IARC MONOGRAPHS

SOME NITROBENZENES AND OTHER INDUSTRIAL CHEMICALS

VOLUME 123

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

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This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 9–16 October 2018

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

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

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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 *IARC Monographs* Group, 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 *IARC Monographs* Group, so that corrections can be reported in future volumes.

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[4] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a Monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[5] At the time of the meeting, John O'Connor was employed by EI du Pont de Nemours and Company, USA.

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-twenty-third volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of several industrial chemicals to which workers or the general population are or can potentially be exposed. They include: *ortho*-phenylenediamine (and its dihydrochloride salt), 2-chloronitrobenzene, 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene, 2,4-dichloro-1-nitrobenzene, 2-amino-4-chlorophenol, *para*-nitroanisole, and *N*,*N*-dimethylacetamide.

These chemicals were recommended for evaluation primarily because new studies of cancer in experimental animals had become available. Epidemiological data for each of the chemicals included in the present volume were either lacking or scant. Mechanistic data were sparse for most of these chemicals, and did not alter the overall evaluations for any agent. 2-Chloronitrobenzene and 4-chloronitrobenzene were both previously evaluated as *not classifiable as to its carcinogenicity to humans* (Group 3) by the Working Group in Volume 65 of the *IARC Monographs* (IARC, 1996); new data that have become available since these evaluations have been included and considered in the present volume. The other chemicals considered in the present volume have not been previously evaluated by the Working Group. A summary of the findings of this volume appears in *The Lancet Oncology* (Van den Berg et al., 2018).

Limitations on data on production and use, and quantification and relative contributions of sources of exposure

Despite the fact that seven of the agents evaluated (*ortho*-phenylenediamine, 2-chloronitrobenzene, 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene, 2,4-dichloro-1-nitrobenzene, *para*-nitroanisole, and *N*,*N*-dimethylacetamide) are "high production volume" chemicals, few quantitative data were available to characterize exposure in the workplace or general population. Occupational exposure is expected during production and use via inhalation, skin contact (the primary exposure route for *N*,*N*-dimethylacetamide), or inadvertent ingestion. Drinking-water and some consumer products might contain residues of some of these agents.

The Working Group particularly noted the poor information available on production and use of, and exposures to these chemicals in workers or in other populations in low- and middle-income countries (especially India and

China). Regarding production and use, data are not generally available in the peer-reviewed literature because of the barriers of language and unfamiliarity with systems for regulating chemicals and recording such data. There are few researchers from low- and middle-income countries publishing data on occupational and environmental exposure to chemicals.

Common effects for structurally related chloronitrobenzenes

Four of the chemicals evaluated in this volume were structurally related chloronitrobenzenes: 2-chloronitrobenzene, 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene, and 2,4-dichloro-1-nitrobenzene. Both similarities and differences were observed in the major toxicological and carcinogenic effects induced by these related compounds. For all four chemicals, hepatocarcinogenicity, hepatotoxicity, and haematotoxicity were observed in rats and mice, and renal toxicity was seen in rats. For 2-chloronitrobenzene and the dichloronitrobenzenes, renal carcinogenicity in rats was also apparent, and hepatocarcinogenicity in mice was characterized by the induction of hepatoblastoma.

2-Chloronitrobenzene appeared to be the most potent hepatotoxicant of the agents considered, while 4-chloronitrobenzene was a potent haematotoxicant, and also induced malignant splenic tumours (e.g. fibrosarcoma, osteosarcoma, and haemangiosarcoma) in rats and liver haemangiosarcoma in mice. 2,4-Dichloro-1-nitrobenzene caused peritoneal haemangiosarcomas in mice.

High-throughput screening data

Of the compounds considered, all except *para*-nitroanisole have been evaluated in at least some of the high-throughput screening assays of the United States Environmental Protection Agency and National Institutes of Health (EPA, 2018). In addition, assay results were available for several key metabolites of these compounds. Data obtained from high-throughput screening have certain strengths, and may provide additional evidence to support mechanistic conclusions made on the basis of other types of assay. However, there are limitations to the applicability and utility of such data for the evaluations made by the *IARC Monographs* Working Group (see also Chiu et al., 2018; Guyton et al., 2018), including that xenobiotic metabolism in these assays is lacking or poor (and uncharacterized), restricting observations primarily to effects elicited by the parent compounds. In addition, for compounds of lower relative molecular mass it may be difficult to attain the concentrations required for bioactivity in these assays (Hopkins, et al., 2004); four of the compounds evaluated by the present Working Group (*N*,*N*-dimethylacetamide, *para*-nitroanisole, *ortho*-phenylenediamine, and 2-amino-4-chlorophenol) have a relative molecular mass of less than 150, with that of the other compounds being slightly above. Furthermore, these large-scale screening programmes were designed to aid prioritization of large chemical libraries for additional toxicity testing rather than to identify the hazards of a specific chemical or chemical group. Thus, presenting the data for all agents under evaluation in one place, as is done in the first monograph of this volume, is useful as it facilitates comparisons. However, in addressing the strength of mechanistic evidence for individual agents, it may be useful to consider the results of high-throughput screening assays in the context of the sensitivity and specificity of

other available in vitro and in vivo assays relevant to the key characteristics of carcinogens.

High-throughput screening results are currently presented as active assay end-points mapped to key characteristics of carcinogens (see Table 4.4 of the monograph on 2-chloronitrobenzene). However, experimental coverage is not consistent across agents or within key characteristics of carcinogens (Chiu et al., 2018). Gaps were significant for the agents evaluated in the present volume; for five of the agents, data were available for only 54 of the 229 assay end-points mapped to the key characteristics (see Table 4.4, and supplementary information available in Annex 1). There was also variability in coverage across assays (the experimental unit), the platform (the type of assay), and the cell type; these factors may contribute to the activity patterns seen. For example, for the 225 assay end-point results available for *ortho*-phenylenediamine, the data spanned 70 individual experiments (assays) performed on eight different platforms in various cell types. Most results were from assays performed across six primary human cell cultures and a number of immortalized human cell lines (including kidney, cervix, ovary, pituitary, intestinal, liver, and breast cell lines), with a few other results from a Chinese hamster ovary cell line (CHO-K1), or from a cell-free platform. Analyses that move beyond counts (or ratios of counts) of active assay end-points may aid further characterization of the variability associated with individual experiments, cell type, and platform. This may also aid in determining whether and when individual or nested high-throughput screening assay results can be more fully integrated with other in vitro and in vivo assays typically evaluated within the section on mechanistic evidence, Section 4, of each monograph.

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2-CHLORONITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

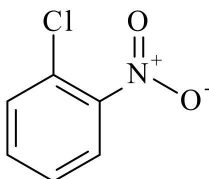
Chem. Abstr. Serv. Reg. No.: 88-73-3

Chem. Abstr. Serv. name:
1-chloro-2-nitrobenzene

IUPAC systematic name:
1-chloro-2-nitrobenzene

Synonyms: 2-chloronitrobenzene; *ortho*-chloronitrobenzene; 2-chloro-1-nitrobenzene; 2-CNB; 2-nitrochlorobenzene; *ortho*-nitrochlorobenzene; 1-nitro-2-chlorobenzene; 2-nitro-1-chlorobenzene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_4ClNO_2$

Relative molecular mass: 157.55 (PubChem, 2018).

1.1.3 Chemical and physical properties

Description: yellow crystals

Boiling point: 246 °C

Melting point: 32–33 °C

Solubility: slightly soluble in water (441 mg/L at 25 °C); soluble in acetone, benzene, toluene, ethanol, and other organic solvents

Volatility: vapour pressure, 0.018 mm Hg [at 25 °C]

Relative vapour density (air = 1): 5.4

Octanol/water partition coefficient (P): log K_{ow} = 2.52 (PubChem, 2018)

Conversion factor: 1 ppm = 6.44 mg/m^3 at normal temperature (25 °C) and pressure (101 kPa)

Technical products and impurities: available commercially at purities of greater than 99% (Sigma-Aldrich, 2018).

1.2 Production and use

1.2.1 Production process

Continuous or batch nitration of chlorobenzene with mixed acids typically gives a 98% yield of an isomer mix comprising 34–36% 2-chloronitrobenzene, 63–65% 4-chloronitrobenzene, and about 1% 3-chloronitrobenzene. The isomers can be separated by a combination of fractional crystallization and distillation (Booth, 2012).

1.2.2 Production volume

2-Chloronitrobenzene is included in the 2007 Organisation for Economic Co-operation and Development list of chemicals with a high production volume (OECD, 2009). For the year 1995, the worldwide (excluding eastern Europe) production of 2-chloronitrobenzene was reported as 111 800 tonnes from approximately 30 producers in western Europe (27 000 tonnes), China (39 000 tonnes), India (15 500 tonnes), Japan (9000 tonnes), the Republic of Korea (2300 tonnes), and the USA (19 000 tonnes) (OECD-SIDS, 2001).

The non-confidential production volumes for 2-chloronitrobenzene for the USA, as submitted to the United States Environmental Protection Agency (EPA) by companies for chemicals under the 1986–2002 Inventory Update Rule and for 2012–2015 under the Chemical Data Reporting Rule, are presented in Table 1.1. Over this period, annual production in the USA decreased from a peak of 23–45 thousand tonnes to less than 11 tonnes.

The European Chemicals Agency reported that 1–10 tonnes of 2-chloronitrobenzene per year are currently manufactured in and/or imported into the European Economic Area (ECHA, 2018).

Table 1.1 Production volumes for 2-chloronitrobenzene, USA[a]

Year	Production volume range, pounds [tonnes]
1986	(> 10–50) × 10^6 [4536–22 680]
1990	(> 50–100) × 10^6 [22 680–45 359]
1994	(> 50–100) × 10^6 [22 680–45 359]
1998	(> 50–100) × 10^6 [22 680–45 359]
2002	(> 10–50) × 10^6 [4536–22 680]
2012	(10–50) × 10^4 [45–227]
2013	(25–100) × 10^3 [11–45]
2014	< 25 × 10^3 [< 11]
2015	< 25 × 10^3 [< 11]

[a] Non-confidential information on production volumes submitted to the United States Environmental Protection Agency by companies for chemicals under the 1986–2002 Inventory Update Rule and for 2012–2015 under the Chemical Data Reporting Rule (HSDB, 2008; EPA, 2018a)

1.2.3 Use

2-Chloronitrobenzene is an important intermediate in the synthesis of colorants and specialty chemicals (Booth, 2012). The products obtained from 2-chloronitrobenzene – 2-chloroaniline and 3,3′-dichlorobenzidine – are important diazo components. 2-Chloronitrobenzene is also used to synthesize 2-nitrophenol, 2-nitroanisole, 2-ethoxynitrobenzene, and 2-nitroaniline. All of these chemicals are used as precursors of the corresponding amines and many other products, including *ortho*-anisidine, *ortho*-phenetidine, 3-amino-4-hydroxybenzenesulfonamide, picric acid, lumber preservatives, diaminophenol hydrochloride (a photographic developer), corrosion inhibitors, pigments, and agricultural chemicals. Sulfonation and chlorosulfonation also give important sulfonic acid and sulfonyl chloride derivatives (Booth, 2012).

1.3 Methods of measurement and analysis

1.3.1 Air

No specific methods have been published for the measurement of 2-chloronitrobenzene in air, although researchers have adapted the published method for 4-chloronitrobenzene (NIOSH, 2005) to measure concentrations of 2-chloronitrobenzene (Jones et al., 2006). The United States National Institute for Occupational Safety and Health (NIOSH) method is based on collecting samples in silica gel tubes, and analysis by gas chromatography combined with mass spectrometry or flame ionization detection.

1.3.2 Other environmental media

EPA Method 8091 is a gas chromatography method that can be used to determine the concentration of nitroaromatics and cyclic ketones, allowing the contamination in water, soil, and wastewater to be measured (EPA, 1996). Method 8091 can detect nitroaromatics in water and soil at concentrations of parts per billion, and in wastewater samples at concentrations of parts per million.

1.3.3 Biomonitoring

There are no published biological monitoring methods for 2-chloronitrobenzene. [The Working Group noted that published methods for monitoring 4-chloronitrobenzene could be adapted for 2-chloronitrobenzene.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

2-Chloronitrobenzene is not known to occur naturally. The major source of environmental release of 2-chloronitrobenzene is the chemical plants at which it is produced and/or used as an intermediate. Minor sources of release into the environment may occur during transport, storage, or disposal to landfills; 2-chloronitrobenzene may also form in the environment through the oxidation of non-natural aromatic amines or the reaction of nitrogen oxides in highly polluted air with chlorinated aromatic hydrocarbons (Howard et al., 1976).

2-Chloronitrobenzene is most likely to be found in air and water, with water being the most contaminated (accounting for 65.4% of 2-chloronitrobenzene found in the environment) (OECD-SIDS, 2001). Measurements of 2-chloronitrobenzene in water identified by the Working Group are summarized in Table 1.2. The compound was measured at concentrations of up to 37 μg/L in the Mississippi river, USA, in the 1960s (Middleton & Lichtenberg, 1960). In the USA, concentrations of up to 1800 mg/L were reported for 2-, 3-, and 4-chloronitrobenzenes in wastewater from a chloronitrobenzene-production plant in the 1970s (Howard et al., 1976). In 1976, an accidental release of chloronitrobenzenes from a plant producing chemicals and dyes was reported in France (Raguet et al., 2010); in the area of the accident, concentrations of 2-chloronitrobenzene of up to 1.8 mg/L in groundwater were measured (Duguet et al., 1988). Elsewhere in Europe, 2-chloronitrobenzene concentrations of up to 0.2 μg/L in German rivers (Feltes et al., 1990) and 0.8 μg/L in Italian rivers (Trova et al., 1991) were measured in the 1990s; more recent measurements in German rivers (2004) measured concentrations of less than 0.06 μg/L (Schäfer et al., 2011). Based on the available experimental data, 2-chloronitrobenzene is not readily biodegradable in water. [The Working Group noted that 4-chloronitrobenzene can be biodegraded by adapted microorganisms. Similar approaches could be successful with 2-chloronitrobenzene, but no confirmatory research was identified.]

1.4.2 Occurrence in food

2-Chloronitrobenzene has been found at low levels in edible portions of various fish species from the Mississippi river in the USA (Yurawecz & Puma, 1983), as well as in fish from the river Main in Germany (Steinwandter, 1987).

1.4.3 Exposure in the general population

No information on the exposure of the general population to 2-chloronitrobenzene was available to the Working Group.

1.4.4 Occupational exposure

Occupational exposure to 2-chloronitrobenzene may occur through inhalation and skin contact at workplaces where this compound

Table 1.2 Environmental occurrence of 2-chloronitrobenzene

Location, collection date	Sampling matrix	Mean exposure concentration (range)	Comments	Reference
France, 1987	Groundwater	1500 (970–1828) µg/L	Accidental pollution from a dye production plant; 2-chloronitrobenzene was the primary pollutant, accounting for 70% of the pollution	Duguet et al. (1988)
France, 2011	Groundwater	10 (maximum, 290) ng/L	0.2% positive measurements; ~500 sites throughout France	Lopez & Laurent (2013)
Mississippi, Cape Girardeau, MO, USA, 1957	River water	NR (4–37 µg/L)	Specific industrial discharge identified	Middleton & Lichtenberg (1960)
Mississippi, New Orleans, LA, USA, 1959	River water	NR (1–2 µg/L)		Middleton & Lichtenberg (1960)
Elbe, Germany, NR	River water	NR (0.04–0.20 µg/L)	Three samples	Feltes et al. (1990)
Bormida river, Italy, 1989–1990	River water	0.21 (0.02–0.84) µg/L	Monthly measurements at five sampling stations	Trova et al. (1991)
Germany, 1994–2004	River water	NR (maximum, 0.06 µg/L)	A total of 110 measurements from the four largest rivers of northern Germany; detection at > 20% of sites	Schäfer et al. (2011)
Netherlands, 1983–1984	Coastal water	11.0 (0.2–50) ng/L	108 measurements throughout the year at nine locations	van de Meent et al. (1986)
Scheldt estuary, Netherlands and/or Belgium, 1986	Estuary water	Median, 1.3 (0.5–2.1) ng/L	Heavy pollution due to large wastewater discharge	van Zoest and van Eck (1991)
USA, early 1970s[a]	Wastewater	NR (1500–1800 mg/L)	Effluent from a 3-chloronitrobenzene production plant	Howard et al. (1976)
India, 1980s	Wastewater	71 (24–93) mg/L	Effluent from a chloronitrobenzene production plant	Swaminathan et al. (1987)

NR, not reported

[a] 3- and 4-Chloronitrobenzene also collected

is produced or used. Exposure may also occur through inadvertent ingestion (CDC, 2016).

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 2900 employees in the USA were potentially exposed to 2-chloronitrobenzene. The estimates were based on a survey of companies and did not involve measurements of exposure (NOES, 1995).

The exposure of workers at a chemical factory in China was reported in 2006. The median concentration of 2-chloronitrobenzene determined from the personal air samples of 19 workers was 0.37 mg/m^3 (Jones et al., 2006). In another study by the same authors in the same factory, the mean 8-hour average exposure level of 2-chloronitrobenzene of 19 workers was 0.49 mg/m^3 (Jones et al., 2007).

1.5 Regulations and guidelines

There are no international occupational exposure limit values available for 2-chloronitrobenzene.

The EPA has set a chronic oral reference dose of 0.003 mg/kg per day for 2-chloronitrobenzene (EPA, 2009). The EPA regional screening levels for 2-chloronitrobenzene are 1.8 mg/kg in resident soil, 1.0 ng/m^3 in resident air, and 0.24 µg/L in tap water (EPA, 2018b).

The French *Agence française de securité sanitaire de l'environnement et du travail* (AFSSET, 2009) proposed two toxicological reference values for ingestion of 2-chloronitrobenzene: a chronic reference toxicological value with a threshold based on the hepatotoxic effects of 0.08 mg/kg per day; and a non-threshold reference toxicological value based on potential carcinogenic effects of 6×10^{-8} mg/kg per day.

For all methaemoglobin inducers such as 2-chloronitrobenzene, a biological exposure index was set by the American Conference of Governmental Industrial Hygienists at 1.5% methaemoglobin in blood (ACGIH, 2008).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Evidence for the carcinogenic activity of 2-chloronitrobenzene was previously reviewed by the Working Group in *IARC Monographs* Volume 65 (IARC, 1996). On the basis of one study in male mice, one study in female mice, and one study in male rats (Weisburger et al., 1978), the Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of chloronitrobenzenes [2-, 3-, and 4-chloronitrobenzene]. An additional study with 2-chloronitrobenzene in male and female rats and mice has since become available for evaluation (Matsumoto et al., 2006b).

See Table 3.1

3.1 Mouse

Oral administration

In the study by Weisburger et al. (1978), groups of 25 male and 25 female CD-1 mice (derived from HaM/ICR mice) (age, 6–8 weeks) were fed diets containing 1-chloro-2-nitrobenzene [2-chloronitrobenzene] (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0 (control), 3000, or 6000 ppm for 8 months. After 8 months, the groups at 3000 and 6000 ppm had their dietary concentrations reduced to 1500 and 3000 ppm, respectively, for a further 10 months. After this 18-month dosing period, all groups were held for an additional 3 months on the control diet before being killed at 21 months. There was also a pooled control group of 99 males and 102 females [no additional details were provided]. Mice that died within the first 6 months of the study were discarded

Table 3.1 Studies of carcinogenicity with 2-chloronitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978)	Oral Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (3000 ppm for 8 mo, 1500 ppm for 10 mo, then held for an additional 3 mo without dosing), higher dose (6000 ppm for 8 mo, 3000 ppm for 10 mo, then held for an additional 3 mo without dosing), 0 (pooled control) 25, 25, 25, 99 NR	*Liver*: hepatoma [hepatocellular carcinoma] 3/18, 7/17*, 3/16, 7/99	 *$P < 0.025$ (vs pooled control)	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details provided; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (F) 6–8 wk 21 mo Weisburger et al. (1978)	Oral Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (3000 ppm for 8 mo, 1500 ppm for 10 mo, then held for an additional 3 mo without dosing), higher dose (6000 ppm for 8 mo, 3000 ppm for 10 mo, then held for an additional 3 mo without dosing), 0 (pooled control) 25, 25, 25, 102 NR	*Liver*: hepatoma [hepatocellular carcinoma] 0/20, 5/22*, 5/19*, 1/102	 *$P < 0.025$ (vs concurrent control and pooled control)	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details provided; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, Crj:BDF$_1$ (M) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 100, 500, 2500 ppm, continuous dosing 50, 50, 50, 50 35, 35, 17, 8	*Liver* Hepatocellular adenoma 19/50, 29/50*, 30/50*, 34/50**	 $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test; **$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study The incidence of hepatoblastoma in all groups of treated male mice exceeded the maximum incidence in the JBRC historical control data for this tumour (3/348; maximum incidence, 2%). Incidence and statistics for combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006a)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF_1 (M) 6 wk 2 yr Matsumoto et al. (2006b) (cont.)		Hepatocellular carcinoma		
		15/50, 14/50, 20/50, 35/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma		
		1/50, 6/50, 35/50*, 44/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)		
		30/50, 36/50, 49/50*, 49/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
Mouse, Crj:BDF_1 (F) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 100, 500, 2500 ppm, continuous dosing 50, 50, 50, 50 29, 34, 26, 5	*Liver* Hepatocellular adenoma		Principal strengths: males and females used; well-conducted GLP study Incidence and statistics for combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006a)
		8/50, 22/50*, 48/50*, 38/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular carcinoma		
		0/50, 3/50, 14/50*, 48/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma		
		0/50, 0/50, 9/50*, 28/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)		
		8/50, 24/50, 50/50*, 50/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (1000 ppm for 6 mo, 500 ppm for 12 mo, then 6 mo without dosing), higher dose (2000 ppm for 6 mo, 1000 ppm for 12 mo, then 6 mo without dosing), 0 (pooled control) 25, 25, 25, 111 NR	*Multiple sites*: multiple tumours 1/22, 7/22*, 1/19, 14/111	 *$P < 0.025$ (vs concurrent control and pooled control)	Principal limitations: limited number of dose groups; limited experimental details provided; limited macroscopic and microscopic evaluation; no study in female rats; small number of rats Histopathology conducted only on rats surviving after 6 mo
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 80, 400, 2000 ppm, continuous dosing 50, 50, 50, 50 40, 40, 39, 0	*Liver* Hepatocellular adenoma 2/50, 3/50 (6%), 7/50, 1/50 Hepatocellular carcinoma 0/50, 0/50, 3/50 (6%), 1/50 Hepatocellular adenoma or carcinoma (combined) 2/50, 3/50, 10/50*, 2/50 *Kidney* Renal cell adenoma 0/50, 1/50, 0/50, 1/50 Renal cell carcinoma 0/50, 0/50, 0/50, 4/50 (8%)	 $P < 0.05$, Peto trend test $P < 0.01$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test [NS] [$P < 0.015$, Cochran–Armitage trend test]	Principal strengths: males and females used; well-conducted GLP study The 2000 ppm exposure in male rats exceeded the maximum tolerated dose; the data for this dose level were not included for statistical analysis The incidence of hepatocellular adenoma in male rats at 400 ppm (7/50, 14%) exceeded the maximum incidence for this tumour in the JBRC historical control data (7/400; maximum incidence, 6%) The incidence of hepatocellular carcinoma in male rats at 400 ppm (3/50, 6%) exceeded the incidence for this tumour in the JBRC historical control data (0/400) Incidence and statistics for a combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006b) The incidence of renal cell carcinoma in the group at 2000 ppm (4/50, 8%) exceeded the incidence in the JBRC historical control data (0/400)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 80, 400, 2000 ppm, continuous dosing 50, 50, 50, 50 41, 42, 45, 39	*Liver* Hepatocellular adenoma 0/50, 0/50, 2/50, 20/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study The incidence of hepatocellular carcinoma in female rats at 2000 ppm (4/50, 8%) exceeded the incidence for this tumour in the JBRC historical control data (0/400) Incidence and statistics for a combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006b) The incidence of renal cell adenoma in the group exposed at 2000 ppm (2/50, 4%) exceeded the maximum incidence in the JBRC historical control data (1/400; maximum incidence, 2%)
		Hepatocellular carcinoma 0/50, 0/50, 0/50, 4/50 (8%)	 $P < 0.01$, Peto trend test	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 0/50, 2/50, 23/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Kidney: renal cell adenoma 0/50, 0/50, 0/50, 2/50 (4%)	 [NS]	

F, female; GLP, good laboratory practice; JBRC, Japan Bioassay Research Centre; M, male; mo, month; NR, not reported; NS, not significant; vs, versus; wk, week; yr, year

without necropsy. Complete gross necropsy was carried out on all other mice. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, and reproductive organs. Information on survival, body weight, or non-neoplastic lesions was not reported. The incidence of hepatocellular carcinoma in males was 3/18 (controls), 7/17 (lower dose), and 3/16 (higher dose); the incidence in the group exposed at the lower dose was significantly increased ($P < 0.025$) compared with the pooled control group (7/99), but was not significant when compared with the concurrent control group (3/18). The incidence of hepatocellular carcinoma in females was significantly increased at the lower and higher doses (control, 0/20; lower dose, 5/22; and higher dose, 5/19; $P < 0.025$) compared with the concurrent control and pooled control (incidence of 1/102) groups (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of mice at the start and the small number necropsied, the use of only two dose groups, and the limited histopathological examination and reporting.]

In a study of carcinogenicity with *ortho*-chloronitrobenzene [2-chloronitrobenzene] (Matsumoto et al., 2006b), groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were fed diets containing 2-chloronitrobenzene (purity, > 99%) at a concentration of 0 (control), 100, 500, or 2500 ppm for 2 years. Based on feed consumption, the estimated dose given to male mice was 0, 11, 54, or 329 mg/kg body weight (bw) per day, and to female mice 0, 14, 69, or 396 mg/kg bw per day, for the groups exposed at 0, 100, 500, or 2500 ppm, respectively. The survival of the male and female mice at 500 and 2500 ppm was reduced, attributed to the development of malignant tumours of the liver. The number surviving until being killed at the termination of the experiment was 35, 35, 17, and 8 (males), and 29, 34, 26, and 5 (females) at 0, 100, 500, and 2500 ppm, respectively. The body weights of male and female mice were significantly reduced for the groups at the two higher doses. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy. There was a dose-related increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in male and female mice. In males at 0, 100, 500, and 2500 ppm, the incidence of hepatocellular tumours was: hepatocellular adenoma, 19/50, 29/50 ($P < 0.05$), 30/50 ($P < 0.05$), and 34/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); hepatocellular carcinoma, 15/50, 14/50, 20/50, and 35/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); and hepatoblastoma, 1/50, 6/50, 35/50 ($P < 0.01$), and 44/50 ($P < 0.01$) ($P < 0.01$, Peto trend test). The combined incidence of hepatocellular tumours was not reported. In females at 0, 100, 500, and 2500 ppm, the incidence of hepatocellular tumours was: hepatocellular adenoma, 8/50, 22/50 ($P < 0.01$), 48/50 ($P < 0.01$), and 38/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); hepatocellular carcinoma, 0/50, 3/50, 14/50 ($P < 0.01$), and 48/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); and hepatoblastoma, 0/50, 0/50, 9/50 ($P < 0.01$), and 28/50 ($P < 0.01$) ($P < 0.01$, Peto trend test). In the original study report (Japan Bioassay Research Center, 2006a), the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was reported as 30/50, 36/50, 49/50 ($P < 0.01$), and 49/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) for males, and 8/50, 24/50 ($P < 0.01$), 50/50 ($P < 0.01$), and 50/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) for females. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice (GLP) and used males and females.]

3.2 Rat

Oral administration

In a study by Weisburger et al. (1978), groups of 25 male Charles River CD rats (derived from Sprague-Dawley rats) (age, 6–8 weeks) were fed diets containing 2-chloronitrobenzene (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0 (control), 1000, or 2000 ppm for 6 months. The dietary concentrations for the groups at 1000 and 2000 ppm were then reduced to 500 and 1000 ppm, respectively, for another 12 months. All rats were then held for a further 6 months on the control diet, until being killed at the termination of the experiment at 24 months. There was a pooled control group of 111 male rats [no additional details provided]. Rats that died within the first 6 months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other rats. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries. Information on survival, body weight, or non-neoplastic lesions was not reported. The number of rats with multiple tumours was increased in male rats at the lowest dose, with an incidence of 1/22 (control), 7/22 (lower dose), and 1/19 (higher dose), respectively. The tumour incidence in the group exposed at the lower dose was significantly increased ($P < 0.025$) compared with that in the concurrent control group (1/22) and the pooled control group (14/111) (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of rats at the start of the experiment, the small number necropsied, the use of only two dose groups and one sex, and the limited histopathological examination and reporting.]

In a study of carcinogenicity with 2-chloronitrobenzene (Matsumoto et al., 2006b), groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diets containing 2-chloronitrobenzene (purity, > 99%) at a concentration of 0 (control), 80, 400, or 2000 ppm for 2 years. Based on feed consumption, the estimated dose given was 0, 4, 19, or 99 mg/kg bw per day (males) and 0, 4, 22, or 117 mg/kg bw per day (females) at 0, 80, 400, or 2000 ppm, respectively. The survival of the male rats at 2000 ppm was significantly lower after 76 weeks and all died before the end of the 2-year dosing period, this being attributed to chronic progressive nephropathy. The exposure of male rats at 2000 ppm exceeded the maximum tolerated dose. [The data for the males at 2000 ppm were not included for statistical analysis.] No significant difference in survival was found in male rats at 80 or 400 ppm compared with controls. The final number of rats surviving until being killed at the termination of the experiment was 40, 40, 39, and 0 for males and 41, 42, 45, and 39 for females for the groups at 0, 80, 400, and 2000 ppm, respectively. The body weight of males at 2000 ppm was decreased after 20 weeks. The body weight of females at 2000 ppm was decreased by 10% at 78 weeks, and by 18% at 2 years. The males at 400 ppm showed significantly decreased terminal body weight (10% decrease), although the females at 400 ppm did not. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

There was a dose-related increase in the incidence of hepatocellular adenoma and hepatocellular carcinoma in male and female rats. In males at 0, 80, and 400 ppm, the incidence of hepatocellular adenoma was 2/50, 3/50, and 7/50 ($P < 0.05$, Peto trend test), and the incidence of hepatocellular carcinoma was 0/50, 0/50, and 3/50 ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma and hepatocellular carcinoma in males at 400 ppm exceeded the maximum tumour incidence (0/400) in the Japan Bioassay Research Center

(JBRC) historical control database. In females at 0, 80, 400, and 2000 ppm, the incidence of hepatocellular adenoma was 0/50, 0/50, 2/50, and 20/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) and the incidence of hepatocellular carcinoma was 0/50, 0/50, 0/50, and 4/50 ($P < 0.01$, Peto trend test). The incidence of hepatocellular carcinoma in females at 2000 ppm exceeded the maximum tumour incidence (0/400) in the JBRC historical control database. An increase in the incidence of non-neoplastic liver lesions was seen in exposed rats, including liver cell foci in male and female rats, and spongiosis hepatis in male rats. In the original study report (Japan Bioassay Research Center, 2006b), the incidence of hepatocellular adenoma or carcinoma (combined) for male rats was 2/50, 3/50, 10/50 ($P < 0.05$), and 2/50 (exposure at 2000 ppm, which exceeded the maximum tolerated dose) ($P < 0.01$, Peto trend test) and for female rats was 0/50, 0/50, 2/50, and 23/50 ($P < 0.01$) ($P < 0.01$, Peto trend test).

Spontaneous, age-related chronic progressive nephropathy was exacerbated in a dose-related manner in male and female rats. Nephropathy was more severe in males than in females, and caused the early death of males in the group at 2000 ppm. The incidence of renal cell adenoma in males was 0/50 (control), 1/50 (80 ppm), 0/50 (400 ppm), and 1/50 (2000 ppm). There was a significant positive trend [$P < 0.015$, Cochran–Armitage trend test] in the incidence of renal cell carcinoma in males: 0/50 (control), 0/50, 0/50, and 4/50. The incidence of renal cell carcinoma in males in historical controls was 0/400. In females, the corresponding incidence of renal cell adenoma was 0/50, 0/50, 0/50, and 2/50. The incidence of renal cell adenoma in females at 2000 ppm (4%) exceeded the maximum incidence in the JBRC historical control database (1/400; maximum incidence, 2%), although it was not statistically significant. There were no renal cell carcinomas in any of the groups of female rats. The renal cell tumours were larger than tumours associated with chronic progressive nephropathy, and the renal cell carcinomas had a histopathologically pleomorphic appearance. In addition, urothelial hyperplasia of the kidney pelvis and the deposit of brown pigment of the proximal tubule were seen in male rats at 400 ppm. Urothelial hyperplasia of the pelvis was seen in females at 2000 ppm, and the deposit of brown pigment of the proximal tubule was observed in females at 400 and 2000 ppm (Matsumoto et al., 2006b). [The Working Group noted that this was a well-conducted study that complied with GLP and used males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Other than the data on haemoglobin adducts discussed in the following paragraph, no data on metabolism in humans were available to the Working Group. [The metabolic profile of 2-chloronitrobenzene resembles that of 4-chloronitrobenzene in rats (Bucher, 1993), and the major metabolites of 4-chloronitrobenzene in rats have been found to occur in humans exposed to 4-chloronitrobenzene (Yoshida, 1994). The Working Group therefore expected the metabolic pathways of 2-chloronitrobenzene in humans to be similar to those of 4-chloronitrobenzene (see Fig. 4.1 in the monograph on 4-chloronitrobenzene in the present volume).]

Haemoglobin adducts were studied in workers exposed to 2-chloronitrobenzene in a chemical factory in China (Jones et al., 2006). Blood samples were obtained from 39 exposed workers, 15 factory controls, and 6 non-factory controls. The mean duration of work-years was about 6 years in the exposed workers. Air exposure concentrations of 2-chloronitrobenzene were

measured for a subset of 19 exposed workers with personal samplers, yielding a mean 8-hour time-weighted average of 0.49 mg/m^3. Hydrolysable haemoglobin adducts of 2-chloroaniline were detected in 38 of the 39 blood samples from workers exposed to 2-chloronitrobenzene (mean, 92.4 pg/mg; median, 82.9 pg/mg), indicating the availability of the 2-chloronitrobenzene metabolite, *N*-hydroxy-2-chloroaniline, a highly reactive intermediate metabolite. The haemoglobin adducts of 2-chloroaniline were also detected (at lower concentrations) in the blood samples from 13 of the 15 factory controls, but not in any of the non-factory control samples. Haemoglobin adduct concentrations did not correlate with 2-chloronitrobenzene concentrations in air, possibly indicating the importance of other routes of exposure (Jones et al., 2006).

4.1.2 Experimental systems

Dermal absorption studies were conducted in three groups of three male Fischer 344 rats exposed to 2-chloronitro[^{14}C]benzene as a single dermal application at a dose of 0.65, 6.5, or 65 mg/kg bw [0.0325, 0.325, or 3.25 mg/cm^2] (Bucher, 1993). Urine and faeces were collected for up to 72 hours. Based upon measurements of eliminated radiolabel, 33–40% of the administered dose of 2-chloronitrobenzene was absorbed from the skin within 72 hours, with absorption increasing nonlinearly with increasing dose. Urinary excretion of radiolabel over 72 hours accounted for 21–28% of the administered dose; faecal excretion accounted for 11–15%.

Bucher (1993) exposed groups of eight male Fischer 344 rats to 2-chloronitro[^{14}C]benzene at a single dose of 2, 20, or 200 mg/kg bw by gavage, and excretions were collected for up to 72 hours. Minimum absorption (determined by the percentage of the administered dose recovered in the urine or tissues) was 61–77%. A comparison of this finding with the Bucher (1993) dermal results discussed above shows greater absorption by oral exposure than by dermal exposure. The 2-chloronitrobenzene was then rapidly metabolized and excreted primarily in the urine. At the lower doses, about 6% and 3% of the total administered radiolabel was found in tissues after 24 and 72 hours, respectively. The highest dose had the greatest absorption (at least 77%) and was eliminated more slowly, with about 20% and 4% retention after 24 and 72 hours, respectively. At 24 hours, the greatest percentage of radiolabel at the lower doses was 4% in the liver, and the highest concentrations were in the liver and kidney. At 24 hours after administration of the highest dose, the greatest percentage of radiolabel was 13% in fat; the highest concentrations occurred in fat, followed by kidney and liver. At 72 hours after administration of the lower doses, the greatest percentage of radiolabel (2%) and the highest concentrations occurred in the liver. At 72 hours after administration of the highest dose, the greatest percentage of radiolabel was also in the liver (1.6%), although the highest concentrations occurred in the kidney. High-performance liquid chromatography analysis of urine revealed the presence of up to 23 metabolites [metabolites unspecified] (Bucher, 1993).

Groups of four young adult (age, 10–12 weeks) or geriatric (age, 19–20 months) male Fischer 344 rats were given repeated gavage doses of 2-chloronitro[^{14}C]benzene at 65 mg/kg bw on days 1, 5, and 9 and unlabelled parent compound on days 2, 3, 4, 6, 7, 8, 10, and 11 (Bucher, 1993). In young adult rats, urinary excretion accounted for 71–74% of the administered dose and faecal excretion 20–27%. Approximately 5% of the administered radiolabel was found in the tissues 72 hours after the day 9 dose, primarily in the liver (3.4%); the highest concentrations of radiolabel occurred in the liver and kidney. In geriatric rats, faecal excretion was similar to that in young adults; however, urinary excretion was greater than in the young adults (85% versus 71%). At later time points in geriatric rats, there

was no change in faecal excretion but urinary excretion decreased to the levels observed in the young adults. Further, the percentage of radiolabel retained in the geriatric rats 72 hours after the day 9 dose (8.2%) was greater than that in the young adults (4.7%). Most of the retention was in the liver (6%). The highest concentrations of radiolabel were found in the liver and kidney (Bucher, 1993).

Rickert & Held (1990) studied the metabolism of radiolabelled 2-chloronitrobenzene by isolated male Fischer 344 rat hepatocytes and hepatic microsomes. Incubation of 2-chloronitro[^{14}C]benzene with rat hepatocytes yielded 2-chloroaniline (19.2% of total radiolabel), 2-chloroaniline-*N*-glucuronide (14.2% of total radioactivity), and *S*-(2-nitrophenyl)glutathione (13.3% of total radiolabel). Incubation of the radiolabelled parent with microsomes demonstrated that the reduction to 2-chloroaniline was mediated by metabolism dependent upon cytochrome P450; formation was inhibited by SKF 525-A, metyrapone, and carbon monoxide.

Bray et al. (1956) examined the metabolism of 2-chloronitrobenzene in female rabbits given 2-chloronitrobenzene [route unspecified] at 0.1 g/kg [whether diet or body weight was not specified]. Urine was collected over 24-hour periods until metabolites were no longer excreted (usually after 48 hours). The main metabolic processes were reduction and hydroxylation. Nearly the entire administered dose was excreted in the urine as either 2-chloroaniline (9% of the administered dose) or derivatives of phenolic metabolites. The phenols formed were excreted mainly as conjugates with sulfuric and glucuronic acids (66% of the administered dose). The formation of mercapturic acid from 2-chloronitrobenzene metabolism appears to be a minor metabolic pathway in rabbits.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether 2-chloronitrobenzene: is genotoxic; induces oxidative stress; induces chronic inflammation; and alters cell proliferation, cell death, or nutrient supply.

4.2.1 Genetic and related effects

See Table 4.1, Table 4.2, and Table 4.3

(a) Humans

Sabbioni et al. (2016) analysed lymphocytes from a subset of the same workers studied by Jones et al. (2006), who were exposed to 2-chloronitrobenzene, to assess the formation of chromosomal aberrations. Samples from 24 exposed workers and 13 factory controls were analysed. These workers were also exposed to other chloronitrobenzenes, and the major isomer was 4-chloronitrobenzene (70%). There was a non-statistically significant increase in the frequency of chromosomal aberrations in the half of the exposed subset with the highest level of 2-chloroaniline–haemoglobin adducts compared with the half with the lowest adduct levels, but not in the group exposed to chloronitrobenzene compared with the unexposed group. [The Working Group noted that the latter comparison could be limited by the small number of workers evaluated, as well as by the observation in the larger group of workers studied by Jones et al. (2006) that the unexposed were of a notably higher average age. Further, the higher exposures to 4-chloronitrobenzene (and other chloronitrobenzenes) in the workers studied meant that effects could not be specifically attributed to 2-chloronitrobenzene.]

Table 4.1 Genetic and related effects of 2-chloronitrobenzene in exposed humans and in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments on study quality	Reference
Chromosomal aberrations	Exposed humans	Lymphocytes	(+)	Mean (± standard deviation) 0.49 ± 0.32 mg/m^3 (8-h TWA)	Mean number of years working in the factory, 5.9 ± 3.5	Effects could not be attributed to 2-chloronitrobenzene specifically, because of concurrent exposures to other chloronitrobenzenes	Sabbioni et al. (2016)
DNA adducts	Rat, Wistar (F)	Liver	–	NR	Gavage, 1×, 0.5 M solutions (0.1 mL per 100 g bw)		Jones & Sabbioni (2003)
DNA strand breaks	Mouse, Swiss (M)	Brain	+	60 mg/kg bw	Intraperitoneal injection, 1×, 60 or 180 mg/kg bw		Cesarone et al. (1980)
DNA strand breaks	Mouse, Swiss (M)	Liver and Kidney	+	60 mg/kg bw	Intraperitoneal injection, NR		Cesarone et al. (1982)

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

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

Table 4.2 Genetic and related effects of 2-chloronitrobenzene in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Chinese hamster ovary	+	–	50 µg/mL	Positive in two trials	Bucher (1993)
Sister-chromatid exchange	Chinese hamster ovary	–	+	500 µg/mL	This is a test in a separate laboratory reported by Bucher (1993); positive results in two trials	Bucher (1993)
Chromosomal aberrations	Chinese hamster ovary	–	+	500 µg/mL	Positive results in two trials	Bucher (1993)
Chromosomal aberrations	Chinese hamster ovary	(+)	–	160 µg/mL	This is a test in a separate laboratory reported by Bucher (1993); only one trial was conducted	Bucher (1993)
Chromosomal aberrations	Chinese hamster lung, CHL/IU	+	NT	NR	Positive for numerical, but not structural, chromosomal aberrations with 24 or 48 h treatments, but not shorter times	JETOC (1996)

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

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

Table 4.3 Genetic and related effects of 2-chloronitrobenzene in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster (adult)	Sex-linked recessive lethal mutations	–	NA	125 mg/kg (feed); 10 000 mg/kg (injection)		Zimmering et al. (1985)
Drosophila melanogaster (larvae)	Sex-linked recessive lethal mutations	–	NA	60 mg/kg (feed)		Zimmering et al. (1989)
Escherichia coli PQ37	DNA strand breaks	–	–	NR	Prophage induction, SOS repair test, cross-links, or related damage were tested with the same result	von der Hude et al. (1988)
Salmonella typhimurium TA100	Reverse mutation	–	+	38 µg/mL		Bucher (1993)
Salmonella typhimurium TA100	Reverse mutation	–	+	38 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	50 µg/mL		Suzuki et al. (1983)
Salmonella typhimurium TA100, TA1535, TA1537	Reverse mutation	–	NT	630 µg/mL		Shimizu et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	–	(+)	50 µg/mL		Bucher (1993)
Salmonella typhimurium TA98	Reverse mutation	–	(+)	77 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98, TA1538	Reverse mutation	+	NT	80 µg/mL		Shimizu et al. (1983)
Salmonella typhimurium TA1535, TA1537	Reverse mutation	–	–	128 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98NR	Reverse mutation	NT	–	5 µg/mL		Suzuki et al. (1987)
Salmonella typhimurium TA98, TA98NR/1,8-DNP6	Reverse mutation	NT	–	2.5 µg/mL		Suzuki et al. (1987)
Salmonella typhimurium TA98, TA100, TA1530, TA1532, TA1535, TA1537, TA1538, TA1950, TA1975, TA1978, or G46	Reverse mutation	–	–	NR		Gilbert et al. (1980)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	NR	TA98 gave positive results with hamster but not rat S9; TA98 and TA100 gave positive results in both trials	JETOC (1996)
Salmonella typhimurium TA1535, TA1537	Reverse mutation	–	–	NR	Negative results in both trials	JETOC (1996)
Escherichia coli WP2 *uvrA*	Reverse mutation	–	+	NR	WP2 *uvrA* gave positive results with hamster but not rat S9; and positive results in one of two trials	JETOC (1996)
Calf thymus DNA	DNA adducts	+	NA	250 µg		Jones & Sabbioni (2003)

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

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

(b) Experimental systems

Jones & Sabbioni (2003) did not observe DNA adducts in hepatic DNA from female Wistar rats exposed to 2-chloronitrobenzene or 2-chloroaniline by gavage, despite the formation of haemoglobin adducts by both compounds. Adducts in calf thymus DNA were seen after exposure to the 2-chloronitrobenzene metabolite, 2-chloroaniline.

In Swiss CD-1 mice exposed to 2-chloronitrobenzene by intraperitoneal injection, DNA single-strand breaks were observed in the liver, kidney, and brain (Cesarone et al., 1980, 1982).

2-Chloronitrobenzene induced sister-chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells (Bucher, 1993). In Chinese hamster lung cells, 2-chloronitrobenzene gave positive results for numerical but not structural chromosomal aberrations after 24 or 48 hours (but not less) of treatment (JETOC, 1996).

2-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in the germ cells of male *Drosophila melanogaster* when given to adults either by feeding or by injection, or to larvae by feeding (Zimmering et al., 1985, 1989).

2-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS chromotest (von der Hude et al., 1988). Results of mutagenic testing in multiple strains of *Salmonella typhimurium* were largely, but not entirely, negative with or without metabolic activation across several studies (e.g. Gilbert et al., 1980; Haworth et al., 1983).

The 2-chloroaniline metabolite of 2-chloronitrobenzene has also been tested for genotoxicity in a variety of assays, and the results are inconsistent. In vivo, 2-chloroaniline induced micronucleus formation in rat bone marrow, but not in mouse bone marrow or peripheral blood cells; in vitro, the metabolite gave positive results for mutagenicity in a mouse lymphoma cell assay, but not in *S. typhimurium* (NTP, 1998).

4.2.2 Oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rats and mice, methaemoglobin formation is a well-established effect of exposure to 2-chloronitrobenzene from multiple routes (Bucher, 1993; Matsumoto et al., 2006a). Methaemoglobin formation is attributed to the *N*-hydroxy-2-chloroaniline metabolite. In erythrocytes, such *N*-hydroxyarylamines can engage in Kiese redox cycling, yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017). The oxidative damage to the erythrocytes is thought to be the underlying cause of many of the toxic effects observed in response to 2-chloronitrobenzene exposure (Bucher, 1993).

Paranich et al. (1993) studied the effects of 2-chloronitrobenzene in the spleen and liver of rats and reported that short-term (5 days), but not longer-term (30 days), exposure caused some changes in lipid peroxidation, antioxidative activity, and vitamin E concentration that were variable across markers and tissues.

4.2.3 Chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 2-week study with 2-chloronitrobenzene administered by inhalation (Bucher, 1993), granulomatous inflammation of the liver was observed in mice. In a 13-week study, inflammation characterized by fibrosis and accumulations of mononuclear inflammatory cells was observed in the livers of mice (Bucher, 1993; Travlos et al., 1996).

4.2.4 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Haematopoietic proliferation arising from the need to replace damaged erythrocytes was observed in several subchronic and chronic studies. In a 4-week inhalation study in rats, extramedullary haematopoiesis in the spleen was reported (Nair et al., 1986). Extramedullary haematopoiesis, primarily erythropoiesis, was observed in the spleens of mice but not rats exposed for 13 weeks by inhalation (Bucher, 1993; Travlos et al., 1996). In a 13-week dietary study (Matsumoto et al., 2006a), extramedullary haematopoiesis in the spleen was found in mice and rats; erythropoiesis in the bone marrow was observed for rats only. In a 2-year dietary study (Matsumoto et al., 2006b), extramedullary haematopoiesis in the spleen was observed in mice and rats.

Other proliferative effects observed in the subchronic and chronic studies with 2-chloronitrobenzene include: hyperplasia of the nasal cavity in rats, considered to be due to irritation, and regeneration of the proximal convoluted tubules in rat kidneys in a 13-week study of exposure by inhalation (Bucher, 1993); capsule hyperplasia in the spleens of rats in a 13-week dietary study (Matsumoto et al., 2006a); and kidney tubule hyperplasia in male rats and capsule hyperplasia in the spleens of rats in a 2-year dietary study (Matsumoto et al., 2006b).

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In rodents fed diets containing 2-chloronitrobenzene at a concentration of 0, 80, 400, or 2000 ppm w/w for Fischer 344 rats and at 0, 100, 500, or 2500 ppm for BDF_1 mice for 2 years, the target tissue for carcinogenicity was the liver (Matsumoto et al., 2006b). The non-neoplastic toxic effects observed in the target tissue in the 2-year study included: hepatotoxicity in mice, as shown by increases in blood markers of liver damage (starting in the intermediate dose group); hepatotoxicity in rats, as indicated by increases in blood markers in females (males were not analysed); and histopathological changes, such as necrosis and hydropic degeneration, in male and female rats at the highest doses (Matsumoto et al., 2006b). In addition, exacerbated chronic progressive nephropathy occurred in male and female rats and caused significant early mortality in the males at the highest dose from week 73. The other main non-neoplastic toxic effects observed in the 2-year study included: haematotoxicity, as indicated by increased reticulocyte counts in mice (starting in the intermediate dose group); a variety of blood parameter perturbations in rats, including decreased erythrocyte counts and haemoglobin levels, and increased reticulocyte counts and methaemoglobin for the intermediate and highest dose; and haemosiderin deposits and extramedullary haematopoiesis in the spleens of mice and rats starting from the lowest or the intermediate dose depending on species (Matsumoto et al., 2006b).

The non-neoplastic toxic effects of 2-chloronitrobenzene exposure were also observed in several subchronic studies. In a small 2-week inhalation study, Bucher (1993) observed early evidence of liver toxicity and haematotoxicity in male and female Fischer 344 rats and $B6C3F_1$ mice. In a 4-week study in Sprague-Dawley rats treated by inhalation, Nair et al. (1986) reported haematotoxicity in both males and females. In a 13-week inhalation study, Bucher (1993) observed

hepatotoxicity in male and female Fischer 344 rats and B6C3F$_1$ mice, with mice being the most sensitive. Rats, particularly males, exhibited some kidney lesions. In a 13-week dietary study, Matsumoto et al. (2006a) reported signs of hepatotoxicity in male and female Fischer 344 rats and BDF$_1$ mice, particularly male mice; however, no kidney effects were reported. Haematotoxicity occurred in male and female rats and mice in both 13-week studies, with rats being more sensitive than mice.

Several assessments of the reproductive effects of exposure to 2-chloronitrobenzene have also been conducted. In the 13-week inhalation study by Bucher (1993), decreased spermatogenesis was observed in male rats in the group at the highest dose. In a continuous breeding study in breeding pairs of CD-1 Swiss mice, Bucher (1993) observed no effects on fertility after exposure to 2-chloronitrobenzene by gavage at doses of up to 160 mg/kg bw.

In a 13-week study of exposure by gavage to the 2-chloronitrobenzene metabolite, 2-chloroaniline, increased levels of methaemoglobin and related haematotoxic effects were observed in rats and mice (NTP, 1998).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-throughput screening programmes

Data from standardized biochemical and cell-based assays can contribute to the understanding of carcinogenicity potential based on comparisons of activity in these assays across several end-points linked to characteristics common to carcinogens. Previous volumes of the *IARC Monographs* (IARC 2017a, b) and other publications (Chiu et al., 2018; Guyton et al., 2018) have described the key characteristics of carcinogens and their link to high-throughput screening (HTS) assays. Currently, 7 of the 10 key characteristics have some coverage in HTS assays used by the EPA and the United States National Institutes of Health (Chiu et al., 2018).

HTS assay results for the eight compounds and key metabolites assessed in the present volume of the *IARC Monographs* are evaluated here; all except *para*-nitroanisole (Chemical Abstracts Service registry number, CAS No. 100-17-4) have been evaluated in at least some of the HTS assays used by the EPA and the United States National Institutes of Health (EPA, 2018c). There are also HTS assay results available for several key metabolites of compounds considered in the present volume. The specific assays tested for each chemical and the mapping to the key characteristics can be found in Annex 1 (http://publications.iarc.fr/584). Findings for assays mapped to key characteristics for the eight compounds and the four metabolites evaluated are summarized in Table 4.4 and Table 4.5, respectively.

It is important to note two caveats that are relevant to the eight compounds evaluated in *IARC Monographs* Volume 123: (i) the assays used either fully lacked or had uncharacterized and generally low xenobiotic metabolism, limiting observations primarily to effects elicited by the parent compounds; and (ii) it may be difficult to reach the concentrations required for bioactivity for compounds of lower molecular mass and/or high vapour pressure in these assays. In particular, *N*,*N*-dimethylacetamide, *para*-nitroanisole, *ortho*-phenylenediamine, and 2-amino-4-chlorophenol have a relative molecular mass of less than 150, and others (2-chloronitrobenzene, 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene, and 2,4-dichloro-1-nitrobenzene) have a relative molecular mass of just over 150. The assay end-points mapped to key characteristics considered in the present volume are described briefly below. Instances in which a quality concern was raised concerning compound purity are noted in Section 4.4.2.

Table 4.4 Summary of activity of agents reviewed in *IARC Monographs* Volume 123 and tested in ToxCast and/or Tox21 high-throughput screening assays[a]

Key characteristic (total no. of assays mapped to characteristic)[b]	Number of positive results out of the number of assays tested							
	2-Chloronitro-benzene (CAS No. 88-73-3)	4-Chloronitro-benzene (CAS No. 100-00-5)	1,4-Dichloro-2-nitrobenzene (CAS No. 89-61-2)	2,4-Dichloro-1-nitrobenzene (CAS No. 611-06-3)	2-Amino-4-chlorophenol (CAS No. 95-85-2)	*ortho*-Phenylene-diamine dihydrochloride (CAS No. 615-28-1)	*ortho*-Phenylene-diamine (CAS No. 95-54-5)	*N*,*N*-Dimethyl-acetamide (CAS No. 127-19-5)
1. Is electrophilic or can be metabolically activated (2)	0 out of 1	0 out of 2	NT	0 out of 1	0 out of 1	0 out of 1	0 out of 2	0 out of 2
2. Is genotoxic (12)	0 out of 7	0 out of 7	0 out of 1	0 out of 6	5 out of 6	2 out of 6	4 out of 9	0 out of 7
4. Induces epigenetic alterations (5)	0 out of 4	0 out of 4	0 out of 4	NT	NT	NT	3 out of 4	0 out of 4
5. Induces oxidative stress (16)	0 out of 7	0 out of 12	0 out of 4	1 out of 3	1 out of 3	1 out of 3	5 out of 15	1 out of 7
6. Induces chronic inflammation (47)	0 out of 2	0 out of 47	0 out of 1	0 out of 1	0 out of 1	0 out of 1	1 out of 47	0 out of 2
8. Modulates receptor-mediated effects (98)	1 out of 58	0 out of 79	3 out of 36	0 out of 22	4 out of 22	4 out of 22	8 out of 79	1 out of 78
10. Alters cell proliferation, cell death, or nutrient supply (119)	0 out of 29	1 out of 52	0 out of 8	1 out of 21	1 out of 21	1 out of 21	9 out of 69	1 out of 29
Total hits out of total number of assays evaluated	1 out of 108	1 out of 203	3 out of 54	2 out of 54	11 out of 54	8 out of 54	31 out of 225	3 out of 152

CAS No., Chemical Abstracts Service number; NT, not tested; Tox21, Toxicology in the 21st Century programme; ToxCast, Toxicity Forecaster programme

[a] No data on high-throughput screening were available for *para*-nitroanisole

[b] Seven of the 10 key characteristics have mapped high-throughput screening assay end-points as described by Chiu et al. (2018). The mapping file with findings for agents considered in Volume 123 is available in the supplementary material (Annex 1). No assay end-points in ToxCast or Tox21 were determined to be applicable to the evaluation of three key characteristics, namely: alters DNA repair or causes genomic instability, is immunosuppressive, and causes immortalization

Table 4.5 Summary of activity of key metabolites of agents reviewed in *IARC Monographs* Volume 123 and tested in ToxCast and/or Tox21 high-throughput screening assays

Key characteristic (total no. of assays mapped to characteristic)[a]	Number of positive results out of the number of assays tested			
	2-Chloroaniline (CAS No. 95-51-2)	**4-Chloroaniline (CAS No. 106-47-8)**	***para*-Nitrophenol (CAS No. 100-02-7)**	**Acetamide (CAS No. 60-35-5)**
1. Is electrophilic or can be metabolically activated (2)	0 out of 1	0 out of 2	0 out of 2	0 out of 2
2. Is genotoxic (12)	0 out of 6	0 out of 9	1 out of 9	0 out of 9
4. Induces epigenetic alterations (5)	NT	0 out of 4	0 out of 4	0 out of 4
5. Induces oxidative stress (16)	0 out of 3	0 out of 15	0 out of 15	0 out of 15
6. Induces chronic inflammation (47)	0 out of 1	0 out of 47	0 out of 47	0 out of 47
8. Modulates receptor-mediated effects (98)	0 out of 22	2 out of 76	2 out of 79	1 out of 79
10. Alters cell proliferation, cell death, or nutrient supply (119)	0 out of 21	3 out of 69	1 out of 61	2 out of 68
Total hits out of total number of assays evaluated	0 out of 54	5 out of 222	4 out of 217	3 out of 224

CAS No., Chemical Abstracts Service number; NT, not tested; Tox21, Toxicology in the 21st Century programme; ToxCast, Toxicity Forecaster programme

[a] Seven of the 10 key characteristics have mapped high-throughput screening assay end-points as described by Chiu et al. (2018). The mapping file with findings for agents considered in Volume 123 is available in the supplementary material (Annex 1). No assay end-points in ToxCast or Tox21 were determined to be applicable to the evaluation of three key characteristics, namely: alters DNA repair or causes genomic instability, is immunosuppressive, and causes immortalization

1. *Is electrophilic or can be metabolically activated*: 2 assay end-points consisting of cytochrome P450 biochemical activity assays, including aromatase.
2. *Is genotoxic*: 12 assay end-points consisting of assays for induction of cellular TP53 protein and DNA repair-sensitive cellular assays. These assay end-points do not directly assess genotoxicity, but rather the downstream effects of DNA damage.
3. *Alters DNA repair or causes genomic instability*: 0 assay end-points.
4. *Induces epigenetic alterations*: 5 assay end-points including biochemical assays targeting histone deacetylases and other enzymes modifying chromatin, as well as cellular transcription factor assays involved in epigenetic regulation.
5. *Induces oxidative stress*: 16 assay end-points, all cellular assays targeting nuclear factor-like 2 and/or the antioxidant response element (ARE), other stress-related transcription factors, and protein upregulation in response to reactive oxygen species.
6. *Induces chronic inflammation*: 47 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*: 98 assay end-points targeting nuclear receptors (including aryl hydrocarbon receptor) 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*: 119 assay end-points measuring cytotoxicity by a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

4.4.2 Outcomes for chemicals tested

(a) 2-Chloronitrobenzene

For 2-chloronitrobenzene (CAS No. 88-73-3), results were available for 108 assay end-points of the 299 assay end-points mapped to key characteristics. 2-Chloronitrobenzene was considered active in only 1 of the 108 assays, an assay that assessed binding to the human retinoid X receptor β transcription factor in the HepG2 human liver cell line. In addition, a key metabolite of 2-chloronitrobenzene, 2-chloroaniline (CAS No. 95-51-2), was not positive in any of the 54 assay end-points evaluated.

(b) 4-Chloronitrobenzene

4-Chloronitrobenzene (CAS No. 100-00-5) was evaluated in 203 of the assay end-points mapped to key characteristics, and was only considered active in 1 assay end-point mapped to the “Alters cell proliferation, cell death, or nutrient supply” key characteristic. This assay is cell based and measures viability based on adenosine triphosphate (ATP) content in a human intestinal cell line (HCT116). 4-Chloroaniline (CAS No. 106-47-8), a key metabolite of 4-chloronitrobenzene, was active in 5 of the 222 assay end-points evaluated. Three assays of proliferative effects mapped to “Alters cell proliferation, cell death, or nutrient supply” and 2 evaluating aryl hydrocarbon receptor (AhR) agonist activity and estrogen receptor α antagonism activity mapped to “Modulates receptor-mediated effects”.

(c) 1,4-Dichloro-2-nitrobenzene

1,4-Dichloro-2-nitrobenzene (CAS No. 89-61-2) was considered active in 3 of the 54 assay end-points evaluated. Concerning “Modulates receptor-mediated effects”, the assay end-points assessed induction of reporter transcripts for the human aryl hydrocarbon receptor response element (AhRE, responsive to AhR), human nuclear receptor subfamily 1, group I, member 2 response element (PXRE, responsive to NR1I2);

and human peroxisome proliferator-activated receptor gamma (PPARγ) transcription factors in the HepG2 human liver cell line. Induction of PPARγ activity was the most sensitive target for 1,4-dichloro-2-nitrobenzene.

(d) 2,4-Dichloro-1-nitrobenzene

2,4-Dichloro-1-nitrobenzene (CAS No. 611-06-3) has been assessed in 54 of the assay end-points mapped to the key characteristics of carcinogens, and was considered active in 2 of those assay end-points: Tox21_ARE_BLA agonist assay end-point mapped to the "Induces oxidative stress" key characteristic; and TP53 viability assay end-point mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. The former assay is a cell-based transcription response element reporter assay regulated by the human nuclear factor erythroid 2-like factor 2 (NFE2L2) changes in the human liver HepG2 cell line, and the latter assay is cell-based and measures viability based on ATP content in a human intestinal cell line (HCT116).

(e) 2-Amino-4-chlorophenol

2-Amino-4-chlorophenol (CAS No. 95-85-2) was considered active in 11 of the 54 assay end-points assessed and mapped to the key characteristics of carcinogens. It was considered active in 5 of the 6 assay end-points evaluated, and mapped to the "Is genotoxic" key characteristic. These 5 assay end-points are cell based using the human intestinal cell line HCT116, and are designed to measure changes in transcriptional gene expression as a result of modulation of TP53. Activity was seen at non-cytotoxic concentrations. 2-Amino-4-chlorophenol was considered active in the Tox21_ARE_BLA agonist assay end-point mapped to the "Induces oxidative stress" key characteristic. 2-Amino-4-chlorophenol was also considered active in 4 assay end-points mapped to the "Modulates receptor-mediated effects" key characteristic and 1 assay end-point mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. The 4 receptor-mediated assay end-points include: (i) transcription as a result of antagonist activity regulated by the human androgen receptor (AR) in the human kidney cell line HEK293T; (ii) transcription as a result of antagonist activity regulated by the human AR in the human breast cell line MDAKB2; (iii) transcription as a result of antagonist activity regulated by the human thyroid hormone receptor α and β (THRA and THRB) in the rat pituitary gland cell line GH3; and (iv) transcription as a result of agonist activity regulated by the human AhR in the human liver cell line HepG2. The Toxicity Testing in the 21st Century (Tox21) matrix metalloproteinases (MMP) ratio assay end-point is mapped to the "Alters cell proliferation, cell death, and nutrient supply", and assesses mitochondrial toxicity by measuring mitochondrial membrane potential in the human liver cell line HepG2.

(f) ortho*-Phenylenediamine dihydrochloride and* ortho*-phenylenediamine*

ortho-Phenylenediamine dihydrochloride (CAS No. 615-28-1) was considered active in 8 of the 54 assay end-points evaluated, and *ortho*-phenylenediamine (CAS No. 95-54-5) was active in 31 of the 225 assay end-points evaluated and mapped to key characteristics. Activity was seen in the absence of cytotoxicity for both *ortho*-phenylenediamine dihydrochloride and *ortho*-phenylenediamine in all mapped assays. For both compounds, there was a quality control concern identified by the testing laboratory.

ortho-Phenylenediamine was considered active in 4 out of the 9 assay end-points under "Is genotoxic", and *ortho*-phenylenediamine dihydrochloride was considered active in 2 assay end-points mapped to the "Is genotoxic" key characteristic. End-points that were active measure transcription regulated by the human ATPase family AAA domain containing 5

(ATAD5) agonist activity in human kidney cell line (HEK293T), and activation and transcription regulated by TP53. *ortho*-Phenylenediamine was considered active in 3 of the 4 assay end-points mapped to "Induces epigenetic alterations". These assay end-points evaluate transcription regulated by Pax6, Sp1, and Sox transcription factors. Consistent with three other nitrobenzenes evaluated, *ortho*-phenylenediamine and *ortho*-phenylenediamine hydrochloride were considered active in the ARE agonist assay end-point in the "Induces oxidative stress" key characteristic, measuring transcription regulated by the human nuclear factor erythroid 2-like factor 2 (NFE2L2) in the human liver cell line (HepG2). Further, *ortho*-phenylenediamine was active in 5 additional assay end-points mapped to "Induces oxidative stress", including assays evaluating oxidative stress and kinase activation in HepG2 cells, and transcription regulated via HIF1α, MRE, and NRF2. *ortho*-Phenylenediamine was considered active in 1 out of 47 assay end-points mapped to the "Induces chronic inflammation" key characteristic. This assay quantifies the level of chemokine (C-C motif) ligand 2 protein (CCL2) as indicative of proinflammation.

ortho-Phenylenediamine dihydrochloride and *ortho*-phenylenediamine were also considered active in 4 and 8 assay end-points, respectively, under the "Modulates receptor-mediated effects" key characteristic. These assays considered transcription regulated by agonist activity of the human AR in the human breast cell line MDAKB2, transcription regulated by human estrogen receptor 1 antagonist activity (ESR1) in the human kidney cell line HEK293T, transcription regulated by human AHR agonist activity in human liver cell line HepG2, and transcription regulated by antagonist activity of the human nuclear receptor subfamily 1, group H, member 4 (NR1H4) in a human kidney cell line (HEK293T). Transcription regulated via additional nuclear receptors, including retinoic acid receptor, vitamin D receptor, and PPAR, were also modulated by *ortho*-phenylenediamine. *ortho*-Phenylenediamine was also active in 9 additional assay end-points, including those that measure mitochondrial membrane potential, cell loss, and mitotic arrest in HepG2 and fibroblast cells, and transcriptional regulation via transcription factors related to cell death and proliferation.

(g) para-*Nitroanisole*

para-Nitroanisole (CAS No. 100-17-4) has not been evaluated in HTS assays; however, there are HTS data available for the key metabolite *para*-nitrophenol (CAS No. 100-02-07). *para*-Nitrophenol was active in 4 out of 217 assay end-points mapped to key characteristics: 1 in "Is genotoxic" assessing TP53-mediated transcription; 2 in "Modulates receptor-mediated effects" assessing androgen-receptor agonist activity; and 1 in "Alters cell proliferation, cell death, or nutrient supply" (MMP ratio assay).

(h) N,N-*Dimethylacetamide*

N,*N*-Dimethylacetamide (CAS No. 127-19-5) was considered active in 3 of the 152 assay end-points evaluated and mapped to key characteristics. Similar to three of the other nitrobenzenes evaluated in this report, it was considered active in the ARE agonist assay end-point under "Induces oxidative stress". *N*,*N*-Dimethylacetamide was active in the human estrogen receptor 1 responsive cell growth assay end-point in the human breast cell line T47DF. This assay end-point is mapped to the "Modulates receptor-mediated effects" key characteristic. Finally, *N*,*N*-dimethylacetamide was active in a viability assay in the human HepG2 liver cell line mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. A key metabolite, acetamide (CAS No. 60-35-5), was considered active in 3 out of the 224 assay end-points evaluated: 1 assessing modulation of PPARγ (mapped to "Modulates receptor-mediated effects"); and 2 assay end-points assessing

cell loss in HepG2 cells and modulation of transforming growth factor-β family (TGFβ), which are mapped to "Alters cell proliferation, cell death, or nutrient supply".

4.4.3 Overall considerations

In summary, there are gaps in coverage in the HTS data with respect to the key characteristics of carcinogens. In addition, with the exception of chemicals relevant to the monographs on 2-amino-4-chlorophenol and on *ortho*-phenylenediamine and *ortho*-phenylenediamine dihydrochloride, all other compounds in the present volume and their key metabolites were active for few (< 10%) HTS assay end-points in the Toxicity Forecaster (ToxCast) and/or Toxicology in the 21st Century (Tox21) programmes mapped to key characteristics in which they had been tested. The HTS data are therefore generally consistent with the conclusions of *weak* or *moderate* evidence for these compounds. 2-Amino-4-chlorophenol was active in 11 of 54 assay end-points. *ortho*-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride were active in 31 of 225 and 8 of 54 assay end-points, respectively; however, confidence in the HTS data available for *ortho*-phenylenediamine was decreased as a result of the quality control concern identified by the testing laboratory.

For the present volume of the *IARC Monographs*, the key characteristics mapping file is provided as supplementary material (see Annex 1, available from http://publications.iarc.fr/584). When comparing with the previous volumes of the *IARC Monograph*s that include HTS data, it is important to note that the assays mapped to each key characteristic have been refined as additional assays have been added and as the relevance of individual assays to key characteristics has been evaluated. All ToxCast/Tox21 data were downloaded from the EPA Dashboard V2 (https://comptox.epa.gov/dashboard) during 4–16 June 2018 for primary compounds and 9–11 October 2018 for metabolites and *ortho*-phenylenediamine.

5. Summary of Data Reported

5.1 Exposure data

2-Chloronitrobenzene is a chemical with a high production volume that is manufactured worldwide. United States annual production has decreased significantly over time (from 23–45 thousand tonnes during 1986–2002 to < 11 tonnes during 2012–2015); similarly, recent European production volumes are low. Data on current production volumes for regions outside of Europe and the USA were not available.

2-Chloronitrobenzene is used as an intermediate in the synthesis of colorants and chemicals; downstream uses include lumber preservatives, corrosion inhibitors, pigments, and agricultural chemicals.

Although not known to occur naturally, 2-chloronitrobenzene can be released to the environment as a by-product of production or manufacturing; release may also occur during transport, storage, or disposal, or accidentally. It has been detected in water and is moderately persistent in the environment; 2-chloronitrobenzene has also been detected at low levels in edible fish.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 2-chloronitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Detectable levels of 2-chloronitrobenzene have been measured in workplace air in chemical factories in China and Japan.

Quantitative information on exposure in the general population was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

2-Chloronitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet in the same laboratory, one in male and female mice and one in male and female rats. In limited studies of oral exposure by diet in another laboratory, 2-chloronitrobenzene was tested in one study in male and female mice and one study in male rats.

In the GLP study of oral exposure by diet in male mice, 2-chloronitrobenzene induced a significant positive trend and a significant increase in the incidence of: hepatocellular adenoma; hepatocellular carcinoma; hepatoblastoma; and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In the limited study of oral exposure by diet in male mice, 2-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma.

In the GLP study of oral exposure by diet in female mice, 2-chloronitrobenzene induced a significant positive trend and a significant increase in the incidence of: hepatocellular adenoma; hepatocellular carcinoma; hepatoblastoma; and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In the limited study of oral exposure by diet in female mice, 2-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma.

In the GLP study of oral exposure by diet in male rats, 2-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or hepatocellular carcinoma (combined); there was also a significant increase in the incidence of hepatocellular adenoma or hepatocellular carcinoma (combined). In the same study, there was a significant positive trend in the incidence of renal cell carcinoma in male rats. In the limited study in male rats, 2-chloronitrobenzene induced a significant increase in the number of rats bearing multiple tumours (of unspecified type).

In the GLP study of oral exposure by diet in female rats, 2-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or hepatocellular carcinoma (combined). 2-Chloronitrobenene also induced a significant increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined); the incidence of hepatocellular carcinoma in the group exposed at the highest dose exceeded the historical control range.

5.4 Mechanistic and other relevant data

In one occupational study, adducts of haemoglobin and the reactive metabolite *N*-hydroxy-2-chloroaniline were detected, but no other human data on absorption, distribution, metabolism or excretion were available. In rats, dermally and orally administered 2-chloronitrobenzene is absorbed, widely distributed to tissues, and excreted as metabolites in urine and faeces. In one study in female rabbits, the primary metabolites excreted were 2-chloroaniline and phenolic derivatives.

Concerning the key characteristics of carcinogens, there is *moderate* evidence that 2-chloronitrobenzene induces oxidative stress. No data in humans were available. In mice and rats, 2-chloronitrobenzene increases methaemoglobin and causes oxidative damage to erythrocytes in subchronic and chronic exposure studies.

There is *moderate* evidence that 2-chloronitrobenzene alters cell proliferation, cell death, or nutrient supply in rodents. No data in humans were available. In multiple studies in mice and rats of different duration up to 2 years, regenerative haematopoietic proliferation, primarily erythropoiesis, occurs in multiple tissues, primarily the spleen. Rat renal and splenic hyperplasia was seen in subchronic and chronic studies.

There is *weak* evidence (scarcity of data) that 2-chloronitrobenzene induces chronic inflammation in the liver in mice.

There is *weak* evidence that 2-chloronitrobenzene is genotoxic. The only study in humans showed a non-statistically significant increase in chromosomal aberrations in workers exposed to various chloronitrobenzenes including 2-chloronitrobenzene. In Swiss mice, DNA single-strand breaks were seen in the liver, kidney, and brain. In Chinese hamster cells, 2-chloronitrobenzene increased sister-chromatid exchanges and chromosomal aberrations in some tests but not others. Several mutagenicity tests in multiple strains of *Salmonella typhimurium* gave largely but not entirely negative results, with or without metabolic activation. 2-Chloroaniline gave inconsistent results in a variety of genotoxicity assays.

Exposure of rodents to 2-chloronitrobenzene resulted in toxicity in the liver, kidney, haematopoietic system, and male reproductive tract.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2-chloronitrobenzene.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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4-CHLORONITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

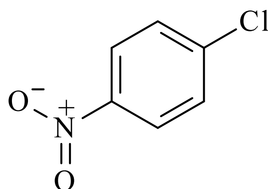
Chem. Abstr. Serv. Reg. No.: 100-00-5

Chem. Abstr. Serv. name: 1-chloro-4-nitrobenzene

IUPAC systematic name: 1-chloro-4-nitrobenzene

Synonyms: 4-chloronitrobenzene; *para*-chloronitrobenzene; 4-chloro-1-nitrobenzene; 4-CNB; 4-nitrochlorobenzene; *para*-nitrochlorobenzene; 1-nitro-4-chlorobenzene; 4-nitro-1-chlorobenzene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_4ClNO_2$

Relative molecular mass: 157.55 (PubChem, 2018).

1.1.3 Chemical and physical properties of the pure substance

Description: yellow, crystalline solid with a sweet odour (PubChem, 2018)

Boiling point: 242 °C (PubChem, 2018)

Melting point: 82–84 °C (PubChem, 2018)

Solubility: slightly soluble in water (225 mg/L at 20 °C) (PubChem, 2018); soluble in acetone, boiling ethanol, diethyl ether, and carbon disulfide; sparingly soluble in cold ethanol (DECOS, 2002)

Volatility: vapour pressure, 0.09 mm Hg at 25 °C (PubChem, 2018)

Relative vapour density (air = 1): 5.44 (PubChem, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 2.39 (PubChem, 2018)

Conversion factor: 1 ppm = 6.44 mg/m^3, at normal temperature (25 °C) and pressure (101 kPa)

Technical products and impurities: available commercially at purities of greater than 99% (Sigma-Aldrich, 2018).

1.2 Production and use

1.2.1 Production process

Continuous or batch nitration of chlorobenzene with mixed acids typically gives a 98% yield of an isomer mix comprising

2-chloronitrobenzene (34–36%), 4-chloronitrobenzene (63–65%), and 3-chloronitrobenzene (~1%). The isomers can be separated by a combination of fractional crystallization and distillation (Booth, 2012).

1.2.2 Production volume

4-Chloronitrobenzene is included in the 2007 Organisation for Economic Co-operation and Development list of chemicals with a high production volume (OECD, 2009).

The worldwide (excluding eastern Europe) production of 4-chloronitrobenzene amounted to 220 900 tonnes in 1995 by approximately 30 producers: about 54 000 tonnes in west Europe, 78 000 tonnes in China, 29 000 tonnes in India, 17 700 tonnes in Japan, 4700 tonnes in the Republic of Korea, and 37 500 tonnes in the USA (OECD-SIDS, 2002).

The production volumes for non-confidential chemicals reported under the 1986–2002 Inventory Update Rule submitted to the United States Environmental Protection Agency (EPA) for 4-chloronitrobenzene are presented in Table 1.1. Production volumes in the USA in more recent years are not publicly available.

About 250 000 tonnes per year of 4-chloronitrobenzene were produced in China in 2003 and 2004, which represented about 60% of the total annual global production (Shen et al., 2008). Production of 4-chloronitrobenzene in India has reportedly grown by 7.3% per year, and it was estimated that the combined production of 2- and 4-chloronitrobenzene would increase to 127 000 tonnes per year by 2010 (INERIS, 2010).

The European Chemicals Agency reports that 1–10 tonnes of 4-chloronitrobenzene per year are currently manufactured in and/or imported into the European Economic Area (ECHA, 2018). Production of 4-chloronitrobenzene ended in France in 2007 (INERIS, 2010).

Table 1.1 Production volumes for 4-chloronitrobenzene, USA[a]

Year	Production range in pounds [tonnes]
1986	(50–100) × 10^6 [22 680–45 359]
1990	(100–500) × 10^6 [45 359–226 796]
1994	(50–100) × 10^6 [22 680–45 359]
1998	(50–100) × 10^6 [22 680–45 359]
2002	(50–100) × 10^6 [22 680–45 359]

[a] Non-confidential production volume information submitted to United States Environmental Protection Agency by companies for chemicals under the 1986–2002 Inventory Update Rule (HSDB, 2008)

1.2.3 Use

4-Chloronitrobenzene and its derivatives are used in many synthetic processes (Booth, 2012). Chemical intermediates produced from 4-chloronitrobenzene include: 4-chloroaniline, 4-nitrophenol, 4-nitroanisole, *para*-anisidine, 4-nitroaniline, 6-chloro-3-nitrobenzenesulfonic acid, 2,4-dinitrochlorobenzene, and 3,4-dichloronitrobenzene. 4-Chloronitrobenzene is a precursor for the synthesis of agricultural chemicals, including the herbicides nitrofen and fluoronitrofor, as well as antioxidants used in the rubber industry, for example, 4-isopropylamino-diphenylamine (Booth, 2012). The compound is also used in the pharmaceutical industry for the synthesis of several antibiotics, anxiolytics, and analgesics, including the widely used acetaminophen (i.e. paracetamol) (INERIS, 2010; Table 1.2).

The use of 4-chloronitrobenzene by activity sector in France was reported in 2010 as: chemistry, 86.49%; textile treatment, 5.33%; paint, pigments, colorants, and plastic production, 2.44%; leather treatments, 2.11%; paper industry, 1.43%; and others such as food processing, less than 1% (INERIS, 2010).

Table 1.2 Some pharmaceutical drugs synthesized from 4-chloronitrobenzene

Drug name	CAS No.	Function
Paracetamol (acetaminophen)	103-90-2	Analgesic
Phenacetin	62-44-2	Analgesic
Dapsone	80-08-0	Antibiotic (to treat leprosy)
Norfloxacin	70 458-96-7	Urinary antibiotic
Albendazole	54 965-21-8	Anti-helminthic
Alprazolam	28 981-97-7	Anxiolytic
Demoxepam	963-39-3	Anxiolytic
Estazolam	29 975-16-4	Sedative
Itraconazole	84 625-61-6	Antifungal
Diazepam	439-14-5	Anxiolytic

CAS, Chemical Abstracts Service
Compiled by the Working Group with data from INERIS (2010)

1.3 Methods of measurement and analysis

1.3.1 Air

The measurement of 4-chloronitrobenzene in air using the United States National Institute for Occupational Safety and Health method involves collecting the sample on silica gel tubes, followed by analysis by gas chromatography and either mass spectrometry or flame ionization detection (NIOSH, 2005). [The Working Group was not able to identify other published methods to measure 4-chloronitrobenzene in air.]

1.3.2 Other environmental media

EPA Method 8091 is a gas chromatography method that can be used to determine the concentration of nitroaromatics and cyclic ketones, allowing the measurement of contamination in water, soil, and waste matrices. Nitroaromatics can be detected in water and soil at concentrations of parts per billion and in waste matrices at concentrations of parts per million (EPA, 1996).

1.3.3 Biomonitoring

Dangwal & Jethani (1980) described a colorimetric method to measure 4-chloronitrobenzene in urine with a limit of detection of 0.6 mg/L, and Lewalter & Ellrich (1991) provided a method for the measurement of nitroaromatic compounds, including 4-chloronitrobenzene, in plasma using gas chromatography with electron capture detection (detection limit, 1 µg/L blood). The method of Lewalter & Ellrich (1991) was approved by the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission).

A recent study compared several urinary biomarkers for 4-chloronitrobenzene exposure in a group of Chinese workers in a chemical factory in Tainjing City, China (Jones et al., 2006, 2007; Sabbioni et al., 2016). Urine samples of the workers and of the factory controls were analysed before and after acid hydrolysis to quantify the free and conjugated metabolites of 4-chloronitrobenzene using high-performance liquid chromatography and ultraviolet spectroscopy. The mercapturic acid *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was the only metabolite detected in non-hydrolysed urine, accounting for approximately 51% of the total metabolites detected. The two remaining metabolites, 4-chloroaniline and 2-chloro-5-nitrophenol, were identified as cleavage products in hydrolysed urine and accounted for approximately 18% and 30% of the total metabolites detected, respectively. No 4-chloronitrobenzene metabolites were found in factory controls. There was a correlation between *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine and 4-chloroaniline, and between *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine and 2-chloro-5-nitrophenol ($r = 0.63$ and $r = 0.68$, respectively; $P < 0.01$, ranks). Creatinine-adjusted *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was correlated with 4-chloronitrobenzene in air ($r = -0.30$; $P > 0.05$) (Jones et al., 2007).

N-acetyl-*S*-(4-nitrophenyl)-L-cysteine would be an appropriate biomarker of exposure in urine

for a recent absorbed dose of 4-chloronitrobenzene; it is the major urinary metabolite and was detected in all exposed workers. Its concentration also correlated well with the concentration of the two other main metabolites (4-chloroaniline and 2-chloro-5-nitrophenol) and with the concentration of 4-chloronitrobenzene in air (Jones et al., 2007). The limit of detection of the method ranged over 0.10–1.0 µmol/L, depending on the analyte.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

4-Chloronitrobenzene is not known to occur naturally.

The major sources of environmental release of 4-chloronitrobenzene are from chemical plants where it is produced or used as an intermediate. Minor sources of release into the environment may occur during transport, storage, or land burial, and the compound may form in the environment through the oxidation of synthetic aromatic amines or the reaction of nitrogen oxides in highly polluted air with chlorinated aromatic hydrocarbons (Howard et al., 1976). 4-Chloronitrobenzene is most likely to be found in air (65%) and water (OECD-SIDS, 2002).

Measurements of 4-chloronitrobenzene in water identified by the Working Group are summarized in Table 1.3.

In the 1970s, concentrations of up to 1800 mg/L were reported for 2-, 3-, and 4-chloronitrobenzenes in wastewater from a chloronitrobenzene production plant in the USA (Howard et al., 1976).

In 1976, an accidental release of chloronitrobenzenes was reported in France (Raguet et al., 2010); in the area of this accident, concentrations of 4-chloronitrobenzene of up to 0.12 mg/L were measured in the groundwater (Duguet et al., 1988).

Elsewhere in Europe, concentrations of 4-chloronitrobenzene of up to 0.3 µg/L in German rivers (Feltes et al., 1990) and of 0.37 µg/L in Italian rivers (Trova et al., 1991) were measured in the 1990s. Concentrations in German rivers decreased to less than 0.06 µg/L in 2004 (Schäfer et al., 2011).

High concentrations of 4-chloronitrobenzene of up to 16.3 mg/L were measured in river water in China in 1990 (Lang et al., 1993). In India, concentrations of the compound of up to 227 mg/L were measured in the wastewater from a chlorobenzene production plant (Swaminathan et al., 1987).

Based on the available experimental data, 4-chloronitrobenzene is not readily biodegradable in water. However, it can be biodegraded by adapted microorganisms; many studies on wastewater treatment and soil remediation have been published (e.g. Xia et al., 2011; Arora et al., 2012; Zhu et al., 2013; Xu et al., 2016).

Recent measurements of 4-chloronitrobenzene from 16 source water reservoirs in the Haihe river basin, China, were on average 0.017 µg/L, with a maximum value of 0.050 µg/L (less than the standard limit in China by a factor of 1000) (Gao et al., 2012).

1.4.2 Occurrence in food

4-Chloronitrobenzene has been reported at low levels in edible portions of various fish species from the Mississippi river in the USA (Yurawecz & Puma, 1983), and in fish from the river Main in Germany (Steinwandter, 1987).

1.4.3 Exposure of the general population

It is possible that the general population could be exposed to 4-chloronitrobenzene from the use of mouth washes containing chlorhexidine gluconate (Below et al., 2004, 2017). Forty-three patients who had orofacial operations were randomized to use a 0.2% chlorhexidine

Table 1.3 Environmental occurrence of 4-chloronitrobenzene

Location, collection date	Sampling matrix	Mean (range) exposure concentration	Comments	Reference
France, 1987	Groundwater	NR (5–123 µg/L)	Accidental pollution from a dye production plant; 2-chloronitrobenzene was the primary pollutant, accounting for 70% of the pollution	Duguet et al. (1988)
France, 2011	Groundwater	26 (maximum, 400) ng/L	0.2% positive measurements; ~500 sites throughout France	Lopez & Laurent (2013)
Elbe, Germany, NR	River water	NR (0.04–0.30 µg/L)		Feltes et al. (1990)
Bormida river, Italy, 1989–1990	River water	0.08 (0.002–0.37) µg/L	Monthly measurements at five sampling stations	Trova et al. (1991)
Songhua river, China, early 1990s	River water	NR (0.17–16.30 mg/L)	Reference in Chinese reported in Men et al. (2011)	Lang et al. (1993)
Daliao river, China, 2006	River water	NR (maximum, 0.896 mg/L)	28 sites in the dry season	Men et al. (2011)
Germany, 1994–2004	River water	NR (maximum, 0.06 µg/L)	110 measurements from the four largest rivers of northern Germany; detection at more than 20% of all sites sampled	Schäfer et al. (2011)
Haihe river, China, NR	River water	16.9 (< 10.5–50.0) ng/L	Detection in 62.5% of the 16 reservoirs of the Haihe river basin	Gao et al. (2012)
Netherlands, 1983–1984	Costal water	6.9 (0.1–31.0) ng/L	108 measurements throughout the year at nine locations	van de Meent et al. (1986)
Scheldt estuary, Netherlands and/or Belgium, 1986	Estuary water	Median, 1.4 (0.5–2.5) ng/L	Heavy pollution due to large wastewater discharges	van Zoest & van Eck (1991)
USA, early 1970s[a]	Wastewater	NR (1500–1800 mg/L)	Effluent from a 3-chloronitrobenzene production plant	Howard et al. (1976)
India, 1980s	Wastewater	166 (112–227) mg/L	Effluent from a chloronitrobenzene production plant	Swaminathan et al. (1987)
France, NR	Industrial wastewater	3.19 µg/L	31 industrial sites were positive out of the 2876 measured	INERIS (2010)
France, NR	Urban wastewater	0.55 µg/L	Three urban wastewater sites were positive out of the 167 measured	INERIS (2010)

NR, not reported

[a] 2-, 3-, and 4-Chloronitrobenzene collected

gluconate (n = 23) or an octenidine-based chlorhexidine-free (n = 20, controls) mouthwash once preoperatively and 3 times per day for 5 days postoperatively (Below et al., 2017). 4-Chloroaniline, which may metabolically transform to 4-chloronitrobenzene, was detectable in saliva at higher concentrations in the chlorhexidine group (0.55 mg/L) than the octenidine group (0.21 mg/L). However, 4-chloronitrobenzene in saliva was not significantly increased in the chlorhexidine group compared with the controls. [The Working Group noted that there is no experimental evidence that 4-chloronitrobenzene exposure arises from using chlorhexidine gluconate mouthwashes.]

1.4.4 Occupational exposure

Occupational exposure to 4-chloronitrobenzene may occur through inhalation and dermal contact at workplaces where this compound is produced or used. Exposure may also occur through inadvertent ingestion (CDC, 2016).

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 2950 employees in the USA were potentially exposed to 4-chloronitrobenzene. The estimates were based on a survey of companies and did not involve measurements of exposure (NOES, 1995).

Long-term occupational exposure to aromatic nitro and amino compounds, including 4-chloronitrobenzene, was studied in 35 male Japanese workers involved in the production of dyes or pharmaceuticals (Yoshida et al., 1989). The workers were routinely exposed to aromatic nitro-amino compounds at concentrations of greater than 0.3 mg/m^3 (reported as 4-chloronitrobenzene), and exposure was described as occurring from skin contact and inhalation (Yoshida et al., 1993).

The exposure of workers from a chemical factory in China was reported by Jones et al. (2006). The median concentration of 4-chloronitrobenzene in air determined from four personal samples was 0.87 mg/m^3. In another study in the same factory by the same authors, the mean 8-hour average 4-chloronitrobenzene exposure of 19 workers was 1.17 mg/m^3. Urine samples from the workers and the factory controls were analysed before and after acid hydrolysis to quantify the free and conjugated metabolites of 4-chloronitrobenzene. The *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine metabolite in post-shift urine samples from the exposed workers (n = 38) was 0.30–34.3 µmol/L, 4-chloroaniline ranged from not detected to 10.5 µmol/L, and 2-chloro-5-nitrophenol ranged from not detected to 11.8 µmol/L (Jones et al., 2007). *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine, 4-chloroaniline, and 2-chloro-5-nitrophenol were not detected in the post-shift urine taken from unexposed control workers, or in other unexposed volunteers. *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine, 4-chloroaniline, and 2-chloro-5-nitrophenol were not detected in pre-shift urine samples (n = 5) from exposed workers (Jones et al., 2007).

1.5 Regulations and guidelines

The international occupational exposure limit values for 4-chloronitrobenzene, as published by the German *Institut für Arbeitsschutz* (IFA), are presented in Table 1.4.

The EPA has set a chronic oral reference dose of 0.0007 mg/kg bw per day for 4-chloronitrobenzene (EPA, 2015), and regional screening levels of 4.4 mg/kg for resident soil, 210 ng/m^3 for resident air, and 1.2 µg/L for tap water (EPA, 2018).

The French *Agence française de securité sanitaire de l'environnement et du travail* (AFSSET, 2009) proposed two toxicological reference values (TRVs) for ingestion of 4-chloronitrobenzene: a chronic TRV with a threshold based on haematotoxic effects of 0.034 mg/kg bw per day; and a non-threshold TRV based on potential carcinogenic effects of 5×10^{-8} mg/kg bw per day.

Table 1.4 International limit values for 4-chloronitrobenzene

Country or region	8-hour limit		Short-term limit	
	(ppm)	(mg/m³)	(ppm)	(mg/m³)
Australia	0.1	0.64		
Austria	0.075	0.5	0.3	2.0
Belgium	0.1	0.65		
Canada, Ontario	0.1			
Canada, Québec (Province)	0.1	0.64		
China		0.6		
Denmark	0.1	0.64	0.2	1.28
Finland		1.0		3.0[a]
Hungary		0.5		2.0
Ireland		1.0		2.0[b]
Japan (JSOH)	0.1	0.64		
New Zealand	0.1	0.64		
Poland		0.6		
Republic of Korea	0.1	0.6		
Romania			0.16[a]	1.0[a]
Singapore	0.1	0.64		
Spain[c]	0.1	0.65		
Switzerland	0.075			
UK		1		2
USA (OSHA)		1		

JSOH, Japan Society for Occupational Health; OSHA, United States Occupational Safety and Health Administration
[a] 15-minute average value
[b] 15-minute reference period
[c] Skin exposure
Reproduced from IFA (2018)

For all methaemoglobin inducers such as 4-chloronitrobenzene, a biological exposure index was set by the American Conference of Governmental Industrial Hygienists at 1.5% methaemoglobin in blood (ACGIH, 2008).

In China, the maximum concentration of 4-chloronitrobenzene is regulated at 500 µg/L in surface water and at 50 µg/L in drinking-water (Shen et al., 2008).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

The evidence for the carcinogenic activity of 4-chloronitrobenzene was previously reviewed by the Working Group in *IARC Monographs* Volume 65 (IARC, 1996). On the basis of one study in male mice, one study in female mice, and one study in male rats (Weisburger et al., 1978), the Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of chloronitrobenzenes [2-, 3-, and 4-chloronitrobenzene]. An additional study with 4-chloronitrobenzene in male and female rats and mice has since become available for evaluation (Matsumoto et al., 2006).

See Table 3.1

Table 3.1 Studies of carcinogenicity with 4-chloronitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), 3000, 6000, 0 (pooled control) ppm for 18 mo 25, 25, 25, 99 NR	*Liver*: hepatoma [hepatocellular carcinoma] 1/14, 4/14*, 0/14, 7/99 *Vascular*: tumours [histology and sites unspecified] 0/14, 2/14, 4/14*, 5/99	 **P* < 0.025 vs pooled controls **P* < 0.025 vs concurrent and pooled controls	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (F) 6–8 wk 21 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), 3000, 6000, 0 (pooled control) ppm for 18 mo 24, 25, 25, 102 NR	*Vascular*: tumours [histology and sites unspecified] 0/15, 2/20, 7/18*, 9/102	 **P* < 0.025 vs concurrent and pooled controls	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, Crj:BDF$_1$ (M) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 125, 500, 2000 ppm 50, 50, 50, 50 47, 49, 42, 38	*Liver* Hepatocellular adenoma 3/50, 2/50, 4/50, 2/50 Hepatocellular carcinoma 1/50, 3/50, 1/50, 6/50 Haemangiosarcoma 2/50, 2/50, 1/50, 1/50 *Lymph node*: malignant lymphoma 2/50, 2/50, 1/50, 8/50	 NS *P* < 0.01, Peto trend test NS *P* < 0.01, Peto trend test	Principal strengths: males and females used; well-conducted GLP study Terminal body weights of males exposed at 2000 ppm were decreased by 5% compared with controls; incidence for combination of hepatocellular tumours not given; except for males exposed at 2000 ppm, there was no significant difference in survival in treated groups (no survival statistics given)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF_1 (F) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-chloronitrobenzene, > 99.9% Diet 0, 125, 500, 2000 ppm 50, 50, 50, 50 32, 35, 35, 29	*Liver* Hepatocellular adenoma 4/50, 1/50, 3/50, 3/50	NS	Principal strengths: well-conducted GLP study; males and females used Incidence for combination of hepatocellular tumours not given
		Hepatocellular carcinoma 2/50, 0/50, 2/50, 5/50	$P < 0.05$, Peto trend test	
		Haemangiosarcoma 0/50, 1/50, 0/50, 5/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Lymph node: malignant lymphoma 11/50, 11/50, 7/50, 12/50	NS	
Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), low dose (2000 ppm for 3 mo, 250 ppm for 2 mo, 500 ppm for 13 mo, 0 for 6 mo), high dose (4000 ppm for 3 mo, 500 ppm for 2 mo, 1000 ppm for 13 mo, 0 for 6 mo), 0 ppm (pooled control) 25, 25, 25, 111 NR	Any tumour type: no significant increase in incidence		Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation Only male rats were studied; histopathology conducted only on rats surviving after 6 mo

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 40, 200, 1000 ppm 50, 50, 50, 50 43, 46, 42, 12	*Spleen* Fibroma 0/50, 0/50, 1/50, 15/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study Incidence for combination of splenic tumours not given; survival was reduced in the group exposed at 1000 ppm; terminal body weight was significantly decreased in the group exposed at 1000 ppm
		Fibrosarcoma 0/50, 1/50, 0/50, 29/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Osteosarcoma 0/50, 0/50, 0/50, 11/50*	$P < 0.01$, Peto trend test; *$P < 0.01$ Fisher exact test	
		Sarcoma [NOS] 0/50, 0/50, 1/50, 6/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Haemangiosarcoma 0/50, 0/50, 5/50*, 7/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Adrenal gland: pheochromocytoma 7/50, 7/50, 6/50, 16/50	$P < 0.01$, Peto trend test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 40, 200, 1000 ppm 50, 50, 50, 50 36, 41, 38, 28	*Spleen*		Principal strengths: males and females used; well-conducted GLP study Incidence for combination of splenic tumours not given; terminal body weight was significantly decreased in groups exposed at 200 and 1000 ppm
		Fibroma 0/50, 0/50, 1/50, 3/50	 $P < 0.05$, Peto trend test	
		Fibrosarcoma 0/50, 0/50, 0/50, 17/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Osteosarcoma 0/50, 0/50, 0/50, 3/50	 $P < 0.01$, Peto trend test	
		Sarcoma [NOS] 0/50, 0/50, 0/50, 1/50	 NS	
		Haemangiosarcoma 0/50, 0/50, 2/50, 4/50	 $P < 0.01$, Peto trend test	
		Adrenal gland: pheochromocytoma 3/50, 6/50, 4/50, 16/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	

F, female; GLP, good laboratory practice; M, male; mo, month; NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; vs, versus; wk, week; yr, year

3.1 Mouse

Oral administration

In a study by Weisburger et al. (1978), groups of 25 male and 25 female CD-1 mice (derived from HaM/ICR mice) (age, 6–8 weeks) were given diets containing 1-chloro-4-nitrobenzene [4-chloronitrobenzene] at a concentration of 0 (control), 3000, or 6000 ppm (21 chemicals were tested in the study; purity of most was 97–99%) for 18 months. The mice were then held for 3 months on the control diet before being killed at 21 months. There was a pooled control group of 99 males and 102 females [no additional details provided]. Mice that died within the first 6 months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other mice. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, and reproductive organs. Information on survival, body weight, or non-neoplastic lesions was not reported.

The incidence of hepatocellular carcinoma was significantly increased in males at the lower dose: 1/14 (control), 4/14 (lower dose; $P < 0.025$ versus pooled controls, 7/99), and 0/14 (higher dose). The incidence of vascular tumours [histology and sites unspecified] was significantly increased in males at the higher dose: 0/14 (control), 2/14 (lower dose), and 4/14 (higher dose; $P < 0.025$ versus concurrent (0/14) and pooled controls (5/99). The incidence of vascular tumours [histology and sites unspecified] was significantly increased in female mice at the higher dose: 0/15 (control), 2/20 (lower dose), and 7/18 (higher dose); $P < 0.025$ versus concurrent (0/15) and pooled controls (9/102) (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of mice at the start, the small number of mice necropsied, the use of only two dose groups, and the limited histopathological examination and reporting.]

In a study of carcinogenicity that complied with good laboratory practice (GLP) (Matsumoto et al., 2006), groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were given diets containing 4-chloronitrobenzene (purity, > 99.9%) at a concentration of 0 (control), 125, 500, or 2000 ppm for 2 years. Based on feed consumption, the estimated amount of chemical given was 0, 15, 60, and 240 mg/kg body weight (bw) per day (males) and 0, 18, 73, and 275 mg/kg bw per day (females) for the groups at 0, 125, 500, and 2000 ppm, respectively. The survival in treated groups of males and females was similar to that of controls except for male mice at 2000 ppm, for which survival was reduced. The final number of mice surviving until termination of the experiment was 47, 49, 42, and 38 for males and 32, 35, 35, and 29 for females. The terminal body weights in treated groups of males and females were similar those of controls, with the terminal body weight of males at 2000 ppm decreased by 5%. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

There were dose-related increases in the incidence of different tumour types in male and female mice. In males, the incidence of liver tumours for groups at 0 (control), 125, 500, and 2000 ppm was: hepatocellular adenoma, 3/50, 2/50, 4/50, and 2/50; hepatocellular carcinoma, 1/50, 3/50, 1/50, and 6/50 ($P < 0.01$, Peto trend test); and haemangiosarcoma, 2/50, 2/50, 1/50, and 1/50. The incidence of malignant lymphoma in males was 2/50, 2/50, 1/50, and 8/50 ($P < 0.01$, Peto trend test).

In females, the incidence of liver tumours was: hepatocellular adenoma, 4/50, 1/50, 3/50, and 3/50; hepatocellular carcinoma, 2/50, 0/50, 2/50, and 5/50 ($P < 0.05$, Peto trend test); and haemangiosarcoma, 0/50, 1/50, 0/50, and 5/50 ($P < 0.05$, Fisher exact test) ($P < 0.01$, Peto trend

test). [The Working Group noted that the incidence of the combination of hepatocellular tumours was not reported in the study.]

In male mice, there were increases in the incidence of splenic non-neoplastic lesions, including congestion and extramedullary haematopoiesis. In female mice, there were increases in the incidence of splenic non-neoplastic lesions, including congestion, deposit of haemosiderin, and ossification (Matsumoto et al., 2006). [The Working Group noted that this was a well-conducted GLP study in males and females.]

3.2 Rat

Oral administration

In the study by Weisburger et al. (1978), groups of 25 male Charles River CD rats (derived from Sprague-Dawley rats) (age, 6–8 weeks) were fed diets containing 4-chloronitrobenzene (21 chemicals were tested in the study; purity for most, 97–99%) at a concentration of 0 (control), 2000, or 4000 ppm for 3 months. Dietary concentrations were then lowered to 0, 250, and 500 ppm for 2 months and then increased to 0, 500, and 1000 ppm for 13 months; rats were held for a further 6 months on the control diet before being killed at 24 months. There was a pooled control group of 111 male rats [no additional details provided]. Rats that died within the first 6 months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries. Information on survival, body weight, or non-neoplastic lesions was not reported. No significant increase in tumour incidence in male rats was reported (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of rats at the start, the small number of rats necropsied, the use of only two dose groups and one sex, and the limited histopathological examination and reporting.]

In a GLP study of carcinogenicity with 4-chloronitrobenzene (Matsumoto et al., 2006), groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diets containing 4-chloronitrobenzene (purity, > 99.9%) at a concentration of 0 (control), 40, 200, or 1000 ppm for 2 years. Based on feed consumption, the estimated amount of chemical given was 0, 1.5, 7.7, and 41.2 mg/kg bw per day (males) and 0, 1.9, 9.8, and 53.8 mg/kg bw per day (females) for the groups at 0, 40, 200, and 1000 ppm, respectively. The number of surviving rats was decreased for males at 1000 ppm; the numbers surviving were 43, 46, 42, and 12 (males) and 36, 41, 38, and 28 (females) for the groups at 0, 40, 200, and 1000 ppm, respectively. Terminal body weight was decreased in the male rats at 1000 ppm and in the female rats at 200 and 1000 ppm. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

There was a dose-related increase in the incidence of splenic tumours in male rats, including spleen fibroma – 0/50, 0/50, 1/50, and 15/50 ($P < 0.01$); $P < 0.01$, Peto trend test – and spleen fibrosarcoma – 0/50, 1/50, 0/50, and 29/50 ($P < 0.01$); $P < 0.01$, Peto trend test. The incidence of splenic osteosarcoma – 0/50, 0/50, 0/50, and 11/50 ($P < 0.01$); $P < 0.01$, Peto trend test – splenic sarcoma (not otherwise specified) – 0/50, 0/50, 1/50, and 6/50 ($P < 0.05$); $P < 0.01$, Peto trend test – and splenic haemangiosarcoma – 0/50, 0/50, 5/50 ($P < 0.05$), and 7/50 ($P < 0.05$); $P < 0.01$, Peto trend test – was also significantly increased.

In female rats, there were also dose-related increases in the incidence of splenic tumours, including splenic fibroma – 0/50, 0/50, 1/50, and 3/50; $P < 0.05$, Peto trend test – and splenic fibrosarcoma – 0/50, 0/50, 0/50, and 17/50 ($P < 0.01$);

$P < 0.01$, Peto trend test. The incidence of splenic osteosarcoma (0/50, 0/50, 0/50, and 3/50; $P < 0.01$, Peto trend test) and of splenic haemangiosarcoma (0/50, 0/50, 2/50, and 4/50; $P < 0.01$, Peto trend test) was also increased.

There were increases in the incidence of splenic non-neoplastic lesions, including capsule hyperplasia, fibrosis, fatty metamorphosis, and extramedullary haematopoiesis, in male and female rats in the groups at 200 and 1000 ppm.

In male rats, there was a significant positive trend in the incidence of adrenal gland pheochromocytoma (7/50, 7/50, 6/50, and 16/50; $P < 0.01$, Peto trend test). In female rats, there was a significant increase in the incidence of adrenal gland pheochromocytoma – 3/50, 6/50, 4/50, and 16/50 ($P < 0.01$); $P < 0.01$, Peto trend test. The incidence of adrenal gland medullary hyperplasia was increased in male rats at 40 ppm and in female rats at 1000 ppm (Matsumoto et al., 2006). [The Working Group noted that this was a well-conducted GLP study in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

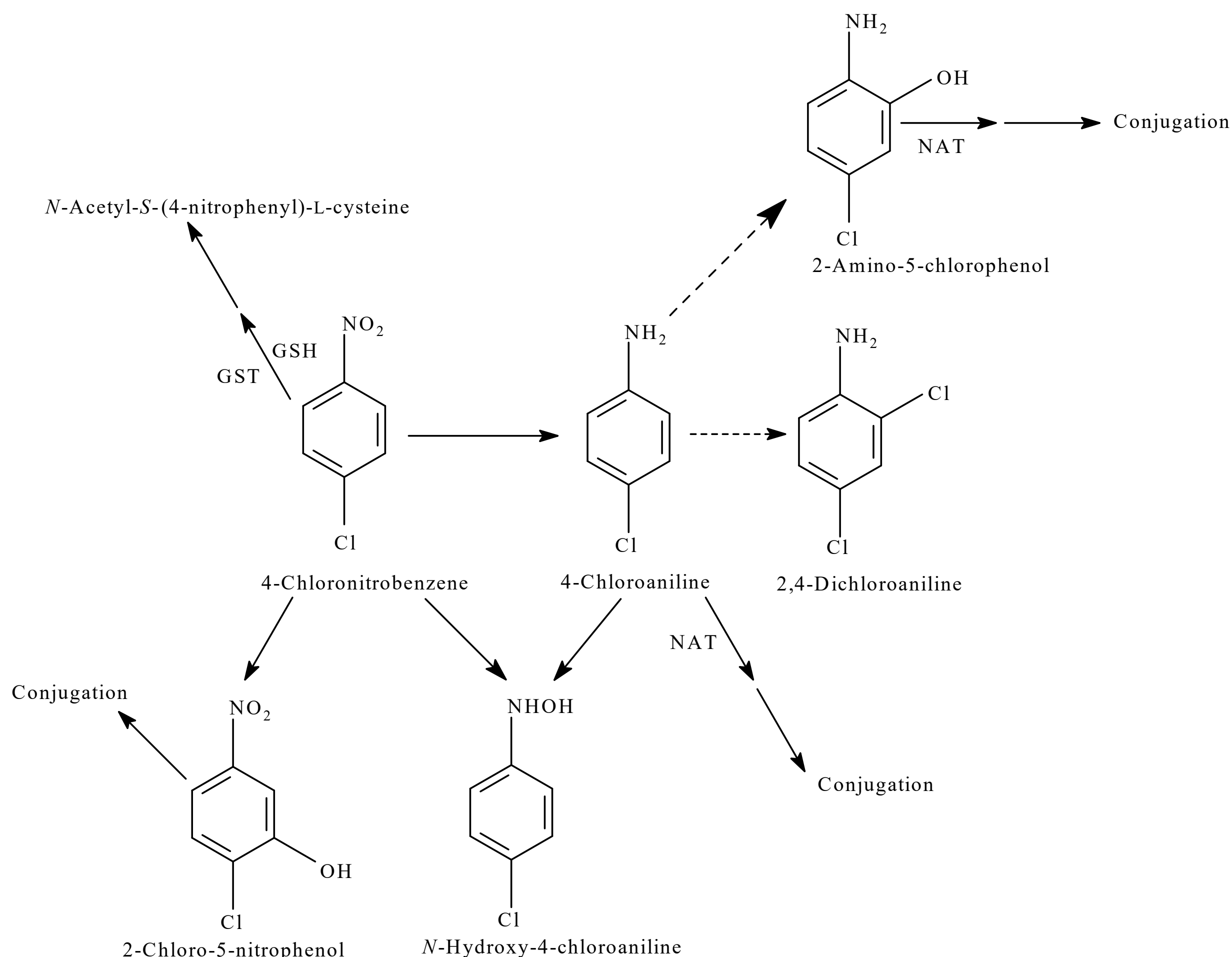
4.1.1 Humans

The excretion of urinary metabolites of 4-chloronitrobenzene has been studied in connection with an episode of accidental poisoning by 4-chloronitrobenzene (Yoshida et al., 1992, 1993). Five major urinary metabolites were identified (Yoshida et al., 1992). In the more extensive study of the poisoning, Yoshida et al. (1993) analysed urinary elimination of the five metabolites for 14–29 days in six exposed workers. The parent compound was not excreted; however, metabolites were eliminated for the total observation period (total metabolites, 179–1076 mg). The urinary elimination of each metabolite appeared to fit into a one-compartment model.

There was a complicated metabolic pattern with considerable inter-individual variation, which had three major pathways (see Fig. 4.1). The most important pathway of metabolism was glutathione conjugation, which resulted in the excretion of the mercapturic acid *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine (48% of the total metabolites, on average). In the second pathway, there was also a slow reduction of the nitro group, resulting in the formation of 4-chloroaniline which was rapidly metabolized further by: (a) fast *N*-acetylation to a variety of *N*-conjugated metabolites (detected in the urine after hydrolysis as 4-chloroaniline, 29.9%); (b) ring-hydroxylation to 2-amino-5-chlorophenol (8.7%), which was also further *N*-acetylated; and (c) chlorination to 2,4-dichloroaniline, which was readily excreted (1.2%). Chlorination was a novel metabolic pathway in humans, hypothesized by Yoshida et al. (1992) to be catalysed by myeloperoxidases from activated leukocytes associated with toxicity induced by 4-chloronitrobenzene. Finally, in the third pathway, there was a slow ring hydroxylation of the parent compound to give 2-chloro-5-nitrophenol (12.2%).

The excretion of urinary metabolites of 4-chloronitrobenzene has also been studied in workers chronically exposed to 4-chloronitrobenzene (Jones et al., 2007). Post-shift urine samples were obtained from 38 exposed workers (36 men, 2 women) in a Chinese chemical factory; five pre-shift samples were also taken from a subset of the workers. Air exposure levels of 4-chloronitrobenzene were measured for 19 workers with personal samplers, yielding a mean 8-hour time-weighted average (TWA) of 1.17 mg/m^3. Three urinary metabolites were detected in the post-shift urine samples of the chronically exposed workers, reflecting each of the three major pathways discussed in the paragraph above: the mercapturic acid *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine (51%); conjugated 4-chloroaniline (18%);

Fig. 4.1 Proposed metabolic pathway of 4-chloronitrobenzene in humans



GSH, glutathione; GST, glutathione *S*-transferase; NAT, *N*-acetyltransferase

and conjugated 2-chloro-5-nitrophenol (30%). *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was detected in all 38 post-shift samples, 4-chloroaniline was detected in 29 of the 38 samples, and 2-chloro-5-nitrophenol was detected in 36 of the 38 samples. As with the accidently exposed workers studied by Yoshida et al. (1992, 1993), no parent compound was observed. In contrast to the accidently exposed workers, 2,4-dichloroaniline was not observed within the limits of quantitation of the assay, and there was no evidence of the *N*-acetylated metabolite of 2-amino-5-chlorophenol. No metabolites were detected in control samples from unexposed workers or in the pre-shift samples. Contrasting with the observation of metabolites during the 14–29 days after exposure of the accidently exposed workers, the absence of detection of two of the urinary metabolites and the absence of detectable metabolites in the pre-shift samples might be a reflection of the different amount of 4-chloronitrobenzene absorbed, as indicated by the 1000-fold higher levels of 4-chloronitrobenzene metabolites reported for the accidently exposed compared with the chronically exposed workers (Jones et al., 2007).

A study of haemoglobin adducts in the same group of Chinese chemical factory workers

exposed to 4-chloronitrobenzene has also been conducted (Jones et al., 2006). In the haemoglobin adduct study, blood samples were obtained from 39 exposed workers. The mean 8-hour TWA from a subset of 19 workers was 1.17 mg/m^3, as reported by Jones et al. (2007), and the median was 0.87 mg/m^3. Hydrolysable haemoglobin adducts of 4-chloroaniline were detected in all 39 blood samples from workers exposed to 4-chloronitrobenzene (mean, 1037 pg/mg; median, 1013 pg/mg), indicating the availability of the reactive intermediate metabolite *N*-hydroxy-4-chloroaniline that was not apparent in the studies of urinary metabolites. The haemoglobin adducts of 4-chloroaniline were also detected, at lower concentrations, in all of the blood samples from factory controls (n = 15) and in 1 of the 6 non-factory control samples. As with the urinary metabolites, haemoglobin adduct concentrations were not correlated with air levels, possibly indicating the importance of other routes of exposure.

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

Dermal absorption studies of groups of three male Fischer 344 rats exposed to 4-chloronitro[^{14}C]benzene by single dermal application at 0.65, 6.5, and 65 mg/kg bw [0.0325, 0.325, and 3.25 mg/cm^2] were conducted (Bucher, 1993). Urine and faeces were collected for up to 72 hours. Based upon measurements of eliminated radiolabel, 51–62% of 4-chloronitrobenzene was absorbed from the skin within 72 hours, with absorption increasing non-significantly with increasing dose. Urinary excretion of radiolabel over 72 hours accounted for 43–45% of the administered dose; faecal excretion accounted for 5–12%, and increased with increasing dose.

Bucher (1993) exposed groups of eight male Fischer 344 rats to 4-chloronitro[^{14}C]benzene as a single gavage dose at 2, 20, or 200 mg/kg bw, and urine and faeces were collected for up to 72 hours. Minimum absorption (determined by the percentage of the administered dose recovered in the urine or tissues) of the 4-chloronitrobenzene was 73–78%. A comparison of this finding with the results for dermal application in Bucher (1993), discussed in the previous paragraph, demonstrates greater absorption by oral exposure than by dermal exposure. 4-Chloronitrobenzene was rapidly metabolized and excreted, primarily in the urine. At the lower doses, about 23% and 5% of the administered radiolabel was found in tissues after 24 and 72 hours, respectively. The highest dose was eliminated more slowly, with about 75% greater tissue retention at both time points. At 24 hours the greatest percentage of radiolabel was in fat, increasing from 15% to 28% across the dose levels. For all dose levels, the highest concentrations of radiolabel at 24 hours were found in fat, followed by blood cells, kidney, liver, and spleen. With the exception of blood cells and spleen, tissue levels of radiolabel declined between 24 and 72 hours. At 72 hours, the greatest percentage of radioactivity at the lower doses was 3% in blood cells; at the highest dose, it was 4% in fat. The highest concentrations at 72 hours occurred in blood cells, followed by fat and spleen. High-performance liquid chromatography analysis of urine revealed the presence of up to 25 metabolites from 4-chloronitrobenzene [metabolites unspecified].

In repeat-dose studies, groups of four young adult (age, 10–12 weeks) or geriatric (age, 19–20 months) male Fischer 344 rats were exposed to 4-chloronitro[^{14}C]benzene at a dose of 65 mg/kg bw by gavage on days 1, 5, and 9, and unlabelled compound on days 2, 3, 4, 6, 7, 8, 10, and 11 (Bucher, 1993). In young adult rats, urinary and faecal excretion accounted for 71–80% and 13–15% of the administered dose, respectively. Approximately 2% of the administered radiolabel was found in the tissues, primarily in blood cells and fat, with the highest concentrations in blood cells and spleen. Urinary excretion in geriatric rats was similar to that of young adults; however,

faecal excretion in geriatric rats was about half of that in the young adults. Furthermore, the percentage of radiolabel retained in the geriatric rats 72 hours after the dose on day 9 (17%) was greater than that in the young adults (2%). Most of the retention was in fat (11%), followed by skeletal muscle and blood cells. The highest concentration of radiolabel was in fat, followed by blood cells, testes, and spleen.

(b) Metabolism

Bray et al. (1956) examined the metabolism of 4-chloronitrobenzene in female rabbits given 4-chloronitrobenzene [exposure route unspecified] at 0.2 g/kg [whether diet or body weight not specified]. Urine was collected over 24-hour periods until metabolites were no longer excreted (usually after 48 hours). The main metabolic processes were reduction and hydroxylation. Nearly the entire dose was excreted in the urine as 4-chloroaniline (9% of the administered dose as free chloroaniline) or derivatives of phenolic metabolites. The phenols formed were excreted mainly as conjugates with sulfuric and glucuronic acids (40% of the administered dose). The formation of mercapturic acid from 4-chloronitrobenzene appears to be a minor metabolic pathway in rabbits.

Yoshida et al. (1991) identified the urinary metabolites of 4-chloronitrobenzene in rats by gas chromatography and mass spectrometry. Male Sprague-Dawley rats were given a single intraperitoneal injection of 4-chloronitrobenzene at 100 mg/kg bw, and urine was collected 8–24 hours after dosing. Rats excreted eight urinary metabolites: 4-chloroaniline, 2,4-dichloroaniline, 4-nitrothiophenol, 2-chloro-5-nitrophenol, 2-amino-5-chlorophenol, 4-chloroformanilide, 4-chloro-2-hydroxyacetanilide, and a small amount of 4-chloroacetanilide. Only trace amounts of unchanged 4-chloronitrobenzene were detected. In a later study, 4-chloro-oxanilic acid was also identified as a metabolite in the urine of rats exposed to 4-chloronitrobenzene (Yoshida, 1994).

Rickert & Held (1990) studied the metabolism of radiolabelled 4-chloronitrobenzene in isolated hepatocytes and hepatic microsomes from male Fischer 344 rats. Incubation of 4-chloronitro[^{14}C]benzene with rat hepatocytes yielded 4-chloroaniline (15.4% of total radioactivity), *S*-(4-nitrophenyl)glutathione (10.4%), and 4-chloroacetaniline (16.3%). Incubation of the radiolabelled parent with microsomes demonstrated that the reduction to 4-chloroaniline was mediated by metabolism dependent on cytochrome P450, as this reduction was inhibited by SKF 525-A, metyrapone, and carbon monoxide.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether 4-chloronitrobenzene: is genotoxic; alters DNA repair; induces oxidative stress; alters cell proliferation, cell death, or nutrient supply; induces chronic inflammation; or is immunosuppressive.

4.2.1 Genetic and related effects

See Table 4.1, Table 4.2, Table 4.3, and Table 4.4

(a) Humans

Sabbioni (2017) assessed the formation of chromosomal aberrations in lymphocytes from a subset of the same workers exposed to 4-chloronitrobenzene studied by Jones et al. (2006). Samples from 24 exposed workers and 13 factory controls were analysed. These workers were also exposed to other chloronitrobenzenes; however, the major isomer was 4-chloronitrobenzene (70%). There was a statistically significant increase in chromosomal aberrations in the half of the exposed subset with the highest levels of 4-chloroaniline–haemoglobin adducts compared with the half

Table 4.1 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in exposed humans and in human cells in vitro

End-point	Tissue, cell type (if specified)	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Exposed humans						
Chromosomal aberrations	Lymphocytes	+	NT	4-Chloronitrobenzene, 0.87 mg/m³ (TWA 8 h)	Effects could not be attributed to 4-chloronitrobenzene specifically, because of concurrent exposures to other chloronitrobenzenes	Sabbioni (2017)
Human cells in vitro						
DNA strand breaks	Exfoliated cells from breast milk (including mammary epithelial and various immune cells)	+	NT	4-Chloroaniline, 0. 71 mM		Martin et al. (2000)
DNA strand breaks	Fibroblast cell line	+	NT	4-Chloroaniline, 0.5 mM in the presence of H_2O_2 (40 µM)		Lueken et al. (2004)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; TWA, time-weighted average

[a] +, positive

Table 4.2 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Reference
DNA adducts	Rat, Wistar (F)	Liver	–	4-Chloronitrobenzene or 4-chloroaniline, 0.5 mmol/kg bw	Gavage 1×, 24 h after dosing	Jones & Sabbioni (2003)
DNA strand breaks	Mouse, Swiss CD-1 (M)	Brain, liver, kidney	+	4-Chloronitrobenzene, 60 mg/kg bw	Intraperitoneal injection 1×, 16 h after dosing	Cesarone et al. (1983)
DNA strand breaks	Rat, Sprague-Dawley (M)	Liver, stomach	+	4-Chloroaniline, 75 and 150 mg/kg bw per day	Gavage 1×/day for 3 days	Barfield & Burlinson (2015)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male

[a] +, positive; –, negative

Table 4.3 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Agent, concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Rat, hepatocytes	+	–	4-Chloronitrobenzene, 50 µM		Cesarone et al. (1984)
Chromosomal aberrations	Chinese hamster lung	–	(+)	4-Chloronitrobenzene, NR	Structural chromosomal aberrations; cytotoxicity, NR	JETOC (1996)
Chromosomal aberrations	Chinese hamster ovary	(+)	(+)	4-Chloronitrobenzene, 600 µg/mL	Without activation, one trial gave negative results (HIC, 500 µg/mL) and two gave positive results (LEC, 700 and 900 µg/mL); with activation, one trial gave negative results (HIC, 5000 µg/mL) and the other gave positive results; cytotoxicity was seen with positive results	Bucher (1993)
Sister-chromatid exchange	Chinese hamster ovary	–	+	4-Chloronitrobenzene, 250 µg/mL	Positive with activation in two trials	Bucher (1993)
Mutation/*Tk*	Mouse, L5178 lymphoma cells	+	+	4-Chloroaniline, NR		NTP (1989)
Chromosomal aberrations	Chinese hamster ovary	–	+	4-Chloroaniline, NR		NTP (1989)
Sister-chromatid exchange	Chinese hamster ovary	+	+	4-Chloroaniline, NR		NTP (1989)

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

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

Table 4.4 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Drosophila melanogaster (adult)	Sex-linked recessive lethal mutations	–	NA	4-Chloronitrobenzene, 100 ppm either by feeding or injection	Zimmering et al. (1985)
Drosophila melanogaster (larvae)	Sex-linked recessive lethal mutations	–	NT	4-Chloronitrobenzene, 80 ppm (feed)	Zimmering et al. (1989)
Salmonella typhimurium TA100, TA1530, TA1535, TA1537, TA98, TA1532, TA1950, TA1975, TA1978, G46	Reverse mutation	–	–	4-Chloronitrobenzene, NR	Gilbert et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	–	+	4-Chloronitrobenzene, 128 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	+/–	–	4-Chloronitrobenzene, 256 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	4-Chloronitrobenzene, 384 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA100, TA98	Reverse mutation	–	–	4-Chloronitrobenzene, 50 µg/mL	Suzuki et al. (1983)
Salmonella typhimurium TA98, TA98NR, TA98NR/1,8-DNP6	Reverse mutation	NT	–	4-Chloronitrobenzene, 50 µg/mL	Suzuki et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	+/–	NT	4-Chloronitrobenzene, 630 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	+	NT	4-Chloronitrobenzene, 315 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA1537, TA1538	Reverse mutation	–	NT	4-Chloronitrobenzene, 630 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA100	Reverse mutation	–	+	4-Chloronitrobenzene, 192 µg/mL	Bucher (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	4-Chloronitrobenzene, 385 µg/mL	Bucher (1993)
Salmonella typhimurium TA1535	Reverse mutation	–	(+)	4-Chloronitrobenzene, 256 µg/mL	Bucher (1993)
Escherichia coli PQ37	DNA strand breaks	–	–	4-Chloronitrobenzene, NR	von der Hude et al. (1988)
Hens' eggs	Micronucleus formation	+	NT	4-Chloroaniline, 0.5 mg per egg	Greywe et al. (2012)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	4-Chloroaniline, NR	NTP (1989)
Salmonella typhimurium TA97, TA1535, TA1537	Reverse mutation	–	–	4-Chloroaniline, NR	NTP (1989)
Calf thymus DNA	DNA adducts	+	NT	4-Chloroaniline, 60 µmol	Jones & Sabbioni (2003)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million

[a] +, positive; –, negative; (+), positive in a study of limited quality; +/–, equivocal (variable response in several experiments within an adequate study)

with the lowest adduct levels, but not in the group exposed to chloronitrobenzene versus the unexposed group. [The Working Group noted that the latter comparison could be limited by the small number of workers evaluated, as well as by the fact that the unexposed workers were of notably higher average age and had some exposure to 2-chloronitrobenzene, as evidenced by the haemoglobin adducts also measured in the unexposed workers.]

The 4-chloroaniline metabolite of 4-chloronitrobenzene caused single-strand DNA breaks in exfoliated cells isolated from human breast milk, which included mammary epithelial cells and various immune cells (Martin et al., 2000). 4-Chloroaniline also acted synergistically with non-cytotoxic doses of H_2O_2 to induce DNA strand breaks in a human fibroblast cell line (Lueken et al., 2004).

(b) Experimental systems

Jones & Sabbioni (2003) did not observe DNA adducts in the liver in female Wistar rats exposed in vivo to 4-chloronitrobenzene or 4-chloroaniline by gavage, despite the formation of haemoglobin adducts by both compounds. Adducts in calf thymus DNA were seen after exposure to 4-chloroaniline (Jones & Sabbioni, 2003).

4-Chloronitrobenzene injected intraperitoneally into male Swiss CD-1 mice induced DNA single-strand breaks in liver, kidney, and brain (Cesarone et al., 1983). 4-Chloronitrobenzene also induced DNA single-strand breaks in rat hepatocytes in vitro (Cesarone et al., 1984).

In Chinese hamster ovary cells, 4-chloronitrobenzene induced chromosomal aberrations with and without S9, and sister-chromatid exchange in the presence of S9, but the positive response for chromosomal aberrations occurred only at doses that were toxic (Bucher, 1993). 4-Chloronitrobenzene induced structural chromosomal aberrations in Chinese hamster lung cells, but information on cytotoxicity was not reported (JETOC, 1996).

4-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when given to adults either by feeding or by injection, or to larvae by feeding (Zimmering et al., 1985, 1989).

Results of mutagenic testing in multiple strains of *Salmonella typhimurium* were largely but not entirely negative with or without metabolic activation across several studies (e.g. Gilbert et al., 1980; Haworth et al. 1983).

4-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS-chromotest (von der Hude et al., 1988).

The metabolite 4-chloroaniline induced DNA damage in vivo in a comet assay in the liver and stomach of male Sprague-Dawley rats (Barfield & Burlinson, 2015), although Jones & Sabbioni (2003) did not observe adducts in hepatic DNA of female Wistar rats. 4-Chloroaniline also exhibited some genotoxic activity in multiple assays in vitro reported by NTP (1989), including mutagenicity in *S. typhimurium* strains TA98 and TA100 with S9, but not in TA97, TA1535, or TA1537. 4-Chloroaniline also tested positive for mutagenicity in mouse lymphoma cells with and without S9, for induction of sister-chromatid exchange with and without S9, and for chromosomal aberrations with S9 in Chinese hamster ovary cells. 4-Chloroaniline gave positive results in a hen's egg test in vitro for micronucleus formation (Greywe et al., 2012).

4.2.2 DNA repair

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Cesarone et al. (1984) measured the repair of single-strand DNA breaks in freshly isolated rat hepatocytes after 3 hours of exposure to

4-chloronitrobenzene in vitro, and observed incomplete DNA repair at 48 hours.

4.2.3 Oxidative stress

Methaemoglobin formation is a well-established effect of exposure to 4-chloronitrobenzene by multiple routes in humans, mice, and rats (Bucher, 1993; Yoshida et al., 1993; Matsumoto et al., 2006a). Methaemoglobin formation is attributed to the *N*-hydroxy-4-chloroaniline metabolite. In erythrocytes, such *N*-hydroxyarylamines can engage in Kiese redox cycling, yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017).

Paranich et al. (1993) studied the effects of 4-chloronitrobenzene in the spleen and liver in rats and reported that short-term (5-day) but not longer-term (30-day) exposure caused increased lipid peroxidation and decreased vitamin E concentration in the spleen, but not liver. No effect was seen on antioxidative activity.

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Haematopoietic proliferation arising from the need to replace damaged erythrocytes was observed in several subchronic and chronic studies with 4-chloronitrobenzene. In a 4-week study in rats treated by inhalation, extramedullary haematopoiesis in the spleen was reported (Nair et al., 1986). In both the Bucher (1993) 13-week study of inhalation exposure and the Matsumoto et al. (2006a) 13-week study of dietary exposure, erythropoiesis in the bone marrow and extramedullary haematopoiesis in the spleen were observed in rats and mice. In the latter study, extramedullary haematopoiesis in the liver of mice and rats was also reported. In a 2-year study of dietary exposure, extramedullary haematopoiesis in the spleen of rats and male mice was observed (Matsumoto et al., 2006b).

Other proliferative effects observed in the subchronic and chronic studies included: squamous cell hyperplasia of the forestomach epithelium in female mice in the Bucher (1993) 13-week study of inhalation exposure, possibly from grooming (Travlos et al., 1996); capsular hyperplasia in the spleens of rats in the Matsumoto et al. (2006a) 13-week study of oral exposure by diet; and fibroblast hyperplasia in the spleens of rats and adrenal gland hyperplasia in rats in the Matsumoto et al. (2006b) 2-year study of oral exposure by diet.

4.2.5 Chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In the Bucher (1993) 13-week study of inhalation exposure to 4-chloronitrobenzene, chronic inflammation of the Harderian gland and capsular fibrosis of the spleen, accompanied by mononuclear inflammatory cell infiltrates, was observed in rats (Bucher, 1993; Travlos et al., 1996).

4.2.6 Immunosuppression

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Li et al. (1998) investigated immunotoxicity in BDF_1 male mouse splenocytes after a single intraperitoneal injection of 300 mg/kg (acute treatment), or intraperitoneal injections of 30 mg/kg bw three times per week for 4 weeks (subchronic treatment). Compared with controls, natural killer cell activity was decreased after both treatments, cytotoxic T-cell activity was

decreased primarily after subchronic treatment, and lipopolysaccharide-stimulated B-cell proliferation was decreased primarily after the acute treatment. In a second study in BDF_1 male mouse splenocytes after a single intraperitoneal injection of 300 mg/kg bw, Li et al. (1999) reported that, compared with controls, B-cells, CD4 and CD8 T-cells, and natural killer cells were significantly decreased, although macrophages and nucleated erythrocytes were increased.

In a study in sheep peripheral blood lymphocytes in vitro, Kačmár et al. (1995) observed that the 4-chloronitrobenzene metabolite, 4-chloroaniline, decreased the mitogenic stimulation of the lymphocytes.

4.3 Other adverse effects

4.3.1 Humans

Eleven longshoremen were poisoned with 4-chloronitrobenzene as a result of the accidental tearing of bags during loading. It was assumed that both inhalation and skin absorption occurred. Symptoms reported included cyanosis, and laboratory tests revealed methaemoglobinaemia, anaemia, reticulocytosis, and Heinz bodies (Yoshida et al., 1992, 1993).

4.3.2 Experimental systems

A 2-year study of Fischer 344 rats and BDF_1 mice exposed orally to 4-chloronitrobenzene by diet indicated that the major target tissue for carcinogenicity in male and female rats is the spleen; in mice, cancers of the liver (in males and females) and lymphoma (in males) were also observed (see Section 3). The non-neoplastic toxic effects observed in the 2-year study included haematotoxicity in the rats exposed at the two higher doses (200 and 1000 ppm) and mice exposed at the two higher doses (500 and 2000 ppm), as indicated by haematology findings and non-neoplastic lesions in the spleen in both rats and mice, and increased incidence of adrenal gland hyperplasia and pheochromocytoma in rats (Matsumoto et al., 2006b).

The non-neoplastic toxic effects of exposure to 4-chloronitrobenzene were also observed in several subchronic studies. In a small 2-week inhalation study, Bucher (1993) observed early evidence of haematotoxicity and spleen effects in Fischer 344 rats and $B6C3F_1$ mice and kidney lesions in rats. In a 4-week inhalation study in Sprague-Dawley rats, Nair et al. (1986) reported haematotoxicity in males and females, including effects associated with methaemoglobinaemia, effects on haematological parameters, and histopathological changes in the spleen. In a 13-week inhalation study, Bucher (1993) observed haematotoxicity and spleen lesions in male and female Fischer 344 rats and $B6C3F_1$ mice, with rats being the most sensitive. In rats, effects on the liver, kidney, and Harderian gland were also observed, as well as testicular atrophy in males. In mice, liver effects were reported, as well as hyperplasia of the forestomach in females. In a 13-week study of dietary exposure, Matsumoto et al. (2006a) also reported haematotoxicity and spleen lesions in male and female Fischer 344 rats and BDF_1 mice, with rats being the most sensitive. Signs of hepatotoxicity were also apparent, particularly in mice.

The United States National Toxicology Program (NTP, 1989) conducted a 2-year bioassay with the 4-chloronitrobenzene metabolite, 4-chloroaniline. Non-neoplastic effects in Fischer 344 rats included an increased incidence of fibrosis of the spleen and dose-related increases in methaemoglobin and other signs of haematotoxicity, such as bone marrow hyperplasia and hepatic haemosiderosis, in males and females, and adrenal medullary hyperplasia in females. Effects in $B6C3F_1$ mice included increased haemosiderin deposits in males and females as well as increased extramedullary haematopoiesis in the liver of females.

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

4-Chloronitrobenzene is a high production volume chemical that is currently produced primarily in China and India. Between 1995 and 2004, production volumes in China increased 3-fold from 78 000 to 250 000 tonnes per annum; the 2004 production volume represented 60% of total annual global production.

4-Chloronitrobenzene is used as an intermediate in the synthesis of various chemicals, including agricultural chemicals and antioxidants used in the rubber industry. It is also used: in the pharmaceutical industry for the synthesis of certain drugs; in the treatment of textiles and leather; in the production of paint, pigments, colorants, and plastics; and in the paper industry.

The compound is not known to occur naturally, but it can be released to the environment as a by-product of production or manufacture; release may also occur during transport, storage, or disposal, or accidentally. It has been detected in various water sources in Asia, Europe, and North America, and is considered moderately persistent in the environment. 4-Chloronitrobenzene has also been detected at low concentrations in edible fish.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 4-chloronitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Detectable levels of 4-chloronitrobenzene have been measured in workplace air in chemical factories in China and Japan, and its metabolites have been detected in the urine of Chinese chemical factory workers.

No quantitative data on exposure to 4-chloronitrobenzene in the general population were available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

4-Chloronitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet, including one study in male and female mice and one study in male and female rats, conducted in the same laboratory. In limited studies of oral exposure by diet in another laboratory, 4-chloronitrobenzene was tested in one study in male and female mice and one study in male rats.

In the GLP study of oral exposure by diet in male mice, 4-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular carcinoma and malignant lymphoma. In the limited study of oral exposure by diet in male mice, 4-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma and vascular tumours.

In the GLP study of oral exposure by diet in female mice, 4-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular carcinoma and liver haemangiosarcoma; 4-chloronitrobenzene also induced a significant increase in the incidence of liver haemangiosarcoma. In the limited study of oral exposure by diet in female mice, 4-chloronitrobenzene induced a significant increase in the incidence of vascular tumours.

In the GLP study of oral exposure by diet in male rats, 4-chloronitrobenzene induced a significant positive trend in the incidence and a significant increase in the incidence of

splenic tumours (fibroma, fibrosarcoma, osteosarcoma, sarcoma (not otherwise specified), and haemangiosarcoma); 4-chloronitrobenzene also induced a significant positive trend in the incidence of adrenal gland pheochromocytoma. In the limited study of oral exposure by diet in male rats, 4-chloronitrobenzene did not induce a significant increase in the incidence of any tumours.

In the GLP study of oral exposure by diet in female rats, 4-chloronitrobenzene induced a significant positive trend in the incidence of splenic tumours (fibroma, fibrosarcoma, osteosarcoma, and haemangiosarcoma) and adrenal gland pheochromocytoma; 4-chloronitrobenzene also induced a significant increase in the incidence of splenic fibrosarcoma and adrenal gland pheochromocytoma.

5.4 Mechanistic and other relevant data

Data on absorption, distribution, metabolism and excretion in humans are available from occupational exposures, including an accidental high-exposure episode. The major metabolic pathways include glutathione conjugation and reduction to 4-chloroaniline, which is further metabolized including to the reactive intermediate *N*-hydroxy-4-chloroaniline. In rats exposed dermally, orally, or by inhalation, 4-chloronitrobenzene is absorbed, widely distributed to tissues, and excreted as metabolites in the urine and faeces.

Concerning the key characteristics of carcinogens, there is *moderate* evidence that 4-chloronitrobenzene is genotoxic. An increased frequency of chromosomal aberrations was observed in workers exposed to various chloronitrobenzenes including 4-chloronitrobenzene. DNA strand breaks were observed in: two studies of human cells in vitro; in mouse liver, kidney, and brain after exposure by intraperitoneal injection; and in rat hepatocytes exposed in vitro. In Chinese hamster cells, 4-chloronitrobenzene increased the frequency of sister-chromatid exchanges, but results on chromosomal aberrations were inconclusive. Mutagenicity tests in multiple strains of *Salmonella typhimurium* gave largely negative results with or without metabolic activation. Genotoxicity results for the 4-chloroaniline metabolite were generally positive in a variety of assays.

There is *moderate* evidence that 4-chloronitrobenzene induces oxidative stress. Oxidative damage to erythrocytes was observed after exposure to 4-chloronitrobenzene. Methaemoglobinaemia was observed in humans and rodents, and toxic effects to erythrocytes were seen in mice and rats in subchronic and chronic exposure studies. In orally exposed rats, 4-chloronitrobenzene elevated lipid peroxidation in the spleen and liver.

There is *moderate* evidence that 4-chloronitrobenzene alters cell proliferation, cell death, or nutrient supply in rodents. No data in humans were available. In subchronic and chronic studies in rodents, erythropoiesis increased in bone marrow, spleen, and liver, and hyperplasia occurred in female mouse forestomach, rat spleen, and rat adrenal gland.

There is *weak* evidence (scarcity of data) that 4-chloronitrobenzene induces chronic inflammation (in the rat spleen) and is immunosuppressive.

Haematotoxicity was seen in highly exposed humans and in rodents, and rodent kidney and liver toxicity was also observed.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 4-chloronitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-chloronitrobenzene.

6.3 Overall evaluation

4-Chloronitrobenzene is *possibly carcinogenic to humans (Group 2B).*

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1,4-DICHLORO-2-NITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

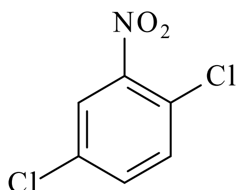
Chem. Abstr. Serv. Reg. No.: 89-61-2

Chem. Abstr. Serv. name: 1,4-dichloro-2-nitrobenzene

IUPAC systematic name: 2,5-dichloronitrobenzene

Synonyms: 2,5-dichloro-1-nitrobenzene; benzene, 1,4-dichloro-2-nitro-; 2,5-DCNB.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_3Cl_2NO_2$

Relative molecular mass: 192.00

1.1.3 Chemical and physical properties of the pure substance

Description: solid, crystalline, and pale yellow plates and prisms with a faint aromatic odour; the substance can react dangerously with strong bases and strong oxidizing agents (IFA, 2018)

Density (at 22 °C*)*: 1.67 g/cm^3 (IFA, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 3.03 (IFA, 2018)

Henry law constant (at 22 °C*)*: 1.52 Pa m^3/mol (HSDB, 2013)

Melting point: 56 °C (IFA, 2018)

Boiling point: 267 °C (IFA, 2018)

Volatility: vapour pressure, 0.38×10^{-2} mm Hg [0.51 Pa] at 25 °C (HSDB, 2013)

Solubility: soluble in ethanol, ether, benzene, carbon disulfide; slightly soluble in carbon tetrachloride; 95 mg/L in water at 25 °C; 83 mg/L in water at 20 °C (HSDB, 2013; IFA, 2018)

Flammable limits: lower explosion limit: 2.4 vol% (191 g/m^3); upper explosion limit: 8.5 vol% (677 g/m^3) (IFA, 2018)

Flash point: 135 °C (IFA, 2018)

Ignition temperature: 465 °C (IFA, 2018).

1.2 Production and use

1.2.1 Production process

Almost-pure 1,4-dichloro-2-nitrobenzene is produced as a chemical intermediate in closed systems by nitrating 1,4-dichlorobenzene at low temperatures (35–65 °C) (HSDB, 2013).

1.2.2 Production volume

1,4-Dichloro-2-nitrobenzene has been listed as a chemical with a high production volume (OECD, 2009). The annual production volume in Japan was 200–1200 tonnes during 1988–1992; in Germany in 1992, it was 2400–2800 tonnes per year (OECD-SIDS, 1996). One manufacturer in the USA is listed in the Hazardous Substances Data Bank as a producer of 1,4-dichloro-2-nitrobenzene (EPA, 2018). Between 1998 and 2002, annual production volume was 10 000–500 000 pounds [~4.5–227 tonnes] in the USA (HSDB, 2013). Annual production volume in and import into Japan was reported to be 2100 tonnes per year in 2000 (Yamazaki et al., 2006). 1,4-Dichloro-2-nitrobenzene is currently manufactured in or imported into the European Economic Area at volumes of 1–10 tonnes per year (ECHA, 2018). No information on current production volumes in other countries or economies was available to the Working Group.

1.2.3 Use

1,4-Dichloro-2-nitrobenzene has been extensively used as an intermediate in the manufacture of diazo pigments. Additional uses have been in the production of agrochemicals and ultraviolet absorbents (OECD-SIDS, 1996). Registered industrial uses in the European Union include the colouring of paper products, chemicals, textiles, leather, and fur (ECHA, 2018).

1.3 Methods of measurement and analysis

1.3.1 Air

No methods have been described for the determination of 1,4-dichloro-2-nitrobenzene in air samples.

1.3.2 Other environmental media

1,4-Dichloro-2-nitrobenzene has been analysed as part of a multianalyte method for the determination of semivolatile nitroaromatics and cyclic ketones by gas chromatography with electron capture detection or nitrogen phosphorus detection in water and soil samples. However, detection might be hampered due to low resolution and potentially accompanying contaminants such as 4-chloro-3-nitrobenzene and 2,4-dichloro-1-nitrobenzene. Sample preparation is usually carried out by ultrasonic extraction using organic solvents such as methylene chloride for water samples. If necessary, further clean-up steps using florisil or size-exclusion (gel permeation) chromatography can be performed. No data on detection or quantitation limits have been reported (EPA, 1996).

1.3.3 Biomarkers

No methods of measurement or analysis have been identified for biomarkers of exposure to 1,4-dichloro-2-nitrobenzene in urine or blood. [The Working Group noted that suitable biomarkers in exposed individuals could include 2,5-dichloroaniline and 4-nitro-2,5-dichlorophenol in urine and haemoglobin adducts of 2,5-dichloroaniline in blood.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

1,4-Dichloro-2-nitrobenzene is not known to occur naturally (HSDB, 2013).

If used in the production of dyestuffs, agrochemicals, and coloured consumer products, 1,4-dichloro-2-nitrobenzene can be released through various waste streams (HSDB, 2013; ECHA, 2018) and is considered moderately persistent in the environment (OECD-SIDS, 1996).

With an analytical limit of detection of 20 μg/L, 1,4-dichloro-2-nitrobenzene has not been detected in surface water samples from 21 areas of Japan since 1982 (OECD-SIDS, 1996). The local concentration of 1,4-dichloro-2-nitrobenzene in a bay close to a Japanese manufacturer has been predicted to be 0.8 μg/L based on an estimated release of 8 tonnes of the compound per year, a total effluent of 1.0×10^{10} L per year, and a dilution factor of 1000 (OECD-SIDS, 1996). If released to water, a generic level III fugacity model suggests that most of the 1,4-dichloro-2-nitrobenzene (> 90%) remains in the aqueous system, and about 8% is transported to soil and sediment (OECD-SIDS, 1996). 1,4-Dichloro-2-nitrobenzene remained stable in an aqueous hydrolysis test to abiotic hydrolysis at pH 4–9 at a temperature of 25 °C. A half-life of 34 days has been calculated as a result of decay by photodegradation from water (OECD-SIDS, 1996). Concentrations in fish are 120–820 times greater than in water, suggesting a moderate potential for bioconcentration in aquatic organisms (Deneer et al., 1987; Niimi et al., 1989; Franke et al., 1994).

No data on the concentration of 1,4-dichloro-2-nitrobenzene and its stability in soil have been found. If released to soil, a generic level III fugacity model suggests that most of the 1,4-dichloro-2-nitrobenzene (> 95%) remains in the soil, and about 3% is transported into the aqueous system (OECD-SIDS, 1996).

No data have been found on the concentration of 1,4-dichloro-2-nitrobenzene in environmental air. If released to air, the compound is susceptible to photodegradation by sunlight as a result of ultraviolet absorption at wavelengths greater than 290 nm (OECD-SIDS, 1996; HSDB, 2013). The half-life in air is estimated to be approximately 1 week. In addition, 1,4-dichloro-2-nitrobenzene is photochemically degraded in the atmosphere in the presence of hydroxyl radicals, with an estimated half-life of approximately 320 days (HSDB, 2013). If released to air, a generic level III fugacity model suggests that approximately 7% and 80% of the released 1,4-dichloro-2-nitrobenzene is transported to water and soil, respectively (OECD-SIDS, 1996).

1.4.2 Occurrence in food

No data on the occurrence of 1,4-dichloro-2-nitrobenzene in food samples were available. However, an average daily intake in humans through drinking-water and consumption of edible fish has been estimated as 2.6×10^{-5} and 1.2×10^{-3} mg/kg per day, respectively (OECD-SIDS, 1996).

1.4.3 Exposure in the general population

Data on exposure of the general population to 1,4-dichloro-2-nitrobenzene were not available to the Working Group. It has previously been reported that 1,4-dichloro-2-nitrobenzene, or dyes and antimicrobials based on the compound, are not known ingredients in consumer products (OECD-SIDS, 1996). However, recent information suggests that manufactured consumer products may contain residues of this substance or coloured products that are based on this substance, including: washing and cleaning products; automobile care products; paints and coatings or adhesives; and long-life materials

for indoor use and with low release rates such as flooring, furniture, toys, curtains, footwear, and paper and cardboard products (ECHA, 2018).

1.4.4 Occupational exposure

Quantitative data on occupational exposure to 1,4-dichloro-2-nitrobenzene were not available to the Working Group; however, the main routes of occupational exposure are expected to be through inhalation in workplaces where 1,4-dichloro-2-nitrobenzene is produced or used as an intermediate in the manufacture of diazo dyes or agrochemical products (HSDB, 2013; IFA 2018). In addition, 1,4-dichloro-2-nitrobenzene is expected to be absorbed via skin; unspecified systemic effects have been observed in workers after repeated dermal exposure (IFA, 2018). Gastrointestinal absorption is also expected based on a kinetic model, and this route might be of importance when inhaled dust particles are transported out of the airways by mucociliary clearance into the gastrointestinal tract (BUA, 1991; IFA, 2018). [The Working Group noted that exposure via ingestion may also arise from inadvertent hand-to-mouth contact.]

Registered industrial uses in the European Union indicate that exposure may also occur in the manufacture of 1,4-dichloro-2-nitrobenzene and its downstream uses, including textile finishing, the manufacture of pulp, paper, and paper products, and as a chemical intermediate (ECHA, 2018).

1.5 Regulations and guidelines

Concerning human health, 1,4-dichloro-2-nitrobenzene is harmful if swallowed (H302, category 4) according to the Globally Harmonized System of Classification and Labelling of Chemicals. The substance may also cause an allergic skin reaction (H317, category 1) and skin and eye irritation (H315, H319, category 2) (ECHA, 2018). Precautionary measures should include avoiding oral uptake and skin contact (IFA, 2018).

No occupational or environmental exposure limit values for 1,4-dichloro-2-nitrobenzene were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were randomized by weight and fed diets containing 1,4-dichloro-2-nitrobenzene (purity, > 98.8%) at a concentration of 0, 320, 800, and 2000 ppm for 2 years (104 weeks) (Yamazaki et al., 2006). All mice (except for one male in the control group) underwent complete necropsy. Although survival analysis did not show a difference between groups exposed to 1,4-dichloro-2-nitrobenzene and control groups, the survival rates of the males and females at 2000 ppm tended to be lower after week 65 of treatment, which was attributed to increased tumour-associated mortality. Survival to 2 years for the groups at 0, 320, 800, and 2000 ppm was 27/49, 35/50, 26/50, and 18/50 in males, and 30/50, 27/50, 28/50, and 23/50 in females, respectively. At termination of treatment, the body weights of the males at 800 and 2000 ppm and of the females at 2000 ppm were significantly decreased relative to their respective control groups.

Table 3.1 Studies of carcinogenicity with 1,4-dichloro-2-nitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (M) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 49, 50, 50, 50 27, 35, 26, 18	*Liver* Hepatocellular adenoma 17/49, 21/50, 20/50, 16/50 Hepatocellular carcinoma 15/49, 15/50, 23/50, 31/50* Hepatoblastoma 1/49, 10/50*, 12/50*, 25/50* Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 26/49, 34/50, 41/50*, 45/50*	 NS $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of mice
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 30, 27, 28, 23	*Liver* Hepatocellular adenoma 5/50, 5/50, 17/50*, 16/50* Hepatocellular carcinoma 1/50, 3/50, 15/50*, 31/50* Hepatoblastoma 0/50, 0/50, 0/50, 2/50 Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 6/50, 8/50, 29/50*, 39/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test NS $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of mice Historical control incidence of hepatoblastoma was 0/1048 female mice

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 40, 44, 41, 39	*Liver* Hepatocellular adenoma 0/50, 1/50, 0/50, 6/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of rats Historical control incidence in 1249 male rats (maximum incidence in any study): hepatocellular carcinoma, 0.2% (2%); renal cell adenoma, 0.16% (2%); renal cell carcinoma, 0.16% (2%); Zymbal gland adenoma, 0.2% (2%)
		Hepatocellular carcinoma 0/50, 0/50, 1/50 (2%), 2/50 (4%)	NS	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 1/50, 1/50, 8/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Kidney Renal cell adenoma 0/50, 0/50, 0/50, 2/50 (4%)	NS	
		Renal cell carcinoma 0/50, 1/50 (2%), 0/50, 1/50 (2%)	NS	
		Renal cell adenoma or carcinoma (combined) 0/50, 1/50, 0/50, 3/50	$P < 0.05$, Peto trend test	
		Zymbal gland: adenoma 0/50, 0/50, 0/50, 4/50 (8%)	$P < 0.01$, Peto trend test	
Rat, F344/DuCrj (F) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 38, 35, 39, 34	Any tumour type: no significant increase		Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of animals

F, female; GLP, good laboratory practice; M, male; NS, not significant; ppm, parts per million; wk, week

In male mice, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase (*P* < 0.01, Peto trend test) in the incidence of hepatocellular carcinoma, hepatoblastoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular carcinoma (15/49, 15/50, 23/50, and 31/50) was significantly (*P* < 0.01, Fisher exact test) increased in males at 2000 ppm. The incidence of hepatoblastoma (1/49, 10/50, 12/50, and 25/50) was significantly (*P* < 0.01, Fisher exact test) increased in all exposed groups of males. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (26/49, 34/50, 41/50, and 45/50) was significantly (*P* < 0.01, Fisher exact test) increased in males at 800 and 2000 ppm.

In female mice, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase (*P* < 0.01, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (5/50, 5/50, 17/50, and 16/50) was significantly (*P* < 0.01, Fisher exact test) increased in females at 800 and 2000 ppm. The incidence of hepatocellular carcinoma (1/50, 3/50, 15/50, and 31/50) was significantly (*P* < 0.01, Fisher exact test) increased in females at 800 and 2000 ppm. The incidence of hepatoblastoma in exposed female mice was not significantly increased (0/50, 0/50, 0/50, and 2/50), although the incidence in females exposed at 2000 ppm (2/50) exceeded the upper bound of the range in historical controls from the laboratory (0%, 0/1048 female mice). The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (6/50, 8/50, 29/50, and 39/50) was significantly (*P* < 0.01, Fisher exact test) increased in female mice at 800 and 2000 ppm.

Dietary exposure to 1,4-dichloro-2-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the liver (hepatocellular hypertrophy with nuclear atypia; centrilobular in males and females at all concentrations, and acidophilic foci in males at 800 and 2000 ppm). [The Working Group noted that the strengths of this well-conducted study that complied with GLP included the use of multiple doses, a large number of mice per group, and testing in males and females.]

3.2 Rat

Oral administration

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were randomized by weight and fed diets containing 1,4-dichloro-2-nitrobenzene (purity, > 98.8%) at a concentration of 0, 320, 800, or 2000 ppm for 2 years (104 weeks) (Yamazaki et al., 2006). All rats underwent complete necropsy. Survival analysis did not show a difference between the groups exposed to 1,4-dichloro-2-nitrobenzene and control groups. Survival to 2 years for the groups at 0, 320, 800, and 2000 ppm was 40/50, 44/50, 41/50, and 39/50 in males, and 38/50, 35/50, 39/50, and 34/50 in females, respectively. At the termination of treatment, the body weights of all exposed males and of the females at 2000 ppm were significantly decreased relative to their respective control groups.

In male rats, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase (*P* < 0.01, Peto trend test) in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). The incidence of hepatocellular adenoma (0/50, 1/50, 0/50, and 6/50) was significantly (*P* < 0.05, Fisher exact test) increased in males at 2000 ppm. The incidence of hepatocellular carcinoma was 0/50, 0/50, 1/50 (2%), and

2/50 (4%), respectively. The incidence of hepatocellular carcinoma in the group at the highest dose (2/50) exceeded the upper bound of the range for historical controls (3/1249, 0.2%) in 25 studies that reported a maximum incidence of 2%. The incidence of hepatocellular adenoma or carcinoma (combined) (0/50, 1/50, 1/50, and 8/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 2000 ppm. Dietary administration of 1,4-dichloro-2-nitrobenzene also caused a significant dose-related increase ($P < 0.05$, Peto trend test) in the incidence of renal cell adenoma or carcinoma (combined) in male rats. By pair-wise comparison, the incidence of renal cell adenoma or carcinoma (combined) (0/50, 1/50, 0/50, and 3/50) was not significantly increased in any exposed group of males. By pair-wise comparison, the incidence of renal cell adenoma (0/50, 0/50, 0/50, and 2/50) was not significantly increased in any exposed group of males. The incidence of renal cell adenoma in males at 2000 ppm (2/50, 4%) exceeded the upper bound of the range for historical controls from this laboratory (4% in this study versus (vs) an upper bound of 2% in historical controls). By pair-wise comparison, the incidence of renal cell carcinoma (0/50, 1/50, 0/50, and 1/50) was not significantly increased in any exposed group of males. The incidence of renal cell carcinoma in any exposed group of males did not exceed the upper bound of the range for historical controls from this laboratory (0–2% in this study vs an upper bound of 2% in historical control groups). Dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of adenoma of the Zymbal gland in males. By pair-wise comparison, the incidence of adenoma of the Zymbal gland (0/50, 0/50, 0/50, and 4/50) was not significantly increased in any exposed group of males, but the incidence in males at 2000 ppm exceeded the upper bound of the range for historical control from this laboratory (8% in this study vs an upper bound of 2% in historical controls).

In female rats, dietary administration of 1,4-dichloro-2-nitrobenzene did not cause a significant increase in the incidence of any type of neoplasm.

In male rats, dietary administration of 1,4-dichloro-2-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the liver (basophilic cell foci in the groups at 800 and 2000 ppm) and in the kidney (chronic progressive nephropathy in all exposed groups; mineralization of the papilla in groups at 800 and 2000 ppm; urothelial hyperplasia of the pelvis in all exposed groups). [The Working Group noted that the strengths of this well-conducted study that complied with GLP included the use of multiple doses, a large number of rats per group, and testing in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

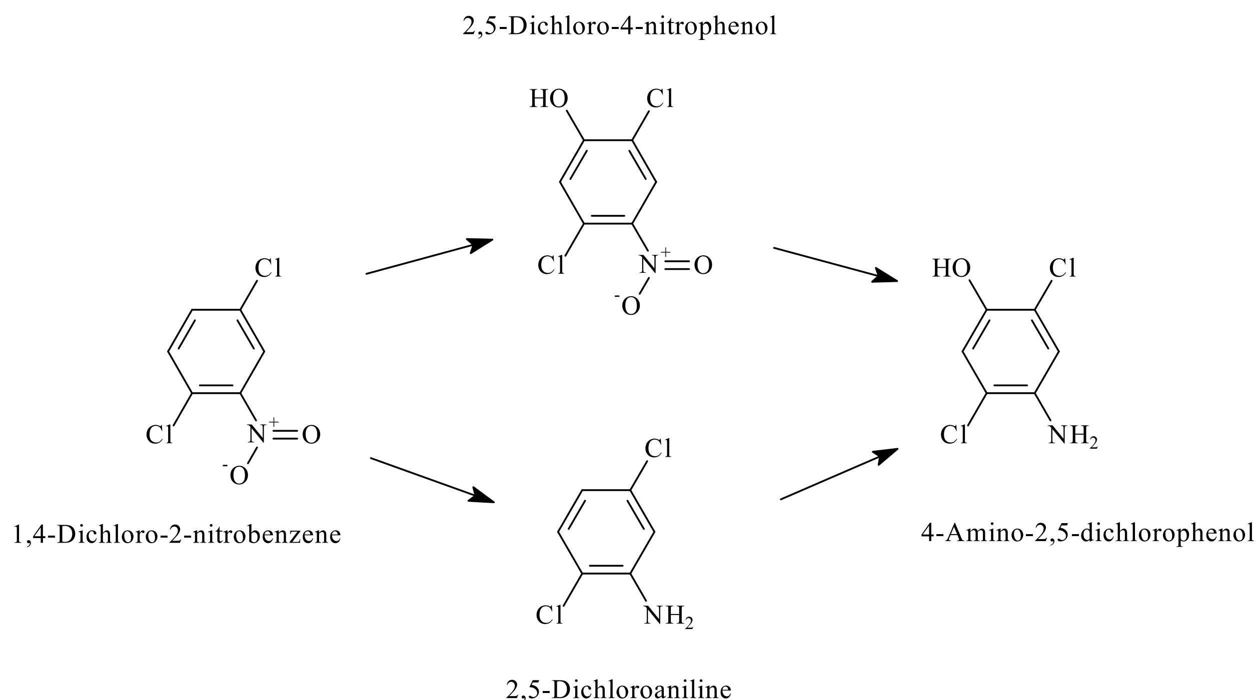
4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Data on the absorption, distribution, metabolism, and excretion of 1,4-dichloro-2-nitrobenzene in experimental systems were sparse. On the basis of studies of metabolism and other studies, gastrointestinal absorption of 1,4-dichloro-2-nitrobenzene occurs. For example, Bray et al. (1957) gave a group of 6–10 doe rabbits (weight, 2–3 kg) an aqueous oral suspension of 1,4-dichloro-2-nitrobenzene at a dose of 0.4 g/kg body weight (bw). After exposure to 1,4-dichloro-2-nitrobenzene, rabbit

Fig. 4.1 Reported urinary metabolites of 1,4-dichloro-2-nitrobenzene observed in rabbits after oral exposure to 1,4-dichloro-2-nitrobenzene



Adapted with permission from *Biochemical Journal*. Bray HG, James SP, Thorpe WV, The metabolism of 2:4-, 2:5- and 3:4-dichloronitrobenzene in the rabbit, 1957; Volume 65, issue 3: 483-490 © The Biochemical Society (Bray et al., 1957)

urine samples were collected and metabolites identified using paper chromatography and absorption spectra. Some oxidative metabolites were excreted as mercapturic acid (9–33%), glucuronide (8–56%), and sulfate (3–21%) metabolites. The main urinary metabolites of 1,4-dichloro-2-nitrobenzene observed in rabbits after exposure were 2,5-dichloroaniline (13%), *N*-acetyl-*S*-(4-chloro-2-nitrophenyl)-L-cysteine (2%), and 4-amino-2,5-dichlorophenol (1%). Fig. 4.1 shows a partial metabolic scheme for 1,4-dichloro-2-nitrobenzene.

Ohnishi et al. (2004) identified the urinary metabolites of 1,4-dichloro-2-nitrobenzene in three male Fischer 344/DuCrj rats given a diet containing 1,4-dichloro-2-nitrobenzene at 1% for 2 days. Individual urine samples were collected for 24 hours using metabolic cages, and samples were subsequently pooled yielding a single sample. Urine samples were analysed using a variety of analytical chemistry methods including liquid chromatography with tandem mass spectrometry. The main urinary metabolite was an *N*-acetylcysteine conjugate, *N*-acetyl-*S*-(4-chloro-3-nitrophenyl)-L-cysteine.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016). 1,4-Dichloro-2-nitrobenzene has only been studied in a small number of assays related to genotoxicity.

Table 4.1 Genetic and related effects of 1,4-dichloro-2-nitrobenzene in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster lung, CHL/IU	(+)	–	LEC, 24 μg/mL (–S9) HIC, 94 μg/mL (+S9)	Cytotoxic at 0.15 mg/mL (–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)

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

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

Table 4.2 Genetic and related effects of 1,4-dichloro-2-nitrobenzene in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA1535/pSK1002	Mutation, other (SOS)	+	NT	1000 μg/mL		Jin & Qian (1991)
Salmonella typhimurium TA1535/pSK1002	Reverse mutation	+	NT	5000 μg/mL		Jin & Qian (1991)
Salmonella typhimurium TA100	Reverse mutation	+	+	5000 μg/plate	Cytotoxic at 1250 μg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Salmonella typhimurium TA1535	Reverse mutation	+	–	LEC, 78 μg/plate (–S9) HIC, 5000 μg/plate (+S9)	Cytotoxic at 1250 μg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	5000 μg/plate	Cytotoxic at 1250 μg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Escherichia coli WP2 *uvrA*	Reverse mutation	–	–	5000 μg/plate	Cytotoxic at 1250 μg/plate (+/–S9)	MHW Japan (1994), cited in OECD SIDS (1996)

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

[a] +, positive; –, negative; NT, not tested

4.2.1 Genetic and related effects

See Table 4.1 and Table 4.2

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data in non-human mammals in vivo were available to the Working Group.

A test for chromosomal aberration was conducted in cultured Chinese hamster lung (CHL/IU) cells (MHW Japan, 1994, as cited in OECD-SIDS, 1996). [The Working Group noted that results were available from a secondary source, but were unable to obtain the primary source documentation to determine whether positive results occurred as a result of cytotoxicity.]

Jin & Qian (1991) reported that 1,4-dichloro-2-nitrobenzene (at concentrations of ≥ 1000 μg/mL) induces *umu* expression and is mutagenic in *Salmonella typhimurium* strain TA1535 with the plasmid pSK1002.

Additional limited data from secondary sources were also available to the Working Group. An assay for reverse gene mutation was conducted, using the pre-incubation method. 1,4-Dichloro-2-nitrobenzene yielded positive results in *S. typhimurium* strain TA100 with and without metabolic activation, and in strain TA1535 without metabolic activation. Negative results were obtained in *S. typhimurium* (strain TA98 and TA1537) and in *Escherichia coli* WP2 *uvrA* with and without metabolic activation, at concentrations of up to 5 mg per plate (MHW Japan, 1994, as cited in OECD-SIDS, 1996). [The Working Group noted that these results were available from a secondary source, but was unable to obtain the primary source documentation to determine whether positive results occurred as a result of cytotoxicity.]

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Other adverse effects of 1,4-dichloro-2-nitrobenzene that may be related to carcinogenicity (see Section 3) include toxicity in the liver, kidney, lymphohaematopoietic system, and the male reproductive tract in rodents. [The Working Group noted that carcinogenic effects were seen in mouse and rat liver.]

The most sensitive end-points observed in mice and rats after 14-day and 13-week exposure to 1,4-dichloro-2-nitrobenzene included hepatic effects in male and female mice and rats, and renal effects in male rats only (Yamazaki et al., 2005a, b). Mice exposed at the highest doses demonstrated a reduced terminal body weight and increased incidence of mortality. Treatment-related histopathological changes were seen in the liver, kidney, testes, and haematopoietic systems. Centrilobular single-cell hepatic necrosis and increased hepatocyte mitosis were seen after exposure to 1,4-dichloro-2-nitrobenzene. Increased incidences of proximal tubule hyaline droplets and granular casts suggestive of α_{2u}-globulin nephropathy were also observed in male rats orally exposed to 1,4-dichloro-2-nitrobenzene. Although these studies suggest that α_{2u}-globulin nephropathy occurs in male rats after exposure to 1,4-dichloro-2-nitrobenzene (Yamazaki et al., 2005a, b), the findings are incomplete with respect to fulfilling all criteria for concluding that 1,4-dichloro-2-nitrobenzene operates through response associated with α_{2u}-globulin in male rats (IARC, 1999). In particular, criteria not met included chemical identification of the protein accumulating in tubule cells as α_{2u}-globulin, and reversible binding of 1,4-dichloro-2-nitrobenzene or a metabolite to α_{2u}-globulin.

Yamazaki et al. (2006) also performed a 2-year study of toxicity in groups of 50 male and 50 female mice and rats fed diet containing 1,4-dichloro-2-nitrobenzene at a concentration of 320, 800, or 2000 ppm. As for the shorter-term studies, relative liver weight was increased in rats at 320 ppm and above and in mice at 800 ppm and above. Centrilobular hepatocyte hyperplasia with nuclear atypia was increased in mice in all dose groups. Relative kidney weights were also increased in exposed male rats and male mice. Lesions consistent with chronic progressive nephropathy were seen more frequently in male rats. An increased incidence of urothelial hyperplasia in the renal pelvis and of renal papilla mineralization was seen in male rats at 320 ppm and above, and 800 ppm and above, respectively. An increased incidence of renal haemosiderin deposition and increased numbers of bone marrow erythroblasts occurred in male mice at 2000 ppm.

4.4 Data related to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

1,4-Dichloro-2-nitrobenzene has been classified as a chemical with a high production volume, although data on current production volumes and locations are limited. It is used as an intermediate in the manufacture of diazo pigments, agrochemicals, and ultraviolet absorbents.

The compound is not known to occur naturally, but it can be released to the environment as a by-product of manufacturing and downstream uses.

Some coloured consumer products may contain residues of 1,4-dichloro-2-nitrobenzene, although quantitative information on exposure in the general population was not found.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 1,4-dichloro-2-nitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Quantitative information on exposure in occupational settings was not available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

1,4-Dichloro-2-nitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet from the same laboratory, one in male and female mice and one in male and female rats.

In male mice, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In female mice, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). Two female mice at the highest dose developed hepatoblastoma; no hepatoblastomas have been reported in female mice in the historical database of the laboratory.

In male rats, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence

and an increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). In male rats exposed at the highest dose, the incidence of hepatocellular carcinoma exceeded the upper bound of the range for historical controls for the laboratory. In male rats, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence of renal cell adenoma or carcinoma (combined) and of adenoma of the Zymbal gland. In male rats exposed at the highest dose, the incidence of adenoma of the Zymbal gland exceeded the upper bound of the range for historical controls for the laboratory.

In female rats exposed to 1,4-dichloro-2-nitrobenzene, there was no significant increase in the incidence of any neoplasm.

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion of 1,4-dichloro-2-nitrobenzene in humans were available. In rodents, 1,4-dichloro-2-nitrobenzene is absorbed after oral exposure and is metabolized to aniline and phenol metabolites; these metabolites can undergo secondary mercapturic acid, glucuronide, sulfate, and *N*-acetylcysteine conjugation.

Concerning the key characteristics of carcinogens, there is *weak* evidence that 1,4-dichloro-2-nitrobenzene is genotoxic. No data in exposed humans or in non-human mammals in vivo were available. In a single test, 1,4-dichloro-2-nitrobenzene gave positive results solely in the absence of metabolic activation for chromosomal aberrations in Chinese hamster lung cells, but concurrent cytotoxicity could not be ruled out. 1,4-Dichloro-2-nitrobenzene gave positive results in some, but not all, tested *Salmonella typhimurium* strains in the absence or presence of metabolic activation.

Exposure to 1,4-dichloro-2-nitrobenzene resulted in toxicity in the liver, kidney, lymphohaematopoietic system, and male reproductive tract in rodents. No data in exposed humans were available. Increased incidences of proximal tubule hyaline droplets and granular casts, suggestive of α_{2u}-globulin nephropathy, were also observed in male rats orally exposed to 1,4-dichloro-2-nitrobenzene by diet; however, the criteria established by IARC for considering the induction of kidney tumours to have occurred by a response associated with α_{2u}-globulin were not met.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1,4-dichloro-2-nitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,4-dichloro-2-nitrobenzene.

6.3 Overall evaluation

1,4-Dichloro-2-nitrobenzene is *possibly carcinogenic to humans (Group 2B).*

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2,4-DICHLORO-1-NITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

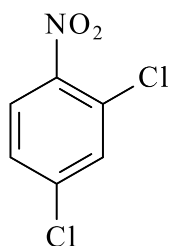
Chem. Abstr. Serv. Reg. No.: 611-06-3

Chem. Abstr. Serv. name: 2,4-dichloronitrobenzene

IUPAC systematic name: 2,4-dichloronitrobenzene

Synonyms: 2,4-dichloro-1-nitrobenzene; 1,3-dichloro-4-nitrobenzene; benzene, 2,4-dichloro-1-nitro-; 2,4-DCNB; nitro-*m*-dichlorobenzene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_3Cl_2NO_2$

Relative molecular mass: 192.00

1.1.3 Chemical and physical properties of the pure substance

Description: solid, crystalline, and pale yellow needles with a faint aromatic odour; the substance can react dangerously with bases, amines, and oxidizing agents (IFA, 2018)

Density: 1.54 g/cm^3 at 15 °C (IFA, 2018)

Relative vapour density (air = 1): 6.6 (PubChem, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 3.07 (IFA, 2018)

Henry law constant (at 25 °C): 3.22×10^{-5} atm m^3/mol [3.26 Pa m^3/mol] (HSDB, 2008)

Melting point: 34 °C (IFA, 2018)

Boiling point: 258 °C (IFA, 2018)

Volatility: vapour pressure, 1.43×10^{-2} mm Hg (estimated) [~190 Pa] at 25 °C (HSDB, 2008)

Solubility: entirely soluble in ethanol and ether, and slightly soluble in chloroform (HSDB, 2008); slightly soluble in water with 188 mg/L at 20 °C (IFA, 2018)

Flammable limits: poorly flammable, prone to combustion (IFA, 2018)

Flash point: 152 °C (IFA, 2018)

Ignition temperature: 500 °C (IFA, 2018).

1.2 Production and use

1.2.1 Production process

2,4-Dichloro-1-nitrobenzene is produced as a chemical intermediate in closed systems by nitrating 1,3-dichlorobenzene with mixed acid, and separating the product from the resulting isomer mixture (HSDB, 2008).

1.2.2 Production volume

In 1990, the production volume of 2,4-dichloro-1-nitrobenzene was about 1500 tonnes in Germany and approximately 50 tonnes in Japan (OECD-SIDS, 1996). It was listed in 2004 and 2007 as a chemical with a high production volume (OECD, 2004, 2009). Manufacturers in Germany and France were registered with the European Chemical Agency (ECHA) as producers of 2,4-dichloro-1-nitrobenzene for the years 2013 and 2017, respectively (ECHA, 2018). No other information on recent production volumes of 2,4-dichloro-1-nitrobenzene was available.

1.2.3 Use

2,4-Dichloro-1-nitrobenzene has been used as an intermediate in the manufacture of diazo pigments (Yamazaki et al., 2006), pharmaceuticals, and agrochemicals (e.g. diazoxide and chlomethoxyfen) (OECD-SIDS, 1996; HSDB, 2008).

1.3 Methods of measurement and analysis

1.3.1 Air

The only method that has been described for the determination of 2,4-dichloro-1-nitrobenzene in air is part of a multianalyte method for the determination of multiple semivolatile organic compounds. Soxhlet extraction of air filters is followed by solid-phase extraction on florisil or size-exclusion (gel permeation) chromatography. The final analysis can be conducted by gas chromatography with either electron capture detection or mass spectrometry. Because 2,4-dichloro-1-nitrobenzene can co-elute with some other compounds, particularly (di/tri) chloronitrobenzenes, analysis by gas chromatography with mass spectrometry is recommended. No data on quantitation limits were available for this method (EPA, 1998).

1.3.2 Other environmental media

The United States Environmental Protection Agency (EPA) multianalyte method for the determination of semivolatile organic compounds in air described above can also be applied to water and soil samples. Estimated quantitation limits were reported as 0.01–0.10 mg/L in water and 0.5–1.0 mg/kg in soil when using gas chromatography with mass spectrometry. Recovery from soil samples was low (20–40%) (EPA, 1998).

1.3.3 Biomarkers

No methods of measurement and analysis have been identified for biomarkers of exposure to 2,4-dichloro-1-nitrobenzene in urine or blood. [The Working Group noted that suitable biomarkers of exposure could include 2,4-dichloroaniline in urine and its corresponding haemoglobin adduct in blood of exposed individuals.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

2,4-Dichloro-1-nitrobenzene is not known to occur naturally (HSDB, 2008).

2,4-Dichloro-1-nitrobenzene can be released to the environment through various waste streams from industry, where it is used as an intermediate in the manufacture of various

agrochemicals, dyes, and pharmaceuticals (Meylan & Howard, 1991; HSDB, 2008).

2,4-Dichloro-1-nitrobenzene was detected but not quantified in drinking-water and in effluent from advanced wastewater treatment that was collected in Seattle and Cincinnati, USA, in 1976 and in 1978, respectively (Lucas, 1984). 2,4-Dichloro-1-nitrobenzene was also detected, but not quantified, in fish collected in the Main river, Germany (Steinwandter, 1989). Average chloronitrobenzene (isomers not specified) concentrations of 17.3 and 19.2 μg/kg fat were measured in zebra mussels and eels, respectively, collected from the Rhine and Meuse delta (Hendriks et al., 1998).

Based on its octanol/water partition coefficient, if released into water 2,4-dichloro-1-nitrobenzene will either be adsorbed to the sediment or released by volatilization from water surfaces into the air (HSDB, 2008). The half-life of the compound in rivers has been predicted as 17 days. For structural reasons (aromaticity), degradation of 2,4-dichloro-1-nitrobenzene by hydrolysis is not likely to be an important process in the environment (HSDB, 2008). A moderate to high bioconcentration factor of between 80 and 150 has been suggested in various fish and algae (Freitag et al., 1985; Niimi et al., 1989; Meylan & Howard, 1991; HSDB, 2008).

2,4-Dichloro-1-nitrobenzene has been qualitatively detected in river sediment in Japan (Tokuda et al., 1985). The compound is expected to have no mobility in soil; biodegradation in soil is estimated to be low (< 0.1%) based on a 5-day biological oxygen demand test and a medium bioaccumulation factor of 310 in activated sludge (Freitag et al., 1985; HSDB, 2008).

2,4-Dichloro-1-nitrobenzene has not been detected in environmental air. However, volatilization of the compound into the atmosphere is expected to be an important emission process based on its air to water partition coefficient (Henry constant). 2,4-Dichloro-1-nitrobenzene contains chromophores that absorb at wavelengths of greater than 290 nm; the compound may therefore degrade by direct photolysis from sunlight (HSDB, 2008). The mass of 2,4-dichloro-1-nitrobenzene adsorbed on silica gel decreased by 11.2% after irradiation at wavelengths of greater than 290 nm for 17 hours (Freitag et al., 1985). Reaction with photochemically generated hydroxyl radicals was also reported as an important alternative pathway of degradation, with an estimated half-life of approximately 130 days (Freitag et al., 1985; HSDB, 2008).

1.4.2 Exposure in the general population

Exposure of the general population to 2,4-dichloro-1-nitrobenzene has not been reported, and the compound is not found in consumer products (OECD-SIDS, 1996). According to the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals regulation No. 1907/2006 Annex XVII, 2,4-dichloro-1-nitrobenzene is prohibited in consumer goods such as decorative objects or games; exposure of the general population is therefore not expected through the use or handling of these consumer products (IFA, 2018).

1.4.3 Occupational exposure

Measurements of occupational exposure to 2,4-dichloro-1-nitrobenzene were not available to the Working Group. However, exposure may occur through both inhalation and dermal contact at workplaces where 2,4-dichloro-1-nitrobenzene is produced or used as an intermediate (HSDB, 2008). [The Working Group noted that exposure by ingestion may also arise from inadvertent hand to mouth contact.]

1.5 Regulations and guidelines

Concerning human health, 2,4-dichloro-1-nitrobenzene is suspected of causing cancer (H351, category 2) and of causing genetic defects (H341, category 2) according to the Globally Harmonized System of Classification and Labelling of Chemicals. The substance is also harmful if swallowed (H302, category 4) and toxic if in contact with skin (H311, category 3), and may cause an allergic skin reaction (H317, category 1B). Precautionary measures should include wearing protective clothing (P280) and, if the skin is contaminated, washing with plenty of soap and water (P302, P352) (IFA, 2018).

No occupational or environmental exposure limit values were available for 2,4-dichloro-1-nitrobenzene in air.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were randomized by weight and fed diet containing 2,4-dichloro-1-nitrobenzene (purity, 99.4%) at a concentration of 0, 750, 1500, or 3000 ppm (males) and 0, 1500, 3000, or 6000 ppm (females) for 2 years (104 weeks) (Kano et al., 2012). All mice (except for one female in the control group) underwent complete necropsy. The survival rates of the males at 3000 ppm and the females at 3000 and 6000 ppm were significantly decreased as a result of increased mortality from tumours attributable to treatment. Survival rates to the end of 2 years for the males at 0, 750, 1500, and 3000 ppm were 35/50, 38/50, 29/50, and 23/50. Survival rates to the end of 2 years for the females at 0, 1500, 3000, and 6000 ppm were 28/49, 28/50, 18/50, and 19/50. Relative to their respective control groups, body weights were significantly decreased in males at 3000 ppm and in females at 3000 and 6000 ppm at the termination of treatment.

In male mice, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (18/50, 34/50, 30/50, and 43/50) was significantly increased in males at 1500 ppm ($P < 0.05$, Fisher exact test) and in males at 750 and 3000 ppm ($P < 0.01$, Fisher exact test). The incidence of hepatocellular carcinoma (7/50, 7/50, 11/50, and 15/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 3000 ppm. The incidence of hepatoblastoma (1/50, 5/50, 16/50, and 27/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 1500 and 3000 ppm. The incidence of hepatoblastoma in male mice at 750 ppm exceeded the upper bound of the range for historical control groups from this laboratory (10% in this study versus an upper bound of 6% in historical control groups). The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (19/50, 39/50, 41/50, and 45/50) was significantly ($P < 0.01$, Fisher exact test) increased in all treated males. Dietary administration of 2,4-dichloro-1-nitrobenzene caused a dose-related increase in peritoneal haemangiosarcoma ($P < 0.01$, Peto trend test) in males. The incidence of peritoneal haemangiosarcoma (1/50, 0/50,

Table 3.1 Studies of carcinogenicity with 2,4-dichloro-1-nitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF_1 (M) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 35, 38, 29, 23	*Liver* Hepatocellular adenoma 18/50, 34/50*, 30/50**, 43/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, **$P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of mice Historical control incidence in 1496 male mice (maximum incidence in any study): hepatoblastoma, 0.7% (6%); peritoneal haemangiosarcoma, 0.2% (4%)
		Hepatocellular carcinoma 7/50, 7/50, 11/50, 15/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Hepatoblastoma 1/50, 5/50 (10%), 16/50*, 27/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 19/50, 39/50*, 41/50*, 45/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Peritoneum: haemangiosarcoma 1/50, 0/50, 2/50 (4%), 5/50 (10%)	$P < 0.01$, Peto trend test	
Mouse, Crj:BDF_1 (F) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 1500, 3000, 6000 ppm 49, 50, 50, 50 28, 28, 18, 19	*Liver* Hepatocellular adenoma 8/49, 25/50*, 42/50*, 45/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of mice Historical control incidence in 1498 female mice: hepatoblastoma, 0%; peritoneal haemangiosarcoma, 0.4% (maximum incidence in any study, 4%)
		Hepatocellular carcinoma 1/49, 2/50, 11/50*, 21/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 0/49, 2/50 (4%), 7/50*, 7/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 8/49, 28/50*, 43/50*, 48/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Kano et al. (2012) (cont.)		*Peritoneum:* haemangiosarcoma 0/49, 3/50 (6%), 7/50*, 17/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
Rat, F344/DuCrj (M) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 39, 42, 40, 40	*Kidney* Renal cell adenoma 0/50, 0/50, 3/50 (6%), 26/50* Renal cell carcinoma 0/50, 0/50, 2/50, 23/50* Renal cell adenoma or carcinoma (combined) 0/50, 0/50, 5/50*, 38/50** *Preputial gland:* adenoma 1/50, 4/50, 2/50, 7/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.05$, **$P < 0.01$, Fisher exact test $P < 0.05$, Peto trend test; *$P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of rats Historical control incidence of renal cell adenoma was 0.1% in 1749 male rats, with a maximum incidence of 2% in any study
Rat, F344/DuCrj (F) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 35, 44, 38, 43	*Kidney* Renal cell adenoma 0/50, 0/50, 3/50 (6%), 26/50* Renal cell carcinoma 0/50, 0/50, 0/50, 12/50* Renal cell adenoma or carcinoma (combined) 0/50, 0/50, 3/50, 32/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of rats Incidence of renal cell adenoma in historical controls was 0.1% in 1597 female rats, with a maximum incidence of 2% in any study

F, female; GLP, good laboratory practice; M, male; ppm, parts per million; wk, week

2/50, and 5/50) was not significantly increased in any exposed males compared with controls. The incidence of peritoneal haemangiosarcoma in males at 3000 ppm exceeded the upper range of historical control groups from this laboratory (10% in this study versus an upper bound of 4% in historical control groups).

In female mice, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (8/49, 25/50, 42/50, and 45/50) was significantly ($P < 0.01$, Fisher exact test) increased in all groups of treated females. The incidence of hepatocellular carcinoma (1/49, 2/50, 11/50, and 21/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm. The incidence of hepatoblastoma (0/49, 2/50, 7/50, and 7/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (8/49, 28/50, 43/50, and 48/50) was significantly ($P < 0.01$, Fisher exact test) increased in all groups of treated females. Dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase in the incidence of peritoneal haemangiosarcoma ($P < 0.01$, Peto trend test) in females. The incidence of peritoneal haemangiosarcoma (0/49, 3/50, 7/50, and 17/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in increases in the incidence of non-neoplastic lesions in the liver (hepatocellular hypertrophy (centrilobular) in all groups of treated males and in females exposed at 6000 ppm; acidophilic foci in females exposed at 3000 and 6000 ppm). [The Working Group noted that the strengths of this well-conducted GLP study included the use of multiple doses, the large number of mice per group, and testing in males and females.]

3.2 Rat

Oral administration

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were randomized by weight and fed diet containing 2,4-dichloro-1-nitrobenzene (purity, 99.4%) at a concentration of 0, 750, 1500, or 3000 ppm for 2 years (104 weeks) (Kano et al., 2012). All rats underwent complete necropsy. Survival analysis did not show a difference between groups exposed to 2,4-dichloro-1-nitrobenzene and control groups. Survival rates to the end of 2 years for the groups at 0, 750, 1500, and 3000 ppm were 39/50, 42/50, 40/50, and 40/50 in males, and 35/50, 44/50, 38/50, and 43/50 in females. Relative to their respective control groups, body weights were significantly decreased in all treated males and in the females exposed at 1500 and 3000 ppm at the termination of treatment.

In male rats, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of renal cell adenoma, renal cell carcinoma, and renal cell adenoma or carcinoma (combined). The incidence of renal cell adenoma (0/50, 0/50, 3/50, and 26/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 3000 ppm. The incidence of renal cell carcinoma (0/50, 0/50, 2/50, and 23/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 3000 ppm. The incidence of renal cell adenoma or carcinoma (combined) (0/50, 0/50, 5/50, and 38/50) was significantly increased in males at 1500 ppm ($P < 0.05$, Fisher exact test) and at 3000 ppm ($P < 0.01$, Fisher exact test). Dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase

($P < 0.05$, Peto test) in the incidence of adenoma of the preputial gland in males. The incidence of adenoma of the preputial gland (1/50, 4/50, 2/50, and 7/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 3000 ppm.

In female rats, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of renal cell adenoma, renal cell carcinoma, and renal cell adenoma or carcinoma (combined). The incidence of renal cell adenoma (0/50, 0/50, 3/50, and 26/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm. The incidence of renal cell carcinoma (0/50, 0/50, 0/50, and 12/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm. The incidence of renal cell adenoma or carcinoma (combined) (0/50, 0/50, 3/50, and 32/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the kidney (atypical tubular hyperplasia in all treated males and females; chronic progressive nephropathy in all treated males; chronic progressive nephropathy in females exposed at 750 and 1500 ppm; eosinophilic droplets in the proximal tubules in all treated males and females; mineralization of the papilla in all treated males; and urothelial hyperplasia of the pelvis in all treated males). [The Working Group noted that the strengths of this well-conducted GLP study included the use of multiple doses, the large number of rats per group, and testing in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

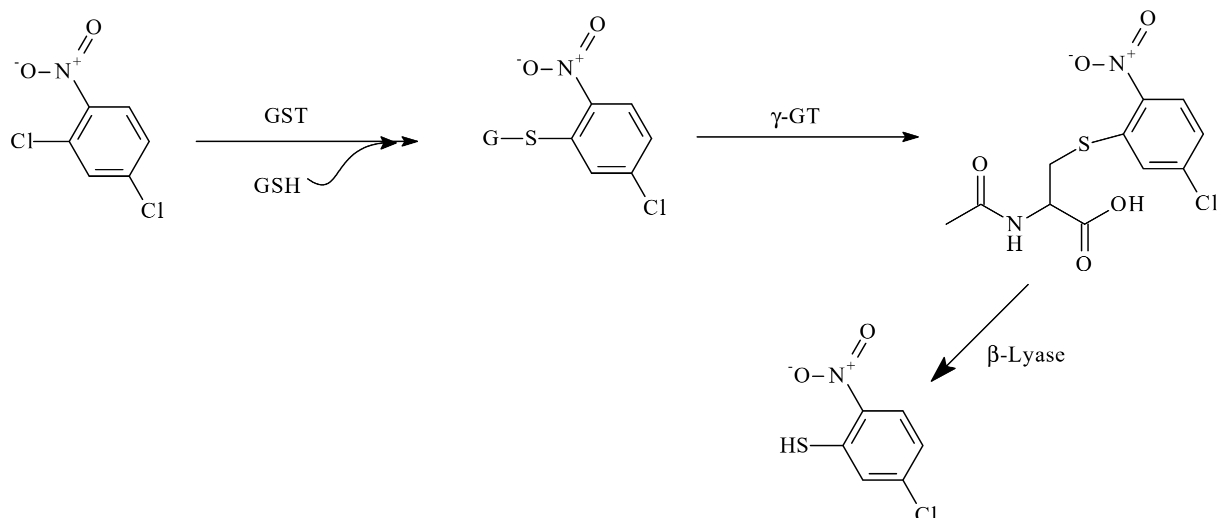
Data concerning the absorption, distribution, metabolism, and excretion of 2,4-dichloro-1-nitrobenzene in experimental systems were sparse.

Botham et al. (1987) evaluated the dermal uptake of ^{14}C-radiolabelled 2,4-dichloro-1-nitrobenzene in mice. Mice were dermally exposed by application of 50 µL of a solution containing 13 µmol of 2,4-dichloro-1-nitrobenzene suspended in acetone:olive oil (4:1). Dermal absorption was estimated to be 45% and 71% of the applied dose after 24 hours and 72 hours, respectively.

Bray et al. (1957) treated three groups of six doe rabbits (weight, 2–3 kg) with 2,4-dichloro-1-nitrobenzene at a dose of 200, 300, or 400 mg/kg body weight (bw) by aqueous oral suspension. Pronounced anorexia was observed in rabbits at the highest dose. After exposure to 2,4-dichloro-1-nitrobenzene, urine samples were collected and metabolites identified using paper chromatography and absorption spectra. Some oxidative metabolites were excreted as mercapturic acid and phenols. The main urinary metabolites observed in rabbits were *N*-acetyl-*S*-(5-chloro-2-nitrophenyl)-L-cysteine (23% of dose) and 2,4-dichloroaniline as the acetyl conjugate (1%).

Ohnishi et al. (2009) identified the urinary metabolites of 2,4-dichloro-1-nitrobenzene in three male Fischer 344/DuCrj rats fed diet containing 2,4-dichloro-1-nitrobenzene at a con-

Fig. 4.1 Putative steps in the metabolism of 2,4-dichloro-1-nitrobenzene in rats



GSH, glutathione; GST, glutathione *S*-transferase; γ-GT, γ-glutamyltransferase
Adapted from Ohnishi et al. (2009)

centration of 1% for 2 days. Individual samples of urine were collected for 24 hours using metabolic cages, and samples were subsequently pooled yielding a single sample. Urine samples were analysed using a variety of analytical chemistry methods including liquid chromatography with tandem mass spectrometry. The main urinary metabolite was the *N*-acetylcysteine conjugate *N*-acetyl-*S*-(5-chloro-2-nitrophenyl)-L-cysteine. Ohnishi et al. (2009) proposed the metabolic scheme depicted in Fig. 4.1 for 2,4-dichloro-1-nitrobenzene in rats.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016). 2,4-Dichloro-1-nitrobenzene has only been studied in a small number of assays related to genotoxicity.

4.2.1 Genetic and related effects

See Table 4.1

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data in non-human mammals in vivo were available to the Working Group.

A chromosomal aberration test was conducted using cultured Chinese hamster lung (CHL/IU) cells. [The Working Group noted that the results were available from a secondary source, but were unable to obtain the primary source documentation.] Structural chromosomal aberrations and polyploidy were not observed in CHL/IU cells after exposure in vitro to 2,4-dichloro-1-nitrobenzene at concentrations of up to 140 μg/mL, whether in the presence or absence of an exogenous metabolic activation system (MHW Japan, 1996, as cited in OECD-SIDS, 1996).

Table 4.1 Genetic and related effects of 2,4-dichloro-1-nitrobenzene in non-human mammalian cells in vitro and in non-mammalian experimental systems

Test system	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Chinese hamster lung, CHL	Chromosomal aberrations	–	–	140 µg/mL	Concentrations used: 0, 40, 70, 140 µg/mL	MHW Japan (1996), cited in OECD-SIDS (1996)
Salmonella typhimurium TA100	Reverse mutation	+	+	3.3 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	–	–	100 µg/plate	Toxicity at 215 µg/plate	Haworth et al. (1983)
Salmonella typhimurium TA1537	Reverse mutation	–	+	10 µg/plate	Toxicity at 215 µg/plate	Haworth et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	–	+	10 µg/plate		Haworth et al. (1983)

HIC, highest ineffective concentration; LEC, lowest effective concentration

[a] +, positive; –, negative

Non-mammalian experimental systems

2,4-Dichloro-1-nitrobenzene gave positive results in *Salmonella typhimurium* strains TA100, TA1537, and TA98, and negative results in strain TA1535 (Haworth et al., 1983).

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Other adverse effects of 2,4-dichloro-1-nitrobenzene that may be related to carcinogenicity (see Section 3) include toxicity in rodent liver and kidney, and in the mouse respiratory system. [The Working Group noted that carcinogenic effects in rodents were seen in mouse liver and rat kidney.]

Kano et al. (2012) evaluated the chronic toxicity of 2,4-dichloro-1-nitrobenzene administered in the diet to male and female Fischer 344/DuCrj rats and Crj:BDF$_1$ mice for 2 years. In rats, survival rate was unaffected by exposure to 2,4-dichloro-1-nitrobenzene. Terminal body weights decreased in all exposed male rats (decreases of 7–15%) and in female rats exposed at 1500 ppm and above (decreases of 8–14%). Increased absolute and relative liver weights were seen in all exposed male rats and in female rats at the two higher doses. Absolute kidney weights were also increased in all groups exposed to 2,4-dichloro-1-nitrobenzene, and relative kidney weight and blood urea nitrogen concentrations were significantly increased in all exposed male rats and in female rats at 1500 ppm and above. Rats exposed to 2,4-dichloro-1-nitrobenzene also developed atypical hyperplasia and eosinophilic droplets in the proximal tubule. The severity of chronic progressive nephropathy increased in a dose-related manner in male rats, and incidence

was increased in female rats at 750 and 1500 ppm. An increased incidence of urothelial hyperplasia in the renal pelvis and renal papilla mineralization was observed in male rats exposed to 2,4-dichloro-1-nitrobenzene.

In mice, unlike in rats, survival rates decreased after chronic exposure to 2,4-dichloro-1-nitrobenzene at 3000 ppm and above, attributed to fatal tumours. Similarly to rats, terminal body weights decreased in mice. This effect was seen in male mice at 1500 ppm and above (decreases of 10–27%) and in females exposed at 1500 ppm and above (decreases of 8–40%). Increased absolute and relative liver weights were observed in male mice exposed at 1500 ppm and above and in all treated female mice. Histological changes observed in male and female mice given 2,4-dichloro-1-nitrobenzene included pigment deposition and respiratory metaplasia (with increased numbers of submucosal glands) in the olfactory epithelium. An increased incidence of eosinophilic globules in the olfactory and respiratory epithelia was reported in exposed female mice, and an increased incidence of eosinophilic globules in the nasopharynx was observed in male and female mice (Kano et al., 2012).

4.4 Data related to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

2,4-Dichloro-1-nitrobenzene has been classified as a chemical with a high production volume, although data on current production volumes and locations are limited. It is used primarily as an intermediate in the manufacture of diazo pigments, pharmaceuticals, and agrochemicals.

The compound is not known to occur naturally, but it can be released to the environment as a by-product of manufacturing and downstream uses. It has been detected in water and soil.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 2,4-dichloro-1-nitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Quantitative information on exposure in occupational settings was not found.

According to European Union regulations, 2,4-dichloro-1-nitrobenzene is not permitted in consumer goods such as decorative objects and games. Quantitative information on exposure in the general population was not found.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

2,4-Dichloro-1-nitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet from the same laboratory, one in male and female mice and one in male and female rats.

In male mice, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In male mice, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence of peritoneal haemangiosarcoma.

In female mice, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence

of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In female mice, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of peritoneal haemangiosarcoma.

In male rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of renal cell adenoma, renal cell carcinoma, and of renal cell adenoma or carcinoma (combined). In male rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of preputial gland adenoma.

In female rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of renal cell adenoma, renal cell carcinoma, and of renal cell adenoma or carcinoma (combined).

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion of 2,4-dichloro-1-nitrobenzene in humans were available. In rodents, 2,4-dichloro-1-nitrobenzene is absorbed after oral or dermal exposure and is metabolized to aniline and phenol metabolites; these metabolites can undergo secondary mercapturic acid and *N*-acetylcysteine conjugation.

Concerning the key characteristics of carcinogens, there is *weak* evidence that 2,4-dichloro-1-nitrobenzene is genotoxic. No data in humans or in non-human mammals in vivo were available. 2,4-Dichloro-1-nitrobenzene gave negative results in the presence or absence of metabolic activation for chromosomal aberrations performed in a single study in Chinese hamster lung cells. In one study, 2,4-dichloro-1-nitrobenzene gave positive results in various *Salmonella typhimurium* strains in the presence or absence of metabolic activation.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in toxicity in rodent liver and kidney, and in the mouse respiratory system. No data for humans were available. In the chronic bioassay, both male and female rats exposed to 2,4-dichloro-1-nitrobenzene by diet developed atypical hyperplasia and eosinophilic droplets in the proximal tubule. Chronic progressive nephropathy occurred in male and female rats exposed to 2,4-dichloro-1-nitrobenzene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2,4-dichloro-1-nitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,4-dichloro-1-nitrobenzene.

6.3 Overall evaluation

2,4-Dichloro-1-nitrobenzene is *possibly carcinogenic to humans (Group 2B)*.

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2-AMINO-4-CHLOROPHENOL

1. Exposure Data

1.1 Identification of the agent

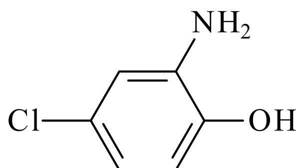
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 95-85-2

Chem. Abstr. Serv. name: 2-amino-4-chlorophenol

Synonyms: 1-amino-2-hydroxy-5-chlorobenzene; 1-hydroxy-2-amino-4-chlorobenzene; 2-amino 4-chloro phenol; phenol, 2-amino-4-chloro-; 5-chloro-2-hydroxyaniline; 4-chloro-2-aminophenol; *para*-chloro-*ortho*-aminophenol.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C_6H_6NOCl

Relative molecular mass: 143.57 (PubChem, 2018).

1.1.3 Chemical and physical properties of the pure substance

Description: brown crystalline powder with characteristic amine-like odour (Merck, 2012; PubChem, 2018)

Melting point: 140 °C (PubChem, 2018)

Volatility: vapour pressure, 0.18 Pa at 25 °C (Merck, 2012)

Density: 1.406 g/cm^3 (Emco Dyestuff Pvt. Ltd, 2018)

Water solubility: 2.3 g/L at 25 °C (Merck, 2012)

Octanol/water partition coefficient (P): log K_{ow} = 1.24 (PubChem, 2018)

Conversion factor: 1 ppm = 5.87 mg/m^3

Relative vapour density (air = 1): 5.0 (NIOSH, 2015)

Stability: does not burn; decomposes when heated or burnt to form toxic and corrosive emissions, including hydrogen chloride and nitrogen oxides; reacts with oxidizing agents (PubChem, 2018)

Impurities: available with a purity of greater than 95% (ThermoFisher Scientific, 2018).

1.2 Production and use

1.2.1 Production

2-Amino-4-chlorophenol is manufactured by converting 2,5-dichloronitrobenzene to 4-chloro-2-nitrophenol in a reaction with sodium hydroxide, followed by reduction with iron, hydrazine, or hydrogen, with Raney nickel or platinum catalyst (Yamazaki et al., 2009).

1.2.2 Production volume

In 2006, the annual production of 2-amino-4-chlorophenol in Japan was reported to be 500 tonnes, and it is currently listed as being produced in volumes of between 1 and 1000 tonnes (Yamazaki et al., 2009; NITE, 2018). 2-Amino-4-chlorophenol is listed as an intermediate with four active producers in Europe; between 1 and 10 tonnes are currently manufactured in or imported into Europe each year (ECHA, 2018). Information on quantities produced and used elsewhere in the world was not available to the Working Group.

1.2.3 Use

2-Amino-4-chlorophenol is used as an intermediate in the production of dyes, which in turn are used to colour textiles and clothing fabrics (e.g. mattresses, curtains, carpets, or textile toys), leather (e.g. gloves, shoes, purses, or furniture), and paper chemicals (e.g. tissues, feminine hygiene products, nappies, books, magazines, or wallpaper), and as inks and toners (ECHA, 2018). The compound is not used directly in consumer products, although trace amounts may be present in such products (Corbett, 1999). 2-Amino-4-chlorophenol has also been used in some hair dyes (Corbett, 1999; Anon., 2004). A United States Cosmetics Ingredients Review Expert Panel concluded that it was safe to use 2-amino-4-chlorophenol in oxidative hair dyes, but there was insufficient information to support its safety in nonoxidative (semipermanent) hair dyes (Anon., 2004). 2-Amino-4-chlorophenol is also used in the manufacture of the muscle relaxant chlorzoxazone (Belal et al., 2011).

1.3 Methods of measurement and analysis

1.3.1 Air

Air samples collected on polytetrafluoroethylene membrane filters with a 5 µm pore can be analysed for 2-amino-4-chlorophenol using high-performance liquid chromatography (HPLC) with ultraviolet detection, based on the United States National Institute for Occupational Safety and Health (NIOSH) analytical method 5013 for dyes (NIOSH, 1994).

1.3.2 Other environmental media

Puig & Barcelo (1996) reviewed the methods available for the analysis of phenols in water. An analytical method for measuring 2-amino-4-chlorophenol in water, based on solid-phase extraction followed by HPLC coupled with tandem mass spectrometry and electrospray ionization, was developed by Mourato (2014). The limit of quantification was 2 mg/L.

Dedhiya et al. (2016) described a method to assess the concentration of the 2-amino-4-chlorophenol contaminant in the drug chlorzoxazone. The method uses reversed-phase HPLC with particle size 5 µm, C18 column with 70:30:1% v/v/v water:acetonitrile:acetic acid as isocratic mobile phase, and ultraviolet detection. The limits of detection and quantification for 2-amino-4-chlorophenol were 0.5 mg/L and 2 mg/L, respectively. Similar analytical techniques described by others (Hassib et al., 2007; Belal et al., 2011) include a colorimetric test to identify 2-amino-4-chlorophenol as an impurity in chlorzoxazone powder using the reaction with 4-aminoantipyrine in the presence of alkaline

oxidizing agent, $K_3(Fe(CN)_6)/NH_3$, and measuring the produced red colour at 520 nm. This method reported detection and quantitation limits of 0.2 mg/L and 0.6 mg/L, respectively (Belal et al., 2011).

1.3.3 Biomarkers

No methods of measurement and analysis for biomarkers of exposure to 2-amino-4-chlorophenol were available to the Working Group.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

If accidentally released to the environment, 2-amino-4-chlorophenol may be found in air, water, or soil. It is also formed from the degradation of other anthropogenic toxic chemicals such as 4-chloro-2-nitrophenol. Degradation in the environment may occur through microbial metabolism, for example (Arora et al., 2012).

1.4.2 Exposure in the general population

Consumer exposure to very low concentrations may occur from residues in products where 2-amino-4-chlorophenol has been used as an intermediate (Belal et al., 2011). These exposures could arise from inhalation or ingestion; for example, low concentrations (< 0.0004–0.3200%) of 2-amino-4-chlorophenol have been identified in commercial formulations of the drug chlorzoxazone (Zhang et al., 2013; Dedhiya et al., 2016).

1.4.3 Occupational exposure

Occupational exposure to 2-amino-4-chlorophenol mainly occurs in industrial plants where the chemical is synthesized or used in the manufacture of other products (Yamazaki et al., 2009). Primary manufacturing is carried out in enclosed systems [the Working Group noted that this would be expected to minimize worker exposure], but higher levels of exposure are possible during packing, cleaning, or maintenance tasks (Yamazaki et al., 2009).

Exposure to 2-amino-4-chlorophenol in the workplace may also occur by inhalation and inadvertent ingestion (NIOSH, 2015). The primary route of exposure is by inhalation; dermal absorption can also be assumed from reports of skin sensitization of workers using this chemical (Anon., 2004).

1.5 Regulations and guidelines

2-Amino-4-chlorophenol is covered by generic occupational health and safety regulations relating to hazardous chemicals in many countries. It is registered under the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals regulations (ECHA, 2018).

According to the Globally Harmonized System of Classification and Labelling of Chemicals, 2-amino-4-chlorophenol is harmful if swallowed (H302, category 4) and may cause skin irritation (H315, category 2). Precautionary measures should therefore include the avoidance of exposure by inhalation (P261) (IFA, 2018).

The use of 2-amino-4-chlorophenol in cosmetic products was banned in 2008 in some south-east Asian countries through the Association of Southeast Asian Nations Cosmetics Directive (Health Sciences Authority, 2007). Its use was also recently banned in the late 2000s: in the European Union under the provision of regulation (European Commission) No. 1223/2009 (European Commission, 2018); in Canada through the Health Canada Cosmetic Ingredient Hotlist; and in New Zealand under the Cosmetic Products Group Standard. 2-Amino-4-chlorophenol is still permitted for use in hair dyes in Australia (NICNAS, 2015), China, and the USA, and in other countries.

No threshold limit values or MAK values (established by the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, or MAK Commission) have been established for 2-amino-4-chlorophenol (NIOSH, 2015; ACGIH, 2018; DFG, 2018).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crlj mice (age, 6 weeks) were fed diets containing 2-amino-4-chlorophenol (purity, > 99.1%) at a concentration of 0 (control), 512, 1280, or 3200 ppm for 104 weeks (2 years) (JBRC, 2008a). All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy. In males and females at 0, 512, 1280, or 3200 ppm, survival was similar to their respective controls; survival rates were 33/50, 34/50, 36/50, and 35/50 for males, and 34/50, 28/50, 28/50, and 30/50 for females. There was no significant difference in final body weight of treated male and female mice compared with their respective controls.

In male mice, the incidence of squamous cell papilloma of the forestomach (0/50, 4/50, 3/50, and 6/50) was significantly (P = 0.0133, Fisher exact test) increased in the group at 3200 ppm, with a significant positive trend (P = 0.0273, Peto trend test). The incidence of squamous cell papilloma of the forestomach in all groups of treated males was higher than the upper bound observed in male mice (incidence, 5/1946; range, 0–2%) in historical control groups in studies from the Japan Bioassay Research Center (JBRC).

In female mice, the incidence of squamous cell papilloma of the forestomach (0/50, 1/50, 1/50, and 3/50) was not significantly increased, but there was a small but significant positive trend (P = 0.0279, Peto trend test). The incidence in all groups was within the range observed for historical controls for JBRC female mice (incidence, 8/1947; range, 0–6%). [The Working Group noted that although all incidences in all groups of female mice were within the range for historical controls, there was also a significant positive trend in the incidence and an increase in the incidence of squamous cell papilloma of the forestomach in male mice.]

There was no treatment-related increase in non-neoplastic lesions in groups of treated males or females. [The Working Group noted that the strengths of this well-conducted GLP study included the use of multiple doses, a large number of mice per group, and testing in males and females.]

3.2 Rat

Oral administration

In a GLP study, groups of 50 male and 50 female Fischer 344/DuCrlCrlj rats (age, 6 weeks) were fed diet containing 2-amino-4-chlorophenol (purity, > 99.1%) at a concentration of 0 (control), 1280, 3200, or 8000 ppm, equivalent to 0, 56, 144, or 373 mg/kg body weight (bw) per day for males, and 0, 75, 184, or 469 mg/kg bw per day for females, for 104 weeks (JBRC, 2008b; Yamazaki et al., 2009). All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

Table 3.1 Studies of carcinogenicity with 2-amino-4-chlorophenol in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6D2F$_1$/Crlj (M) 6 wk 104 wk JBRC (2008a)	Oral > 99.1% Diet 0, 512, 1280, 3200 ppm 50, 50, 50, 50 33, 34, 36, 35	*Forestomach:* squamous cell papilloma 0/50, 4/50 (8%), 3/50 (6%), 6/50 (12%)*	P = 0.0273, Peto trend test; P = 0.0365, Cochran–Armitage trend test; *P = 0.0133, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; use of multiple doses; adequate number of mice Incidence in historical controls: forestomach squamous cell papilloma, 5/1946 (range, 0–2%)
Mouse, B6D2F$_1$/Crlj (F) 6 wk 104 wk JBRC (2008a)	Oral > 99.1% Diet 0, 512, 1280, 3200 ppm 50, 50, 50, 50 34, 28, 28, 30	*Forestomach:* squamous cell papilloma 0/50, 1/50 (2%), 1/50 (2%), 3/50 (6%)	P = 0.0279, Peto trend test	Principal strengths: well-conducted GLP study; males and females used; use of multiple doses; adequate number of mice Incidence in historical controls: forestomach squamous cell papilloma, 8/1947 (range, 0–6%)
Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2008b), Yamazaki et al. (2009)	Oral > 99.1% Diet 0, 1280, 3200, 8000 ppm (0, 56, 144, 373 mg/kg bw) 50, 50, 50, 50 33, 38, 39, 39	*Forestomach* Squamous cell papilloma 0/50, 2/50, 11/50*, 39/50**	P < 0.0001, Peto trend test; *P = 0.0003, **P < 0.0001, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; use of multiple doses; adequate number of rats Incidence in historical controls: forestomach squamous cell papilloma, 5/2249 (range, 0–2%); forestomach squamous cell carcinoma, 4/2249 (0–2%); urinary bladder transitional cell papilloma, 11/2249 (0–4%); urinary bladder transitional cell carcinoma, 0/2249
		Squamous cell carcinoma 0/50, 0/50, 0/50, 12/50*	P < 0.0001, Peto trend test; *P = 0.0001, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined) 0/50, 2/50, 11/50*, 43/50**	P < 0.0001, Peto trend test; *P = 0.0003, **P < 0.0001, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2008b), Yamazaki et al. (2009) (cont.)		*Urinary bladder*		
		Transitional cell papilloma		
		0/50, 0/50, 1/50, 0/50	NS	
		Transitional cell carcinoma		
		0/50, 0/50, 0/50, 7/50*	$P < 0.0001$, Peto trend test; *$P = 0.0062$, Fisher exact test	
		Transitional cell papilloma or carcinoma (combined)		
		0/50, 0/50, 1/50, 7/50*	$P < 0.0001$, Peto trend test; *$P = 0.0062$, Fisher exact test	
Rat, F344/DuCrlCrlj (F) 6 wk 104 wk JBRC (2008b), Yamazaki et al. (2009)	Oral > 99.1% Diet 0, 1280, 3200, 8000 ppm (0, 75, 184, 469 mg/kg bw) 50, 50, 50, 50 42, 45, 46, 40	*Forestomach*		Principal strengths: well-conducted GLP study; males and females used; use of multiple doses; adequate number of rats Incidence in historical controls: forestomach squamous cell papilloma, 5/2097 (range, 0–2%); forestomach squamous cell carcinoma, 0/2097
		Squamous cell papilloma		
		1/50, 1/50, 1/50, 25/50*	$P < 0.0001$, Peto trend test; *$P < 0.0001$, Fisher exact test	
		Squamous cell carcinoma		
		0/50, 0/50, 0/50, 2/50 (4%)	NS	
		Squamous cell papilloma or carcinoma (combined)		
		1/50, 1/50, 1/50, 25/50*	$P < 0.0001$, Peto trend test; *$P < 0.0001$, Fisher exact test	

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

Survival in groups of treated males and females was similar to that in their respective controls; survival rates were 33/50, 38/50, 39/50, and 39/50 in males, and 42/50, 45/50, 46/50, and 40/50 in females. There was a significant decrease in the body weights of females at 3200 and 8000 ppm of 7% and 13%, respectively, compared with female controls.

In male rats, there were significant increases in the incidence of squamous cell papilloma of the forestomach in those exposed at 3200 ppm (11/50; $P = 0.0003$, Fisher exact test) and at 8000 ppm (39/50; $P < 0.0001$, Fisher exact test) compared with controls (0/50), with a significant positive trend ($P < 0.0001$, Peto trend test). There was a significant increase in the incidence of squamous cell carcinoma of the forestomach at the highest dose (12/50, 24%) versus controls (0/50) ($P = 0.0001$, Fisher exact test), with a significant positive trend ($P < 0.0001$, Peto trend test). There was a significant increase in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in males at 3200 ppm (11/50; $P = 0.0003$, Fisher exact test) and at 8000 ppm (43/50; $P < 0.0001$, Fisher exact test) compared with controls, with a significant positive trend. As potential pre-neoplastic lesions, the incidence of squamous cell hyperplasia of the forestomach was significantly increased in males at 3200 and 8000 ppm. There was a significant increase in the incidence of transitional cell carcinoma of the urinary bladder in males at 8000 ppm (7/50; $P = 0.0062$, Fisher exact test) compared with controls (0/50), with a significant positive trend ($P < 0.0001$, Peto trend test).

In female rats, there was a significant increase in the incidence of squamous cell papilloma of the forestomach of females at 8000 ppm (25/50; $P < 0.0001$, Fisher exact test) compared with controls (1/50, 2%), with a significant positive trend ($P < 0.0001$, Peto test). The occurrence of squamous cell carcinoma of the forestomach was observed at the highest dose (2/50, 4%) compared with none in 50 controls. Squamous cell carcinoma of the forestomach was not observed in female rats (0/2097) in the JBRC historical control database. [On the basis of comparison with the historical control database, the Working Group concluded that the increase in the incidence of squamous cell carcinoma of the forestomach in females was related to treatment.] There was a significant increase ($P < 0.0001$, Fisher exact test) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach at the highest dose (25/50), with a significant positive trend ($P < 0.0001$, Peto trend test). As potential pre-neoplastic lesions, the incidence of squamous cell hyperplasia of the forestomach was significantly increased in females at 8000 ppm. [The Working Group noted that the strengths of this well-conducted GLP study included the use of multiple doses, the large number of rats per group, and testing in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The only available study reported that partially oxidized haemoglobin or methaemoglobin was elevated in circulating erythrocytes in the blood samples of all 21 workers who had handled 2-amino-4-chlorophenol (and other compounds) (Tomoda et al., 1989).

4.1.2 Experimental systems

No data were available to the Working Group.

[The Working Group noted that, on the basis of its hydrophilicity, orally administered 2-amino-4-chlorophenol is expected to be absorbed and eliminated via the kidneys.]

Table 4.1 Genetic and related effects of 2-amino-4-chlorophenol in non-human mammalian cells in vitro and in non-mammalian experimental systems

Test system	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Chinese hamster lung cells, CHL	Chromosomal aberrations	+	+	NR	JETOC (1997b)
Salmonella typhimurium TA1535	Reverse mutation	–	+	333 μg/plate	Zeiger et al. (1988)
Salmonella typhimurium TA97, TA98, TA100	Reverse mutation	–	–	1000 μg/plate	Zeiger et al. (1988)
Salmonella typhimurium TA100, TA1537	Reverse mutation	–	+	NR	JETOC (1997a)
Salmonella typhimurium TA98, TA1535, WP2*uvr*A	Reverse mutation	–	–	625 μg/plate	JETOC (1997a)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported
[a] +, positive; –, negative

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether 2-amino-4-chlorophenol: is genotoxic; or alters cell proliferation, cell death, or nutrient supply.

4.2.1 Genetic and related effects

See Table 4.1

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data in experimental animals in vivo were available to the Working Group. Exposure to 2-amino-4-chlorophenol gave a positive result in hamster lung cells in vitro, increasing the percentage of cells with structural chromosomal aberrations at some of the tested concentrations (JETOC, 1997b).

2-Amino-4-chlorophenol produced statistically significant mutagenic effects with (but not without) metabolic activation in *Salmonella typhimurium* strains TA100, TA1535, and TA1537, but not in other tested strains (Zeiger et al., 1988; JETOC, 1997a).

Because there is *weak* evidence of genotoxicity, including some positive results in vitro, the human relevance of forestomach tumours observed in rodents could not be ruled out (IARC, 2003).

4.2.2 Altered cell proliferation, cell death or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 2-year bioassay with 2-amino-4-chlorophenol in rats, forestomach nodules and squamous cell hyperplasia were observed (Yamazaki et al., 2009). Similar effects were not observed in mice (JBRC, 2008a).

In a 13-week test for oral toxicity in rats, 2-amino-4-chlorophenol induced proliferative lesions in the forestomach and urinary bladder (Yamazaki et al., 2009). Simple and papillary and/or nodular types of transitional cell hyperplasia

were observed in the urinary bladder of male rats fed diets containing 2-amino-4-chlorophenol.

4.3 Other adverse effects

4.3.1 Humans

As noted in Section 4.1.1 above, partially oxidized haemoglobin or methaemoglobin were elevated in circulating erythrocytes in the blood samples of all 21 workers who handled 2-amino-4-chlorophenol (and other compounds) (Tomoda et al., 1989).

4.3.2 Experimental systems

In a chronic bioassay in rats, 2-amino-4-chlorophenol decreased erythrocyte counts, haemoglobin, and haematocrit, indicating erythrocyte toxicity. Methaemoglobin concentrations and reticulocyte counts were concurrently increased (Yamazaki et al., 2009). In a chronic bioassay in mice, the effects observed included eosinophilic changes in the mouse nasal respiratory epithelium, and clinical chemistry changes indicating renal and hepatic toxicity in males (JBRC, 2008a).

Nephrotoxic effects were observed when male Fischer 344 rats were exposed to 2-amino-4-chlorophenol by intraperitoneal injection, inducing mild effects on renal function and marked proximal tubular damage; hepatotoxicity was not observed (Hong et al., 1996). In vitro, 2-amino-4-chlorophenol affected organic ion accumulation, pyruvate-stimulated gluconeogenesis, and lactate dehydrogenase leakage in a renal cortical slice system (Hong et al., 1996).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

2-Amino-4-chlorophenol is manufactured in relatively small quantities in Japan and Europe; information on production elsewhere in the world was unavailable. It is used as an intermediate in the manufacture of dyes used in textiles and other consumer products, and in the manufacture of the muscle relaxant chlorzoxazone. It is also used in some hair dyes, although its use in this way has been banned in several countries.

The compound is not known to occur naturally, but can be released to the environment as a by-product of manufacturing and downstream uses. Quantitative information on levels in the environment was not available.

Some consumer products may contain residues of 2-amino-4-chlorophenol, and low levels have been detected in the drug chlorzoxazone. However, quantitative information on exposure in the general population was not available.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 2-amino-4-chlorophenol is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Quantitative information on exposure in occupational settings was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

2-Amino-4-chlorophenol was tested for carcinogenicity in two well-conducted good laboratory practice (GLP) studies of oral exposure by diet from the same laboratory: one in male and female mice, and one in male and female rats.

In male mice, 2-amino-4-chlorophenol induced a significant positive trend in the incidence and a significant increase in the incidence of squamous cell papilloma of the forestomach.

In female mice, 2-amino-4-chlorophenol induced a significant positive trend in the incidence of squamous cell papilloma of the forestomach.

In male rats, 2-amino-4-chlorophenol induced a significant positive trend in the incidence and a significant increase in the incidence of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach. In male rats, 2-amino-4-chlorophenol induced a significant positive trend in the incidence and a significant increase in the incidence of transitional cell carcinoma of the urinary bladder.

In female rats, 2-amino-4-chlorophenol induced a significant positive trend in the incidence and a significant increase in the incidence of squamous cell papilloma, and squamous cell papilloma or carcinoma (combined) of the forestomach. Two animals developed a squamous cell carcinoma of the forestomach at the highest dose tested; this cancer was not observed in the historical database of the laboratory.

5.4 Mechanistic and other relevant data

No studies evaluating absorption, distribution, metabolism, or excretion in humans or in experimental animals were available.

Concerning the key characteristics of carcinogens, there is *weak* evidence that 2-amino-4-chlorophenol is genotoxic; available data were scarce and there were inconsistencies in the reported results. No data in humans or experimental animals in vivo were available. In one study, 2-amino-4-chlorophenol induced chromosomal damage in Chinese hamster lung cells in the presence or absence of metabolic activation. In bacteria, 2-amino-4-chlorophenol induced mutations in some strains, but only with metabolic activation.

There is *moderate* evidence that 2-amino-4-chlorophenol alters cell proliferation, cell death, or nutrient supply. Data were available for hyperplasia, but not for other relevant end-points. In 13-week and 2-year studies of oral exposure, hyperplastic lesions in the forestomach were observed in male and female rats; similar results were not seen in mice. Hyperplasia was observed in the urinary bladder of male rats in the 13-week study.

Haematotoxicity, especially methaemoglobin and other erythrocyte effects, was reported in exposed workers and in the rat (but not the mouse) chronic toxicity bioassay. In an acute toxicity study of intraperitoneal exposure in male rats, nephrotoxicity was seen.

Because there is *weak* evidence of genotoxicity, including some positive results in vitro, the human relevance of forestomach tumours observed in rodents could not be ruled out.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2-amino-4-chlorophenol.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-amino-4-chlorophenol.

6.3 Overall evaluation

2-Amino-4-chlorophenol is *possibly carcinogenic to humans (Group 2B).*

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ORTHO-PHENYLENEDIAMINE AND *ORTHO*-PHENYLENEDIAMINE DIHYDROCHLORIDE

1. Exposure Data

ortho-Phenylenediamine, the parent compound of *ortho*-phenylenediamine dihydrochloride, is a basic compound and will undergo acid–base reactions. *ortho*-Phenylenediamine and its dihydrochloride salt will undergo a pH-dependent acid–base equilibrium in the body.

1.1 *ortho*-Phenylenediamine

1.1.1 Identification of the agent

(a) Nomenclature

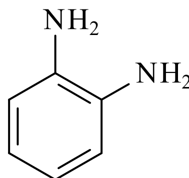
Chem. Abstr. Serv. Reg. No.: 95-54-5

Chem. Abstr. Serv. name: *ortho*-phenylenediamine

IUPAC systematic name: *ortho*-phenylenediamine

Synonyms: 2-aminoaniline; 1,2-benzenediamine; 1,2-diaminobenzene; 1,2-phenylenediamine; 2,3-diaminobenzene; orthamine; *o*-phenylenediamine.

(b) Structural and molecular formula, and relative molecular mass



Molecular formula: $C_6H_8N_2$

Relative molecular mass: 108.14

(c) Chemical and physical properties of the pure substance

Description: solid, crystalline powder, colourless to weak red; can react dangerously with oxidizing agents and concentrated acid (IFA, 2018); colourless monoclinic crystals if pure; technical-grade brownish-yellow crystals or a sandy brown solid when containing oxidized impurities (DFG, 1999)

Density (at 20 °C): 1.14 g/cm³ (IFA, 2018)

Relative vapour density (air = 1): 3.73 (PubChem, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 0.15 (HSDB, 2013)

Melting point: 99–102 °C (IFA, 2018); 102.1 °C (HSDB, 2013)

Boiling point: 257 °C (IFA, 2018)

Volatility: vapour pressure, 2.06×10^{-3} mm Hg at 25 °C (HSDB, 2013)

Solubility: 4.07×10^4 mg/L in water at 35 °C; soluble in water and freely soluble in alcohol, chloroform, ether, and benzene (HSDB, 2013)

Flammable limits: lower explosion limit, 1.5 vol% (ILO, 2017)

Flash point: 110 °C (IFA, 2018)

Ignition temperature: 540 °C (IFA, 2018)

Purity (technical grade): 99% or greater (HSDB, 2013)

Dissociation constants (of the conjugated acids BH^+): $pK_a(1) < 2$; $pK_a(2) = 4.47$ (at 25 °C) (HSDB, 2013).

1.1.2 Production and use

(a) Production process

ortho-Phenylenediamine is produced by hydrogenation of 2-nitroaniline, which is obtained by the amination of 2-chloronitrobenzene with ammonia. Commercial hydrogenation is achieved by using palladium catalysts, but iron, hydrazine, or hydrogen sulfide can also be used (Smiley, 2002).

(b) Production volume

ortho-Phenylenediamine was listed as a chemical with a high production volume in 1990, with a production volume in or import into the USA of greater than 1 million pounds [~453 tonnes] (HSDB, 2013). In the USA, the production range was 1–10 million pounds [~453–4535 tonnes] between 1986 and 2002 and in 2006 (HSDB, 2013). Two manufacturers are currently listed in the USA (HSDB, 2013). *ortho*-Phenylenediamine was not listed as a chemical with a high production volume in 2004 (OECD, 2004), but was listed as such in 2009 (OECD, 2009). Annual production and import of *ortho*-phenylenediamine in 2008 in Japan was approximately 2300 tonnes (Matsumoto et al., 2012). It is currently manufactured in or imported into the European Economic Area, but the European Chemicals Agency has no information on annual production or import volume; the registration requirements suggest that this volume is less than 1 tonne (ECHA, 2018). Quantities produced and used elsewhere in the world were not available to the Working Group.

(c) Use

ortho-Phenylenediamine is used as an important precursor (intermediate) in the production of a wide variety of heterocyclic compounds, including synthetic dyes and pigments that are used as colorants for furs and hair dye (HSDB, 2013). Heterocyclic compounds based on *ortho*-phenylenediamine are also widely used in the manufacture of agrochemicals, antioxidants in rubber products, corrosion inhibitors, polyamides, ultraviolet absorbers, and pharmaceuticals (HSDB, 2013).

1.1.3 Methods of measurement and analysis

(a) Air

Early methods for the determination of *ortho*-phenylenediamine in air samples (stationary sampling) relied on its direct collection in acetic anhydride in fritted bubblers, its conversion to the corresponding diacetamide derivative, and analysis by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection (Burg et al., 1980). To make the method applicable for personal air sampling, Tenax tubes have been used to collect *ortho*-phenylenediamine followed by desorption and derivatization to the corresponding diacetamide derivative (Elia et al., 1982). The limits of detection using HPLC-UV were dependent on the sampling capacity of the Tenax tubes and sampling flow rates. Air concentrations as low as 0.05 mg/m³ have been analysed using this method (Elia et al., 1982). Another method for the measurement of *ortho*-phenylenediamine in

air samples is available, and relies on the chemical conversion of aromatic amines to amine salts when collected on fibreglass filters coated with dilute sulfuric acid (OSHA, 1991). The filters are extracted with an aqueous ethylenediaminetetraacetic acid (EDTA) solution and the extracts are analysed for the free amine by HPLC-UV. A similar method has been described by the Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK commission) of the German Research Foundation (DFG, 2002) by using a fibreglass filter impregnated with hydrochloric acid rather than sulfuric acid. The adsorbed *ortho*-phenylenediamine is desorbed with a mixture of acetonitrile and aqueous ammonia and analysed by HPLC-UV.

(b) Other environmental media

Several methods are capable of analysing *ortho*-phenylenediamine in water samples. A method based on the induction of chemiluminescence by *ortho*-phenylenediamine in the presence of the superoxide anion radical has been developed to analyse *ortho*-phenylenediamine in shampooing wastewater after dying hair (Zhou et al., 2004). A colorimetric method for the determination of *ortho*-phenylenediamine in water samples, which relies on the formation of silver nanoparticles by the reduction of silver ions in the presence of *ortho*-phenylenediamine and the use of an UV–visible spectrophotometer, has also been described (Li et al., 2015); the limit of detection was approximately 0.2 µmol/L. A method using surface-enhanced Raman spectroscopy has also been developed for the analysis of *ortho*-phenylenediamine in water samples, based on its chemical cyclization with nitrite to benzotriazole (Ma et al., 2015); the limit of detection was reported as 30 nmol/L.

Multiple analytical methods have been described for the determination of *ortho*-phenylenediamine in hair dyes and cosmetic products, including the use of HPLC-UV or diode array detection (Rastogi, 2001; Zhou et al., 2004; Lai et al., 2012) and electrophoresis with amperometric detection (Wang & Huang, 2005; Dong et al., 2008). To prevent oxidation of the target compound, the addition of sodium dithionite has been suggested (Lai et al., 2012). Depending on the method, limits of detection as low as 10 µg/L can be achieved (Dong et al., 2008).

(c) Biomarkers

No biomonitoring methods for the assessment of exposure to *ortho*-phenylenediamine were identified in the literature. [The Working Group noted that measurement of *ortho*-phenylenediamine in urine, although not entirely specific, might serve as a biomarker of exposure; similar to the determination of *para*-phenylenediamine in urine (Gube et al., 2011; Bhandari et al., 2016; Mohamed & Steenkamp, 2016).]

Similar to other amino- and nitroaromatic compounds, *ortho*-phenylenediamine induces the formation of methaemoglobin in blood. Methaemoglobin levels above 1.5% (ACGIH, 2006) or 5% (Leng & Bolt, 2008) have previously been suggested as a general and non-specific biomarker of occupational exposure to aromatic amino- and nitroaromatic compounds.

1.1.4 Occurrence and exposure

(a) Environmental occurrence

ortho-Phenylenediamine is not known to occur naturally in the environment.

Release of *ortho*-phenylenediamine to the environment can occur via multiple waste streams from its industrial use, including in the manufacture of the substance and its use as an intermediate in downstream uses (ECHA, 2018). However, the European Chemicals Agency has no public registered data on routes of release of this substance into the environment (ECHA, 2018).

ortho-Phenylenediamine has been detected in China in samples of tap water and lake water (taken from Lake Xuanwu) at concentrations of 1.8–6.9 μmol/L (Li et al., 2015); however, the concentrations of *ortho*-phenylenediamine were below the limit of detection (30 nmol/L) in tap, lake, reservoir, and river water in Changchun, China (Ma et al., 2015). If released to the aquatic system, the estimated volatilization from water surfaces is low based on its low vapour pressure, moderate solubility in water, and its pK_a values of < 2 and 4.47, indicating that *ortho*-phenylenediamine will exist partially in its cation form at pH values of 5–9 (HSDB, 2013). However, the low octanol/water partition coefficient of *ortho*-phenylenediamine suggests a low potential for bioconcentration in aquatic organisms (HSDB, 2013); treatment of wastewater by ozone has been shown to effectively remove *ortho*-phenylenediamine (Arowo et al., 2016).

No data on the concentrations of *ortho*-phenylenediamine could be found for other environmental media. Based on a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, if released to air *ortho*-phenylenediamine is expected to exist solely as a vapour in the ambient atmosphere (Bidleman, 1988). It is susceptible to photodegradation by sunlight as a result of UV absorption at wavelengths greater than 290 nm, and can degrade in the atmosphere with an estimated half-life of 0.7 hours (HSDB, 2013). If released to soil, *ortho*-phenylenediamine is expected to have a low mobility as a result of the high reactivity of the aromatic amino group towards mineral contents (Bollag et al., 1978; Adrian et al., 1989). *ortho*-Phenylenediamine is prone to microbial degradation in experimental studies. After 5 days of incubation in an aerobic screening study using activated sludge, 33% of the substance was degraded (Pitter, 1976). A decomposition period of longer than 64 days was determined in a screening test using soil microflora (Alexander & Lustigman, 1966).

(b) Exposure in the general population

The presence of *ortho*-phenylenediamine in some hair dyes indicates that selected individuals within the general population will be exposed to the compound. Exposure is more likely in countries outside the European Union, where the use of *ortho*-phenylenediamine as an ingredient of hair dyes was banned in 2007 (EPA, 2016). The occurrence of *ortho*-phenylenediamine in hair dye has been evaluated in several studies in China and Italy; no *ortho*-phenylenediamine was detected in the majority of these studies (Tokuda et al., 1986; Gennaro et al., 1990; Wu et al., 2011; Lai et al., 2012). However, the analysis of hair dye samples of different colours from a local supermarket in Shanghai, China yielded concentrations of up to 109 μmol/L in six out of the eight samples (Dong et al., 2008). *ortho*-Phenylenediamine was consistently below the limit of quantification in 30 hair dye creams (15 each from Japan and China, six different colorants) (Zhong et al., 2012).

ortho-Phenylenediamine occurs as a degradation product of thiophanate methyl and benomyl, two benzimidazole fungicides (HSDB, 2013). [The Working Group noted that individuals may be potentially exposed to *ortho*-phenylenediamine in agricultural settings in which these fungicides have been applied.]

The United States Environmental Protection Agency (EPA) estimated a median daily intake of 3.62×10^{-8} mg/kg body weight (bw) per day for *ortho*-phenylenediamine in the general population, with a 95th percentile of 3.98×10^{-6} mg/kg bw per day (EPA, 2018). [The Working Group noted that the data forming the basis of this estimate (diet, drinking-water, etc.) could not be located.]

(c) Occupational exposure

Quantitative occupational exposures to *ortho*-phenylenediamine have not been reported in the literature. However, approximately 3000

workers who participated in the National Occupational Exposure Survey of the United States National Institute of Occupational Safety and Health (NIOSH), conducted between 1981 and 1983, were potentially exposed to *ortho*-phenylenediamine (HSDB, 2013). According to the 2006 Toxic Substances Control Act Inventory Update Reporting data, the number of people potentially exposed to *ortho*-phenylenediamine in United States workplaces is 1000 or greater (HSDB, 2013).

Occupational exposure is expected to occur primarily through inhalation in workplaces where *ortho*-phenylenediamine is produced or used as an intermediate in the manufacture of other products, such as heterocyclic compounds used in dyes, agrochemicals, and corrosion inhibitors (HSDB, 2013; IFA, 2018). Inhalation may be particularly likely if the substance is heated, as a result of release vapours or sublimation dusts (IFA, 2018). Skin intake may also occur, although a low rate of penetration of the epidermis is expected based on a permeability constant of 0.45 mm per hour (Bronaugh & Congdon, 1984). [The Working Group noted that inadvertent ingestion may also occur.]

Clinical records over a 7-year period (1975–1982) were evaluated for 27 workers in a phenylenediamine manufacturing chemical plant in the USA (EPA, 2016). Haemoglobin and oxygen saturation levels among these employees did not differ from normal levels. However, specific exposure data in this workplace, such as air concentrations of the individual phenylenediamine isomer(s), were unknown.

1.1.5 Regulations and guidelines

According to the Globally Harmonized System of Classification and Labelling of Chemicals, *ortho*-phenylenediamine is suspected of causing cancer (H351, category 2) and mutagenic defects (H341, category 2). *ortho*-Phenylenediamine is also toxic if swallowed (H301, category 3), inhaled (H332, category 4), or taken up via the skin (H312, category 4), and can cause skin sensitization (H317, category 1) and eye irritation (H319, category 2) (ECHA, 2018).

A threshold limit value averaged over 8 hours for *ortho*-phenylenediamine of 0.1 mg/m³ has been assigned by several national authorities worldwide (ACGIH, 2001; Matsumoto et al., 2012; IFA, 2018). Short-term limit values of 0.4, 0.2, and 10 mg/m³ have been assigned in Austria, Denmark, and Romania (15-minute average value), respectively (IFA, 2018).

1.2 *ortho*-Phenylenediamine dihydrochloride

1.2.1 Identification of the agent

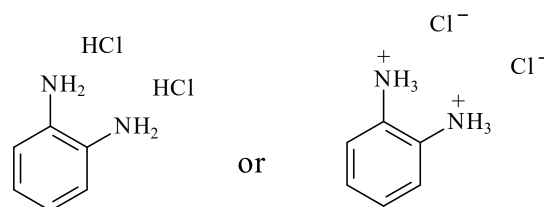
(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 615-28-1

Chem. Abstr. Serv. name: *ortho*-phenylenediamine dihydrochloride

Synonyms: 1,2-phenylenediamine dihydrochloride; benzene-1,2-diamine dihydrochloride; *o*-phenylenediamine dihydrochloride; 1,2-benzenediamine dihydrochloride.

(b) Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_{10}Cl_2N_2$

Relative molecular mass: 181.06

(c) Chemical and physical properties of the pure substance

Description: slightly yellow crystalline solid (IFA, 2018)

Melting point: 258 °C (Chemspider, 2018)

Density: 1.17 g/cm^3 (EPA, 2018)

Solubility: very soluble in water, 0.405 mol/L (EPA, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 0.131 (EPA, 2018)

Stability: combustible substance, poorly flammable; the substance decomposes to hydrogen chloride and nitrogen oxides, and can react dangerously with oxidizing agents (IFA, 2018)

Impurities: none known.

1.2.2 Production and use

ortho-Phenylenediamine dihydrochloride is used as a chemical laboratory reagent, and as an intermediate in the manufacture of dyes, coatings, and photographic chemicals (Matsumoto et al., 2012; OEHHA, 2018).

Less than 1 tonne of *ortho*-phenylenediamine dihydrochloride is manufactured or used in the European Union (ECHA, 2018). Quantities produced and used elsewhere in the world are unknown.

1.2.3 Methods of measurement and analysis

No specific measurement methods are available for *ortho*-phenylenediamine dihydrochloride.

1.2.4 Occurrence and exposure

ortho-Phenylenediamine dihydrochloride does not occur naturally. Accidental environmental releases of this chemical are likely to accumulate in water; contamination of air and soil are less likely. Exposure outside the workplace is unlikely to occur (HSDB, 2013).

Occupational exposure may arise from inhalation of aerosols, skin contact, and inadvertent ingestion (HSDB, 2013).

1.2.5 Regulations and guidelines

ortho-Phenylenediamine dihydrochloride is covered by generic regulations relating to hazardous chemicals, although it is not registered under the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals regulations (ECHA, 2018). There are no occupational exposure limits for *ortho*-phenylenediamine dihydrochloride.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

3.1.1 Oral administration in drinking-water

To assess the carcinogenicity of *ortho*-phenylenediamine, in a study that complied with good laboratory practice (GLP), randomized groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were given drinking-water containing *ortho*-phenylenediamine dihydrochloride (purity, 99.5%) at a concentration of 0, 500, 1000, or 2000 ppm (0, 46, 94, or 177 mg/kg bw per day) in males and 0, 1000, 2000, or 4000 ppm (0, 106, 200, or 391 mg/kg bw per day) in females for 2 years (104 weeks). All animals underwent complete necropsy. The survival of male and female mice was not affected in exposed groups;

Table 3.1 Studies of carcinogenicity with *ortho*-phenylenediamine dihydrochloride in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (M) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 500, 1000, 2000 ppm 50, 50, 50, 50 38, 38, 42, 39	*Liver* Hepatocellular adenoma 12/50, 25/50*, 34/50*, 35/50* Hepatocellular carcinoma 6/50, 9/50, 12/50, 10/50 Hepatocellular adenoma or carcinoma (combined) 18/50, 29/50*, 39/50**, 38/50** *Gall bladder*: papillary adenoma 0/46, 2/50, 4/49, 5/47*	 *P* < 0.01, Peto trend test; *P < 0.01, Fisher exact test NS *P* < 0.01, Peto trend test; *P < 0.05, **P < 0.01, Fisher exact test *P* < 0.05, Peto trend test; *P < 0.05, Fisher exact test	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1296 mice (range): hepatocellular adenoma, 17.8% (4–34%); hepatocellular carcinoma, 20.4% (2–42%) (JBRC, 2004a) Gall bladder papillary adenoma was not observed in 1296 male historical controls
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 1000, 2000, 4000 ppm 50, 50, 50, 50 24, 29, 28, 34	*Liver* Hepatocellular adenoma 6/50, 22/50*, 23/50*, 34/50* Hepatocellular carcinoma 1/50, 4/50, 11/50*, 17/50* Hepatocellular adenoma or carcinoma (combined) 6/50, 23/50*, 31/50*, 41/50* *Gall bladder*: papillary adenoma 0/50, 1/50, 5/50*, 3/50	 *P* < 0.01, Peto trend test; *P < 0.01, Fisher exact test *P* < 0.01, Peto trend test; *P < 0.01, Fisher exact test *P* < 0.01, Peto trend test; *P < 0.01, Fisher exact test *P < 0.05, Fisher exact test	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1298 mice (range): hepatocellular adenoma, 5.1% (0–10%); hepatocellular carcinoma, 2.5% (0–8%) (JBRC, 2004a) Gall bladder papillary adenoma was not observed in 1298 female historical controls

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, albino CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978), Sontag (1981)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 6872, 13 743 (time-weighted average), 0 (pooled control) mg/kg diet for 18 mo, then control diet for 3 mo 25, 25, 25, 99 NR	*Liver* Hepatocellular carcinoma 0/14, 5/17*, 3/14, 7/99	 *$P < 0.025$ (vs concurrent or pooled controls), Fisher exact test	Principal strengths: mice randomly allocated to groups; males and females used Principal limitations: only two dose groups; lack of reported details on histopathology; only 25 mice per group The doses were increased from 4000 and 8000 mg/kg diet after 5 mo to 8000 and 16 000 mg/kg diet for the next 13 mo Histopathology was only conducted on mice surviving until 6 mo
Mouse, albino CD-1 derived from HaM/ICR mice (Charles River) (F) 6–8 wk 21 mo Weisburger et al. (1978), Sontag (1981)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 6872, 13 743 (time-weighted average), 0 (pooled control) mg/kg diet for 18 mo, then control diet for 3 mo 25, 25, 25, 102 NR	*Liver*: hepatocellular carcinoma 1/15, 6/18*, 6/15**, 1/102	 *$P < 0.025$ (vs pooled control), **$P < 0.025$ (vs concurrent or pooled controls), Fisher exact test	Principal strengths: mice randomly allocated to groups; males and females used Principal limitations: only two dose groups; lack of reported details on histopathology; only 25 mice per group The doses were increased from 4000 or 8000 mg/kg diet after 5 mo to 8000 or 16 000 mg/kg diet for the next 13 mo Histopathology was only conducted on mice surviving until 6 mo

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 500, 1000, 2000 ppm 50, 50, 50, 50 41, 36, 42, 42	*Liver*		Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1499 rats (range): hepatocellular adenoma, 1.4% (0–6%); hepatocellular carcinoma, 0.3% (0–2%); hepatocellular adenoma or carcinoma (combined), 1.7% (0–6%) Historical control incidence of urinary bladder transitional cell papilloma, 6/1498 (maximum of 2% in any study) Historical control incidence of thyroid follicular adenoma (1493 animals), 0.8% (range, 0–4%) (JBRC, 2004b) In rats at the highest dose, the incidence of urinary bladder transitional cell papilloma and transitional cell carcinoma exceeded the historical control range
		Hepatocellular adenoma		
		3/50, 2/50, 12/50*, 15/50**	$P < 0.01$, Peto trend test; *$P < 0.05$, **$P < 0.01$, Fisher exact test	
		Hepatocellular carcinoma		
		1/50, 1/50, 6/50, 10/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined)		
		4/50, 3/50, 16/50*, 22/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Urinary bladder		
		Transitional cell papilloma		
		1/50, 0/50, 0/50, 6/50 (12%)	$P < 0.01$, Peto trend test	
		Transitional cell carcinoma		
		1/50, 0/50, 0/50, 4/50 (8%)	$P < 0.05$, Peto trend test	
		Transitional cell papilloma or carcinoma (combined)		
		2/50, 0/50, 0/50, 10/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Thyroid		
		Follicular adenoma		
		0/50, 1/50, 0/50, 4/50 (8%)	$P < 0.01$, Peto trend test	
		Follicular adenocarcinoma		
		1/50, 0/50, 1/50, 1/50	NS	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 250, 500, 1000 ppm 50, 50, 50, 50 41, 38, 44, 41	*Liver*		Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1447 rats (range): hepatocellular adenoma, 1.2% (0–6%); hepatocellular carcinoma, 0.1% (0–2%); hepatocellular adenoma or carcinoma (combined), 1.3% (0–8%) Historical control incidence of urinary bladder transitional cell papilloma is 8/1445 with a maximum of 2% in any study (JBRC, 2004b)
		Hepatocellular adenoma		
		1/50, 3/50, 15/50*, 36/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular carcinoma		
		0/50, 0/50, 4/50, 18/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined)		
		1/50, 3/50, 19/50*, 44/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Thyroid		
		Follicular adenoma		
		1/50, 0/50, 1/50, 0/50	NS	
		Follicular adenocarcinoma		
		0/50, 0/50, 1/50, 0/50	NS	
		Urinary bladder		
		Transitional cell papilloma		
		1/50, 0/50, 1/50, 1/50	NS	
		Transitional cell carcinoma		
		0/50, 0/50, 0/50, 0/50	–	

Table 3.1 (continued)

Species, strain (sex) **Age at start** **Duration** **Reference**	**Route** **Agent tested, purity** **Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**Incidence of tumours**	**Significance**	**Comments**
Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 2000, 4000, 0 (pooled control) mg/kg diet for 18 mo, then control diet for 6 mo 25, 25, 25, 111 NR	*Liver*: hepatocellular carcinoma 0/16, 0/14, 5/16*, 2/111	 *$P < 0.025$ (vs simultaneous or pooled control), Fisher exact test	Principal strengths: rats randomly allocated to groups Principal limitations: only males were used; only two dose groups; lack of reported details on histopathology; only 25 rats per group Histopathology conducted only on rats surviving until 6 mo

F, female; GLP, good laboratory practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; vs, versus; wk, week

survival rates were 38/50, 38/50, 42/50, and 39/50 in males, and 24/50, 29/50, 28/50, and 34/50 in females. Terminal body weights were decreased significantly in male and female mice for all exposed groups (Matsumoto et al., 2012; see also JBRC, 2004a).

There was a statistically significant ($P < 0.01$, Peto trend test) positive trend and significant increase in the incidence of hepatocellular adenoma in all exposed male mice (12/50, 25/50, 34/50, and 35/50; $P < 0.01$, Fisher exact test) and in all exposed female mice (6/50, 22/50, 23/50, and 34/50; $P < 0.01$, Fisher exact test). The incidence of hepatocellular adenoma or carcinoma (combined) (18/50, 29/50, 39/50, and 38/50) was significantly increased in male mice at 500 ppm ($P < 0.05$, Fisher exact test) and at 1000 and 2000 ppm ($P < 0.01$, Fisher exact test), with a significant positive trend ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma or carcinoma (combined) (6/50, 23/50, 31/50, and 41/50) was significantly ($P < 0.01$, Fisher exact test) increased in all exposed female mice, with a significant positive trend ($P < 0.01$, Peto trend test). Female mice also showed a significant ($P < 0.01$, Peto trend test) positive trend for hepatocellular carcinoma (1/50, 4/50, 11/50, and 17/50), the incidence of which was significantly ($P < 0.01$, Fisher exact test) increased in those exposed at 2000 and 4000 ppm. The increased incidence of hepatocellular carcinoma in exposed male mice (6/50, 9/50, 12/50, and 10/50) was not significant. In male mice, the incidence of hepatocellular adenoma in historical controls (1296 animals) (JBRC, 2004a) was 17.8% (range, 4–34%), and the incidence of hepatocellular carcinoma in historical controls was 20.4% (range, 2–42%). [The Working Group noted that the incidence of hepatocellular carcinoma in males did not exceed the range for historical controls.]

In both male and female mice, *ortho*-phenylenediamine dihydrochloride caused a significant increase in the incidence of papillary adenoma of the gall bladder for males exposed at the highest dose (0/46, 2/50, 4/49, and 5/47; $P < 0.05$, Fisher exact test) and for females exposed at the intermediate dose (5/50 compared with 0/50 controls; $P < 0.05$, Fisher exact test) with a significant positive trend ($P < 0.05$, Peto trend test). Matsumoto et al. (2012) stated that papillary adenoma of the gall bladder was not observed in historical controls of the JBRC database of 1296 male and 1298 female Crj:BDF$_1$ mice.

Significant increases in the incidence of non-neoplastic lesions were observed for most of the treated groups in the liver (acidophilic cell foci and basophilic cell foci for male and female mice, and clear cell foci in female mice) and, for some of the treated groups, in the gall bladder (papillary hyperplasia in male and female mice). [The Working group noted that this was a well-conducted GLP study, with stratified randomization of the animals and the use of historical controls for tumours.]

3.1.2 Oral administration in diet

In a study by Weisburger et al. (1978), randomized groups of 25 male and 25 female albino CD-1 mice (age, 6–8 weeks) were fed diet containing *ortho*-phenylenediamine dihydrochloride (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0, 6872, or 13 743 mg/kg diet (time-weighted average) (Sontag, 1981) for 18 months. Mice were first exposed at 0, 4000, or 8000 mg/kg diet for 5 months, then at 0, 8000, or 16 000 mg/kg diet for another 13 months, and were then kept for 3 months on control diet. Only mice older than 6 months were necropsied, resulting in reported groups of 14, 17, and 14 males, and of 15, 18, and 15 females. There was a pooled control group of 99 males and 102 females [no additional details provided]. [No information on body weights was provided.] Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, and

reproductive organs (Weisburger et al., 1978). For male mice, the incidence of hepatocellular carcinoma in the group exposed at the lower dose (5/17, 29%) was significantly increased compared with controls (0/14 concurrent controls and 7/99 (7%) pooled controls; $P < 0.025$, Fisher exact test for both comparisons). For female mice, there was a significant increase in the incidence of hepatocellular carcinoma for the group exposed at the lower dose – 6/18 (33%) compared with 1/102 (1%) pooled controls; $P < 0.025$, Fisher exact test – and for the group exposed at the higher dose – 6/15 (40%) compared with 1/15 (7%) concurrent or 1/102 (1%) pooled controls; $P < 0.025$ for both comparisons, Fisher exact test. [The Working Group noted that the limitations of this study included the small number of mice at the start and the small number necropsied; the use of only two dose groups; and the limited histopathological examination and reporting.]

3.2 Rat

3.2.1 Oral administration in drinking-water

To assess the carcinogenicity of *ortho*-phenylenediamine, in a GLP study Matsumoto et al. (2012) (see also JBRC, 2004b) gave randomized groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) drinking-water containing *ortho*-phenylenediamine dihydrochloride (purity, 99.5%) at a concentration of 0, 500, 1000, or 2000 ppm in males (0, 22, 42, or 86 mg/kg bw per day), and 0, 250, 500, or 1000 ppm in females (0, 18, 33, or 58 mg/kg bw per day) for 2 years (104 weeks). All animals underwent complete necropsy. The survival of male and female rats was not affected in exposed groups; survival rates in males were 41/50, 36/50, 42/50, and 42/50, and in females were 41/50, 38/50, 44/50, and 41/50. Terminal body weights were decreased significantly in male rats in all exposed groups and in female rats exposed at the two higher concentrations.

There was a statistically significant positive trend ($P < 0.01$, Peto trend test) and significant increase in the incidence of hepatocellular adenoma in male rats – 3/50, 2/50, 12/50 ($P < 0.05$, Fisher exact test), and 15/50 ($P < 0.01$, Fisher exact test) – and in female rats – 1/50, 3/50, 15/50 ($P < 0.01$, Fisher exact test), and 36/50 ($P < 0.01$, Fisher exact test). The incidence of hepatocellular carcinoma was significantly ($P < 0.01$, Fisher exact test) increased in males (1/50, 1/50, 6/50, and 10/50) and females (0/50, 0/50, 4/50, and 18/50) at the highest dose, with a significant positive trend ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma or carcinoma (combined) was significantly ($P < 0.01$, Fisher exact test) increased in males (4/50, 3/50, 16/50, and 22/50) and females (1/50, 3/50, 19/50, and 44/50) at the two higher doses, with a significant positive trend ($P < 0.01$, Peto trend test).

In male rats only, there were statistically significant positive trend for transitional cell papilloma of the urinary bladder (1/50, 0/50, 0/50, and 6/50 (12%); $P < 0.01$, Peto trend test), transitional cell carcinoma of the urinary bladder (1/50, 0/50, 0/50, and 4/50; $P < 0.05$, Peto trend test), and transitional cell papilloma or carcinoma (combined) (2/50, 0/50, 0/50, and 10/50; $P < 0.01$, Peto trend test); the incidence of transitional cell papilloma or carcinoma (combined) at the highest dose (10/50, 20%) was significantly ($P < 0.05$, Fisher exact test) increased compared with controls (2/50, 4%). JBRC historical control incidence in males for transitional cell papilloma of the urinary bladder (JBRC, 2004b) was 6/1498 (0.4%; range, 0–2%). [The Working Group noted that, although Matsumoto et al. (2012) stated that the incidence of transitional cell carcinoma of the urinary bladder exceeded the historical control range in the group exposed at the highest dose, no numerical values were provided.]

For male rats, the incidence of follicular adenoma of the thyroid (0/50, 1/50, 0/50, and 4/50 (8%)) showed a statistically significant positive trend ($P < 0.01$, Peto trend test). For follicular adenoma of the thyroid in males, the historical incidence was 13/1493 (0.8%; range, 0–4%) (JBRC, 2004b).

Significant increases in the incidence of non-neoplastic lesions in the liver were observed for some of the dose groups (basophilic cell foci for male and female rats, and clear cell foci in male rats) and urinary bladder (papillary and/or nodular hyperplasia in male rats). [The Working group noted that this was a well-conducted GLP study, with stratified randomization of the animals and the availability of historical controls for tumours.]

3.2.2 Oral administration in diet

Randomized groups of 25 male Charles River CD rats (age, 6–8 weeks) were fed diet (Purina laboratory chow) containing *ortho*-phenylenediamine dihydrochloride (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0, 2000, or 4000 mg/kg diet for 18 months, then 6 months on control diet (Weisburger et al., 1978). Only rats older than 6 months were necropsied, resulting in reported groups of 16, 14, and 16 males. There was a pooled control group of 111 male rats [no additional details provided]. [No information on body weights was provided.] Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries (Weisburger et al., 1978). The incidence of hepatocellular carcinoma in the group at the higher dose (5/16, 31%) was significantly increased ($P < 0.025$, Fisher exact test) compared with concurrent (0/16) and pooled (2/111, 2%) controls. The incidence of hepatocellular carcinoma in the group at the lower dose was 0/14. [The Working Group noted that the limitations of the study included the small number of animals at the start and the small number necropsied; the use of only two dose groups; and the limited histopathological examination and reporting.]

3.2.3 Subcutaneous injection

Four groups of five Wistar-King rats [age and sex not reported] were injected subcutaneously with *ortho*-phenylenediamine [purity not reported] in 0.5 mL distilled water at a dose of 0 (control) or 45 mg/kg bw in one experiment, and 0 (control) or 90 mg/kg bw in a second experiment, every second day, for 17 months. No tumours of the skin or subcutaneous tissue were observed in any of the groups (Saruta et al., 1962). [The Working Group noted the very limited reporting of the study, the use of only one dose, and the very low number of animals, making the study impossible to interpret. The Working Group considered the study inadequate for the evaluation.]

4. Mechanistic and Other Relevant Data

No data were available on *ortho*-phenylenediamine dihydrochloride, other than as reported from the chronic bioassays (see Section 4.3, “Other adverse effects”).

Data available on *ortho*-phenylenediamine are summarized in the following sections.

4.1 Absorption, distribution, metabolism, and excretion

No data in humans or in experimental animals were available.

A study in vitro using human abdominal skin showed that *ortho*-phenylenediamine binds and is absorbed by skin (Bronaugh & Congdon, 1984).

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether *ortho*-phenylenediamine: is genotoxic, and induces oxidative stress.

4.2.1 Genetic and related effects

See Table 4.1, Table 4.2, Table 4.3, and Table 4.4

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

DNA damage (as assessed using the comet assay), chromosomal aberrations, and sister-chromatid exchanges were induced by *ortho*-phenylenediamine (0–40 mM) in human lymphocytes in a study by Cebulska-Wasilewska et al. (1998).

(b) Experimental systems

ortho-Phenylenediamine did not increase somatic gene mutations in mouse embryos as assessed using the mammalian spot test (Gocke et al., 1983). Exposure to *ortho*-phenylenediamine by intraperitoneal injection induced micronuclei in the bone marrow of mice, Chinese hamsters, and guinea-pigs (Wild et al., 1980). A similar result was also seen in mice after oral exposure to *ortho*-phenylenediamine (Wild et al., 1980).

In rat hepatocytes, *ortho*-phenylenediamine gave positive results in an assay for unscheduled DNA synthesis (Thompson et al., 1983). Asgård et al. (2013) performed several assays for genotoxicity in mouse lymphoma cells with *ortho*-phenylenediamine and reported positive results in assays for DNA damage and mutation.

In plants, *ortho*-phenylenediamine induced DNA damage, detectable using the comet assay, or a change in phenotypic expression (Gichner et al., 1994, 2001; Xiao & Ichikawa, 1998; Gichner, 2003).

Numerous studies have assessed the mutagenicity of *ortho*-phenylenediamine in bacteria. Positive results have been seen in *Salmonella typhimurium* with (but not without) metabolic activation in strains TA98 (Voogd et al., 1980; Thompson et al., 1983; Gentile et al., 1987; Watanabe et al., 1990), TA100 (Thompson et al., 1983; Gentile et al., 1987), and TA1538 (Ames et al., 1975; Thompson et al., 1983), as well as in strains TA1537, D3052, and G46 (Thompson et al., 1983), and YG1024 (Wagner et al., 1997). Negative results were observed in strains TA100 and TA1537 (with metabolic activation) and in *Klebsiella pneumoniae* (tested only without metabolic activation) in a study by Voogd et al. (1980).

4.2.2 Oxidative stress

In the presence of Cu(II), *ortho*-phenylenediamine (100 µM) induced DNA damage in a *TP53* gene fragment. These lesions occurred at cytosine and guanine residues in a site reported to be a mutation hotspot region of the *TP53* gene (Murata et al., 2006).

ortho-Phenylenediamine caused Cu(II)-mediated damage to calf thymus DNA, measured by 8-oxo-7,8-dihydro-2-deoxyguanosine formation, and superoxide dismutase enhanced this oxidative DNA damage (Murata et al., 2006).

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In chronic bioassays with *ortho*-phenylenediamine dihydrochloride (Matsumoto et al., 2012) and a study with *ortho*-phenylenediamine (Saruta et al., 1962), effects that may be related

Table 4.1 Genetic and related effects of *ortho*-phenylenediamine in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Lymphocytes	+	NT	NR	Cytotoxicity, NR	Cebulska-Wasilewska et al. (1998)
Chromosomal aberrations	Lymphocytes	+	NT	NR	Cytotoxicity, NR; treated whole blood, then analysed the lymphocytes	Cebulska-Wasilewska et al. (1998)
Sister-chromatid exchange	Lymphocytes	+	NT	NR	Cytotoxicity, NR; treated whole blood, then analysed the lymphocytes	Cebulska-Wasilewska et al. (1998)

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

[a] +, positive

Table 4.2 Genetic and related effects of *ortho*-phenylenediamine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Mutation (spot test)	Mouse, C57BL/6JHan × T hybrid (M, F)	Whole body hair	–	196 mg/kg bw	Single intraperitoneal injection on the 10th day of pregnancy	Gocke et al. (1983)
Micronucleus formation	Mouse, NMRI (M, F)	Bone marrow (total PE)	+	108 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval or oral, 2×, 24 h interval	Wild et al. (1980)
Micronucleus formation	Chinese hamster (M, F)	Bone marrow (total PE)	+	216 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval	Wild et al. (1980)
Micronucleus formation	Guinea-pig, albino (M, F)	Bone marrow (total PE)	+	108 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval	Wild et al. (1980)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; PE, polychromatic erythrocytes

[a] +, positive; –, negative

Table 4.3 Genetic and related effects of *ortho*-phenylenediamine in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, Fischer F334, primary hepatocytes	+	NT	50 µM	Positive results were at non-cytotoxic concentrations	Thompson et al. (1983)
DNA oxidation	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM	Under the condition of hOGG1 treatment	Asgård et al. (2013)
DNA strand breaks	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM (–S9) and 0.12 mM (+S9)		Asgård et al. (2013)
Mutation/*Tk*	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM		Asgård et al. (2013)

HIC, highest ineffective concentration; hOGG1, human 8-oxoguanine DNA *N*-glycosylase; HPLC, high-performance liquid chromatography; LEC, lowest effective concentration; NT, not tested

[a] +, positive

Table 4.4 Genetic and related effects of *ortho*-phenylenediamine in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Tradescantia clone BNL4430, stamen	DNA damage/other	+	NT	10 mM		Xiao & Ichikawa (1998)
Tradescantia clone 4430, stamen	DNA damage/other	+	NT	50 mM		Gichner et al. (1994)
Nicotiana tabacum var. Petit Havana SR1-wildtype, *CAT1AS* mutant	DNA damage/other	–	NT	8 mM		Gichner (2003)
Nicotiana tabacum var. Petit Havana SR1-wildtype, *CAT1AS* mutant	DNA damage/other	+	NT	8 mM		Gichner (2003)
Nicotiana tabacum var. xanthi, chlorophyll-deficient	DNA damage/other	+	NT	0.01 mM		Gichner et al. (2001)
Nicotiana tabacum var. xanthi	DNA damage/other	+	NT	0.01 mM		Gichner et al. (2001)
Salmonella typhimurium TA98	Reverse mutation	–	+	1 mg/L		Voogd et al. (1980)
Salmonella typhimurium TA98, TA100, TA1537, TA1538, D3052, G46	Reverse mutation	–	+	0.1–100 µg/mL		Thompson et al. (1983)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	10 µg per plate	Both mammalian S9 and plant S9 were used for metabolic activation; DMSO was the solvent	Gentile et al. (1987)
Salmonella typhimurium TA98	Reverse mutation	–	+	10 µg per plate		Watanabe et al. (1990)
Salmonella typhimurium TA100, TA1537	Reverse mutation	–	–	1 mg/L		Voogd et al. (1980)
Salmonella typhimurium TA1538	Reverse mutation	NT	+	10–100 µg per plate		Ames et al. (1975)
Salmonella typhimurium YG1024	Reverse mutation	NT	+	20–100 µM	This mutation activity was dose-dependently increased, and enhanced by paraoxon	Wagner et al. (1997)
Klebsiella pneumoniae	Reverse mutation	–	NT	4.6 mM		Voogd et al. (1980)

8-oxodG, 8-oxo-7,8-dihydro-2-deoxyguanosine; DMSO, dimethyl sulfoxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[a] +, positive; –, negative

to carcinogenicity were observed in the liver, kidney, and the haematological system (see also Section 3).

In the study in rats with chronic exposure to drinking-water containing *ortho*-phenylenediamine dihydrochloride, Matsumoto et al. (2012) observed increased absolute and relative liver weight, haematological changes including decreased mean corpuscular volume, mean corpuscular haemoglobin, and haemoglobin concentration, as well as clinical chemistry alterations suggestive of renal and hepatic injury. Gross pathology revealed liver nodule formation, and microscopic evaluations showed renal changes including chronic nephropathy, renal papillary necrosis with mineralization, and urothelial hyperplasia of the renal pelvis.

In the study in mice with chronic exposure to drinking-water containing *ortho*-phenylenediamine dihydrochloride, Matsumoto et al. (2012) observed haematological changes and clinical chemistry changes suggestive of hepatic injury. Mice also developed hepatic nodules in addition to hydronephrosis. Non-neoplastic hepatic lesions, including the presence of increased numbers of clear, acidophilic, and basophilic cell foci, were observed in treated mice. Hepatocellular hyperplasia was not observed in mice. Increased eosinophilic changes in the respiratory and olfactory epithelium, and nasopharynx and nasal glandular metaplasia, were observed in female mice. Hydronephrosis was increased in all exposed females.

Saruta et al. (1962) exposed rats to *ortho*-phenylenediamine by subcutaneous injection and observed decreased haemoglobin values and decreased numbers of erythrocytes and leukocytes in blood after 10 months. Histopathological alterations occurred in the liver, spleen, kidney, and lung. [The Working Group noted that the manuscript implies a total dose of 90 mg/kg bw in the form of two injections at 45 mg/kg bw given 24 hours apart, although this is not explicitly stated.]

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

ortho-Phenylenediamine, the parent compound of *ortho*-phenylenediamine dihydrochloride, is a basic compound and will undergo acid–base reactions. *ortho*-Phenylenediamine and its dihydrochloride salt will undergo a pH-dependent acid–base equilibrium in the body.

ortho-Phenylenediamine has been listed as a chemical with a global high production volume. *ortho*-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride are manufactured in and/or imported into the European Economic Area in small amounts; information on quantities produced and used elsewhere in the world was not available. *ortho*-Phenylenediamine is used as an intermediate in the production of chemicals used to produce agrochemicals, antioxidants in rubber products, corrosion inhibitors, ultraviolet adsorbers, pharmaceuticals, and dyes and pigments used for colouring furs and hair dyes. *ortho*-Phenylenediamine dihydrochloride is primarily used as a chemical laboratory reagent and as an intermediate in the manufacture of dyes, coatings, and photographic chemicals.

ortho-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride are not known to occur naturally, although they may be released to the environment as a by-product of production and downstream uses; accumulation in water is most likely. *ortho*-Phenylenediamine has been detected in tap and lake water in China.

Occupational exposure to both agents is expected to occur primarily through inhalation in workplaces where they are produced or

used as an intermediate in the manufacture of other products; exposure through inadvertent ingestion may also occur. Exposure to *ortho*-phenylenediamine may also occur through skin contact.

The general population may be exposed to *ortho*-phenylenediamine through the use of hair dyes that contain this agent. Such exposure is more likely in countries outside the European Union, where the use of *ortho*-phenylenediamine in hair dyes has been banned since 2007.

No other quantitative data on exposure in the general population were available for these agents.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

ortho-Phenylenediamine was tested as its dihydrochloride for carcinogenicity in the same laboratory in two well-conducted good laboratory practice (GLP) oral exposure studies by drinking-water: one in male and female mice, and one in male and female rats. There were also two limited oral exposure studies by diet from another laboratory: one in male and female mice, and one in male rats.

In male mice, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and an increase in the incidence of hepatocellular adenoma, hepatocellular adenoma or carcinoma (combined), and papillary adenoma of the gall bladder in the study of oral exposure by drinking-water. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In female mice, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water; there was also a significant increase in the incidence of papillary adenoma of the gall bladder. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In male rats, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water. In the same study, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence of transitional cell papilloma, transitional cell carcinoma, and transitional cell papilloma or carcinoma (combined) of the urinary bladder, and of follicular adenoma of the thyroid. A significant increase in the incidence of transitional cell papilloma or carcinoma (combined) of the urinary bladder was also observed. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In female rats, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water.

5.4 Mechanistic and other relevant data

No studies evaluating absorption, distribution, metabolism, or excretion of *ortho*-phenylenediamine dihydrochloride or *ortho*-phenylenediamine in humans or experimental animals were available. No mechanistic studies of exposure to

ortho-phenylenediamine dihydrochloride were available.

Concerning the key characteristics of carcinogens, there is *strong* evidence that *ortho*-phenylenediamine is genotoxic based upon positive results in mammals, in mammalian cells in vitro, and in non-mammalian experimental systems including plants and prokaryotes. No data in exposed humans were available. In cultured human lymphocytes (one study), DNA strand breaks, chromosomal aberrations, and sister-chromatid exchanges were observed. In one study in non-human mammals conducted in vivo, the frequency of bone marrow micronuclei was increased after exposure by intraperitoneal injection (hamster, guinea-pig, and mouse) or orally (mouse). *ortho*-Phenylenediamine increased the frequency of unscheduled DNA synthesis in a study in rat hepatocytes, and gave positive results in assays for DNA damage and mutation in mouse lymphoma cells. Metabolic activation of *ortho*-phenylenediamine resulted in mutagenicity in multiple strains of *Salmonella typhimurium*, although in one study negative results were reported in two strains. *ortho*-Phenylenediamine induced DNA damage in multiple plant species. In one study of oxidative stress providing *weak* evidence, *ortho*-phenylenediamine in the presence of Cu(II) induced DNA damage in a human *TP53* gene fragment and calf thymus DNA.

Long-term effects observed in rats after acute exposure to *ortho*-phenylenediamine by subcutaneous injection included toxicity to the haematological, hepatic, and renal systems. Long-term effects of oral exposure to *ortho*-phenylenediamine dihydrochloride by drinking-water in rats and mice included toxicity to the haematological, hepatic, and renal systems. Histological changes were also seen in the mouse upper respiratory tract.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *ortho*-phenylenediamine and its dihydrochloride salt.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-phenylenediamine dihydrochloride.

6.3 Overall evaluation

ortho-Phenylenediamine and its dihydrochloride salt are *possibly carcinogenic to humans (Group 2B).*

6.4 Rationale

ortho-Phenylenediamine is the parent compound of *ortho*-phenylenediamine dihydrochloride. A pH-dependent acid–base equilibrium exists between the two compounds, and the Working Group considered that in vivo studies on either compound were informative about the carcinogenic hazard of both.

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PARA-NITROANISOLE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

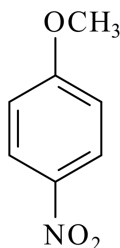
Chem. Abstr. Serv. Reg. No.: 100-17-4

Chem. Abstr. Serv. name: *para*-nitroanisole

IUPAC systematic name: 4-nitroanisole

Synonyms: 1-methoxy-4-nitrobenzene; *para*-methoxynitrobenzene.

1.1.2 Structural and molecular formula, and relative molecular mass



Molecular formula: $C_7H_7NO_3$

Relative molecular mass: 153.14

1.1.3 Chemical and physical properties of the pure substance

Description: solid, yellowish-grey substance (IFA, 2018)

Density (at 25 °C*)*: 1.233 g/cm³ (IFA, 2018)

Octanol/water partition coefficient: log K_{ow} = 2.03 (IFA, 2018)

Melting point: 52 °C (IFA, 2018)

Boiling point: 258–260 °C (IFA, 2018)

Solubility: slightly soluble in water, 468 mg/L at 20 °C (IFA, 2018)

Flammable limits: lower explosion limit, 1.0 vol% (66 g/m³) (IFA, 2018)

Flash point: 130 °C (IFA, 2018)

Impurities: available with purity of greater than 99% (ThermoFisher Scientific, 2018).

1.2 Production and use

1.2.1 Production process

para-Nitroanisole can be prepared from *para*-nitrophenol by etherification with dimethyl sulfate. Alternatively, reaction of *para*-chloronitrobenzene with sodium methoxide can also be performed (Booth, 2005).

1.2.2 Production volume

para-Nitroanisole has not been listed as a chemical with a high production volume (OECD, 2009). Global production in 2002 was approximately 10 000 tonnes per year, with Japan importing approximately 400 tonnes (JBRC, 2005).

para-Nitroanisole is in Annex III of the European Union Registration, Evaluation,

Authorisation and Restriction of Chemicals regulations (ECHA, 2018), which lists substances with low production volumes (1–10 tonnes per year) or no known human exposure scenarios. Information on production volumes elsewhere in the world was not available to the Working Group.

1.2.3 Use

para-Nitroanisole is used as an intermediate in the manufacture of *para*-anisidine (4-methoxyaniline), a precursor to synthetic colorants (e.g. in cosmetics), and may therefore occur as a residue in coloured products. *para*-Nitroanisole can also be used as a chemical to detect oxidation products in fats and oils, particularly unsaturated aldehydes (Dijkstra, 2016).

1.3 Methods of measurement and analysis

1.3.1 Air

No specific methods have been described for the determination of *para*-nitroanisole in air samples.

1.3.2 Other environmental media

No specific methods have been described for the detection and measurement of *para*-nitroanisole in water and soil samples. A method based on gas chromatography with mass spectrometric confirmation was recently described for the quantitative determination of *para*-nitroanisole in cosmetics. Sample preparation was carried out by a combination of liquid-liquid and dispersive solid-phase extraction, depending on the matrix, and the limits of detection were reported as 8.6 μg/kg for aqueous cosmetics, and 13.9 μg/kg for cream and powdery cosmetics (Huang et al., 2017).

1.3.3 Biomonitoring

Early methods of measurement of *para*-nitroanisole in alkaline solutions applied semi-quantitative polarography in pharmacological studies (Burgschat & Netter, 1977a). [The Working Group noted that, on the basis of the observed demethylation of *para*-nitroanisole by cytochrome P450 (CYP) 2A6 and 2E1 in human liver microsomes (Jones et al., 1997; Sai et al., 1999), *para*-nitrophenol may qualify as a biomarker in the urine of individuals and populations exposed to *para*-nitroanisole. There are multiple reliable analytical methods available to quantify *para*-nitrophenol in human urine samples (Hill et al., 1995; Sancho et al., 2002; Olsson et al., 2003; Hernández et al., 2004; Babina et al., 2012). However, measurement of *para*-nitrophenol in urine is not specific to the exposure to *para*-nitroanisole, as several other environmental and occupational pollutants can form this metabolite in humans; co-exposures to chemicals that are capable of forming *para*-nitrophenol (e.g. parathion, nitrobenzene) may therefore affect interpretation of the data (e.g. Ikeda & Kita, 1964; Padungtod et al., 2000).]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

para-Nitroanisole is not known to occur in the environment naturally. If used as an intermediate in the production of dyes, *para*-nitroanisole can be released through various waste streams. Additionally, *para*-nitroanisole may be formed by microbial degradation in munition-contaminated soil (Torralba-Sanchez et al., 2017).

No data on the concentrations of *para*-nitroanisole have been reported in environmental media or food samples. If released to air, *para*-nitroanisole is expected to be susceptible to photodegradation by sunlight and to photochemically degrade in the atmosphere in the

presence of hydroxyl radicals (Wubbels et al., 2010). Photodegradation of *para*-nitroanisole to *para*-methoxyphenol and *para*-nitrophenol has also been observed in the presence of hydroxide ions in water samples (Wubbels et al., 2010). If released to soil, *para*-nitroanisole may be susceptible to microbial degradation, forming *para*-nitrophenol (Schäfer et al., 1996). Additionally, *para*-nitroanisole has been reported to be taken up by plants with steady state reached after 3 weeks; an experimental bioconcentration factor of 3.3 in plants has been determined from contaminated soil (Torralba-Sanchez et al., 2017).

1.4.2 Exposure in the general population

Exposure to *para*-nitroanisole in the general population has not been reported. *para*-Nitroanisole was not detected in 15 cosmetic samples collected from local markets in China. The limit of detection was 7 µg/kg for aqueous cosmetics, and 12 µg/kg for cream and powdery cosmetics (Huang et al., 2017).

para-Nitrophenol, a potential metabolite of *para*-nitroanisole in humans, is routinely assessed in urine samples of the general population in many countries including the USA. However, *para*-nitrophenol is not entirely specific for exposure to *para*-nitroanisole; *para*-nitrophenol is also a human metabolite of nitrobenzene, a common environmental pollutant, and a metabolite of selected crop protection agents (such as *O*-ethyl-*O*-(4-nitrophenyl)phenylthiophosphonate, ethyl-parathion, and methyl-parathion). The median concentration of *para*-nitrophenol in urine of the general population of the USA in 2009/2010 was reported to be 0.51 µg/L (CDC, 2009). Median concentrations of *para*-nitrophenol in urine samples of 2.9 µg/L in farmers in Thailand (Panuwet et al., 2009) and of 7.9 µg/L in preschool children in an area of southern Australia where methyl-parathion was used (Babina et al., 2012) were reported.

1.4.3 Occupational exposures

Occupational exposures to *para*-nitroanisole have not been reported in the literature. However, occupational exposures may occur through inhalation and dermal absorption where *para*-nitroanisole is produced or used as an intermediate in the manufacture of dyes. [The Working Group noted that exposure may also occur through inadvertent ingestion.]

1.5 Regulations and guidelines

Concerning human health, *para*-nitroanisole is suspected of causing mutagenic defects (H341, category 2) according to the Globally Harmonized System of Classification and Labelling of Chemicals (ECHA, 2018). On the basis of this classification, the use of personal protective equipment in occupational settings is required (IFA, 2018). Ireland, the Republic of Latvia, and Romania have reported occupational exposure limits of 1, 3, and 5 mg/m^3, respectively (IFA, 2018).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were fed diet containing *para*-nitroanisole (purity, 99.72%; containing 0.28% *meta*-chloronitrobenzene) at a

Table 3.1 Studies of carcinogenicity with *para*-nitroanisole in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (M) 6 wk 104 wk JBRC (2000a,b, 2004a)	Oral 99.72% Diet 0, 5000, 10 000, 20 000 ppm 50, 50, 50, 50 36, 35, 27, 16	*Liver* Hepatocellular adenoma 12/50, 17/50, 18/50, 3/50	NS (for increase)	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in male historical controls: hepatocellular carcinoma, 20.1% (2–42%); hepatoblastoma, 0.6% (0–6%); hepatocellular adenoma, carcinoma, or hepatoblastoma, 35.5% (8–72%)
		Hepatocellular carcinoma 16/50, 11/50, 14/50, 39/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 1/50, 12/50*, 18/50*, 38/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 22/50, 27/50, 33/50*, 43/50**	$P < 0.01$, Peto trend test, Cochran-Armitage trend test; *$P < 0.05$, **$P < 0.01$, Fisher exact test	
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk JBRC (2000a,b, 2004a)	Oral 99.72% Diet 0, 5000, 10 000, 20 000 ppm 50, 50, 50, 50 23, 27, 30, 13	*Liver* Hepatocellular adenoma 5/50, 18/50*, 13/50**, 4/50	*$P < 0.01$, **$P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in female historical controls: hepatocellular adenoma, 5.3% (0–10%); hepatocellular carcinoma, 2.4% (0–12%); hepatoblastoma, 0.0%; hepatocellular adenoma, carcinoma, or hepatoblastoma, 7.5% (2–14%); liver histiocytic sarcoma, 1.3% (19/1448) (0–4%)
		Hepatocellular carcinoma 2/50, 12/50*, 41/50*, 46/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 0/50, 0/50, 8/50*, 38/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk JBRC (2000a,b, 2004a) (cont.)		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)		
		7/50, 24/50*, 45/50*, 48/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Histiocytic sarcoma		
		1/50 (2%), 0/50, 0/50, 3/50 (6%)	$P < 0.05$, Peto trend test	
		All sites: histiocytic sarcoma		
		18/50, 17/50, 15/50, 15/50	NS	
Rat, F344/DuCrj (M) 6 wk 104 wk JBRC (2000c,d, 2004b)	Oral 99.72% Diet 0, 2000, 4000, 8000 ppm 50, 50, 50, 50 37, 39, 32, 2	*Liver*		Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in male historical controls: hepatocellular adenoma, 1.6% (0–6%); testis, interstitial cell tumour, 85.6% (56–98%)
		Hepatocellular adenoma		
		0/50, 1/50, 13/50*, 11/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular carcinoma		
		0/50, 0/50, 0/50, 0/50	–	
		Testis: Interstitial cell tumour, benign		
		34/50 (68%), 45/50 (90%)*, 48/50 (96%)*, 48/50 (96%)*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) 6 wk 104 wk JBRC (2000c,d, 2004b)	Oral 99.72% Diet 0, 2000, 4000, 8000 ppm 50, 50, 50, 49 45, 38, 35, 31	*Liver* Hepatocellular adenoma 0/50, 0/50, 0/50, 5/49*	 *P* < 0.01, Peto trend test, Cochran–Armitage trend test; **P* < 0.05, Fisher exact test	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in female historical controls: hepatocellular adenoma, 1.3% (0–6%); uterine adenocarcinoma, 0.4% (0–4%)
		Hepatocellular carcinoma 0/50, 0/50, 0/50, 0/49	 –	
		Uterus: adenocarcinoma 1/50, 4/50, 8/50*, 8/49*	 *P* < 0.01, Peto trend test; *P* < 0.05, Cochran–Armitage trend test; **P* < 0.05, Fisher exact test	

F, female; GLP, good laboratory practice; M, male; NS, not significant; ppm, parts per million; wk, week

concentration of 0, 5000, 10 000, or 20 000 ppm for 104 weeks (JBRC, 2000a,b, 2004a). Mice were housed alone. Mean daily intake of the test compound given at 5000, 10 000, and 20 000 ppm was estimated from food consumption and body weight to be 599, 1328, and 3314 mg/kg body weight (bw) per day in males and 745, 1663, and 3496 mg/kg bw per day in females, respectively. A decreased survival rate attributed to tumours of the liver was observed in males at 10 000 and 20 000 ppm and in females at 20 000 ppm; survival rates were 36/50, 35/50, 27/50, and 16/50 in males, and 23/50, 27/50, 30/50, and 13/50 in females. A significant reduction in final body weight was observed in males and females at 10 000 and 20 000 ppm. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, the incidence of hepatocellular carcinoma (16/50, 11/50, 14/50, and 39/50) was significantly increased in mice at 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatoblastoma (1/50, 12/50, 18/50, and 38/50) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (22/50, 27/50, 33/50, and 43/50) was significantly increased in mice at 10 000 ppm ($P < 0.05$, Fisher exact test) and at 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma, of hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) occurred with significant positive trends in males ($P < 0.01$, Peto trend test, Cochran–Armitage trend test).

In females, the incidence of hepatocellular adenoma (5/50, 18/50, 13/50, and 4/50) was significantly increased in mice at 5000 ppm ($P < 0.01$, Fisher exact test) and 10 000 ppm ($P < 0.05$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma (2/50, 12/50, 41/50, and 46/50) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatoblastoma (0/50, 0/50, 8/50, and 38/50) was significantly increased in mice at 10 000 and 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (7/50, 24/50, 45/50, and 48/50 ppm) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma, of hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) occurred with significant positive trends ($P < 0.01$, Peto trend test, Cochran–Armitage trend test). The incidence of histiocytic sarcoma in the liver of female mice at 5000 ppm (0/50), 10 000 ppm (0/50), and 20 000 ppm (3/50, 6%) was not significantly increased compared with controls (1/50, 2%), but showed a significant positive trend ($P < 0.05$, Peto trend test) and a slightly higher incidence in the group at the highest dose compared with that in historical controls (19/1448 (1.3%); range, 0–4%). However, the incidence of histiocytic sarcoma (all sites) in the groups at 5000 ppm (17/50), 10 000 ppm (15/50), and 20 000 ppm (15/50) was not significantly increased compared with controls (18/50). [The Working Group concluded that the incidence of histiocytic sarcoma of the liver was not increased by the treatment.]

Regarding non-neoplastic lesions, a significant increase was observed in the incidence of hepatocellular hypertrophy in the centrilobular area in all groups of treated males and in females at 20 000 ppm, and in the incidence of hepatocytes with nuclear atypia in the centrilobular area in males at 10 000 and 20 000 ppm (JBRC, 2000a, b, 2004a). [The Working Group noted that this was a well-conducted GLP study that used multiple doses, a high number of mice per group, and males and females.]

3.2 Rat

Oral administration

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diet containing *para*-nitroanisole (purity, 99.72%; containing 0.28% *meta*-chloronitrobenzene) at a concentration of 0, 2000, 4000, or 8000 ppm for 104 weeks (JBRC, 2000c, d, 2004b). Rats were housed alone. Mean daily intake of the test compound given at 2000, 4000, and 8000 ppm was estimated from food consumption and body weight as 92, 191, and 413 mg/kg bw per day in males and 119, 229, and 475 mg/kg bw per day in females, respectively. A decreased survival rate was observed in males and females at 8000 ppm, attributed to chronic progressive nephropathy in males and females and to uterine tumours (adenocarcinomas) in females. Survival rates were 37/50, 39/50, 32/50, and 2/50 in males, and 45/50, 38/50, 35/50, and 31/49 in females. A significant reduction in final body weight was observed in males at 4000 ppm and in all treated females. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, although no significantly increased incidence in malignant tumours was observed, the incidence of hepatocellular adenoma (0/50, 1/50, 13/50, and 11/50) was significantly increased in rats at 4000 and 8000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of benign interstitial cell tumour of the testis (34/50 (68%), 45/50 (90%), 48/50 (96%), and 48/50 (96%)) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma and of benign interstitial cell tumours of the testis occurred with significant positive trends ($P < 0.01$, Peto trend test, Cochran–Armitage trend test). [The Working Group noted that the incidence of interstitial cell tumour in this study was within the range for historical controls, 1368/1598 (85.6%) (range, 56–98%).]

In females, the incidence of adenocarcinoma of the uterus (1/50 (2%), 4/50 (8%), 8/50 (16%), and 8/49 (16%)) was significantly increased in rats at 4000 and 8000 ppm ($P < 0.05$, Fisher exact test) compared with controls, with a significant positive trend ($P < 0.01$, Peto trend test; $P < 0.05$, Cochran–Armitage trend test). The incidence of hepatocellular adenoma (0/50, 0/50, 0/50, and 5/49) was significantly increased in rats at 8000 ppm ($P < 0.05$, Fisher exact test) compared with controls, with a significant positive trend ($P < 0.01$, Peto trend test, Cochran–Armitage trend test).

Regarding non-neoplastic lesions, a significant increase in incidence and/or grade was observed for spongiosis hepatis and basophilic cell focus in the liver of males at 4000 and 8000 ppm, and for chronic nephropathy in all treated males and in females at 4000 and 8000 ppm (JBRC, 2000c, d, 2004b). [The Working Group noted that this was a well-conducted GLP study conducted with multiple doses, a high number of rats per group, and in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 *Absorption, distribution, and excretion*

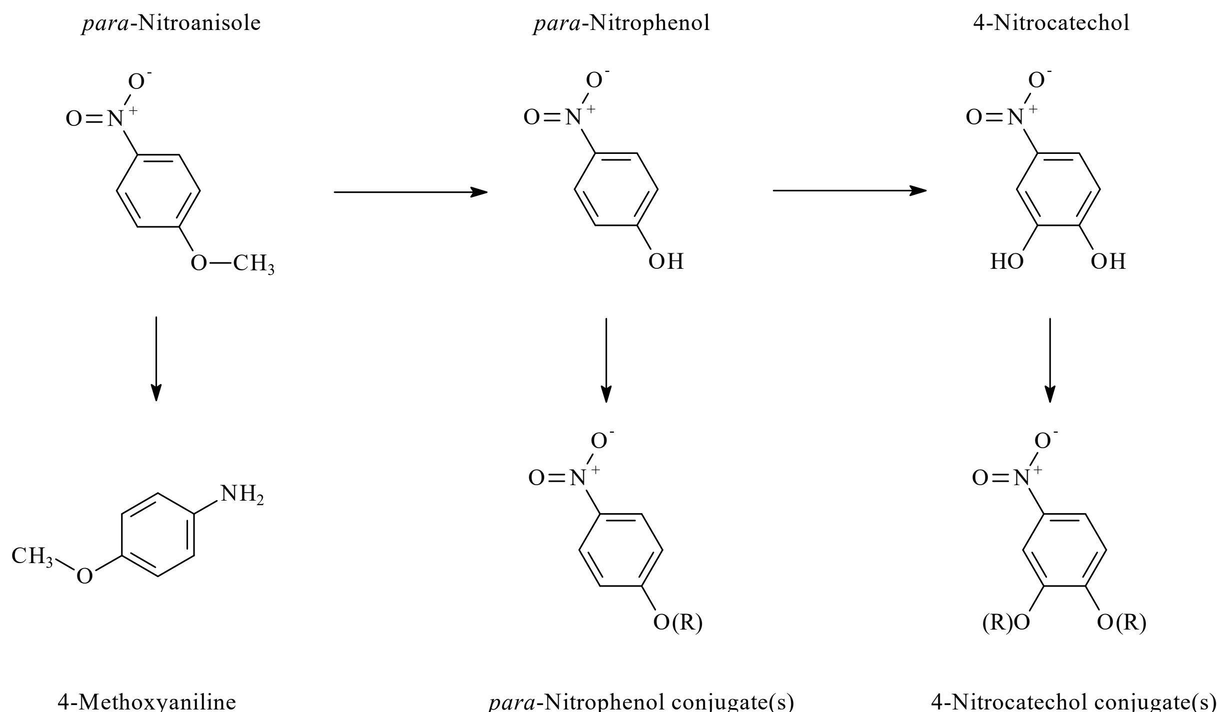
(a) *Humans*

No data were available to the Working Group.

(b) *Experimental systems*

Only a few studies on the absorption, distribution, and excretion of *para*-nitroanisole have been published. One study in rabbits has indicated that *para*-nitroanisole can be transported

Fig. 4.1 Metabolic scheme for *para*-nitroanisole for humans and experimental animals



Compiled by the Working Group

across the skin membrane and rapidly metabolized to *para*-nitrophenol and *para*-nitrophenol conjugates (Henrikus et al., 1991).

A study in mice of a single intravenous dose or dermal application of *para*-nitrophenol provided detailed toxicokinetic information for the primary metabolite of *para*-nitroanisole, showing few differences in various toxicokinetic parameters between males and females (Eichenbaum et al., 2009).

4.1.2 Metabolism

(a) Humans

See Fig. 4.1

O-Demethylation of *para*-nitroanisole into *para*-nitrophenol in human liver microsomes is mediated by both CYP2A6 and CYP2E1 (Jones et al. 1997; Gelboin & Krausz, 2006). CYP2A6 appears to be the major enzyme involved in humans, although the major enzyme in the rat is predominantly CYP2E1. Sai et al. (1999) found that human CYP2A6 further metabolized *para*-nitrophenol into *para*-nitrocatechol. A study that compared male and female hepatic metabolism of *para*-nitroanisole in humans and rats did not detect sex-specific differences (Kremers et al., 1981).

(b) Experimental systems

In rat primary hepatocytes, *para*-nitroanisole can be metabolized by CYP with subsequent glucuronide and sulfate conjugation (Eacho & Weiner, 1980; Eacho et al., 1981). In the rat liver, the metabolism of *para*-nitroanisole by CYP enzyme(s) and glucuronidation appears to be concentrated in the midzonal and periportal zones (James et al., 1981). Studies in vitro with rat hepatic microsomal fractions showed that *para*-nitroanisole is rapidly metabolized into *para*-nitrophenol, after which further hydroxylation at the *ortho* position can take place to produce

para-nitrocatechol (Burgschat & Netter, 1977b). No sex differences were observed in the rat for the sulfation and glucuronidation of *para*-nitrophenol, the primary metabolite of *para*-nitroanisole (Meerman et al., 1987). Metabolism of this compound to conjugates of *para*-nitrophenol was also observed after dermal application to the rabbit ear (Henrikus et al., 1991). Colonic mucosa of the rat also contains CYP enzymes that are able to efficiently metabolize *para*-nitroanisole (Strobel et al., 1980).

The metabolic rates of *para*-nitroanisole differ between rat tissues. Compared with the liver and lungs, the nasal epithelial membrane was found to have a higher metabolic capacity for this compound (Hadley & Dahl, 1982). These tissue-specific differences in the metabolism of *para*-nitroanisole were also observed in the rabbit. In pulmonary and hepatic microsomal fractions of this species, *para*-nitroanisole was found to be metabolized by the same CYP isoenzyme; however, from a quantitative point of view this metabolic rate differed between the types of tissues (Croft et al., 1986).

Based on total CYP content in rat primary hepatocytes, the metabolism of *para*-nitroanisole to *para*-nitrophenol was slower in females than males. After induction by phenobarbital, no sex-specific differences in metabolism were observed (Mazur & Petrenko, 1997). Moreover, it was shown that in pregnant and lactating female rats, the metabolism of *para*-nitroanisole increased during lactation (Borlakoglu et al., 1993). The metabolism of *para*-nitroanisole in rats was also found to be dependent on time during the postnatal period, with increasing O-demethylation activity until age 20 days followed by a decrease at age 30 days (Sonawane et al., 1981). Such a time dependency in the metabolism of *para*-nitroanisole was also found in the postnatal period of the beagle dog (Tanaka et al., 1998).

Several studies have addressed the specific role of certain isoforms of CYP in the O-demethylation of *para*-nitroanisole. Two studies in mice and rats, using specific antibodies against CYP3A3/4 and CYP2E1, indicated that *para*-nitroanisole is metabolized in these rodents by both CYP isoforms (Gelboin et al., 1995, 1996). In addition, CYP2B was also found to play a metabolic role in the rat (Sequeira et al., 1994). The role of CYP1A2 and CYP2E1 in the metabolism of *para*-nitroanisole in the rat was also identified using specific monoclonal antibodies against these isoforms of CYP. In the beagle dog, CYP1A1 was reported to be the major CYP isoenzyme responsible for the metabolism of *para*-nitroanisole (Tanaka et al., 1998).

It can therefore be concluded that several isoforms of CYP are involved in the primary metabolism of *para*-nitroanisole, in which CYP2A6 (humans) and CYP2E1 (humans and rats) play a dominant role.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether *para*-nitroanisole is genotoxic; and alters cell proliferation, cell death, or nutrient supply.

4.2.1 Genetic and related effects

See Table 4.1

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rat hepatocytes exposed to *para*-nitroanisole, unscheduled DNA synthesis was not increased (Probst et al., 1981). Mutagenicity was observed in *Salmonella typhimurium* strain TA100, without metabolic activation, in a study by Probst et al. (1981); two other studies in strains TA98, TA100, TA1535, TA1537 or TA1538

Table 4.1 Genetic and related effects of *para*-nitroanisole and its major metabolite *para*-nitrophenol in experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
para-*Nitroanisole*						
Rat, primary hepatocytes	Unscheduled DNA synthesis	–	NT	0.050 µmol/mL [7.5 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	+	NT	6.5 nmol/mL [1 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	100 µg/plate		Suzuki et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	NT	5000 µg/plate		Shimizu & Yano (1986)
para-*Nitrophenol*						
Mouse, Crl:CD-1, bone marrow, polychromatic erythrocytes, in vivo (M, F)	Micronucleus formation	–	NA	30 mg/kg bw, gavage, 1×		Eichenbaum et al. (2009)
Mouse, L5178Y, lymphoma cells	*Tk* gene mutation	NT	–	440 µg/mL		Amacher & Turner (1982)
Rat hepatocytes	Unscheduled DNA synthesis	–	NT	0.1 µmol/mL [14 µg/mL]		Probst et al. (1981)
Chinese hamster lung V79 cells	DNA strand breaks, comet assay	–	–	100 µM [14 µg/mL]	Cytotoxicity was specifically taken into account, but negative results across the whole concentration range were found	Hartmann & Speit (1997)
Chinese hamster ovary, CHO-K1 cell line	*Hprt* gene mutation	–	–	800 µg/mL		Oberly et al. (1990)
Chinese hamster ovary cells (CHO)	Sister-chromatid exchange	–	–	1500 µg/mL		NTP (1993)
Chinese hamster lung cells	Chromosomal aberrations	(+)	+	800 µg/mL (–S9), 600 µg/mL (+S9)	Induced chromosomal aberrations at non-toxic concentrations (+S9); cytotoxic at 800 µg/mL (–S9)	Noda (1995)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	7500 ppm by feeding 1500 ppm by injection		NTP (1993)

Table 4.1 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium G46	Reverse mutation; host-mediated assay (mouse)	–	NA	75 mg/kg bw		Buselmaier (1972)
Serratia marcescens	Reverse mutation; host-mediated assay (mouse)	–	NA	75 mg/kg bw		Buselmaier (1972)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	500 µg/plate		McCann et al. (1975)
Salmonella typhimurium TA1538	Reverse mutation	–	NT	1 µg/plate		Commoner (1976)
Salmonella typhimurium TA98, TA100, TA1537, TA1538, D3052, G46, C3076	Reverse mutation	–	–	0.1 µmol/mL [14 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA1535, TA1537, TA98, TA100	Reverse mutation	–	–	1000 µg/plate		Haworth et al. (1983); NTP (1993)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	100 µg/plate		Suzuki et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	1000 µg/plate		Shimizu & Yano (1986)

bw, body weight; F, female; HIC, highest ineffective concentration; *Hprt*, hypoxanthine (guanine) phosphoribosyltransferase; LEC, lowest effective concentration; M, male; NA, not applicable; NT, not tested; S9, 9000 × *g* supernatant; *Tk*, thymidine kinase

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

reported negative results (Suzuki et al., 1983; Shimizu & Yano, 1986).

The genotoxicity of *para*-nitrophenol, the major metabolite of *para*-nitroanisole, has also been studied. *para*-Nitrophenol gave negative results in an in vivo mouse micronucleus assay (Eichenbaum et al., 2009). In studies of mouse, rat and Chinese hamster cells in vitro, *para*-nitrophenol gave negative results for various end-points including unscheduled DNA synthesis, DNA strand breaks, gene mutation, (Probst et al., 1981; Amacher & Turner, 1982; Oberly et al., 1990; NTP, 1993; Noda, 1995; Hartmann & Speit, 1997). *para*-Nitrophenol was not mutagenic in *Drosophila melanogaster* (NTP, 1993), in the host-mediated assay in mice (Buselmaier, 1972) or in various *Salmonella typhimurium* strains including TA98, TA100, TA1535, and TA1537, with or without metabolic activation (McCann et al., 1975; Commoner, 1976; Probst et al., 1981; Haworth et al., 1983; Suzuki et al., 1983; Shimizu & Yano, 1986; NTP, 1993).

4.2.2 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Esmaeili et al. (2006) did not report activity with *para*-nitroanisole in a host-mediated assay conducted in mice. The role of β-catenin, an essential contributor to Wnt signalling, was studied in the formation of hepatocellular neoplasms that occurred during the 104-week study in Crj:BDF$_1$ mice exposed to *para*-nitroanisole. No functional abnormalities in β-catenin could be detected in this particular mouse strain (Kushida et al., 2006).

4.3 Other adverse effects

Male and female Fischer 344/DuCrj rats exposed to *para*-nitroanisole at 8000 ppm demonstrated an increased incidence of chronic progressive nephropathy. Decreased erythrocyte counts and haemoglobin concentrations were observed in male rats (JBRC, 2004b).

In Crj:BDF$_1$ mice, an increased incidence of centrilobular hepatocyte hypertrophy was observed. Dose-related increases in the incidence of non-neoplastic lesions in the nasal cavity, nasopharynx, and lung were also noted, as well as haemosiderin deposition in the spleen and kidneys in males and females (JBRC, 2004a).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

para-Nitroanisole is a nitrobenzene. Global production quantities are unknown, although production volumes in and import into Europe are known to be low. It is used as an intermediate in the manufacture of synthetic dyes used for cosmetics and other consumer products.

para-Nitroanisole is not known to occur naturally. It may be released to the air or water during its production and downstream use, or be found as a degradation product in munition-contaminated soil. Quantitative information on levels in the environment was not available.

Occupational exposure to *para*-nitroanisole has not been reported, although this would be expected to occur through inhalation and dermal absorption in workplaces where *para*-nitroanisole

is produced or used. Quantitative information on exposure to *para*-nitroanisole in occupational settings or in the general population was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

para-Nitroanisole was tested for carcinogenicity in the same laboratory in one well-conducted good laboratory practice (GLP) study of oral exposure by diet in male and female mice, and one well-conducted GLP study of oral exposure by diet in male and female rats.

In male mice, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In female mice, *para*-nitroanisole induced a significant positive trend in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined), and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In male rats, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma. In addition, there was a significant positive trend in the incidence and a significant increase in the incidence of interstitial tumours of the testis.

In female rats, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of adenocarcinoma of the uterus. In addition, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma.

5.4 Mechanistic and other relevant data

Information about the absorption, distribution, and excretion of *para*-nitroanisole in humans and experimental animals was sparse, but the occurrence of dermal uptake has been reported. In humans and rodents, *para*-nitroanisole is rapidly metabolized, predominantly by cytochrome P450 2A6 and 2E1, to *para*-nitrophenol, followed by catechol formation, conjugation, and excretion. In rodents, tissue-specific differences in metabolism were observed in the liver, lungs, and nasal epithelium. No differences in metabolic rate were detected between sexes in rodents.

Concerning the key characteristics of carcinogens, there is *weak* evidence that *para*-nitroanisole and its primary metabolite *para*-nitrophenol are genotoxic. No data in humans or in experimental animals in vivo were available. Results were largely negative in multiple studies in vitro in rodent cells and in bacteria for end-points including DNA strand breaks, mutation, and unscheduled DNA synthesis.

In chronic studies with *para*-nitroanisole, an increased incidence of chronic progressive nephropathy was observed in male and female rats, and hepatotoxicity was observed in male and female mice.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *para*-nitroanisole.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *para*-nitroanisole.

6.3 Overall evaluation

para-Nitroanisole is *possibly carcinogenic to humans (Group 2B)*.

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N,N-DIMETHYLACETAMIDE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

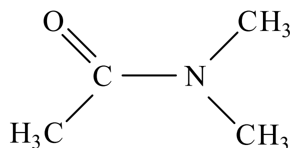
Chem. Abstr. Serv. Reg. No.: 127-19-5

Chem. Abstr. Serv. name: acetamide, *N*,*N*-dimethyl-

IUPAC systematic name: *N*,*N*-dimethylacetamide

Synonyms: acetic acid dimethylamide; acetic acid *N*,*N*-dimethylamide; dimethyl-acetamide; dimethylacetone amide; acetyl-dimethylamine.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C_4H_9NO

Relative molecular mass: 87.12

1.1.3 Chemical and physical properties of the pure substance

Description: at room temperature, colourless liquid with a weak ammonia- or fish-like odour; stable; combustible; incompatible with strong oxidizing agents (PubChem, 2019)

Boiling point: 166 °C (Gescher & Threadgill, 1990)

Melting point: –20 °C (Gescher & Threadgill, 1990)

Volatility: vapour pressure, 2 mm Hg (0.27 kPa) at 25 °C (ChemSpider, 2015)

Flash point: 70 °C (Gescher & Threadgill, 1990)

Relative liquid density (water = 1): 0.9366 (25 °C/4 °C) (Gescher & Threadgill, 1990)

Flammable limits: upper explosion limit, 320 °F (160 °C), 11.5%; lower explosion limit, 212 °F (100 °C), 1.8% (NIOSH, 2016)

Solubility: soluble in water and most organic solvents (Gescher & Threadgill, 1990)

Conversion factor: 1 ppm = 3.563 mg/m^3 at normal temperature (25 °C) and pressure (101 kPa) (Gescher & Threadgill, 1990)

Technical products and impurities: available at purities of greater than 95% (ThermoFisher Scientific, 2018). Okuda et al. (2006) used *N*,*N*-dimethylacetamide of reagent grade (purity, > 99.9%) in a study in rats.

1.2 Production and use

1.2.1 Production

N,*N*-Dimethylacetamide is usually produced by the reaction of acetic acid with dimethylamine in a closed system at elevated temperature and pressure; the subsequent distillation purifies the product (ECHA, 2012).

1.2.2 Production volume

N,*N*-Dimethylacetamide has been listed as a chemical with a high production volume (OECD, 2009). In 2000, worldwide production of *N*,*N*-dimethylacetamide was between 50 000 and 60 000 tons per year [~45 360–54 430 metric tonnes per year] (OECD-SIDS, 2012).

In 2010, European production was reported to be around 15 000–20 000 tonnes, of which 85% was consumed in Europe (ECHA, 2012). In 1997, the consumption of *N*,*N*-dimethylacetamide in Japan was about 5000 tonnes per year (Nomiyama et al., 2000).

1.2.3 Use

In France, 1510 tonnes of *N*,*N*-dimethylacetamide are used per year in the preparation of chemical compounds, particularly for textile fibres and in the pharmaceutical sector (Honnert & Grzebyk, 2010). Worldwide, *N*,*N*-dimethylacetamide is mostly used (65–70%) as an intermediate in the production of agrochemicals, pharmaceuticals, fine chemicals, and as an excipient in human and veterinary pharmaceuticals (ECHA, 2012).

Approximately 20–25% of *N*,*N*-dimethylacetamide produced globally is used in the manufacture of synthetic textile fibres. It is also used as a solvent for several resins, including polyacrylonitrile, polyamides, and cellulose derivates, and in the manufacture of coatings, films, and other miscellaneous products (ECHA, 2012).

1.3 Methods of measurement and analysis

1.3.1 Air

N,*N*-Dimethylacetamide has been quantified in workplace air using a solid sorbent tube containing silica gel. Air is sampled at 0.01–1.0 L/min to give a sample volume of 15–80 L. Analysis was performed using gas chromatography (GC) with flame ionization detector. Silica gel has a high affinity for water, meaning that high relative humidity in the workplace may limit the use of this method (NIOSH, 1994).

Tanaka et al. (2002) described the measurement of *N*,*N*-dimethylacetamide in workplace air using liquid passive–diffusive samplers with analysis using GC with mass spectrometry or a flame thermionic detector. This method yielded data comparable to the United States National Institute of Occupational Safety and Health (NIOSH) silica gel tube method.

Diffusive samplers with activated charcoal adsorbent have also been used to measure exposure to *N*,*N*-dimethylacetamide (Spies et al., 1995a).

[The Working Group noted that the concentrations of *N*,*N*-dimethylacetamide in the air of working environments may not correctly express the intensity of the solvent uptake, as a substantial part occurs percutaneously. Consequently, the biological monitoring of exposure to *N*,*N*-dimethylacetamide is preferred to the study of the uptake of *N*,*N*-dimethylacetamide in different working facilities.]

1.3.2 Other environmental media

The measurement of *N*,*N*-dimethylacetamide at very low concentrations in water can be performed using a cartridge containing activated carbon fibre felt that is used for solid-phase extraction of different compounds in water, including *N*,*N*-dimethylacetamide.

The minimum detectable concentration of *N*,*N*-dimethylacetamide in water was reported to be 0.02 µg/L (Kawata et al., 2001).

1.3.3 Biomonitoring

Urinary concentration of *N*-methylacetamide has been shown to reflect systematic exposure to *N*,*N*-dimethylacetamide that may have entered the body by inhalation or through the skin, and is considered as a good biomarker for biological monitoring in professional exposure to *N*,*N*-dimethylacetamide (HSL, 2018). A good linear correlation between urinary concentrations of *N*-methylacetamide and urinary concentrations of *N*,*N*-dimethylacetamide has been demonstrated (Kawai et al., 1997; Perbellini et al., 2003). As shown in Section 4.1, Fig. 4.1, the first oxidation phase of *N*,*N*-dimethylacetamide yields *N*-hydroxymethyl-*N*-methylacetamide, which is slowly demethylated to *N*-methylacetamide. In practice, most of the *N*-hydroxymethyl-*N*-methylacetamide measured in urine is demethylated to *N*-methylacetamide during analysis by GC, because the GC injection port is maintained at a temperature of 250 °C or greater. Biological monitoring is normally based on the total urinary *N*-methylacetamide measured using this procedure (HSL, 2018). In addition, *N*-acetyl-*S*-(acetamidomethyl)-L-cysteine (another metabolite of *N*,*N*-dimethylacetamide) has been measured in the urine by Perbellini et al. (2003) and Princivalle et al. (2010).

Measurement of *N*,*N*-dimethylacetamide in plasma was proposed by Oechtering et al. (2006) and Cendana et al. (2017) to monitor exposure from drug therapies containing *N*,*N*-dimethylacetamide as a solvent. Oechtering et al. (2006) used a rapid and selective method involving plasma protein precipitation with trichloroacetic acid, followed by analysis by liquid chromatography with mass spectrometry. Results were accurate, precise, and reproducible in the range 0.25–150.0 mg/L of *N*,*N*-dimethylacetamide in plasma (Oechtering et al., 2006). Cendana et al. (2017) proposed the measurement of *N*,*N*-dimethylacetamide in plasma by precipitation of plasma proteins with acetonitrile, followed by high-performance liquid chromatography and ultraviolet detection. The limits of detection and quantification were 1 and 5 µg/mL respectively, with linearity between 1 and 1000 µg/mL.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

The release of *N*,*N*-dimethylacetamide to the environment during its production and processing occurs primarily as liquid effluent and gaseous emissions by venting (OECD-SIDS, 2001).

A global distribution model for persistent organic chemicals suggests that any release of *N*,*N*-dimethylacetamide into the air is relocated into water and soil (OECD-SIDS, 2012).

The principal sources of environmental release and possible population exposure in Europe are: to the air, about 650 tonnes per year related to fibre production; and liquid emissions, 350 tonnes per year related to water treatment, 950 tonnes per year from waste products or during maintenance or cleaning, and 450 tonnes per year as a result of residues in fibres (ECHA, 2012).

Despite using a very sensitive analytical method with a minimum detectable concentration of 0.02 µg/L in water, Kawata et al. (2001) were not able to find a detectable amount of *N*,*N*-dimethylacetamide in groundwater and in four river water samples in the Niigata Prefecture, Japan.

N,*N*-Dimethylacetamide undergoes rapid photochemical degradation in the atmosphere, with a half-life of 6.1 hours. Its bioaccumulation in aquatic species is very low because of its bioconcentration factor of 0.008. A low adsorption in soils and sediments can be assumed from

a calculated soil adsorption coefficient (K_{oc}) of 9.1. Several tests in sludges confirmed the biodegradability of *N*,*N*-dimethylacetamide in about 1 month. In soil, *N*,*N*-dimethylacetamide is easily mobilized by water without any possibility of volatilization (OECD-SIDS, 2001).

1.4.2 Exposure in the general population

N,*N*-Dimethylacetamide is used as a carrier in the intravenous formulation of some drugs. The compound was detected in the plasma of eight children who were given a single daily dose of intravenous busulfan, an alkylating agent used in blood transfusion or bone-marrow transplantation. Plasma concentrations of *N*,*N*-dimethylacetamide ranging from 110 to 198 μg/mL were measured 8 hours after the end of a 3-hour infusion, and peak plasma concentrations ranged from 158 to 438 μg/mL (Cendana et al., 2017).

Consumer exposure was reported to be negligible as suggested by results from migration tests with simulated sweating on textile articles containing residual *N*,*N*-dimethylacetamide (at 0.010–0.001%) (OECD-SIDS, 2001).

1.4.3 Occupational exposure

Human exposure to *N*,*N*-dimethylacetamide occurs mostly in workplaces where the substance is used downstream of its primary manufacture. [The Working Group noted that the manufacture of *N*,*N*-dimethylacetamide in a closed system does not favour the dispersion of the product in the workplace.]

In occupational settings, particularly downstream of production, the main routes of exposure are by inhalation and skin uptake. Table 1.1 and Table 1.2 summarize the workplace exposure to *N*,*N*-dimethylacetamide as measured in air and urine, respectively, reported in the literature.

Occupational inhalation exposures associated with the manufacture and processing of *N*,*N*-dimethylacetamide in Alabama, USA, were measured at up to 2.49 mg/m³. Workers involved in resin preparation were exposed to *N*,*N*-dimethylacetamide at average concentrations of 9.30 mg/m³ (maximum, 51.35 mg/m³), and spinning operators had an average exposure of 7.02 mg/m³ (maximum, 35.53 mg/m³) (OECD-SIDS, 2001).

Spies et al. (1995a) monitored the exposure to *N*,*N*-dimethylacetamide of 127 employees from seven job classes in two departments of an acrylic-fibre manufacturing facility in the USA. *N*,*N*-Dimethylacetamide in workplace air was sampled with passive dosimeters worn on workers' lapels for the 12-hour shift. A total of 419 measurements of *N*,*N*-dimethylacetamide were made during 1 year. The arithmetic and geometric means were 2.0 and 1.45 ppm [7.13 and 5.17 mg/m³] (averaged over 12 hours), respectively, with concentrations ranging from 0.2 to 25 ppm [0.71–89.08 mg/m³]. The geometric standard deviation was 2.25 ppm [8.02 mg/m³]. Results obtained on different working days were not significantly different. A positive association was observed between workplace *N*,*N*-dimethylacetamide air concentration and its metabolite *N*-methylacetamide in urine collected at the end of a 12-hour shift.

Perbellini et al. (2003) described workers exposed occupationally to low concentrations of *N*,*N*-dimethylacetamide (median not exceeding 1.5 ppm [< 5.3 mg/m³]) while wearing light clothes (undershirt and shorts), which quickly became damp because of high humidity and temperature. In these conditions the skin uptake of *N*,*N*-dimethylacetamide was very high; about 20% of 233 urine samples provided by the workers at the end of their work shift had *N*-methylacetamide concentrations higher than 30 mg/g creatinine. The solvent adsorbed by sweat, which acted as a reservoir for skin uptake, continued throughout and after the work shift; a shower and a change of clothing at the end of the work shift ensured that dermal absorption of *N*,*N*-dimethylacetamide did not

Table 1.1 Workplace exposure to *N*,*N*-dimethylacetamide measured in air

Industry, country, year	Job and/or process	Mean (range) (mg/m³)[a]	Comments/additional data	Reference
Prefabricated synthetic products, Netherlands, 1997	Mechanical processing of the synthetic substance, produced elsewhere	52.52 (42.08–61.43)	Stationary monitoring with an infrared analyser for 7 days	Borm et al. (1987)
		Average exposure of eight different workers: 21.73 (2.49–41.33), 33.49 (2.49–147.50), 47.74 (3.56–112.23), 51.66 (3.56–131.12), 79.10 (2.85–184.92), 78.39 (6.06–163.18), 48.46 (3.21–118.29), 39.19 (3.21–128.27)	Personal sampling for six workers over an 8-h shift and two workers over a 4-h shift, over 5 subsequent working days	
Production of synthetic fibres, USA, 1989	Area where fibres containing *N*,*N*-dimethylacetamide were processed	Average exposure of five different workers: 4.56 (0.89–12.29), 5.20 (0.82–8.02), 3.81 (0.89–10.05), 3.35 (0.81–8.84), 3.81 (0.89–8.66)	Personal sampling for 5 days/week, over 4 subsequent working weeks	Kennedy & Pruett (1989)
Acrylic-fibre manufacturing facility, USA, 1995	Seven job classes in two departments	7.16 (0.71–89.08)	Data collected over 1 year from 30 workers who wore passive dosimeters during 12-h shifts	Spies et al. (1995a)

[a] Air concentrations given in ppm in the original publications were converted to mg/m³ by the Working Group using the conversion factor 1 ppm = 3.563 mg/m³

Table 1.2 Workplace exposure to *N*,*N*-dimethylacetamide as indicated by measurement of *N*-methylacetamide in urine

Industry, country, year	Job and/or process	Mean (range)	Comments/additional data	Reference
Production of synthetic fibres, USA, 1989	Area where fibres containing *N*,*N*-dimethylacetamide were processed	Average exposure of five different workers: 12.2 (4–31), 16.8 (6–42), 8.8 (4–15), 18.5 (7–42), 9.2 (1–20) mg/L	Urine samples provided at the end of 8-h work shifts for 5 days/week, for 4 subsequent working weeks	Kennedy & Pruett (1989)
Acrylic-fibre manufacturing facility, USA, 1995	Seven job classes in two departments	First day, end of 8-h shift: 15.4 (~1.8–280) mg/g creatinine Second day, end of 8-h shift: 18.9 mg/g creatinine	Data collected over 1 year from 30 workers who wore passive dosimeters during 12-h shifts	Spies et al. (1995a)
Elastane-fibre factories, Republic of Korea, 2002–2004	440 new workers were included as study subjects	19.6 (2.2–196.5) mg/g creatinine 5.2 (0.1–79.2) mg/g creatinine	503 urine samples from the eight departments in which 28 hepatic injuries induced by *N*,*N*-dimethylacetamide were found 464 urinary *N*-methylacetamide results from the other 11 departments without any hepatic injuries	Lee et al. (2006)
Factory producing acrylic fibres, Italy, 2003	223 workers from a chemical industry producing synthetic acrylic fibres (acrylic resin blended with *N*,*N*-dimethylacetamide was spun through about 600 small holes over an area of 100 cm^2 to produce acrylic fibres)	Median, 20.5 (1.5–173.6) mg/g creatinine	*N*-Methylacetamide concentrations were high in the urine of workers who had recently started up machinery; those attending the production control always had urinary *N*-methylacetamide < 30 mg/g creatinine	Perbellini et al. (2003)

continue, and reduced urinary concentrations of *N*-methylacetamide were measured the following morning.

1.5 Regulations and guidelines

Table 1.3 reports the occupational exposure limits for *N*,*N*-dimethylacetamide. A "skin" notation is identified in all regulations. Most countries propose the limit of 10 ppm (36 mg/m^3); exceptions to this include France and Germany (Deutsche Forschungsgemeinschaft), which propose 2 ppm (7.2 mg/m^3) and 5 ppm (18 mg/m^3), respectively (IFA, 2018).

Table 1.4 summarizes the biological monitoring limits for *N*-methylacetamide as a biological indicator index of exposure to *N*,*N*-dimethylacetamide.

1.6 Critical review of exposure assessment in key epidemiological studies

There were no studies in the literature specifically reporting on exposure assessment in key epidemiological studies. Spies et al. (1995a, b) and Lee et al. (2006) reported data on exposure to *N*,*N*-dimethylacetamide, but without any relationship to studies of cancer in humans. The single study of cancer in workers exposed to *N*,*N*-dimethylacetamide (Mastrangelo et al., 1993) used qualitative exposure categorization based on job tasks, work areas, and employment duration. [The Working Group considered this method insufficient to adequately characterize exposure in relation to risk of cancer.]

2. Cancer in Humans

2.1 Cohort studies

Mastrangelo et al. (1993) described a retrospective study of the mortality of 671 workers employed in an acrylic-fibre manufacturing facility in Venice, Italy. The process produced fibre for hosiery, clothing, and upholstery by dissolving polymerized acrylonitrile using *N*,*N*-dimethylacetamide, and then spinning the paste to form the fibre. The facility began manufacturing in 1959, and workers employed for more than 1 year before 1988 were recruited into the cohort; clerks and those with past exposure to vinyl chloride or benzidine (classified as Group 1 by IARC, as causes of cancer of the liver and bladder, respectively) were excluded. Workers were grouped according to work area and job task, reflecting presumed differences in exposure levels, and into categories of duration of exposure and time since first exposure. Workers were followed until 1990.

There were a small number of deaths (32 observed, 31.2 expected), and statistically significant increased risks were observed only for cancers of the intestine and colon (4 deaths, 0.38 expected [standardized mortality rate, 10.5; 95% confidence interval (CI), 2.9–27.0]) or for death from "ill-defined symptoms". The number of deaths from cancers of the intestine and colon was significantly increased in the subgroup with a short duration of exposure (< 4 years) and in the subgroup with less than 9 years since first exposure.

Perbellini et al. (2003) investigated the exposure in the workforce in this factory around 10 years after completion of the epidemiological study. Concentrations of *N*,*N*-dimethylacetamide in air were low (< 1.5 ppm) at this time, but median concentration of *N*-methylacetamide in urine was 20.5 mg/g creatinine with about 20% of measurements greater than 30 mg/g creatinine. [The Working Group noted that these

Table 1.3 International occupational exposure limit values for *N,N*-dimethylacetamide

Country or region	8-h limit		Short-term limit	
	ppm	mg/m³	ppm	mg/m³
Australia	10	36		
Austria	10	36	20	72
Belgium	10[a]	36	20[a]	72[a]
Canada, Ontario	10			
Canada, Province of Québec	10	36		
China		20		
Denmark	10	35	20	70
European Union[b]	10	36	20[a]	72[a]
Finland	10	36	20[a]	72[a]
France[c]	2	7.2	10[a]	36[a]
Germany (AGS)	5	18	10[a]	36[a]
Germany (DFG)	5	18	10[a]	36[a]
Hungary		36		72
Ireland	10	36	20[c]	72[c]
Italy[d]	10	36	20	72
Japan (JSOH)	10	36		
Latvia	10	36	20[a]	72[a]
Netherlands		36		72
New Zealand	10	36		
Republic of Korea	10	35		
Romania	10	36	20[a]	72[a]
Singapore	10	36		
Spain	10	36	20	72
Sweden	10	35	20[a]	70[a]
Switzerland	10	35	20	70
Turkey	10	36	20[a]	72[a]
UK	10	36	20	72
USA (NIOSH)	10	35		
USA (OSHA)	10	35		

AGS, Ausschuss für Gefahrstoffe; DFG, Deutsche Forschungsgemeinschaft; JSOH, Japan Society for Occupational Health; NIOSH, United States National Institute for Occupational Safety and Health; OSHA, United States Occupational Safety and Health Administration

[a] 15-minute average value

[b] Indicative occupational exposure limit value (IOELV)

[c] 15-minute reference period

[d] Skin

From IFA (2018)

Table 1.4 Occupational exposure to *N*,*N*-dimethylacetamide: biological exposure limits for urinary *N*-methylacetamide

Country	Year	Concentration	Sampling time and notation
Germany (DFG)	2017	30 mg/g creatinine (*N*-methylacetamide plus *N*-hydroxymethyl-*N*-methylacetamide)	End of exposure after several shifts
UK	2017	64 mg/g creatinine[a]	
USA (ACGIH)	2017	30 mg/g creatinine	End of shift at the end of the working week

ACGIH, American Conference of Governmental Industrial Hygienists; DFG, Deutsche Forschungsgemeinschaft
[a] 100 mmol *N*-methylacetamide per mol creatinine (conversion, 1 mmol/mol = 0.646 mg/g)
Compiled from HSL (2018)

findings suggest high dermal absorption of *N*,*N*-dimethylacetamide.]

[The Working Group noted that the study by Mastrangelo et al. (1993) found an excess of some cancers in this workforce. However, the association between these cancers and *N*,*N*-dimethylacetamide exposure is unclear, given the study's crude exposure assessment methodology. The Working Group also noted that the study population was relatively young and the number of expected deaths from cancer was small. It is unclear whether data from the Perbellini et al. (2003) study are representative of historical conditions in the factory.]

2.2 Case–control studies

No case–control studies of occupational exposure to *N*,*N*-dimethylacetamide were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Inhalation

Groups of 78 male and 78 female Crl:CD-1 (ICR) mice (age, 49 days) were exposed to *N*,*N*-dimethylacetamide (purity, 99.9%) at a concentration of 0 (control), 25, 100, or 350 ppm by whole-body inhalation for 6 hours per day, 5 days per week, for 18 months (Malley et al., 1995). Five males and five females per group were killed at 2–3 weeks, 3 months, and 12 months. There was a slight decrease in survival in females at 350 ppm. Survival rates in males were 46% (control), 60% (25 ppm), 54% (100 ppm), and 41% (350 ppm); respective survival rates in females were 80%, 77%, 76%, and 60%. Male mice at 100 and 350 ppm had a tendency towards higher body weight over the course of the 18-month exposure period. Female mice at 350 ppm had a significantly higher body weight compared with the control group for the first 9 months; body weights after 9 months were comparable to those of the control group. Full histopathology was performed on all major organs in the groups exposed at 0 and 350 ppm; lungs, liver, kidneys, and all gross lesions from mice in the groups at 25 and 100 ppm were also examined microscopically. There was no significant increase in the incidence of any tumour in either male or female mice. Regarding non-neoplastic lesions, there was a significant increase in the incidence of centrilobular hepatocellular hypertrophy

Table 3.1 Studies of carcinogenicity with *N,N*-dimethylacetamide in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Crl:CD-1 (ICR) (M) 49 d 18 mo Malley et al. (1995)	Whole-body inhalation 99.9% Air 0, 25, 100, 350 ppm for 6 h/d, 5 d/wk 78, 78, 78, 78 46%, 60%, 54%, 41%	*Liver* Hepatocellular adenoma 14/64, 12/64, 10/64, 8/65 Hepatocellular carcinoma 2/64, 5/64, 4/64, 1/65 Haemangiosarcoma 0/64, 1/64, 0/64, 2/65 *Testis:* interstitial cell tumour 0/64, 0/30, 1/39, 1/65	 NS NS NS NS	Principal strengths: covered most of the lifespan; multiple dose study; males and females used; high number of mice per group Principal limitations: inconsistencies in numbers of mice reported throughout the article The denominator represents the number of mice investigated for this tumour type; five males and five females per group were killed at 2–3 wk, 3 mo, and 12 mo
Full carcinogenicity Mouse, Crl:CD-1 (ICR) (F) 49 d 18 mo Malley et al. (1995)	Whole-body inhalation 99.9% Air 0, 25, 100, 350 ppm for 6 h/d, 5 d/wk 78, 78, 78, 78 80%, 77%, 76%, 60%	*Liver* Hepatocellular adenoma 0/63, 0/64, 0/63, 1/65 Haemangiosarcoma 0/63, 2/64, 0/63, 1/65 *Mammary gland:* adenocarcinoma 0/59, 1/17, 0/15, 1/59	 NS NS NS	Principal strengths: covered most of the lifespan; multiple dose study; males and females used; high number of mice per group Principal limitations: inconsistencies in numbers of mice reported throughout the article The denominator represents the number of mice investigated for this tumour type; five males and five females per group were killed at 2–3 wk, 3 mo, and 12 mo
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (M) 6 wk 104 wk JBRC (2013b)	Whole-body inhalation 99.9% Air 0, 12, 60, 300 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 35, 40, 33, 32	*Liver* Hepatocellular adenoma 10/50, 8/50, 7/50, 28/50* Hepatocellular carcinoma 7/50, 4/50, 2/50, 3/50 Hepatocellular adenoma or carcinoma (combined)	 $P \leq 0.0001$, Peto trend test prevalence method, Peto trend test combined analysis, Cochran–Armitage trend test; NS, Peto trend test standard method; *$P = 0.0002$, Fisher exact test NS	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Historical control incidence (range) in B6D2F$_1$/Crlj male mice: hepatocellular adenoma or carcinoma, 35.2% (8–68%); lymph node, malignant lymphoma, 12.5% (2–28%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, $B6D2F_1$/Crlj (M) 6 wk 104 wk JBRC (2013b) (cont.)		16/50 (32%), 12/50 (24%), 9/50 (18%), 29/50 (58%)*	$P \leq 0.0001$, Peto trend test prevalence method, Peto trend test combined analysis, Cochran–Armitage trend test; NS, Peto trend test standard method; *$P < 0.01$, Fisher exact test	
		Histiocytic sarcoma		
		1/50, 4/50, 4/50, 3/50	NS	
		Haemangiosarcoma		
		0/50, 3/50, 2/50, 0/50	NS	
		Lymph node: malignant lymphoma		
		7/50 (14%), 8/50 (16%), 8/50 (16%), 12/50 (24%)	$P < 0.05$, Peto trend test combined analysis; NS, Peto trend test standard method, Peto trend test prevalence method, Cochran–Armitage trend test	
Full carcinogenicity Mouse, $B6D2F_1$/Crlj (F) 6 wk 104 wk JBRC (2013b)	Whole-body inhalation 99.9% Air 0, 12, 60, 300 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 21, 25, 21, 22	*Liver*		Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence in historical controls (range): pituitary adenoma, 14.4% (2–34%); pituitary adenocarcinoma, 0.6% (0–4%); pituitary adenoma or adenocarcinoma, 15.0% (2–34%)
		Hepatocellular adenoma		
		2/50, 2/50, 4/50, 35/50*	$P < 0.0001$, Peto trend test prevalence method, Cochran–Armitage trend test; *$P < 0.0001$, Fisher exact test	
		Hepatocellular carcinoma		
		0/50, 1/50, 0/50, 8/50*	$P < 0.0001$, Peto trend test prevalence method, Cochran–Armitage trend test; *$P = 0.0029$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined)		
		2/50, 3/50, 4/50, 37/50*	$P < 0.0001$, Peto trend test prevalence method, Cochran–Armitage trend test; *$P < 0.0001$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, $B6D2F_1$/Crlj (F) 6 wk 104 wk JBRC (2013b) (cont.)		*Pituitary gland*		
		Adenoma		
		8/48 (16.7%), 6/50 (12%), 11/50 (22%), 8/50 (16%)	*P* = 0.0027, Peto trend test standard method; NS, Peto trend test prevalence method, Peto trend test combined analysis, Cochran–Armitage trend test	
		Adenocarcinoma		
		0/48, 0/50, 1/50, 1/50	NS	
		Adenoma or adenocarcinoma (combined)		
		8/48 (16.7%), 6/50 (12%), 12/50 (24%), 9/50 (18%)	*P* = 0.0027, Peto trend test standard method; NS, Peto trend test prevalence method, Peto trend test combined analysis, Cochran–Armitage trend test	
Full carcinogenicity Rat, Crl:CD (M) 43 d 24 mo Malley et al. (1995)	Whole-body inhalation 99.9% Air 0, 25, 100, 350 ppm for 6 h/d, 5 d/wk 78, 78, 78, 78 28%, 25%, 29%, 40%	*Liver*		Principal strengths: covers most of the lifespan; multiple dose study; males and females used; high number of rats per group Principal limitations: inconsistencies in numbers of rats reported throughout the article. The article reported that the number of animals per group at start was 87, which the Working Group believed was a misprint of 78 The denominator represents the number of rats investigated for this tumour type; five males and five females per group killed at 2–3 wk, 3 mo, and 12 mo
		Hepatocellular adenoma		
		3/65, 3/63, 2/63, 1/62	NS	
		Hepatocellular carcinoma		
		1/65, 0/63, 1/63, 1/62	NS	
		Testis: interstitial cell adenoma		
		7/65, 6/53, 8/50, 4/62	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Crl:CD (F) 43 d 23.5 mo Malley et al. (1995)	Whole-body inhalation 99.9% Air 0, 25, 100, 350 ppm for 6 h/d, 5 d/wk 78, 78, 78, 78 18%, 29%, 26%, 32%	*Liver* Hepatocellular adenoma 0/62, 0/62, 1/62, 0/64 Hepatocellular carcinoma 0/62, 0/62, 0/62, 0/64	 NS NA	Principal strengths: covers most of the lifespan; multiple dose study; males and females used; high number of rats per group Principal limitations: inconsistencies in numbers of rats reported throughout the article. The article reported that the number of animals per group at start was 87, which the Working Group believed was a misprint of 78 The denominator represents the number of rats investigated for this tumour type; five males and five females per group killed at 2–3 wk, 3 mo, and 12 mo
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2013d)	Whole-body inhalation 99.9% Air 0, 18, 90, 450 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 38, 41, 39, 40	*Liver* Hepatocellular adenoma 1/50, 1/50, 1/50, 9/50* Hepatocellular carcinoma 0/50, 0/50, 0/50, 4/50 (8%) Hepatocellular adenoma or carcinoma (combined)	 $P < 0.0001$, Peto trend test prevalence method, Cochran–Armitage trend test; *$P = 0.0078$, Fisher exact test $P \leq 0.0006$, Peto trend test prevalence method, Cochran–Armitage trend test	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence in historical controls (range): hepatocellular adenoma, 2.1% (0–12%); hepatocellular carcinoma, 0.5% (0–4%); hepatocellular adenoma or carcinoma, 2.5% (0–14%); adrenal gland, pheochromocytoma, 326/2847 (11.5%) (0–40%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2013d) (cont.)		1/50, 1/50, 1/50, 12/50*	$P < 0.0001$, Peto trend test prevalence method, Cochran–Armitage trend test; *$P = 0.0009$, Fisher exact test	
		Adrenal gland		
		Pheochromocytoma		
		2/50 (4%), 1/50 (2%), 1/50 (2%), 5/50 (10%)	$P < 0.04$, Peto trend test prevalence method, Cochran–Armitage trend test	
		Pheochromocytoma or malignant pheochromocytoma (combined)		
		4/50, 3/50, 1/50, 6/50	NS	
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6 wk 104 wk JBRC (2013d)	Whole-body inhalation 99.9% Air 0, 18, 90, 450 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 41, 39, 36, 45	*Liver*		Principal strengths: well-conducted GLP study; multiple dose study; males and females used
		Hepatocellular adenoma		
		2/50, 0/50, 2/50, 0/50	NS	
		Hepatocellular carcinoma		
		0/50, 0/50, 0/50, 1/50	NS	
Initiation–promotion (tested as promoter) Hamster, Syrian golden (F) ~1 mo 6 wk McGaughey & Jensen (1980)	Application on the cheek pouch NR DMSO 0.2% DMBA 3×/wk for 4 wk, followed by 0.05 M retinyl acetate plus 0 (control) or 0.1 M *N*,*N*-dimethylacetamide 3×/wk for 6 wk 10, 10 10, 10	*Oral mucosa*		Principal limitations: use of a single dose; low dose used; short duration of exposure; only one sex used; exact amount of chemicals applied unknown Plaques are putative non-neoplastic lesions
		Total "tumours" (including plaques)		
		10/10, 10/10	NS	
		5.70 ± 1.19, 3.90 ± 0.67	NS	
		Advanced "tumours" (excluding plaques)		
		7/10, 5/10	NS	
		2.20 ± 0.55, 1.00 ± 0.39	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. (or %) of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Hamster, Syrian golden (F) ~1 mo 6 wk McGaughey & Jensen (1980)	Application on the cheek pouch NR DMSO 0.2% DMBA 3×/wk for 4 wk, followed by 0.5% croton oil plus 0 (control) or 0.1 M *N*,*N*-dimethylacetamide 3×/wk for 6 wk 10, 10 10, 9	*Oral mucosa* Total "tumours" (including plaques) 10/10, 7/9 6.00 ± 1.05, 3.00 ± 1.00 Advanced "tumours" (excluding plaques) 5/10, 1/9 1.00 ± 0.42, 0.22 ± 0.22	 NS NS NS NS	Principal limitations: use of single dose; low dose used; short duration of exposure; only one sex used; exact amount of chemicals applied unknown Plaques are putative non-neoplastic lesions

d, day; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; 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

and hepatic Kupffer cell pigment accumulation in males at 350 ppm, and of hepatic single-cell necrosis in females at 350 ppm (Malley et al., 1995). [The Working Group noted that the study covered most of the lifespan, was conducted using multiple doses in males and females, and used a high number of mice per group. However, a limitation of the study was the inconsistency in the numbers of mice reported throughout the article.]

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crlj mice (age, 6 weeks) were exposed to *N*,*N*-dimethylacetamide (purity, 99.9%) at a concentration of 0 (control), 12, 60, or 300 ppm by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2013a, b). The survival rate was not affected by the treatment. Survival rates in males were 35/50 (control), 40/50 (12 ppm), 33/50 (60 ppm), and 32/50 (300 ppm); respective survival rates in females were 21/50, 25/50, 21/50, and 22/50. A significant decrease in body-weight gain was observed in males at 300 ppm throughout the exposure period (9% lower at the end of the exposure period). No significant difference in body-weight gain was observed in females. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, the incidence of hepatocellular adenoma (10/50, 8/50, 7/50, and 28/50) was significantly increased in mice at 300 ppm ($P = 0.0002$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma or carcinoma (combined) – 16/50 (32%), 12/50 (24%), 9/50 (18%), and 29/50 (58%) – was significantly increased in mice at 300 ppm ($P < 0.01$, Fisher exact test) compared with controls, but within the historical control range (incidence, 35.2%; range, 8–68%). The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred with significant positive trends ($P \leq 0.0001$, Peto trend test prevalence method, Peto trend test combined analysis, and Cochran–Armitage trend test; not significant by Peto trend test standard method). The incidence of hepatocellular carcinoma (7/50, 4/50, 2/50, and 3/50) was not significantly increased. [Although the Working Group considered the increased incidence of hepatocellular adenoma to be related to treatment, the increased incidence of hepatocellular adenoma or carcinoma (combined) was not considered to be related to treatment because it was driven by the increase in the incidence of hepatocellular adenoma.] There was a significant positive trend in the incidence of malignant lymphoma of the lymph node (7/50 (14%), 8/50 (16%), 8/50 (16%), and 12/50 (24%); $P < 0.05$, Peto trend test combined analysis; not significant by Peto trend test standard method, Peto trend test prevalence method, and Cochran–Armitage trend test); all these incidences were within the range for historical controls (incidence, 12.5%; range, 2–28%). [The Working Group considered that the positive trend in the incidence of malignant lymphoma could not be linked to the treatment because statistical analyses were inconclusive and all incidences were within the range for historical controls.]

In females, the incidence of hepatocellular adenoma (2/50, 2/50, 4/50, and 35/50) was significantly increased in mice at 300 ppm ($P < 0.0001$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma (0/50, 1/50, 0/50, and 8/50) was significantly increased in mice at 300 ppm ($P = 0.0029$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma or carcinoma (combined) (2/50, 3/50, 4/50, and 37/50) was significantly increased in mice at 300 ppm ($P < 0.0001$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) occurred with significant positive trends ($P < 0.0001$, Peto trend test prevalence method and Cochran–Armitage trend test).

The incidence of adenoma of the pituitary gland (8/48 (16.7%), 6/50 (12%), 11/50 (22%), and 8/50 (16%)) was not significantly increased in treated-females (not significant, Fisher exact test). There was a significant positive trend in the incidence of adenoma of the pituitary gland (*P* = 0.0027, Peto trend test standard method) that was not detected by other trend tests (not significant by Peto trend test prevalence method, Peto trend test combined analysis, and Cochran–Armitage trend test); all incidences were within the range for historical controls (incidence, 14.4%; range, 2–34%). The incidence of adenocarcinoma of the pituitary gland (0/48, 0/50, 1/50, and 1/50) was not significantly increased. The incidence of adenoma or adenocarcinoma (combined) of the pituitary gland – 8/48 (16.7%), 6/50 (12%), 12/50 (24%), and 9/50 (18%) – was not significantly (Fisher exact test) increased in treated females compared with controls. However, there was a significant positive trend in the incidence of adenoma or adenocarcinoma (combined) of the pituitary gland (*P* = 0.0027, Peto trend test standard method) that was not detected by other trend tests (not significant by Peto trend test prevalence method, Peto trend test combined analysis, and Cochran–Armitage trend test), but all incidences were within the range for historical controls (incidence, 15.0%; range, 2–34%). [The Working Group considered that the positive trend in the incidence of adenoma and of adenoma or adenocarcinoma (combined) of the pituitary gland could not be linked to the treatment because statistical analyses were inconclusive and all incidences were within the range for historical controls.]

Regarding non-neoplastic lesions, there was a significant increase in the incidence of eosinophilic foci in the liver in males and females at 300 ppm (JBRC, 2013a, b). [The Working Group noted that this was a well-conducted GLP study with the use of multiple doses, a high number of mice per group, and males and females.]

3.2 Rat

Inhalation

Groups of 87 male and 87 female [the Working Group believed 87 was a misprint of 78 in both cases] Crl:CD rats (age, 43 days) were exposed to *N*,*N*-dimethylacetamide (purity, 99.9%) at a concentration of 0 (control), 25, 100, or 350 ppm by whole-body inhalation for 6 hours per day, 5 days per week, for 24 months in males and 23.5 months in females (Malley et al., 1995). Five males and five females per group were killed at 2–3 weeks, 3 months, and 12 months. There was a slight non-significant increase in survival in males at 350 ppm and in all treated females. Survival rates in males were 28% (control), 25% (25 ppm), 29% (100 ppm), and 40% (350 ppm); respective survival rates in females were 18%, 29%, 26%, and 32%. A compound-related decrease in body weight and body-weight gain was observed in males (body-weight gain, 16% lower) and females (body-weight gain, 17% lower) at 350 ppm. Full histopathology was performed on all major organs in the rats at 0 and 350 ppm; lungs, liver, kidneys, and all gross lesions from rats in the groups at 25 and 100 ppm were also examined microscopically. There was no significant compound-related increase in the incidence of any tumours in either male or female rats. Regarding non-neoplastic lesions, there was a significant increase in the incidence of hepatic focal cystic degeneration in males at 100 and 350 ppm, of biliary hyperplasia and hepatic peliosis in males at 350 ppm, and of hepatic accumulation of lipofuscin and/or haemosiderin in males and females at 350 ppm (Malley et al., 1995). [The Working Group noted that the study covered most of the lifespan and was conducted with multiple doses, in both males and females, and with a high number of rats per group; data on survival rate and body weight throughout the study were provided in graphic form. However, a limitation of the study was the inconsistencies

in the numbers of rats reported throughout the article.]

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrlCrlj rats (age, 6 weeks) were exposed to *N*,*N*-dimethylacetamide (purity, 99.9%) at a concentration of 0 (control), 18, 90, or 450 ppm by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2013c, d). The survival rate was not affected by the treatment. Survival rates in males were 38/50 (control), 41/50 (18 ppm), 39/50 (90 ppm), and 40/50 (450 ppm); respective survival rates in females were 41/50, 39/50, 36/50, and 45/50. A significant decrease in body weight was observed throughout the exposure period in males (16% lower at the end of the exposure period) and females (9% lower at the end of the exposure period) at 450 ppm. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, the incidence of hepatocellular adenoma (1/50, 1/50, 1/50, and 9/50; $P < 0.0001$, Peto trend test prevalence method and Cochran–Armitage trend test) was significantly increased in rats at 450 ppm ($P = 0.0078$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma (0/50, 0/50, 0/50, and 4/50) was not statistically different (by Fisher exact test), but the increase in the incidence in the group exposed at the highest dose was above the upper bound of the range for historical controls (incidence, 13/2848 (0.5%); range, 0–4%). The incidence of hepatocellular adenoma or carcinoma (combined) – 1/50 (2%), 1/50 (2%), 1/50 (2%), and 12/50 (24%) – was significantly increased in rats at 450 ppm ($P = 0.0009$, Fisher exact test) compared with controls; the incidence in the group at the highest dose was above the upper bound of the range for historical controls (incidence, 2.5%; range, 0–14%). The incidence of hepatocellular adenoma, hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) occurred with significant positive trends ($P \leq 0.0006$, Peto trend test prevalence method and Cochran–Armitage trend test). There was a significant positive trend ($P < 0.04$, Peto trend test prevalence method and Cochran–Armitage trend test) in the incidence of pheochromocytoma of the adrenal gland – 2/50 (4%), 1/50 (2%), 1/50 (2%), and 5/50 (10%) – but all were below the average reported in the historical control database (326/2847 (11.5%); range; 0–40%). [The Working Group considered that the incidence of pheochromocytoma of the adrenal gland was not increased by the treatment because the incidences were below the average reported in the historical control database.]

In females, there was no significant increase in tumour incidence in any of the treated groups compared with controls.

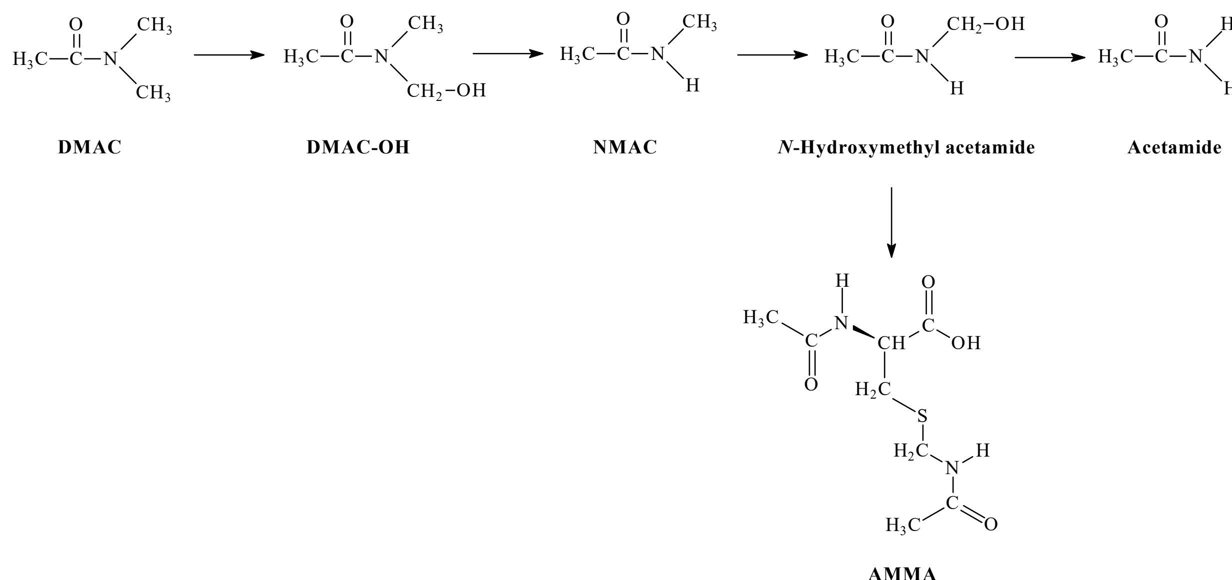
Regarding non-neoplastic lesions, there was a significant increase in the incidence of eosinophilic foci in the liver in males at 450 ppm, of hepatic clear cell foci in females at 450 ppm, of hepatic Kupffer cell pigment accumulation in males at 450 ppm and in females at 90 and 450 ppm, and of focal fatty degeneration in the liver in males at 90 and 450 ppm (JBRC, 2013c, d). [The Working Group noted this was a well-conducted GLP study with the use of multiple doses, a high number of rats per group, and males and females.]

3.3 Hamster

Initiation–promotion (tested as promoter)

A total of four groups of 10 female Syrian golden hamsters (age, ~1 month) were treated by painting the right cheek pouches with 0.2% 7,12-dimethylbenz[*a*]anthracene (DMBA) in dimethyl sulfoxide (DMSO) using cotton-tipped applicator sticks, 3 times per week for 4 weeks. This was followed by painting either with 0.05 M retinyl acetate or 0.5% croton oil in DMSO, plus painting either with DMSO (con-

Fig. 4.1 Proposed metabolic scheme for *N*,*N*-dimethylacetamide



AMMA, *S*-(acetamidomethyl)mercapturic acid; DMAC, *N*,*N*-dimethylacetamide; DMAC-OH, *N*-hydroxymethyl-*N*-methylacetamide; NMAC, *N*-methylacetamide

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trol) or 0.1 M *N*,*N*-dimethylacetamide [purity, not reported] in DMSO, 3 times per week, for another 6 weeks. *N*,*N*-Dimethylacetamide did not increase the incidence or multiplicity of total oral mucosa "tumours" and of advanced oral mucosa "tumours" (all oral mucosa "tumours" except plaques [putative preneoplastic lesions]) compared with the incidence and multiplicity for the groups treated with DMBA plus retinyl acetate only or DMBA plus croton oil only (McGaughey & Jensen, 1980). [The Working Group noted that the limitations of the study included the short-duration two-stage design, the small number of hamsters per group, the use of only one sex and a single dose, and the lack of precise information about the exact amount of chemicals applied.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

See Fig. 4.1

Biomonitoring studies of exposed workers have provided information about the metabolism of *N*,*N*-dimethylacetamide (Spies et al., 1995a; Perbellini et al., 2003; Yamamoto et al., 2018). [Because biomonitoring studies using GC can be confounded by thermal degradation of certain metabolites during analysis (Perbellini et al., 2003; Yamamoto et al., 2018), the Working Group considered other analytical methods to evaluate possible *N*,*N*-dimethylacetamide urinary metabolites.] Analysis of urine using liquid chromatography and tandem mass spectrometry has shown that *N*,*N*-dimethylacetamide

is metabolized to *N*-hydroxymethyl-*N*-methylacetamide, *N*-methylacetamide, *N*-hydroxymethylacetamide, acetamide, and *S*-(acetamidomethyl)mercapturic acid, with *N*-hydroxymethyl-*N*-methylacetamide being the most commonly identified metabolite (Yamamoto et al., 2018). Nomiyama et al. (2000) exposed healthy men to *N*,*N*-dimethylacetamide at 6.1 ± 1.3 (mean ± standard deviation, SD) ppm for 4 hours under one of two sets of experimental conditions: whole-body while breathing fresh air (to estimate dermal absorption) or via a face mask (to estimate inhalation absorption). Mean dermal and lung absorption were estimated to be 40.4% and 59.6% of the total *N*,*N*-dimethylacetamide uptake, respectively. Mean biological half-lives of urinary *N*-methylacetamide were 9.0 ± 1.4 and 5.6 ± 1.3 hours via skin and lung, respectively (Nomiyama et al., 2000). Maxfield et al. (1975) also performed inhalation (10 ppm for 6 hours via face mask) and dermal exposure studies with human subjects. *N*,*N*-Dimethylacetamide absorption was estimated to be 30% via the skin and 70% via the lung (Maxfield et al., 1975). Dermal absorption of *N*,*N*-dimethylacetamide also occurs in workers and has been estimated as 13–30% (Borm et al., 1987). Most ($n = 6$) workers studied ($n = 8$) excreted about 13% of the calculated inhaled dose as metabolite in urine, and the half-life of urinary *N*-methylacetamide was evaluated as 16 hours (Borm et al., 1987). Half-lives of *N*-methylacetamide and *S*-(acetamidomethyl) mercapturic acid in 13 workers were estimated as 8.7 ± 1.9 and 29.4 ± 6.6 hours, respectively (Princivalle et al., 2010).

Several studies have also examined the pharmacokinetics of *N*,*N*-dimethylacetamide in children receiving an intravenous formulation of the DNA-alkylating drug busulfan, which uses *N*,*N*-dimethylacetamide as a vehicle (Oechtering et al., 2006; Hempel et al., 2007; Trame et al., 2013). The pharmacokinetics of *N*,*N*-dimethylacetamide in children could be described using a one-compartment model (Hempel et al., 2007; Trame et al., 2013) with a mean initial half-life of 3.74 hours, which decreased to 0.83 hours after 96 hours (Hempel et al., 2007). [The Working Group noted that these studies also had co-exposure to busulfan and other therapeutic agents.]

4.1.2 Experimental systems

Rats given *N*,*N*-dimethylacetamide excrete some of the same metabolites observed in humans. For example, rats exposed by subcutaneous injection to *N*,*N*-dimethylacetamide at 300 mg per day for 2 days excreted *N*-methylacetamide and acetamide in urine, suggesting that successive N-demethylation of *N*,*N*-dimethylacetamide occurs in rats (Barnes & Ranta, 1972). In rats, a single oral administration of ^{14}C-labelled *N*,*N*-dimethylacetamide resulted in metabolism to *N*-methylacetamide (60–70%), *N*-hydroxymethylacetamide (7–10%), and acetamide (7–10%) within 72 hours (EPA, 1995).

The plasma half-life of *N*,*N*-dimethylacetamide was between 0.6 and 1.5 hours in rats exposed by whole-body inhalation (Hundley et al., 1994). Half-lives of *N*-methylacetamide and *S*-(acetamidomethyl)mercapturic acid in urine were about 2.5 and 6.5 hours, respectively, in rats after exposure to *N*,*N*-dimethylacetamide at 200 mg/kg bw by gavage (Princivalle et al., 2010).

Incubation of liver microsomes from pyridine-induced rats with *N*,*N*-dimethylacetamide has shown that it is metabolized in vitro by cytochrome P450 (CYP), probably CYP2E1, to free radical metabolites that attack the haem prosthetic group, leading to enzyme inactivation (Tolando et al., 2001).

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence on the key characteristics of carcinogens (Smith et al., 2016), on whether *N*,*N*-dimethylacetamide: is genotoxic; induces oxidative stress; and alters cell proliferation, cell death, or nutrient supply.

N,*N*-Dimethylacetamide gave negative results in multiple test systems with respect to genotoxicity (see Table 4.1). For example, McGregor (1980) performed the following assays with *N*,*N*-dimethylacetamide: unscheduled DNA synthesis in human diploid fibroblasts (in vitro), tests for dominant lethal mutation in male rats and sperm morphology in male mice after exposure by inhalation, cytogenetic evaluations in male and female rat bone marrow after exposure by inhalation, and the sex-linked recessive lethal test in *Drosophila melanogaster* using atmospheric exposure. The results were negative for each of these assays. Terada et al. (1978) exposed mouse erythroleukaemia cells to *N*,*N*-dimethylacetamide, and inferred that DNA damage occurred based on changes in DNA sucrose sedimentation rates.

Acetamide, a metabolite of *N*,*N*-dimethylacetamide, has also been investigated for genotoxicity in several *D. melanogaster* assays with conflicting results (Mitchell et al., 1981; Valencia et al., 1985; Batiste-Alentorn et al., 1991, 1995; Muñoz & Barnett, 2003).

Other data relevant to the key characteristics of carcinogens were also available. Liu et al. (2016) found that *N*,*N*-dimethylacetamide increased reactive oxygen species (ROS) formation and decreased glutathione concentrations in cultured human hepatocytes at concentrations that were also associated with decreased cell viability and/or apoptosis. Apoptosis was the result of ROS-mediated activation of the TP53-BCL2 signalling pathway (Liu et al., 2016). *N*,*N*-Dimethylacetamide also promoted cell differentiation of several mammalian cell types in vitro (Tanaka et al., 1975; Ohta et al., 1976; Porter, 1979; Speers et al., 1979; Dutko & Oldstone, 1981; Andrews et al., 1986; Moore et al., 1986; Meng et al., 1995). Other studies in vitro have shown that *N*,*N*-dimethylacetamide can inhibit DNA synthesis in cultured human lymphocytes (Novogrodsky et al., 1980), and increase the cell proliferation of murine fibroblasts and protozoa (Sauvant et al., 1995).

4.3 Other adverse effects

4.3.1 Humans

Weiss et al. (1962) treated patients with metastatic tumours with *N*,*N*-dimethylacetamide by intravenous injection at up to 500 mg/kg bw per day for up to 5 consecutive days, and observed clinical chemistry changes consistent with hepatotoxicity and neurotoxicity. Occupational exposure to *N*,*N*-dimethylacetamide has also been associated with hepatotoxicity and effects on the central nervous system in workers (Corsi, 1971; Marino et al., 1994; Baum & Suruda, 1997; Su et al., 2000; Lee et al., 2006; Jung et al., 2007; see also the review by Kennedy, 2012). For example, two cases of hepatitis in women as a result of dermal exposure, in addition to “minor respiratory exposures”, were reported among 25 workers exposed to *N*,*N*-dimethylacetamide in an acrylic-fibre production line (Baum & Suruda, 1997). Of 30 workers exposed to *N*,*N*-dimethylacetamide in the spinning department of an acrylic-polymer factory for 2–10 years, abnormalities in tests for liver function and hepatobiliary excretion were found in 19 cases (63%) (Corsi, 1971). Hepatic injuries were reported in 28 cases in 440 newly employed *N*,*N*-dimethylacetamide-exposed workers, with an observation period of 313.3 person-years, in two elastane-fibre plants. After controlling for confounders, urinary *N*-methylacetamide estimates of 20 or 30 mg/g creatinine were positively associated with hepatic injury with odds ratios of 3.70 (95% CI,

Table 4.1 Genetic and related effects of *N,N*-dimethylacetamide and its metabolite acetamide in in human cells in vitro and in experimental systems

Test system (species, strain, sex)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Human embryonic intestinal cells	Unscheduled DNA synthesis	–	(–)	*N,N*-Dimethylacetamide, 9366 μg/mL	The incorporation of radiolabel was insufficient to permit analysis of results in the presence of S9	McGregor (1980)
Rat, CD (M, F)	Chromosomal aberrations	–	NA	*N,N*-Dimethylacetamide, inhalation, 700 ppm, 7 h/d for 5 d		McGregor (1980)
Rat, CD (M)	Dominant lethal test	–	NA	*N,N*-Dimethylacetamide, inhalation, 700 ppm, 7 h/d for 5 d		McGregor (1980)
Rat, CD (M, F)	DNA damage	–	NA	*N,N*-Dimethylacetamide, inhalation, 700 ppm, 7 h/d for 5 d		McGregor (1980)
Mouse erythroleukaemia cell, strain 745A	DNA damage	+	NT	*N,N*-Dimethylacetamide, 30 mM		Terada et al. (1978)
Drosophila melanogaster	Sex-linked recessive lethal mutations	–	NA	*N,N*-Dimethylacetamide, 200 ppm, 95 min in atmosphere		McGregor (1980)
Drosophila melanogaster (sc z w sn)*	Somatic mutation and recombination test (SMART)	–	NA	Acetamide, 4500 ppm after feeding		Mitchell et al. (1981)
Drosophila melanogaster	Sex-linked recessive lethal mutations	–	NA	Acetamide, 50 000 ppm, after feeding or injection		Valencia et al. (1985)
Drosophila melanogaster, unstable Zeste–White (UZ)	SMART	+	NA	Acetamide, 10 mM	Doses tested: 0, 10, 20, and 30 mM; positive result at 10 mM	Batiste-Alentorn et al. (1991)
Drosophila melanogaster, trans-heterozygous (*mwh +/+flr*[3])	SMART	+	NA	Acetamide, 50 mM	Doses tested: 0, 20, 30, and 50 mM; positive result at 50 mM	Batiste-Alentorn et al. (1995)

Table 4.1 (continued)

Test system (species, strain, sex)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	Chromosomal damage	+	NA	Acetamide, 0.5%, oral exposure	Doses tested: 0.5, 1, 1.5, 2, and 4%; females heterozygous for the genotype *y w f Df(1) n20/y w f Df(1)n49* were mated to males of the genotype *y Df(1)n23/B*^{S} *Y y*$^+$; decreased viability at ≥ 2%; nondisjunction may be due to toxicity	Muñoz & Barnett (2003)

d, day; F, female; HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; min, minute; NA, not applicable; NT, not tested; ppm, parts per million

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

1.33–10.26) or 4.67 (95% CI, 1.66–13.15), respectively (Lee et al., 2006).

4.3.2 Experimental systems

Liver effects induced by short-term exposure to *N*,*N*-dimethylacetamide were consistently seen in mice (Malley et al., 1995; Valentine et al., 1997; JBRC, 2013a, b) and rats (Horn, 1961; Kinney et al., 1993; JBRC, 2013c, d).

Other tissues, including kidney, lymphoid organs, bone marrow, adrenal gland, and testis, were also affected in short-term studies in rodents (Horn, 1961; Valentine et al., 1997).

Non-neoplastic hepatic lesions seen in studies of chronic exposure by inhalation in mice and rats include centrilobular hepatocellular necrosis and hypertrophy. Hepatic cell proliferation was not seen in mice or rats after chronic exposure by inhalation. Other effects seen in rodents exposed to *N*,*N*-dimethylacetamide include chronic progressive nephropathy, renal papillary necrosis, and retinal atrophy (Malley et al., 1994; JBRC, 2013a, b, c, d).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

N,*N*-Dimethylacetamide is an amphiphilic chemical that is soluble in both water and organic solvents. It is a chemical with a high production volume, with global annual production estimated to be approximately 45 000 to 55 000 tonnes in 2000. The compound is used in the preparation of textile fibres, agrochemicals, pharmaceuticals, and fine chemicals. It is also used as a solvent for resins and in the manufacture of coatings and films.

N,*N*-Dimethylacetamide is not known to occur naturally, but it may be released to the environment during its production and downstream use, primarily as liquid effluent and gaseous emissions by venting.

The primary routes of exposure in human populations are inhalation and dermal uptake; dermal exposure is likely predominant in occupational settings, meaning that biomonitoring is preferred to air monitoring when estimating exposure.

Occupational exposures have been assessed in cross-sectional studies of workers in synthetic-fibre production facilities, using both air and biomonitoring methods. Occupational inhalation exposures were measured at a facility in the USA; workers involved in resin preparation and spinning operations were exposed to higher concentrations of *N*,*N*-dimethylacetamide than those involved in its manufacture. Some of these inhalation exposures were above the current occupational exposure limit. In a separate study (the only epidemiological study that was reviewed), a large number of air and urinary metabolite concentrations were measured in workers at an acrylic-fibre factory. Air concentrations of *N*,*N*-dimethylacetamide were generally low, although metabolites in urine were relatively high, indicating substantial uptake via the dermal route.

Low concentrations of *N*,*N*-dimethylacetamide have been measured in the plasma of children who were intravenously injected with a formulation of busulfan (an alkylating agent used in blood transfusion and bone-marrow transplantation) that contained *N*,*N*-dimethylacetamide as a solvent. Exposure to the compound in the general population from textile articles containing residual *N*,*N*-dimethylacetamide was reported to be negligible.

5.2 Cancer in humans

A small cohort study evaluating exposure to *N*,*N*-dimethylacetamide was conducted at a plant manufacturing acrylic fibre for hosiery, clothing, and upholstery in the Venice administrative region, Italy. The cohort included industrial workers employed for at least 1 year. Workers with past exposure to vinyl chloride or benzidine (classified as Group 1 by IARC, as causes of cancer of the liver and bladder, respectively) and administrative staff were excluded. Workers were grouped according to work area or job task, reflecting presumed differences in exposure levels, and into categories of duration of exposure and time since first exposure. Cancer mortality for the cohort was compared with the general regional population and found to be significantly elevated for cancers of the intestine and colon (4 cases, 0.38 expected), but the association between this elevation and exposure to *N*,*N*-dimethylacetamide was unclear. The study was limited by the young age of the cohort, leading to a low number of expected cancer deaths, and the relatively crude exposure assessment methodology.

5.3 Cancer in experimental animals

In the same laboratory, *N*,*N*-dimethylacetamide was tested for carcinogenicity in one well-conducted study that complied with good laboratory practice (GLP) in male and female mice exposed by inhalation, and in one well-conducted GLP study in male and female rats exposed by inhalation. *N*,*N*-Dimethylacetamide was tested in another laboratory in one study in male and female mice exposed by inhalation, and in one study in male and female rats exposed by inhalation. *N*,*N*-Dimethylacetamide was tested as a promoter in a limited initiation–promotion study by application to the cheek pouch of female Syrian golden hamsters.

In the GLP study in male mice, *N*,*N*-dimethylacetamide induced a significant positive trend in the incidence of and a significant increase in the incidence of hepatocellular adenoma.

In the GLP study in female mice, *N*,*N*-dimethylacetamide induced a significant positive trend in the incidence of and a significant increase

in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined).

In the other study in male and female mice, there was no significant increase in the incidence of any tumour.

In the GLP study in male rats, *N*,*N*-dimethylacetamide induced a significant positive trend in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), and a significant increase in the incidence of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined). The incidence of hepatocellular carcinoma in male rats exposed to the highest dose exceeded the upper bound of the range for historical controls.

In the GLP study in female rats, there was no significant increase in the incidence of any tumour.

In the other study in male and female rats, there was no significant increase in the incidence of any tumour.

In the study in hamsters, there was no increase in the incidence or multiplicity of any tumour.

5.4 Mechanistic and other relevant data

Information about the absorption, distribution, metabolism, and excretion of *N*,*N*-dimethylacetamide was derived from workers, human subjects, children undergoing chemotherapy, and experimental animals. *N*,*N*-Dimethylacetamide is absorbed from the skin, lung, and gastrointestinal tract. In humans, metabolites include acetamide. Rats excrete many of the same metabolites observed in humans.

Concerning the key characteristics of carcinogens, there is *weak* evidence that *N*,*N*-dimethylacetamide and its metabolite acetamide are genotoxic. No data in exposed humans were available. *N*,*N*-Dimethylacetamide gave negative results in tests for genotoxicity in human diploid fibroblasts, in rats exposed by inhalation (dominant lethal mutation test, bone marrow cytogenetics), and in *Drosophila melanogaster* (sex-linked recessive lethal test). In *D. melanogaster*, acetamide yielded conflicting results.

In vitro, *N*,*N*-dimethylacetamide promotes differentiation of several mammalian cell types, inhibits DNA synthesis in human lymphocytes, and increases cell proliferation in cultured murine fibroblasts and protozoa. There is *weak* evidence that *N*,*N*-dimethylacetamide alters cell proliferation, cell death, or nutrient supply.

Humans exposed to *N*,*N*-dimethylacetamide develop hepatotoxicity. In rats and mice, hepatotoxicity, including centrilobular hepatocellular necrosis and hypertrophy, was observed.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *N*,*N*-dimethylacetamide.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

N,*N*-dimethylacetamide is *possibly carcinogenic to humans (Group 2B).*

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LIST OF ABBREVIATIONS

AhR	aryl hydrocarbon receptor
AR	androgen receptor
ARE	antioxidant response element
ATP	adenosine triphosphate
CAS No.	Chemical Abstracts Service registry number
CI	confidence interval
CYP	cytochrome P450
DMBA	7,12-dimethylbenz[*a*]anthracene
DMSO	dimethyl sulfoxide
EPA	United States Environmental Protection Agency
GC	gas chromatography
GLP	good laboratory practice
HPLC	high-performance liquid chromatography
HTS	high-throughput screening
JBRC	Japan Bioassay Research Center
OECD	Organisation for Economic Co-operation and Development
PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
Tox21	Toxicity Testing in the 21st Century
ToxCast	Toxicity Forecaster
TWA	time-weighted average
UV	ultraviolet
vs	versus

ANNEX 1. SUPPLEMENTARY MATERIAL FOR TOXCAST/TOX21

This supplementary material (which is available online at: http://publications.iarc.fr/584) comprises a spreadsheet (123-Mono01-Section4.4-spreadsheet.xlsx) analysed by the Working Group for Volume 123 of the *IARC Monographs*. The spreadsheet lists the Toxicity Forecaster (ToxCastTM) and Toxicity Testing in the 21st Century (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 (Smith et al., 2016), and the decision as to whether each chemical was “active” or “inactive” (EPA, 2018).

References

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M IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of *ortho*-phenylenediamine dihydrochloride and its parent compound *ortho*-phenylenediamine, 2- and 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene and 2,4-dichloro-1-nitrobenzene, 2-amino-4-chlorophenol, *para*-nitroanisole, and *N,N*-dimethylacetamide.

ortho-Phenylenediamine dihydrochloride is used in the manufacture of dyes, coatings, and photographic chemicals, while *ortho*-phenylenediamine is used in the production of pharmaceuticals and agrochemicals, and in dyes and pigments for colouring hair and furs. 2-Chloronitrobenzene is used to make colorants and various other chemicals. 4-Chloronitrobenzene is a chemical that is used in the production of agricultural chemicals, pharmaceuticals, paints, pigments, colorants, plastics, and paper, and in the treatment of textiles and leather. 1,4-Dichloro-2-nitrobenzene and 2,4-dichloro-1-nitrobenzene are intermediates in the manufacture of diazo pigments, agrochemicals, ultraviolet absorbents, and pharmaceuticals. 2-Amino-4-chlorophenol is a chemical used in the manufacture of pharmaceuticals and of dyes for textiles and other consumer products. *para*-Nitroanisole is used to make synthetic dyes used for cosmetics and other consumer products. Finally, *N,N*-dimethylacetamide is used in the manufacture of textile fibres, agrochemicals, pharmaceuticals, fine chemicals, coatings and films, and as a solvent for resins.

Exposure to all the agents considered may occur in different occupational settings and in the general population.

The *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.

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