12)	Muscle	Norway	1.0–13.7	Schlabach et al. (2004)

Table 1.3 (continued)

Species (No. of samples)	Matrix or tissue	Location	Concentration Mean (range) or range (ng/g lipid weight)[a]	Reference
Bull shark (13)	Muscle	Florida, USA	0.03–35.6	Johnson-Restrepo et al. (2008)
Atlantic sharpnose shark (3)	Muscle	Florida, USA	0.87 (0.5–1.4)	
African penguins (3)	Muscle	Gdansk zoo, Poland	2.7–8.9	Reindl & Falkowska (2015)
	Liver		4–9.3	
	Adipose		3–12	
	Brain		7–15	
	Egg		11.4 ± 2.6	
Cormorant (2)	Liver	Wales and England, UK	2.5–14	Morris et al. (2004)
Common tern (10)	Egg	Western Scheldt	< 2.9	
Predatory bird (62)	Egg	Norway	< 0.003–0.013	Herzke et al. (2005)
Harbour seal (2)	Blubber	Wadden Sea	< 14	Morris et al. (2004)
Harbour porpoise (9)	Blubber	North Sea	83 (0.1–418)	
Harbour porpoise (1)	Blubber	Tyne/Tees rivers, UK	0.31	
Harbour porpoise (82)	Blubber	UK	< 5–35	Law et al. (2006a)
Bottlenose dolphin (15)	Blubber	Florida, USA	0.05–8.48	Johnson-Restrepo et al. (2008)

[a] Unless otherwise indicated
LOD, limit of detection; ND, not detected

1.4.4 Exposure of the general population

As a reactive flame retardant, the only potential for exposure is from unreacted tetrabromobisphenol A, which may exist where an excess has been added during the production process. When used as an additive (up to 22% by weight), the potential for the migration of tetrabromobisphenol A out of the matrix is greater, due to abrasion, weathering and high temperatures (ECHA, 2006).

Exposure of the general population predominantly occurs through the diet and through ingestion of indoor dust. While intake by very young children is predominantly via ingestion of indoor dust, intake by adults occurs mainly via the diet. Very young children are estimated to have a higher daily intake than adults. Exposure may occur prenatally, and tetrabromobisphenol A has been measured in breast milk (see Table 1.3).

Average estimated exposures of the population in the United Kingdom to tetrabromobisphenol A via inhalation of outdoor and indoor air from different microenvironments were 100–300 pg per day (Abdallah et al., 2008). In Japan, adults were reported to inhale tetrabromobisphenol A at 67–210 pg per day, while the exposure of children was 37–114 pg per day (Takigami et al., 2009). The daily intake of tetrabromobisphenol A in a Chinese population via inhalation and ingestion of indoor dust particles of different particle sizes accumulated in air-conditioner filters was estimated. The results revealed that approximately 28.7 pg/kg bw per day particulate matter $(PM)_{2.5}$-bound tetrabromobisphenol A can be inhaled deep into the lungs, while 14.5 pg/kg bw per day PM_{10}-bound tetrabromobisphenol A tends to be

deposited in the upper parts of the respiratory system. The average adult intake of tetrabromobisphenol A was 17.6 pg/kg bw per day via dust inhalation and 966.2 pg/kg bw per day via dust ingestion (Ni & Zeng, 2013).

Several studies have highlighted the importance of the accidental ingestion of indoor dust as a pathway of human exposure to tetrabromobisphenol A. The significance of this pathway increases for toddlers who ingest more dust (due to increased hand-to-mouth behaviour) than adults, spend more time in close proximity to the floor and have lower personal hygiene standards. Moreover, the body weight of toddlers and children results in higher exposure to tetrabromobisphenol A when estimated on a per-kilogram of body weight basis (Harrad et al., 2010). Human exposure to tetrabromobisphenol A via the ingestion of indoor dust was estimated in 12 countries (Table 1.2). The highest estimated daily intake in dust was reported for infants and toddlers in Japan (median, 820 and 430 pg/kg bw per day, respectively), the Republic of Korea (median, 500 and 260 pg/kg bw per day, respectively) and China (median, 140 and 70 pg/kg bw per day, respectively). The estimated daily intake values for these three countries were several times higher than those found for other countries (Wang et al., 2015b).

In the United Kingdom, average estimated daily intakes of tetrabromobisphenol A from the ingestion of dust were 1600 and 4400 pg/day for adults and toddlers, respectively, contributing 34% and 90% of their overall daily intake of tetrabromobisphenol A from the air, dust and diet (Abdallah et al., 2008).

The average dietary intake of tetrabromobisphenol A by adults and toddlers in the United Kingdom was 1600 and 5400 pg/kg bw per day, respectively, from 19 food groups (FSA, 2006). [The Working Group noted that these estimates should be regarded with caution due to the large number of "non-detects" in the samples analysed. The concentration of tetrabromobisphenol A in the non-detects was assumed to be half the limit of detection, hence the estimates of overall dietary intake are largely dependent on the limit of detection of the method (0.36 µg/kg whole weight).]

In a smaller study on six food groups, the total average dietary intake of tetrabromobisphenol A by the Dutch population was 40 pg/kg bw per day (de Winter-Sorkina et al., 2003).

Tetrabromobisphenol A was measured in 48 Chinese total diet study samples collected in 2007. The medium bound estimated daily intake of tetrabromobisphenol A for an average adult from foods of animal origin was 256 pg/kg bw per day (Shi et al., 2009). In Japan, the daily adult intake of tetrabromobisphenol A ranged from 4 to 40 ng in 10 duplicate diet samples collected in 2004 from Okinawa. In 2009, the estimated intake of tetrabromobisphenol A from an analysis of another 10 duplicate diet studies from the same area decreased to 0.5–7.5 ng per day. The average estimated dietary intake of a Japanese adult was 185 pg/kg bw per day (Fujii et al., 2014b).

Dietary exposure to tetrabromobisphenol A among nursing infants via breast milk is well documented. The average estimated daily intake of tetrabromobisphenol A for a Japanese nursing infant was 3.4 ng/kg bw per day (Fujii et al., 2014b). The average intake of tetrabromobisphenol A of a baby aged 1 month in the United Kingdom via breast milk was estimated at 1000 pg/kg bw per day (Abdallah & Harrad, 2011). In China, the average estimated daily intake of tetrabromobisphenol A via human milk was 5094 pg/kg bw per day for nursing infants aged 1–6 months (Shi et al., 2009).

Tetrabromobisphenol A was also detected in all umbilical cord samples from 16 Japanese mothers (16 ± 5.5 pg/g wet weight), indicating potential prenatal exposure (Kawashiro et al., 2008).

[The Working Group noted that, in the above studies, the dust PM_{10} fraction, rather than the higher inhalable fraction, was reported.]

1.5 Regulations and guidelines

There are no current restrictions on the production of tetrabromobisphenol A or its derivatives in the European Union or worldwide. The only exposure limit value for tetrabromobisphenol A was provided by the United Kingdom Committee on Toxicology as a tolerable daily intake of 1 mg/kg bw per day (COT, 2004). According to the Global Harmonized System of Classification and Labelling of Chemicals, tetrabromobisphenol A is very toxic to aquatic life (H400), with long-lasting effects (H410) (ECHA, 2016).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were given tetrabromobisphenol A (purity, > 99%) in corn oil by gavage at doses of 0 (control), 250, 500 or 1000 mg/kg bw on 5 days per week for up to 105 weeks (NTP, 2014). Survival of males and females at 1000 mg/kg bw was significantly lower than that of their respective vehicle-control group. Mean body weights of females at 1000 mg/kg bw were more than 10% lower than those of the vehicle controls after week 25. This decrease in survival at 1000 mg/kg bw was attributed to forestomach toxicity, which consisted of ulcers, inflammation, and/or hyperplasia. Because of the large decrease in survival of mice at 1000 mg/kg bw, this dose was not used in the statistical analysis for treatment-related tumour formation.

In male mice, increases in the incidence of hepatoblastoma were observed at both 250 and 500 mg/kg bw (2/50 controls, 11/50 at 250 mg/kg bw ($P = 0.006$), and 8/50 at 500 mg/kg bw [not significant]). The incidence of hepatoblastoma in the treated groups exceeded the upper bound of the range for historical controls for this tumour in studies with gavage in corn oil and for all routes. The historical incidence of hepatoblastoma in male mice for studies with gavage in corn oil was 9/250 (3.6% ± 2.6%; range, 0–6%) and for all routes was 40/949 (4.2% ± 3.5%; range, 0–12%). [Hepatoblastomas are uncommon spontaneous neoplasms that may occur after chemical administration, and have been seen after other chemical treatments (Bhusari et al., 2015).] An increased incidence of liver foci (clear cell and eosinophilic foci) and a significant increase in the incidence of hepatocellular adenoma (multiple) were seen in treated males (12/50 controls, 20/50 at 250 mg/kg bw, and 28/50 at 500 mg/kg bw ($P \leq 0.05$)). However, the incidence of hepatocellular adenoma (including multiple) was not increased in treated males (32/50 controls, 33/50 at 250 mg/kg bw, and 38/50 at 500 mg/kg bw). The historical incidence of hepatocellular adenoma (including multiple) in male mice in studies in which tetrabromobisphenol A was administered by gavage in corn oil was 145/250 (58.0% ± 5.1%; range, 52–64%), and for administration by all routes was 594/949 (62.6% ± 9.1%; range, 48–78%). The incidence of hepatocellular carcinoma was not significantly increased in male mice (11/50 controls, 15/50 at 250 mg/kg bw, and 17/50 at 500 mg/kg bw) and was within the historical ranges; the historical incidence of hepatocellular carcinoma in male mice in gavage studies (in corn oil) was 87/250 (34.8% ± 10.9%; range, 22–44%), and for administration by all routes was 348/949 (36.7% ± 11.4%; range, 22–56%). However, the incidence of hepatocellular carcinoma or hepatoblastoma (combined) was significantly increased at 250 mg/kg bw (12/50 controls, 24/50 at 250 mg/kg bw ($P = 0.008$), and 20/50

Table 3.1 Studies of carcinogenicity in experimental animals given tetrabromobisphenol A by oral gavage in corn oil

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$/N (M) 5–6 wks 105 wks NTP (2014)	Purity, > 99% 0, 250, 500 mg/kg bw 5 days/wk for 105 wks 50/group 33, 26, 39	*Liver* Hepatoblastoma: 2/50, 11/50*, 8/50 Hepatocellular carcinoma: 11/50, 15/50, 17/50 Hepatocellular carcinoma or hepatoblastoma (combined): 12/50, 24/50*, 20/50 Hepatocellular adenoma: 32/50, 33/50, 38/50 *Large intestine (caecum or colon)* Adenoma or carcinoma (combined): 0/50, 0/50, 3/50 *All organs* Haemangioma: 2/50, 0/50, 1/50 Haemangiosarcoma: 1/50, 5/50, 8/50* Haemangioma or haemangiosarcoma (combined): 3/50, 5/50, 9/50	 *P = 0.006 NS *P = 0.008 NS Trend: P = 0.039 NS Trend: P = 0.014; *P = 0.019 Trend: P = 0.047	GLP study Due to the large decrease in survival of mice at 1000 mg/kg bw, this dose was not used in the statistical analysis for treatment-related tumour formation The historical incidence of hepatoblastoma in male mice was: corn oil gavage studies: 9/250 (3.6% ± 2.6%; range, 0–6%); all routes: 40/949 (4.2% ± 3.5%; range; 0–12%) The historical incidence of caecum or colon adenoma or carcinoma (combined) in male mice was: corn oil gavage studies: 0/250; all routes: 4/950 (0.4% ± 0.8%; range, 0–2%)
Mouse, B6C3F$_1$/N (F) 5–6 wks 105 wks NTP (2014)	Purity, > 99% 0, 250, 500 mg/kg bw 5 days/wk for 105 wks 50/group 40, 31, 36	*All sites* No treatment-related tumorigenic effects		GLP study Due to the large decrease in survival of mice at 1000 mg/kg bw, this dose was not used in the statistical analysis for treatment-related tumour formation
Rat, Wistar Han [Crl:WI(Han)] (M) 6–7 wks 104 wks NTP (2014)	Purity, > 99% 0, 250, 500, 1000 mg/kg bw 5 days/wk for 104 wks 50/group 33, 28, 38, 39	*Testis* Interstitial cell adenoma, bilateral: 0/50, 0/50, 1/50, 0/50 Interstitial cell adenoma (includes bilateral): 0/50, 0/50, 1/50, 3/50	 NS Trend: P = 0.023	GLP study Historical incidence of interstitial cell adenoma in male rats (all routes): 4/150 (2.7%)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, Wistar Han [Crl:WI(Han)] (F) 6–7 wks 105 wks NTP (2014)	Purity, > 99% 0, 250, 500, 1000 mg/kg bw 5 days/wk for 104 wks 50, 50, 50, 50 35, 34, 29, 33	*Uterus* (original transverse review)		GLP study Historical incidence of uterine adenocarcinoma (all routes): 7/150 (4.7%) in studies involving an original transverse examination; historical incidence of malignant mixed Müllerian tumour (all routes): 0/150 in studies involving an original transverse examination
		Adenoma: 0/50, 0/50, 3/50, 4/50	Trend: $P = 0.010$	
		Adenocarcinoma: 3/50, 3/50, 8/50, 9/50	Trend: $P = 0.016$	
		Malignant mixed Müllerian tumour: 0/50, 4/50, 0/50, 2/50	NS (rare tumour)	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour (combined): 3/50, 7/50, 11/50*, 13/50**	Trend: $P = 0.003$; *$P = 0.013$; **$P = 0.005$	
		Uterus (residual longitudinal review)		
		Adenoma: 3/50, 2/50, 1/50, 3/50	NS	
		Adenocarcinoma: 4/50, 9/50, 15/50*, 15/50**	Trend: $P = 0.003$; *$P = 0.002$; **$P = 0.005$	
		Malignant mixed Müllerian tumour: 0/50, 0/50, 0/50, 1/50	NS (rare tumour)	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour (combined): 6/50, 10/50, 16/50*, 16/50**	Trend: $P = 0.008$; *$P = 0.007$; **$P = 0.015$	
		Uterus (original transverse and residual longitudinal review combined)		
		Adenoma: 3/50, 2/50, 4/50, 6/50	NS	
		Adenocarcinoma: 4/50, 10/50, 15/50*, 16/50**	Trend: $P = 0.002$; *$P = 0.002$; **$P = 0.002$	
		Malignant mixed Müllerian tumour: 0/50, 4/50, 0/50, 2/50	NS	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour: 6/50, 11/50, 16/50*, 19/50**	Trend: $P < 0.001$; *$P = 0.007$; **$P = 0.002$	

bw, body weight; F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week

at 500 mg/kg bw). The historical incidence of hepatocellular carcinoma or hepatoblastoma (combined) in male mice in gavage studies (in corn oil) was 93/250 (37.2% ± 10.0%; range, 24–48%) and for administration by all routes was 371/949 (39.1% ± 11.6%; range, 22–54%). The incidence of caecum or colon tumours (adenoma or carcinoma, combined) in male mice was 0/50 controls, 0/50 at 250 mg/kg bw, and 3/50 at 500 mg/kg bw, which was statistically significantly increased by the trend test ($P = 0.039$), but not by pairwise comparison. For the three male mice at 500 mg/kg bw with tumours of the large intestine, one had a caecum carcinoma, one had a colon carcinoma and one had a colon adenoma. The incidence of these tumours (3/50) exceeded the range for historical controls in gavage studies (in corn oil) and for administration by all routes. The historical incidence for caecum or colon adenoma or carcinoma (combined) in male mice in gavage studies (in corn oil) was 0/250 and for administration by all routes was 4/950 (0.4% ± 0.8%; range, 0–2%). A significant positive trend ($P = 0.014$) in the incidence of haemangiosarcoma (all organs) (1/50 controls, 5/50 at 250 mg/kg bw, and 8/50 at 500 mg/kg bw) was observed in males and the incidence at 500 mg/kg bw was also significantly increased ($P = 0.019$). The incidence of haemangiosarcoma (all organs) in historical controls in gavage studies (in corn oil) was 28/250 (11.2% ± 6.4%; range, 2–18%).

No significant increase in the incidence of tumours was observed in female mice treated with tetrabromobisphenol A. Treatment-related non-neoplastic lesions were found in the forestomach in male and female mice, and in the kidney in male mice (NTP, 2014).

[The strengths of this study, which complied with good laboratory practice, included the use of multiple doses, the large number of animals per group, and the treatment of males and females.]

3.2 Rat

Groups of 50 male and 50 female Wistar Han [Crl:WI(Han)] rats (age, 6–7 weeks) were given tetrabromobisphenol A (purity, > 99%) by gavage at doses of 0 (control), 250, 500, or 1000 mg/kg bw on 5 days per week for up to 104 (males) or 105 (females) weeks (NTP, 2014). Mean body weights of the males at 500 and 1000 mg/kg bw were at least 10% lower than those of the vehicle-control group after week 25. The mean body weights in the other groups of treated males and all groups of treated females were similar to those of the corresponding controls.

3.2.1 Original transverse examination of the uterus

In treated female rats, an increase in the incidence of uterine epithelial tumours was observed. The incidence of uterine adenoma was significantly increased according to trend statistics ($P = 0.010$), but not pairwise statistics (0/50 controls, 0/50 at 250 mg/kg bw, 3/50 at 500 mg/kg bw, and 4/50 at 1000 mg/kg bw), and the historical incidence in female rats was 0/150 (all routes). The incidence of uterine adenocarcinoma was also increased according to trend statistics ($P = 0.016$), but not pairwise statistics (3/50 controls, 3/50 at 250 mg/kg bw, 8/50 at 500 mg/kg bw, and 9/50 at 1000 mg/kg bw), and the historical incidence (all routes) in female rats was 7/150 (including one carcinoma of the endometrium). Malignant mixed Müllerian tumours were present in treated groups (0/50 controls, 4/50 at 250 mg/kg bw, 0/50 at 500 mg/kg bw, and 2/50 at 100 mg/kg bw), but were not seen in historical control data (all routes of exposure, 0/150). The incidence of uterine adenoma, adenocarcinoma, or malignant mixed Müllerian tumour (combined) was increased in treated groups (3/50 controls, 7/50 at 250 mg/kg bw, 11/50 at 500 mg/kg bw ($P = 0.013$), and 13/50 at 1000 mg/kg bw ($P = 0.005$)), with a statistically positive trend ($P = 0.003$), and the

historical incidence for these uterine tumours (combined) by all routes was 7/150 (4.7% ± 2.3%; range, 2–6%). In addition, cystic endometrial hyperplasia was reported in this initial evaluation (8/50 controls, 13/50 at 250 mg/kg bw, 11/50 at 500 mg/kg bw, and 18/50 at 1000 mg/kg bw ($P \leq 0.05$)). These findings were based on the traditional histopathology review of the uterus from the United States National Toxicology Program (NTP), with a transverse section through each uterine horn approximately 0.5 cm from the cervix of the uterus. The cervix and vagina were not investigated in most animals in this original review of uterine pathology.

3.2.2 Residual longitudinal examination of the uterus

While the initial (original) review of the uterus showed a treatment-related carcinogenic effect, an additional examination of the uterus called the "residual longitudinal" examination was conducted to examine all remaining parts of the uterus, cervix, and vagina more completely. This residual longitudinal examination consisted of trimming, embedding and sectioning the remaining uterine tissue, cervix, and vagina (remaining in the formalin-fixed samples) longitudinally. Additional non-neoplastic and neoplastic uterine lesions were found in this review that supported the original carcinogenic findings. The findings in Table 3.1 are presented as: the original carcinogenic findings; the carcinogenic findings of the residual longitudinal examination; and the combined carcinogenic findings of the original and residual longitudinal examinations. There were no historical control data for the residual or residual and original (combined) uterine tumour findings (NTP, 2014).

After the residual longitudinal examination of the uterus, the incidence of atypical endometrial hyperplasia was significantly increased in all treated groups (2/50 controls, 13/50 at 250 mg/kg bw ($P \leq 0.01$), 11/50 at 500 mg/kg bw ($P \leq 0.01$) and 13/50 at 1000 mg/kg bw ($P \leq 0.01$)). This lesion had not been identified in the original transverse review. [This lesion is considered to be preneoplastic (Bartels et al., 2012; van der Zee et al., 2013; NTP, 2014).] The incidence of uterine tumours in the residual longitudinal examination was: uterine adenoma – 3/50 controls, 2/50 at 250 mg/kg bw, 1/50 at 500 mg/kg bw, and 3/50 at 1000 mg/kg bw; uterine adenocarcinoma (P for trend, 0.003) – 4/50 controls, 9/50 at 250 mg/kg bw, 15/50 at 500 mg/kg bw ($P = 0.002$), and 15/50 at 1000 mg/kg bw ($P = 0.005$); malignant mixed Müllerian tumours – 0/50 controls; 0/50 at 250 mg/kg bw, 0/50 at 500 mg/kg bw, and 1/50 at 1000 mg/kg bw; and uterine adenoma, adenocarcinoma, or malignant mixed Müllerian tumour (combined) (P for trend, 0.008) – 6/50 controls, 10/50 at 250 mg/kg bw, 16/50 at 500 mg/kg bw ($P = 0.007$), and 16/50 at 1000 mg/kg bw ($P = 0.015$) (NTP, 2014).

3.2.3 Original transverse and residual longitudinal examinations of the uterus (combined)

The incidence of uterine tumours in the original and residual examinations (combined) was: uterine adenoma – 3/50 controls, 2/50 at 250 mg/kg bw, 4/50 at 500 mg/kg bw, and 6/50 at 1000 mg/kg bw; uterine adenocarcinoma (P for trend, 0.002) – 4/50 controls, 10/50 at 250 mg/kg bw, 15/50 at 500 mg/kg bw ($P = 0.002$), and 16/50 at 1000 mg/kg bw ($P = 0.002$); malignant mixed Müllerian tumours – 0/50 controls, 4/50 at 250 mg/kg bw, 0/50 at 500 mg/kg bw, and 2/50 at 1000 mg/kg bw; and uterine adenoma, adenocarcinoma or malignant mixed Müllerian tumours (combined) (P trend, < 0.001) – 6/50 controls, 11/50 at 250 mg/kg bw, 16/50 at 500 mg/kg bw ($P = 0.007$), and 19/50 at 1000 mg/kg bw ($P = 0.002$). The incidence of endometrium hyperplasia (atypical) was 2/50 controls, 13/50 at 250 mg/kg bw ($P \leq 0.01$), 11/50 at 500 mg/kg bw

($P \leq 0.01$), and 13/50 at 1000 mg/kg bw ($P \leq 0.01$). These uterine tumours were highly metastatic, with metastasis found in 24% (11/45) of the rats with uterine adenocarcinomas and 66% (4/6) of those with malignant mixed Müllerian tumours. Metastases of uterine tumours were found in the intestine, liver, mesentery, pancreas, glandular stomach, adrenal cortex, lymph nodes, spleen, thymus, skeletal muscle, lung, kidney and/or urinary bladder (NTP, 2014; Dunnick et al., 2015).

Based on current knowledge of the histogenesis of malignant mixed Müllerian tumours, the epithelial component is considered to be the primary component in these tumours, and the mesenchymal component is derived from the carcinoma. In the current study all the metastases were carcinomas, which supports this hypothesis. For this reason, the malignant mixed Müllerian tumours were combined with the epithelial tumours (NTP, 2014).

3.2.4 Other findings

The incidence of testicular (interstitial cell) adenoma in male rats occurred with a significant ($P = 0.023$) positive trend (0/50 controls, 0/50 at 250 mg/kg bw, 1/50 at 500 mg/kg bw, and 3/50 at 1000 mg/kg bw), and the incidence at the highest dose exceeded the incidence of this tumour in historical controls by all routes of administration (4/150). Treatment-related non-neoplastic lesions of the ovary (rete ovarii, cyst) were observed in female rats (NTP, 2014).

[The strengths of this study, which complied with good laboratory practice, included the use of multiple doses, large numbers of animals per group, and the use of males and females.]

3.3 Co-carcinogenicity

In one study (Imai et al., 2009), groups of six Fischer 344 rat dams [age not reported] received diets containing tetrabromobisphenol A [purity not reported, chemical grade at 0% (control), 0.01%, 0.1% or 1%, or drinking-water containing 0.01% of potassium perchlorate, for 3 weeks after parturition. Pups were selected randomly at 4 days after birth, to give approximately four males and four females in each litter, to maximize the uniformity of the growth rates of the offspring. The weaned offspring in each group were treated for 2 weeks in the same manner as their dams. All offspring (age, 6 weeks) received drinking-water containing *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) (0.08% DHPN for males, and 0.2% DHPN for females) for 4 weeks. In addition, the female offspring (age, 7 weeks) received a single dose of 7,12-dimethylbenz[*a*]anthracene (50 mg/kg bw in 5 mL sesame oil) by gavage. All surviving male offspring were killed at age 39 weeks, and all surviving female offspring were killed at age 47 weeks. The liver, kidneys, lungs, oesophagus, thyroid, testes, epididymides, ovaries, urinary bladder, skin with mammary tissue, any subcutaneous nodules and other macroscopic abnormalities were fixed in formalin and routinely processed for histological examination. The incidence of thyroid follicular cell adenoma in female offspring treated with 1% tetrabromobisphenol A, and the incidence of transitional cell papilloma of the urinary bladder in female offspring treated with 0.01%, 0.1%, and 1% tetrabromobisphenol A, were increased compared with controls. The incidence of thyroid gland follicular cell adenoma in female offspring was 13/22 (59%) controls, 12/14 (86%) with potassium perchlorate, 9/13 (69%) with 0.01% tetrabromobisphenol A, 11/17 (65%) with 0.1% tetrabromobisphenol A, and 12/13 (92%; $P < 0.05$) with 1% tetrabromobisphenol A. The incidence of transitional cell papilloma of the urinary bladder in female offspring was: 0/23 (0%) in controls, 1/15 (7%) with potassium perchlorate, 3/13 (23%; $P < 0.05$) with 0.01% tetrabromobisphenol A, 4/17 (24%; $P < 0.05$) with 0.1% tetrabromobisphenol A, and 4/13 (31%; $P < 0.05$) with 1% tetrabromobisphenol

A. Treatment with tetrabromobisphenol A had no significant effect on tumour incidence in male offspring. [The Working Group noted the lack of control groups treated with tetrabromobisphenol A only. No historical data were available for this study, and no explanation was given as to why the group size varied. In addition, these data were not analysed to determine whether a litter effect occurred. The study was judged to be inadequate for the evaluation of the carcinogenicity of tetrabromobisphenol A.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Absorption, distribution, and excretion

(a) Humans

After administration of tetrabromobisphenol A as a single oral dose at 0.1 mg/kg bw in human subjects, conjugated metabolites were detected in the blood, with maximum concentrations observed between 2 and 6 hours. A small amount of the administered dose was excreted in the urine. The parent compound was below the limit of detection in all blood samples collected from 1 to 178 hours. The longest-lived conjugate reached its limit of detection in the blood at 124 hours (Schauer et al., 2006).

Tetrabromobisphenol A has been detected in the serum, tissues, and milk, as a result of environmental or occupational exposure. It has been detected in milk in surveys of the general population conducted in France, Japan, the United Kingdom, and the USA (Cariou et al., 2008; Abdallah & Harrad, 2011; Carignan et al., 2012; Akiyama et al., 2015), and in the serum of the general population in France, Japan, and Norway, as well as from exposed workers in Norway and Sweden (Hagmar et al., 2000b; Thomsen et al., 2001; Jakobsson et al., 2002; Thomsen et al., 2002; Hayama et al., 2004; Cariou et al., 2008). In many studies, mean concentrations of free tetrabromobisphenol A in tissues and fluids were < 1 ng/g of lipid, and were below the limit of detection in some subjects. In one study, adipose tissue obtained from cosmetic surgery contained about 0.05 ng/g of lipid (Johnson-Restrepo et al., 2008).

The half-life of tetrabromobisphenol A was estimated to be 2.2 days in a study of four occupationally exposed humans (Hagmar et al., 2000b).

In a study in vitro, human skin penetration was about 3.5% (of a total dose of 100 nmol/cm^2) within 24 hours of application (Knudsen et al., 2015).

(b) Experimental systems

Tetrabromobisphenol A was readily absorbed after oral administration of [^{14}C]-labelled doses in male or female rats (Hakk et al., 2000; Kuester et al., 2007; Knudsen et al., 2014). After dermal application in female rats, about 8% of the total dose (100 nmol/cm^2) reached the systemic circulation within 24 hours (Knudsen et al., 2015). The extent of absorption was calculated to be 3–11% of a total dose to male rats by dermal exposure (6 hours per day for 90 days) to up to 600 mg/kg bw (Yu et al., 2016).

Systemic bioavailability was <5% in female Wistar Han rats. The elimination half-life from plasma was 133 minutes following intravenous administration of 25 mg/kg bw, and about 290 minutes after oral administration of 250 mg/kg bw (Knudsen et al., 2014).

After intravenous administration of a 20 mg/kg bw dose in male Fischer 344 rats, tetrabromobisphenol A disappeared rapidly from the blood, with distribution and elimination half-lives of 5 and 82 minutes, respectively (Kuester et al., 2007). A similar dose administered by gavage resulted in a peak concentration in the blood at 30 minutes, with an elimination half-life similar to that after intravenous administration.

The level of tetrabromobisphenol A was below the limit of quantitation after 4 hours after either oral or intravenous administration. There was evidence of enterohepatic circulation, as also supported by Hakk et al. (2000) and Knudsen et al. (2014).

After oral administration of a dose of 250 mg/kg bw, tetrabromobisphenol A-derived radiolabel was detected in all assayed tissues 1 hour after treatment of female Wistar Han rats. Over 24 hours, the greatest amount of radiolabel was detected in the tissues and/or contents of the gastrointestinal tract. Of the other tissues assayed, the liver and pancreas contained the highest concentrations. No significant sex-specific differences in cumulative 72-hour oral disposition data were observed (Knudsen et al., 2014). In studies of repeated dosing in rats, accumulation of the administered dose was not observed in any of the tissues assayed after oral administration of up to 1000 mg/kg bw per day for 14 consecutive days (Kuester et al., 2007; Kang et al., 2009).

The major route of excretion of a dose of 20 mg/kg bw administered intravenously in male Fischer 344 rats was in the faeces, which contained about 75% of the total dose at 24 hours (Kuester et al., 2007). More than 90% of orally administered doses of 25, 250, or 1000 mg/kg bw in female Wistar Han rats was excreted in the faeces, up to 2% was excreted in the urine, and < 1% remained in tissues at 72 hours (Knudsen et al., 2014). After oral administration to male Sprague-Dawley rats, > 90% of a total dose of 2 mg/kg bw of tetrabromobisphenol A was excreted in the faeces within 72 hours, and about 3% of the total dose was recovered in the tissues and cumulative urine (Hakk et al., 2000). In male Fischer 344 rats that received tetrabromobisphenol A as a single gavage dose of 2, 20, or 200 mg/kg bw, reduced excretion of the highest dose in the faeces indicated the saturation of absorption and/or elimination processes; however, the cumulative data on faecal elimination were similar for the lowest and highest doses at 72 hours. The total dose remaining in tissues was < 1% (Kuester et al., 2007). In gavaged (2.0 mg/kg bw per day) bile duct-cannulated male Sprague-Dawley rats, > 70% of the total radioactivity was excreted in the bile, mostly within 24 hours (Hakk et al., 2000). About 50% of a dose of 20 mg/kg bw was excreted in the bile of male Sprague-Dawley rats within 2 hours after administration by gavage (Kuester et al., 2007).

The disposition of [^{14}C]-labelled tetrabromobisphenol A (250 or 1000 mg/kg bw) administered intraperitoneally to female rats differed from that of an oral dose. The total dose excreted in the faeces was lower and the tissue burdens and elimination half-lives were longer after intraperitoneal injection (Szymańska et al., 2001).

4.1.2 Metabolism

See Figure 4.1

(a) Humans

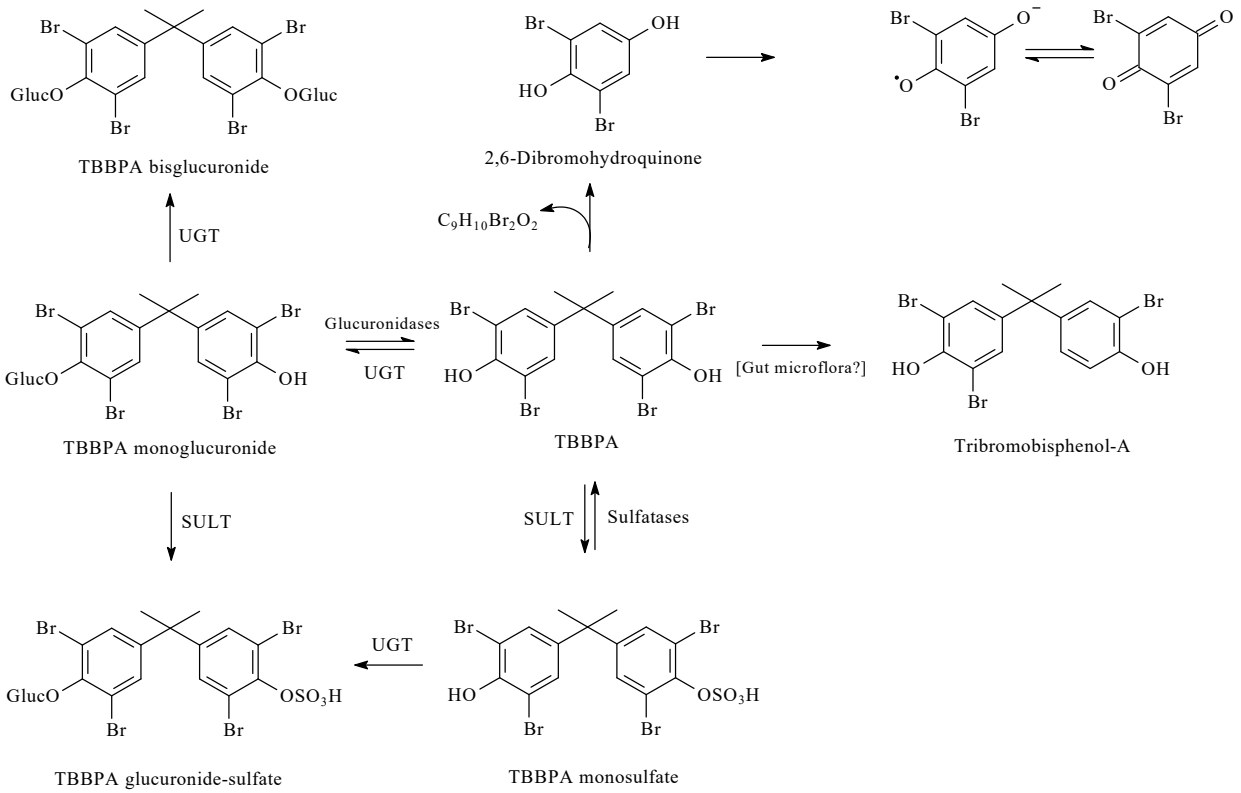
A monoglucuronide and/or monosulfate were detected in the blood and urine after oral administration of tetrabromobisphenol A in humans (Schauer et al., 2006).

The metabolism of tetrabromobisphenol A in human liver microsomes and metabolic activation preparations was described as being qualitatively similar to that observed in rat subcellular liver fractions. A glucuronide was formed in the metabolic activation system. Oxidative cleavage of the tetrabromobisphenol A molecule was a major pathway in microsomes (Zalko et al., 2006).

(b) Experimental systems

In male and female rats, tetrabromobisphenol A undergoes rapid metabolism catalysed by UDP glucuronosyltransferase and sulfotransferase isozymes to form glucuronide and sulfate conjugates (Hakk et al., 2000; Schauer et al., 2006; Kuester et al., 2007; Knudsen et al., 2014). The specific structures (mono, di/

Fig. 4.1 Metabolic scheme for tetrabromobisphenol A in rats



Gluc, $C_6H_9O_6$; SULT, sulfotransferase; TBBPA, tetrabromobisphenol A; UGT, uridine 5′-diphospho-glucuronosyltransferase
Adapted from NTP (2014)

bis, and mixed), presence and relative abundance of these conjugates in the blood, bile and/or excreta were sex- and strain-dependent and may also be dose- and species-dependent (Schauer et al., 2006; Dunnick et al., 2015). Tetrabromobisphenol A was identified in the plasma and faeces at low concentrations and its monoglucuronide was detected in the urine of Sprague-Dawley rats treated by gavage (Schauer et al., 2006). A glucuronide was formed in metabolic activation systems after incubation. Rat hepatocytes metabolized tetrabromobisphenol A to a monoglucuronide and monosulfate conjugate (Nakagawa et al., 2007). Glucuronide and sulfate conjugates were detected in *Xenopus laevis* tadpoles exposed to tetrabromobisphenol A in water (Fini et al., 2012). Oxidative cleavage of tetrabromobisphenol A is possible in vivo. The 2,6-dibromobenzenosemiquinone radical, derived from 2,6-dibromohydroquinone, was identified in the bile of male Sprague-Dawley rats that received tetrabromobisphenol A by intraperitoneal injection (Chignell et al., 2008). In rat microsomal preparations, the major metabolites of tetrabromobisphenol A were products of oxidative cleavage (Zalko et al., 2006).

4.2 Mechanisms of carcinogenesis

The evidence on the "key characteristics" of carcinogens (Smith et al., 2016) concerning whether tetrabromobisphenol A modulates receptor-mediated effects, induces oxidative stress, induces chronic inflammation, is immunosuppressive, alters cell proliferation, cell death, or nutrient supply, and is genotoxic, is summarized below.

4.2.1 Receptor-mediated effects

(a) Thyroid hormone pathway

(i) Exposed humans

Serum concentrations of tetrabromobisphenol A were positively correlated with serum concentrations of free thyroxine (T_4), but negatively correlated with serum triiodothyronine (T_3) in mothers of infants diagnosed with congenital hypothyroidism. No correlation was observed in the infants with congenital hypothyroidism, but they also had therapeutic T_4 supplementation (Kim & Oh, 2014).

Serum concentrations of tetrabromobisphenol A were not correlated with measures of thyroid function in another study of 515 adolescents (age, 13–17 years). These measurements included serum concentrations of free T_4, T_3 and thyroid-stimulating hormone (Kiciński et al., 2012).

To determine whether tetrabromobisphenol A interferes with measures of T_4 in human serum, McIver et al. (2013) tested several commercial immunoassays for serum total or free T_4 or T_3. The results indicated that high concentrations of tetrabromobisphenol A could displace tracer T_4 in an in-house serum total T_4 assay, but none of the other assays were disturbed. [This indicated that tetrabromobisphenol A may produce erroneously high T_4 readings in some assays.]

(ii) Human cells in vitro

Tetrabromobisphenol A exhibited a potent interaction with human transthyretin, and had greater avidity for binding than T_4 (displacing ^{125}I-T_4) (Meerts et al., 2000).

Tetrabromobisphenol A demonstrated both agonist and antagonist activity on thyroid hormone receptor activation in HepG2 cells, activating a transiently transfected thyroid hormone-responsive reporter at or above 10 µM and also inhibiting transactivation of the reporter by T_3 at 1 µM (Hofmann et al., 2009).

In contrast, tetrabromobisphenol A did not show agonist or antagonist activity on human thyroid hormone receptor TRα1 or TRβ1 in human embryonic kidney HEK293 cells. This assay was a transient transfection paradigm using a palindromic thyroid hormone-responsive element (TRE) to avoid the requirement for a TR:retinoid X receptor heterodimer formation on the TRE (Oka et al., 2013).

Tetrabromobisphenol A inhibited luciferase expression induced by T_3 in human embryonic kidney HEK293 cells stably transfected with a construct that would allow the detection of changes in intracellular free T_3 by one or more of several potential pathways. In a follow-up experiment using a murine cerebellar cell line expressing the TRα1 receptor, tetrabromobisphenol A significantly interfered with TRα1-mediated gene expression using a genome-wide RNa-Seq approach (Guyot et al., 2014).

In human liver microsomes, tetrabromobisphenol A inhibited the ativity of type 1 deiodinase, which converts T_4 to the more biologically active T_3, in the micromolar concentration range; 1 µM of tetrabromobisphenol A inhibited the activity by 20% and 10 µM inhibited the activity by 80% (Butt et al., 2011).

Tetrabromobisphenol A did not increase cell proliferation in human cervical cancer HeLa cells stably transfected with human TRα1, and did not appear to reduce the induction of luciferase activity by T_3 driven by a death receptor-4 promoter in a transient transfection paradigm (Yamada-Okabe et al., 2005). [The Working Group noted that the authors did not appear to correct for the efficiency of transfection of the HeLa-TR cells, which may have altered the outcome.]

(iii) Non-human mammalian systems in vivo

Tetrabromobisphenol A (100, 1000, or 10 000 ppm) given to pregnant rats throughout lactation did not affect serum T_4 or thyroid-stimulating hormone, but did slightly decrease levels of serum T_3 (Saegusa et al., 2009).

In a study of reproductive toxicity, tetrabromobisphenol A significantly reduced levels of serum T_4 in male and female rats exposed orally to various concentrations throughout fetal life, lactation, and the end of the experiment at age 12 weeks. Reduced levels of serum T_4 correlated with a cluster of measures related to thyroid function, including delayed onset of puberty and hearing deficits. However, in a short-term study, these effects were not observed in relation to the decrease in serum T_4 (Van der Ven et al., 2008).

Serum T_4 was significantly reduced in males and females exposed to tetrabromobisphenol A at 100 and 1000 mg/kg bw, and in both the parental and F_1 generations in a large multigeneration study of reproductive toxicity in Sprague-Dawley rats that complied with good laboratory practice. No effects were observed on serum T_3 or thyroid-stimulating hormone (Cope et al., 2015). [Because the units of serum T_4 were reported as ng/dL, the Working Group was unable to draw any conclusions on this study.]

No effect was seen on serum T_3 or thyroid-stimulating hormone in CD/SD rats (age, 8 weeks) administered 0, 100, 300, and 1000 mg/kg bw of tetrabromobisphenol A by gavage in corn oil daily. In contrast, mean serum T_4 concentrations (reported as ng/dL and ng/mL) were reduced at day 33 in males (4.96, 3.66, 3.42 and 3.39 at 0, 100, 300, and 1000 mg/kg bw, respectively) and females (4.27, 3.31, 3.24, and 3.33 at 0, 100, 300, and 1000 mg/kg bw, respectively) (Osimitz et al., 2016). [Because the units of serum T_4 were reported as both ng/dL and ng/mL, the Working Group was unable to draw any conclusions about this study.]

(iv) Non-human mammalian systems in vitro

Tetrabromobisphenol A (10^{-6} to 10^{-4} M) markedly inhibited the binding of T_3 to the TR in isolated nuclei from the rat pituitary MtT/E-2 cell line, and also stimulated proliferation and growth hormone production of rat pituitary GH3 cells. Tetrabromobisphenol A enhanced T_3-induced GH3 proliferation (at 10^{-4} M) and growth hormone production (at both 10^{-5} and 10^{-4} M). These data were interpreted to indicate that tetrabromobisphenol A could act on the TR as an agonist (Kitamura et al., 2002).

In contrast, tetrabromobisphenol A was antagonistic to the human TRα1 receptor in a transient transfection assay using CHO cells, and inhibited the effect of 10^{-8} M T_3 on luciferase activity in the 4–50 μM concentration range, at which it was not cytotoxic. However, tetrabromobisphenol A did not antagonize the TRβ1 receptor at concentrations that were not cytotoxic and did not exhibit agonistic action on TRα1 or TRβ1 (Kitamura et al., 2005a). This group later confirmed their original observation that tetrabromobisphenol A could stimulate growth hormone production in GH3 cells (Kitamura et al., 2005b).

Sun et al. (2009) reported that tetrabromobisphenol A did not exhibit TR agonist action, but suppressed transactivation by 10 nM T_3 at 10^{-4}M in CV-1 cells (African green monkey kidney cells). As CV-1 cells do not express TRs, this transient transfection system employed a GAL4/hTRβ1 fusion protein with a 4×UAS/luciferase construct.

Tetrabromobisphenol A inhibited T_3-induced luciferase expression at concentrations above 10 μM in rat pituitary GH3 cells stably transfected with a 2×DR4/luciferase construct, a system that is highly sensitive and can detect picomolar concentrations of T_3. Extensive validation was carried out and tetrabromobisphenol A did not induce an agonistic effect in this system (Freitas et al., 2011).

Tetrabromobisphenol A produced a TR agonist effect in the 10^{-5} to 10^{-4} M range in a yeast two-hybrid assay, an effect enhanced by prior incubation with a microsomal metabolic activation system. To detect chemical effects on the TR, this assay employed yeast cells transfected with human TRα and co-factor TIF2. The reporter gene was β-galactosidase (Terasaki et al., 2011).

Lévy-Bimbot et al. (2012) evaluated the effect of tetrabromobisphenol A on structural changes within the TRα receptor using a co-regulator recruitment assay. Tetrabromobisphenol A decreased the affinity of the TR ligand-binding domain for the NCoR-binding peptide, but did not simultaneously increase the affinity of the TR for the SRC2-binding peptide. The effective concentration of tetrabromobisphenol A was in the 1 μM range.

Grasselli et al. (2014) evaluated tetrabromobisphenol A in a rat hepatoma cell line (FaO) that does not express TRs but accumulates lipid droplets. Treatment with T_3 can cause a depletion of the lipids, which was mimicked by 10–6 M tetrabromobisphenol A. However, tetrabromobisphenol A induced the expression of genes related to lipid accumulation. [The Working Group noted that, in the absence of an antagonist to block T_3-dependent processes (that are also TR-independent), this study was difficult to interpret.]

(v) Non-mammalian experimental systems

Tetrabromobisphenol A inhibited T_3-induced tail resorption in *Rana rugosa* tadpoles at a concentration of 10^{-6} M, a similar range to that used in TR-binding studies, but exerted no effect on tail length in the absence of T_3 (Kitamura et al., 2005a). This observation was confirmed by Goto et al. (2006), who reported that both 10^{-7} and 10^{-6} M tetrabromobisphenol A inhibited T_3-induced tail resorption in *Rana rugosa* tadpoles, but again had no effect in the absence of T_3. During T_3-induced tail resorption, DNA becomes highly fragmented; Goto et al. (2006) also demonstrated that 10^{-6} M tetrabromobisphenol A could inhibit this fragmentation and block T_3-induced hind limb growth. Finally, they demonstrated in transgenic *Xenopus laevis* carrying a TRE-linked green fluorescent protein that T_3-induced fluorescence was blocked by tetrabromobisphenol A at 10^{-7} M. [The Working Group noted that, taken together, these data consistently demonstrated the antagonist action of tetrabromobisphenol A in amphibians and in the range of its binding to TR.]

In the tree frog, tetrabromobisphenol A was antagonistic to the TR. Veldhoen et al. (2006) reported that it was agonistic to metamorphic changes in *Pseudacris regilla*. At 10 nmol/L, tetrabromobisphenol A suppressed T_3-induced TRβ expression in the frog *Pelophylax nigromaculatu*, coincident with a suppression of several thyroid hormone-regulated genes (Zhang et al., 2015b).

(b) Other pathways

(i) Nuclear receptors and steroidogenesis

Several studies have addressed the possible agonistic or antagonistic properties of tetrabromobisphenol A on relevant nuclear receptors in various human cell lines, such as human mammary carcinoma MCF-7 and human cervical carcinoma HeLa cells. For the estrogen and progesterone receptors, no significant agonistic or antagonistic properties of tetrabromobisphenol A were detected (Samuelsen et al., 2001; Hamers et al., 2006; Molina-Molina et al., 2013). This lack of direct estrogenicity of tetrabromobisphenol A has also been shown in vivo in the mouse uterotrophic assay (Ohta et al., 2012). The androgen receptor antagonistic activity of tetrabromobisphenol A was detected at the lower micromolar levels in MDA-kb2 cells (Christen et al., 2010).

Tetrabromobisphenol A does not have any significant agonistic activity on the aryl hydrocarbon receptor (AhR), and several studies in vitro and in vivo showed that it does not induce AhR-mediated cytochrome P450 (CYP) A1 enzymes (Behnisch et al., 2003; Germer et al., 2006; Hamers et al., 2006). In addition to the data mentioned above, ToxCast identified interactions between tetrabromobisphenol A and the glucocorticoid receptor, the farnesyl X receptor, and the xenobiotic receptor PXR.

In stably transfected human HeLa cells and monkey Cos-7 kidney cells, significant agonistic activity for human peroxisome proliferator-activated receptor-γ1 and -γ2 was found below 1 μM (Christen et al., 2010; Watt & Schlezinger, 2015). In human choriocarcinoma JEG-3 cells, low nanomolar levels of tetrabromobisphenol A also increased peroxisome proliferator-activated receptor-γ, as well as increasing progesterone and β-human chorionic gonadotrophin (Honkisz & Wójtowicz, 2015). Furthermore, tetrabromobisphenol A (0.1 and 1 μM) induced aromatase (CYP19) in these JEG-3 cells (Honkisz & Wójtowicz, 2015), but not in human adenocarcinoma H295R cells (Song et al., 2008).

(ii) Neurotoxicity

The possible neurotoxic mechanisms of action of tetrabromobisphenol A were studied in SH-SY5Y human neuroblastoma cells in vitro. It was both neurotoxic and amyloidogenic, as demonstrated by increased intracellular calcium levels and a release of β-amyloid peptide (Aβ-42) at micromolar levels (Al-Mousa & Michelangeli, 2012). As further evidence of its potential neurotoxicity, tetrabromobisphenol A inhibited the plasma membrane uptake of the neurotransmitters dopamine, glutamate, and gamma-amino butyric acid in rat brain synaptosomes (Mariussen & Fonnum, 2003). However, in spite of the interactions of tetrabromobisphenol A in vitro with various neurotransmitters, neonatal exposure of mice did not induce any neurobehavioural changes (Eriksson et al., 2001; Viberg & Eriksson, 2011).

(iii) Other effects

Several studies in vitro have addressed the direct interaction of tetrabromobisphenol A with estrogen sulfotransferase (e.g. SULT1E1) and concluded that it strongly inhibits the estradiol binding process with half-maximal inhibitory concentrations between 12 and 33 nM (Kester et al., 2002; Hamers et al., 2006; Gosavi et al., 2013). In addition, many authors hypothesized that, at high concentrations, the conjugation of tetrabromobisphenol A to form tetrabromobisphenol A sulfate could possibly saturate the sulfation pathway (Kester et al., 2002; Hamers et al., 2006; Gosavi et al., 2013; Dunnick et al., 2015 Lai et al., 2015; Wikoff et al., 2016). [This competitive inhibition of estrogen sulfotransferases could lead to an increase in systemic and target tissue levels of estrogens.]

A follow-up 28-day study of tetrabromobisphenol A in rats was carried out by Borghoff et al. (2016) using the same dose levels as those in the 2-year NTP carcinogenicity bioassay (NTP, 2014). At the highest dose levels (250, 500, and 1000 mg/kg bw per day), a decrease in the ratio of tetrabromobisphenol A sulfates to tetrabromobisphenol A glucuronides occurred. These results demonstrated that the saturation of tetrabromobisphenol A sulfation also occurs in vivo at these dose levels, which were associated with uterine tumours in the 2-year NTP study. [The Working Group noted that neither the NTP (2014) nor Borghoff et al. (2016) studies took measurements that provided information on estrogen homeostasis.]

In addition, several other mechanistic explanations have been postulated to explain the uterine tumours in the 2-year NTP study (Dunnick et al., 2015; Lai et al., 2015), one of which is the interaction of tetrabromobisphenol A with dopamine and a subsequent decrease in prolactin levels that is considered to be a rat-specific mechanism (Neumann, 1991; Harleman et al., 2012). Tetrabromobisphenol A inhibited the cellular uptake of dopamine with a half-maximal inhibitory concentration of 9 μM (Mariussen & Fonnum, 2003). [At present, insufficient data were available to evaluate its relevance to the tetrabromobisphenol A-induced uterine tumours in the NTP (2014) study.]

4.2.2 Oxidative stress

(a) Humans

Treatment of isolated human neutrophil granulocytes with tetrabromobisphenol A at 1–12 µM for 60 minutes induced significant dose-dependent increases in the production of reactive oxygen species (ROS) and increased intracellular calcium concentrations (Reistad et al., 2005). ROS production was determined using the fluorescent probe 2,7-dichlorofluorescein diacetate or by lucigenin-amplified chemiluminescence. Production of ROS was inhibited by pretreatment with diphenyleneiodonium (a nicotinamide adenine dinucleotide phosphate oxidase inhibitor), U0126 (an inhibitor of mitogen-activated protein kinase kinases MEK1 and MEK2, i.e. MAPK/ERK kinase), bisindolylmaleimide (a protein kinase C inhibitor), erbstatin A (a tyrosine kinase inhibitor), or verapamil (a Ca^{2+} channel blocker), or by incubation in calcium-free media. A decrease in tetrabromobisphenol A-induced ROS by diethyldithiocarbamate, an inhibitor of superoxide dismutase (SOD), confirmed the involvement of the superoxide anion in the production of ROS by tetrabromobisphenol A (Reistad et al., 2005).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

Chignell et al. (2008) administered tetrabromobisphenol A (100 or 600 mg/kg bw) to Sprague-Dawley rats together with the spin-trapping agent α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone and detected the α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone/•CH_3 spin adduct by electron paramagnetic resonance in the bile. Also measured in the bile was the 2,6-dibromobenzosemiquinone radical; reaction of the latter compound with oxygen could generate the superoxide anion.

Daily treatment of male Sprague-Dawley rats with tetrabromobisphenol A (500 mg/kg bw for 30 days, beginning on postnatal day 18), induced a significant increase in the levels of 8-hydroxy-2′-deoxyguanosine (a biomarker of oxidative DNA damage) in the testis and kidney. No increase in the levels of malondialdehyde was observed in the liver of exposed rats compared with controls (Choi et al., 2011).

Daily administration of tetrabromobisphenol A (750 or 1125 mg/kg bw) for 7 days to Wistar rats decreased the levels of reduced glutathione in females at both doses and increased the levels of malondialdehyde in male rats at the higher dose (Szymańska et al., 2000).

A single oral dose of tetrabromobisphenol A in Sprague-Dawley rats produced increases in kidney levels of thiobarbituric acid reactive substances (TBARS) at 1000 mg/kg bw and in SOD activity at 250–1000 mg/kg bw, but no significant changes in urine analysis parameters. These parameters were not increased in a 14-day repeated-dose experiment with the same doses of tetrabromobisphenol A (Kang et al., 2009).

(ii) Non-human mammalian systems in vitro

Exposure of hepatocytes isolated from Fischer 344/Jcl rats to tetrabromobisphenol A at 0.25–1.0 mM for up to 3 hours decreased the reduced glutathione content with concomitant increases in oxidized glutathione (GSSG), and increased malondialdehyde levels (TBARS). Treatment with tetrabromobisphenol A also reduced the mitochondrial membrane potential and had an uncoupling effect on mitochondrial oxidative phosphorylation (Nakagawa et al., 2007). [Based on the longer time needed to induce lipid peroxidation compared with the rapid reduction in cellular adenosine triphosphate levels, the results suggested that lipid peroxidation induced by tetrabromobisphenol A was due to impaired mitochondrial function.]

Incubation of primary cultures of cerebellar granule cells from Wistar rats with tetrabromobisphenol A at 2.5–7.5 µM produced significant increases in ROS production, with reductions in ^{45}Ca uptake, increases in

intracellular concentrations of ^{45}Ca, and a slight decrease in the mitochondrial membrane potential. The production of ROS was reduced by co-treatment with 0.1 mM ascorbic acid or 1 mM glutathione (Ziemińska et al., 2012). Reistad et al. (2007) also observed concentration-dependent increases in ROS, phosphorylation of ERK1/2 and intracellular calcium in primary cultures of rat cerebellar granule cells exposed to tetrabromobisphenol A. ROS formation was inhibited by pretreatment with the MAPK/ERK kinase inhibitor U0126, the tyrosine kinase inhibitor erbstatin A, the SOD inhibitor diethyldithiocarbamate or by eliminating calcium from the culture medium.

(iii) Fish and other species

In goldfish (*Carassius auratus*) given a single intraperitoneal injection of tetrabromobisphenol A (100 mg/kg bw), ROS were increased in the liver and bile, an effect inhibited by the hydroxyl radical scavenger mannitol. Lipid peroxidation products (TBARS) and protein carbonyl levels, indicators of oxidative damage, were significantly increased in the liver at 1–3 days after treatment with tetrabromobisphenol A (Shi et al., 2005). Tetrabromobisphenol A in aquarium water (3 mg/L for 7 days) significantly decreased reduced glutathione levels and antioxidant enzyme activites (SOD and catalase) in fish livers (He et al., 2015). In *Carassius auratus*, intraperitoneal injections of tetrabromobisphenol A (10 or 100 mg/kg bw for 14 days) decreased the activities of antioxidant enzymes (SOD, catalase, and glutathione peroxidase), decreased reduced glutathione levels and increased the levels of malondialdehyde (a marker of lipid peroxidation) in the liver (Feng et al., 2013).

In zebrafish embryos, tetrabromobisphenol A (0.05, 0.25, or 0.75 mg/mL for 96 hours) increased SOD activity, lipid peroxidation (TBARS), and the expression of heat-shock protein 70 (Hsp70) (Hu et al., 2009). Significant decreases in the activities of the antioxidant enzymes SOD, catalase, and glutathione peroxidase were observed in embryos and zebrafish larvae exposed to tetrabromobisphenol A at 0.4–1.0 mg/L in holding tanks for 3, 5, or 8 days post-fertilization (Wu et al., 2015). Similarly, increases in ROS production were observed in zebrafish embryos and larvae exposed to tetrabromobisphenol A at 0.1, 0.5, or 1.0 mg/L for 96 hours; the increases in ROS production were inhibited by co-incubation with puerarin (1 mg/L), an antioxidant free-radical scavenger. ROS production was measured with a fish ROS enzyme-linked immunosorbent assay kit using a horseradish peroxidase-labelled fish ROS antibody (Yang et al., 2015). Hepatic oxidative stress and general stress was induced in zebrafish exposed to tetrabromobisphenol A (0.75 or 1.5 μM) for 14 days and evaluated for hepatic changes in gene and protein expression (De Wit et al., 2008). [The Working Group noted that tetrabromobisphenol A induced oxidative stress, based on antioxidant-related responses, and general stress responses, based on stimulation of Hsp70 protein in the liver of zebrafish.]

Tetrabromobisphenol A also induced hydroxyl radical formation and oxidative stress in earthworms (*Eisenia fetida*). Lipid peroxidation was increased while the reduced glutathione/GSSG ratio was decreased (Xue et al., 2009). Exposure of earthworms to tetrabromobisphenol A at 50–400 mg/kg dry soil for 14 days resulted in an increased expression of genes encoding SOD and Hsp70 (Shi et al., 2015).

In scallops (*Chlamys farreri*), exposure to tetrabromobisphenol A in seawater tanks (0.2, 0.4, and 0.8 mg/L for up to 10 days) increased SOD activity, the reduced glutathione levels, and malondialdehyde levels in the gill and digestive gland (Hu et al., 2015a).

(iv) Plant systems

Tetrabromobisphenol A increased total free radical generation and enhanced lipid peroxidation in plants (*Ceratophyllum demersum L.*) exposed at 0.05–1.0 mg/L in growth solution.

In addition, levels of GSH were decreased (Sun et al., 2008). ROS were also induced in green alga (*Chlorella pyrenoidosa*) cultures exposed to tetrabromobisphenol A at 2.7–13.5 mg/L for 4–216 hours (Liu et al., 2008).

[The Working Group noted that the induction of oxidative stress by tetrabromobisphenol A has been well established on studies in human cells and in numerous experimental systems.]

4.2.3 Inflammation and immunosuppression

Studies in human cells and in several experimental systems have demonstrated immunosuppressive effects caused by exposures to tetrabromobisphenol A.

(a) Humans

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

The lytic and binding functions of isolated human natural killer (NK) cells were decreased when they were incubated with tetrabromobisphenol A at 0.1–5 µM for 1, 2, or 6 days. The effects of treatment with tetrabromobisphenol A on NK cells were dependent on both the concentration and duration of exposure. Exposure of NK cells to tetrabromobisphenol A at 1–10 µM for 1 hour resulted in a decrease in lytic function that persisted for at least 6 days. The loss of lytic function was more sensitive than the decrease in binding function to the treatment with tetrabromobisphenol A (Kibakaya et al., 2009).

Exposure of human NK cells to tetrabromobisphenol A (2.5 µM for 24 or 48 hours) caused significant decreases in the expression of cell surface proteins that are involved in NK cell binding and/or the lysis of target cells. The analysis was done by flow cytometry after reactions with anti-CD2, anti-CD11a, anti-CD16, anti-CD18, or anti-CD56 antibodies (Hurd & Whalen, 2011).

Phospho-p44/42 and phospho-p38 MAPKs were activated in isolated human NK cells exposed to tetrabromobisphenol A at 0.5–10 µM for 10 minutes, but not after exposures of 1 or 6 hours. Phosphorylation of MEK1/2 and MKK3/6, upstream activators of p44/42 and p38, respectively, was also increased in NK cells exposed to tetrabromobisphenol A at 5 or 10 µM for 10 minutes (Cato et al., 2014). This group had shown previously (Kibakaya et al., 2009) that tetrabromobisphenol A decreased the ability of human NK cells to lyse tumour cells, and that the activation of p44/42 can decrease the lytic function of NK cells. Thus, the aberrant activation of MAPKs by tetrabromobisphenol A may result in NK cells becoming unresponsive to subsequent encounters with tumour cells or virally infected cells.

Tetrabromobisphenol A also activates inflammatory pathways in the human first trimester placental cell line HTR-8/SVneo (Park et al., 2014). Trophoblast cells were cultured for 8, 16, or 24 hours in media containing tetrabromobisphenol A at 5, 10, 20, or 50 µM and analysed for cytokine release (interleukin-(IL)-6, IL-8 and tumour growth factor-β) and prostaglandin E2 (PGE2) production by enzyme-linked immunosorbent assay. Exposure to tetrabromobisphenol A increased the release of PGE2 and the proinflammatory cytokines IL-6 and IL-8, and reduced the release of the anti-inflammatory cytokine tumour growth factor-β. Treatment with NS-398, a cyclooxygenase-2 (COX-2)-specific inhibitor, suppressed the tetrabromobisphenol A-stimulated release of PGE2. Quantitative mRNA analyses by the reverse transcriptase polymerase chain reaction showed that exposure to tetrabromobisphenol A at 10 µM increased the expression of genes encoding prostaglandin-endo-peroxide synthase 2, COX-2, and IL-6 and IL-8. Thus, exposure to tetrabromobisphenol A activates inflammatory pathways in human placental cells (Park et al., 2014).

(b) Experimental systems

The pulmonary viral titer was significantly increased in BALB/c mice fed diets containing 1% tetrabromobisphenol A for 28 days and then intranasally infected with the A2 strain of respiratory syncytial virus. The viral titres were increased two- to threefold in tetrabromobisphenol A-treated mice compared with controls on day 5 after infection. Bronchoalveolar fluid from respiratory syncytial virus-infected mice treated with tetrabromobisphenol A showed enhanced production of tumour necrosis factor-α, IL-6 and interferon-γ, and reduced production of IL-4 and IL-10 (Watanabe et al., 2010).

In a study of immune/allergic responses in vitro to brominated flame retardants, exposure of splenocytes from NC/Nga mice to tetrabromobisphenol A at 1 or 10 μg/mL for 24 hours increased the expression of surface proteins on antigen presenting cells (major histocompatibility complex class II and CD86), and increased the expression of the T-cell receptor and the production of cytokine IL-4 in splenic T-cells. Exposure of isolated mouse bone marrow cells to tetrabromobisphenol A at 1 μM for 6 days did not affect bone marrow-derived dendritic cell activation or differentiation (Koike et al., 2013).

In splenocytes isolated from C57Bl/6 mice that had been incubated with tetrabromobisphenol A at 3 μM and concanavalin A (2 μg/mL) for 48 hours, the expression of the IL-2 receptor α chain (CD25), essential for proliferation of activated T-cells during the immune response, was suppressed (Pullen et al., 2003).

Exposure of the mouse macrophage cell line RAW 264.7 to tetrabromobisphenol A at 1–50 μM increased the mRNA expression and protein levels of COX-2, enhanced the production of PGE2 (a major metabolite of COX-2), and increased the mRNA expression and production of proinflammatory cytokines including tumour necrosis factor-α, IL-6 and IL-1β. Pretreatment of the cells with tetrabromobisphenol A and NS-398, a COX-2-specific inhibitor, inhibited the tetrabromobisphenol A-induced increase in PGE2 production, indicating that the effect of tetrabromobisphenol A is mediated by COX-2 activity. Thus, exposure to tetrabromobisphenol A may promote inflammation by transcriptionally activating the macrophage COX-2 gene and protein expression and increasing the expression and secretion of proinflammatory cytokines (Han et al., 2009).

Tetrabromobisphenol A activated MAPKs and protein kinase C in mussel haemocytes. The observed increase in extracellular superoxide production was reduced by pretreatment with kinase inhibitors specific for protein kinase C and MAPKs (Canesi et al., 2005).

4.2.4 Altered cell proliferation or death

The studies reviewed below indicated neither enhanced cell proliferation nor suppression of apoptosis after exposure to tetrabromobisphenol A, which was associated with an increase in apoptosis in several experimental systems.

(a) Humans

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

In human A549 epithelial alveolar lung cells and the human thyroid cell line Cal-62, tetrabromobisphenol A decreased the rates of DNA synthesis. A549 cells tended to arrest in the G1 phase, while Cal-62 cells tended to arrest in the G2 phase. MAPK cascades were also affected, but not in association with an increase in cell proliferation (Strack et al., 2007; see also Cagnol & Chambard, 2010).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

Apoptosis was induced in the testes of CD-1 mice exposed to drinking-water containing tetrabromobisphenol A at a concentration of 200 μg/L during gestation, lactation, and up to

age 70 days. In addition, expression of the pro-apoptotic *Bax* gene was increased, while expression of the anti-apoptotic *Bcl-2* gene was decreased in tetrabromobisphenol A-exposed mice compared with controls (Zatecka et al., 2013).

Although increased incidences of atypical endometrial hyperplasia were observed in the uterus of female Wistar Han rats exposed to tetrabromobisphenol A (250 mg/kg bw per day) in a 2-year study of carcinogenicity (NTP, 2014; Dunnick et al., 2015), this effect was considered to be a preneoplastic lesion rather than an early event in the development of uterine cancer. [The Working Group noted that, in the 3-month study at doses (5 times per week) of up to 1000 mg/kg bw (NTP, 2014), no treatment-related lesions were observed in the uterus of Wistar Han rats, Fischer 344/NTac rats, or B6C3F$_1$/N mice treated with tetrabromobisphenol A.]

(ii) Non-human mammalian systems in vitro

In a non-transformed rat kidney (NRK) cell line, tetrabromobisphenol A decreased rates of DNA synthesis. NRK cells tended to arrest in the G1 phase. MAPK cascades were also affected, but not in association with an increase in cell proliferation (Strack et al., 2007; see also Cagnol & Chambard, 2010).

Tetrabromobisphenol A induced cell death in mouse TM4 cells, a cell line derived from mouse testicular Sertoli cells, via apoptosis involving mitochondrial depolarization due to increases in cytosolic Ca^{2+} levels. Intracellular levels of Ca^{2+} were elevated in TM4 cells within 1–3 minutes of incubation with tetrabromobisphenol A at 30 µM; after 18 hours, cell viability was < 50%. Tetrabromobisphenol A also caused rapid mitochondrial membrane depolarization. The loss of cell viability by tetrabromobisphenol A was suppressed by the caspase inhibitor Ac-DEVD-CMK, indicating that this loss was due in part to apoptosis. Tetrabromobisphenol A also inhibited Ca^{2+}-adenosine triphosphatase activity in rabbit muscle sarcoplasmic reticulum vesicles and in pig cerebellar microsomes at concentrations as low as 0.5 µM (Ogunbayo et al., 2008).

The treatment of primary cultured neurons from rat cerebellum with tetrabromobisphenol A at 5 µM for 24 hours induced apoptosis-like nuclear changes, characterized by condensed chromatin and DNA fragmentation; however, other hallmarks of apoptosis, including activation of caspase-3, were not observed. Tetrabromobisphenol A induced a concentration-dependent increase in the phosphorylation of ERK1/2 (Reistad et al., 2007).

(iii) Other experimental systems

Apoptotic cells were detected in the brain, heart, and tail of zebrafish embryos and larvae exposed to tetrabromobisphenol A at 1.0 mg/L in holding tanks for 96 hours (Wu et al., 2015); exposures to tetrabromobisphenol A at 0.1–1.0 mg/L induced the expression of three proapoptotic genes – *Tp53*, *Bax*, and caspase 9 – and decreased the expression of the anti-apoptotic gene *Bcl2* (Yang et al., 2015).

4.2.5 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammalian systems in vivo

See Table 4.1

No increase in DNA damage in the alkaline comet assay was observed in the testicular cells of CD-1 mice given tetrabromobisphenol A in corn oil twice (24 hours apart) at doses of 500, 1000, or 2000 mg/kg bw (Hansen et al., 2014). Tetrabromobisphenol A did not increase the frequency of micronucleated erythrocytes in the peripheral blood of male and female B6C3F$_1$ mice exposed by gavage (10–1000 mg/kg bw in corn oil on 5 days per week for 14 weeks) (NTP, 2014).

Table 4.1 Genetic and related effects of tetrabromobisphenol A in non-human mammals in vivo

Species, strain, sex	Tissue	End-point	Test	Results	Dose (LED/HID)	Route, duration, dosing regimen	Reference
Mouse, Swiss CD-1, M	Testis	DNA damage	DNA strand breaks (comet assay)	–	2000 mg/kg bw	Gavage; twice (24 h apart)	Hansen et al. (2014)
Mouse, B6C3F$_1$, M/F	Peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	–	1000 mg/kg bw	Gavage; 14 wk, 5 days/wk	NTP (2014)
Rat, Wistar Han, F	Uterine carcinoma	Mutation	*Tp53* mutation frequency	+	250 mg/kg bw	Gavage; 2 years, 5 days/wk	Harvey et al. (2015)

+, positive;–, negative; bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; wk, week

Uterine carcinomas that were induced in female Wistar Han rats in a study of carcinogenicity (NTP, 2014) were examined for molecular alterations in genes relevant to human endometrial cancer (Harvey et al., 2015). This study identified a marked increase in the frequency of *Tp53* mutations and increased human growth factor receptor 2 gene expression in tetrabromobisphenol A-associated uterine carcinomas compared with spontaneous uterine carcinomas in vehicle controls. [The Working Group noted that it was not clear if the increased frequency of tumours with *Tp53* mutations was due to a genotoxic effect of tetrabromobisphenol A or to enhanced proliferation of cells with spontaneous mutations in the *Tp53* gene.]

(ii) Experimental systems in vitro

See Table 4.2

Tetrabromobisphenol A did not induce intragenic recombination in Sp5 or SPD8 cell lines (mutants isolated from V79 Chinese hamster cells) when tested at doses of 5–40 µg/mL. These cell lines have a partial duplication of the *Hprt* gene that results in a non-functional hypoxanthine-guanine phosphoribosyltransferase protein (Helleday et al., 1999).

In scallops (*Chlamys farreri*), tetrabromobisphenol A (0.2, 0.4 and 0.8 mg/L for up to 10 days) induced DNA damage in the gills and digestive gland tissues in a time- and dose-dependent manner (Hu et al., 2015b).

Studies on the genetic toxicology of tetrabromobisphenol A (up to 10 000 µg/plate) conducted by the NTP (2014) showed negative results for bacterial gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, in the presence or absence of metabolic activation by S9 from induced Syrian hamster or Sprague-Dawley rat liver, and in *Escherichia coli* strain WP2 *uvrA*/pKM101, in the presence or absence of metabolic activation by S9 mix from Sprague-Dawley rat liver.

(c) Acellular systems

The binding of tetrabromobisphenol A to calf thymus DNA was studied by ultraviolet-visible absorption, fluorometric competition with DNA-bound ethidium bromide, circular dichroism, and molecular modelling (Wang et al., 2014). Tetrabromobisphenol A intercalated into DNA [through an interaction involving hydrogen binding and hydrophobic interaction].

Table 4.2 Genetic and related effects of tetrabromobisphenol A in experimental systems in vitro

Phylogenetic class	End-point	Test	Results	Dose (LED or HID)	Reference
Chinese hamster Sp5 and SPD8 cell lines	DNA damage	Mutation	–	40 µg/mL; 24 h	Helleday et al. (1999)
Chlamys farreri (scallops)	DNA damage	DNA strand breaks (alkaline unwinding assay)	+	0.2 mg/L, 10 days	Hu et al. (2015b)
Salmonella typhimurium TA1535, TA1537, TA98, TA100	Mutation	Reverse mutation	–[a]	10 000 µg/plate	NTP (2014)
Escherichia coli WP2 *uvrA*	Mutation	Reverse mutation	–[a]	6000 µg/plate	NTP (2014)
Calf thymus DNA	DNA damage	DNA intercalation	+	100 µM	Wang et al. (2014)

[a] With and without metabolic activation
+, positive;–, negative; HID, highest ineffective dose; LED, lowest effective dose

4.3 Data relevant to comparisons across agents and end-points

For all compounds evaluated in the present volume of the *IARC Monographs*, including tetrabromobisphenol A, analyses of high-throughput screening data generated by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) are presented in the *Monograph* on 1-bromopropane, in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

No other adverse effects were identified in exposed humans.

In a NTP 2-year bioassay in rats, the incidence of rete ovarii cyst was significantly increased in the group at the highest dose of tetrabromobisphenol A (1000 mg/kg bw) (NTP, 2014; Dunnick et al., 2015).

Brainstem auditory evoked potentials thresholds and latency were increased in females and latency was increased in males in a developmental study of rats (Lilienthal et al., 2008). [The Working Group noted that these findings may reflect an effect of tetrabromobisphenol A on thyroid hormone-regulated developmental events, including hearing and testis weight. However, no experimental group was available that would have tested this directly (e.g. tetrabromobisphenol A + T_4).] This group later published an additional study showing that tetrabromobisphenol A decreased serum total T_4 (Van der Ven et al., 2008) (see also Section 4.2.1).

Behl et al. (2015) reported that tetrabromobisphenol A was active in assays in vitro that were indicative of [potential] developmental toxicity and neurotoxicity in the low micromolar range. The assays used evaluated the effects of tetrabromobisphenol A on the differentiation of mouse embryonic stem cells, human neural stem cell proliferation and growth, and rat neuronal growth and network activity.

5. Summary of Data Reported

5.1 Exposure data

Tetrabromobisphenol A is a flame retardant with a high production volume that is applied in a wide variety of consumer products. The most common use is for printed circuit boards, whereby tetrabromobisphenol A is chemically bonded to the polymer matrix. Tetrabromobisphenol A is also applied as an additive compound in the manufacture of acrylonitrile–butadiene–styrene resins and high-impact polystyrene. It has been detected in almost all biotic and abiotic compartments worldwide. Occupational exposures to tetrabromobisphenol A have been measured in facilities manufacturing electronic products, and at higher concentrations in recycling facilities. Exposure of the general population predominantly occurs through the diet and through the ingestion of indoor dust. While intake by very young children is predominantly via the ingestion of indoor dust, intake by adults occurs mainly via the diet. Very young children are estimated to have a higher daily intake than adults. Exposure may occur prenatally, and tetrabromobisphenol A has been measured in breast milk.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3. Animal carcinogenicity data

Tetrabromobisphenol A was tested for carcinogenicity after oral administration by gavage in one study in male and female mice, and in one study in male and female rats. One co-carcinogenicity study of transplacental/perinatal exposure in rats was found to be inadequate for the evaluation of the carcinogenicity of tetrabromobisphenol A.

In the study in male mice, tetrabromobisphenol A caused significantly increases in the incidence of hepatoblastoma, of hepatocellular adenoma (multiple), and of hepatocellular carcinoma or hepatoblastoma (combined). Tetrabromobisphenol A significantly increased the incidence of haemangiosarcoma (all organs), with a significant positive trend. A significant positive trend in the incidence of adenoma or carcinoma (combined) of the large intestine (caecum or colon) was also observed; the incidence in mice receiving the highest dose exceeded the range for historical controls. Tetrabromobisphenol A did not cause any significant increases in tumour incidence in female mice.

In the study in male rats, tetrabromobisphenol A caused a significant positive trend in the incidence of interstitial cell adenoma of the testes. In female rats, tetrabromobisphenol A caused a significant increase in the incidence of adenocarcinoma of the uterus, with a significant positive trend. Several rats in the groups exposed to tetrabromobisphenol A were diagnosed with the rare uterine tumour, malignant mixed Müllerian tumour; this tumour was not reported in the data for historical controls. A significant increase was observed in the incidence of adenoma, adenocarcinoma, or malignant mixed Müllerian tumour (combined) of the uterus, with a significant positive trend.

5.4 Mechanistic and other relevant data

After oral administration in humans and rats, tetrabromobisphenol A is readily absorbed and widely distributed among tissues, is extensively metabolized to glucuronide and sulfate conjugates, and was shown to be excreted primarily in the faeces in rats. Tetrabromobisphenol A has been detected in human milk in surveys of the general population. In rats, tetrabromobisphenol A and its metabolites do not accumulate in the

tissues. A metabolic minor pathway involving radical formation has been demonstrated in vivo in rats. Quantitative species-, sex- and strain-dependent differences in conjugated metabolites have been observed.

With respect to the "key characteristics" of human carcinogens, there is *strong* evidence that tetrabromobisphenol A modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive; there is *moderate* evidence that tetrabromobisphenol A induces chronic inflammation; and there is *weak* evidence that tetrabromobisphenol A is electrophilic, is genotoxic or alters cell proliferation, cell death or nutrient supply.

There is *strong* evidence that tetrabromobisphenol A can alter thyroid hormone receptor function both directly and indirectly. Studies in experimental animals demonstrated that tetrabromobisphenol A reduces serum levels of thyroxine. Cell-based assays that included the use of human cells, and biochemical studies showed that tetrabromobisphenol A can interact with thyroid hormone receptors directly, the outcome being dependent on several variables. Tetrabromobisphenol A is a potent inhibitor of sulfotransferases. ToxCast data supported the conclusion that tetrabromobisphenol A can interact with nuclear receptors, in particular peroxisome proliferator-activated receptor-γ, as well as inhibit aromatase and alter steroid biosynthesis in a human cell line.

There is *strong* evidence that tetrabromobisphenol A induces oxidative stress. No data were available in exposed humans. Oxidative stress was induced by tetrabromobisphenol A in vivo in rats (testis and kidney), goldfish, zebrafish, earthworms, and scallops. In vitro, tetrabromobisphenol A also induced oxidative stress in human neutrophils and granulocytes, and rat hepatocytes and cerebellar cells, with activation of the mitogen-activated protein kinase pathway. ToxCast data also supported the conclusion that tetrabromobisphenol A induces oxidative stress. Tetrabromobisphenol A activated inflammatory pathways in a human placental cell line.

There is *strong* evidence that tetrabromobisphenol A is immunosuppressive. Tetrabromobisphenol A decreased the lytic and binding functions of isolated human natural killer cells, and reduced the expression of cell-surface proteins needed for the attachment of human natural killer cells to target cells. The effects observed in vitro were supported by the observation in vivo of the reduced ability of mice exposed to tetrabromobisphenol A to suppress a respiratory virus.

In a mouse macrophage cell line, tetrabromobisphenol A increased the expression and production of proinflammatory cytokines. However, because of the absence of data on chronic effects in vivo, the evidence that tetrabromobisphenol A induces chronic inflammation is *moderate*.

There is *weak* evidence that tetrabromobisphenol A is genotoxic. Tetrabromobisphenol A interacted with calf thymus DNA by intercalation. An increased frequency of *Tp53* mutation was observed in uterine carcinomas induced by tetrabromobisphenol A in female rats.

There were few data on the other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, or causes immortalization).

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

In making its overall evaluation, a majority of the Working Group considered that the strong mechanistic evidence that tetrabromobisphenol A can operate through three key characteristics of carcinogens and that these can be operative in humans warranted an upgrade to *Group 2A*. Specifically, the evidence was strong for the modulation of receptor-mediated effects, for the induction of oxidative stress, and for the induction of immunosuppression:

- Tetrabromobisphenol A interacts directly with several human nuclear receptors relevant to human cancers, including thyroid hormone and peroxisome proliferator-activated receptor-γ. Tetrabromobisphenol A modulates enzymes relevant for the endocrine system, inhibits aromatase, and is a potent inhibitor of sulfotransferase.
- In multiple species in vivo and in human cells in vitro, tetrabromobisphenol A causes oxidative stress.
- Immunosuppressive effects were observed in mice exposed in vivo. Multiple experiments in human natural killer cells exposed in vitro also showed effects consistent with immunosuppression.

However, a minority of the Working Group judged that these data did not support a mechanistic upgrade.

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LIST OF ABBREVIATIONS

4-OH-TEMPO	4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl
AcPrCys	*N*-acetyl-*S*-(*n*-propyl)-L-cysteine
AHR	aromatic hydrocarbon receptor
AhR	aryl hydrocarbon receptor
AMCC	*N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine
AUC	area under the concentration curve
bw	body weight
CI	confidence interval
CoQ10	coenzyme Q_{10}
CYP	cytochrome P450
DHPN	*N*-bis(2-hydroxypropyl)nitrosamine
EPA	United States Environmental Protection Agency
GABA	gamma-aminobutyric acid
GC	gas chromatography
GLP	good laboratory practice
GM	geometric mean
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione *S*-transferase
GSPrCys	globin-*S*-propylcysteine
HHE	health hazard evaluation
HMF	*N*-hydroxymethylformamide
HMMF	*N*-hydroxymethyl-*N*-methylformamide
HPLC	high-performance liquid chromatography
MAK	Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
NIOSH	United States National Institute for Occupational Safety and Health
NK	natural killer
NRF2/ARE	nuclear erythroid-related factor 2/antioxidant response element
NTP	United States National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PGE2	prostaglandin E2

PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
RR	relative risk
SEER	Surveillance, Epidemiology and End Results
SMR	standardized mortality ratio
SOD	superoxide dismutase
SRR	standardized relative risk
T_3	triiodothyronine
T_4	thyroxine
TBARS	thiobarbituric acid reactive substances
TR	thyroid hormone receptor
TRE	thyroid hormone-responsive element
UNEP	United Nations Environment Programme

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: http://publications.iarc.fr/563) comprises a spreadsheet (.xlsx) analysed by the Working Group for Volume 115 of the IARC Monographs. The spreadsheet lists 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 the decision as to whether each chemical was "active" or "inactive" (EPA, 2015).

References

EPA (2015). ToxCast & Tox21 Summary Files from invitrodb_v1. Washington (DC): Office of Research and Development. United States Environmental Protection Agency. Retrieved from https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data on 30 November 2015. Data released December 2014.

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of: *N*,*N*-dimethylformamide, a solvent produced in high volumes and commonly used in many industrial processes; 2-mercaptobenzothiazole, a rubber accelerant and preservative; the rocket fuel hydrazine; the widely used fire retardant tetrabromobisphenol A; 1-bromopropane, a solvent used in dry cleaning, degreasing and adhesive resins; the seed fumigant 3-chloro-2-methylpropene; and *N*,*N*-dimethyl-*p*-toluidine, a hardening agent in dental and bone adhesives.

Exposure to all seven agents considered may occur in the general population as well as in different occupational settings.

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