IARC MONOGRAPHS

SOME AROMATIC AMINES AND RELATED COMPOUNDS

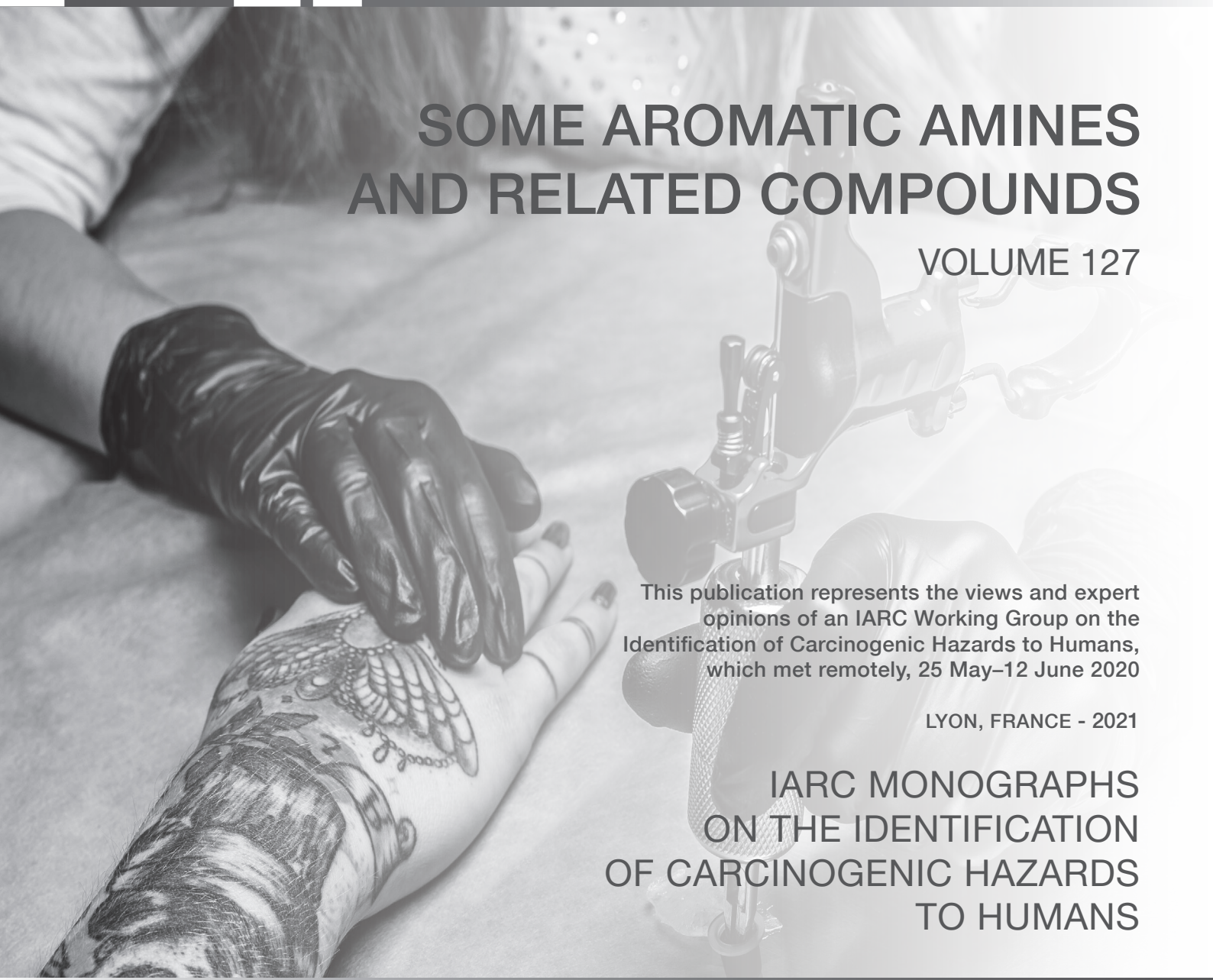
VOLUME 127

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



SOME AROMATIC AMINES AND RELATED COMPOUNDS

VOLUME 127

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 25 May–12 June 2020

LYON, FRANCE - 2021

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

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IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic hazard of chemicals to humans, involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic hazards 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 cancer hazard to humans with the help of international working groups of experts in carcinogenesis and related fields; and to identify gaps in evidence. The lists of IARC evaluations are regularly updated and are available on the internet at https://monographs.iarc.fr/.

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The *IARC Monographs* Working Group alone is responsible for the views expressed in this publication.

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About the cover: A tattoo artist at work. Aniline and *ortho*-anisidine are used in the synthesis of pigments that are ingredients of tattoo inks, in which both agents have been detected.

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NOTE TO THE READER

The evaluations of carcinogenic hazard in the *IARC Monographs on the Identification of Carcinogenic Hazards to Humans* series are made by international working groups of independent scientists. The *IARC Monographs* classifications do not indicate the level of risk associated with a given level or circumstance of exposure. The *IARC Monographs* do not make recommendations for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic hazard of an agent to humans is encouraged to make this information available to the *IARC Monographs* programme, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, or via email at imo@iarc.fr, 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* programme. Corrigenda are published online on the relevant webpage for the volume concerned (IARC Publications: https://publications.iarc.fr/).

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PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, general principles and procedures, and scientific review and evaluations. The *IARC Monographs* embody principles of scientific rigour, impartial evaluation, transparency, and consistency. The Preamble should be consulted when reading a *Monograph* or a summary of a *Monograph*'s evaluations. Separate Instructions for Authors describe the operational procedures for the preparation and publication of a volume of the *Monographs*.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after the International Agency for Research on Cancer (IARC) was established in 1965, it started to receive frequent requests for advice on the carcinogenicity of chemicals, including requests for lists of established and suspected human carcinogens. In 1970, an 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 next year, the IARC Governing Council adopted a resolution that IARC should prepare "monographs on the evaluation of carcinogenic risk of chemicals to man", which became the initial title of the series.

In succeeding years, the scope of the programme broadened as *Monographs* were developed for complex mixtures, occupational exposures, physical agents, biological organisms, pharmaceuticals, and other exposures. In 1988, "of chemicals" was dropped from the title, and in 2019, "evaluation of carcinogenic risks" became "identification of carcinogenic hazards", in line with the objective of the programme.

Identifying the causes of human cancer is the first step in cancer prevention. The identification of a cancer hazard may have broad and profound implications. National and international authorities and organizations can and do use information on causes of cancer in support of actions to reduce exposure to carcinogens in the workplace, in the environment, and elsewhere. Cancer prevention is needed as much today as it was when IARC was established, because the global burden of cancer is high and continues to increase as a result of population growth and ageing and upward trends in some exposures, especially in low- and middle-income countries (https://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports).

IARC's process for developing *Monographs*, which has evolved over several decades, involves

the engagement of international, interdisciplinary Working Groups of expert scientists, the transparent synthesis of different streams of evidence (exposure characterization, cancer in humans, cancer in experimental animals, and mechanisms of carcinogenesis), and the integration of these streams of evidence into an overall evaluation and classification according to criteria developed and refined by IARC. Since the *Monographs* programme was established, the understanding of carcinogenesis has greatly deepened. Scientific advances are incorporated into the evaluation methodology. In particular, strong mechanistic evidence has had an increasing role in the overall evaluations since 1991.

The Preamble is primarily a statement of the general principles and procedures used in developing a *Monograph*, to promote transparency and consistency across *Monographs* evaluations. In addition, IARC provides Instructions for Authors (https://monographs.iarc.fr/preamble-instructions-for-authors/), which specify more detailed working procedures. IARC routinely updates these Instructions for Authors to reflect advances in methods for cancer hazard identification and accumulated experience, including input from experts.

2. Objective and scope

The objective of the programme is to prepare, with the engagement of international, interdisciplinary Working Groups of experts, scientific reviews and evaluations of evidence on the carcinogenicity of a wide range of agents.

The *Monographs* assess the strength of the available evidence that an agent can cause cancer in humans, based on three streams of evidence: on cancer in humans (see Part B, Section 2), on cancer in experimental animals (see Part B, Section 3), and on mechanistic evidence (see Part B, Section 4). In addition, the exposure to each agent is characterized (see Part B, Section 1). In this Preamble, the term "agent" refers to any chemical, physical, or biological entity or exposure circumstance (e.g. occupation as a painter) for which evidence on the carcinogenicity is evaluated.

A cancer *hazard* is an agent that is capable of causing cancer, whereas a cancer *risk* is an estimate of the probability that cancer will occur given some level of exposure to a cancer hazard. The *Monographs* assess the strength of evidence that an agent is a cancer hazard. The distinction between hazard and risk is fundamental. The *Monographs* identify cancer hazards even when risks appear to be low in some exposure scenarios. This is because the exposure may be widespread at low levels, and because exposure levels in many populations are not known or documented.

Although the *Monographs* programme has focused on hazard identification, some epidemiological studies used to identify a cancer hazard are also used to estimate an exposure–response relationship within the range of the available data. However, extrapolating exposure–response relationships beyond the available data (e.g. to lower exposures, or from experimental animals to humans) is outside the scope of *Monographs* Working Groups (IARC, 2014). In addition, the *Monographs* programme does not review quantitative risk characterizations developed by other health agencies.

The identification of a cancer hazard should trigger some action to protect public health, either directly as a result of the hazard identification or through the conduct of a risk assessment. Although such actions are outside the scope of the programme, the *Monographs* are used by national and international authorities and organizations to inform risk assessments, formulate decisions about preventive measures, motivate effective cancer control programmes, and choose among options for public health decisions. *Monographs* evaluations are only one part of the body of information on which decisions to

control exposure to carcinogens may be based. Options to prevent cancer vary from one situation to another and across geographical regions and take many factors into account, including different national priorities. Therefore, no recommendations are given in the *Monographs* with regard to regulation, legislation, or other policy approaches, which are the responsibility of individual governments or organizations. The *Monographs* programme also does not make research recommendations. However, it is important to note that *Monographs* contribute significantly to the science of carcinogenesis by synthesizing and integrating streams of evidence about carcinogenicity and pointing to critical gaps in knowledge.

3. Selection of agents for review

Since 1984, about every five years IARC convenes an international, interdisciplinary Advisory Group to recommend agents for review by the *Monographs* programme. IARC selects Advisory Group members who are knowledgeable about current research on carcinogens and public health priorities. Before an Advisory Group meets, IARC solicits nominations of agents from scientists and government agencies worldwide. Since 2003, IARC also invites nominations from the public. IARC charges each Advisory Group with reviewing nominations, evaluating exposure and hazard potential, and preparing a report that documents the Advisory Group's process for these activities and its rationale for the recommendations.

For each new volume of the *Monographs*, IARC selects the agents for review from those recommended by the most recent Advisory Group, considering the availability of pertinent research studies and current public health priorities. On occasion, IARC may select other agents if there is a need to rapidly evaluate an emerging carcinogenic hazard or an urgent need to re-evaluate a previous classification. All evaluations consider the full body of available evidence, not just information published after a previous review.

A *Monograph* may review:

(a) An agent not reviewed in a previous *Monograph*, if there is potential human exposure and there is evidence for assessing its carcinogenicity. A group of related agents (e.g. metal compounds) may be reviewed together if there is evidence for assessing carcinogenicity for one or more members of the group.

(b) An agent reviewed in a previous *Monograph*, if there is new evidence of cancer in humans or in experimental animals, or mechanistic evidence to warrant re-evaluation of the classification. In the interests of efficiency, the literature searches may build on previous comprehensive searches.

(c) An agent that has been established to be carcinogenic to humans and has been reviewed in a previous *Monograph*, if there is new evidence of cancer in humans that indicates new tumour sites where there might be a causal association. In the interests of efficiency, the review may focus on these new tumour sites.

4. The Working Group and other meeting participants

Five categories of participants can be present at *Monographs* meetings:

(i) *Working Group* members are responsible for all scientific reviews and evaluations developed in the volume of the *Monographs*. The Working Group is interdisciplinary and comprises subgroups of experts in the fields of (a) exposure characterization, (b) cancer in humans, (c) cancer in experimental animals, and (d) mechanistic evidence. IARC selects Working Group members on the basis of

expertise related to the subject matter and relevant methodologies, and absence of conflicts of interest. Consideration is also given to diversity in scientific approaches and views, as well as demographic composition. Working Group members generally have published research related to the exposure or carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Since 2006, IARC also has encouraged public nominations through its Call for Experts. IARC's reliance on experts with knowledge of the subject matter and/or expertise in methodological assessment is confirmed by decades of experience documenting that there is value in specialized expertise and that the overwhelming majority of Working Group members are committed to the objective evaluation of scientific evidence and not to the narrow advancement of their own research results or a pre-determined outcome (Wild & Cogliano, 2011). Working Group members are expected to serve the public health mission of IARC, and should refrain from consulting and other activities for financial gain that are related to the agents under review, or the use of inside information from the meeting, until the full volume of the *Monographs* is published.

IARC identifies, from among Working Group members, individuals to serve as Meeting Chair and Subgroup Chairs. At the opening of the meeting, the Working Group is asked to endorse the selection of the Meeting Chair, with the opportunity to propose alternatives. The Meeting Chair and Subgroup Chairs take a leading role at all stages of the review process (see Part A, Section 7), promote open scientific discussions that involve all Working Group members in accordance with normal committee procedures, and ensure adherence to the Preamble.

(ii) *Invited Specialists* are experts who have critical knowledge and experience but who also have a conflict of interest that warrants exclusion from developing or influencing the evaluations of carcinogenicity. Invited Specialists do not draft any section of the *Monograph* that pertains to the description or interpretation of cancer data, and they do not participate in the evaluations. These experts are invited in limited numbers when necessary to assist the Working Group by contributing their unique knowledge and experience to the discussions.

(iii) *Representatives of national and international health agencies* may attend because their agencies are interested in the subject of the meeting. They do not draft any section of the *Monograph* or participate in the evaluations.

(iv) *Observers* with relevant scientific credentials may be admitted in limited numbers. Attention is given to the balance of Observers from constituencies with differing perspectives. Observers are invited to observe the meeting and should not attempt to influence it, and they agree to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers do not draft any section of the *Monograph* or participate in the evaluations.

(v) The *IARC Secretariat* consists of scientists who are designated by IARC and who have relevant expertise. The IARC Secretariat coordinates and facilitates all aspects of the evaluation and ensures adherence to the Preamble throughout development of the scientific reviews and classifications (see Part A, Sections 5 and 6). The IARC Secretariat organizes and announces the meeting, identifies and recruits the Working Group members, and assesses the declared interests of all meeting participants. The IARC Secretariat supports the activities of the Working Group (see Part A, Section 7) by

Table 1 Roles of participants at *IARC Monographs* meetings

Category of participant	Role			
	Prepare text, tables, and analyses	Participate in discussions	Participate in evaluations	Eligible to serve as Chair
Working Group members	✓	✓	✓	✓
Invited Specialists	✓[a]	✓		
Representatives of health agencies		✓[b]		
Observers		✓[b]		
IARC Secretariat	✓[c]	✓	✓[d]	

[a] Only for the section on exposure characterization.
[b] Only at times designated by the Meeting Chair and Subgroup Chairs.
[c] When needed or requested by the Meeting Chair and Subgroup Chairs.
[d] Only for clarifying or interpreting the Preamble.

searching the literature and performing title and abstract screening, organizing conference calls to coordinate the development of pre-meeting drafts and discuss cross-cutting issues, and reviewing drafts before and during the meeting. Members of the IARC Secretariat serve as meeting rapporteurs, assist the Meeting Chair and Subgroup Chairs in facilitating all discussions, and may draft text or tables when designated by the Meeting Chair and Subgroup Chairs. Their participation in the evaluations is restricted to the role of clarifying or interpreting the Preamble.

All participants are listed, with their principal affiliations, in the front matter of the published volume of the *Monographs*. Working Group members and Invited Specialists serve as individual scientists and not as representatives of any organization, government, or industry (Cogliano et al., 2004).

The roles of the meeting participants are summarized in Table 1.

5. Working procedures

A separate Working Group is responsible for developing each volume of the *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year before the meeting of a Working Group, a preliminary list of agents to be reviewed, together with a Call for Data and a Call for Experts, is announced on the *Monographs* programme website (https://monographs.iarc.fr/).

Before a meeting invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests form to report financial interests, employment and consulting (including remuneration for serving as an expert witness), individual and institutional research support, and non-financial interests such as public statements and positions related to the subject of the meeting. IARC assesses the declared interests to determine whether there is a conflict that warrants any limitation on participation (see Table 2).

Approximately two months before a *Monographs* meeting, IARC publishes the names and affiliations of all meeting participants together with a summary of declared interests, in the interests of transparency and to provide an opportunity for undeclared conflicts of interest to be brought to IARC's attention. 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).

Table 2 Public engagement during *Monographs* development

Approximate timeframe	Engagement
Every 5 years	IARC convenes an Advisory Group to recommend high-priority agents for future review
~1 year before a *Monographs* meeting	IARC selects agents for review in a new volume of the *Monographs* IARC posts on its website: Preliminary List of Agents to be reviewed Call for Data and Call for Experts Request for Observer Status WHO Declaration of Interests form
~8 months before a *Monographs* meeting	Call for Experts closes
~4 months before a *Monographs* meeting	Request for Observer Status closes
~2 months before a *Monographs* meeting	IARC posts the names of all meeting participants together with a summary of declared interests, and a statement discouraging contact of the Working Group by interested parties
~1 month before a *Monographs* meeting	Call for Data closes
~2–4 weeks after a *Monographs* meeting	IARC publishes a summary of evaluations and key supporting evidence
~9 months after a *Monographs* meeting	IARC Secretariat publishes the verified and edited master copy of plenary drafts as a *Monographs* volume

The Working Group meets at IARC for approximately eight days to discuss and finalize the scientific review and to develop summaries and evaluations. At the opening of the meeting, all participants update their Declaration of Interests forms, which are then reviewed by IARC. Declared interests related to the subject of the meeting are disclosed to the meeting participants during the meeting and in the published volume (Cogliano et al., 2004). The objectives of the meeting are peer review and consensus. During the first part of the meeting, subgroup sessions (covering exposure characterization, cancer in humans, cancer in experimental animals, and mechanistic evidence) review the pre-meeting drafts, develop a joint subgroup draft, and draft subgroup summaries. During the last part of the meeting, the Working Group meets in plenary session to review the subgroup drafts and summaries and to develop the consensus evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections. After the meeting, the master copy is verified by the IARC Secretariat and is then edited and prepared for publication. The aim is to publish the volume within approximately nine months of the Working Group meeting. A summary of the evaluations and key supporting evidence is prepared for publication in a scientific journal or is made available on the *Monographs* programme website soon after the meeting.

In the interests of transparency, IARC engages with the public throughout the process, as summarized in Table 2.

6. Overview of the scientific review and evaluation process

The Working Group considers all pertinent epidemiological studies, cancer bioassays in experimental animals, and mechanistic evidence, as well as pertinent information on exposure in humans. In general, for cancer in humans, cancer in experimental animals, and mechanistic evidence, only studies that have been published or accepted for publication in the openly available scientific literature are reviewed. Under some circumstances, materials

that are publicly available and whose content is final may be reviewed if there is sufficient information to permit an evaluation of the quality of the methods and results of the studies (see Step 1, below). Such materials may include reports and databases publicly available from government agencies, as well as doctoral theses. The reliance on published and publicly available studies promotes transparency and protects against citation of premature information.

The principles of systematic review are applied to the identification, screening, synthesis, and evaluation of the evidence related to cancer in humans, cancer in experimental animals, and mechanistic evidence (as described in Part B, Sections 2–4 and as detailed in the Instructions for Authors). Each *Monograph* specifies or references information on the conduct of the literature searches, including search terms and inclusion/exclusion criteria that were used for each stream of evidence.

In brief, the steps of the review process are as follows:

Step 1. Comprehensive and transparent identification of the relevant information: The IARC Secretariat identifies relevant studies through initial comprehensive searches of literature contained in authoritative biomedical databases (e.g. PubMed, PubChem) and through a Call for Data. These literature searches, designed in consultation with a librarian and other technical experts, address whether the agent causes cancer in humans, causes cancer in experimental systems, and/or exhibits key characteristics of established human carcinogens (in humans or in experimental systems). The Working Group provides input and advice to IARC to refine the search strategies, and identifies literature through other searches (e.g. from reference lists of past *Monographs*, retrieved articles, and other authoritative reviews).

For certain types of agents (e.g. regulated pesticides and pharmaceuticals), IARC also provides an opportunity to relevant regulatory authorities, and regulated parties through such authorities, to make pertinent unpublished studies publicly available by the date specified in the Call for Data. Consideration of such studies by the Working Group is dependent on the public availability of sufficient information to permit an independent evaluation of (a) whether there has been selective reporting (e.g. on outcomes, or from a larger set of conducted studies); (b) study quality (e.g. design, methodology, and reporting of results), and (c) study results.

Step 2. Screening, selection, and organization of the studies: The IARC Secretariat screens the retrieved literature for inclusion based on title and abstract review, according to pre-defined exclusion criteria. For instance, studies may be excluded if they were not about the agent (or a metabolite of the agent), or if they reported no original data on epidemiological or toxicological end-points (e.g. review articles). The Working Group reviews the title and abstract screening done by IARC, and performs full-text review. Any reasons for exclusion are recorded, and included studies are organized according to factors pertinent to the considerations described in Part B, Sections 2–4 (e.g. design, species, and end-point). Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results.

Step 3. Evaluation of study quality: The Working Group evaluates the quality of the included studies based on the considerations (e.g. design, methodology, and reporting of results) described in Part B, Sections 2–4. Based on these considerations, the Working Group may accord greater weight to some of the included studies. Interpretation of the

results and the strengths and limitations of a study are clearly outlined in square brackets at the end of study descriptions (see Part B).

Step 4: *Report characteristics of included studies, including assessment of study quality*: Pertinent characteristics and results of included studies are reviewed and succinctly described, as detailed in Part B, Sections 1–4. Tabulation of data may facilitate this reporting. This step may be iterative with Step 3.

Step 5: *Synthesis and evaluation of strength of evidence*: The Working Group summarizes the overall strengths and limitations of the evidence from the individual streams of evidence (cancer in humans, cancer in experimental animals, and mechanistic evidence; see Part B, Section 5). The Working Group then evaluates the strength of evidence from each stream of evidence by using the transparent methods and defined descriptive terms given in Part B, Sections 6a–c. The Working Group then develops, and describes the rationale for, the consensus classification of carcinogenicity that integrates the conclusions about the strength of evidence from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic evidence (see Part B, Section 6d).

7. Responsibilities of the Working Group

The Working Group is responsible for identifying and evaluating the relevant studies and developing the scientific reviews and evaluations for a volume of the *Monographs*. The IARC Secretariat supports these activities of the Working Group (see Part A, Section 4). Briefly, the Working Group's tasks in developing the evaluation are, in sequence:

(i) Before the meeting, the Working Group ascertains that all appropriate studies have been identified and selected, and assesses the methods and quality of each individual study, as outlined above (see Part A, Section 6). The Working Group members prepare pre-meeting working drafts that present accurate tabular or textual summaries of informative studies by extracting key elements of the study design and results, and highlighting notable strengths and limitations. They participate in conference calls organized by IARC to coordinate the development of working drafts and to discuss cross-cutting issues. Pre-meeting reviews of all working drafts are generally performed by two or more subgroup members who did not participate in study identification, data extraction, or study review for the draft. Each study summary is written or reviewed by someone who is not associated with the study.

(ii) At the meeting, within subgroups, the Working Group members critically review, discuss, and revise the pre-meeting drafts and adopt the revised versions as consensus subgroup drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. A proposed classification of the strength of the evidence reviewed in the subgroup using the *IARC Monographs* criteria (see Part B, Sections 6a–c) is then developed from the consensus subgroup drafts of the evidence summaries (see Part B, Section 5).

(iii) During the plenary session, each subgroup presents its drafts for scientific review and discussion to the other Working Group members, who did not participate in study identification, data extraction, or study review for the drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary.

After review, discussion, and revisions as needed, the subgroup drafts are adopted as a consensus Working Group product. The summaries and classifications of the strength of the evidence, developed in the subgroup in line with the *IARC Monographs* criteria (see Part B, Sections 6a–c), are considered, revised as needed, and adopted by the full Working Group. The Meeting Chair proposes an overall evaluation using the guidance provided in Part B, Section 6d.

The Working Group strives to achieve consensus evaluations. Consensus reflects broad agreement among the Working Group, but not necessarily unanimity. The Meeting Chair may poll the Working Group to determine the diversity of scientific opinion on issues where consensus is not apparent.

Only the final product of the plenary session represents the views and expert opinions of the Working Group. The entire *Monographs* volume is the joint product of the Working Group and represents an extensive and thorough peer review of the body of evidence (individual studies, synthesis, and evaluation) by an interdisciplinary expert group. Initial working papers and subsequent revisions are not released, because they would give an incomplete and possibly misleading impression of the consensus developed by the Working Group over a full week of deliberation.

B. SCIENTIFIC REVIEW AND EVALUATION

This part of the Preamble discusses the types of evidence that are considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations. In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and any key issues encountered during the meeting.

1. Exposure characterization

This section identifies the agent and describes its occurrence, main uses, and production locations and volumes, where relevant. It also summarizes the prevalence, concentrations in relevant studies, and relevant routes of exposure in humans worldwide. Methods of exposure measurement and analysis are described, and methods of exposure assessment used in key epidemiological studies reviewed by the Working Group are described and evaluated.

Over the course of the *Monographs* programme, concepts of exposure and dose have evolved substantially with deepening understanding of the interactions of agents and biological systems. The concept of exposure has broadened and become more holistic, extending beyond chemical, physical, and biological agents to stressors as construed generally, including psychosocial stressors (National Research Council, 2012; National Academies of Sciences, Engineering, and Medicine, 2017). Overall, this broader conceptualization supports greater integration between exposure characterization and other sections of the *Monographs*. Concepts of absorption, distribution, metabolism, and excretion are considered in the first subsection of mechanistic evidence (see Part B, Section 4a), whereas validated biomarkers of internal exposure or metabolites that are routinely used for exposure assessment are reported on in this section (see Part B, Section 1b).

(a) Identification of the agent

The agent being evaluated is unambiguously identified. Details will vary depending on the type of agent but will generally include physical and chemical properties relevant to the agent's identification, occurrence, and biological activity.

If the material that has been tested in experimental animals or in vitro systems is different from that to which humans are exposed, these differences are noted.

For chemical agents, the Chemical Abstracts Service Registry Number is provided, as well as the latest primary name and other names in common use, including important trade names, along with available information on the composition of common mixtures or products containing the agent, and potentially toxic and/or carcinogenic impurities. Physical properties relevant to understanding the potential for human exposure and measures of exposure used in studies in humans are summarized. These might include physical state, volatility, aqueous and fat solubility, and half-life in the environment and/or in human tissues.

For biological agents, taxonomy and structure are described. Mode of replication, life-cycle, target cells, persistence, latency, and host responses, including morbidity and mortality through pathologies other than cancer, are also presented.

For foreign bodies, fibres and particles, composition, size range, relative dimensions, and accumulation, persistence, and clearance in target organs are summarized. Physical agents that are forms of radiation are described in terms of frequency spectrum and energy transmission.

Exposures may result from, or be influenced by, a diverse range of social and environmental factors, including components of diet, sleep, and physical activity patterns. In these instances, this section will include a description of the agent, its variability across human populations, and its composition or characteristics relevant to understanding its potential carcinogenic hazard to humans and to evaluating exposure assessments in epidemiological studies.

(b) Detection and analysis

Key methods of detection and quantification of the agent are presented, with an emphasis on those used most widely in surveillance, regulation, and epidemiological studies. Measurement methods for sample matrices that are deemed important sources of human exposure (e.g. air, drinking-water, food, residential dust) and for validated exposure biomarkers (e.g. the agent or its metabolites in human blood, urine, or saliva) are described. Information on detection and quantification limits is provided when it is available and is useful for interpreting studies in humans and in experimental animals. This is not an exhaustive treatise but is meant to help readers understand the strengths and limitations of the available exposure data and of the epidemiological studies that rely on these measurements.

(c) Production and use

Historical and geographical patterns and trends in production and use are included when they are available, to help readers understand the contexts in which exposures may occur, both within key epidemiological studies reviewed by the Working Group and in human populations generally. Industries that produce, use, or dispose of the agent are described, including their global distribution, when available. National or international listing as a high-production-volume chemical or similar classification may be included. Production processes with significant potential for occupational exposure or environmental pollution are indicated. Trends in global production volumes, technologies, and other data relevant to understanding exposure potential are summarized. Minor or historical uses with significant exposure potential or with particular relevance to key epidemiological studies are included. Particular effort may be directed towards finding data on production in low- and middle-income countries, where rapid

economic development may lead to higher exposures than those in high-income countries.

(d) Exposure

A concise overview of quantitative information on sources, prevalence, and levels of exposure in humans is provided. Representative data from research studies, government reports and websites, online databases, and other citable, publicly available sources are tabulated. Data from low- and middle-income countries are sought and included to the extent feasible; information gaps for key regions are noted. Naturally occurring sources of exposure, if any, are noted. Primary exposure routes (e.g. inhalation, ingestion, skin uptake) and other considerations relevant to understanding the potential for cancer hazard from exposure to the agent are reported.

For occupational settings, information on exposure prevalence and levels (e.g. in air or human tissues) is reported by industry, occupation, region, and other characteristics (e.g. process, task) where feasible. Information on historical exposure trends, protection measures to limit exposure, and potential co-exposures to other carcinogenic agents in workplaces is provided when available.

For non-occupational settings, the occurrence of the agent is described with environmental monitoring or surveillance data. Information on exposure prevalence and levels (e.g. concentrations in human tissues) as well as exposure from and/or concentrations in food and beverages, consumer products, consumption practices, and personal microenvironments is reported by region and other relevant characteristics. Particular importance is placed on describing exposures in life stages or in states of disease or nutrition that may involve greater exposure or susceptibility.

Current exposures are of primary interest; however, information on historical exposure trends is provided when available. Historical exposures may be relevant for interpreting epidemiological studies, and when agents are persistent or have long-term effects. Information gaps for important time periods are noted. Exposure data that are not deemed to have high relevance to human exposure are generally not considered.

(e) Regulations and guidelines

Regulations or guidelines that have been established for the agent (e.g. occupational exposure limits, maximum permitted levels in foods and water, pesticide registrations) are described in brief to provide context about government efforts to limit exposure; these may be tabulated if they are informative for the interpretation of existing or historical exposure levels. Information on applicable populations, specific agents concerned, basis for regulation (e.g. human health risk, environmental considerations), and timing of implementation may be noted. National and international bans on production, use, and trade are also indicated.

This section aims to include major or illustrative regulations and may not be comprehensive, because of the complexity and range of regulatory processes worldwide. An absence of information on regulatory status should not be taken to imply that a given country or region lacks exposure to, or regulations on exposure to, the agent.

(f) Critical review of exposure assessment in key epidemiological studies

Epidemiological studies evaluate cancer hazard by comparing outcomes across differently exposed groups. Therefore, the type and quality of the exposure assessment methods used are key considerations when interpreting study findings for hazard identification. This section summarizes and critically reviews the exposure assessment methods used in the individual epidemiological studies that contribute data relevant to the *Monographs* evaluation.

Although there is no standard set of criteria for evaluating the quality of exposure assessment methods across all possible agents, some concepts are universally relevant. Regardless of the agent, all exposures have two principal dimensions: intensity (sometimes defined as concentration or dose) and time. Time considerations include duration (time from first to last exposure), pattern or frequency (whether continuous or intermittent), and windows of susceptibility. This section considers how each of the key epidemiological studies characterizes these dimensions. Interpretation of exposure information may also be informed by consideration of mechanistic evidence (e.g. as described in Part B, Section 4a), including the processes of absorption, distribution, metabolism, and excretion.

Exposure intensity and time in epidemiological studies can be characterized by using environmental or biological monitoring data, records from workplaces or other sources, expert assessments, modelled exposures, job-exposure matrices, and subject or proxy reports via questionnaires or interviews. Investigators use these data sources and methods individually or in combination to assign levels or values of an exposure metric (which may be quantitative, semi-quantitative, or qualitative) to members of the population under study.

In collaboration with the Working Group members reviewing human studies (of cancer and of mechanisms), key epidemiological studies are identified. For each selected study, the exposure assessment approach, along with its strengths and limitations, is summarized using text and tables. Working Group members identify concerns about exposure assessment methods and their impacts on overall quality for each study reviewed (see Part B, Sections 2d and 4d). In situations where the information provided in the study is inadequate to properly consider the exposure assessment, this is indicated. When adequate information is available, the likely direction of bias due to error in exposure measurement, including misclassification (overestimated effects, underestimated effects, or unknown) is discussed.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part B, Section 2b) that include cancer as an outcome. These studies encompass certain types of biomarker studies, for example, studies with biomarkers as exposure metrics (see Part B, Section 2) or those evaluating histological or tumour subtypes and molecular signatures in tumours consistent with a given exposure (Alexandrov et al., 2016). Studies that evaluate early biological effect biomarkers are reviewed in Part B, Section 4.

(a) Types of study considered

Several types of epidemiological studies contribute to the assessment of carcinogenicity in humans; they typically include cohort studies (including variants such as case–cohort and nested case–control studies), case–control studies, ecological studies, and intervention studies. Rarely, results from randomized trials may be available. Exceptionally, case reports and case series of cancer in humans may also be reviewed. In addition to these designs, innovations in epidemiology allow for many other variants that may be considered in any given *Monographs* evaluation.

Cohort and case–control studies typically have the capacity to 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. Well-conducted cohort and case–control studies provide most of the evidence of cancer in humans evaluated by Working Groups. Intervention studies are much less common, but when available can provide strong evidence for making causal inferences.

In ecological 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 in the population under study. In ecological studies, data on individual exposure and outcome are not available, which renders this type of study more prone to confounding and exposure misclassification. In some circumstances, however, ecological studies may be informative, especially when the unit of exposure is most accurately measured at the population level (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

Exceptionally, case reports and case series may provide compelling evidence about the carcinogenicity of an agent. In fact, many of the early discoveries of occupational cancer hazards came about because of observations by workers and their clinicians, who noted a high frequency of cancer in workers who share a common occupation or exposure. Such observations may be the starting point for more structured investigations, but in exceptional circumstances, when the risk is high enough, the case series may in itself provide compelling evidence. This would be especially warranted in situations where the exposure circumstance is fairly unusual, as it was in the example of plants containing aristolochic acid (IARC, 2012a).

The uncertainties that surround the interpretation of case reports, case series, and ecological studies typically make them inadequate, except in rare instances as described above, to form the sole basis for inferring a causal relationship. However, when considered together with cohort and case–control studies, these types of study may support the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, pre-neoplastic lesions, malignant precursors, and other end-points are also reviewed when they relate to the agents reviewed. On occasion they can strengthen inferences drawn from studies of cancer itself. For example, benign brain tumours may share common risk factors with those that are malignant, and benign neoplasms (or those of uncertain behaviour) may be part of the causal path to malignancies (e.g. myelodysplastic syndromes, which may progress to acute myeloid leukaemia).

(b) Identification of eligible studies of cancer in humans

Relevant studies of cancer in humans are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Eligible studies include all studies in humans of exposure to the agent of interest with cancer as an outcome. Multiple publications on the same study population are identified so that the number of independent studies is accurately represented. Multiple publications may result, for example, from successive follow-ups of a single cohort, from analyses focused on different aspects of an exposure–disease association, or from inclusion of overlapping populations. Usually in such situations, only the most recent, most comprehensive, or most informative report is reviewed in detail.

(c) Assessment of study quality and informativeness

Epidemiological studies are potentially susceptible to several different sources of error, summarized briefly below. Qualities of individual studies that address these issues are also described below.

Study quality is assessed as part of the structured expert review process undertaken by the Working Group. A key aspect of quality assessment is consideration of the possible roles of chance and bias in the interpretation of epidemiological studies. Chance, which is also called

random variation, can produce misleading study results. This variability in study results is strongly influenced by the sample size: smaller studies are more likely than larger studies to have effect estimates that are imprecise. Confidence intervals around a study's point estimate of effect are used routinely to indicate the range of values of the estimate that could easily be produced by chance alone.

Bias is the effect of factors in study design or conduct that lead an association to erroneously appear stronger or weaker than the association that really exists between the agent and the disease. Biases that require consideration are varied but are usually categorized as selection bias, information bias (e.g. error in measurement of exposure and diseases), and confounding (or confounding bias), (Rothman et al., 2008). Selection bias in an epidemiological study occurs when inclusion of participants from the eligible population or their follow-up in the study is influenced by their exposure or their outcome (usually disease occurrence). Under these conditions, the measure of association found in the study will not accurately reflect the association that would otherwise have been found in the eligible population (Hernán et al., 2004). Information bias results from inaccuracy in exposure or outcome measurement. Both can cause an association between hypothesized cause and effect to appear stronger or weaker than it really is. Confounding is a mixing of extraneous effects with the effects of interest (Rothman et al., 2008). An association between the purported causal factor and another factor that is associated with an increase or decrease in incidence of disease can lead to a spurious association or absence of a real association of the presumed causal factor with the disease. When either of these occurs, confounding is present.

In assessing study quality, the Working Group consistently considers the following aspects:

- **Study description:** Clarity in describing the study design and its implementation, and the completeness of reporting of all other key information about the study and its results.
- **Study population:** Whether the study population was appropriate for evaluating the association between the agent and cancer. Whether the study was designed and carried out to minimize selection bias. Cancer cases in the study population must have been identified in a way that was independent of the exposure of interest, and exposure assessed in a way that was not related to disease (outcome) status. In these respects, completeness of recruitment into the study from the population of interest and completeness of follow-up for the outcome are essential measures.
- **Outcome measurement:** The appropriateness of the cancer outcome measure (e.g. mortality vs incidence) for the agent and cancer type under consideration, outcome ascertainment methodology, and the extent to which outcome misclassification may have led to bias in the measure(s) of association.
- **Exposure measurement:** The adequacy of the methods used to assess exposure to the agent, and the likelihood (and direction) of bias in the measure(s) of association due to error in exposure measurement, including misclassification (as described in Part B, Section 1f).
- **Assessment of potential confounding:** To what extent the authors took into account in the study design and analysis other variables (including co-exposures, as described in Part B, Section 1d) that can influence the risk of disease and may have been related to the exposure of interest. Important sources of potential confounding by such variables should have been addressed either in the design of the study, such as by matching or restriction, or in the analysis, by statistical adjustment. In some instances, where direct information on confounders is unavailable,

use of indirect methods to evaluate the potential impact of confounding on exposure–disease associations is appropriate (e.g. Axelson & Steenland, 1988; Richardson et al., 2014).

- **Other potential sources of bias:** Each epidemiological study is unique in its study population, its design, its data collection, and, consequently, its potential biases. All possible sources of bias are considered for their possible impact on the results. The possibility of reporting bias (i.e. selective reporting of some results and the suppression of others) should be explored.
- **Statistical methodology:** Adequacy of the statistical methods used and their ability to obtain unbiased estimates of exposure–outcome associations, confidence intervals, and test statistics for the significance of measures of association. Appropriateness of methods used to investigate confounding, including adjusting for matching when necessary and avoiding treatment of probable mediating variables as confounders. Detailed analyses of cancer risks in relation to summary measures of exposure such as cumulative exposure, or temporal variables such as age at first exposure or time since first exposure, are reviewed and summarized when available.

For the sake of economy and simplicity, in this Preamble the list of possible sources of error is referred to with the phrase "chance, bias, and confounding", but it should be recognized that this phrase encompasses a comprehensive set of concerns pertaining to study quality.

These sources of error do not constitute and should not be used as a formal checklist of indicators of study quality. The judgement of experienced experts is critical in determining how much weight to assign to different issues in considering how all of these potential sources of error should be integrated and how to rate the potential for error related to each of these considerations.

The informativeness of a study is its ability to show a true association, if there is one, between the agent and cancer, and the lack of an association, if no association exists. Key determinants of informativeness include: having a study population of sufficient size to obtain precise estimates of effect; sufficient elapsed time from exposure to measurement of outcome for an effect, if present, to be observable; presence of an adequate exposure contrast (intensity, frequency, and/or duration); biologically relevant definitions of exposure; and relevant and well-defined time windows for exposure and outcome.

(d) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to inconsistent results that are difficult to interpret or reconcile. Combined analyses of data from multiple studies may be conducted as a means to address this ambiguity. 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 & O'Rourke, 2008).

The strengths of combined analyses are increased precision because of increased sample size and, in the case of pooled analyses, the opportunity to better control for potential confounders and to explore in more detail interactions and modifying effects that may explain heterogeneity among studies. A disadvantage of combined analyses is the possible lack of comparability of data from various studies, because of differences in population characteristics, subject recruitment, procedures of data collection, methods of measurement, and effects of unmeasured covariates that may differ among studies. These differences in study methods and quality can influence

results of either meta-analyses or pooled analyses. If published meta-analyses are to be considered by the Working Group, their adequacy needs to be carefully evaluated, including the methods used to identify eligible studies and the accuracy of data extracted from the individual studies.

The Working Group may conduct ad hoc meta-analyses during the course of a *Monographs* meeting, when there are sufficient studies of an exposure–outcome association to contribute to the Working Group's assessment of the association. The results of such unpublished 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, the following key considerations apply: the same criteria for data quality must be applied as for individual studies; sources of heterogeneity among studies must be carefully considered; and the possibility of publication bias should be explored.

(e) Considerations in assessing the body of epidemiological evidence

The ability of the body of epidemiological evidence to inform the Working Group about the carcinogenicity of the agent is related to both the quantity and the quality of the evidence. There is no formulaic answer to the question of how many studies of cancer in humans are needed from which to draw inferences about causality, although more than a single study in a single population will almost always be needed. The number will depend on the considerations relating to evidence described below.

After the quality of individual epidemiological studies of cancer has been assessed and the informativeness of the various studies on the association between the agent and cancer has been evaluated, a judgement is made about the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several aspects of the body of evidence (e.g. Hill, 1965; Rothman et al., 2008; Vandenbroucke et al., 2016).

A strong association (e.g. a large relative risk) is more likely to indicate causality than is a weak association, because it is more difficult for confounding to falsely create a strong association. However, it is recognized that estimates of effect of small magnitude do not imply lack of causality and may have impact on public health if the disease or exposure is common. Estimates of effect of small magnitude could also contribute useful information to the assessment of causality if level of risk is commensurate with level of exposure when compared with risk estimates from populations with higher exposure (e.g. as seen in residential radon studies compared with studies of radon from uranium mining).

Associations that are consistently observed in several studies of the same design, or in studies that use different epidemiological approaches, or under different circumstances of exposure are more likely to indicate a causal relationship than are isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (e.g. differences in study informativeness because of latency, exposure levels, or assessment methods). Results of studies that are judged to be of high quality and informativeness are given more weight than those of studies judged to be methodologically less sound or less informative.

Temporality of the association is an essential consideration: that is, the exposure must precede the outcome.

An observation that cancer risk increases with increasing exposure is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship, and there are several reasons why the shape of the exposure–response

association may be non-monotonic (e.g. Stayner et al., 2003). 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.

Confidence in a causal interpretation of the evidence from studies of cancer in humans is enhanced if it is coherent with physiological and biological knowledge, including information about exposure to the target organ, latency and timing of the exposure, and characteristics of tumour subtypes.

The Working Group considers whether there are subpopulations with increased susceptibility to cancer from the agent. For example, molecular epidemiology studies that identify associations between genetic polymorphisms and inter-individual differences in cancer susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. Such studies may be particularly informative if polymorphisms are found to be modifiers of the exposure–response association, because evaluation of polymorphisms may increase the ability to detect an effect in susceptible subpopulations.

When, in the process of evaluating the studies of cancer in humans, the Working Group identifies several high-quality, informative epidemiological studies that clearly show either no positive association or an inverse association between an exposure and a specific type of cancer, a judgement may be made that, in the aggregate, they suggest evidence of lack of carcinogenicity for that cancer type. Such a judgement requires, first, that the studies strictly meet 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 ruled out with reasonable confidence. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of relative effect of unity (or below unity) for any observed level of exposure, (b) when considered together, provide a combined estimate of relative risk that is at or below unity, and (c) have a narrow confidence interval. Moreover, neither any individual well-designed and well-conducted 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 must be noted that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the exposure levels reported and the timing and route of exposure studied, to the intervals between first exposure and disease onset observed in these studies, and to the general population(s) studied (i.e. there may be susceptible subpopulations or life stages). Experience from studies of cancer in humans indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; therefore, latency periods substantially shorter than about 30 years cannot provide evidence of lack of carcinogenicity. Furthermore, there may be critical windows of exposure, for example, as with diethylstilboestrol and clear cell adenocarcinoma of the cervix and vagina (IARC, 2012a).

3. Studies of cancer in experimental animals

Most human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species. For some agents, carcinogenicity in experimental animals was demonstrated before epidemiological studies identified their carcinogenicity in humans. Although this observation 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) present

a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, such as strong evidence that a given agent causes cancer in experimental animals through a species-specific mechanism that does not operate in humans (see Part B, Sections 4 and 6; Capen et al., 1999; IARC, 2003), these agents are considered to pose a potential carcinogenic hazard to humans. The inference of potential carcinogenic hazard to humans does not imply tumour site concordance across species (Baan et al., 2019).

(a) Types of studies considered

Relevant studies of cancer in experimental animals are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (or possibly metabolites or derivatives of the agent) (see Part A, Section 7) after a thorough evaluation of the study features (see Part B, Section 3b). Those studies 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, 2018).

In addition to conventional long-term bioassays, alternative studies (e.g. in genetically engineered mouse models) may be considered in assessing carcinogenicity in experimental animals, also after a critical evaluation of the study features. For studies of certain exposures, such as viruses that typically only infect humans, use of such specialized experimental animal models may be particularly important; models include genetically engineered mice with targeted expression of viral genes to tissues from which human cancers arise, as well as humanized mice implanted with the human cells usually infected by the virus.

Other types of studies can provide supportive evidence. These include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies); studies in which the end-point was not cancer but a defined precancerous lesion; and studies of cancer in non-laboratory animals (e.g. companion animals) exposed to the agent.

(b) Study evaluation

Considerations of importance in the interpretation and evaluation of a particular study include: (i) whether the agent was clearly characterized, including the nature and extent of impurities and contaminants and the stability of the agent, and, in the case of mixtures, whether the sample characterization was adequately reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration and frequency of treatment, duration of observation, and route of exposure were appropriate; (iv) whether appropriate experimental animal species and strains were evaluated; (v) whether there were adequate numbers of animals per group; (vi) whether animals were allocated randomly to groups; (vii) whether the body weight, food and water consumption, and survival of treated animals were affected by any factors other than the test agent; (viii) whether the histopathology review was adequate; and (ix) whether the data were reported and analysed adequately.

(c) Outcomes and statistical analyses

An assessment of findings of carcinogenicity in experimental animals involves consideration of (i) study features such as route, doses, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age, and duration of follow-up; (ii) the spectrum of neoplastic response, from

pre-neoplastic lesions and benign tumours to malignant neoplasms; (iii) the incidence, latency, severity, and multiplicity of neoplasms and pre-neoplastic lesions; (iv) the consistency of the results for a specific target organ or organs across studies of similar design; and (v) the possible role of modifying factors (e.g. diet, infection, stress).

Key factors for statistical analysis include: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type or lesion, and (iii) duration of survival.

Benign tumours may be combined with malignant tumours in the assessment of tumour incidence when (a) they occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) they appear to represent a stage in the progression to malignancy (Huff et al., 1989). The occurrence of lesions presumed to be pre-neoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.

Evidence of an increased incidence of neoplasms with increasing level 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, including non-linearity, depending on the particular agent under study and the target organ. The dose–response relationship can also be affected by differences in survival among the treatment groups.

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 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 and a survival-adjusted analysis would be warranted. 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 that 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–Haenszel 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).

The concurrent control group is generally the most appropriate comparison group for statistical analysis; however, for uncommon tumours, 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, sex, 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). 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.

Meta-analyses and pooled analyses may be appropriate when the experimental protocols are sufficiently similar.

4. Mechanistic evidence

Mechanistic data may provide evidence of carcinogenicity and may also help in assessing the relevance and importance of findings of cancer in experimental animals and in humans (Guyton et al., 2009; Parkkinen et al., 2018) (see Part B, Section 6). Mechanistic studies have gained in prominence, increasing in their volume, diversity, and relevance to cancer hazard evaluation, whereas studies pertinent to other streams of evidence evaluated in the *Monographs* (i.e. studies of cancer in humans and lifetime cancer bioassays in rodents) may only be available for a fraction of agents to which humans are currently exposed (Guyton et al., 2009, 2018). Mechanistic studies and data are identified, screened, and evaluated for quality and importance to the evaluation by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below.

The Working Group's synthesis reflects the extent of available evidence, summarizing groups of included studies with an emphasis on characterizing consistencies or differences in results within and across experimental designs. Greater emphasis is given to informative mechanistic evidence from human-related studies than to that from other experimental test systems, and gaps are identified. Tabulation of data may facilitate this review. The specific topics addressed in the evidence synthesis are described below.

(a) Absorption, distribution, metabolism, and excretion

Studies of absorption, distribution, metabolism, and excretion in mammalian species are addressed in a summary fashion; exposure characterization is addressed in Part B, Section 1. The Working Group describes the metabolic fate of the agent in mammalian species, noting the metabolites that have been identified and their chemical reactivity. A metabolic schema may indicate the relevant metabolic pathways and products and whether supporting evidence is from studies in humans and/or studies in experimental animals. Evidence on other adverse effects that indirectly confirm absorption, distribution, and/or metabolism at tumour sites is briefly summarized when direct evidence is sparse.

(b) Evidence relevant to key characteristics of carcinogens

A review of Group 1 human carcinogens classified up to and including *IARC Monographs* Volume 100 revealed several issues relevant to improving the evaluation of mechanistic evidence for cancer hazard identification (Smith et al., 2016). First, it was noted that human carcinogens often share one or more characteristics that are related to the multiple mechanisms by which agents cause cancer. Second, different human carcinogens may exhibit a different spectrum of these key characteristics and operate through distinct mechanisms. Third, for many carcinogens evaluated before Volume 100, few data were available on some mechanisms of recognized importance in carcinogenesis, such as epigenetic alterations (Herceg et al., 2013). Fourth, there was no widely accepted method to search systematically for relevant mechanistic evidence, resulting in a lack of uniformity in the scope of mechanistic topics addressed across *IARC Monographs* evaluations.

To address these challenges, the key characteristics of human carcinogens were introduced to facilitate systematic consideration of mechanistic evidence in *IARC Monographs* evaluations (Smith et al., 2016; Guyton et al., 2018). The key characteristics described by Smith et al. (2016) (see Table 3), such as "is genotoxic", "is immunosuppressive", or "modulates receptor-mediated

Table 3 The key characteristics of carcinogens

Ten key characteristics of carcinogens	
1.	Is electrophilic or can be metabolically activated to an electrophile
2.	Is genotoxic
3.	Alters DNA repair or causes genomic instability
4.	Induces epigenetic alterations
5.	Induces oxidative stress
6.	Induces chronic inflammation
7.	Is immunosuppressive
8.	Modulates receptor-mediated effects
9.	Causes immortalization
10.	Alters cell proliferation, cell death, or nutrient supply

From Smith et al. (2016).

effects", are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by the *IARC Monographs* programme up to and including Volume 100. The list of key characteristics and associated end-points may evolve, based on the experience of their application and as new human carcinogens are identified. Key characteristics are distinct from the "hallmarks of cancer", which relate to the properties of cancer cells (Hanahan & Weinberg, 2000, 2011). Key characteristics are also distinct from hypothesized mechanistic pathways, which describe a sequence of biological events postulated to occur during carcinogenesis. As such, the evaluation approach based on key characteristics, outlined below, "avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence" (National Academies of Sciences, Engineering, and Medicine, 2017).

Studies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available. For each key characteristic with adequate evidence for evaluation, studies are grouped according to whether they involve (a) humans or human primary cells or tissues or (b) experimental systems; further organization (as appropriate) is by end-point (e.g. DNA damage), duration, species, sex, strain, and target organ as well as strength of study design. Studies investigating susceptibility related to key characteristics of carcinogens (e.g. of genetic polymorphisms, or in genetically engineered animals) can be highlighted and may provide additional support for conclusions on the strength of evidence. Findings relevant to a specific tumour type may be noted.

(c) Other relevant evidence

Other informative evidence may be described when it is judged by the Working Group to be relevant to an evaluation of carcinogenicity and to be of sufficient importance to affect the overall evaluation. Quantitative structure–activity information, such as on specific chemical and/or biological features or activities (e.g. electrophilicity, molecular docking with receptors), may be informative. In addition, evidence that falls outside of the recognized key characteristics of carcinogens, reflecting emerging knowledge or important novel scientific developments on carcinogen mechanisms, may also be included. Available evidence relevant to criteria provided in authoritative publications (e.g. Capen et al., 1999; IARC, 2003) on thyroid, kidney, urinary

bladder, or other tumours in experimental animals induced by mechanisms that do not operate in humans is also described.

(d) Study quality and importance to the evaluation

Based on formal considerations of the quality of the studies (e.g. design, methodology, and reporting of results), the Working Group may give greater weight to some included studies.

For observational and other studies in humans, the quality of study design, exposure assessment, and assay accuracy and precision are considered, in collaboration with the Working Group members reviewing exposure characterization and studies of cancer in humans, as are other important factors, including those described above for evaluation of epidemiological evidence (García-Closas et al., 2006, 2011; Vermeulen et al., 2018) (Part B, Sections 1 and 2).

In general, in experimental systems, studies of repeated doses and of chronic exposures are accorded greater importance than are studies of a single dose or time-point. Consideration is also given to factors such as the suitability of the dosing range, the extent of concurrent toxicity observed, and the completeness of reporting of the study (e.g. the source and purity of the agent, the analytical methods, and the results). Route of exposure is generally considered to be a less important factor in the evaluation of experimental studies, recognizing that the exposures and target tissues may vary across experimental models and in exposed human populations. Non-mammalian studies can be synthetically summarized when they are considered to be supportive of evidence in humans or higher organisms.

In vitro test systems can provide mechanistic insights, but important considerations include the limitations of the test system (e.g. in metabolic capabilities) as well as the suitability of a particular test article (i.e. because of physical and chemical characteristics) (Hopkins et al., 2004). For studies on some end-points, such as for traditional studies of mutations in bacteria and in mammalian cells, formal guidelines, including those from the Organisation for Economic Co-operation and Development, may be informative in conducting the quality review (OECD, 1997, 2016a, b). However, existing guidelines will not generally cover all relevant assays, even for genotoxicity. Possible considerations when evaluating the quality of in vitro studies encompass the methodology and design (e.g. the end-point and test method, the number of replicate samples, the suitability of the concentration range, the inclusion of positive and negative controls, and the assessment of cytotoxicity) as well as reporting (e.g. of the source and purity of the agent, and of the analytical methods and results). High-content and high-throughput in vitro data can serve as an additional or supportive source of mechanistic evidence (Chiu et al., 2018; Guyton et al., 2018), although large-scale screening programmes measuring a variety of end-points were designed to evaluate large chemical libraries in order to prioritize chemicals for additional toxicity testing rather than to identify the hazard of a specific chemical or chemical group.

The synthesis is focused on the evidence that is most informative for the overall evaluation. In this regard, it is of note that some human carcinogens exhibit a single or primary key characteristic, evidence of which has been influential in their cancer hazard classifications. For instance, ethylene oxide is genotoxic (IARC, 1994), 2,3,7,8-tetrachlorodibenzo-*para*-dioxin modulates receptor-mediated effects (IARC, 1997), and etoposide alters DNA repair (IARC, 2012a). Similarly, oncogenic viruses cause immortalization, and certain drugs are, by design, immunosuppressive (IARC, 2012a, b). Because non-carcinogens can also induce oxidative stress, this key characteristic should be interpreted with caution unless it is found in combination

with other key characteristics (Guyton et al., 2018). Evidence for a group of key characteristics can strengthen mechanistic conclusions (e.g. "induces oxidative stress" together with "is electrophilic or can be metabolically activated to an electrophile", "induces chronic inflammation", and "is immunosuppressive"); see, for example, 1-bromopropane (IARC, 2018).

5. Summary of data reported

(a) Exposure characterization

Exposure data are summarized to identify the agent and describe its production, use, and occurrence. Information on exposure prevalence and intensity in different settings, including geographical patterns and time trends, may be included. Exposure assessment methods used in key epidemiological studies reviewed by the Working Group are described and evaluated.

(b) Cancer in humans

Results of epidemiological studies pertinent to an evaluation of carcinogenicity in humans are summarized. The overall strengths and limitations of the epidemiological evidence base are highlighted to indicate how the evaluation was reached. The target organ(s) or tissue(s) in which a positive association between the agent and cancer was observed are identified. Exposure–response and other quantitative data may be summarized when available. When the available epidemiological studies pertain to a mixed exposure, process, occupation, or industry, the Working Group seeks to identify the specific agent considered to be most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data permit.

(c) Cancer in experimental animals

Results pertinent to an evaluation of carcinogenicity in experimental animals are summarized to indicate how the evaluation was reached. For each animal species, study design, and route of administration, there is a statement about whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or pre-neoplastic lesions was observed, and the tumour sites are indicated. Special conditions resulting in tumours, such as prenatal exposure or single-dose experiments, are mentioned. Negative findings, inverse relationships, dose–response patterns, and other quantitative data are also summarized.

(d) Mechanistic evidence

Results pertinent to an evaluation of the mechanistic evidence on carcinogenicity are summarized to indicate how the evaluation was reached. The summary encompasses the informative studies on absorption, distribution, metabolism, and excretion; on the key characteristics with adequate evidence for evaluation; and on any other aspects of sufficient importance to affect the overall evaluation, including on whether the agent belongs to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans, and on criteria with respect to tumours in experimental animals induced by mechanisms that do not operate in humans. For each topic addressed, the main supporting findings are highlighted from exposed humans, human cells or tissues, experimental animals, or in vitro systems. When mechanistic studies are available in exposed humans, the tumour type or target tissue studied may be specified. Gaps in the evidence are indicated (i.e. if no studies were available in exposed humans, in in vivo systems, etc.). Consistency or differences of effects across different experimental systems are emphasized.

6. Evaluation and rationale

Consensus evaluations of the strength of the evidence of cancer in humans, the evidence of cancer in experimental animals, and the mechanistic evidence are made using transparent criteria and defined descriptive terms. The Working Group then develops a consensus overall evaluation of the strength of the evidence of carcinogenicity for each agent under review.

An evaluation of the strength of the evidence is limited to the agents under review. When multiple agents being evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single and unified evaluation of the strength of the evidence.

The framework for these evaluations, described below, may not encompass all factors relevant to a particular evaluation of carcinogenicity. After considering all relevant scientific findings, the Working Group may exceptionally assign the agent to a different category than a strict application of the framework would indicate, while providing a clear rationale for the overall evaluation.

When there are substantial differences of scientific interpretation among the Working Group members, the overall evaluation will be based on the consensus of the Working Group. A summary of the alternative interpretations may be provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

The categories of the classification refer to the strength of the evidence that an exposure is carcinogenic and not to the risk of cancer from particular exposures. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used as descriptors of different strengths of evidence of carcinogenicity in humans; *probably carcinogenic* signifies a greater strength of evidence than *possibly carcinogenic*.

(a) Carcinogenicity in humans

Based on the principles outlined in Part B, Section 2, the evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:** A causal association between exposure to the agent and human cancer has been established. That is, a positive association has been observed in the body of evidence on exposure to the agent and cancer in studies in which chance, bias, and confounding were ruled out with reasonable confidence.

***Limited evidence of carcinogenicity*:** A causal interpretation of the positive association observed in the body of evidence on exposure to the agent and cancer is credible, but chance, bias, or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence regarding carcinogenicity*:** The available studies are of insufficient quality, consistency, or statistical precision to permit a conclusion to be drawn about the presence or the absence of a causal association between exposure and cancer, or no data on cancer in humans are available. Common findings that lead to a determination of inadequate evidence of carcinogenicity include: (a) there are no data available in humans; (b) there are data available in humans, but they are of poor quality or informativeness; and (c) there are studies of sufficient quality available in humans, but their results are inconsistent or otherwise inconclusive.

***Evidence suggesting lack of carcinogenicity*:** There are several high-quality 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 the studied cancers at any

observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit below or close to the null value (e.g. a relative risk of unity). Bias and confounding were ruled out with reasonable confidence, and the studies were considered informative. A conclusion of *evidence suggesting lack of carcinogenicity* is limited to the cancer sites, populations and life stages, 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.

When there is *sufficient evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a causal interpretation has been established. When there is *limited evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a positive association between exposure to the agent and the cancer(s) was observed in humans. When there is *evidence suggesting lack of carcinogenicity*, a separate sentence identifies the target organ(s) or tissue(s) where evidence of lack of carcinogenicity was observed in humans. Identification of a specific target organ or tissue as having *sufficient evidence* or *limited evidence* or *evidence suggesting lack of carcinogenicity* does not preclude the possibility that the agent may cause cancer at other sites.

(b) Carcinogenicity in experimental animals

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

***Sufficient evidence of carcinogenicity*:** A causal relationship has been established between exposure to the agent and cancer in experimental animals based on 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 and/or under different protocols. An increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices (GLP), can also provide *sufficient evidence*.

Exceptionally, a single study in one species and sex may 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 marked 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, for example, (a) the evidence of carcinogenicity is restricted to a single experiment and does not meet the criteria for *sufficient evidence*; (b) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; (c) the agent increases tumour multiplicity or decreases tumour latency but does not increase tumour incidence; (d) the evidence of carcinogenicity is restricted to initiation–promotion studies; (e) the evidence of carcinogenicity is restricted to observational studies in non-laboratory animals (e.g. companion animals); or (f) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the available studies.

***Inadequate evidence regarding carcinogenicity*:** The studies cannot be interpreted as showing either the presence or the absence

of a carcinogenic effect because of major qualitative or quantitative limitations, or no data are available on cancer in experimental animals.

***Evidence suggesting lack of carcinogenicity*:** Well-conducted studies (e.g. conducted under GLP) involving both sexes of at least two species are available showing that, within the limits of the tests used, the agent was not carcinogenic. The conclusion of *evidence suggesting lack of carcinogenicity* is limited to the species, tumour sites, age at exposure, and conditions and levels of exposure covered by the available studies.

(c) Mechanistic evidence

Based on the principles outlined in Part B, Section 4, the mechanistic evidence is classified into one of the following categories:

***Strong mechanistic evidence*:** Results in several different experimental systems are consistent, and the overall mechanistic database is coherent. Further support can be provided by studies that demonstrate experimentally that the suppression of key mechanistic processes leads to the suppression of tumour development. Typically, a substantial number of studies on a range of relevant end-points are available in one or more mammalian species. Quantitative structure–activity considerations, in vitro tests in non-human mammalian cells, and experiments in non-mammalian species may provide corroborating evidence but typically do not in themselves provide strong evidence. However, consistent findings across a number of different test systems in different species may provide strong evidence.

Of note, "strong" relates not to potency but to strength of evidence. The classification applies to three distinct topics:

(a) Strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans. The considerations can go beyond quantitative structure–activity relationships to incorporate similarities in biological activity relevant to common key characteristics across dissimilar chemicals (e.g. based on molecular docking, –omics data).

(b) Strong evidence that the agent exhibits key characteristics of carcinogens. In this case, three descriptors are possible:

1. The strong evidence is in exposed humans. Findings relevant to a specific tumour type may be informative in this determination.
2. The strong evidence is in human primary cells or tissues. Specifically, the strong findings are from biological specimens obtained from humans (e.g. ex vivo exposure), from human primary cells, and/or, in some cases, from other humanized systems (e.g. a human receptor or enzyme).
3. The strong evidence is in experimental systems. This may include one or a few studies in human primary cells and tissues.

(c) Strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Certain results in experimental animals (see Part B, Section 6b) would be discounted, according to relevant criteria and considerations in authoritative publications (e.g. Capen et al., 1999; IARC, 2003). Typically, this classification would not apply when there is strong mechanistic evidence that the agent exhibits key characteristics of carcinogens.

***Limited mechanistic evidence*:** The evidence is suggestive, but, for example, (a) the studies cover a narrow range of experiments, relevant end-points, and/or species; (b) there are unexplained inconsistencies in the studies of similar design; and/or (c) there is unexplained incoherence across studies of different end-points or in different experimental systems.

***Inadequate mechanistic evidence*:** Common findings that lead to a determination of inadequate mechanistic evidence include: (a) few or no data are available; (b) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the studies; (c) the available results are negative.

(d) Overall evaluation

Finally, the bodies of evidence included within each stream of evidence are considered as a whole, in order to reach an overall evaluation of the carcinogenicity of the agent to humans. The three streams of evidence are integrated and the agent is classified into one of the following categories (see Table 4), indicating that the Working Group has established that:

The agent is carcinogenic to humans (Group 1)

This category applies whenever there is *sufficient evidence of carcinogenicity* in humans.

In addition, this category may apply when there is both *strong evidence in exposed humans that the agent exhibits key characteristics of carcinogens* and *sufficient evidence of carcinogenicity* in experimental animals.

The agent is probably carcinogenic to humans (Group 2A)

This category generally applies when the Working Group has made at least *two of the following* evaluations, *including at least one* that involves either exposed humans or human cells or tissues:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,
- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

If there is *inadequate evidence regarding carcinogenicity* in humans, there should be *strong evidence in human cells or tissues that the agent exhibits key characteristics of carcinogens*. If there is *limited evidence of carcinogenicity in humans*, then the second individual evaluation may be from experimental systems (i.e. s*ufficient evidence of carcinogenicity* in experimental animals or *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens).*

Additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2A.

Separately, this category generally applies if there is *strong evidence that the agent 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*.

The agent is possibly carcinogenic to humans (Group 2B)

This category generally applies when only one of the following evaluations has been made by the Working Group:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,

Table 4 Integration of streams of evidence in reaching overall classifications (the evidence in *bold italic* represents the basis of the overall evaluation)

Stream of evidence			Classification based on strength of evidence
Evidence of cancer in humans[a]	**Evidence of cancer in experimental animals**	**Mechanistic evidence**	
Sufficient	Not necessary	Not necessary	**Carcinogenic to humans (Group 1)**
Limited or Inadequate	***Sufficient***	***Strong (b)(1) (exposed humans)***	
Limited	***Sufficient***	Strong (b)(2–3), Limited, or Inadequate	**Probably carcinogenic to humans (Group 2A)**
Inadequate	***Sufficient***	***Strong (b)(2) (human cells or tissues)***	
Limited	Less than Sufficient	***Strong (b)(1–3)***	
Limited or Inadequate	Not necessary	***Strong (a) (mechanistic class)***	
Limited	Less than Sufficient	Limited or Inadequate	**Possibly carcinogenic to humans (Group 2B)**
Inadequate	***Sufficient***	Strong (b)(3), Limited, or Inadequate	
Inadequate	Less than Sufficient	***Strong b(1–3)***	
Limited	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	
Inadequate	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	**Not classifiable as to its carcinogenicity to humans (Group 3)**
All other situations not listed above			

[a] Human cancer(s) with highest evaluation

[b] The *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* must specifically be for the tumour sites supporting the classification of *sufficient evidence in experimental animals*.

• *Strong evidence that the agent exhibits key characteristics of carcinogens.*

Because this category can be based on evidence from studies in experimental animals alone, there is **no** requirement that the strong mechanistic evidence be in exposed humans or in human cells or tissues. This category may be based on *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens.*

As with Group 2A, additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2B.

The agent is not classifiable as to its carcinogenicity to humans (Group 3)

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

This includes the case when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites in experimental animals, the remaining tumour sites do not support an evaluation of *sufficient evidence in experimental animals*, and other categories are not supported by data from studies in humans and mechanistic studies.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that the agent is of unknown carcinogenic potential and that there are significant gaps in research.

If the evidence suggests that the agent exhibits no carcinogenic activity, either through *evidence suggesting lack of carcinogenicity* in both humans and experimental animals, or through

evidence suggesting lack of carcinogenicity in experimental animals complemented by strong negative mechanistic evidence in assays relevant to human cancer, then the Working Group may add a sentence to the evaluation to characterize the agent as well-studied and without evidence of carcinogenic activity.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is summarized so that the basis for the evaluation offered is transparent. This section integrates the major findings from studies of cancer in humans, cancer in experimental animals, and mechanistic evidence. It includes concise statements of the principal line(s) of argument that emerged in the deliberations of the Working Group, the conclusions of the Working Group on the strength of the evidence for each stream of evidence, an indication of the body of evidence that was pivotal to these conclusions, and an explanation of the reasoning of the Working Group in making its evaluation.

References

Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, et al. (2016). Mutational signatures associated with tobacco smoking in human cancer. *Science*, 354(6312):618–22. doi:10.1126/science.aag0299 PMID:27811275

Axelson O, Steenland K (1988). Indirect methods of assessing the effects of tobacco use in occupational studies. *Am J Ind Med*, 13(1):105–18. doi:10.1002/ajim.4700130107 PMID:3344750

Baan RA, Stewart BW, Straif K, editors (2019). Tumour site concordance and mechanisms of carcinogenesis (IARC Scientific Publication No. 165). Lyon, France: International Agency for Research on Cancer. Available from: https://publications.iarc.fr/578.

Bieler GS, Williams RL (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics*, 49(3):793–801. doi:10.2307/2532200 PMID:8241374

Capen CC, Dybing E, Rice JM, Wilbourn JD, editors (1999). Species differences in thyroid, kidney and urinary bladder carcinogenesis (IARC Scientific Publication No. 147). Lyon, France: IARC Press. Available from: https://publications.iarc.fr/302 PMID:10627184

Chiu WA, Guyton KZ, Martin MT, Reif DM, Rusyn I (2018). Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. *ALTEX*, 35(1):51–64. doi:10.14573/altex.1703231 PMID:28738424

Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. (2005). Transparency in IARC Monographs. *Lancet Oncol*, 6(10):747 doi:10.1016/S1470-2045(05)70380-6

Cogliano VJ, Baan RA, Straif K, Grosse Y, Secretan MB, El Ghissassi F, et al. (2004). The science and practice of carcinogen identification and evaluation. *Environ Health Perspect*, 112(13):1269–74. doi:10.1289/ehp.6950 PMID:15345338

Dunson DB, Chen Z, Harry J (2003). A Bayesian approach for joint modeling of cluster size and subunit-specific outcomes. *Biometrics*, 59(3):521–30. doi:10.1111/1541-0420.00062 PMID:14601753

Fung KY, Krewski D, Smythe RT (1996). A comparison of tests for trend with historical controls in carcinogen bioassay. *Can J Stat*, 24(4):431–54. doi:10.2307/3315326

García-Closas M, Vermeulen R, Cox D, Lan Q, Caporaso N, Hunter D, et al. (2011). Population-based study designs in molecular epidemiology. In: Rothman N, Hainaut P, Schulte P, Smith M, Boffetta P, Perera FP, editors. Molecular epidemiology: principles and practices. Lyon, France: International Agency for Research on Cancer; pp. 241–59.

García-Closas M, Vermeulen R, Sherman ME, Moore LE, Smith MT, Rothman N (2006). Application of biomarkers in cancer epidemiology. In: Schottenfeld D, Fraumeni JF Jr, editors. Cancer epidemiology and prevention. 3rd ed. New York (NY), USA: Oxford University Press; pp. 70–88. doi:10.1093/acprof:oso/9780195149616.003.0006

Gart JJ, Krewski D, Lee PN, Tarone RE, Wahrendorf J (1986). Statistical methods in cancer research, Volume III: The design and analysis of long-term animal experiments (IARC Scientific Publication No. 79). Lyon, France: International Agency for Research on Cancer.

Greenland S, O'Rourke K (2008). Meta-analysis. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA), USA: Wolters Kluwer Health/Lippincott Williams & Wilkins; pp. 652–682.

Greim H, Gelbke H-P, Reuter U, Thielmann HW, Edler L (2003). Evaluation of historical control data in carcinogenicity studies. *Hum Exp Toxicol*, 22(10):541–9. doi:10.1191/0960327103ht394oa PMID:14655720

Guyton KZ, Kyle AD, Aubrecht J, Cogliano VJ, Eastmond DA, Jackson M, et al. (2009). Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat Res*, 681(2–3):230–40. doi:10.1016/j.mrrev.2008.10.001 PMID:19010444

Guyton KZ, Rusyn I, Chiu WA, Corpet DE, van den Berg M, Ross MK, et al. (2018). Application of the key characteristics of carcinogens in cancer hazard identification. *Carcinogenesis*, 39(4):614–22. doi:10.1093/carcin/bgy031 PMID:29562322

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. *Cell*, 100(1):57–70. doi:10.1016/S0092-8674(00)81683-9 PMID:10647931

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5):646–74. doi:10.1016/j.cell.2011.02.013 PMID:21376230

Haseman JK, Huff J, Boorman GA (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol Pathol*, 12(2):126–35. doi:10.1177/019262338401200203 PMID:11478313

Herceg Z, Lambert MP, van Veldhoven K, Demetriou C, Vineis P, Smith MT, et al. (2013). Towards incorporating epigenetic mechanisms into carcinogen identification and evaluation. *Carcinogenesis*, 34(9):1955–67. doi:10.1093/carcin/bgt212 PMID:23749751

Hernán MA, Hernández-Díaz S, Robins JM (2004). A structural approach to selection bias. *Epidemiology*, 15(5):615–25. doi:10.1097/01.ede.0000135174.63482.43 PMID:15308962

Hill AB (1965). The environment and disease: association or causation? *Proc R Soc Med*, 58:295–300. PMID:14283879

Hopkins AL, Groom CR, Alex A (2004). Ligand efficiency: a useful metric for lead selection. *Drug Discov Today*, 9(10):430–1. doi:10.1016/S1359-6446(04)03069-7 PMID:15109945

Huff JE, Eustis SL, Haseman JK (1989). Occurrence and relevance of chemically induced benign neoplasms in long-term carcinogenicity studies. *Cancer Metastasis Rev*, 8(1):1–22. doi:10.1007/BF00047055 PMID:2667783

IARC (1994). Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 60:1–560. Available from: https://publications.iarc.fr/78 PMID:7869568

IARC (1997). Polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans. *IARC Monogr Eval Carcinog Risks Hum*, 69:1–631. Available from: https://publications.iarc.fr/87 PMID:9379504

IARC (2003). Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans (IARC Technical Publication No. 39). Lyon, France: IARC Press.

IARC (2004). Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr Eval Carcinog Risks Hum*, 84:1–477. Available from: https://publications.iarc.fr/102 PMID:15645577

IARC (2012a). Pharmaceuticals. *IARC Monogr Eval Carcinog Risks Hum*, 100A:1–437. Available from: https://publications.iarc.fr/118 PMID:23189749

IARC (2012b). Biological agents. *IARC Monogr Eval Carcinog Risks Hum*, 100B:1–441. Available from: https://publications.iarc.fr/119 PMID:23189750

IARC (2014). Report of the IARC Advisory Group to Recommend on Quantitative Risk Characterization (IARC Internal Report 14/001). Available from: https://monographs.iarc.fr/wp-content/uploads/2018/06/14-001.pdf.

IARC (2018). Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 115:1–292. Available from: https://publications.iarc.fr/563.

National Academies of Sciences, Engineering, and Medicine (2017). Using 21st century science to improve risk-related evaluations. Washington (DC), USA: National Academies Press. doi:10.17226/24635 PMID:28267305

National Research Council (2012). Exposure science in the 21st century: a vision and a strategy. Washington (DC), USA: National Academies Press. doi:10.17226/13507 PMID:24901193

OECD (1997). Test no. 471: Bacterial reverse mutation test, OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: Organisation for Economic Co-operation and Development. doi:10.1787/9789264071247-en

OECD (2016a). Test no. 476: *In vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes, OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: Organisation for Economic Co-operation and Development. doi:10.1787/9789264264809-en

OECD (2016b). Test no. 490: *In vitro* mammalian cell gene mutation tests using the thymidine kinase gene, OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: Organisation for Economic Co-operation and Development. doi:10.1787/9789264264908-en

OECD (2018). Test no. 451: Carcinogenicity studies, OECD Guidelines for the Testing of Chemicals, Section 4: Health effects. Paris, France: Organisation for Economic Co-operation and Development. doi:10.1787/9789264071186-en

Parkkinen VP, Wallmann C, Wilde M, Clarke B, Illari P, Kelly MP, et al. (2018). Evaluating evidence of mechanisms in medicine: principles and procedures. Cham, Switzerland: Springer. doi:10.1007/978-3-319-94610-8

Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, et al. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long-term and short-term screening assays for carcinogens: a critical appraisal. *IARC Monogr Eval Carcinog Risk Chem Hum* Suppl. 2. Lyon, France: International Agency for Research on Cancer;

pp. 311–426. Available from: https://publications.iarc.fr/134 PMID:6935176

Portier CJ, Bailer AJ (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam Appl Toxicol*, 12(4):731–7. doi:10.1016/0272-0590(89)90004-3 PMID:2744275

Richardson DB, Laurier D, Schubauer-Berigan MK, Tchetgen Tchetgen E, Cole SR (2014). Assessment and indirect adjustment for confounding by smoking in cohort studies using relative hazards models. *Am J Epidemiol*, 180(9):933–40. doi:10.1093/aje/kwu211 PMID:25245043

Rothman KJ, Greenland S, Poole C, Lash TL (2008). Causation and causal inference. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA), USA: Wolters Kluwer Health/Lippincott Williams & Wilkins; pp. 5–31.

Sherman CD, Portier CJ, Kopp-Schneider A (1994). Multistage models of carcinogenesis: an approximation for the size and number distribution of late-stage clones. *Risk Anal*, 14(6):1039–48. doi:10.1111/j.1539-6924.1994.tb00074.x PMID:7846311

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Stayner L, Steenland K, Dosemeci M, Hertz-Picciotto I (2003). Attenuation of exposure-response curves in occupational cohort studies at high exposure levels. *Scand J Work Environ Health*, 29(4):317–24. doi:10.5271/sjweh.737 PMID:12934726

Vandenbroucke JP, Broadbent A, Pearce N (2016). Causality and causal inference in epidemiology: the need for a pluralistic approach. *Int J Epidemiol*, 45(6):1776–86. doi:10.1093/ije/dyv341 PMID:26800751

Vermeulen R, Bell DA, Jones DP, Garcia-Closas M, Spira A, Wang TW, et al. (2018). Application of biomarkers in cancer epidemiology. In: Thun MJ, Linet MS, Cerhan JR, Haiman CA, Schottenfeld D, editors. Schottenfeld and Fraumeni cancer epidemiology and prevention. 4th ed. New York (NY), USA: Oxford University Press; pp. 77–96.

Wild CP, Cogliano VJ (2011). A plea on behalf of expert evaluation and the experts involved. *Int J Epidemiol*, 40(1):253–61. doi:10.1093/ije/dyq038 PMID:20223796

GENERAL REMARKS

This one-hundred-and-twenty-seventh volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of some aromatic amines and related compounds, including *ortho*-nitroanisole, *ortho*-anisidine (and its salt, *ortho*-anisidine hydrochloride), aniline (and its salt, aniline hydrochloride), and cupferron. Due to the coronavirus disease (COVID-19) pandemic, this meeting, which was scheduled to be held in Lyon, France, was held remotely.

ortho-Nitroanisole was considered previously by the *IARC Monographs* programme in Volume 65 of the *IARC Monographs* (IARC, 1996), when it was evaluated as *possibly carcinogenic to humans (Group 2B)* because of *sufficient evidence* in experimental animals. *ortho*-Anisidine, a High Production Volume chemical and metabolite of *ortho*-nitroanisole, was considered previously in Supplement 7 (IARC, 1987) and Volume 73 of the *IARC Monographs* (IARC, 1999), when it was evaluated as *Group 2B* because of *sufficient evidence* in experimental animals. Aniline is a High Production Volume chemical that was considered previously in Volume 27 (IARC, 1982) and Supplement 7 of the *IARC Monographs* (IARC, 1987), when it was evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)* because of *limited evidence* in experimental animals and *inadequate evidence* in humans. Cupferron has not been previously evaluated by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* recommended that *ortho*-anisidine (together with the structurally similar *ortho*-nitroanisole), aniline, and cupferron be evaluated with high priority (Marques et al., 2019). New data have become available, primarily bioassay and mechanistic evidence, and these data have been included and considered in the present volume.[1]

A summary of the findings of this volume appears in *The Lancet Oncology* (DeMarini et al., 2020).

[1] Standardized searches of the PubMed database were conducted for each agent and for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). The literature trees, including the full set of search terms for the agent name and each outcome type, are available at: https://hawcproject.iarc.fr/assessment/623/ (*ortho*-anisidine and its hydrochloride salt), https://hawcproject.iarc.fr/assessment/624/ (*ortho*-nitroanisole), https://hawcproject.iarc.fr/assessment/625/ (aniline and its hydrochloride salt), and https://hawcproject.iarc.fr/assessment/627/ (cupferron).

Information gaps in exposure characterization

For all the agents in this volume, the Working Group noted substantial data gaps regarding production and use, as well as environmental and occupational exposure levels. Data gaps exist for high-income countries but are particularly notable in low- and middle-income countries. The Working Group has noted in the monograph on aniline that these data gaps were especially surprising given that aniline is a High Production Volume chemical with widespread occupational use, and for which there is long-standing concern about toxicity and potential carcinogenicity.

Considerations relating to studies of cancer in humans

The body of literature related to cancer in humans exposed to these agents was sparse. Exceptionally, case reports or case series were considered for *ortho*-anisidine and aniline as potentially providing information on rare cancers among exposed workers, as well as historical context. However, the paucity of exposure information and co-exposures to other carcinogenic agents limited the informativeness of these reports. The few available case–control or cohort studies of occupational exposure to aniline mostly investigated cancer of the urinary bladder. Most of these studies could not distinguish the effects of aniline from those of co-exposures to known bladder carcinogens, or had other important limitations.

Mechanistic class considerations

Three of the agents evaluated (*ortho*-nitroanisole, *ortho*-anisidine, and aniline) were classified in IARC Group 2A on the basis of *strong* mechanistic evidence. In view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, and mechanistic considerations for all three agents, *ortho*-nitroanisole, *ortho*-anisidine, and aniline were classified on the basis of belonging to a class of aromatic amines for which several members (i.e. 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine) have been classified as *carcinogenic to humans* (IARC Group 1). *ortho*-Nitroanisole, *ortho*-anisidine, and aniline are similar to this class of aromatic amines with respect to the formation of common DNA-reactive moieties, genotoxicity, and target organs of carcinogenicity in animal bioassays for chronic toxicity. No data on DNA adducts in humans were available for *ortho*-nitroanisole, *ortho*-anisidine, or aniline. However, data on metabolism and DNA-adduct formation from human in vitro systems and studies of exposed rodents supported the view that *ortho*-nitroanisole, *ortho*-anisidine, and aniline are metabolically activated to reactive electrophiles and undergo binding to DNA in a manner that parallels the established paradigm for carcinogenic aromatic amines. *ortho*-Nitroanisole, *ortho*-anisidine, and aniline are genotoxic. The urinary bladder is a common target organ of carcinogenicity for several of these aromatic amines in experimental animals. In the rat urinary bladder, *ortho*-nitroanisole and *ortho*-anisidine form DNA adducts, induce DNA damage, and cause malignant tumours when administered orally. The Group 1 agents *ortho*-toluidine and 2-naphthylamine similarly cause malignant bladder tumours in the rat, and 4-aminobiphenyl causes malignant tumours of the urinary bladder when administered orally to dogs and mice (IARC, 2012). Other common target organs

of carcinogenicity in experimental animals are the spleen and testis. DNA binding and malignant tumours are seen in the spleen after oral administration of aniline to male Fischer 344 rats. The Group 1 agent *ortho*-toluidine similarly induces malignant splenic tumours in these rats, and an increased incidence of mesothelioma of the tunica vaginalis of the testis is seen with both aniline and *ortho*-toluidine (IARC, 2012). Overall, the mechanistic considerations that strongly support classification of *ortho*-nitroanisole, *ortho*-anisidine, and aniline in Group 2A go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

Data gaps regarding cupferron

Cupferron (*N*-nitroso-*N*-phenylhydroxylamine) is a *N*-nitroso hydroxylamine that has a unique chemical structure (Hrabie & Keefer, 2002). Such chemicals have attracted research interest for pharmaceutical applications because of their capacity to release nitric oxide (NO) under physiological conditions. Several experimental studies in acellular and non-mammalian systems support the view that cupferron can generate NO. For instance, in an acellular system, Alston et al. (1985) showed that cupferron is an excellent substrate for horseradish peroxidase (with $k > 10^7$ M^{-1} s^{-1}) and generated NO and nitrosobenzene. Similarly, Hou et al. (1999) demonstrated that the *O*-alkyl derivatives of cupferron could function as NO photoreleasing donor compounds, as also supported by Thomsen et al. (2018). The exposure of *Vicia faba* roots to cupferron and visible light in the absence of oxygen caused the induction of chromosomal aberrations, as did NO in this test system (Kihlman, 1959). Overall, these results indicate that cupferron and its derivatives can be oxidized to the unstable oxy radical, which then spontaneously decomposes to nitrosobenzene. Considering that many mammalian peroxidases (e.g. thyroid peroxidase, lactoperoxidase, eosinophil peroxidase) have a similar function to that of plant horseradish peroxidase (Josephy, 1986; Vlasova, 2018), it is reasonable to assume that in mammalian species cupferron is likely to be oxidized to nitrosobenzene and produce NO by certain peroxidases, especially in the presence of oxygen. Interestingly, nitrosobenzene is a reactive metabolite of aniline, supporting mechanistic commonalities between aniline and cupferron. However, no data on absorption, distribution, metabolism, or excretion in humans or in other mammalian systems (in vivo or in vitro) were available for the main metabolites formed from cupferron to inform conclusions about any commonalities with aniline or other aromatic amines (e.g. *ortho*-toluidine).

The available information from tests for genotoxicity with cupferron in experimental systems indicated that cupferron is mutagenic and clastogenic. Quantitative structure–activity relationship (QSAR) modelling predicted the mutagenic potential of cupferron. The findings in animal cancer bioassays that cupferron induces tumours at multiple sites in both sexes of rats and mice are consistent with expectations for chemicals that are mutagenic and clastogenic. However, there are significant gaps in evidence for cupferron including a lack of information in humans and other in vivo mammalian systems relevant to key characteristics of carcinogens. As noted above, studies on absorption, distribution, metabolism, or excretion of cupferron in mammalian species or cells were not available, although chemistry information and acellular data support the inference that cupferron can be metabolized to nitrosobenzene, especially in the presence of oxygen. Overall, there is strong evidence from experimental systems that cupferron exhibits key characteristics of carcinogens; cupferron is genotoxic. Cupferron was

carcinogenic in both rats and mice, inducing tumours of the forestomach and liver. Even in the absence of the observed *sufficient evidence* for carcinogenicity in experimental animals, the available *strong* mechanistic evidence alone supports the classification of cupferron in Group 2B.

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of several of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). The results from these assays were uninformative regarding the carcinogenicity of all these agents. Although neither programme includes assays for mutagenicity or DNA-adduct formation, they do include a few assays to detect end-points encompassed by the key characteristics of carcinogens (Smith et al., 2016), such as DNA repair, altered gene expression, oxidative stress, and modulated receptor-mediated effects. Nonetheless, a recent analysis of data from five such assays in Tox21 showed < 40% sensitivity for agents that are direct-acting genotoxicants in standard assays (i.e. Ames test, chromosomal aberrations in vitro, micronucleus formation in vivo) (Hsieh et al., 2019). These programmes are constantly being improved and new assays are included over time. However, at present, the general lack of metabolic activation and the small number of genotoxicity assays restricts the value of these high-throughput screening programmes for carcinogenicity assessments of genotoxic and other chemicals.

Methaemoglobinaemia

Methaemoglobinaemia is an adverse outcome commonly seen after exposure to many toxicants, including *ortho*-nitroanisole, *ortho*-anisidine, aniline, and various other aromatic amino- and nitroaromatic compounds classified by the *IARC Monographs* programme (e.g. the Group 2B agents 2-chloronitrobenzene, 4-chloronitrobenzene, 2-amino-4-chlorophenol, and *N*,*N*-dimethyl-*p*-toluidine). For several such agents, methaemoglobin formation has been attributed to the formation of *N*-hydroxyarylamine and other metabolites that engage in Kiese redox cycling yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017). In methaemoglobinaemia, oxidation of the haem iron reduces the oxygen-carrying capacity of the blood. As a biological marker of exposure, methaemoglobin in blood is used as a basis for exposure indices, such as those set by the American Conference of Governmental Industrial Hygienists (ACGIH, 2008).

References

ACGIH (2008). American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

Alston TA, Porter DJ, Bright HJ (1985). Generation of nitric oxide by enzymatic oxidation of *N*-hydroxy-*N*-nitrosamines. *J Biol Chem*. 260(7):4069–74. doi:10.1016/S0021-9258(18)89232-8 PMID:3980468

DeMarini DM, Carreón-Valencia T, Gwinn WM, Hopf NB, Sandy MS, Bahadori T, et al.; IARC Monographs Vol 127 group (2020). Carcinogenicity of some aromatic amines and related compounds. *Lancet Oncol*. 21(8):1017–8. doi:10.1016/S1470-2045(20)30375-2 PMID:32593317

Hou YC, Janczuk A, Wang PG (1999). Current trends in the development of nitric oxide donors. *Curr Pharm Des*. 5(6):417–41. PMID:10390607

Hrabie JA, Keefer LK (2002). Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. *Chem Rev.* 102(4):1135–54. doi:10.1021/cr000028t PMID:11942789

Hsieh JH, Smith-Roe SL, Huang R, Sedykh A, Shockley KR, Auerbach SS, et al. (2019). Identifying compounds with genotoxicity potential using Tox21 high-throughput screening assays. *Chem Res Toxicol.* 32(7):1384–401. doi:10.1021/acs.chemrestox.9b00053 PMID:31243984

IARC (1982). Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking-water and dental preparations. *IARC Monogr Eval Carcinog Risk Chem Hum*, 27:1–341. Available from: https://publications.iarc.fr/45 PMID:6955259

IARC (1987). Overall evaluations of carcinogenicity: an updating of *IARC Monographs* volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7:1–440. Available from: https://publications.iarc.fr/139 PMID:3482203

IARC (1996). Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65:1–578. Available from: https://publications.iarc.fr/83 PMID:9148039

IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. *IARC Monogr Eval Carcinog Risks Hum*, 73:1–674. Available from: https://publications.iarc.fr/91.

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: https://publications.iarc.fr/123 PMID:23189753

Josephy PD (1986). Benzidine: mechanisms of oxidative activation and mutagenesis. *Fed Proc.* 45(10):2465–70. PMID:3091404

Kihlman BA (1959). Studies on the production of chromosomal aberrations by visible light: the effects of cupferron, nitric oxide and wavelength. *Exp Cell Res.* 17(3):590–3. doi:10.1016/0014-4827(59)90096-5 PMID:13672232

Marques MM, Berrington de Gonzalez A, Beland FA, Browne P, Demers PA, Lachenmeier DW, et al.; IARC Monographs Priorities Group (2019). Advisory Group recommendations on priorities for the IARC Monographs. *Lancet Oncol.* 20(6):763–4. doi:10.1016/S1470-2045(19)30246-3 PMID:31005580

Sabbioni G (2017). Hemoglobin adducts and urinary metabolites of arylamines and nitroarenes. *Chem Res Toxicol.* 30(10):1733–66. doi:10.1021/acs.chemrestox.7b00111 PMID:28933159

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect.* 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: A strategic and operational plan for continued leadership. *ALTEX.* 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

Thomsen H, Marino N, Conoci S, Sortino S, Ericson MB (2018). Confined photo-release of nitric oxide with simultaneous two-photon fluorescence tracking in a cellular system. *Sci Rep.* 8(1):9753. doi:10.1038/s41598-018-27939-4 PMID:29950654

Vlasova II (2018). Peroxidase activity of human hemoproteins: keeping the fire under control. *Molecules.* 23(10):2561. doi:10.3390/molecules23102561 PMID:30297621

ORTHO-ANISIDINE AND *ORTHO*-ANISIDINE HYDROCHLORIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

(a) ortho-*Anisidine*

Chem. Abstr. Serv. Reg. No.: 90-04-0

Chem. Abstr. Serv. name: benzenamine, 2-methoxy

EC No.: 201-963-1

IUPAC systematic name: 2-methoxyaniline

Synonyms: *o*-anisidine; 2-anisidine; 2-amino-anisole, 2-methoxyaniline; 1-amino-2-meth-oxybenzene; 2-methoxy-1-aminobenzene; *ortho*-methoxyaniline; 2-methoxybenzen-amine; *ortho*-methoxyphenylamine; 2-meth-oxyphenylamine; *o*-anisylamine.

(b) ortho-*Anisidine hydrochloride*

Chem. Abstr. Serv. Reg. No.: 134-29-2

Chem. Abstr. Serv. name: benzenamine, 2-methoxy-, hydrochloride

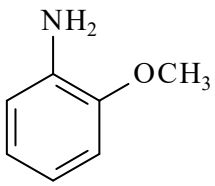
EC No.: 603-807-1

IUPAC systematic name: 2-methoxyaniline hydrochloride

Synonyms: *o*-anisidine.HCl; Fast Red BB Base; 2-aminoanisole hydrochloride; 2-meth-oxyaniline HCl; 2-anisidine hydrochloride; *o*-anisidine, hydrochloride; *o*-anisylamine hydrochloride.

1.1.2 Structural and molecular formulae, and relative molecular mass

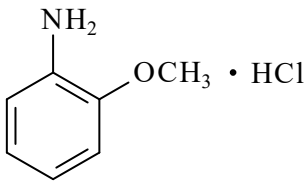
(a) ortho-*Anisidine*



Molecular formula: C_7H_9NO

Relative molecular mass: 123.15 (NCBI, 2020a).

(b) ortho-*Anisidine hydrochloride*



Molecular formula: $C_7H_{10}ClNO$

Relative molecular mass: 159.61 (NCBI, 2020b).

1.1.3 Chemical and physical properties of the pure substance

ortho-Anisidine is a basic compound and will undergo acid–base reactions. *ortho*-Anisidine and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

(a) ortho-*Anisidine*

Description: *ortho*-anisidine appears as clear, yellowish to reddish or brown liquid with an amine (fishy) odour (NCBI, 2020a)

Boiling point: 224 °C (NCBI, 2020a)

Melting point: 6.2 °C (NCBI, 2020a)

Density: 1.09 g/cm^3 at 20 °C (NCBI, 2020a)

Vapour density: 4.25 (air = 1) (NCBI, 2020a)

Vapour pressure: 10 Pa at 20 °C (ECHA, 2020)

Solubility: 14 g/L at 25 °C in water; miscible with ethanol, diethyl ether, acetone, and benzene (NTP, 2016)

Flash point: 107 °C, closed cup (NCBI, 2020a)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, 1.18 (NTP, 2016)

Dissociation constant: pK_a, 4.53 (NTP, 2016)

Conversion factor: 1 ppm = 5.037 mg/m^3; 1 mg/m^3 = 0.199 ppm at 25 °C (European Commission, 2011).

(b) ortho-*Anisidine hydrochloride*

Description: *ortho*-anisidine hydrochloride is a grey-black crystalline solid or light grey powder (NCBI, 2020b)

Melting point: 225 °C (NTP, 2016)

Vapour pressure: 55 Pa at 25 °C (NTP, 2016)

Solubility: 10–50 g/L at 21 °C in water (NCBI, 2020b).

1.1.4 Technical grade and impurities

The purity of commercial *ortho*-anisidine is ≥ 99.0% and typically ≥ 99.4%. Possible impurities are aniline (≤ 0.4% w/w), *ortho*-chloranisole (≤ 0.2% w/w), *ortho*-chloraniline (≤ 0.4% w/w), and water (≤ 0.1% w/w) (European Chemicals Bureau, 2002).

1.2 Production and use

1.2.1 Production process

ortho-Anisidine is produced from *ortho*-nitroanisole (2-methoxy-nitrobenzene) by catalytic reduction with hydrogen under pressure in an inert liquid medium (European Commission, 2011). *ortho*-Anisidine hydrochloride is derived from *ortho*-anisidine (NCBI, 2020b).

1.2.2 Production volume

Production and imports of *ortho*-anisidine in the USA were in the range of 500 000 to less than 1 million pounds [230 to < 450 tonnes] in both 2015 and 2014, less than 1 million pounds [450 tonnes] in 2013, and 100 000–500 000 pounds [45–230 tonnes] in 2012 in the Chemical Data Reporting (CDR) database (US EPA, 2016). At least three companies in the USA manufacture *ortho*-anisidine. One company in Europe was listed as a Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) registrant, with *ortho*-anisidine listed only for use as an intermediate (ECHA, 2020).

It is estimated that less than 1000 tonnes of *ortho*-anisidine are produced annually within the European Union (European Commission, 2011). Information available indicated the presence of other manufacturer or supplier sites in India (Nandosal Chem Industries, 2020), China, and Japan (ChemNet, 2020). Historical data indicated that production plus imports of *ortho*-anisidine in the USA totalled 500 000

to 1 million pounds [230–450 tonnes] in 1986, 1990, and 2006; 1 million to 10 million pounds [450–4500 tonnes] in 1990 and 1998; and 10 000–500 000 pounds [4.5–230 tonnes] in 2002 (NTP, 2016). Additionally, information available in 1995 indicated that *ortho*-anisidine was produced in Armenia, China, France, Germany, India, Japan, Ukraine, and the United Kingdom (Chemical Information Services, 1995).

[The Working Group understood that *ortho*-anisidine hydrochloride is not produced in significant quantities commercially. For example, Sigma-Aldrich provides this product to early discovery researchers as part of a collection of unique chemicals (Merck, 2020).]

1.2.3 Uses

ortho-Anisidine is used as a chemical intermediate in the synthesis of azo pigments and dyes in consumer products, pharmaceuticals, and fragrances (for example, yellow/red azo pigments in hair dyes, tattoo ink, print ink, polymer dyes, and packaging foils, and in the manufacture of guaiacol and vanillin) (ECHA, 2011; Danish Environmental Protection Agency, 2012; NCBI, 2020a). About 90% of the dyes produced from *ortho*-anisidine are used in textiles, whereas the pigments are used mainly for printing paper and cardboard (European Commission, 2011). It is also used as a corrosion inhibitor and metal colourant in the automobile industry and in the production of steel storage tanks (IARC, 1999; Chaudhari et al., 2006; US EPA, 2019a). It is estimated that less than 850 tonnes of *ortho*-anisidine were used in the European Union in 1997 and this quantity declined in the early 2000s (European Commission, 2011).

ortho-Anisidine hydrochloride is used as a chemical intermediate to produce dyes, pigments, and pharmaceuticals, as a corrosion inhibitor, and as an antioxidant for polymercaptan resins (OEHHA, 1992).

1.3 Measurement and analysis

The presence of *ortho*-anisidine can be determined in water, soil, air, and solid waste samples using gas chromatography-mass spectrometry (GC-MS) with a quantitation limit of 10 µg/L in ground water (NEMI, 1998; EPA Method 8270D). The National Institute for Occupational Safety and Health (NIOSH) applies a method based on high-performance liquid chromatography with ultraviolet detection for the determination of *ortho*-anisidine in air samples, with a detection limit of 0.35 µg/sample (NIOSH, 2016; Method 2514 – Issue 3). Several GC-MS methods have been developed for the quantification of urinary *ortho*-anisidine: as reported, the limit of quantification was 0.05 µg/L [50 ng/L] (Kütting et al., 2009) and the limit of detection was between 7 ng/L (Mazumder et al., 2019) and 50 ng/L (Weiss & Angerer, 2002). Similarly, GC-MS was used for the measurement of *ortho*-anisidine and other aromatic amines in mainstream cigarette smoke, with a limit of detection of 7 pg/cigarette (Stabbert et al., 2003).

1.4 Occurrence and exposure

The primary routes of exposure to *ortho*-anisidine and *ortho*-anisidine hydrochloride are inhalation, skin absorption, ingestion, and eye contact (NCBI, 2020a).

1.4.1 Environmental occurrence

The United States Environmental Protection Agency (US EPA) Toxics Release Inventory (TRI) reported that 243 pounds [110 kg] of *ortho*-anisidine were released in the USA across all industries in 2018: 217 pounds [98 kg] to air emissions and 26 pounds [12 kg] to water discharges. There was little variation in the level of releases between 2012 and 2018 (US EPA, 2020). Monitoring data from 1993–1997 for German and Dutch rivers in most cases yielded *ortho*-anisidine levels

below or slightly above the limit of detection of 0.5 µg/L. Only peak concentrations in a highly polluted German river were equal to or above 5 µg/L (European Chemicals Bureau, 2002).

1.4.2 Occupational exposure

Regarding numbers of workers in publicly accessible exposure registries, the only information available to the Working Group was for the USA and Finland. The National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that 705 workers classified as being employed in the chemicals and allied products industry were potentially exposed to *ortho*-anisidine and 1108 workers in the same industry were potentially exposed to *ortho*-anisidine hydrochloride (CDC, 2011). Six persons exposed to *ortho*-anisidine and its salts were recorded in the Finnish national register of workers exposed to carcinogenic substances and processes (Saalo et al., 2016). [The Working Group noted that information was sparse regarding numbers and global distribution of exposed workers.]

The following workplace 8 hour time-weighted average (TWA) concentrations were measured during production and processing of *ortho*-anisidine: production, 0.06–0.07 mg/m^3; long-term measurements of processing, 0.05–0.15 mg/m^3 (≥ 1 hour, shift average); and short-term measurements of processing, 0.05–0.09 mg/m^3 (< 1 hour). For the formulation of pigments (especially printing inks), the estimated exposure concentrations were between 0.07 and 28 ng/m^3. Dermal exposure concentrations for *ortho*-anisidine at the workplace were calculated using the EASE (Estimation and Assessment of Substance Exposure Physico-chemical properties) model. Significant exposure concentrations were derived only for the installation of gas compensation pipes, resulting in a maximum calculated body burden of 0.6 mg/kg body weight (bw) per day. The maximum calculated body burden for the formulation and use of *ortho*-anisidine–based printing inks was in the range of 6×10^{-5} to 1.5×10^{-3} mg/kg bw per day (European Chemicals Bureau, 2002).

1.4.3 Exposure of the general population

ortho-Anisidine has been detected in textiles, consumer products, cosmetics, and substances and products to which children may be exposed. In some cases, *ortho*-anisidine may be absent from the "ingredient list" for the product, but unexpectedly detected in product-testing studies (ECHA 2011; US EPA, 2019a). With the frequency of tattooing increasing while relevant regulations remain inconsistent, consumer use of tattoo inks is expected to be a growing source of population exposure to *ortho*-anisidine (ECHA 2011; European Commission, 2011; Danish Environmental Protection Agency, 2012; JRC, 2017)

ortho-Anisidine is also present in tobacco smoke. Mean concentrations of *ortho*-anisidine in mainstream tobacco smoke were reported to range from < 0.2 to 5.12 ng/cigarette (Stabbert et al., 2003). Health Canada reported an average of 4 ng/cigarette in mainstream tobacco smoke (Health Canada, 2009). The compound was detected in urine from 20 study participants in the general population without known exposure in Germany; the median was 0.22 µg/L, the range was < 0.05 to 4.2 µg/L, and 95% of participants had a detectable result (Weiss & Angerer, 2002). In a population-based cross-sectional study with more than 1000 volunteers in Bavaria, Germany, *ortho*-anisidine was detected at concentrations of up to 8.66 µg/L in urine samples, with median values of 0.23 µg/L. Although the sources of these exposures are not known, reported *ortho*-anisidine values in women were significantly higher in the urine of smokers than of non-smokers (Kütting et al., 2009) (see also Section 4.1.1(a)).

[The Working Group noted that these studies, together with the haemoglobin adduct study described in Section 4.2.1, provide an

inconsistent picture of the importance of tobacco smoking in contributing to levels of exposure to *ortho*-anisidine detected by biomonitoring.]

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) US EPA

The reportable quantity under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) is 100 pounds [45 kg] for *ortho*-anisidine and is not indicated for *ortho*-anisidine hydrochloride (US EPA, 2019a). Releases of CERCLA hazardous substances, in quantities equal to or greater than their reportable quantity, are subject to reporting to the National Response Center under CERCLA and are also reportable under Emergency Planning and Community Right-To-Know Act (Section 313), or the US EPA's TRI (US EPA, 2019a). Emissions, transfers, and waste management data must be reported annually. Waste from the production of certain dyes, pigments, and food, drug, and cosmetic colourants – produced at a dye- or pigment-manufacturing site – is listed as US EPA hazardous waste K181. Under the K181 listing, *ortho*-anisidine is one of seven constituents whose presence in waste at a threshold amount (110 kg/year for *ortho*-anisidine) can serve as the basis for classifying the waste as hazardous (US EPA, 2003).

(b) OSHA

For *ortho*-anisidine, the current United States Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is 0.5 mg/m^3 of air as an 8-hour TWA. Absorption through the skin may be a significant source of exposure (NTP, 2016; OSHA, 2019).

(c) NIOSH

NIOSH lists an immediately dangerous to life and health (IDLH) concentration of 50 mg/m^3 for *ortho*-anisidine (NIOSH, 1994). It also lists a recommended exposure limit (REL) of 0.5 mg/m^3 TWA [skin]. NIOSH considers *ortho*-anisidine to be a potential occupational carcinogen as defined by the OSHA carcinogen policy (OSHA, 1990; NIOSH, 2018).

(d) European Chemicals Agency (ECHA)

ECHA has classified *ortho*-anisidine as carcinogenic (Category 1B), mutagenic (Category 2) and causing acute toxicity (Category 3). The use of *ortho*-anisidine is banned in any cosmetic products marketed for sale or use in the European Union (ECHA, 2020). It is on the candidate list of substances of very high concern (SVHC) for authorization – in accordance with Article 59(10) of the REACH Regulation – on the basis of carcinogenicity classification (ECHA, 2011). Workers who are aged < 18 years, pregnant, or breastfeeding, may not be exposed to *ortho*-anisidine. Employers are obliged to minimize other workers' exposure to *ortho*-anisidine as far as possible, and must arrange for medical surveillance of exposed workers (ECHA, 2020).

(e) Other international guidelines and limits

An occupational exposure limit (OEL), TWA, threshold limit value (TLV), and PEL of 0.5 mg/m^3 have also been adopted by many countries, including Argentina, Australia, Austria, Belgium, Bulgaria, Canada, China, Croatia, Denmark, France, Finland, Germany, Iceland, India, Indonesia, Ireland, Italy, Japan, Malaysia, Mexico, the Netherlands, New Zealand, Nicaragua, Norway, the Philippines, Poland, Portugal, the Republic of Korea, South Africa, Spain, Switzerland, and the United Arab Emirates. Romania has set an 8-hour limit value of 0.3 mg/m^3. In some countries, a short-term (15-minute average) limit value of 0.5–1.5 mg/m^3

has been adopted (European Chemicals Bureau, 2002; NICNAS, 2014; IFA, 2019). It is important to note that the United Kingdom Advisory Committee on Toxic Substances has expressed concern about the scientific basis for the derivation of the 0.5 mg/m^3 PEL and the adequacy of health protection it provides. This value was omitted from its 2005 OEL list and henceforth (HSE, 2020). The European Scientific Committee on Occupational Exposure Limits did not assign a TWA or short-term OEL for *ortho*-anisidine, citing insufficiency of evidence (European Commission, 2011). The California Environmental Protection Agency lists no-significant-risk levels (NSRLs) of 5 and 7 µg/day for *ortho*-anisidine and *ortho*-anisidine hydrochloride, respectively (OEHHA, 1992).

1.5.2 Reference values for biological monitoring of exposure

No reference values were available to the Working Group.

2. Cancer in Humans

Case reports

Nakano et al. (2018) reported data on 10 cases of cancer of the bladder in male Japanese workers exposed primarily to *ortho*-toluidine and employed at two plants producing organic dye and pigment intermediates. Of these 10 cases, 3 cases were also exposed to *ortho*-anisidine. Surrogate levels of exposure to six aromatic amines were calculated based on number of years and proportion of time spent on each of four production processes (preparation and reaction by mixing *ortho*-toluidine and diketene in organic solvent; filtering and rinsing the product with organic solvent; drying and packing the product; and distillation of waste organic solvent) each month. Two of the three *ortho*-anisidine-exposed cases were tobacco smokers. All 10 affected workers were hired between 1987 and 1997 and had been primarily engaged in drying and packing the product made from *ortho*-toluidine. Mean age at diagnosis for all cases of bladder cancer combined was 56 years (range, 41–71 years). [The Working Group considered that this study was not informative since all 3 *ortho*-anisidine-exposed cases were in workers co-exposed to *ortho*-toluidine and 2 of these cases were also in tobacco smokers; *ortho*-toluidine and tobacco smoking are both *carcinogenic to humans* (IARC Group 1) with *sufficient evidence* in humans for bladder cancer.]

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (feed)

Groups of 55 male and 55 female B6C3F$_1$ mice (age, 41 days) were given feed containing *ortho*-anisidine hydrochloride (purity, > 99%) at a concentration of 0 (controls), 2500, or 5000 mg/kg, 7 days per week, for 103 weeks (105 weeks for the controls), followed by an additional observation period of 1–2 weeks (males at the lower dose) or 2 weeks (NCI, 1978). There was a slight (non-significant) dose-related positive trend in mortality among male and female mice. Numbers surviving to the end of the study were 44/55 (control group), 43/55 (lower dose), and 43/55 (higher dose) for males; and 44/55, 38/55, and 42/55, respectively, for females [sufficient numbers of mice of each sex were at risk of developing tumours]. There was a significant dose-related decrease in mean body weight in treated male and female mice compared with controls over the course of the

Table 3.1 Studies of carcinogenicity with *ortho*-anisidine and *ortho*-anisidine hydrochloride in mice and rats

Study design Species, strain (sex) Age at start Duration Reference	Route Agent, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 41 days 104–105 wk NCI (1978)	Oral *ortho*-Anisidine hydrochloride, > 99% Feed 0, 2500, 5000 mg/kg, 7 days/wk, 103 wk 55, 55, 55 44, 43, 43	*Urinary bladder* Transitional cell papilloma 0/48, 2/55, 7/53* Transitional cell carcinoma 0/48, 0/55, 15/53* Transitional cell papilloma or carcinoma (combined) 0/48, 2/55, 22/53*	[$P = 0.010$ (Cochran–Armitage trend test); *$P < 0.02$ (Fisher exact test)] $P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test) $P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test)	Principal strengths: adequate number of mice used, randomly allocated in groups; adequate duration of exposure and observation and schedule of exposure; use of males and females. Controls put on study 3 wk earlier than treated groups. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on mice that survived at least as long as the mouse in which the tumour was found.
Full carcinogenicity Mouse, B6C3F$_1$ (F) 41 days 105 wk NCI (1978)	Oral *ortho*-Anisidine hydrochloride, > 99% Feed 0, 2500, 5000 mg/kg, 7 days/wk, for 103 wk 55, 55, 55 44, 38, 42	*Urinary bladder* Transitional cell carcinoma 0/50, 0/51, 18/50* Transitional cell papilloma or carcinoma (combined) 0/50, 1/51, 22/50*	$P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test) $P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test)	Principal strengths: adequate number of mice used, randomly allocated in groups; adequate duration of exposure and observation and schedule of exposure; use of males and females. Controls put on study 3 wk earlier than treated groups. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on mice that survived at least as long as the mouse in which the tumour was found.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 41 days 88 wk (high dose) or 104–106 wk NCI (1978)	Oral *ortho*-anisidine hydrochloride, > 99% Feed 0, 5000, 10 000 mg/kg, 7 days/wk, for 106 wk (control), 103 wk (low dose) or 88 wk (high dose) 55, 55, 55 39, 7, 0	*Urinary bladder* Transitional cell carcinoma 0/51, 50/54*, 51/52*	$P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test)	Principal strengths: adequate number of rats used, randomly allocated in groups; adequate duration of exposure and observation and schedule of exposure; use of males and females. Positive dose-related trend in mortality. Controls put on study 3 wk earlier than treated groups. Statistical analyses were performed on rats that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on rats that survived at least as long as the rat in which the tumour was found. In the historical control data, the incidence of thyroid follicular cell tumours in male rats was 3/250 (1.2%).
		Transitional cell papilloma or carcinoma (combined) 0/51, 52/54*, 52/52*	$P < 0.001$ (Cochran–Armitage trend test); *$P < 0.001$ (Fisher exact test)	
		Renal pelvis: transitional cell carcinoma 0/53, 3/55, 7/53*	$P = 0.005$ (Cochran–Armitage trend test); *$P = 0.006$ (Fisher exact test)	
		Thyroid Follicular cell adenoma, cystadenoma, or papillary cystadenoma (combined) 0/53, 4/40*, 4/40*	$P = 0.030$ (Cochran–Armitage trend test); *$P = 0.031$ (Fisher exact test)	
		Follicular cell carcinoma or papillary cystadenocarcinoma (combined) 0/53, 3/40 (7.5%), 2/40 (5.0%)	NS	
		Follicular cell carcinoma, adenoma, cystadenoma, papillary cystadenoma, or papillary cystadenocarcinoma (combined) 0/53, 7/40 (17.5%)*, 6/40 (15.0%)**	$P = 0.009$ (Cochran–Armitage trend test); *$P = 0.002$ (Fisher exact test); **$P = 0.005$ (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (F) 41 days 83 wk (high dose) or 103–107 wk NCI (1978)	Oral *ortho*-Anisidine hydrochloride, > 99% Feed 0, 5000, 10 000 mg/kg, 7 days/wk, for 107 wk (control), 103 wk (low dose) or 83 wk (high dose) 55, 55, 55 36, 0, 0	*Urinary bladder* Transitional cell papilloma 0/49, 5/49*, 0/51 Transitional cell carcinoma 0/49, 41/49*, 50/51* Transitional cell papilloma or carcinoma (combined) 0/49, 46/49*, 50/51*	 *P = 0.028 (Fisher exact test) P < 0.001 (Cochran–Armitage trend test); *P < 0.001 (Fisher exact test) P < 0.001 (Cochran–Armitage trend test); *P < 0.001 (Fisher exact test)	Principal strengths: adequate number of rats used, randomly allocated in groups; adequate duration of exposure and observation and schedule of exposure; use of males and females. Positive dose-related trend in mortality. Controls put on study 3 wk earlier than treated groups. Statistical analyses were performed on rats that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on rats that survived at least as long as the rat in which the tumour was found.
Initiation–promotion (tested as promoter) Rat, F344 (M) 6 wk 36 wk Ono et al. (1992)	Oral *ortho*-Anisidine, NR Feed 0 (control), 1700 mg/kg (for 2 wk) then 425 mg/kg (for 30 wk) 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking-water for 4 wk, then control feed or *ortho*-anisidine 15, 16 13, 16	*Urinary bladder* Papilloma 0/13, 3/16 Carcinoma 0/13, 2/16 Papillary or nodular hyperplasia 2/13, 13/16*	 NS NS *P < 0.01 (Fisher exact test and Student *t*-test)	Principal strengths: adequate duration and schedule of exposure and duration of observation. Principal limitation: no untreated control group was available. No urinary bladder lesions were observed in a group of 10 male rats receiving *ortho*-anisidine only.

F, female; M, male; NR, not reported; NS, not significant; wk, week.

study. An anatomopathological investigation was performed, and full histopathological examination was carried out on major organs, including some regional lymph nodes.

In male and female mice, there was a significant positive trend ($P < 0.001$) and a significant increase ($P < 0.001$, at the higher dose) in the incidence of transitional cell carcinoma and of transitional cell papilloma or carcinoma (combined) of the urinary bladder, compared with untreated male and female controls. In male mice, there was also a significant positive trend [$P = 0.010$] and a significant increase [$P < 0.02$] in the incidence of transitional cell papilloma of the urinary bladder at the higher dose compared with untreated controls. There was a significant increase in the incidence of focal hyperplasia of the urinary bladder in male and female mice at the higher dose. [The Working Group considered local hyperplasia of the urinary bladder to be a pre-neoplastic lesion.]

[The Working Group noted the adequate number of animals used, the random allocation in groups, the use of males and females, the adequate duration of exposure and observation, and the adequate schedule of exposure.]

3.2 Rat

3.2.1 Oral administration (feed)

Groups of 55 male and 55 female Fischer 344 rats (age, 41 days) were given feed containing *ortho*-anisidine hydrochloride (purity, > 99%) at a concentration of 0 (controls), 5000, or 10 000 mg/kg, 7 days per week, for 106–107 weeks for the controls, for 103 weeks for groups of males and females at the lower dose, for 88 weeks for males at the higher dose, and for 83 weeks for females at the higher dose. For males at the lower dose, there was an additional observation period of 1 week (NCI, 1978). There was a dose-related positive trend in mortality that was significant in male and female rats compared with controls. Numbers surviving to the end of the study were 39/55 (control group), 7/55 (lower dose), and 0/55 (higher dose) for males; and 36/55, 0/55, and 0/55, respectively, for females; however, 49/55 (89%) of males at the higher dose and 44/55 (80%) of females at the higher dose were still alive at week 52. All 55 rats in the groups of males and females at the lower dose and all 55 rats in the control groups of males and females lived beyond week 52. [The Working Group considered that sufficient numbers of male and female rats were at risk of developing tumours.] There was a significant dose-related decrease in mean body weight in treated male and female rats compared with controls. An anatomopathological investigation was performed, and full histopathological examination was carried out on major organs, including some regional lymph nodes.

There was a significant positive trend ($P = 0.005$) and a significant increase ($P = 0.006$) in the incidence of transitional cell carcinoma of the kidney/pelvis [renal pelvis] in male rats at the higher dose compared with male untreated controls. There was a significant positive trend ($P < 0.001$) and significant increase ($P < 0.001$) in the incidence of transitional cell carcinoma, and of transitional cell papilloma or carcinoma (combined) of the urinary bladder in treated groups of male and female rats at the lower and higher dose compared with untreated controls. There was also a significant increase ($P = 0.028$) in the incidence of transitional cell papilloma of the urinary bladder in females at the lower dose. In males, there was a significant positive trend ($P = 0.030$) and significant increase ($P = 0.031$) in the incidence of follicular cell adenoma, cystadenoma, or papillary cystadenoma (combined) of the thyroid at the lower and higher dose. In males, there was also a significant positive trend ($P = 0.009$) and significant increase ($P \leq 0.005$, lower and higher dose) in the incidence of follicular cell carcinoma, adenoma, cystadenoma, papillary cystadenoma, or papillary cystadenocarcinoma (combined) of the thyroid; the

incidence was: controls, 0/53; lower dose, 7/40 (17.5%); and higher dose, 6/40 (15.0%), respectively. The incidence of thyroid follicular cell carcinoma or papillary cystadenocarcinoma (combined) in the treated groups – controls, 0/53; lower dose, 3/40 (7.5%); and higher dose, 2/40 (5.0%) – was not significantly increased, but was higher than the incidence of thyroid follicular cell tumours reported for historical controls, which was 3/250 (1.2%) (NCI, 1978). [The Working Group noted the adequate number of animals used, the random allocation in groups, the use of males and females, the adequate duration of exposure and observation, and the adequate schedule of exposure.]

[The Working Group noted that the aromatic amines *ortho*-toluidine and 2-naphthylamine, which are *carcinogenic to humans* (IARC Group 1), also caused malignant tumours of the urinary bladder when administered orally in rats, and that the aromatic amine 4-aminobiphenyl (IARC Group 1) caused malignant tumours of the urinary bladder when administered orally in mice as well as in dogs (IARC, 2012).]

3.2.2 Initiation–promotion

Two groups of 15–16 male Fischer 344 rats (age, 6 weeks) were given drinking-water containing 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) for 4 weeks. They were then given feed containing *ortho*-anisidine [purity not reported] at a concentration of 0 or 1700 mg/kg for the first 2 weeks and 425 mg/kg thereafter for an additional 30 weeks. A third group of 10 rats received *ortho*-anisidine without prior administration of BBN (Ono et al., 1992). At experimental week 36, the rats were killed and urinary bladders were examined histologically. The incidence of papillary or nodular hyperplasia of the urinary bladder was significantly higher ($P < 0.01$) in the group treated with BBN plus *ortho*-anisidine than in the group treated with BBN alone, but there was no significant increase in the incidence of papilloma or carcinoma of the urinary bladder. No lesions of the urinary bladder were observed in the group exposed to *ortho*-anisidine alone. [The Working Group noted that the duration of exposure and observation, and the schedule of exposure were adequate. No untreated control group was available.]

3.3 Synthesis

In one study in male and female B6C3F$_1$ mice treated by oral administration (in feed), *ortho*-anisidine hydrochloride caused a significant increase, with 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 in males, and of transitional cell carcinoma and transitional cell papilloma or carcinoma (combined) of the urinary bladder in females (NCI, 1978).

In one study in male and female Fischer 344 rats treated by oral administration (in feed), *ortho*-anisidine hydrochloride caused a significant increase, with a significant positive trend, in the incidence of transitional cell carcinoma and transitional cell papilloma or carcinoma (combined) of the urinary bladder, transitional cell carcinoma of the renal pelvis, and of follicular cell adenoma, cystadenoma, or papillary cystadenoma (combined) and follicular cell carcinoma, adenoma, cystadenoma, papillary cystadenoma, or papillary cystadenocarcinoma (combined) of the thyroid in males. *ortho*-Anisidine hydrochloride also caused a significant increase, with a significant positive trend, in the incidence of transitional cell carcinoma, and transitional cell papilloma or carcinoma (combined) of the urinary bladder in females. In addition, there was a significant increase in the incidence of transitional cell papilloma of the urinary bladder in treated female rats (NCI, 1978).

In one initiation–promotion study in which *ortho*-anisidine was tested as a promoter in

Fischer 344 male rats, there was no significant increase in the incidence of papilloma or carcinoma of the urinary bladder (Ono et al., 1992).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

ortho-Anisidine was detected in 95% of urine samples in a study of 20 participants without known exposure. The concentration was in the range of 0.05 to 4.2 µg/L, with a median value of 0.22 µg/L and a 95th percentile of 0.68 µg/L (Weiss & Angerer, 2002).

In a cross-sectional study, urinary *ortho*-anisidine was quantified in 1004 volunteers aged 3–84 years. *ortho*-Anisidine was detected in 90% of the population at a concentration range of 0.03–8.66 µg/L, with a median value of 0.23 µg/L and a 95th percentile of 1.12 µg/L (Kütting et al., 2009).

No data on absorption after occupational exposure to *ortho*-anisidine were available to the Working Group.

(b) Human hepatic microsomes

See Fig. 4.1.

Stiborová et al. (2005) reported that *ortho*-anisidine (0.1–0.5 mM) incubated with human hepatic microsomes in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) produced two metabolites, one of which was identified as *N*-(2-methoxyphenyl)hydroxylamine. In addition, Stiborová et al. (2005) demonstrated the involvement of human cytochrome P450 (CYP) enzymes in the *ortho*-anisidine oxidation, mainly CYP2E1 and CYP1A2. These findings were supported by the results of three independent assays: (i) the highly significant correlation found between the rate of chlorzoxazone 6-hydroxylation, a marker for CYP2E1, and the levels of *N*-(2-methoxyphenyl)hydroxylamine in human hepatic microsomes; (ii) the inhibition of *N*-(2-methoxyphenyl)hydroxylamine formation by diethyldithiocarbamate, an inhibitor of CYP2E1 in human hepatic microsomes; and (iii) the oxidation of *ortho*-anisidine by recombinant human CYP enzymes in Supersomes, demonstrating that the enzymes CYP1A2, followed by CYP2B6 and CYP2E1, are the most efficient enzymes catalysing the metabolism of *ortho*-anisidine (Stiborová et al., 2005).

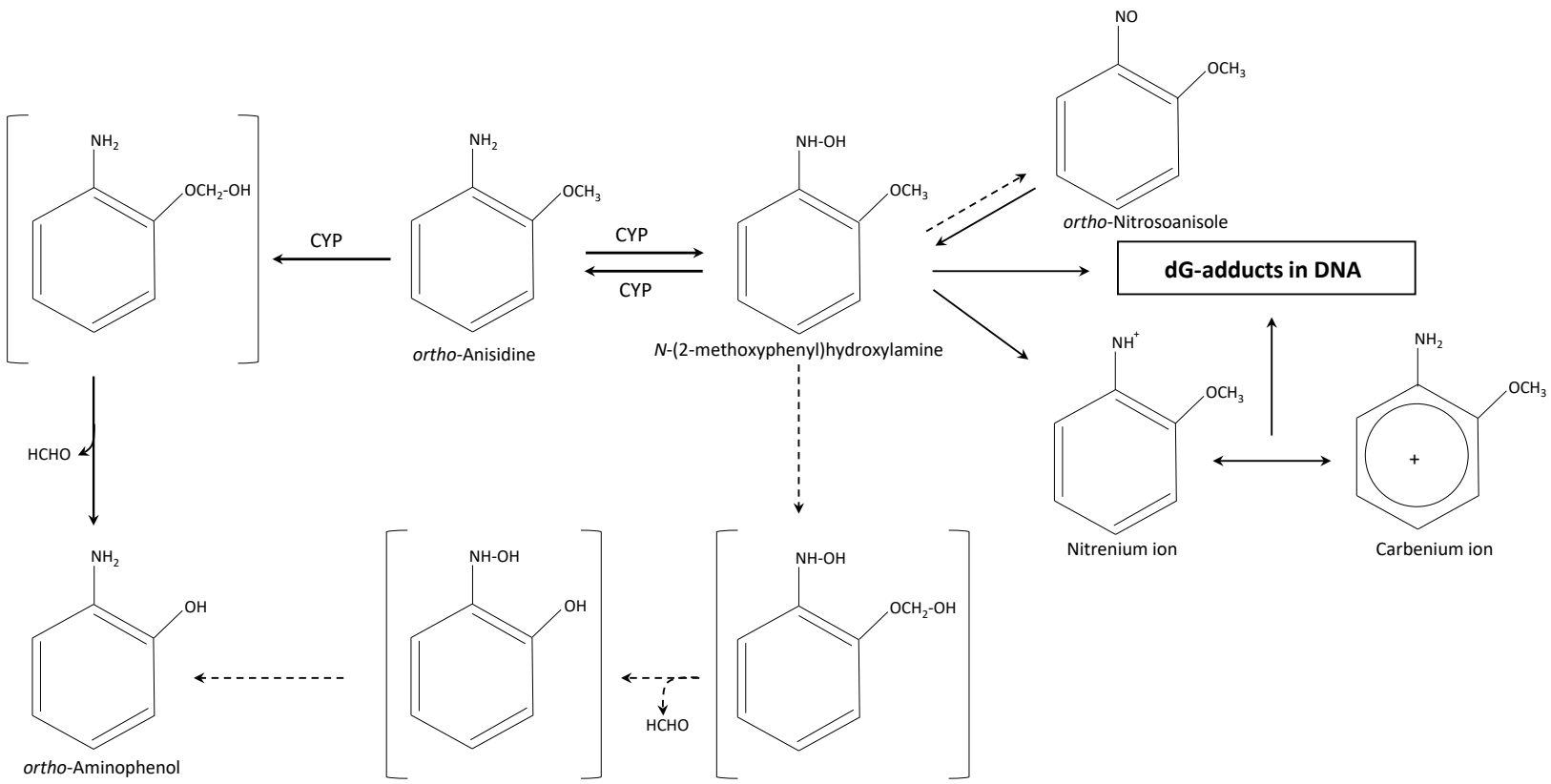
Naiman et al. (2011) reported that human CYP enzymes catalyse the further oxidative and reductive metabolism of *N*-(2-methoxyphenyl) hydroxylamine, the main metabolite of *ortho*-anisidine. Using human hepatic microsomes and human recombinant CYP enzymes incubated with *N*-(2-methoxyphenyl)hydroxylamine, the parent compound *ortho*-anisidine (major product), the isomer *ortho*-aminophenol, and two other metabolites were identified. In addition, the human CYP2E1, CYP3A4, and CYP2C enzymes were found to be important in catalysing the reduction of *N*-(2-methoxyphenyl) hydroxylamine to *ortho*-anisidine (Naiman et al., 2011).

4.1.2 Experimental systems

(a) In vivo

Sapota et al. (2003) reported on the tissue distribution, excretion, and metabolism of *ortho*-anisidine in male IMP:WIST rats given a single intraperitoneal dose of *ortho*-anisidine-ring-U-^{3}H (as a free base, dissolved in olive oil; 10 mg/kg bw). The blood plasma biphasic half-lives for fast and slow phases were about 1.5 hours and 80 hours, respectively, and the erythrocyte biphasic half-lives for fast and slow phases were about 1 hour and 116 hours,

Fig. 4.1 Pathways of *ortho*-anisidine metabolism



CYP, cytochrome P450; dG, deoxyguaonsine.
The compounds showed in square brackets were not detected under experimental conditions. The reactions shown in Fig. 4.1 that are more frequent, not as frequent, or infrequent are indicated by bold, normal, and dashed arrows, respectively.
Adapted from *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, Volume 726, issue 2, Naiman et al. (2011). Human cytochrome-P450 enzymes metabolize *N*-(2-methoxyphenyl)hydroxylamine, a metabolite of the carcinogens *o*-anisidine and *o*-nitroanisole, thereby dictating its genotoxicity, pages 160–188, copyright (2011), with permission from Elsevier.

respectively. *ortho*-Anisidine was widely distributed to tissues, with the highest levels found in the liver, kidneys, and muscle tissue. In all examined tissues except for kidney and fat, the highest concentration of radiolabel was found 12 hours after injection. Urine was the main route of excretion. Almost 72% was excreted during the first 72 hours, about 6% of this in the faeces (Sapota et al., 2003). The main urinary metabolites were: (i) *N*-acetyl-2-methoxyaniline (almost 97% of the total amount excreted in the urine); and (ii) *N*-acetyl-4-hydroxy-2-methoxyaniline (about 1.5% of the total amount excreted in the urine) (Sapota et al., 2003).

ortho-Anisidine induced methaemoglobinaemia in CBA mice and Alpk:APfSD rats treated by oral administration (Ashby et al., 1991), indicating that *ortho*-anisidine is distributed and N-oxidized in rodents.

(b) In vitro

See Fig. 4.1.

Oxidation of *ortho*-anisidine by peroxidases, including the mammalian peroxidases and prostaglandin H synthase (from ram seminal vesicles), has been reported (Thompson & Eling, 1991; Stiborová et al., 2001, 2002). The pattern of metabolites formed after incubation of *ortho*-anisidine (0.1–1.0 mM) with peroxidases was dependent on the concentration of *ortho*-anisidine, concentration of peroxidases, incubation time, and pH. Peroxidases oxidized *ortho*-anisidine to a diimine metabolite, which subsequently hydrolysed to form a quinone imine (Stiborová et al., 2002). [The Working Group noted that diimine and quinone imine are electrophilic species.]

Three studies identified the metabolites formed in incubations of *ortho*-anisidine (0.1–2.0 mM) with rat and rabbit hepatic microsomes (Rýdlová et al., 2005; Naiman et al., 2008a, b). These studies showed that *ortho*-anisidine is subject to redox cycling reactions. It is primarily oxidized to *N*-(2-methoxyphenyl)hydroxylamine (major metabolite), *ortho*-aminophenol, and an additional metabolite. *N*-(2-methoxyphenyl) hydroxylamine is either further oxidized to *ortho*-nitrosoanisole (2-methoxynitrosobenzene) or reduced to parental *ortho*-anisidine, which can be oxidized again to produce *ortho*-aminophenol (Rýdlová et al., 2005; Naiman et al., 2008a, b). Using purified rat and rabbit hepatic CYP enzymes, reconstituted with NADPH:P450 reductase, the ability of CYP1A1, 1A2, 2B2, 2B4, 2E1, and 3A6 to catalyse the oxidation of o*rtho*-anisidine was observed (Naiman et al., 2008b). The involvement of CYP2C, CYP2E1, CYP2D, and CYP2A, was observed in the reduction of *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine (Naiman et al., 2010).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether *ortho*-anisidine is electrophilic or can be metabolically activated to an electrophile; is genotoxic; or alters cell proliferation, cell death, or nutrient supply. For the evaluation of other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

Two studies detected the presence of haemoglobin adducts of *ortho*-anisidine in blood samples. Richter et al. (2001) detected haemoglobin adducts of *ortho*-anisidine, using capillary GC-MS, in blood samples of children from three different regions of southern Germany. The levels of *ortho*-anisidine–haemoglobin adducts were statistically significantly higher in children from an urban area (Munich, 1.3

million inhabitants) than in children from a less urban area (Augsburg, 250 000 inhabitants) and in children from a rural area (Eichstatt, 13 000 inhabitants). The regional differences in levels of *ortho*-anisidine–haemoglobin adducts were not related to tobacco exposure, since there were no major differences between children from smoking and non-smoking households (Richter et al., 2001). Haemoglobin adducts of *ortho*-anisidine, analysed by capillary GC-MS, were detected at similar levels in the blood of smoking and non-smoking pregnant women in Germany (Branner et al., 1998).

(ii) Human cells in vitro

No data from studies in human cells in vitro were available to the Working Group.

In two studies it was observed that *ortho*-anisidine is activated by human hepatic microsomes to form DNA adducts. Stiborová et al. (2005) used two techniques, [^{14}C]-labelled *ortho*-anisidine and ^{32}P-postlabelling, to show that after activation by human hepatic microsomes *ortho*-anisidine forms *N*-(2-methoxyphenyl) hydroxylamine and binds to DNA. Using the ^{32}P-postlabelling technique, Naiman et al. (2011) reported DNA-adduct formation induced by *N*-(2-methoxyphenyl)hydroxylamine, the main metabolite of *ortho*-anisidine, when incubated with human hepatic microsomes.

(b) Experimental systems

Covalent binding to DNA was undetectable in B6C3F$_1$ mouse bladder or liver cells in vivo, after a single oral dose of *ortho*-anisidine hydrochloride (Ashby et al., 1994; see Table 4.1). *ortho*-Anisidine–DNA adducts, detected by ^{32}P-postlabelling, were observed in the urinary bladder, liver, kidney, and spleen (but not in the lung, heart, or brain) of Wistar rats treated with more than one intraperitoneal dose of *ortho*-anisidine (Stiborová et al., 2005; Naiman et al., 2012; see Table 4.1). The highest total DNA-adduct levels were found in the urinary bladder. The level of adducts in the bladder declined with time, but 39% of the initial level of binding remained even after 36 weeks (Naiman et al., 2012). Covalent binding was much less persistent in the liver, kidney, and spleen. *N*-(Deoxyguanosin-8-yl)-2-methoxyaniline was the major DNA adduct formed by *ortho*-anisidine. [The Working Group noted that guanine is also the predominant deoxynucleotide target within DNA for covalent binding by other aromatic amines, such as 4-aminobiphenyl, which is classified in IARC Group 1 (IARC, 2012).] There was formation of DNA adducts, detected by ^{32}P-postlabelling, in the urinary bladder of Wistar rats exposed orally to *ortho*-anisidine hydrochloride for 4 weeks (Iatropoulos et al., 2015; see Table 4.1).

ortho-Anisidine (1 mM) underwent covalent binding to calf thymus DNA (Thompson & Eling, 1991). Metabolites of *ortho*-anisidine (diimine and quinone imine) were consistently more reactive with protein and glutathione than were metabolites of *para*-anisidine (Thompson & Eling, 1991). Two subsequent studies using [^{14}C]-labelled *ortho*-anisidine and ^{32}P-postlabelling assays observed that, after peroxidation to diimine and quinone imine, *ortho*-anisidine binds to calf thymus DNA in the presence of microsomes from ram seminal vesicles (Stiborová et al., 2001, 2002). Using [^{14}C]-labelled *ortho*-anisidine, Stiborová et al. (2002) observed substantial peroxidase-dependent covalent binding of *ortho*-anisidine to DNA, tRNA, and polydeoxynucleotides. Using the ^{32}P-postlabelling assay, and enzymatic digestion with three times higher concentrations of micrococcal nuclease and spleen phosphodiesterase than in the standard procedure, *ortho*-anisidine activated by peroxidases was bound to poly(dG)–poly(dC) and to a lesser extent to poly(dA), but binding to poly(dC) or poly(dT) was not detectable, suggesting specificity for purine adduct formation (Stiborová et al., 2002).

Table 4.1 Genetic and related effects of *ortho*-anisidine and *ortho*-anisidine hydrochloride in non-human mammals in vivo

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA adducts (^{14}C-labelling assay)	Mouse, B6C3F$_1$ (F)	Bladder and liver	–	[^{14}C]-Labelled *ortho*-anisidine hydrochloride, 750 mg/kg bw	Oral, 1×		Ashby et al. (1994)
DNA adducts (^{32}P-post-labelling assay)	Mouse, B6C3F$_1$ (F)	Bladder and liver	–	*ortho*-Anisidine hydrochloride, 750 mg/kg bw	Oral, 1×		Ashby et al. (1994)
DNA adducts (^{32}P-post-labelling assay)	Rat, Wistar (M)	Bladder, liver, kidney and spleen	+	*ortho*-Anisidine, 0.15 mg/kg bw	Intraperitoneal, 1×/day for 5 days	DNA adducts detected using nuclease P1 version, but not with standard procedure	Stiborová et al. (2005)
DNA adducts (^{32}P-post-labelling assay)	Rat, Wistar (M)	Lung, heart and brain	–	*ortho*-Anisidine, 0.15 mg/kg bw	Intraperitoneal, 1×/day for 5 days		Stiborová et al. (2005)
DNA adducts (^{32}P-post-labelling assay)	Rat, Wistar (M)	Bladder, liver, kidney and spleen	+	*ortho*-Anisidine: day 1, 0.15 mg/kg bw; day 2, 0.18 mg/kg bw; and day 3, 0.2 mg/kg bw	Intraperitoneal, 1×/day for 3 days; total dose, 0.53 mg/kg bw	DNA adducts detected using nuclease P1 version and standard procedure under ATP-deficient conditions.	Naiman et al. (2012)
DNA adducts (^{32}P-post-labelling assay)	Rat, Wistar (M)	Bladder	+	*ortho*-Anisidine hydrochloride, 17 mg/kg bw	Oral, 3×/wk for 4 wk	DNA adducts detected using nuclease P1 version and HLB columns	Iatropoulos et al. (2015)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Bladder	+	*ortho*-Anisidine hydrochloride, 17 mg/kg bw	Oral, 3×/wk for 4 wk		Iatropoulos et al. (2015)
DNA single-strand break (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver, thymus, and testis	–	*ortho*-Anisidine, 700 mg/kg bw	Oral, 1×		Ashby et al. (1991)
DNA single-strand break (automated alkaline elution system)	Rat, Wistar (M)	Liver, kidney, spleen and bladder	–	*ortho*-Anisidine, 500 mg/kg bw	Oral, 1×		Ashby et al. (1991)
DNA single-strand break (automated alkaline elution system)	Rat, Wistar (M)	Liver and bladder	–	*ortho*-Anisidine, 750 mg/kg bw	Intraperitoneal, 1×		Ashby et al. (1991)

Table 4.1 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow	–	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 0 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Colon, kidney, bladder, and lung	+	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 3 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Stomach, liver, brain, and bone marrow	–	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 3 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Stomach, colon, kidney, bladder, lung, and brain	+	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 8 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Liver and bone marrow	–	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 8 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Colon	+	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Wistar (M)	Stomach, liver, kidney, bladder, lung, brain, and bone marrow	–	*ortho*-Anisidine, 1000 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Rat, Sprague-Dawley (M) and (F)	Bladder	–	*ortho*-Anisidine, 700 mg/kg bw	Oral, 1×/day for 2 days		Wada et al. (2012)
DNA single-strand break (alkaline comet assay)	Rat, Crl:CD(SD) (M)	Liver		*ortho*-Anisidine, 600 mg/kg bw	Oral, 1×		Uno & Omori (2015)
DNA strand breaks (alkaline comet assay)	Rat, Sprague-Dawley (M)	Liver	±	*ortho*-Anisidine, 600 mg/kg bw	Oral, 1×/day, for 3 days	No dose–response relationship observed	Hobbs et al. (2015)
DNA strand breaks (alkaline comet assay)	Rat, Sprague-Dawley (M)	Stomach	–	*ortho*-Anisidine, 600 mg/kg bw	Oral, 1×/day, for 3 days		Hobbs et al. (2015)

Table 4.1 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (phosphorylated histone γ-H2AX)	Rat, Fischer/DuCrl-Crlj (M)	Bladder	+	*ortho*-Anisidine hydrochloride, 1% in feed (563.1 mg/kg bw)	Oral, for 2 days, 2 wk and 4 wk		Toyoda et al. (2019)
Unscheduled DNA synthesis	Rat, F344 (M)	Kidney	–	*ortho*-Anisidine, 500 mg/kg bw	Intraperitoneal, 1×/day		Tyson & Mirsalis (1985)
Unscheduled DNA synthesis	Rat, AP (M)	Liver	–	*ortho*-Anisidine, 1104 mg/kg bw	Oral, 1×		Ashby et al. (1991)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Stomach, colon, liver, kidney, bladder, lung, brain and bone marrow	–	*ortho*-Anisidine, 690 mg/kg bw	Oral, in olive oil; 1×; sampled after 0 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Colon and bladder	+	*ortho*-Anisidine, 690 mg/kg bw	Oral, in olive oil; 1×; sampled after 3 h and 8 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Stomach, liver, kidney, lung, brain and bone marrow	–	*ortho*-Anisidine, 690 mg/kg bw	Oral, in olive oil; 1×; sampled after 3 h and 8 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Bladder	+	*ortho*-Anisidine, 690 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Stomach, colon, liver, kidney, lung, brain and bone marrow	–	*ortho*-Anisidine, 690 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h		Sekihashi et al. (2002)
DNA strand breaks (alkaline comet assay)	Mouse, CD-1 (M)	Bladder	+	*ortho*-Anisidine, 690 mg/kg bw	Oral, 1×	Using modified comet assay; nuclei isolated by homogenization instead whole cells	Sasaki et al. (1998)
DNA strand breaks (alkaline comet assay)	Mouse, CD-1 (M)	Colon	±	*ortho*-Anisidine, 690 mg/kg bw	Oral, 1×	Using modified comet assay; nuclei isolated by homogenization instead of whole cells; results positive 3 h, but not 24 h, after *ortho*-anisidine administration	Sasaki et al. (1998)

Table 4.1 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Gene mutation (*lacI*⁻ transgenic model)	Mouse, Big Blue™ (M)	Bladder	±	*ortho*-Anisidine hydrochloride, 750 mg/kg bw	Oral, 1×/day for 3 days	Statistical significance only after 14 days of administration using one-sided Student's *t*-test. Results were not confirmed in a repeat of the experiment	Ashby et al. (1994)
Gene mutation (*lacI*⁻ transgenic model)	Mouse, Big Blue™, (M)	Liver	–	*ortho*-Anisidine hydrochloride, 750 mg/kg bw	Oral, 1×/day for 10 days		Ashby et al. (1994)
Micronucleus formation	Rat, AP (M) and F344 (M)	Liver	–	*ortho*-Anisidine, 1104 mg/kg bw (AP); and 690 mg/kg bw (F344)	Oral, 1×		Ashby et al. (1991)
Micronucleus formation	Rat, AP (M)	Bone marrow	–	*ortho*-Anisidine, 1380 mg/kg bw	Oral, 1×		Ashby et al. (1991)
Micronucleus formation	Mouse, BDF_1 (M)	Bone marrow	±	*ortho*-Anisidine, 800 mg/kg bw	Intraperitoneal, 1×/day	The administered dose was lethal to CD-1 mice	Morita et al. (1997)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow	–	*ortho*-Anisidine, 500 mg/kg bw	Intraperitoneal, 1×/day for 3 days		Ashby et al. (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	–	*ortho*-Anisidine, 690 mg/kg bw	Oral, 1×/day for 3 days		Ashby et al. (1991)
DNA repair (host-mediated assay)	Mouse, NMRI (M)	*Escherichia coli* K-12 in blood	–	*ortho*-Anisidine, 1300 mg/kg bw	Oral, 1×/day		Hellmér & Bolcsfoldi (1992b)
DNA repair (host-mediated assay)	Mouse, NMRI (M)	*Escherichia coli* K-12 in blood and kidney	+	*ortho*-Anisidine, 310 mg/kg bw	Intraperitoneal, 1×/day		Hellmér & Bolcsfoldi (1992b)

bw, body weight; d, day; F, female; HID, highest ineffective dose; HLB, hydrophilic–lipophilic-balanced; LED, lowest effective dose; M, male; wk, week.

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study).

Rat and rabbit hepatic microsomal CYP enzymes catalyse both O-demethylation and N-hydroxylation of *ortho*-anisidine to form a reactive metabolite, *N*-(2-methoxyphenyl) hydroxylamine (Naiman et al., 2008a, b). As shown in Fig. 4.1, studies using human, rabbit, or rat hepatic microsomes reported CYP-dependent oxidation of *ortho*-anisidine to its major metabolite, *N*-(2-methoxyphenyl) hydroxylamine. This *N*-hydroxy compound can be further oxidized to *ortho*-nitrosoanisole or reduced back to *ortho*-anisidine. Moreover, studies in vitro using mammalian peroxidases showed CYP-dependent formation of the electrophilic species diimine and quinone imine. [The Working Group noted that the bioactivation of *ortho*-anisidine to electrophilic species involves N-oxidation by CYP-associated enzymes, and parallels an established paradigm for aromatic amines such as 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine, which have been classified as *carcinogenic to humans* (IARC Group 1) (IARC, 2010, 2012).]

4.2.2 Is genotoxic

Table 4.1, Table 4.2, and Table 4.3 summarize the available studies on the genetic and related effects of *ortho*-anisidine and *ortho*-anisidine hydrochloride.

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

DNA damage, analysed by the quantification of phosphorylated histone H2AX (γ-H2AX) in protein extracts of cells and by biased sinusoidal field-gel electrophoresis assay, was detected in 1T1 cells (human ureter epithelial cells immortalized by transfection with the human papillomavirus *E6* and *E7* genes) treated with *ortho*-anisidine (10 mM) for 4 hours. The generation of γ-H2AX increased in a dose-dependent manner after treatment of 1T1 cells with *ortho*-anisidine (5 mM) for 4 hours (Qi et al., 2020). Induction of γ-H2AX was also reported in human liver carcinoma HepG2 cells treated with *ortho*-anisidine (5 mM) for 4 hours. [The Working Group noted that the authors did not present quantification values for induction of γ-H2AX in HepG2 cells. HepG2 cells have low metabolic competence.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

The effect of *ortho*-anisidine on DNA damage was evaluated in several studies in rodents, and positive results in several tissues, including urinary bladder, were observed. Iatropoulos et al. (2015) observed a statistically significant increase in the mean percentage values of tail DNA, assessed by comet assay, in the urinary bladder of Wistar rats treated with *ortho*-anisidine hydrochloride for 1 month. Ashby et al. (1991) did not observe DNA strand breaks in the liver, thymus, testes, kidney, spleen, or urinary bladder of Wistar or Sprague-Dawley rats, after a single oral or intraperitoneal dose of *ortho*-anisidine. On the other hand, in Wistar rats, *ortho*-anisidine induced DNA strand breaks in the colon, kidney, bladder, and lung at the 3-hour sampling time; in the stomach, colon, kidney, bladder, lung, and brain at the 8-hour sampling time; and in the colon at the 24-hour sampling time after a single oral dose (Sekihashi et al., 2002). Wada et al. (2012) did not observe an increase in the frequency of DNA strand breaks in the urinary bladder of male and female Sprague-Dawley rats given a single dose of *ortho*-anisidine. Data from an international validation study on the comet assay technique indicated that a single dose of *ortho*-anisidine administered to Crl:CD(SD) male rats induced DNA strand breaks in the liver when the median percentage tail DNA, instead of the mean percentage tail DNA, was analysed

Table 4.2 Genetic and related effects of *ortho*-anisidine in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
DNA strand breaks (alkaline unwinding test)	Mouse lymphoma L5178Y cells	–	+	150 µg/mL	Garberg et al. (1988)
Gene mutation, *Tk*$^{+/-}$ locus	Mouse lymphoma L5178Y cells	+	+	123 µg/mL	Wangenheim & Bolcsfoldi (1988)
Chromosomal aberrations	Chinese hamster ovary cells	+	+	1200 µg/mL	Galloway et al. (1987)
Chromosomal aberrations	Chinese hamster lung	+	+	1000 µg/mL	JETOC (1997)
Sister-chromatid exchange	Chinese hamster ovary cells	+	+	38 µg/mL	Galloway et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration; Tk, thymidine kinase.

[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of *ortho*-anisidine and *ortho*-anisidine hydrochloride in non-mammalian and acellular experimental systems

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	–	+	*ortho*-Anisidine, 10 µg/plate	No information on source of *ortho*-anisidine, no details on methodology	Shimizu & Takemura (1983)
Salmonella typhimurium TA98	Reverse mutation	–	+	*ortho*-Anisidine hydrochloride, 10 000 µg/plate	4 positive findings with RLI, MNL, MLI, and HLI	Dunkel et al. (1985)
Salmonella typhimurium TA100	Reverse mutation	–	+	*ortho*-Anisidine hydrochloride, 10 000 µg/plate	1 positive finding with MLI	Dunkel et al. (1985)
Salmonella typhimurium TA1535	Reverse mutation	–	–	*ortho*-Anisidine hydrochloride, 10 000 µg/plate		Dunkel et al. (1985)
Salmonella typhimurium TA1537	Reverse mutation	+	–	*ortho*-Anisidine hydrochloride, 10 000 µg/plate	1 positive finding with MLN	Dunkel et al. (1985)
Salmonella typhimurium TA1538	Reverse mutation	–	+	*ortho*-Anisidine hydrochloride, 10 000 µg/plate	6 positive findings with MLI and HLI	Dunkel et al. (1985)
Salmonella typhimurium WP2*uvrA*	WP2*uvrA*	–	–	*ortho*-Anisidine hydrochloride, 10 000 µg/plate		Dunkel et al. (1985)
Escherichia coli WP2*uvrA*	Reverse mutation	–	–	*ortho*-Anisidine, 10 000 µg/plate		Dunkel et al. (1985)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	*ortho*-Anisidine, 100 µg/plate for TA98 and 33 µg/plate for TA100		Zeiger et al. (1992)
Salmonella typhimurium YG1012 (TA1538 with *N*-acetyltransferase gene)	Reverse mutation	–	–	*ortho*-Anisidine, NR		Thompson et al. (1992)
Salmonella typhimurium YG1029 (TA100 with *N*-acetyltransferase gene)	Reverse mutation	–	+	*ortho*-Anisidine, 62 µg/plate		Thompson et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation	–	–	*ortho*-Anisidine, 100 µg/plate		Ferretti et al. (1977)
Salmonella typhimurium TA1538	Reverse mutation	–	–	*ortho*-Anisidine, 100 µg/plate		Garner & Nutman (1977)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	*ortho*-Anisidine, 10 800 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052,	Reverse mutation	–	–	*ortho*-Anisidine, 10 000 µg/plate	No clear statement about dose range	Thompson et al. (1983)
Escherichia coli WP2, WP2*uvrA*	Reverse mutation	–	–	*ortho*-Anisidine, 10 000 µg/plate	No clear statement about dose range	Thompson et al. (1983)
Salmonella typhimurium TA102, TA2638	Reverse mutation	–	–	*ortho*-Anisidine, 5000 µg/plate		Watanabe et al. (1996)
Salmonella typhimurium NM2009 (with *O*-acetyltransferase gene)	SOS/*umuC* gene expression (DNA damage)	NR	–	*ortho*-Anisidine, 1000 µg/mL		Oda et al. (1995)
Salmonella typhimurium NM6001 (with *N*-acetyltransferase 1 gene or with *N*-acetyltransferase 2 gene)	SOS/*umuC* gene expression (DNA damage)	NR	+	*ortho*-Anisidine, 100 µM	Dosing not clearly reported	Oda (2004)
Escherichia coli K-12 343	DNA repair	+	–	*ortho*-Anisidine, 94.9 mM [11 687 µg/mL]		Hellmér & Bolcsfoldi (1992a)
Drosophila melanogaster	Sex-linked recessive lethal mutation assay	NA	– –	*ortho*-Anisidine, 500 ppm [500 µg/g], feeding *ortho*-Anisidine, 2000 ppm [2000 µg/mL], injection		Yoon et al. (1985)
Drosophila melanogaster	Interchromosomal mitotic recombination (somatic w/w+ eye assay)	+	NA	*ortho*-Anisidine hydrochloride, 0.5 mM [61.5 µg/mL]		Rodriguez-Arnaiz & Aranda (1994)
Drosophila melanogaster	Interchromosomal mitotic recombination (somatic w/w+ eye assay)	+	NA	*ortho*-Anisidine, 1 mM [123 µg/mL]	Negative results in insecticide-resistant strain	Rodriguez-Arnaiz & Téllez (2002)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Saccharomyces cerevisiae strain RS112	DNA deletion by intrachromosomal recombination (DEL assay)	+	NT	*ortho*-Anisidine, 5 mg/mL		Brennan & Schiestl (1999)
Acellular system, [^{14}C]-labelled *ortho*-anisidine	Calf thymus DNA covalent binding	NA	+	*ortho*-Anisidine, 1 mM		Thompson & Eling (1991)
Acellular system, [^{14}C]-labelled *ortho*-anisidine	Calf thymus DNA covalent binding	NA	+	*ortho*-Anisidine, 1 mM [123 µg/mL]		Stiborová et al. (2002)
Acellular system, ^{32}P-postlabelling	Calf thymus DNA covalent binding	NA	+	*ortho*-Anisidine, 1 mM [123 µg/mL]	Positive when 3× higher amounts of MN/SPD were used	Stiborová et al. (2002)

HIC, highest ineffective concentration; HLI, hamster liver induced S9; LEC, lowest effective concentration; MLI, mouse liver induced S9; MLN, mouse liver S9, not induced; MN, micrococcal nuclease; NA, not applicable; NR, not reported; NT, not tested; RLI, rat liver induced S9; S9, 9000 × *g* supernatant; SPD, spleen phosphodiesterase.

[a] +, positive; –, negative.

(Uno & Omori, 2015). [The Working Group noted that the comet assay resulted in equivocal responses in this study, i.e. positive depending on the choice of statistical evaluation method.] Hobbs et al. (2015) reported that *ortho*-anisidine induced DNA strand breaks in the liver, without a corresponding significant dose–response relationship, but did not induce DNA strand breaks in the stomach of Sprague-Dawley rats. Toyoda et al. (2019) reported that administration of *ortho*-anisidine hydrochloride to Fischer/DuCrl-Crlj rats for 1 month induced DNA damage, as analysed by the quantification of γ-H2AX-positive epithelial cells in the urinary bladder.

ortho-Anisidine or its hydrochloride form did not induce unscheduled DNA synthesis in Fischer 344 or AP rat liver or kidney (Tyson & Mirsalis, 1985; Ashby et al., 1991).

In ddY mice, *ortho*-anisidine induced DNA strand breaks in the colon and urinary bladder at the 3- and 8-hour sampling times and in the urinary bladder at the 24-hour sampling time (Sekihashi et al., 2002). In CD-1 mice, a single dose of *ortho*-anisidine induced DNA damage, assessed by a modified comet assay, in the urinary bladder and colon but not in the stomach, kidney, liver, lung, brain, or bone marrow (Sasaki et al., 1998).

ortho-Anisidine hydrochloride induced gene mutation in the *lacI* transgene; in the Big Blue™ mouse, there was a modest effect in the bladder and no effect in the liver (Ashby et al., 1994). *ortho*-Anisidine did not induce micronucleus formation in AP rat bone marrow or liver (Ashby et al., 1991) or in BDF1 or B6C3F$_1$ mouse bone marrow (Morita et al., 1997; Ashby et al., 1991).

ortho-Anisidine induced DNA repair in *Escherichia coli* in a host-mediated assay in male NMRI mice treated by intraperitoneal administration but not when treated by gavage (Hellmér & Bolcsfoldi, 1992b).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

ortho-Anisidine did not induce unscheduled DNA synthesis in primary cultured rat hepatocytes (Thompson et al., 1983; Yoshimi et al., 1988).

Although DNA strand breaks were observed only in the presence of an exogenous metabolic system (Garberg et al., 1988), *ortho*-anisidine induced gene mutations in mouse lymphoma L5178Y cells in vitro both with and without exogenous metabolic activation (Wangenheim & Bolcsfoldi, 1988). Chromosomal aberrations and sister-chromatid exchange were induced in Chinese hamster ovary cells in vitro both with and without exogenous metabolic activation (Galloway et al., 1987). Structural chromosomal aberrations were observed in Chinese hamster lung cells both with and without exogenous metabolic activation (JETOC, 1997).

(iii) Non-mammalian experimental systems

See Table 4.3.

Three studies were conducted in *Drosophila melanogaster* (Yoon et al., 1985; Rodriguez-Arnaiz & Aranda, 1994; Rodriguez-Arnaiz & Téllez, 2002). *ortho*-Anisidine did not induce sex-linked recessive lethal mutations in *Drosophila* (Yoon et al., 1985), but this compound and its hydrochloride form induced, in a dose-dependent manner, the frequency of light spots in the eyes of an insecticide-sensitive *Drosophila* strain (white/white somatic assay), suggesting loss of heterozygosity by mitotic recombination (Rodriguez-Arnaiz & Aranda, 1994; Rodriguez-Arnaiz & Téllez, 2002).

ortho-Anisidine induced genotoxic effects by increasing recombination frequency, analysed by DEL recombination assay in *Saccharomyces cerevisiae* strain RS112 (Brennan & Schiestl, 1999).

ortho-Anisidine induced reverse mutations in *Salmonella typhimurium* strains TA98, TA100, TA1537, and TA1538, with exogenous metabolic activation (Shimizu & Takemura, 1983; Dunkel et al., 1985; Zeiger et al., 1992). In the presence of exogenous metabolic activation, *ortho*-anisidine

induced reverse mutations in strain YG1029 (but not in strain YG1012, both YG strains having elevated levels of *N*-acetyltransferase) (Thompson et al., 1992). *ortho*-Anisidine or its hydrochloride form did not induce reverse mutation in *E. coli* or in *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, TA1538, TA2638, G46, C3076, D3052, or YG1012 (Ferretti et al., 1977; Garner & Nutman, 1977; Haworth et al., 1983; Thompson et al., 1983, 1992; Dunkel et al., 1985; Zeiger et al., 1992; Watanabe et al., 1996). *ortho*-Anisidine induced dose-dependent expression of the *umuC* gene in *S. typhimurium* overexpressing *N*-acetyltransferase type 1 and *N*-acetyltransferase type 2 (Oda, 2004), but not in *S. typhimurium* overexpressing *O*-acetyltransferase (Oda et al., 1995).

ortho-Anisidine, without exogenous metabolic activation, preferentially killed DNA repair-deficient *E. coli* strains rather than repair-proficient strains (Hellmér & Bolcsfoldi, 1992a).

4.2.3 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Cell proliferation was not induced in the urinary bladder of Wistar rats treated with *ortho*-anisidine hydrochloride (17 mg/kg bw per day, 3 days per week, for 4 weeks) (Iatropoulos et al., 2015). Histopathological evaluation of the bladder of F344/DuCrl-Crlj rats treated with *ortho*-anisidine hydrochloride (1.0% in the feed, for 2 or 4 weeks) showed hyperplasia with an increase in the frequency of cells that tested positive for the cell proliferation marker, Ki67 (Toyoda et al., 2019).

Male and female Fischer 344 rats given feed containing *ortho*-anisidine hydrochloride at a concentration of 5000 or 10 000 mg/kg for up to 103 weeks developed non-neoplastic lesions of the thyroid gland and kidney more frequently than did control animals (NCI, 1978).

In female B6C3F$_1$ mice that received feed containing *ortho*-anisidine hydrochloride at a concentration of 2500 or 5000 mg/kg for up to 103 weeks, the incidence of cystic hyperplasia of the uterine endometrium was higher than in control mice. There was an increased incidence of hyperplasia of the bladder in male and female B6C3F$_1$ mice at 5000 mg/kg relative to controls (NCI, 1978).

4.2.4 Evidence relevant to other key characteristics of carcinogens

(a) Humans

In human liver carcinoma cell lines HepG2 and Huh-7 single cultured and co-cultured with human monocytic THP-1 cells, *ortho*-anisidine induced reactive oxygen species in a concentration-dependent manner, as measured by the 2′,7′-dichlorodihydrofluorescein diacetate assay (Wewering et al., 2017).

(b) Experimental systems

In the absence of exogenous metabolic activation, *ortho*-anisidine hydrochloride at the lowest effective dose (500 µg/mL) induced cell transformation in Syrian hamster embryo cells in vitro (Kerckaert et al., 1998).

Treatment of primary murine hepatocytes with *ortho*-anisidine at 2.5 or 10 mM led to a significant increase in levels of reactive oxygen species after 3 hours of incubation (Wewering et al., 2017). Brennan & Schiestl (1999) reported that the genotoxic effects of *ortho*-anisidine in yeast were reduced in the presence of the free radical scavenger and antioxidant *N*-acetyl cysteine. In addition, comparing yeast strains with different capacities for the detoxification of oxygen radicals, it was shown that a strain with an inactivating disruption in the superoxide dismutase genes *SOD1* and *SOD2* was hypersensitive

to the lethal effects of *ortho*-anisidine, which implies a role of the superoxide anion (O_2^-) in its cytotoxicity (Brennan & Schiestl, 1999). *ortho*-Anisidine also induced the production in yeast of reactive oxygen species, measured by the oxidation of the free radical-sensitive reporter compound 2′,7′-dichlorodihydrofluorescein diacetate (Brennan & Schiestl, 1999).

The *ortho*-anisidine metabolite, *ortho*-aminophenol, induced Cu(II)-dependent DNA damage. This result was achieved using ^{32}P-labelled human DNA fragments (of *c-Ha-RAS* and *TP53)* and calf thymus DNA. In the presence of Cu(II), *ortho*-aminophenol induced formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) in calf thymus DNA (Ohkuma & Kawanishi, 2001).

ortho-Anisidine (1232 µg/mL) inhibited gap-junctional intercellular communication in mouse keratinocytes in the absence of an exogenous metabolic system, which was related to the decreased intensity of immunocytochemical staining for protein connexin 43 on the cell membrane (Jansen et al., 1996), but did not inhibit gap-junctional intercellular communication in Syrian hamster embryo cells exposed to 0.03–10 mM *ortho*-anisidine for up to 24 hours (Rivedal et al., 2000).

4.3 Data relevant to comparisons across agents and end-points

The analysis of the in vitro bioactivity of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). *ortho*-Anisidine was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 26 April 2020. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). [The Working Group noted that the metabolic capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 676 assays in which *ortho*-anisidine (at concentrations up to 100 µM) was tested, it was found to be inactive in almost all assays. Active responses were observed in 18 assays (US EPA, 2019b). Upregulation of the aryl hydrocarbon receptor (AHR) in the hepatocellular carcinoma-derived cell line, HepG2, was reported in two assays at the half-maximal activity concentration (AC_{50}) of 74.5 µM, and with borderline effects at an AC_{50} concentration of 63.5 µM. Inhibition of thyroid peroxidase was reported in two assays at AC_{50} concentrations of 0.318 and 27.8 µM in rat and pig thyroid gland cell lines, respectively. Activation of the pregnane X receptor, PXR (NR1I2), was reported in one assay at an AC_{50} concentration of 60.3 µM in HepG2 cells. Activation of the rat adrenoceptor alpha 2B (Adra2b) was reported in one assay at an AC_{50} concentration of 8.22 µM.

Borderline activity was reported for human retinoid X receptor beta (RXRB), protein tyrosine phosphatase non-receptor type 6 (PTPN6), protein tyrosine phosphatase receptor type C (PTPRC), protein phosphatase 1 catalytic subunit alpha isozyme (PPP1CA), mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2) and sirtuin 1 (SIRT1) at AC_{50} concentrations ranging from 0.164 to 41.3 µM. Mitochondrial depolarization was reported in one assay in HepG2 cells, at an AC_{50} concentration of 32.5 µM.

4.4 Other relevant evidence

ortho-Anisidine induced methaemoglobinaemia in CBA mice and Alpk:APfSD rats treated by oral administration (Ashby et al., 1991).

5. Summary of Data Reported

5.1 Exposure characterization

ortho-Anisidine, the parent compound of *ortho*-anisidine hydrochloride, is a basic compound and will undergo acid–base reactions. *ortho*-Anisidine and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

ortho-anisidine is a substituted aniline compound with the formula $NH_2C_6H_4OCH_3$. It is produced from *ortho*-nitroanisole (see the monograph on *ortho*-nitroanisole in the present volume). It is not a High Production Volume chemical, and its use has been declining in Europe and the USA. Little information is available about production or use in other regions. Its main use is as a chemical intermediate in the synthesis of azo pigments and dyes. These are then used in consumer products, textiles, and for printing paper and cardboard.

The salt, *ortho*-anisidine hydrochloride, is derived from *ortho*-anisidine and is not produced in significant quantities. It is also used as a chemical intermediate for production of dyes and pigments, and has some minor industrial uses.

Exposure to both *ortho*-anisidine and its hydrochloride form may occur through inhalation, ingestion, and skin and eye contact. Time-weighted average occupational exposure limits for *ortho*-anisidine have been established in a number of countries. Sparse measurements in production plants have shown exposure near the exposure limit values, with measured exposures in pigment plants being much lower.

ortho-Anisidine has been detected during product testing of textiles and consumer products and has also been found in tattoo inks and cigarette smoke, and in urine samples from the general population.

5.2 Cancer in humans

The research available related to cancers in humans was limited to one case series of bladder cancer occurring in plants producing organic dye and pigment intermediates. All 3 cases exposed to *ortho*-anisidine were co-exposed to other known bladder carcinogens (*ortho*-toluidine, tobacco smoking).

The available study did not permit a conclusion to be drawn about the presence of a causal association between *ortho*-anisidine and urinary bladder cancer.

5.3 Cancer in experimental animals

ortho-Anisidine hydrochloride caused an increase in the incidence of malignant neoplasms in two species.

In $B6C3F_1$ mice, *ortho*-anisidine hydrochloride administered orally (in feed) in one study caused an increase in the incidence of transitional cell carcinoma of the urinary bladder in males and females.

In Fischer 344 rats, *ortho*-anisidine hydrochloride administered orally (in feed) in one study caused an increase in the incidence of transitional cell carcinoma of the urinary bladder in males and females, and of transitional cell carcinoma of the renal pelvis in males. In addition, *ortho*-anisidine hydrochloride caused an increase in the incidence of a combination of benign and malignant neoplasms (follicular cell carcinoma, adenoma, cystadenoma, papillary cystadenoma, or papillary cystadenocarcinoma, combined) of the thyroid in male rats.

5.4 Mechanistic evidence

No studies characterizing the absorption, distribution, metabolism, or excretion of *ortho*-anisidine or *ortho*-anisidine hydrochloride in humans were available. In two studies of people without known exposure, *ortho*-anisidine

was detected in the urine. Studies using human, rabbit, or rat hepatic microsomes reported cytochrome P450-dependent oxidation of *ortho*-anisidine to its major metabolite, *N*-(2-methoxyphenyl)hydroxylamine. This *N*-hydroxy compound can be further oxidized to *ortho*-nitrosoanisole or reduced back to *ortho*-anisidine. *ortho*-Anisidine can also be metabolized to *ortho*-aminophenol via demethylation. Studies in vitro using mammalian peroxidases showed cytochrome P450-dependent formation of electrophilic species, diimine and quinone imine. In male IMP:WIST rats with intraperitoneal exposure, *ortho*-anisidine was readily absorbed, widely distributed to tissues, and excreted primarily via the urine as *N*-acetyl-2-methoxyaniline.

There is consistent and coherent evidence that *ortho*-anisidine or its hydrochloride form exhibit key characteristics of carcinogens in experimental systems. *ortho*-Anisidine is metabolically activated to electrophiles. Haemoglobin adducts have been detected in blood samples from exposed humans in two studies. No data on DNA adducts in exposed humans were available. In studies in vitro, *ortho*-anisidine is activated by human hepatic microsomes to form DNA adducts. The major adduct formed was *N*-(deoxyguanosin-8-yl)-2-methoxyaniline. In experimental systems, *ortho*-anisidine DNA adducts were observed in several tissues of rats, with the highest level found in the urinary bladder. After peroxidation to diimine and quinone imine, *ortho*-anisidine binds to calf thymus DNA, in the presence of mammalian microsomes. *ortho*-Anisidine is genotoxic, based on the results of multiple studies demonstrating the formation of DNA strand breaks in the bladder of rodents, and mutagenic in base-pair substitution strains of bacteria, both in the presence and in the absence of exogenous metabolic activation.

ortho-Anisidine hydrochloride alters cell proliferation, cell death, or nutrient supply. *ortho*-Anisidine hydrochloride induced hyperplasia in the bladder of rodents – in male F344/DuCrl-Crlj rats after short-term exposure, and in male and female B6C3F$_1$ mice after chronic exposure. It also induced cystic hyperplasia of the uterine endometrium of female B6C3F$_1$ mice.

ortho-Anisidine was mostly without effects in the assay battery of the Tox21 and ToxCast research programmes.

Overall, the evidence is consistent and coherent that *ortho*-anisidine belongs, on the basis of mechanistic considerations, to a class of aromatic amines. Members of this class, including 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine have been classified previously by the *IARC Monographs* programme as *carcinogenic to humans* (IARC Group 1). *ortho*-Anisidine is structurally similar to these aromatic amines. *ortho*-Anisidine is also similar to these aromatic amines with respect to its mechanism of bioactivation to electrophiles, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays. The urinary bladder is a common target organ of carcinogenicity for these aromatic amines in experimental animals. For instance, *ortho*-anisidine causes malignant tumours of the urinary bladder when administered orally to rats, as do *ortho*-toluidine and 2-naphthylamine. 4-Aminobiphenyl causes malignant tumours of the urinary bladder when administered orally to dogs and mice. Therefore, these mechanistic considerations go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of *ortho*-anisidine and *ortho*-anisidine hydrochloride.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-anisidine hydrochloride.

6.3 Mechanistic evidence

There is *strong evidence* that *ortho*-anisidine belongs, based on mechanistic considerations, to a class of aromatic amines for which several members have been classified as carcinogenic to humans. There is also *strong evidence* that *ortho*-anisidine exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

ortho-Anisidine and *ortho*-anisidine hydrochloride are *probably carcinogenic to humans (Group 2A)*.

6.5 Rationale

The Group 2A evaluation is based on *strong* mechanistic evidence that *ortho*-anisidine, on the basis of mechanistic considerations, belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans. *ortho*-Anisidine bears structural similarity to other members of this class, and there is close concordance with respect to the bioactivation mechanism to DNA-reactive moieties, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays.

There is also *sufficient evidence of carcinogenicity* in experimental animals on the basis of increased incidence of malignant neoplasms in two species.

In addition, there is *strong evidence* that *ortho*-anisidine exhibits key characteristics of carcinogens in experimental systems. *ortho*-Anisidine is metabolically activated to electrophiles, it is genotoxic, and it alters cell proliferation, cell death, or nutrient supply.

The evidence for cancer in humans is *inadequate* as the only data available were from a single case series of bladder cancer in workers who were co-exposed to other bladder carcinogens (*ortho*-toluidine, tobacco smoking).

ortho-Anisidine hydrochloride is in equilibrium with *ortho*-anisidine; therefore, the classification of carcinogenic hazard applies to both *ortho*-anisidine and its hydrochloride form.

References

Ashby J, Lefevre PA, Tinwell H, Brunborg G, Schmezer P, Pool-Zobel B, et al. (1991). The non-genotoxicity to rodents of the potent rodent bladder carcinogens *o*-anisidine and *p*-cresidine. *Mutat Res*. 250(1–2):115–33. doi:10.1016/0027-5107(91)90168-N PMID:1719389

Ashby J, Short JM, Jones NJ, Lefevre PA, Provost GS, Rogers BJ, et al. (1994). Mutagenicity of *o*-anisidine to the bladder of lacI⁻ transgenic B6C3F$_1$ mice: absence of ^{14}C or ^{32}P bladder DNA adduction. *Carcinogenesis*. 15(10):2291–6. doi:10.1093/carcin/15.10.2291 PMID: 7955069

Branner B, Kutzer C, Zwickenpflug W, Scherer G, Heller W-D, Richter E (1998). Haemoglobin adducts from aromatic amines and tobacco specific nitrosamines in pregnant smoking and non smoking women. *Biomarkers*. 3(1):35–47. doi:10.1080/135475098231354 PMID:23899255

Brennan RJ, Schiestl RH (1999). The aromatic amine carcinogens *o*-toluidine and *o*-anisidine induce free radicals and intrachromosomal recombination in *Saccharomyces cerevisiae*. *Mutat Res*. 430(1):37–45. doi:10.1016/S0027-5107(99)00118-9 PMID:10592316

CDC (2011). National Occupational Exposure Survey (NOES) 1981–83. United States Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. Available from: https://web.archive.org/web/20111026200522/http://www.cdc.gov/noes/noes1/x8302sic.html, accessed 16 November 2020.

Chaudhari S, Mandale AB, Patil KR, Sainkar SR, Patil PP (2006). Formation of poly(*o*-anisidine) coatings on copper from aqueous salicylate solution. *Surf Coat Tech*. 200(18–19):5557–65. doi:10.1016/j.surfcoat.2005.07.071

Chemical Information Services (1995). Directory of World Chemical Producers. 1995/96 standard edition, Dallas (TX), USA: Chemical Information Services, Inc; p. 66.

ChemNet (2020). *o*-Anisidine, suppliers. Global Chemical Network. Available from: http://www.chemnet.com/Japan/Products/c7h9no%20oanisidine/Suppliers-0-0.html, accessed 16 November 2020.

Danish Environmental Protection Agency (2012). Chemical substances in tattoo ink. Survey of chemical substances in consumer products no. 116, Denmark. Odense, Denmark: Danish Environmental Protection Agency. Available from: https://www2.mst.dk/Udgiv/publications/2012/03/978-87-92779-87-8.pdf.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. (1985). Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen*. 7(S5):1–248. doi:10.1002/em.2860070902 PMID:3905369

ECHA (2011). Annex XV dossier. Proposal for identification of a substance as a category 1A or 1B, CMR, PBT, vPvB or a substance of an equivalent level of concern. *o*-Anisidine. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/documents/10162/8679db03-ce69-47a7-b57b-b39cfc2237a6, accessed 16 November 2020.

ECHA (2020). *o*-Anisidine. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/substance-information/-/substanceinfo/100.001.785, accessed 14 April 2021.

European Commission (2011). Recommendation from the Scientific Committee on Occupational Exposure Limits on Occupational Exposure Limits for *o*-anisidine. SCOEL/ SUM/144, July 2011. Scientific Committee on Occupational Exposure Limits. Available from: http://ec.europa.eu/social/BlobServlet?docId=7422&langId=en, accessed 16 November 2020.

European Chemicals Bureau (2002). European Union risk assessment report. o-Anisidine. CAS No. 90-04-0, EINECS No. 201-963-1. Luxembourg: Office for Official Publications of the European Communities. Available from: https://publications.europa.eu/en/publication-detail/-/publication/03c04c2b-a7e6-11e7-837e-01aa75ed71a1/language-en/format-PDF/source-105828224, accessed 16 November 2020.

Ferretti JJ, Lu W, Liu MB (1977). Mutagenicity of benzidine and related compounds employed in the detection of hemoglobin. *Am J Clin Pathol*. 67(6):526–7. doi:10.1093/ajcp/67.6.526 PMID:326025

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*. 10(S10):1–175. doi:10.1002/em.2850100502 PMID:3319609

Garberg P, Akerblom EL, Bolcsfoldi G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. *Mutat Res*. 203(3):155–76. doi:10.1016/0165-1161(88)90101-X PMID:2836728

Garner RC, Nutman CA (1977). Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA1538. *Mutat Res*. 44(1):9–19. doi:10.1016/0027-5107(77)90110-5 PMID:331098

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*. 5(S1):3–142. doi:10.1002/em.2860050703 PMID:6365529

Health Canada (2009). Aromatic amines: contribution to the mutagenic activity of tobacco smoke. Available from: https://www.canada.ca/content/dam/canada/health-canada/migration/healthy-canadians/publications/healthy-living-vie-saine/aromatic-amines-aromatiques/alt/aromatic-amines-aromatiques-eng.pdf, accessed 16 November 2020.

Hellmér L, Bolcsfoldi G (1992a). An evaluation of the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. *Mutat Res*. 272(2):145–60. doi:10.1016/0165-1161(92)90043-L

Hellmér L, Bolcsfoldi G (1992b). An evaluation of the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated assay. II. In vivo results for 36 compounds tested in the mouse. *Mutat Res*. 272(2):161–73. doi:10.1016/0165-1161(92)90044-M PMID:1383748

Hobbs CA, Recio L, Streicker M, Boyle MH, Tanaka J, Shiga A, et al. (2015). Comet assay evaluation of six chemicals of known genotoxic potential in rats. *Mutat Res Genet Toxicol Environ Mutagen*. 786–788:172–81. doi:10.1016/j.mrgentox.2015.03.003 PMID:26212309

HSE (2020). EH40/2005 workplace exposure limits. Containing the list of workplace exposure limits for use with the Control of Substances Hazardous to Health Regulations 2002 (as amended). London, England: The Stationery Office, Health and Safety Executive. TSO EH40/2005. 4th ed. Available from: https://www.hse.gov.uk/pubns/priced/eh40.pdf.

IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. *IARC Monogr Eval Carcinog Risks Hum*, 73:1–674. Available from: https://publications.iarc.fr/91.

IARC (2010). Some aromatic amines, organic dyes, and related exposures. *IARC Monogr Eval Carcinog Risks Hum*, 99:1–658. Available from: https://publications.iarc.fr/117 PMID:21528837

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: https://publications.iarc.fr/123 PMID:23189753

Iatropoulos MJ, Duan J-D, Schmuck G, Williams GM (2015). The urinary bladder carcinogen propoxur does not produce genotoxic effects in the urinary bladder of Wistar male rats. *Exp Toxicol Pathol*. 67(9):453–8. doi:10.1016/j.etp.2015.06.002 PMID:26164753

IFA (2019). 2-Methoxyaniline. GESTIS substance database. Germany: Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance). Available from: https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp, accessed 2 March 2020.

Jansen L, Mesnil M, Koeman J, Jongen W (1996). Tumor promoters induce inhibition of gap junctional intercellular communication in mouse epidermal cells by affecting the localization of connexin43 and E-cadherin. *Environ Toxicol Pharmacol*. 1(3):185–92. doi:10.1016/1382-6689(96)00005-1 PMID:21781680

JETOC (1997). *o*-Methoxyaniline. mutagenicity test data of existing chemical substances based on the Toxicity Investigation System of the Industrial Safety and Health law. Judgement for chromosomal aberration in CHL; positive. Tokyo, Japan: Chemical Industry Ecology-Toxicology and Information Center. Available from: https://anzeninfo.mhlw.go.jp/user/anzen/kag/pdf/C/C90-04-0.pdf, accessed May 2020.

JRC (2017). Safety of tattoos and permanent make-up. Final Report. JRC science for policy report. Joint Research Centre, European Commission. Available from: https://op.europa.eu/en/publication-detail/-/publication/e08d57a8-4fd0-11e6-89bd-01aa75ed71a1/language-en, accessed 16 November 2020.

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, et al. (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*. 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kerckaert GA, LeBoeuf RA, Isfort RJ (1998). Assessing the predictiveness of the Syrian hamster embryo cell transformation assay for determining the rodent carcinogenic potential of single ring aromatic/nitroaromatic amine compounds. *Toxicol Sci*. 41(2):189–97. doi:10.1093/toxsci/41.2.189 PMID:9520355

Kütting B, Göen T, Schwegler U, Fromme H, Uter W, Angerer J, et al. (2009). Monoarylamines in the general population–a cross-sectional population-based study including 1004 Bavarian subjects. *Int J Hyg Environ Health*. 212(3):298–309. doi:10.1016/j.ijheh.2008.07.004 PMID:18789761

Mazumder S, Ahamed RA, McGahee E, Wang L, Seyler TH (2019). A new automated method for the analysis of aromatic amines in human urine by GC-MS/MS. *J Anal Toxicol*. 43(1):25–35. doi:10.1093/jat/bky045 PMID:30010885

Merck (2020). 2-Methoxyaniline hydrochloride. Available from: https://www.sigmaaldrich.com/catalog/product/aldrich/cds000192?lang=fr&region=FR.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, et al. (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. *Mutat Res*. 389(1):3–122. doi:10.1016/S1383-5718(96)00070-8 PMID:9062586

Naiman K, Dračínská H, Dračínský M, Martínková M, Martínek V, Hodek P, et al. (2008a). CYP-mediated metabolism of *N*-(2-methoxyphenyl)-hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Interdiscip Toxicol*. 1(3–4):218–24. doi:10.2478/v10102-010-0045-8 PMID:21218119

Naiman K, Dracínská H, Martínková M, Sulc M, Dracínský M, Kejíková L, et al. (2008b). Redox cycling in the metabolism of the environmental pollutant and suspected human carcinogen *o*-anisidine by rat and rabbit hepatic microsomes. *Chem Res Toxicol*. 21(8):1610–21. doi:10.1021/tx8001127 PMID:18624415

Naiman K, Dracínský M, Hodek P, Martínková M, Schmeiser HH, Frei E, et al. (2012). Formation, persistence, and identification of DNA adducts formed by the carcinogenic environmental pollutant *o*-anisidine in rats. *Toxicol Sci*. 127(2):348–59. doi:10.1093/toxsci/kfs104 PMID:22403159

Naiman K, Frei E, Stiborová M (2010). Identification of rat cytochromes P450 metabolizing *N*-(2-methoxyphenyl) hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Neuro Endocrinol Lett*. 31(Suppl 2):36–45. PMID:21187827

Naiman K, Martínková M, Schmeiser HH, Frei E, Stiborová M (2011). Human cytochrome-P450 enzymes metabolize *N*-(2-methoxyphenyl)hydroxylamine, a metabolite of the carcinogens *o*-anisidine and *o*-nitroanisole, thereby dictating its genotoxicity. *Mutat Res*. 726(2):160–8. doi:10.1016/j.mrgentox.2011.09.010 PMID:21946300

Nakano M, Omae K, Takebayashi T, Tanaka S, Koda S (2018). An epidemic of bladder cancer: ten cases of bladder cancer in male Japanese workers exposed to *ortho*-toluidine. *J Occup Health*. 60(4):307–11. doi:10.1539/joh.2017-0220-OA PMID:29743389

Nandosal Chem Industries (2020). ortho Anisidine. Available from: http://www.nandosal.com/ortho-anisidine.html, accessed 18 January 2021.

NCI (1978). Bioassay of *o*-anisidine hydrochloride for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser*. 89:1–149. PMID:12806402

NCBI (2020a). *o*-Anisidine. PubChem compound summary for CID 7000. Bethesda (MD), USA: National Center for Biotechnology Information. United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/o-anisidine, accessed 1 October 2020.

NCBI (2020b). *o*-Anisidine hydrochloride. PubChem compound summary for CID 8638. Bethesda (MD), USA: National Center for Biotechnology Information. United States National Library of Medicine.

Available from: https://pubchem.ncbi.nlm.nih.gov/compound/8638, accessed 1 October 2020.

NEMI (1998). Semivolatile organic compounds by GC/MS. Method EPA-RCA: 8270D. Revision 4. National Environmental Methods Index. Available from: https://www.nemi.gov/methods/method_summary/4726/, accessed 16 November 2020.

NICNAS (2014). Benzenamine, 2-methoxy-: Human health tier II assessment CAS Number: 90-04-0. Inventory Multi-tiered Assessment and Prioritisation. Sydney, Australia: Australian Government, National Industrial Chemicals Notifications and Assessment Scheme. Available from: https://www.industrialchemicals.gov.au/sites/default/files/Benzenamine%2C%202-methoxy-_Human%20health%20tier%20II%20assessment.pdf, accessed 18 January 2021.

NIOSH (1994). *o*-Anisidine. Immediately Dangerous to Life or Health concentrations (IDLH). National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/idlh/90040.html, accessed 16 November 2020.

NIOSH (2016). Anisidine. NMAM method 2514. Issue 3. 26 February 2016. NIOSH Manual of Analytical Methods, 5th ed. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/docs/2014-151/pdfs/methods/2514.pdf, accessed 16 November 2020.

NIOSH (2018). *o*-Anisidine. NIOSH pocket guide to chemical hazard. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/npg/npgd0034.html, accessed 16 November 2020

NTP (2016). *o*-Anisidine and its hydrochloride. 14th Report on carcinogens. National Toxicology Program, United States Department of Health and Human Services. Available from: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/anisidine.pdf, accessed 16 November 2020.

Oda Y (2004). Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/*umu* assay system. *Mutat Res*. 554(1–2):399–406. doi:10.1016/j.mrfmmm.2004.06.033 PMID:15450435

Oda Y, Yamazaki H, Watanabe M, Nohmi T, Shimada T (1995). Development of high sensitive *umu* test system: rapid detection of genotoxicity of promutagenic aromatic amines by *Salmonella typhimurium* strain NM2009 possessing high *O*-acetyltransferase activity. *Mutat Res*. 334(2):145–56. doi:10.1016/0165-1161(95)90005-5 PMID:7885366

OEHHA (1992). *o*-Anisidine hydrochloride. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Available from: https://oehha.ca.gov/chemicals/o-anisidine-hydrochloride.

Ohkuma Y, Kawanishi S (2001). Oxidative DNA damage induced by a metabolite of carcinogenic *o*-anisidine: enhancement of DNA damage and alteration in its sequence specificity by superoxide dismutase. *Arch Biochem Biophys*. 389(1):49–56. doi:10.1006/abbi.2001.2302 PMID:11370671

Ono S, Kurata Y, Shichino Y, Sano M, Fukushima S (1992). Synergism of environmental carcinogens and promoters on bladder cancer development initiated by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in F344 rats. *Jpn J Cancer Res*. 83(9):955–63. doi:10.1111/j.1349-7006.1992.tb02007.x PMID:1429206

OSHA (1990). Regulations (Standards 29 CFR). Washington (DC), USA: Occupational Safety and Health Administration. Available from: https://www.osha.gov/laws-regs/regulations/standardnumber/1990, accessed 16 November 2020.

OSHA (2019). Anisidine (*o*-, p-isomers). OSHA Occupational Chemical Database. Last updated, 19 February 2019. Washington (DC), USA: Occupational Safety and Health Administration, United States Department of Labor. Available from: https://www.osha.gov/chemicaldata/chemResult.html?recNo=732, accessed 5 June 2020.

Qi Y, Toyooka T, Nie J, Ohta H, Koda S, Wang R-S (2020). Comparative γ-H2AX analysis for assessment of the genotoxicity of six aromatic amines implicated in bladder cancer in human urothelial cell line. *Toxicol In Vitro*. 66:104880. doi:10.1016/j.tiv.2020.104880 PMID:32387221

Richter E, Rösler S, Scherer G, Gostomzyk JG, Grübl A, Krämer U, et al. (2001). Haemoglobin adducts from aromatic amines in children in relation to area of residence and exposure to environmental tobacco smoke. *Int Arch Occup Environ Health*. 74(6):421–8. doi:10.1007/s004200100243 PMID:11563605

Rivedal E, Mikalsen SO, Sanner T (2000). Morphological transformation and effect on gap junction intercellular communication in Syrian hamster embryo cells as screening tests for carcinogens devoid of mutagenic activity. *Toxicol In Vitro*. 14(2):185–92. doi:10.1016/S0887-2333(00)00006-0 PMID:10793297

Rodriguez-Arnaiz R, Aranda JH (1994). Activity of aromatic amines in the eye: w/w+ somatic assay of *Drosophila melanogaster*. *Environ Mol Mutagen*. 24(1):75–9. doi:10.1002/em.2850240110 PMID:8050419

Rodriguez-Arnaiz R, Téllez GO (2002). Structure-activity relationships of several anisidine and dibenzanthracene isomers in the w/w+ somatic assay of *Drosophila melanogaster*. *Mutat Res*. 514(1–2):193–200. doi:10.1016/S1383-5718(01)00339-4 PMID:11815257

Rýdlová H, Miksanová M, Ryslavá H, Stiborová M (2005). Carcinogenic pollutants *o*-nitroanisole and *o*-anisidine are substrates and inducers of cytochromes P450. *Biomed Pap Med Fac Univ Palacky Olomouc*

Czech Repub. 149(2):441–7. doi:10.5507/bp.2005.077 PMID:16601807

Saalo A, Soosaar A, Mikkola J (2016). ASA 2014. Syöpäsairauden vaaraa aiheuttaville aineille ja menetelmille ammatissaan altistuneiksi ilmoitetut Suomessa. Helsinki, Finland: Työterveyslaitos. Available from: http://www.julkari.fi/bitstream/handle/10024/131073/ASA_2014.pdf?sequence=1&isAllowed=y, accessed 16 November 2020. [Finnish]

Sapota A, Czerski B, Jedrzejczak M (2003). Tissue distribution, excretion and metabolism of *o*-anisidine in rats. *Int J Occup Med Environ Health.* 16(4):351–7. PMID:14964645

Sasaki YF, Nishidate E, Su YQ, Matsusaka N, Tsuda S, Susa N, et al. (1998). Organ-specific genotoxicity of the potent rodent bladder carcinogens *o*-anisidine and *p*-cresidine. *Mutat Res.* 412(2):155–60. doi:10.1016/S1383-5718(97)00183-6 PMID:9539969

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, et al. (2002). Comparative investigation of multiple organs of mice and rats in the comet assay. *Mutat Res.* 517(1–2):53–75. doi:10.1016/S1383-5718(02)00034-7 PMID:12034309

Shimizu H, Takemura N (1983). Mutagenicity of some aniline derivatives. Proceedings of the 11th International Congress on Occupational Health in the Chemical Industry, 26–29 September 1983, Calgary, Alberta, Canada; 497–506.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect.* 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Stabbert R, Schäfer KH, Biefel C, Rustemeier K (2003). Analysis of aromatic amines in cigarette smoke. *Rapid Commun Mass Spectrom.* 17(18):2125–32. doi:10.1002/rcm.1161 PMID:12955743

Stiborová M, Miksanová M, Havlícek V, Schmeiser HH, Frei E (2002). Mechanism of peroxidase-mediated oxidation of carcinogenic *o*-anisidine and its binding to DNA. *Mutat Res.* 500(1–2):49–66. doi:10.1016/S0027-5107(01)00295-0 PMID:11890934

Stiborová M, Miksanová M, Sulc M, Rýdlová H, Schmeiser HH, Frei E (2005). Identification of a genotoxic mechanism for the carcinogenicity of the environmental pollutant and suspected human carcinogen *o*-anisidine. *Int J Cancer.* 116(5):667–78. doi:10.1002/ijc.21122 PMID:15828049

Stiborová M, Schmeiser HH, Breuer A, Frei E (2001). Evidence for activation of carcinogenic *o*-anisidine by prostaglandin H synthase: ^{32}P-postlabelling analysis of DNA adduct formation. *Gen Physiol Biophys.* 20(3):267–79. PMID:11765217

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: a strategic and operational plan for continued leadership. *ALTEX.* 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

Thompson CZ, Hill LE, Epp JK, Probst GS (1983). The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. *Environ Mutagen.* 5(6):803–11. doi:10.1002/em.2860050605 PMID:6653503

Thompson DC, Eling TE (1991). Reactive intermediates formed during the peroxidative oxidation of anisidine isomers. *Chem Res Toxicol.* 4(4):474–81. doi:10.1021/tx00022a012 PMID:1912336

Thompson DC, Josephy PD, Chu JWK, Eling TE (1992). Enhanced mutagenicity of anisidine isomers in bacterial strains containing elevated *N*-acetyltransferase activity. *Mutat Res.* 279(2):83–9. doi:10.1016/0165-1218(92)90249-Y PMID:1375342

Toyoda T, Matsushita K, Morikawa T, Yamada T, Miyoshi N, Ogawa K (2019). Distinct differences in the mechanisms of mucosal damage and γ-H2AX formation in the rat urinary bladder treated with *o*-toluidine and *o*-anisidine. *Arch Toxicol.* 93(3):753–62. doi:10.1007/s00204-019-02396-8 PMID:30656379

Tyson CK, Mirsalis JC (1985). Measurement of unscheduled DNA synthesis in rat kidney cells following in vivo treatment with genotoxic agents. *Environ Mutagen.* 7(6):889–99. doi:10.1002/em.2860070610 PMID:4065062

Uno Y, Omori T (2015). Re-analysis results using medians of the data from the JaCVAM-organized international validation study of the in vivo rat alkaline comet assay. *Mutat Res Genet Toxicol Environ Mutagen.* 786-788:182–7. doi:10.1016/j.mrgentox.2015.06.005 PMID:26212310

US EPA (2003). Hazardous waste management system; Identification and listing of hazardous waste; Dyes and/or pigments production wastes; Land disposal restrictions for newly identified wastes; CERCLA Hazardous Substance Designation and Reportable Quantities; Designation of five chemicals as Appendix VIII Constituents; Addition of five chemicals to the Treatment Standards of F039 and the Universal Treatment Standards. *Fed Regist.* 68(227):66164–230.

US EPA (2016). Benzenamine, 2-methoxy-. Chemical Data Reporting (CDR). ChemView [online database]. Washington (DC), USA: United States Environmental Protection Agency. Available from: https://chemview.epa.gov/chemview/?tf=0&ch=90-04-0&su=2-5-6-7-37574985&ma=4-11-1981377&tds=0&tdl=10&tas1=1&tas2=asc&tas3=undefined&tss=&modal=template&modalId=90250&modalSrc=5-6-4-11-1981377&modalDetailId=&modalCdr=90250, accessed January 2020.

US EPA (2019a). List of lists. Consolidated list of chemicals subject to the Emergency Planning and Community Right To-Know Act (EPCRA), Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and Section 112(r) of the Clean Air Act. EPA 550-B-19-003m. Washington (DC), USA: United States Environmental Protection Agency, Office of Land and Emergency Management. Available from: https://www.epa.gov/sites/production/files/2015-03/documents/list_of_lists.pdf, accessed 17 November 2020.

US EPA (2019b). 2-Anisidine. DSSTox Substance. Distributed Structure-Searchable Toxicity (DSSTox) database. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=o-anisidine, accessed 5 May 2020.

US EPA (2020). *O*-Anisidine. Releases: trends report. TRI Explorer. Toxics Release Inventory program. 2018 National Analysis Data set released 12 November 2019. United States Environmental Protection Agency. Available from: https://enviro.epa.gov/triexplorer/release_trends?p_view=USYR&trilib=TRIQ1&sort=_VIEW_&sort_fmt=1&state=All+states&county=All+counties&chemical=000090040&core_year=&industry=ALL&year=All+years&tab_rpt=1&fld=AIRLBY&fld=E1&fld=E2&fld=E3&fld=E4&fld=E41&fld=E42&fld=E5&fld=E52&fld=E53&fld=E53A&fld=E53B&fld=E54&fld=E51&fld=E51A&fld=E51B&fld=TSFDSP&fld=TSFDSP&fld=m10&fld=m41&fld=m62&fld=potwmetl&fld=m71&fld=m81&fld=m82&fld=m72&fld=m63&fld=m64&fld=m65&fld=m66&fld=m67&fld=m73&fld=m79&fld=m90&fld=m94&fld=m99&fld=RELLBY, accessed 17 November 2020.

US EPA (2021). 2-Anisidine. Chemical activity summary. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID5023877#bioactivity, accessed 18 January 2021.

Wada K, Ohnuma A, Kojima S, Yoshida T, Matsumoto K (2012). A comparison of cell-collecting methods for the comet assay in urinary bladders of rats. *Mutat Res.* 742(1–2):26–30. doi:10.1016/j.mrgentox.2011.11.008 PMID:22155339

Wangenheim J, Bolcsfoldi G (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis.* 3(3):193–205. doi:10.1093/mutage/3.3.193 PMID:3045481

Watanabe K, Sakamoto K, Sasaki T (1996). Comparisons on chemically-induced mutagenicity among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 *uvrA*/pKM101: collaborative study I. *Mutat Res.* 361(2–3):143–55. doi:10.1016/S0165-1161(96)90249-6 PMID:8980700

Weiss T, Angerer J (2002). Simultaneous determination of various aromatic amines and metabolites of aromatic nitro compounds in urine for low level exposure using gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 778(1–2):179–92. doi:10.1016/S0378-4347(01)00542-4 PMID:12376125

Wewering F, Jouy F, Caliskan S, Kalkhof S, von Bergen M, Luch A, et al. (2017). Hepatic co-cultures in vitro reveal suitable to detect Nrf2-mediated oxidative stress responses on the bladder carcinogen *o*-anisidine. *Toxicol In Vitro.* 40:153–60. doi:10.1016/j.tiv.2017.01.006 PMID:28089782

Yoon JS, Mason JM, Valencia R, Woodruff RC, Zimmering S (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen.* 7(3):349–67. doi:10.1002/em.2860070310 PMID:3930235

Yoshimi N, Sugie S, Iwata H, Niwa K, Mori H, Hashida C, et al. (1988). The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. *Mutat Res.* 206(2):183–91. doi:10.1016/0165-1218(88)90159-0 PMID:3173389

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ Mol Mutagen.* 19(S21):2–141. doi:10.1002/em.2850190603 PMID:1541260

ORTHO-NITROANISOLE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

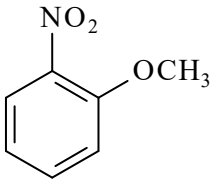
Chem. Abstr. Serv. Reg. No.: 91-23-6

EC No.: 202-052-1

IUPAC systematic name: 1-methoxy-2-nitrobenzene

Synonyms and abbreviations: 2-methoxy-nitrobenzene; 2-methoxy-l-nitrobenzene; *ortho*-nitroanisole; 2-nitroanisole; *ortho*-nitrobenzene methyl ether; 2-nitromethoxybenzene; *ortho*-nitromethoxybenzene; 1-nitro-2-methoxybenzene; *ortho*-nitrophenyl methyl ether.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_7H_7NO_3$

Relative molecular mass: 153.14 (NCBI, 2020)

1.1.3 Chemical and physical properties of the pure substance

Description: *ortho*-nitroanisole is a colourless to yellow-red liquid (NCBI, 2020)

Boiling point: 277 °C (NCBI, 2020)

Melting point: 10.5 °C (NCBI, 2020)

Flash point: 142 °C, closed cup (NCBI, 2020)

Density: 1.254 g/cm^3 at 20 °C (NCBI, 2020)

Vapour density: 5.29 (air = 1) (NCBI, 2020)

Stability and reactivity: stable under normal temperatures and pressures; explosively reactive with sodium hydroxide and zinc (NTP, 2016)

Vapour pressure: 0.004 kPa at 30 °C (ILO, 2017); 3.6×10^{-3} mm Hg at 25 °C (NCBI, 2020)

Water solubility: none at 20 °C; 1.69 g/L at 30 °C. Miscible with ethanol, ethyl ether; soluble in carbon tetrachloride (NCBI, 2020)

Octanol/water partition coefficient (P): log K_{ow}, 1.73 (NCBI, 2020)

Conversion factor: 1 ppm = 6.26 mg/m^3 (IARC, 1996) [calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

1.1.4 Technical grade and impurities

ortho-Nitroanisole is commercially available, with a purity ranging from 98% to 99% (IARC, 1996).

1.2 Production and use

1.2.1 Production process

ortho-Nitroanisole is prepared by slowly adding methanolic sodium hydroxide to a solution of 2-chloronitrobenzene in methanol at 70 °C and then gradually heating the mixture under pressure to 95 °C. After dilution with water, the product is separated as an oil, at a 90% yield; methanol can be recovered from the aqueous layer (Booth, 1991; Lewis, 1993). This process has been optimized by applying pressure earlier and keeping the temperature at 30–60 °C, before filtering and then washing with methanol. The final step is distillation (Xia et al., 2010).

1.2.2 Production volume

ortho-Nitroanisole is produced by several manufacturers in China and in India (HSDB, 2011; Aarti Industries, 2020; LookChem, 2020). In 2020, *ortho*-nitroanisole was available from 28 suppliers worldwide, including 4 in the USA and 18 in China (Chemical Register, 2020). USA imports of *ortho*-nitroanisole totalled more than 319 000 kg in 1976 and 246 000 kg in 1978 (HSDB, 2011). No more recent data on USA imports or exports of *ortho*-nitroanisole were available to the Working Group.

1.2.3 Uses

ortho-Nitroanisole is used primarily as a precursor for *ortho*-anisidine [see the monograph on *ortho*-anisidine in the present volume for a description of its uses].

1.3 Measurement and analysis

Several analytical methods are available. Gas chromatography-mass spectrometry for *ortho*-nitroanisole in cosmetics was reported with a detection limit of 25.4 ng/g (Huang et al., 2017). Sludge samples were analysed for *ortho*-nitroanisole content using both gas chromatography and liquid chromatography coupled with mass spectrometry (Liang et al., 2019). Several studies reported the use of high-performance liquid chromatography coupled to detection by ultraviolet light (HPLC-UV) for determination of *ortho*-nitroanisole in studies of metabolism in vitro conducted using human, rat, rabbit, and porcine hepatic microsomes and cytosol (Mikšanová et al., 2004a, b; Dracínska et al., 2006).

1.4 Occurrence and exposure

ortho-Nitroanisole is not known to occur naturally.

1.4.1 Environmental occurrence

ortho-Nitroanisole can be released into the environment by dye and pharmaceutical manufacturing facilities through various waste streams. Airborne *ortho*-nitroanisole will remain in the vapour phase and will be degraded by reactions with photochemically produced hydroxyl radicals, with an estimated half-life of 109 hours (NTP, 2016).

When released to water, *ortho*-nitroanisole may adsorb to sediments and suspended solids. Volatilization is very slow, with a half-life of 105 days in a model river and 772 days in a model pond (NTP, 2016). *ortho*-Nitroanisole has been detected in water samples in Japan (0.7 µg/L) and from the Rhine river in the Netherlands (0.3–1.0 µg/L) in 1978–1983. Water samples from the Rhine river in Germany were obtained in 1983–1984 and contained concentrations of all nitroanisole isomers combined ranging from 0.1 to 0.9 µg/L (BUA, 1987). *ortho*-Nitroanisole has been identified in drinking-water (NTP, 2016).

When released to soil, *ortho*-nitroanisole has moderate mobility. It is not expected to bioaccumulate in aquatic organisms. *ortho*-Nitroanisole has been detected in sediment samples taken in

Japan (0.01 µg/L) (BUA, 1987). *ortho*-Nitroanisole was among the 16 known, presumed, or suspected human carcinogens identified in several water and sediment samples in China (Greenpeace, 2017).

1.4.2 Occupational exposure

Occupational exposure to *ortho*-nitroanisole can occur via inhalation or skin contact during production and application. Information on number of workers exposed to *ortho*-nitroanisole was available only for Finland and Poland. No workers were registered with occupational exposure in Finland (Saalo et al., 2016). In Poland, 203 workers were exposed to *ortho*-nitroanisole in 2016 (Starek, 2019). No estimates of occupational exposure to *ortho*-nitroanisole in other countries were available (NTP, 2016; IFA, 2019).

1.4.3 Exposure of the general population

The Working Group found no measurements of exposure to *ortho*-nitroanisole within the general population. The general population can be exposed to *ortho*-nitroanisole via the environment either from drinking contaminated water or breathing contaminated air.

1.5 Regulations and guidelines

ortho-Nitroanisole is considered a hazardous material, and there are special requirements for marking, labelling, and transporting this material (NTP, 2016; ECHA, 2019a).

In the European Union, *ortho*-nitroanisole is regulated under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, Annex III: criteria for 1–10 tonne registered substances (ECHA, 2019a). The European Chemicals Agency (ECHA) has classified *ortho*-nitroanisole as carcinogenic (Category 1B) and causing acute toxicity (Category 4) (ECHA, 2019b). It is banned from use in any cosmetic products marketed for sale or use in the European Union. Workers who are aged < 18 years, pregnant, or breastfeeding may not be exposed to *ortho*-nitroanisole. Employers are obliged to minimize other workers' exposure to *ortho*-nitroanisole as far as possible and must arrange for medical surveillance of exposed workers. *ortho*-Nitroanisole is also included in Classification, Labelling and Packaging (CLP) legislation, which lists classification and labelling data that have been notified to the ECHA by manufacturers or importers (ECHA, 2019a, b).

In the USA, *ortho*-nitroanisole is regulated under the Toxic Substances Control Act (TSCA), which requires the United States Environmental Protection Agency (US EPA) to compile, keep current, and publish a list of each chemical substance that is manufactured or processed, including imports, in the USA for uses under TSCA tracked in the Toxic Release Inventory (TRI). Three US EPA compliance-monitoring regulations for *ortho*-nitroanisole are: Emergency Planning and Community Right-To-Know Act (EPCRA 313), Standards of Performance for New Stationary Sources of Air Pollutants – Equipment Leaks Chemical List (CAA 111), and Organic Hazardous Air Pollutants National Emission Standards (CAA 112 (b) HON) (NCBI, 2020).

ortho-Nitroanisole is labelled according to the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as acute toxic category 4 for oral intake with hazard phrase H302: "Harmful if swallowed and a carcinogen, Category 1B with hazard phrase H350: May cause cancer 302." *ortho*-Nitroanisole is highly restricted in products destined for the general public but permitted for use by professionals (NCBI, 2020).

1.5.1 Exposure limits and guidelines

In the Russian Federation in 1993 the short-term exposure limit (STEL) for *ortho*-nitroanisole was set at 1 mg/m^3 (IARC, 1996). In Poland, a maximal admissible concentration of 1.6 mg/m^3, based on the no-observed-effect level of 8 mg/kg body weight (bw) per day and an uncertainty factor of 36, was adopted in 2018 (Starek, 2019). In other countries, occupational limit values or standards have not been set for this compound (ACGIH, 2018; DFG, 2019; IFA, 2019).

1.5.2 Reference values for biological monitoring of exposure

No reference values were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

ortho-Nitroanisole was previously evaluated by the *IARC Monographs* programme in 1995 (IARC, 1996). In its evaluation at that time, the Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-nitroanisole

See Table 3.1.

3.1 Mouse

Oral administration (feed)

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 40 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration of 0 (controls), 666, 2000, or 6000 mg/kg for 103 weeks (NTP, 1993; see also Irwin et al., 1996). Survival did not significantly differ between treated males and controls (controls, 35/50; 666 mg/kg, 43/50; 2000 mg/kg, 39/50; and 6000 mg/kg, 40/50), or between treated females and controls (38/50, 26/50, 33/50, and 45/50). The mean body weights of male and female mice at 2000 and 6000 mg/kg were significantly lower than those of controls throughout the study. Full histopathology was performed on grossly visible lesions and major organs and tissues.

In male mice, the incidence of hepatocellular adenoma at 0 (controls), 666, 2000, and 6000 mg/kg, respectively, was 14/50, 26/50, 41/50, and 29/50; the incidence of hepatocellular carcinoma was 7/50 (14%), 12/50 (24%), 11/50 (22%), and 7/50 (14%); the incidence of hepatoblastoma was 0/50, 3/50, 17/50, and 9/50; and the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was 21/50, 33/50, 46/50, and 34/50. There was a significant positive trend in the incidence of hepatocellular adenoma (P = 0.022), of hepatoblastoma (P = 0.015), and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (P = 0.049). The incidence of hepatocellular adenoma was significantly higher in males at 666 mg/kg (P = 0.012), 2000 mg/kg (P < 0.001), and 6000 mg/kg (P = 0.002) than in controls. The incidence of hepatoblastoma was significantly higher in males at 2000 mg/kg (P < 0.001) and 6000 mg/kg (P = 0.001) than in controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was significantly higher in males at 666 mg/kg (P = 0.013), 2000 mg/kg (P < 0.001), and 6000 mg/kg (P = 0.008) than in controls. The incidence of hepatocellular carcinoma in historical controls for male B6C3F$_1$ mice was 122/865 (mean, 14.1%; range, 3–27%).

In female mice, the incidence of hepatocellular adenoma in the control group and at 666, 2000, and 6000 mg/kg, respectively, was

Table 3.1 Studies of carcinogenicity with *ortho*-nitroanisole in mice and rats

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, B6C3F$_1$ (M) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 666, 2000, 6000 mg/kg for 103 wk 50, 50, 50, 50 35, 43, 39, 40	*Liver* Hepatocellular adenoma 14/50, 26/50*, 41/50**, 29/50***	$P = 0.022$, Cochran–Armitage trend test; *$P = 0.012$, Fisher exact test; **$P < 0.001$, Fisher exact test; ***$P = 0.002$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; adequate number of animals; the duration of exposure and observation was adequate; use of males and females. Historical controls: hepatocellular carcinoma, 122/865 (14.1%, 3–27%).
		Hepatocellular carcinoma 7/50 (14%), 12/50 (24%), 11/50 (22%), 7/50 (14%)	NS	
		Hepatoblastoma 0/50, 3/50, 17/50*, 9/50**	$P = 0.015$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test; **$P = 0.001$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 21/50, 33/50*, 46/50**, 34/50***	$P = 0.049$, Cochran–Armitage trend test; *$P = 0.013$, Fisher exact test; **$P < 0.001$, Fisher exact test; ***$P = 0.008$, 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, B6C3F$_1$ (F) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 666, 2000, 6000 mg/kg for 103 wk 50, 50, 50, 50 38, 26, 33, 45	*Liver* Hepatocellular adenoma 14/50, 20/50, 36/50*, 18/50	[$P < 0.001$, Cochran–Armitage trend test]; *$P < 0.001$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; adequate number of animals; the duration of exposure and observation was adequate; use of males and females. Historical controls: hepatocellular carcinoma, 28/863 (3.2%, 0–10%); hepatoblastoma, 1/863 (0.1%, 0–2%).
		Hepatocellular carcinoma 5/50 (10%), 2/50 (4%), 8/50 (16%), 3/50 (6%)	NS	
		Hepatoblastoma 1/50 (2%), 1/50 (2%), 2/50 (4%), 0/50	NS	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 17/50, 22/50, 37/50*, 20/50	[$P < 0.001$, Cochran–Armitage trend test]; *$P < 0.001$, Fisher exact test	
Rat, F344 (M) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 222, 666, 2000 mg/kg for 103 wk 50, 50, 50, 50 32, 34, 24, 9	*Haematopoietic system*: mononuclear cell leukaemia 26/50, 25/50, 42/50*, 34/50 (68%)	$P = 0.041$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females; adequate number of animals; adequate duration of exposure and observation. Principal limitations: low survival rate (9/50) in the group at the highest dose (2000 mg/kg). Historical controls: mononuclear cell leukaemia, 385/800 (48.1%, 32–62%).
Rat, F344 (F) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 222, 666, 2000 mg/kg for 103 wk 50, 50, 50, 50 33, 41, 26, 33	*Haematopoietic system*: mononuclear cell leukaemia 14/50, 11/50, 14/50, 26/50*	$P < 0.001$, Cochran–Armitage trend test; *$P = 0.012$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; the duration of exposure and observation was adequate; adequate number of animals; use of males and females. Historical controls: mononuclear cell leukaemia, 213/800 (26.6%, 14–52%).

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 (M) 41 days 28 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 28 wk) 10, 10, 10 10, 10, 10	*Urinary bladder*: transitional cell carcinoma 0/10, 0/10, 10/10*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (F) 41 days 28 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 28 wk) 10, 10, 10 10, 10, 10	*Urinary bladder*: transitional cell carcinoma 0/10, 0/10, 10/10*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (M) 41 days 40 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 40 wk) 10, 10, 10 10, 10, 6	*Urinary bladder*: transitional cell carcinoma 0/10, 3/10, 6/6* *Large intestine*: adenoma (adenomatous polyp) 0/10, 2/10, 4/6*	*$P \leq 0.01$, Fisher exact test *$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.

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 (F) 41 days 40 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 40 wk) 10, 10, 10 10, 10, 6	*Urinary bladder*: transitional cell carcinoma 0/10, 1/9, 6/6*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (M) 41 days 65 wk NTP (1993)	Oral > 99% Feed 0, 6000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 65 wk) 10, 10 9, 3	*Large intestine*: adenoma (adenomatous polyp) 0/9, 3/3*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (F) 41 days 65 wk NTP (1993)	Oral > 99% Feed 0, 6000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 65 wk) 10, 10 8, 10	*Urinary bladder*: transitional cell carcinoma 0/8, 9/10*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.

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 (M) 41 days 103 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with evaluation at 103 wk) 20, 20, 20 13, 1, 0	*Urinary bladder* Transitional cell carcinoma 0/21, 23/27*, 33/34* Sarcoma 0/21, 1/27, 7/34* *Kidney*: transitional cell carcinoma 0/21, 1/27, 6/34* *Large intestine*: adenoma (adenomatous polyp) 0/21, 21/27*, 24/34*	 *$P \leq 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test *$P \leq 0.01$, Fisher exact test	Principal strengths: GLP study; use of males and females. Principal limitations: low survival rate. At the end of week 103, 13/20 control rats predesignated for evaluation and 1/20 rats predesignated for evaluation from the group at 6000 mg/kg were alive, and all of 20 rats predesignated for evaluation from the group at 18 000 mg/kg had died before study termination. Rats surviving at least 28 wk but that died before the end of study, and rats alive at the end of the study were included in the histopathological examination of this 2-year stop-exposure study (total number of males: 21 control, 27 lower-dose, and 34 higher-dose animals).
Rat, F344 (F) 41 days 103 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with evaluation at 103 wk) 20, 20, 20 14, 4, 0	*Urinary bladder* Transitional cell carcinoma 0/20, 18/20*, 32/34* Sarcoma 0/20, 2/20, 12/34* *Large intestine*: adenoma (adenomatous polyp) 0/22, 5/20*, 17/34**	 *$P \leq 0.01$, Fisher exact test *$P \leq 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test; **$P \leq 0.01$, Fisher exact test	Principal strengths: GLP study, use of males and females. Principal limitations: low survival rate. At the end of week 103, 14/20 control rats predesignated for evaluation and 4/20 rats predesignated for evaluation from the group at 6000 mg/kg were alive, and all of 20 rats predesignated for evaluation from the group at 18 000 mg/kg had died before study termination. Rats surviving at least 28 wk but that died before the end of study, and rats alive at the end of the study were included in the histopathological examination of this 2-year stop-exposure study (total number of females: 22 control, 20 lower-dose, and 34 higher-dose animals).

F, female; GLP, Good Laboratory Practice; M, male; NS, not significant; wk, week.

14/50, 20/50, 36/50, and 18/50; the incidence of hepatocellular carcinoma was 5/50 (10%), 2/50 (4%), 8/50 (16%), and 3/50 (6%); the incidence of hepatoblastoma was 1/50 (2%), 1/50 (2%), 2/50 (4%), and 0/50; and the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was 17/50, 22/50, 37/50, and 20/50. There was a significant positive trend in the incidence of hepatocellular adenoma [$P < 0.001$] and in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) [$P < 0.001$]. The incidence of hepatocellular adenoma ($P < 0.001$) and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) ($P < 0.001$) was significantly higher in females at 2000 mg/kg than in controls. The incidence of tumours in historical controls for female B6C3F$_1$ mice was: hepatocellular carcinoma, 28/863 (mean, 3.2%; range, 0–10%); and hepatoblastoma, 1/863 (mean, 0.1%; range, 0–2%).

Regarding non-neoplastic lesions in the liver, there was a significant increase in the incidence of cytological alterations and eosinophilic foci in male and female mice, and of necrosis, haemorrhage, and Kuppfer cell pigmentation in males (NTP, 1993). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, and males and females were used. In addition, *ortho*-nitroanisole caused tumours derived from tissues with different embryological differentiation pathways (epithelial and mesenchymal) in males and females.]

3.2 Rat

3.2.1 Oral administration (feed)

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344 rats (age, 40 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration of 0 (controls), 222, 666, or 2000 mg/kg for 103 weeks (NTP, 1993; see also Irwin et al., 1996). Survival at the end of the study was 32/50, 34/50, 24/50, and 9/50 in males, and 33/50, 41/50, 26/50, and 33/50 in females, at 0, 222, 666, and 2000 mg/kg, respectively. Survival of males at 2000 mg/kg was significantly lower than that of controls. For female rats, there were no significant differences in survival rates between control and treatment groups. The final mean body weights of male and female rats at 2000 mg/kg were significantly lower than those of controls. Full histopathology was performed on grossly visible lesions and major organs and tissues.

The incidence of mononuclear cell leukaemia was 26/50, 25/50, 42/50, and 34/50 in male rats, and 14/50, 11/50, 14/50, and 26/50 in female rats, in the control group and at 222, 666, and 2000 mg/kg, respectively. There was a significant positive trend in the incidence of mononuclear cell leukaemia in males ($P = 0.041$) and females ($P < 0.001$). The incidence of mononuclear cell leukaemia was significantly higher in males at 666 mg/kg ($P < 0.001$) and in females at 2000 mg/kg ($P = 0.012$) than in controls. The incidence in males at 2000 mg/kg (68%) was slightly higher than in the control group (52%), but the difference was not significantly different by pairwise comparison [possibly due to the high mortality rate and corresponding early death of these animals]; however, this incidence was still above the upper bound of the historical control range for male Fischer 344 rats (32–62%) (NTP, 1993). [The Working Group noted this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, and males and females were used.]

3.2.2 Oral administration (stop-exposure study)

In a study that complied with GLP, groups of 60 male and 60 female Fischer 344 rats (age, 41 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration

of 0 (controls), 6000, or 18 000 mg/kg for a predetermined duration of 27 weeks, and thereafter received feed only for up to an additional 76 weeks, for a total study duration of up to 103 weeks (NTP, 1993). Ten males and ten females per group were scheduled for interim evaluations at experimental weeks 13, 28, 40, and 65; the remaining rats were killed at experimental week 103. Full histopathology was performed on grossly visible lesions and major organs and tissues.

At experimental week 28 (1 week after the completion of *ortho*-nitroanisole administration), the incidence of transitional cell carcinoma of the urinary bladder was 0/10, 0/10, and 10/10 in males, and 0/10, 0/10, and 10/10 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls.

At experimental week 40 (13 weeks after cessation of *ortho*-nitroanisole administration), 6 males and 6 females out of the 10 males and 10 females predesignated for interim evaluation were alive in the groups at 18 000 mg/kg. The incidence of transitional cell carcinoma of the urinary bladder was 0/10, 3/10, and 6/6 in males, and 0/10, 1/9, and 6/6 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls. In addition, the incidence of adenoma of the large intestine was significantly higher ($P \leq 0.01$) in males at 18 000 mg/kg (4/6) than in controls (0/10).

At experimental week 65 (38 weeks after the completion of *ortho*-nitroanisole administration), all males (10/10) and females (10/10) predesignated for interim evaluation had died before evaluation in groups at 18 000 mg/kg. Three out of 10 males and 10 out of 10 females predesignated for interim evaluation were alive in the groups at 6000 mg/kg. Survival in the controls was 9/10 in males and 8/10 in females. The incidence of transitional cell carcinoma of the urinary bladder was: 0/9 and 1/3 in males, and 0/8 and 9/10 in females, in the control group and at 6000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher ($P \leq 0.01$) in females at 6000 mg/kg than in controls. In addition, the incidence of adenoma of the large intestine was significantly higher ($P \leq 0.01$) in males at 6000 mg/kg (3/3) than in controls (0/9).

At experimental week 103, 13/20 males and 14/20 females predesignated for evaluation in the control groups, and 1/20 males and 4/20 females predesignated for evaluation in the groups at 6000 mg/kg were alive. All 20 male and 20 female rats predesignated for evaluation in the groups at 18 000 mg/kg died before week 103. Rats surviving at least 28 weeks but dying before the end of study and rats alive at the end of the study were combined for histopathological examination (total number of males: controls, 21; lower dose, 27; and higher dose, 34; total number of females: controls, 22; lower dose, 20; and higher dose, 34). The incidence of transitional cell carcinoma of the urinary bladder was 0/21, 23/27, and 33/34 in males, and 0/20, 18/20, and 32/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 6000 and 18 000 mg/kg than in controls. The incidence of sarcoma of the urinary bladder was 0/21, 1/27, and 7/34 in males, and 0/20, 2/20, and 12/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of sarcoma of the urinary bladder was significantly higher in males ($P < 0.05$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls. The incidence of transitional cell carcinoma of the kidney was significantly higher ($P < 0.05$) in males at 18 000 mg/kg (6/34) than in controls (0/21). The

incidence of adenoma of the large intestine was 0/21, 21/27, and 24/34 in males, and 0/22, 5/20, and 17/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of adenoma of the large intestine was significantly higher in males ($P \leq 0.01$) at 6000 and 18 000 mg/kg, and in females at 6000 and 18 000 mg/kg ($P < 0.05$, $P \leq 0.01$, respectively) than in controls.

Regarding non-neoplastic lesions, the incidence of transitional cell hyperplasia of the urinary bladder and of transitional cell hyperplasia of the kidney was significantly higher in many of the treated groups of male and female rats than in controls [transitional cell hyperplasia of the urinary bladder and transitional cell hyperplasia of the kidney are considered to be pre-neoplastic lesions] (NTP, 1993).

[The Working Group noted this was a well-conducted study that complied with GLP, and males and females were used. The high incidence (100%) of transitional cell carcinoma of the urinary bladder after a short duration of exposure in treated males and females was also noted. In addition, *ortho*-nitroanisole caused tumours derived from tissues with different embryological differentiation pathways (epithelial and mesenchymal) in males and females.]

[The Working Group noted that the aromatic amines *ortho*-toluidine and 2-naphthylamine (both IARC Group 1, *carcinogenic to humans*) also caused malignant tumours of the urinary bladder when administered orally in rats, and that the aromatic amine 4-aminobiphenyl (IARC Group 1) caused malignant tumours of the urinary bladder in mice as well as in dogs when administered orally (IARC, 2012).]

3.3 Synthesis

In one well-conducted GLP study in male and female B6C3F$_1$ mice treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase, with a significant positive trend, in the incidence of hepatocellular adenoma, hepatoblastoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in males; and of hepatocellular adenoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in females (NTP, 1993).

In one well-conducted GLP study in male and female Fischer 344 rats treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase, with a significant positive trend, in the incidence of mononuclear cell leukaemia in males and females (NTP, 1993).

In a series of stop-exposure experiments in one well-conducted GLP study in male and female Fischer 344 rats treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase in the incidence of: transitional cell carcinoma of the urinary bladder in males and females in a first stop-exposure experiment; transitional cell carcinoma of the urinary bladder in males and females, and of adenoma of the large intestine in males in a second stop-exposure experiment; transitional cell carcinoma of the urinary bladder in females, and of adenoma of the large intestine in males in a third stop-exposure experiment; and transitional cell carcinoma of the urinary bladder, sarcoma of the urinary bladder, and adenoma of the large intestine in males and females, and of transitional cell carcinoma of the kidney in males in a fourth stop-exposure experiment (NTP, 1993).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

In 1993, an accident (an explosion) in a chemical plant in Frankfurt, Germany, resulted in the emission of various chlorinated and azo compounds, as well as *ortho*-nitroanisole (Heudorf et al., 1994; Hengstler et al., 1995). The median levels of 2-nitrophenol [*ortho*-nitrophenol], a major metabolite of *ortho*-nitroanisole, in urine samples collected from inhabitants of the contaminated area a few days after the accident were three times higher (25.2 µg/L) than in the controls (8.2 µg/L) (Heudorf et al., 1994).

(b) Human hepatic microsomes and cytosols

An O-demethylated metabolite of *ortho*-nitroanisole, *ortho*-nitrophenol and two oxidation products of this metabolite, 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene, were obtained in vitro by human hepatic microsomes and cytochrome P450 (CYP) in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (Mikšanová et al., 2004a; Dračínská et al., 2006). Two reductive metabolites of *ortho*-nitroanisole, *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine, were obtained in vitro by incubation with human hepatic cytosolic samples and xanthine oxidase, particularly in the presence of hypoxanthine (Mikšanová et al., 2004b). The nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine was not detected after incubation with human hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a). *N*-(2-Methoxyphenyl)hydroxylamine was metabolized by human hepatic microsomes predominantly to *ortho*-anisidine, whereas 2-aminophenol [*ortho*-aminophenol] and two metabolites were detected as minor products (Naiman et al., 2011).

In studies in vitro using human recombinant CYPs and purified rodent CYPs, 2E1, 1A1, and 2B6 were the most efficient isoforms oxidizing *ortho*-nitroanisole to *ortho*-nitrophenol (Mikšanová et al., 2004a), whereas 2E1 and 1A1 were the most effective in the formation of 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene (Dračínská et al., 2006). In a study in vitro using selective inhibitors of microsomal CYPs, 3A4, 2E1, and 2C were the most important in the metabolism of *N*-(2-methoxyphenyl) hydroxylamine to *ortho*-anisidine by human hepatic microsomes (Naiman et al., 2011).

4.1.2 Experimental systems

(a) In vivo

The absorption, distribution, and excretion of *ortho*-nitroanisole were studied in male Fischer 344 rats by Miller et al. (1985). Three dose levels of [^{14}C]-labelled *ortho*-nitroanisole (5, 50, or 500 mg/kg bw) were administered orally to rats, and daily excreta were analysed for radiolabel. *ortho*-Nitroanisole was readily absorbed from the stomach. Peak blood concentrations in vitro reflected the dose-dependence of absorption, with parent *ortho*-nitroanisole reaching maximal concentrations at 3 hours after a dose of 50 mg/kg bw and at 6 hours after a dose of 500 mg/kg bw. Within 7 days, 7% of the administered dose had been excreted in the faeces for all dose levels and about 70% of the administered dose had been eliminated in the urine. The predominant route of elimination was through metabolism to *ortho*-nitrophenol, subsequent sulfation to *ortho*-nitrophenyl sulfate, and glucuronidation to *ortho*-nitrophenyl glucuronide. Seven days after oral administration of *ortho*-nitroanisole, less than 0.5% of the administered dose remained in the carcass.

The distribution of *ortho*-nitroanisole–derived ^{14}C to tissues (muscle, 20%; skin, 10%; fat, 6.8%; blood, 6.5%; liver, 4.8%; plasma, 3.1%; kidney, 2.8%; and small intestine, 1.9%) occurred rapidly after intravenous administration of [^{14}C]-labelled *ortho*-nitroanisole at 25 mg/kg bw. Urinary and faecal elimination patterns were similar to those found after oral administration. The subsequent elimination of ^{14}C was rapid and biphasic. The initial elimination phase in all tissues had a half-life of 1–2 hours, and the terminal phase half-lives for all tissues ranged from 2.5 to 6.2 days. Elimination of parent *ortho*-nitroanisole from the blood was biphasic with initial and terminal half-lives of 30 minutes and 2.2 hours, respectively. Monophasic elimination of *ortho*-nitroanisole from the liver, kidneys, and small intestine occurred with half-lives of 0.35, 0.55, and 0.68 hour, respectively. Biliary excretion was similar to faecal elimination, indicating a lack of enterohepatic recirculation (Miller et al., 1985).

(b) In vitro

See Fig. 4.1.

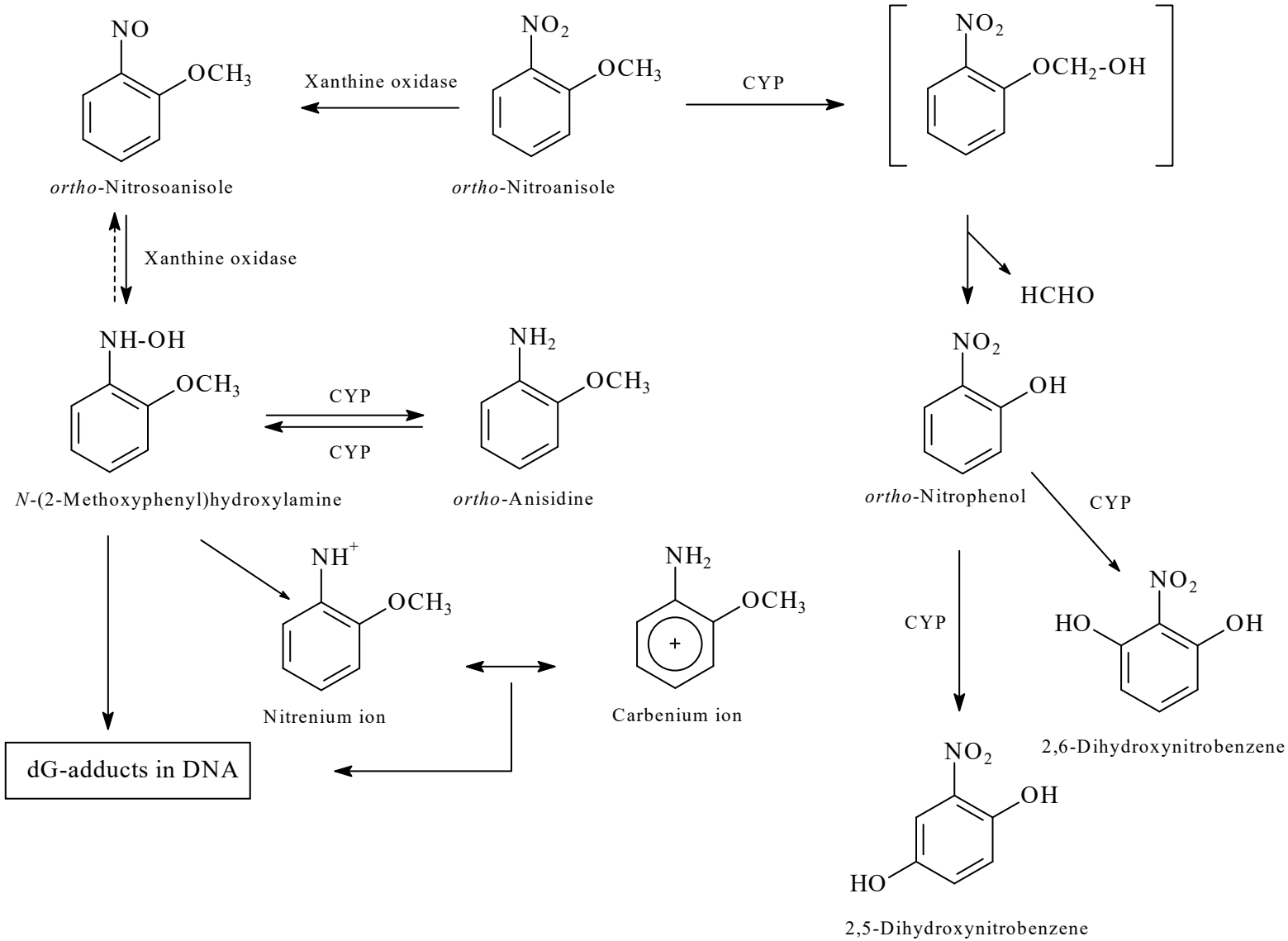
Three metabolites of *ortho*-nitroanisole obtained by incubation with human hepatic microsomes – *ortho*-nitrophenol, 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene – were produced in vitro by incubation with rat and rabbit hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a; Dračínská et al., 2006). 2,5-Dihydroxynitrobenzene was the predominant product of metabolism by human microsomes, whereas *ortho*-nitrophenol was the major metabolite generated by rat and rabbit microsomes (Dračínská et al., 2006). Oxidation of *ortho*-nitrophenol by rat hepatic microsomes to 2,5-dihydroxynitrobenzene was detected by HPLC, whereas reduction of *ortho*-nitrophenol to *ortho*-aminophenol was not observed (Svobodová et al., 2009). Two reductive metabolites of *ortho*-nitroanisole, *N*-(2-methoxyphenyl) hydroxylamine and *ortho*-anisidine, were obtained in vitro after incubation with rat, rabbit, and pig hepatic cytosolic samples and xanthine oxidase, particularly in the presence of hypoxanthine (Mikšanová et al., 2004b). The nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine was not detected after incubation with rat and rabbit hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a). *N*-(2-Methoxyphenyl)hydroxylamine was metabolized by rat and rabbit hepatic microsomes mainly to *ortho*-aminophenol and *ortho*-anisidine, whereas *ortho*-nitrosoanisole was formed as a minor metabolite (Naiman et al., 2008). [Naiman et al. (2010) reported that *N*-(2-methoxyphenyl) hydroxylamine was metabolized by rat hepatic microsomes mainly to *ortho*-anisidine, whereas *ortho*-aminophenol and two other metabolites were minor products.]

Rat recombinant enzymes CYP 2E1, 2D2, 2B2, 2C6, and 1A1 efficiently metabolized *ortho*-nitroanisole (Svobodová et al., 2008). In a study in vitro using selective CYP inhibitor and hepatic microsomes of rats pre-treated with specific CYP inducers, most oxidation of *ortho*-nitrophenol was produced by CYP 2E1 and 3A, followed by 2D and 2C (Svobodová et al., 2009). Rat hepatic CYP 2E1, 1A subfamily and, to a lesser extent, those of a 2B subfamily were capable of metabolizing *N*-(2-methoxyphenyl) hydroxylamine to *ortho*-aminophenol (Naiman et al., 2008). Rat CYP 2C, followed by 2E1, 2D, and 2A were the major enzymes metabolizing *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine (Naiman et al., 2010).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) including whether *ortho*-nitroanisole is electrophilic or can be metabolically activated;

Fig. 4.1 Metabolic pathways for *ortho*-nitroanisole



CYP, cytochrome P450; dG, deoxyguanosine.
The compound shown in square brackets was not detected under experimental conditions.
Compiled by the Working Group using information from Stiborová et al. (2009).

is genotoxic; induces oxidative stress; or alters cell proliferation, cell death or nutrient supply. For the evaluation of other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

Table 4.1, Table 4.2, and Table 4.3 summarize the available studies on the genetic and related effects of *ortho*-nitroanisole.

(a) Humans

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

A study in vitro using [^{3}H]-labelled *ortho*-nitroanisole and the ^{32}P-post-labelling technique showed that the deoxyguanosine adducts formed from *N*-(2-methoxyphenyl)hydroxylamine were detected in calf thymus DNA incubated with *ortho*-nitroanisole and human hepatic cytosolic samples in the presence of hypoxanthine (Stiborová et al., 2004). In contrast, no DNA-adduct formation mediated by *ortho*-nitroanisole oxidation in human hepatic microsomes was detectable (Mikšanová et al., 2004a; see Table 4.3).

(b) Experimental systems

See Table 4.1 and Table 4.3.

In male Wistar rats treated intraperitoneally with *ortho*-nitroanisole (0.15 mg/kg bw per day) for 5 consecutive days, deoxyguanosine adducts formed from *N*-(2-methoxyphenol)hydroxylamine were detected in DNA from the urinary bladder and, to a lesser extent, the kidney, liver, and spleen by ^{32}P-post-labelling assay, whereas no adduct formation was observed in the lung, heart, or brain (Stiborová et al., 2004).

A study in vitro using [^{3}H]-labelled *ortho*-nitroanisole and the ^{32}P-post-labelling technique did not detect DNA-adduct formation mediated by *ortho*-nitroanisole oxidation in rabbit hepatic microsomes incubated with calf thymus DNA (Mikšanová et al., 2004a).

4.2.2 Is genotoxic

Table 4.1, Table 4.2, and Table 4.3 summarize the available studies of the genetic and related effects of *ortho*-nitroanisole.

(a) Humans

In 1993, an accident in a chemical plant in Frankfurt, Germany, resulted in the emission of various chlorinated and azo compounds, including *ortho*-nitroanisole (Heudorf et al., 1994; Hengstler et al., 1995). Levels of DNA single-strand breaks (by alkaline elution) in blood mononuclear cells were slightly but statistically significantly higher 19 days after the accident in 16 firefighters who participated in mechanically removing the precipitate in the contaminated area for about 8 hours than in two reference groups (19 unexposed firefighters living in the same town, and 28 people without any apparent occupational exposure to genotoxic substances) (Hengstler et al., 1995). [The Working Group noted that data on the firefighters' exposure to *ortho*-nitroanisole and co-exposures were lacking and that there were co-exposures to other genotoxicants.] After 3 months, the level of DNA single-strand breaks was no longer increased in comparison with the levels in the reference groups. [Limited data were reported on air concentrations in the community.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

In male rats treated by gavage, *ortho*-nitroanisole induced DNA strand breaks (as measured by the standard alkaline comet assay) in kidney cells in Sprague-Dawley rats (Nesslany et al., 2007), but not in liver and urinary bladder cells in male Sprague-Dawley and Fischer 344 rats (Wada et al., 2014, 2017). In male Fischer 344 rats

Table 4.1 Genetic and related effects of *ortho*-nitroanisole in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
DNA adducts	Rat, Wistar (M)	Liver, kidney, spleen, and urinary bladder	+	0.15 mg/kg bw per day	Intraperitoneal, 5 consecutive days	Stiborová et al. (2004)
DNA adducts	Rat, Wistar (M)	Lung, heart and brain	–	0.15 mg/kg bw per day	Intraperitoneal, 5 consecutive days	Stiborová et al. (2004)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Kidney cells	+	250 mg/kg bw	Gavage, 2 doses, sampling 3–6 h and 22–26 h after dosing	Nesslany et al. (2007)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Urinary bladder and liver cells	–	700 mg/kg bw per day	Gavage, 2 doses on 2 consecutive days, sampling 3 h after final dosing	Wada et al. (2014)
DNA strand breaks (comet assay)	Rat, F344 (M)	Urinary bladder and liver cells	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (modified comet assay, with hOGG1)	Rat, F344 (M)	Urinary bladder cells	+	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (modified comet assay, with hOGG1)	Rat, F344 (M)	Liver cells	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (phosphorylated histone γ-H2AX)	Rat, F344 (M)	Urinary bladder epithelial cell	+	1.8% or 1048 mg/kg bw per day	Feed, 4 wk with and without a 2-wk recovery period	Toyoda et al. (2015)
DNA strand breaks (phosphorylated histone γ-H2AX)	Mouse, $B6C3F_1$ (M)	Urinary bladder epithelial cell	–	0.6% or 638 mg/kg bw per day	Feed, 4 wk with and without a 2-wk recovery period	Sone et al. (2019)
Micronucleus formation	Rat, F344 (M)	Bone marrow	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)

bw, body weight; h, hour; HID, highest ineffective dose; hOGG1, human 8-oxoguanine DNA-glycosylase 1; LED, lowest effective dose; M, male; wk, week.

[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of *ortho*-nitroanisole in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Gene mutation, *Tk* locus	Mouse lymphoma L5178Y cells	+	NT	250 µg/mL	NTP (1993)
Chromosomal aberrations	Chinese hamster ovary cells	–	+	1060 µg/mL	Galloway et al. (1987)
Sister-chromatid exchange	Chinese hamster ovary cells	+	+	123 µg/mL	Galloway et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; *Tk*, thymidine kinase.
[a] +, positive; –, negative.

given feed containing *ortho*-nitroanisole, there was a significant increase in levels of phosphorylated histone H2AX (γ-H2AX), a marker of DNA damage, in epithelial cells of the urinary bladder (Toyoda et al., 2015). In contrast, in male B6C3F$_1$ mice given feed containing *ortho*-nitroanisole, there was no increase in levels of γ-H2AX in bladder epithelial cells (Sone et al., 2019).

No increase in the frequency of micronucleus formation was seen in the bone marrow of male Fischer 344 rats given *ortho*-nitroanisole as three daily doses (up to 500 mg/kg bw per day) by gavage (Wada et al., 2017).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

In single studies in cultured mammalian cells, *ortho*-nitroanisole induced mutation at the *Tk* locus of mouse lymphoma L5178Y cells (NTP, 1993), as well as chromosomal aberrations and sister-chromatid exchange in Chinese hamster ovary cells (Galloway et al., 1987). The clastogenic activity was modest and observed only in the presence of S9, whereas sister-chromatid exchange and *Tk* mutations were induced in the absence of S9.

(iii) Non-mammalian experimental systems

See Table 4.3.

ortho-Nitroanisole was tested in several laboratories for the induction of gene mutations in *Salmonella typhimurium*. Positive responses were obtained consistently with strains TA100 (Chiu et al., 1978; Haworth et al., 1983; Shimizu & Yano, 1986; Dellarco & Prival, 1989; NTP, 1993; JETOC, 1996; Wada et al., 2017) and YG3008 (Wada et al., 2017). Results were generally negative in strains used for detecting frameshift mutations (Chiu et al., 1978; Haworth et al., 1983; Shimizu & Yano, 1986; NTP, 1993; JETOC, 1996).

ortho-Nitroanisole gave positive results in the SOS/*umu* genotoxicity assay in *Salmonella typhimurium* strain TA1535/pSK1002 (Reifferscheid & Heil, 1996). *ortho*-Nitroanisole gave positive results in the rec assay in *Bacillus subtilis* strains H17 and M45 (Shimizu & Yano, 1986). *ortho*-Nitroanisole did not induce reverse mutation in the WP2*uvrA* strain of *Escherichia coli* in the presence or absence of exogenous metabolic activation (JETOC, 1996).

4.2.3 Induces oxidative stress

(a) Humans

No data were available to the Working Group.

Table 4.3 Genetic and related effects of *ortho*-nitroanisole 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 TA100	Reverse mutation	+	NT	1530 µg/mL		Chiu et al. (1978)
Salmonella typhimurium TA100	Reverse mutation	+	+	666 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA100, TA98 and TA1538	Reverse mutation	+	NT	1 µL/plate		Shimizu & Yano (1986)
Salmonella typhimurium TA100	Reverse mutation	NT	+	580 µg/mL		Dellarco & Prival (1989)
Salmonella typhimurium TA100	Reverse mutation	+	+	333 µg/plate		NTP (1993)
Salmonella typhimurium TA100	Reverse mutation	+	+	1000 µg/plate	Detailed methods were not available.	JETOC (1996)
Salmonella typhimurium TA100 and YG3008	Reverse mutation	+	+	313 µg/plate		Wada et al. (2017)
Salmonella typhimurium TA1535, TA1537 and TA98	Reverse mutation	–	–	1000 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA1535 and TA1537	Reverse mutation	–	NT	5 µL/plate		Shimizu & Yano (1986)
Salmonella typhimurium TA1535	Reverse mutation	+	–	1000 µg/plate		NTP (1993)
Salmonella typhimurium TA1535, TA1537, and TA98	Reverse mutation	–	–	1000 µg/plate	Detailed methods were not available.	JETOC (1996)
Salmonella typhimurium TA98	Reverse mutation	+	NT	765 µg/mL		Chiu et al. (1978)
Salmonella typhimurium TA98 and TA1537	Reverse mutation	–	–	1000 µg/plate		NTP (1993)
Salmonella typhimurium TA97	Reverse mutation	–	–	3333 µg/plate		NTP (1993)
Salmonella typhimurium TA1535/pSK1002	SOS/*umu* genotoxicity	+	NT	NR		Reifferscheid & Heil (1996)
Escherichia coli WP2*uvrA*	Reverse mutation	–	–	2000 µg/plate	Detailed methods were not available.	JETOC (1996)
Bacillus subtilis rec H17 and M45 strains	Differential toxicity	+	NT	625 µg/mL		Shimizu & Yano (1986)

Table 4.3 Genetic (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA isolated from calf thymus	DNA adducts	–	+	[^{3}H]-labelled *ortho*-nitroanisole, 20 µL/0.75 mL [0.5 mM]	Metabolic activation by human hepatic cytosolic protein in the presence of hypoxanthine.	Stiborová et al. (2004)
DNA isolated from calf thymus	DNA adducts	–	–	[^{3}H]-labelled *ortho*-nitroanisole, 20 µL/0.75 mL [0.5 mM]	Metabolic activation by human or rabbit hepatic microsomes and NADPH.	Mikšanová et al. (2004a)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NT, not tested; NR, not reported.
[a] +, positive; –, negative.

(b) Experimental systems

In male Fischer 344 rats treated with *ortho*-nitroanisole (500 mg/kg bw per day) by gavage for 3 consecutive days, oxidative damage to DNA in the urinary bladder (as measured by the modified comet assay) was significantly increased only in the presence of human 8-oxoguanine DNA-glycosylase 1 (hOGG1) which can recognize 8-oxoguanine and convert it into a DNA break. 8-Oxoguanine is not detectable by the standard comet assay. In contrast to the findings in the urinary bladder, no increase in the frequency of DNA damage was detected in liver cells regardless of the presence of hOGG1 (Wada et al., 2017).

In a test for reverse mutation in bacteria, strain YG3008 (which is sensitive to oxidative mutagens owing to a lack of the *mutM*$_{st}$ gene encoding an enzyme, formamidopyrimidine DNA-glycosylase, that recognizes and repairs oxidative DNA damage) was more sensitive to *ortho*-nitroanisole than the parent strain TA100 (Wada et al., 2017).

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a dose-finding study for a carcinogenicity test during which Fischer 344 rats were given feed containing *ortho*-nitroanisole at concentrations of 200–18 000 mg/kg (10–720 mg/kg bw per day) for 13 weeks, the incidence of urothelial hyperplasia in the urinary bladder was significantly increased in male and female rats at 6000 and 18 000 mg/kg (NTP, 1993).

In Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 6000 or 18 000 mg/kg (340 or 1100 mg/kg bw per day) for 27 weeks followed by maintenance on feed only for additional 77 weeks (stop-exposure study), the incidence of urothelial hyperplasia in the urinary bladder was significantly increased in males and females from week 13. In addition, there was a significant increase in the incidence of hyperplasia of transitional epithelial cells in the renal pelvis in males at week 28, 40, and 103, and in females at week 103. Subacute inflammation and proliferation of connective tissue in the lamina propria of the urinary bladder were also observed. The lesions were characterized by scattered inflammatory cells, principally neutrophils and macrophages, and increased numbers of fibroblasts with immature collagen (NTP, 1993; Irwin et al., 1996).

In male Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 18 000 mg/kg (1048 mg/kg bw per day) for 4 weeks, there was a significant increase in the expression of Ki67 (a marker of cell proliferation activity) in the bladder urothelium (Toyoda et al., 2015). In contrast, in male B6C3F$_1$ mice given feed containing *ortho*-nitroanisole at a concentration of 6000 mg/kg (638 mg/kg bw per day) for 4 weeks, there was no increase in the frequency of Ki67-positive cells in the bladder urothelium (Sone et al., 2019).

In Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 222, 666, or 2000 mg/kg (10–90 mg/kg bw per day) for 2 years, the incidence of focal hyperplasia of epithelial cells in the forestomach was significantly increased in male rats of all dose groups and in female rats at the highest dose (NTP, 1993; Irwin et al., 1996).

4.2.5 Data on other key characteristics of carcinogens

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a host-mediated in vivo/in vitro assay using male NMRI mice, the transformation of peritoneal macrophages was induced by a single dose of *ortho*-nitroanisole (1.3 mg/kg bw, or 0.1% of the median lethal dose, 1300 mg/kg bw) (Esmaeili et al., 2006).

4.3 Other relevant evidence

Methaemoglobin concentrations were significantly increased in male Fischer 344 rats fed *ortho*-nitroanisole (at concentrations at or above 1166 mg/kg in feed) for 14 days (NTP, 1993). In 13-week studies, increases in methaemoglobin concentrations were observed in male and female Fischer 344 rats (at 6000 and 18 000 mg/kg in feed) and in male $B6C3F_1$ mice (at 6000 mg/kg in feed).

Erythrocyte counts, haematocrit values, and haemoglobin concentrations were significantly lower in male Fischer 344 rats exposed to *ortho*-nitroanisole in the feed (583, 1166, 2332, 4665, or 9330 mg/kg) for 14 days. With the exception of depressed body-weight gain, no such treatment-related effect was observed in $B6C3F_1$ mice (NTP, 1993).

In 13-week feeding studies, observed haemoglobin and haematocrit values were lower in male and female Fischer 344 rats receiving *ortho*-nitroanisole at a concentration of 2000, 6000, or 18 000 mg/kg, and in male and female $B6C3F_1$ mice at 2000 or 6000 mg/kg than in controls (NTP, 1993).

5. Summary of Data Reported

5.1 Exposure characterization

ortho-Nitroanisole is an anisole compound with the formula $C_7H_7NO_3$, which is produced from methanolic sodium hydroxide, 2-chloronitrobenzene, and methanol.

Information on the production of *ortho*-nitroanisole was sparse, and indicated that manufacturing occurs in Asia. *ortho*-Nitroanisole is used primarily as a precursor for *ortho*-anisidine (see the monograph on *ortho*-anisidine in the present volume]. Waste containing *ortho*-nitroanisole can contaminate water and soil, but *ortho*-nitroanisole is not expected to bioaccumulate in aquatic organisms.

Scant information was found on occupational exposure and no information was identified on general population exposure, but *ortho*-nitroanisole has been detected in drinking-water. *ortho*-Nitroanisole is considered to be a hazardous material in the European Union and USA, and is labelled as such under the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals. Only two countries (Poland and the Russian Federation) have established occupational exposure limits.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

ortho-Nitroanisole increased the incidence of malignant neoplasms in two species.

In $B6C3F_1$ mice, *ortho*-nitroanisole administered orally (in feed) in one study caused an increase in the incidence of hepatoblastoma in males. In addition, *ortho*-nitroanisole caused an increase in the incidence of a combination of benign and malignant liver tumours

(hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma) in male and female mice.

In Fischer 344 rats, *ortho*-nitroanisole administered orally (in feed) in one study caused an increase in the incidence of mononuclear cell leukaemia in male and female rats.

In Fischer 344 rats, *ortho*-nitroanisole administered orally (in feed) in four stop-exposure experiments caused an increase in the incidence of transitional cell carcinoma of the urinary bladder and sarcoma of the urinary bladder in male and female rats, and of transitional cell carcinoma of the kidney in male rats.

5.4 Mechanistic evidence

No studies characterizing the absorption, distribution, metabolism or excretion of *ortho*-nitroanisole in humans were available. *ortho*-Nitrophenol, a major metabolite of *ortho*-nitroanisole, was detected in the urine of individuals exposed to contaminated air after an explosion that released *ortho*-nitroanisole, as well as various chlorinated and azo compounds. Human, rabbit, or rat hepatic microsomes catalysed cytochrome P450-dependent oxidation of *ortho*-nitroanisole to *ortho*-nitrophenol and further oxidation to 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene. Human, rabbit, rat, or pig hepatic cytosol catalysed nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl) hydroxylamine and *ortho*-anisidine. Human and rat hepatic microsomes reversibly reduced *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine. In rats exposed orally, *ortho*-nitroanisole is readily absorbed, widely distributed to tissues, and excreted primarily via the urine as sulfate and glucuronide conjugates of *ortho*-nitrophenol.

There is consistent and coherent evidence that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems. *ortho*-Nitroanisole is metabolically activated to electrophiles. No data on DNA adducts in exposed humans were available. In in vitro studies, *ortho*-nitroanisole is activated by human hepatic cytosol in the presence of hypoxanthine to form *N*-(2-methoxyphenyl)hydroxylamine-derived DNA adducts. In experimental systems, these DNA adducts were observed in several tissues of male Wistar rats exposed to *ortho*-nitroanisole, with the highest level found in the urinary bladder.

ortho-Nitroanisole is genotoxic, inducing mutations at the *Tk* locus in mouse lymphoma L5178Y cells in the absence of metabolic activation, and in multiple studies in base-pair substitution strains of bacteria, both in the presence and in the absence of metabolic activation. *ortho*-Nitroanisole also induces DNA damage, with positive findings in the comet assay in Sprague-Dawley rat kidney, but not urinary bladder; positive findings in Fischer 344 rat urinary bladder in the presence of human 8-oxoguanine DNA-glycosylase 1 in the comet assay; increased levels of γ-H2AX in urinary bladder epithelial cells in exposed Fischer 344 rats; and positive findings in two strains of *Bacillus subtilis* in the rec assay.

ortho-Nitroanisole also alters cell proliferation, cell death, or nutrient supply. *ortho*-Nitroanisole induced urothelial hyperplasia in the urinary bladder in male and female Fischer 344 rats, an effect that persisted after cessation of exposure. *ortho*-Nitroanisole also increased expression of Ki67 in bladder urothelial cells in male Fischer 344 rats.

Overall, the evidence is consistent and coherent that, in view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, *ortho*-nitroanisole belongs within a mechanistic class of aromatic amines. Members of this class, including 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine, have been classified previously by the *IARC Monographs* programme as *carcinogenic to humans* (IARC Group 1). *ortho*-Nitroanisole

exhibits concordance with aromatic amines with respect to the formation of common DNA-reactive moieties; genotoxicity; and target organs of carcinogenicity in chronic animal bioassays. The urinary bladder is a common target organ of carcinogenicity for these aromatic amines in experimental animals. For instance, *ortho*-nitroanisole causes malignant tumours of the urinary bladder when administered orally to rats, as do *ortho*-anisidine, *ortho*-toluidine, and 2-naphthylamine. 4-Aminobiphenyl causes malignant tumours of the urinary bladder when administered orally to dogs and mice. Therefore, these mechanistic considerations go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

6. Evaluation and Rationale

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that, in view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, *ortho*-nitroanisole belongs, based on mechanistic considerations, to a class of aromatic amines for which several members have been classified as carcinogenic to humans. There is also *strong evidence* that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

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

6.5 Rationale

The Group 2A evaluation is based on *strong* mechanistic evidence. In view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, there is *strong* evidence that *ortho*-nitroanisole, based on mechanistic considerations, belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans. *ortho*-Nitroanisole exhibits concordance with aromatic amines with respect to the formation of common DNA-reactive moieties, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays.

There is also *sufficient evidence* of carcinogenicity in experimental animals, based on an increased incidence of malignant neoplasms in two species.

In addition, there is *strong evidence* that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems. *ortho*-Nitroanisole is metabolically activated to electrophiles, it is genotoxic, and it also alters cell proliferation, cell death, or nutrient supply.

The evidence on cancer in humans was *inadequate* as no data were available.

References

Aarti Industries (2020). Ortho nitro anisole (ONA). Available from: https://www.aarti-industries.com/products/chemicals/detail/129/?name=Ortho-Nitro-Anisole-(ONA)(91-23-6), accessed 18 January 2021.

ACGIH (2018). American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

Booth G (1991). Nitro compounds, aromatic. In: Elvers B, Hawkins S, Schulz G, editors. Ullmann's encyclopedia of industrial chemistry. Volume A17. 5th rev. New York (NY), USA: VCH Publishers; pp. 411–55.

BUA (1987). GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA): *o*-Nitroanisole (1-Methoxy-2-nitrobenzene) (BUA Report 9) (June 1987). Weinheim, Germany: VCH Verlagsgesellschaft mbH.

Chemical Register (2020). 2-Nitroanisole (CAS No. 91-23-6) Suppliers. Chemical Register. Available from: https://www.chemicalregister.com/2-Nitro_Anisole/Suppliers/pid27982.htm, accessed 7 October 2020.

Chiu CW, Lee LH, Wang CY, Bryan GT (1978). Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat Res*. 58(1):11–22. doi:10.1016/0165-1218(78)90090-3 PMID:362191

Dellarco VL, Prival MJ (1989). Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ Mol Mutagen*. 13(2):116–27. doi:10.1002/em.2850130206 PMID:2645131

DFG (2019). List of MAK and BAT values 2019: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 55, Deutsche Forschungsgemeinschaft (German Research Foundation). Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9783527826155, accessed May 2020.

Dračínská H, Miksanová M, Svobodová M, Smrcek S, Frei E, Schmeiser HH, et al. (2006). Oxidative detoxication of carcinogenic 2-nitroanisole by human, rat and rabbit cytochrome P450. *Neuro Endocrinol Lett*. 27(Suppl 2):9–13. PMID:17159769

ECHA (2019a). Substance information. 2-nitroanisole. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.001.866.

ECHA (2019b). Summary of classification and labelling. 2-nitroanisole. Harmonised classification – Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation). Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/61842, accessed 12 January 2021.

Esmaeili A, Schlatterer K, Demirhan I, Schlatterer B, Nauck M, Chandra P, et al. (2006). Tumorigenic potential and the molecular mechanism of the carcinogenic effect exerted by 2-nitroanisole. *Anticancer Res*. 26(6B):4203–12. PMID:17201134

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*. 10(Suppl 10):1–175. doi:10.1002/em.2850100502 PMID:3319609

Greenpeace (2017). Hazardous chemical releases at large: an investigation at the Lianyungang Chemical Industrial Park, Jiangsu Province, China. Available from: https://www.greenpeace.org/static/planet4-eastasia-stateless/2019/11/f5588785-f5588785-big-bang-report_en-final.pdf.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*. 5(Suppl 1):1–142. doi:10.1002/em.2860050703 PMID:6365529

Hengstler JG, Fuchs J, Bolm-Audorff U, Meyer S, Oesch F (1995). Single-strand breaks in deoxyribonucleic acid in fire fighters accidentally exposed to *o*-nitroanisole and other chemicals. *Scand J Work Environ Health*. 21(1):36–42. doi:10.5271/sjweh.6 PMID:7784863

Heudorf U, Neumann HG, Peters M (1994). [Accident in the Hoechst AG company 22 February 1993. 2. Public health evaluation]. *Gesundheitswesen*. 56(7):405–10. [German] PMID:7919705

HSDB (2011). 2-Nitroanisole. Hazardous Substances Data Bank. PubChem. Bethesda (MD), USA: United States National Library of Medicine, National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/5186, accessed 18 February 2020.

Huang J, Zhou L, Xun Z, Wang Q, Lin S, Guo X, et al. (2017). Simultaneous determination of seven nitrogen-containing phenyl ethers in cosmetics by gas chromatography with mass spectrometry and dispersive solid-phase extraction. *J Sep Sci*. 40(8):1718–23. doi:10.1002/jssc.201601192 PMID:28230323

IARC (1996). 2-Nitroanisole. In: Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65:369–80. Available from: https://publications.iarc.fr/83 PMID:9097112

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: https://publications.iarc.fr/123 PMID:23189753

IFA (2019). 2-Nitroanisole. GESTIS substance database. Germany: Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance). Available from: https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp, accessed 2 March 2020.

ILO (2017). 2-Nitroanisole. Physical and chemical information. International Labour Organization. Available from: https://www.ilo.org/dyn/icsc/showcard.display?p_card_id=1520&p_edit=&p_version=2&p_lang=en.

Irwin RD, Chhabra R, Eustis S, Pinter A, Prejean JD (1996). Tumors of the bladder, kidney, and intestine of F344 rats and liver of B6C3F$_1$ mice administered *o*-nitroanisole in feed. *Fundam Appl Toxicol*. 30(1):1–12. doi:10.1006/faat.1996.0037 PMID:8812201

JETOC (1996). *o*-Nitroanisole. Mutagenicity test data of existing chemical substances based on the Toxicity Investigation System of the Industrial Safety and Health Law. Judgement for chromosomal aberration in CHL; positive. Tokyo, Japan: Chemical Industry Ecology-Toxicology and Information Center. Available from: https://anzeninfo.mhlw.go.jp/user/anzen/kag/pdf/C/C91-23-6.pdf.

Lewis RS (1993). Hawley's condensed chemical dictionary. 12th ed. New York (NY), USA: Van Nostrand Reinhold Co.; pp. 824–5.

Liang J, Ning XA, Song J, Lu X, Sun J, Zhang Y (2019). Treatment of 3,3-dimethoxybenzidine in sludge by advance oxidation process: Degradation products and toxicity evaluation. *J Environ Manage*. 238:102–9. doi:10.1016/j.jenvman.2018.11.135 PMID:30849594

LookChem (2020). 2-Nitroanisole. Hangzhou, China: LookChem Network Technology Co., Ltd. Available from: https://www.lookchem.com/newsell/search.aspx?p=2&key=91-23-6&businessType=Manufacturers.

Mikšanová M, Novák P, Frei E, Stiborová M (2004a). Metabolism of carcinogenic 2-nitroanisole in rat, rabbit, porcine and human hepatic cytosol. *Collect Czech Chem Commun*. 69(3):589–602. doi:10.1021/tx0499721

Mikšanová M, Šulc M, Rýdlová H, Schmeiser HH, Frei E, Stiborová M (2004b). Enzymes involved in the metabolism of the carcinogen 2-nitroanisole: evidence for its oxidative detoxication by human cytochromes P450. *Chem Res Toxicol*. 17(5):663–71. doi:10.1021/tx0499721 PMID:15144223

Miller MJ, Sipes IG, Perry DF, Carter DE (1985). Pharmacokinetics of *o*-nitroanisole in Fischer 344 rats. *Drug Metab Dispos*. 13(5):527–31. PMID:2865097

Naiman K, Dračínská H, Dračínský M, Martínková M, Martínek V, Hodek P, et al. (2008). Cytochrome P450-mediated metabolism of *N*-(2-methoxyphenyl)-hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Interdiscip Toxicol*. 1(3–4):218–24. doi:10.2478/v10102-010-0045-8 PMID:21218119

Naiman K, Frei E, Stiborová M (2010). Identification of rat cytochromes P450 metabolizing *N*-(2-methoxyphenyl) hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Neuro Endocrinol Lett*. 31(Suppl 2):36–45. PMID:21187827

Naiman K, Martínková M, Schmeiser HH, Frei E, Stiborová M (2011). Human cytochrome-P450 enzymes metabolize *N*-(2-methoxyphenyl)hydroxylamine, a metabolite of the carcinogens *o*-anisidine and *o*-nitroanisole, thereby dictating its genotoxicity. *Mutat Res*. 726(2):160–8. doi:10.1016/j.mrgentox.2011.09.010 PMID:21946300

NCBI (2020). 2-Nitroanisole. PubChem Compound Summary for CID 7048. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/7048, accessed 1 October 2020.

Nesslany F, Zennouche N, Simar-Meintières S, Talahari I, Nkili-Mboui EN, Marzin D (2007). In vivo comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds. *Mutat Res*. 630(1–2):28–41. doi:10.1016/j.mrgentox.2007.02.010 PMID:17507283

NTP (1993). NTP toxicology and carcinogenesis studies of *o*-nitroanisole (CAS No. 91-23-6) in F344 rats and B6C3F$_1$ mice (feed studies). *Natl Toxicol Program Tech Rep Ser*. 416:1–482. PMID:12616295

NTP (2016). *o*-Nitroanisole. Report on carcinogens, 14th Ed. Research Triangle Park (NC), USA: U.S. Department of Health and Human Services, Public Health Service. Available from: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/nitroanisole.pdf, accessed February 2020.

Reifferscheid G, Heil J (1996). Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. *Mutat Res*. 369 3–4:129–45. doi:10.1016/S0165-1218(96)90021-X PMID:8792833

Saalo A, Soosaar A, Mikkola J (2016). ASA 2014. Syöpäsairauden vaaraa aiheuttaville aineille ja menetelmille ammatissaan altistuneiksi ilmoitetut Suomessa. Helsinki, Finland: Työterveyslaitos, Available from: http://www.julkari.fi/bitstream/handle/10024/131073/ASA_2014.pdf?sequence=1&isAllowed=y, accessed 16 November 2020. [Finnish]

Shimizu M, Yano E (1986). Mutagenicity of mono-nitrobenzene derivatives in the Ames test and rec assay. *Mutat Res*. 170(1–2):11–22. doi:10.1016/0165-1218(86)90077-7 PMID:3083245

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of

carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Sone M, Toyoda T, Cho YM, Akagi JI, Matsushita K, Mizuta Y, et al. (2019). Immunohistochemistry of γ-H2AX as a method of early detection of urinary bladder carcinogenicity in mice. *J Appl Toxicol*. 39(6):868–76. doi:10.1002/jat.3775 PMID:30701581

Starek A (2019). 2-Nitroanisole. Documentation of occupational exposure limits (OELs). *Podstawy i Metody Oceny Środowiska Pracy*. 2(100):83–99. doi:10.5604/01.3001.0013.2534

Stiborová M, Miksanová M, Smrček S, Bieler CA, Breuer A, Klokow KA, et al. (2004). Identification of a genotoxic mechanism for 2-nitroanisole carcinogenicity and of its carcinogenic potential for humans. *Carcinogenesis*. 25(5):833–40. doi:10.1093/carcin/bgh061 PMID:14729594

Stiborová M, Naiman K, Martínková M, Martínek V, Svobodová M, Schmeiser HH, et al. (2009). Genotoxic mechanisms for the carcinogenicity of the environmental pollutants and carcinogens *o*-anisidine and 2-nitroanisole follow from adducts generated by their metabolite *N*-(2-methoxyphenyl)-hydroxylamine with deoxyguanosine in DNA. *Interdiscip Toxicol*. 2(1):24–7. doi:10.2478/v10102-009-0004-4 PMID:21217841

Svobodová M, Dračínská H, Martínková M, Hudeček J, Hodek P, Frei E, et al. (2008). Oxidation of carcinogenic 2-nitroanisole by rat cytochromes P450 - similarity between human and rat enzymes. *Interdiscip Toxicol*. 1(2):182–5. doi:10.2478/v10102-010-0035-x PMID:21218109

Svobodová M, Martínková M, Dračínská H, Frei E, Stiborová M (2009). Rat cytochromes P450 oxidize 2-nitrophenol, a human metabolite of carcinogenic 2-nitroanisole. *Neuro Endocrinol Lett*. 30(Suppl 1):46–51. PMID:20027144

Toyoda T, Cho YM, Akagi J, Mizuta Y, Hirata T, Nishikawa A, et al. (2015). Early detection of genotoxic urinary bladder carcinogens by immunohistochemistry for γ-H2AX. *Toxicol Sci*. 148(2):400–8. doi:10.1093/toxsci/kfv192 PMID:26338220

Wada K, Katoh Y, Ohnuma-Koyama A, Takahashi N, Yamada M, Matsumoto K (2017). 2-Nitroanisole-induced oxidative DNA damage in *Salmonella typhimurium* and in rat urinary bladder cells. *Mutat Res Genet Toxicol Environ Mutagen*. 816–817:18–23. doi:10.1016/j.mrgentox.2017.03.002 PMID:28464992

Wada K, Yoshida T, Takahashi N, Matsumoto K (2014). Effects of seven chemicals on DNA damage in the rat urinary bladder: a comet assay study. *Mutat Res Genet Toxicol Environ Mutagen*. 769:1–6. doi:10.1016/j.mrgentox.2014.04.015 PMID:25344106

Xia E, Xia E, Yue Y, Xia N (2010). Method for preparing *o*-nitroanisole. Publication of CN101643419A. Google patents. Available from: https://patents.google.com/patent/CN101643419A/en.

ANILINE AND ANILINE HYDROCHLORIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

(a) Aniline

Chem. Abstr. Serv. Reg. No.: 62-53-3

EC No.: 200-539-3

IUPAC systematic name: aniline

Synonyms and abbreviations: benzenamine; phenylamine; aminobenzene; aminophen; aniline oil.

(b) Aniline hydrochloride

Chem. Abstr. Serv. Reg. No.: 142-04-1

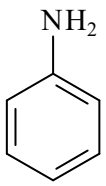
EC No.: 205-519-8

IUPAC systematic name: aniline hydrochloride

Synonyms: aniline chloride; anilinium chloride; benzenamine hydrochloride; aniline. HCl; phenylamine hydrochloride; phenylammonium chloride.

1.1.2 Structural and molecular formulae, and relative molecular mass

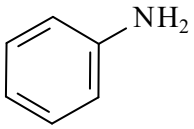
(a) Aniline



Molecular formula: C_6H_7N

Relative molecular mass: 93.13 (NCBI, 2020a).

(b) Aniline hydrochloride



• HCl

Molecular formula: C_6H_8ClN

Relative molecular mass: 129.59 (NCBI, 2020b).

1.1.3 Chemical and physical properties of the pure substance

Aniline is a basic compound and will undergo acid–base reactions. Aniline and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

(a) Aniline

Description: aniline appears as a yellowish to brownish oily liquid with a musty fishy odour (NCBI, 2020a), detectable at 1 ppm [3.81 mg/m³] (European Commission, 2016; NIOSH, 2019)

Boiling point: 184.1 °C (NCBI, 2020a)

Melting point: −6 °C (NCBI, 2020a)

Flash point: 70 °C, closed cup (NCBI, 2020a)

Density: 1.02 g/cm³ (NCBI, 2020a)

Vapour density: 3.2 (air = 1) (NCBI, 2020a)

Vapour pressure: 40 Pa at 20 °C; 0.67 mm Hg at 25 °C (NCBI, 2020a)

Solubility: 36 g/L at 25 °C in water; soluble in alcohol, ether, benzene, ethyl ether, and carbon tetrachloride (NCBI, 2020a)

Dissociation constant: pK_a, 4.6 (at 25 °C; aniline conjugate acid) (NCBI, 2020a)

Octanol/water partition coefficient (P): log K_{ow}, 0.9 (US EPA, 2020a)

Conversion factor: 1 ppm = 3.81 mg/m³ (NIOSH, 2019) [calculated from: mg/m³ = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

(b) Aniline hydrochloride

Description: aniline hydrochloride appears as a white to greenish coloured crystalline solid (NCBI, 2020b)

Boiling point: 245 °C (NCBI, 2020b)

Melting point: 198 °C (NCBI, 2020b)

Flash point: 193 °C, open cup (NCBI, 2020b)

Density: 1.22 g/cm³ (NCBI, 2020b)

Vapour density: 4.46 (air = 1) (NCBI 2020b)

Solubility: soluble in water: 1070 g/L at 20 °C (NCBI, 2020b)

Dissociation constant: pK_a, 4.6 (at 25 °C; aniline conjugate acid)

Octanol/water partition coefficient (P): log $K_{ow,}$ 0.936, predicted median (US EPA, 2020b)

Conversion factor: 1 ppm = 5.3 mg/m³ [calculated from: mg/m³ = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

1.2 Production and use

1.2.1 Production process

Historically, aniline was produced by Otto Unverdorben in 1826 by dry distillation from the leaves of the indigo plant, genus *Indigofera* (most probably *Indigofera tinctoria*). Aniline was isolated from coal tar in 1834. Aniline was first synthesized in 1842 by reducing nitrobenzene with sodium sulfates (Zinin reaction; Zinin, 1842) and first manufactured commercially in 1847 (Kouris & Northcott, 1963). Aniline was made by the Bechamp process of reduction of nitrobenzene with iron and hydrochloric acid and, until 1966, by amination of chlorobenzene with ammonia (IARC, 1982). Presently, aniline is produced by catalytic reduction of nitrobenzene. The process can be divided into three main parts: nitrobenzene hydrogenation, dehydration, and purification. Nitrobenzene is converted to aniline (nearly 100%) in a single pass by feeding it with hydrogen into a reactor containing the catalyst metal. The excess hydrogen is removed, and the liquid product is dehydrated. The crude aniline is purified by distillation. The final product obtained is aniline with a purity of > 99.95 wt% containing less than 0.1 ppm [0.1 mg/kg] of nitrobenzene by weight. The conversion of nitrobenzene to aniline remains the most common pathway for aniline production. An alternative production pathway for aniline uses phenol and ammonia as the starting raw materials (Intratec Solutions, 2016).

Aniline hydrochloride is prepared by reacting aniline vapour and hydrogen chloride gas at temperatures of > 250 °C (Holt & Daudt, 1935).

1.2.2 Production volume

Aniline is a High Production Volume chemical (OECD, 2009; US EPA, 2020c). It is used as a starting material in several industries, including for the manufacture of a variety of plastics, rubber additives, colourants, and drugs (Käfferlein et al., 2014).

Global capacity for aniline production was 4.98 million tonnes per year in 2006, with the major producers being in western Europe, the USA, and the Asia-Pacific region (ICIS, 2008; see Table 1.1). Total volume for manufacture and use in the European Union is 1 million to 10 million tonnes per year (ECHA, 2020a). In an updated assessment report on aniline under the Canadian Environmental Protection Act of 1999 (Health Canada, 2011), Health Canada reported that more than 28 tonnes of aniline and its salts were synthesized as a by-product of chemical manufacturing in Canada in 2007. Additionally, between 13 and 48 tonnes of aniline and aniline salts were imported into Canada in 2000–2007 (Health Canada, 2011). During 2014–2019, global consumption of aniline grew at an average annual rate of 4.8%, reaching more than 6.7 million tonnes in 2019 (IHS Markit, 2020).

North-eastern Asia was the largest producer of aniline during 2013–2018, accounting for more than half of the global production of aniline. Western Europe and the USA were the next-largest suppliers. Most increases in production capacity for aniline were in China during 2013–2018, along with expansions in the Middle East and western Europe. In 2018, China accounted for almost 50% of the world's aniline production capacity, followed by western Europe and the USA (IHS Markit, 2019).

The national aggregate production volume in the USA in 2011 was approximately 1956 million pounds [887 000 tonnes], whereas in 2012–2015, it was between 1000 million and 5000 million pounds [450 000–2 300 000 tonnes] (ChemView, 2016). According to another source, annual aniline production in the USA since 1990 has varied between 449 000 and 1 065 000 tonnes; in 2019, it was 845 000 tonnes (Statista, 2020a). The production volume of aniline in India during 2013–2019 varied between 34 470 and 48 230 tonnes (Statista, 2020b).

Table 1.1 Global production of aniline in 2006, by geographical region

Region	Aniline production (tonnes per year)
Western Europe	1 620 000
USA	1 380 000
Asia-Pacific (excluding Japan)	1 150 000
Japan	474 000
Eastern Europe	316 500
Latin America	70 000
Asia or Middle East	64 000

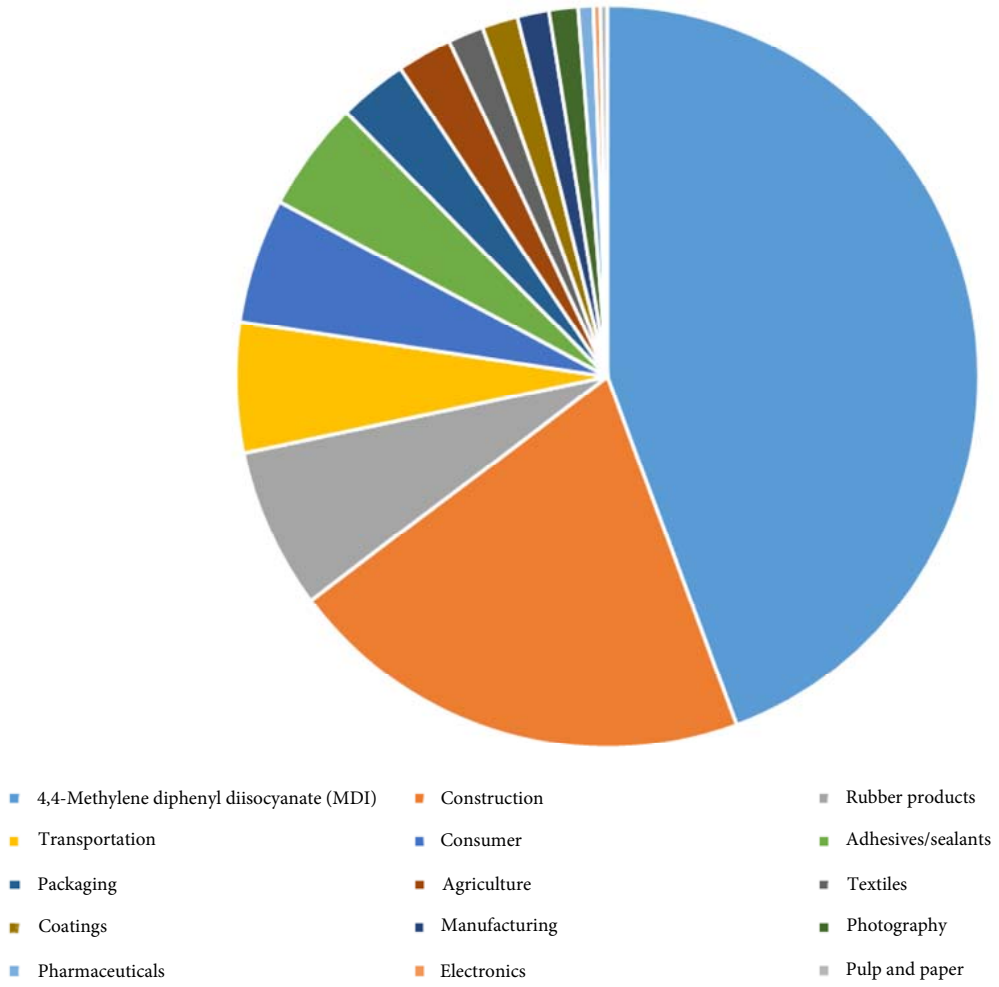
Data from ICIS (2008).

1.2.3 Uses

In the chemical industry, aniline is used as a parent substance for the synthesis of many compounds, including isocyanates, dyes and pigments, antioxidants, and accelerators in rubber processing, pharmaceuticals, varnishes, perfumes, photographic chemicals, herbicides, and fungicides (aniline is no longer approved for pesticide use in the European Union) (ICIS, 2008; European Commission, 2016; see Fig. 1.1).

China is the world's largest consumer of aniline and accounted for 37% of global demand in 2018. Western Europe is the second-largest consumer of aniline, accounting for 23% of global aniline demand in 2018. The USA follows as the third-largest consumer, accounting for 17% of global aniline consumption (IHS Markit, 2019). [The Working Group noted that, despite the major demand for aniline in Asia, few data

Fig. 1.1 Global consumption of aniline, by industry



From Independent Commodity Intelligence Service (ICIS, 2008). Reproduced with permission from www.icis.com.

on uses and exposure levels were available for this region.]

(a) 4,4-Methylene diphenyl diisocyanate

Production of 4,4-methylene diphenyl diisocyanate (MDI) accounts for more than 90% of aniline use (IHS Markit, 2019). Aniline and formaldehyde react in an acid-catalysed reaction, which is followed by liquid-phase phosgenation of diphenylmethane diamine to MDI (Lynch & Ryan, 2012). MDI is used to produce polyurethane foam. Rigid polyurethane is used in walls and roofs of new residential and commercial constructions as well as in the renovation of older buildings for insulating purposes. Flexible polyurethane foam is primarily used in furniture and transportation (IHS Markit, 2019; American Chemistry Council, 2020; GlobeNewswire, 2020).

(b) Rubber processing

Production of chemicals used in rubber processing is the second largest sector of aniline use (Amini & Lowenkron, 2003). Among the rubber-processing agents made from aniline are several important vulcanization accelerators and antioxidants, including aldehyde–aniline condensates (e.g. *N*-butyraldehyde condensate), guanidines (e.g. 1,3-diphenylguanidine), *N*-phenyl-2-naphthylamine (IARC, 1978), thiazoles (e.g. 2,2′-dithiobisbenzothiazole and other derivatives of 2-mercaptobenzothiazole, MBT), and a variety of derivatives of diphenylamine (which is made from aniline) (IARC, 1982). In new and used tyres, aniline was detected in concentrations near the detection limit of 100 mg/kg rubber (ECB, 2004).

(c) Dyes and colouring agents

Some 174 dyes can be prepared from aniline and more than 700 dyes can be prepared from aniline derivatives; however, very few are produced in commercially significant quantities (Northcott, 1978). Of 33 typical dyes derived from aniline (Kouris & Northcott, 1963), only 16 were reported to be produced commercially in the USA in 1979. Production data were published for six of these; those produced in the largest volumes were Basic Orange 2 (275 tonnes), Solvent Yellow 14 (159 tonnes), Acid Red 1 (128 tonnes) and Acid Black 1 (169 tonnes). Another dye made from aniline in significant quantities is Direct Orange 102: production by five companies in the USA in 1979 amounted to 192 tonnes (US International Trade Commission, 1980a; IARC, 1982). As of 2011, 50 000 tons [45 000 tonnes] of Indigo Blue have been synthesized per year, of which 95% was used to dye the more than 4 billion denim garments manufactured annually (Hsu et al., 2018; Nature, 2018). [The Working Group noted that no information was found on occupational or consumer-product exposure levels related to production and use of denim garments.]

(d) Pharmaceutical products

Aniline is used as an intermediate in the production of a variety of pharmaceutical products. A major derivative is acetanilide, which is used in the production of phenazopyridine and several sulfa drugs, including sulfafurazole and sulfamethoxazole (previously evaluated as IARC Group 3, *not classifiable as to its carcinogenicity to humans*, by the *IARC Monographs* programme; IARC, 1980), and also as a precursor in the synthesis of penicillin and other drugs (TMR, 2020).

(e) Consumer uses

Aniline is used in many products, including fabrics, textiles and apparel (e.g. clothing, mattresses, curtains or carpets, textile toys), leather (e.g. gloves, shoes, purses, furniture), paper (e.g. tissues, feminine hygiene products, diapers, books, magazines, wallpaper) and plastic (e.g. food packaging and storage, toys, mobile phones) (ECHA, 2020b). In a Danish survey of hobby products used by children, aniline was detected in samples of certain green and pink marker pens at concentrations of 0.22 and 0.11 mg/g (0.022% and 0.011%), respectively (Hansen et al., 2008). A study of inks in commonly used pens and markers conducted by Health Canada indicated that the level of aniline was below the limit of quantification (LOQ, 67 mg/kg) in all markers intended for children. Overall, inks in 94% of the 86 pens and markers sampled contained aniline at levels below the LOQ. Additionally, samples of black printer ink tested in Canada did not contain aniline at significant levels (Health Canada, 2011).

Table 1.2 Analytical methods for the quantitative determination of aniline

Sample matrix	Analytical technique	Limit of detection	Limit of quantification	Reference
Air	GC-FID	0.03 mg/m³	0.09 mg/m³	OSHA (1994)
Air	GC-FID	0.004 mg/sample	NR	NIOSH (1998)
Ground water	GC-MS	NR	10 µg/L	NEMI (1998)
Soil/sediment	GC-MS	NR	660 µg/kg	NEMI (1998)
Water	GC-MS	10 µg/L	NR	NEMI (2001)
River and tap water	Thin silica-coated and assembled silver nanoparticles-SERS	93 ppm [0.35 mg/L]	NR	Cha et al. (2017)
Water pipe smoke	LC-MS/MS	0.31 mg/m³	0.88 mg/m³	Schubert et al. (2011)
Mainstream cigarette smoke	HPLC-MS/MS	0.52 ng/cigarette	1.72 ng/cigarette	Xie et al. (2013)
Surface water	CZE	0.29 µg/L	NR	Liu et al. (2012)
River and sea water	HPLC-MS/MS	NR	0.016 µg/L	Furukawa et al. (2017)
Food contact materials	HPLC-MS/MS	0.8 µg/kg	2.6 µg/kg	Trier et al. (2010)
Urine	HPLC-EC	1.4 µg/L	NR	NIOSH (2003)
Blood	HPLC-FD	NR	3 µg/L	Chen et al. (2015)
Blood	GC-MS	1 ng/L	NR	Lewalter et al. (2012)

CZE, capillary zone electrophoresis; EC, electrochemical detector; FD, fluorescence detector; FID, flame ionization detector; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NR, not reported; ppm, parts per million; SERS, surface-enhanced Raman scattering.

(f) Tattoo inks

Aniline is an ingredient in tattoo inks (Hauri et al., 2005). Most tattoo inks on the European Union market are manufactured in the USA (Piccinini et al., 2015). At present, since the tattoo ink market represents only a marginal fraction of the global production of colourants, the pigments used in tattoo inks are not specifically produced for such purposes. More than 100 colourants and 100 additives are in use in tattoo inks, with numerous impurities found (Piccinini et al., 2016). Approximately 162 800 L of tattoo inks and permanent make-up products are estimated to be placed on the market in the European Economic Area each year (ECHA, 2017).

1.3 Measurement and analysis

An overview of the analytical methods for quantitative determination of aniline in various sample types is provided in Table 1.2. Several methods have been reported for the analysis of aniline, its derivatives, and other primary aromatic amines in various matrices. The most common techniques applied currently for quantitative determination of aniline and its derivatives are based on gas chromatography (GC) or high-performance liquid chromatography coupled to different types of detectors, depending on the type of sample matrix and required sensitivity.

Primary aromatic amines and colourants have been determined in polyurethane, nylon, and textile toys by extraction in water for 30 minutes at 40 °C, followed by acidification and filtration. The filtered extract was analysed with liquid chromatography-tandem mass spectrometry and the reported detection limit was

0.2 µg/g (limit of quantification not reported). Residual aniline was detected in one toy (0.4 µg/g) (the number of toys tested was not stated) (Abe et al., 2016).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

(a) Water and sediments

The available studies show that aniline is photolytically degraded within about 4–11 hours under spring or summer conditions in the top layer of surface waters (ECHA, 2019a).

Aniline can enter the environment from anthropogenic sources during any stage in the production, storage, transport, use, and disposal of aniline itself or of aniline-containing materials, or possibly by atmospheric and waterborne transport from other countries (Government Canada, 1994). No aniline release data were reported in the Canadian National Pollutant Release Inventory in 2000–2007, indicating that facilities were manufacturing, processing, or otherwise using less than 10 tonnes annually (Health Canada, 2011).

Data for aniline are available for drinking-water, groundwater, fresh surface water, and effluents. Aniline has been reported at concentrations of 0.5–3.7 µg/L in surface water samples taken in the 1970s from some rivers in Germany (Neurath et al., 1977) and in the Netherlands (Greve & Wegman, 1975; Meijers & van der Leer, 1976). The aniline concentrations found in Germany were attributed to industrial chemical waste (Herzel & Schmidt, 1977). Aniline has been identified in the USA in the late 1970–80s in industrial effluents from oil shale recovery and oil refineries, and from chemical and coal conversion plants (Shackelford & Keith, 1976; Hushon et al., 1980). In surveys of groundwater conducted in Ontario, Canada, in the 1980s and 1990s, the concentration of aniline in samples collected near a landfill site and in the vicinity of a chemical company was 0.01 mg/L and up to 300 mg/L, respectively. Levels of aniline ranged up to 20 000 mg/L in samples of dense non-aqueous–phase liquid collected from an area located beneath former containment areas of a chemical plant in Ontario, Canada (Government Canada, 1994). Point source pollution assessments for aniline in groundwater have been modelled for the United Kingdom to be 3.6 mg/L and 0.04 mg/L at 50 m and at 100 m, respectively. Concentrations of aniline ranged from 0 to 0.18 µg/L in drinking-water, 0 to 720 µg/L in groundwater, and 0 to 12 µg/L in fresh surface water, whereas aniline was identified but not quantifiable in effluents. No data were available for aniline in marine surface waters (Rockett et al., 2014). In samples collected from a Chinese reservoir polluted by aniline, maximum aniline content was 1.9 mg/L in water (13 samples) and 0.06 mg/kg in fish (12 samples) (He et al., 2014).

In sediment and soil, there are two competing processes: biodegradation and the formation of non-hydrolysable covalent bonds to humic substances. This binding leads to long biodegradation half-lives for bound aniline of 350 and 3500 days for soil and sediment, respectively. In the European Commission Risk Assessment Report, it was assumed that approximately 80% of aniline is covalently bound in soil (European Commission, 2004). Measurements in sediment tests gave a similar observation (ECHA, 2019a).

(b) Food, beverages, and animal feeds

Information on levels of aniline in food for the general population was available from a study conducted in Germany more than 40 years ago; aniline was quantified in samples of selected fruit and vegetables (range, < 0.1–30.9 mg/kg), with the highest concentration found in carrots (30.9 mg/kg) (Neurath et al., 1977). Among animal feeds, rapeseed cake contained aniline at 120 mg/kg (Neurath et al., 1977). In the early 1990s, average concentrations of aniline were reported for apples (0.16 mg/100 g), cabbage

(0.25 mg/100 g), carrots (3.1 mg/100 g), and garlic (1.00 mg/100 g) (Duke, 2004). Other food sources in which aniline was expected but was not quantified included: cows' milk, tea (black, green, herbal, red), and corn (Duke, 2004). Aniline has also been found as a volatile component of black tea (Vitzthum et al., 1975). No aniline was detected in the 23 vegetable samples from the 2005 Canadian Total Diet Study and, of the 16 fruit samples, it was detected only in apples at an average concentration of 0.278 mg/kg (Cao et al., 2009). In crops treated with the pesticide buprofezin, aniline is generated during specific food-processing methods. Aniline was below the LOQ (0.01 mg/kg) in all processed fractions containing tomato (juice, puree, ketchup, paste, and canned tomato). Tomato puree, ketchup, and paste contained aniline at levels above the limit of detection (LOD) (0.0029–0.0036 mg/kg). No data were available on the possible presence of aniline in other food commodities derived from fruit treated in accordance with authorized uses of buprofezin (e.g. citrus, pome fruits, stone fruits, grapes, strawberries). As a main degradation product of the herbicide desmedipham, aniline is a relevant impurity, with a maximum content of 0.5 g/kg. The European Food Safety Authority (EFSA) concluded, based on the available data, that dietary exposure of consumers and/or livestock to residues containing free and/or conjugated aniline could not be excluded (EFSA, 2015, 2018).

(c) Tobacco and tobacco smoke

Zhu & Aikawa (2004) investigated the presence of aniline in indoor air in selected homes in Canada, and recorded concentrations of 0.011 μg/m^3 for non-smokers and 0.034 μg/m^3 for smokers. [The Working Group noted that this is four orders of magnitude lower than mean levels found in occupational settings.] Luceri et al. (1993) reported aniline concentrations of 35 ng/m^3 in an office with one smoker, 75 ng/m^3 in an office with two smokers, 150 ng/m^3 in a card-playing room, 170 ng/m^3 in a non-smoking train compartment, and 190 ng/m^3 in a hairdresser's salon. Palmiotto et al. (2001) reported the highest values of aniline in two hospital wards (351 and 483 ng/m^3), and other air concentrations obtained were 400–500 ng/m^3 in hospital waiting rooms (n = 2), 60–1650 ng/m^3 in a bar, two clubs, and a discotheque (n = 4), and 10–100 ng/m^3 in office spaces (n = 7).

Average aniline concentrations from cigarettes of nine different brands were 430–9570 ng/cigarette in mainstream smoke, and 5250–15 930 ng/cigarette in sidestream smoke (Goniewicz & Czogała, 2005). Another analysis of aniline in cigarette smoke also showed that levels of aniline were about 20–80 times higher in sidestream smoke than in mainstream smoke (120–809 ng/cigarette for mainstream versus 9467–18 100 ng/cigarette for sidestream smoke) (Luceri et al., 1993). Xie et al. (2013) measured aniline in mainstream cigarette smoke and found aniline concentrations in the range of 129 to 838 ng/cigarette (depending on method used and cigarettes chosen).

1.4.2 Occupational exposure

The main scenarios for occupational exposure to aniline are during the production and distribution of aniline, when used as a chemical intermediate in the production of other chemicals and products, and when handling and using products containing residual aniline. The Working Group searched for information in publicly available exposure registries and identified data only for the USA and Finland. In the United States National Occupational Exposure Survey (NOES), nearly 42 000 workers (15 000 women) were identified as having potential aniline exposure in 1981–1983, with the majority in machine-operator occupations. In the NOES survey, 82 000 workers (47 000 women) were identified as potentially exposed to aniline hydrochloride in 1981–1983, the majority in assembly (notably electrical and

electronic equipment assembly), soldering, or janitorial and cleaning occupations (CDC, 2011). [The Working Group noted that aniline hydrochloride can be used as a flux in brazing and/or soldering operations (Horowitz & Hallock, 2019), but no information in the NOES was identified to support the reported uses of aniline hydrochloride. The Working Group found no information on current uses.] In Finland, fewer than 100 workers were identified as having exposure to aniline or its salts (Saalo et al., 2016). [The Working Group noted that Finland is not involved in aniline production; most of these workers were in research and development and other non-manufacturing settings.] The available quantitative data on aniline exposure were insufficient to draw any conclusions on air concentrations generated, the degree of dermal exposure, and amount absorbed, for all these exposure scenarios. These main scenarios together with the available data on human exposure to aniline are described below. [The Working Group noted that data on occupational exposure to aniline hydrochloride were lacking.]

(a) Manufacture and distribution of aniline

Aniline is synthesized in closed systems, but there is potential for exposure during sampling (mainly enclosed) and analysis of the product, and during checks on fill levels, as well as when the system is opened for cleaning, maintenance, and repair work (ECB, 2004). In the risk assessment of aniline performed by the European Chemicals Bureau, exposure measurements for aniline production were provided by the manufacturers from three production sites (countries not specified) (ECB, 2004). Some additional information was reported by the Health and Safety Executive in the United Kingdom (HSE, 1997). The exposure estimates are given in Table 1.3.

For the hydrogenation method of production of aniline, a geometric mean (GM) exposure to aniline for all jobs combined (personal, 8-hour time-weighted average, TWA) was reported as below 0.04 mg/m^3 in the unspecified western European countries (during 1990–96), whereas the arithmetic mean was 0.45 mg/m^3 (range, ≤ 2.8 mg/m^3) in the United Kingdom (1991–1996) (ECB, 2004; Table 1.3). Mean aniline exposure was highest in production and pilot plants (0.08 mg/m^3 and 0.07 mg/m^3, respectively), whereas the highest reported exposure measurement (2.7 mg/m^3) was measured in the workshop (no information on task was given). Whether this is representative of other production sites and time periods is not known. Nevertheless, aniline exposure measurements provided by the producers for operations performed during the 1980s indicate 8-hour TWA exposures ranging between non-detectable (detection limit not specified) and up to 1.4 ppm [5.3 mg/m^3] (Van Wagenen, 1985). The highest levels were reported for "maintenance" and "operations" (1.4 ppm [5.3 mg/m^3] and 0.86 ppm [3.3 mg/m^3], respectively). No short-term measurements were identified. [The Working Group noted that sampling times were infrequently reported in these publications. Whether any of the workers performed work with a potential for exposure as described above (e.g. opening of the vessels) was not reported.]

Urinary concentrations of aniline have been reported for 43 chemical-plant workers primarily synthesizing and processing aniline and 4-chloroaniline (Riffelmann et al., 1995; Table 1.4). The median urinary concentration of aniline was 7.7 µg/L and 9.6 µg/L for smokers and non-smokers, respectively. Although the range of aniline concentrations released from haemoglobin appeared to be wider among non-smokers (200–7000 ng/L) than smokers (320–1100 ng/L), the concentration did not differ statistically significantly between the two. The concentration of 4-aminobiphenyl released from haemoglobin differed statistically significantly between smokers and non-smokers (median, 17.5 ng/L versus 7.0 ng/L, $P = 0.0001$), being lowest among the non-smokers (Riffelmann et al., 1995).

Table 1.3 Occupational exposure to aniline in air: personal measurements

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Production of aniline							
ECB (2004)[a]	1990–1994, NR	Aniline production, reduction using H_2	Workplace or occupational group not defined	27	8-h TWA	NR	< 0.8
	1990–1996, NR	Aniline production, reduction using H_2	All workplaces	238		GM, < 0.04	< 1
			Production	53		GM, 0.08	0–0.9
			Pilot plants	9		GM, 0.07	0–0.56
			Filling area/store	3		NR	0.01–0.4
			Workshop	3		NR	0.02–2.7
ECB (2004), HSE (1997)	1991–1996, UK	Aniline production, reduction, using H_2	NR	152	8-h TWA	AM, 0.45	≤ 2.8
ECB (2004), HSE (1997)	1991–1996, UK	Maintenance	Maintenance	29	8-h TWA	AM, 0.4	≤ 1.8
ECB (2004)[a]	1990–1994, NR	Aniline production, reduction using Fe (Bechamp process)	NR	9	8-h TWA	NR	< 0.8
				6		NR	0.95–1.5
Van Wagenen (1985)[a]	1984, USA	Tank car loading	Operator (task-based)	1	83 min	< 0.4 ppm [< 1.52]	NR
			Area sample (1 ft [0.3 m] downward of the open tank hatch)	1		2 ppm [7.62]	NR
	1982, USA Data provided by USS Chemicals	Production of aniline (by high-pressure catalysed reaction of phenol with ammonia)	Aniline operator (field)	8	8-h samples	All non-detectable	NR
			Aniline technician	3			
			Aniline foreman	3			
			Aniline maintenance man (working in processing area)	12			
			Aniline chemist (laboratory)	3			
			Aniline analyst (laboratory)	3			

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Van Wagenen (1985)[a] (cont.)	1983, USA Data provided by SOCMA, 5 producers (1982 operations)		Operations Maintenance Quality control Warehouse/shipping Other (incl. supervisory and engineering personnel)	184 215 112 47 78	8-h TWA	NR	0.001–0.86 ppm [0.004–3.28] 0.001–1.4 ppm [0.004–5.33] 0.001–0.4 ppm [0.004–1.5] 0.01–0.6 ppm [0.04–2.3] 0.01 ppm [0.04]
Manufacture of rubber chemicals							
Hanley et al. (2012)	1990, USA	Rubber-chemical manufacturing plant Data collected by NIOSH	All jobs combined	45		GM (GSD), 0.032 (1.83) ppm [0.12 (6.97)]	0.014–0.19 ppm [0.053- 0.72]
			Antioxidant process	17		GM (GSD), 0.041 (2.00) ppm [0.16 (7.62)]	0.016–0.19 ppm [0.061–0.72]
			Maintenance	7		GM (GSD), 0.037 (1.60) ppm [0.14 (6.09)]	0.022–0.083 ppm [0.08–0.32]
			Recycle process	3		GM (GSD), 0.046 (2.72) ppm [0.18 (10.4)]	0.015–0.10 ppm [0.06–0.38]
			Accelerant process	18		GM (GSD), 0.023 (1.36) ppm [0.09 (5.18)]	0.014–0.042 ppm [0.05–0.16]
Hanley et al. (2012)[a]	1976–1979	Rubber-chemical manufacturing plant, USA Data collected by company	Rubber chemicals	36		GM (GSD), 0.081 (5.3) ppm [0.31 (20.2)]	0.026–1.7 ppm [0.10–6.48]
	1980–1994			200		GM (GSD), 0.015 (3.9) ppm [0.06 (14.9)]	0.0010–1.8 ppm [0.004–6.86]
	1995–2004			127		GM (GSD), 0.0021 (4.0) ppm [0.008 (15.2)]	0.000091–0.27 ppm [0.0003–1.03]
	1980–1994		Maintenance	43		GM (GSD), 0.0042 (3.5) ppm [0.016 (13.3)]	0.00069–0.39 ppm [0.003–1.49]
	1995–2004			63		GM (GSD), 0.00091 (4.7) ppm [0.003 (17.9)]	ND (< 0.00013) to 0.25 ppm [ND (0.0005) to 0.95]
	1980–1994		Laboratory	4		NR	0.0018–0.0038 ppm [0.007–0.014]
	1980–1994		Warehouse	2		NR	ND (< 0.096) ppm to ND (< 0.10) [< 0.366–0.38]
	1995–2004			11		NR	ND (< 0.0002) to 0.0020 ppm [< 0.0008–0.008]

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Hanley et al. (2012)[a] (cont.)	1980–1994		Vinyl chemicals	38		NR	ND (< 0.0005) to 0.00095 ppm [< 0.002–0.004]
Manufacture of dyes and colouring agent							
ECB (2004)[a, b]	NR	Further processing, initial dye products	NR	14	8-h TWA	NR	< 0.8
ECB (2004) HSE (1997)	NR, United Kingdom	Further processing of aniline to dyes	NR	NR	8-h TWA	NR	< 2 0.32 < 0.08
Further processing of aniline to other chemical agents							
ECB (2004)[a]	1990–1994, NR	Further processing to MDA	NR	20 15	8-h TWA	NR	< 0.8 < 0.08
	1993–1994, NR	Further processing to NaMBT	NR	5		NR	< 0.08
	1990–1994, NR	Further processing to organic products	NR	82		NR	< 0.8
	NR	Further processing to phenylhydrazine	NR	4 2		NR	< 0.8 < 0.1
	NR	Further processing to acetoacetic anilide	NR	13		NR	< 0.8
	1988–1994, NR	Further processing of aniline	NR	NR		NR	0.01–0.2
	1990–1996, NR	Further processing to dyes, plant protection and initial pharmaceutical products	NR	141		GM, 0.04	0–0.8
ECB (2004) HSE (1997)	1993, UK	Further processing of fine chemicals	NR	26	8-h TWA	AM, 1	0.16–3.6
	1992–1995, UK	Further processing of aniline to rubber chemicals		176 1 62	1–30 min	GM, 0.04–0.12	< 2 2.4 < 0.5–6
	1992–1996, UK			277 2	8-h TWA	GM, 0.04–0.12	< 2 2.4, 4.7
	NR, UK	Laboratory	NR	4		NR	< 0.64

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Use of products containing residual aniline							
Menichini et al. (1989)	NR, Italy	Production of remoulded tyres	Vulcanization of various tyres	10	6–7 h	AM, 0.005 [calculated from reported measurements]	0.0003–0.0098
Renman et al. (1986)	NR, Sweden Area samples (stationary)	Iron and steel foundries (use of polyurethane as core binder)	Casting, pouring station	8	NR	AM, 0.025	0.004–0.087
			Casting, operator's cabin	8		AM, 0.015	0.009–0.019
			Casting, manual raking of mould overflow	8		AM, 0.033	0.007–0.087
			Cooling	8		AM, 0.037	0.007–0.098
			Mould shake-out	8		AM, 0.032	0.009–0.078
		Aluminium foundry (use of polyurethane as core binder)	Manual pouring	2		NR	0.16–0.37
			Cooling, stationary sampling	2		NR	0.51–1.8
Westberg et al. (2001)	Sweden, 1992–1995	Aluminium foundry	All combined	33	Daily TWA	GM, 0.65	< 0.1–6.4
		Aluminium sand foundries	Moulding	4		GM, 2.0	1.3–2.6
			Pouring	4		GM, 2.2	2.0–0.6
			Shake-out	5		GM, 3.9	0.20–6.4
		Aluminium static die casting	Static die casting	10		GM, 0.31	0.1–1.3
			Core knock out	2		GM, 0.21; GSD, 1.1	< 0.27 to < 0.31

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; MDA, 4,4′-methylenedianiline; NaMBT, sodium 2-mercaptobenzothiazole; ND, not detected; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; ppm, parts per million; TWA, time-weighted average.

[a] Data provided by the producer.

[b] Workplace measurements from three production sites were submitted by industry. Workplaces, activities, durations of exposure and collectives of exposed persons were not described by all of the companies in a sufficiently differentiated manner.

Table 1.4 Studies on biological monitoring of aniline and its adducts after occupational exposure

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Käfferlein et al. (2014)	NR, Germany	Experimental study (volunteers; imitation of aniline manufacturing-industry conditions but without gloves or breathing masks; 4 × 20 min exercise to individually predetermined aerobic/anaerobic threshold during the 4 × 2 h exposure) Main study (volunteers; imitation of aniline manufacturing-industry conditions but without gloves or breathing masks; 3 × 20 min exercise to individually predetermined workload representing ventilation ~30 L/min during the 3 × 2 h exposure)	Pilot study: 4 volunteers	2 ppm [7.6]	Mean post-shift: 168.9 (SD, 80.2) Range, 138.9–305.6	NR	NR	1.21 (SD, 0.29) Range, 0.90–1.57 (maximum after 6 h, plateau)
			Main study: 19 volunteers	2 ppm [7.6]	Mean post-shift: 168.0 (SD, 51.8) Range, 79.5–418.3			1.21 (SD, 0.29) Range, 0.80–2.07 (maximum after 6 h, plateau)
Production of aniline								
Thier et al. (2001)	NR, Germany	Nitrobenzene reduction plant Low ambient exposure, potential for significant dermal exposure	80 workers	Previous mean concentrations 1987–1999: 1.20 (SD, 1.21)	NR	All (n = 75): 5180 (SD, 5192)	All (n = 75): 6.39 (SD, 5.48)	NR
			Non-smokers (n = 15)			4589 (SD, 4609)	3.96 (SD, 5.17)	

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Thier et al. (2001) (cont.)			Smokers (n = 18)			6872 (SD, 5003)	10.3 (SD, 3.66)	
Riffelmann et al. (1995)	NR, Germany	Primarily synthesis and processing of aniline and 4-chloroaniline	Smokers (n = 22)	NR	Mean, 13.9 (SD, 17.3); median, 7.7 (range, 1.1–58.7)	Mean, 680.9 (SD, 213.4); median, 700 (range, 320–1100)	Mean 19.9 (SD, 7.1); median, 17.5 (range, 10–35)	NR
			Non-smokers (n = 21)		Mean, 21.9 (SD, 31.4); median, 9.6 (range, 0.0–134.0)	Mean, 1025.2 (SD, 1432.7); median, 650 (range, 200–7000)	Mean, 7.3 (SD, 3.6); median, 7.0 (range, 3.0–20)	
Manufacture of rubber chemicals								
Ward et al. (1996)	1990, USA	Manufacture of rubber chemicals	Exposed (n = 42 post-shift)	Measurements on a subset of the workers during week before sampling: I. AM, 0.187 (SD, 0.181) II. AM, 0.153 (SD, 0.095)	Pre-shift: AM, 14.1 (SD, 16.6) Post-shift: AM, 29.8 (SD, 25.7)	AM, 17 441 (SD, 8867) pg/g Hb	AM, 81.7 (SD, 106.1) pg/g Hb	NR
			Non-smokers (n = 27)		Pre-shift: AM, 11.3 (SD, 11.9) Post-shift: AM, 22.6 (SD, 11.9)	AM, 16 072 (SD, 7422) pg/g Hb		
			Smokers (n = 15)		Pre-shift: AM, 19.4 (SD, 22.4) Post-shift: AM, 42.9 (SD, 37.3)	AM, 19 776 (SD, 10 748) pg/g Hb		

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m^3)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Ward et al. (1996) (cont.)			Non-exposed (n = 25 post-shift)	NR	Pre-shift: AM, 2.6 (SD, 2.4) Post-shift: AM, 3.9 (SD, 2.8)	AM, 3163 (SD, 1302) pg/g Hb	AM, 74.5 (SD, 63.8) pg/g Hb	
			Non-smokers (n = 16)		Pre-shift: AM, 1.6 (SD, 1.1) Post-shift: AM, 2.6 (SD, 1.8)	AM, 3118 (SD, 1513) pg/g Hb	AM, 48.2 (SD, 52.3) pg/g Hb	
			Smokers (n = 9)		Pre-shift: AM, 4.2 (SD, 3.1) Post-shift: AM, 6.2 (SD, 2.9)	AM, 3240 (SD, 905) pg/g Hb	AM, 119.3 (SD, 58.1) pg/g Hb	
Manufacturing of rubber and rubber goods								
Korinth et al. (2007)	NR, Germany	Supplier for the automobile industry Manufacturing of rubber products (mixing raw materials, semi-finishing/assembling, curing, deburring and final inspection of the products) 51 workers	Non-smokers (n = 15)	Range, 0.001–0.0374 Median, 0.0025 Mean, 0.0066 95th percentile, 0.0374	Range, 3.2–37.6 Median, 12.2 Mean, 12.7 95th percentile, 37.6	Range, 367–2662 Median, 1112 Mean, 1213 95th percentile, 2662	NR	NR
			Smokers (n = 36)	Range, 0.0003–0.0483 Median, 0.0033 Mean, 0.0067 95th percentile, 0.0335	Range, 2.2–37.0 Median, 10.2 Mean, 11.8 95th percentile, 36.4	Range, 351–2584 Median, 933 Mean, 1042 95th percentile, 2578		

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
NIOSH (1981)	1980, USA	Production of sporting goods (baseball gloves and basketballs):		NR		NR	NR	NR
		Lacer	*n* = 13		Range, 1.0–5.8			
		Machine operator	*n* = 7		Range, 1–19.6			
		Sorter	*n* = 1		Range, 1.0			
		Cementer	*n* = 2		Range, 1–2.1			
		Packing	*n* = 1		Range, 1.2–2.1			
		Welder	*n* = 2		Range, 1.7–6.3			
		Inspector	*n* = 1		Range, 1.0–1.5			
Manufacture of dyes								
Beyerbach et al. (2006)	India, 1993	Manufacture of benzidine dihydrochloride (4 factories)	15 workers	NR	NR	Mean, 284 (SD, 221) pmol/100 mg Hb	Mean, 392 (SD, 452) pmol/100 mg Hb	NR
		Manufacture of Direct Black 38 (benzidine-dihydrochloride and aniline)	18 workers			Mean, 90.1 (SD, 87.6) pmol/100 mg Hb	Mean, 35.2 (SD, 43.9) pmol/100 mg Hb	
		Building construction company (reference)	15 workers			Mean, 1.37 (SD, 1.04) pmol/100 mg Hb	Mean, 0.12 (SD, 0.06) pmol/100 mg Hb	

4-ABP–Hb, 4-aminobiphenyl–haemoglobin adducts; AM, arithmetic mean; aniline–Hb, aniline–haemoglobin adducts; met-Hb, methaemoglobin; NR, not reported; ppm, parts per million; SD, standard deviation.

A second study reported on 75 workers having a mean concentration of aniline–haemoglobin adducts of 5180 ng/L (standard deviation, SD, 5192 ng/L), being somewhat higher in a subgroup of smokers than in non-smokers (Thier et al., 2001). The corresponding concentration for 4-aminobiphenyl–haemoglobin was 6.39 ng/L (SD, 5.48 ng/L). The study did not measure ambient aniline exposure, but aniline concentration in the workplace air of the aniline-production plant measured periodically between 1987 and 1999 was reported to be 1.20 mg/m^3 (SD, 1.21 mg/m^3). No information on the time lag between the exposure measurements and the biological monitoring was provided.

(b) Chemical industry

(i) Rubber-chemical manufacturing

Aniline is one of the agents used as a vulcanization accelerator and antioxidant during rubber processing. Although the accelerators and antioxidants are synthesized in confined systems, there is potential for inhalation and dermal exposure during several work tasks during which the processing equipment is opened. These tasks include changing sparkler filters, unclogging frozen recycle pipelines, repairing and maintaining pumps and pipes, collecting raw material and recycle samples, laboratory testing, and packing of the finished product (Hanley et al., 2012).

Although manufacture of rubber chemicals is the best-characterized scenario with respect to aniline exposure, information on exposure level is still scarce (Table 1.3). In a retrospective exposure assessment performed at a rubber-chemical manufacturing plant, mean (GM) exposure to aniline for all jobs combined was 0.032 ppm [0.12 mg/m^3] in 1990 (Hanley et al., 2012). The exposure assessment was performed by the National Institute for Occupational Safety and Health (NIOSH) and was based on historical exposure monitoring data provided by the company (1976–2004) and on data collected by NIOSH in 1990. The exposure was highest for the antioxidant process and recycle process (GM, 0.041 ppm [0.16 mg/m^3] and 0.046 ppm [0.18 mg/m^3], respectively). The highest measured value did not exceed 0.2 ppm [0.76 mg/m^3] for any of the processes. Historically, exposure measurements provided by the company indicated that the exposure level to aniline in the rubber-chemical departments (all jobs combined) declined over the decades: GM, 0.081 ppm [0.31 mg/m^3] (1976–1979); GM, 0.015 ppm [0.057 mg/m^3] (1980–1994); and GM, 0.0021 ppm [0.008 mg/m^3] (1995–2004). This is in line with the changes of procedures and implementation of exposure-reducing measures as outlined in Hanley et al. (2012). In 1992–1995, short-term exposure measurements (n = 62; sampling time, 1–30 minutes) indicated a potential for higher exposure over a shorter time period (range, < 0.5–6 mg/m^3; mean not reported) (ECB, 2004).

No data on exposure before 1976 were available to the Working Group.

Biological monitoring of workers employed in the manufacture of rubber additives in the 1980s and 1990s has demonstrated a statistically significant increase in urinary aniline levels from pre-shift to end of shift (Ruder et al., 1992; Stettler et al., 1992; Ward et al., 1996; Table 1.4). Furthermore, the exposed workers had a significantly higher post-shift mean level than the assumed non-exposed workers (32.3 versus 3.8 µg/L) (Ruder et al., 1992). Correspondingly, exposed workers had a significantly higher level of aniline–haemoglobin adducts than non-exposed workers (17 441 pg/g haemoglobin versus 3163 pg/g haemoglobin). There were no significant differences in respect to 4-aminobiphenyl–haemoglobin adducts between the two groups. The mean ambient aniline concentration measured the week before sampling for seven study participants who had personal air samplers was below 0.2 mg/m^3 (Ward et al., 1996).

[The Working Group noted that the studies by Ruder et al. (1992), Stettler et al. (1992), Teass et al. (1993), Ward et al. (1996), and Hanley et al. (2012) were performed in the same plant as a cohort study (Carreón et al., 2014) of incident cases of bladder cancer, as described in Sections 1.6.1 and 2.1.] Some of these studies also reported on the impact of tobacco smoking on biomarker levels (Ruder et al., 1992; Riffelmann et al., 1995; Ward et al., 1996; Thier et al., 2001; Korinth et al., 2007; Table 1.4). The results were conflicting with respect to the relative contribution of smoking to overall exposure to aniline.

(ii) Manufacture of rubber and rubber goods

Aniline is used as a vulcanization accelerator and antioxidant during rubber processing. Data on ambient air exposure stratified on job groups or work task during manufacture of rubber are lacking. Non-smoking workers manufacturing rubber products for the automobile industry (three plants, all jobs combined) had a median aniline exposure of 2.5 μg/m^3 (range, 1.0–37.4 μg/m^3) in the breathing zone, and a median concentration of aniline in urine post-shift of 12.2 μg/L (range, 3.2–37.6 μg/L). The work tasks included mixing raw materials, semi-finishing/assembling, curing, deburring, and final inspection of the products. Exposure did not differ significantly for smokers, with a median aniline exposure in the breathing zone of 3.3 μg/m^3 (range, 0.3–48.3 μg/m^3) and a median aniline concentration in urine post-shift of 10.2 μg/L (range, 2.2–37.0 μg/L) (Table 1.4; Korinth et al., 2007).

(iii) Processing of aniline to other chemical products, including dyes

Aniline is used as an intermediate in the production of other chemical products, including dyes and colouring agents, methylenediamine and related compounds (methylene diphenyl diisocyanate), pesticides, and pharmaceutical products.

For manufacturing of dyes and other chemical products, the data on aniline exposure are scarce. The limited available data provided by the manufacturers indicated that full-shift exposure is mainly below 1 mg/m^3 (ECB, 2004; Table 1.3).

Short-term exposure measurements (1990–1995) during further processing of aniline into organic products [not specified] involving tasks such as filling of drums, work at the filter press, sampling, container-closing work, and sieve cleaning (n = 96; duration, < 60 minutes) ranged between 0.8 and 12 mg/m^3 (ECB, 2004). [The Working Group noted that the lack of specificity about the organic products that were processed makes the utility of these reports uncertain.] In 1950, workers in the indigo-production area of an aromatic amine dye-manufacturing plant (Ott & Langner, 1983) were exposed to 8-hour TWA aniline concentrations of between 2.0 ppm [7.6 mg/m^3] and 8.4 ppm [32 mg/m^3] (this study is described further in Section 2).

Workers in India in 1993 had a mean aniline adduct level of 284 pmol/100 mg haemoglobin (SD, 221 pmol/100 mg haemoglobin) for those involved in the manufacture of benzidine dihydrochloride, and 90.1 pmol/100 mg haemoglobin (SD, 87.6 pmol/100 mg haemoglobin) for those working with Direct Black 38 dye (Beyerbach et al., 2006). [Ambient air or urinary aniline concentrations were not measured.]

(c) Use of products containing residual aniline

Exposures may occur during the handling of formulations with residual aniline contents, e.g. use of dyes containing residual aniline (e.g. textile industry), use of adhesives containing residual aniline (engineering, device- and tool-construction industries), or if aniline appears during further processing as a result of decomposition, e.g. in foundries where polyurethane is used as a core binder, during vulcanization of rubber plastics, and rubber processing and electrical engineering (ECB, 2004); however, there is a lack of data on exposure to aniline for all these exposure

scenarios, except for aniline exposure in foundries. For iron, steel, and aluminium foundries, reported 8-hour TWA exposure measurements between 1988 and 1995 ranged from 0.004 to 6.4 mg/m^3 (ECB, 2004), whereas all measurements collected during production of remoulded tyres in one study in Italy were below 0.01 mg/m^3 (Menichini et al., 1989).

One possible source of aniline exposure is through contact with synthetic turf made from crumb rubber. There are more than 12 000 synthetic turf fields in the USA. The United States National Toxicology Program (NTP) conducted a study to improve characterization of potential human exposure to and biological activity of aniline. A crumb rubber lot prepared by combining material from multiple commercial sources was analysed using a variety of techniques to generate information on chemical and physical characteristics. Data from a combination of analyses for volatile organic compounds identified 33 compounds totalling ~0.0007% by weight in crumb rubber, with an average contribution of aniline of 1 ppm [3.81 mg/m^3] by headspace gas chromatography-mass spectrometry (GC-MS) (NTP, 2019).

(d) Information gaps

Although aniline has been considered a potential occupational carcinogen since the 1970s, exposure data for aniline are scarce for all industries and scenarios where there is a significant potential for exposure to aniline. In particular, there is a lack of detailed exposure assessments stratified on production processes, job categories, and tasks. This information is needed to better define high-risk processes and individuals. Information on exposure to aniline hydrochloride was very sparse and inconsistent.

The database is insufficient to determine the magnitude of aniline exposure for workers with a potential exposure during: (i) production of aniline; (ii) manufacturing of rubber chemicals; (iii) manufacturing of rubber and rubber goods; and (iv) manufacturing of dyes from aniline. Data on aniline exposure could not be found for workers with a potential exposure during down-stream distribution of aniline, or further processing of aniline to other chemical products other than dye. Information on dermal exposure in any occupational exposure setting was not available to the Working Group. Although the production of MDI accounts for more than 90% of aniline use (see Section 1.2.3), the only available data on this scenario comprised 35 exposure measurements from 1990–1994 for processing of a starting material for production of polyurethane plastics (4,4′-methylenedianiline, MDA), provided by the industry (Table 1.3; ECB, 2004).

The majority of the available data on aniline exposure are reported in the USA and in European countries. Except for a study on haemoglobin adducts in a group of Indian workers producing azo dyes and benzidine (Beyerbach et al., 2006), no data were retrieved from the Asia-Pacific region, one of the major producers and consumers of aniline. No data on occupational exposure to aniline were reported from Africa, South America, Canada, or Australia.

Studies investigating occupational exposure to aniline have mainly included men, and hence the database is insufficient to conclude whether sex-specific differences exist with respect to aniline exposure burden; however, in a controlled study with 19 volunteers of which 9 were women, no statistically significant sex-specific differences in the urinary concentration of aniline or methaemoglobin were found after environmental exposure to aniline at 2 ppm [7.6 mg/m^3] for 6 hours (Käfferlein et al., 2014).

1.4.3 Co-exposure in the workplace

Table 1.5 summarizes the agents that have been reviewed by the *IARC Monographs* Working Group and are found in the same industries as aniline. There are nine agents for which there is *sufficient* or *limited* evidence for bladder

Table 1.5 Chemicals co-occurring occupationally with aniline that have *limited* or *sufficient* evidence of bladder carcinogenicity in humans (or structural similarity to such chemicals)

Chemical (CAS No.) or process	Use				IARC Group (Volume, year)	Cohort studies	Case series and reports[a]
	Aniline production	Rubber chemicals	Rubber goods	Dye production			
Chemicals that have limited *or* sufficient *evidence of bladder carcinogenicity in humans*							
2-Mercaptobenzothiazole (149-30-4)		✓			2A (Vol. 115, 2018)	Sorahan (2008)	
2-Naphthylamine (91-59-8)	✓			✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Anon. (1921); Gehrmann (1936); Hueper (1938); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
4-Aminobiphenyl (92-67-1)				✓	1 (Vol. 100F, 2012)		
4-Chloro-*ortho*-toluidine (95-69-2)				✓	2A (Vol. 99, 2010)		
Auramine production				✓	1 (Vol. 100F, 2012)	Case et al. (1954)	
Benzidine (92-87-5)	✓			✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Anon (1921); Gehrmann (1936); Hueper (1938); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
Magenta production				✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Rehn (1895)
ortho-Toluidine (95-53-4)	✓	✓	✓	✓	1 (Vol. 100F, 2012)	Ott & Langner (1983); Sorahan (2008); Hanley et al. (2012); Carreón et al. (2014)	Rehn (1895); Hueper (1938); Aboulker & Smagghe (1953); Nakano et al. (2018)
Rubber production			✓		1 (Vol 100F, 2012)		
Other chemicals (structurally similar to known bladder carcinogens)							
Magenta (632-99-5)				✓	2B (Vol 100F, 2012)	Case et al. (1954); Case & Pearson (1954)	
Nitrobenzene (98-95-3)	✓	✓			2B (Vol. 65, 1996)	Hanley et al. (2012); Carreón et al. (2014)	Rehn (1895)
N-Phenyl-2-naphthylamine (135-88-6)		✓			3 (Suppl. 7, 1987)	Sorahan (2008)	
ortho-Anisidine (90-04-0)				✓	2B (Vol. 99, 2012)		
2,4-Xylidine (95-68-1)				✓	3 (Suppl. 7, 1987)		Hueper (1938)

Table 1.5 (continued)

Chemical (CAS No.) or process	Use				IARC Group (Volume, year)	Cohort studies	Case series and reports[a]
	Aniline production	Rubber chemicals	Rubber goods	Dye production			
ortho-Chloroaniline (95-51-2)				✓	1 (Vol. 100F, 2012)		
1-Naphthylamine (134-32-7)				✓	3 (Suppl. 7, 1987)	Case et al. (1954)	Gehrmann (1936); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
Acenaphthene (83-32-9)				✓	3 (Vol. 92, 2010)	Ott & Langner (1983)	
2-Amino benzoic acid (118-92-3)				✓	3 (Suppl. 7, 1987)	Ott & Langner (1983)	

CAS, Chemical Abstracts Service; Suppl., Supplement; Vol., Volume.

[a] These case series and reports mention co-exposure of aniline with the listed agent, and are further described in Section 2.3.

carcinogenicity from studies in humans, and a further nine agents that have been previously reviewed by the *IARC Monographs* programme, but do not have *sufficient* or *limited* evidence for bladder carcinogenicity from studies in humans. For each agent, the industrial circumstances in which they could occur as co-exposures with aniline, the *IARC Monographs* classification, and the cohort or case-series publications in which they have been mentioned are reported. [The Working Group noted that it was not always clear whether the participants in the studies were co-exposed to aniline and these agents.]

Table 1.6 summarizes environmental and biological monitoring of occupational co-exposure to other bladder carcinogens among workers with aniline exposure.

(a) Primary aniline production

As noted in Section 1.4.2 above, work in primary production of aniline has been reported to be associated with increases in urinary concentrations of *ortho*-toluidine, 2-naphthylamine, and benzidine (Riffelmann et al., 1995). Workers in primary production of aniline are also exposed to nitrobenzene, which has not been determined to be a bladder carcinogen by the *IARC Monographs* programme.

(b) Manufacture of rubber chemicals

Workers in rubber-chemical production have potential co-exposure to two known or suspected bladder carcinogens: 2-mercaptobenzothiazole (IARC Group 2A) and *ortho*-toluidine (IARC Group 1). These workers may also be exposed to nitrobenzene (IARC Group 2B) and *N*-phenyl-2-naphthylamine (IARC Group 3) (Teass et al., 1993; Ward et al., 1996; Hanley et al., 2012). Reported levels of *ortho*-toluidine are given in Table 1.6.

In the retrospective exposure assessment performed at a rubber-chemical manufacturing plant by Hanley et al. (2012), the mean (GM) exposure to *ortho*-toluidine for all jobs combined in 1990 was 0.070 ppm [0.41 mg/m^3] (range, 0.020–0.37 ppm [0.18–2.17 mg/m^3]) (Table 1.6). The exposure was highest in the antioxidant process and recycle process (GM, 0.096 ppm [0.56 mg/m^3] and 0.086 ppm [0.50 mg/m^3], respectively). In a biomonitoring study on workers manufacturing rubber chemicals (n = 43), an exposure to *ortho*-toluidine the week before collection of biological samples was reported to be 0.412 mg/m^3 (SD, 0.37 mg/m^3). Among the exposed workers, the urinary *ortho*-toluidine concentration increased from 15.4 µg/L (SD, 27.1 µg/L) to 98.7 µg/L (SD, 119.4 µg/L) during the work shift (Ward et al. 1996). In a second study on rubber-chemical producers (n = 46), the mean urinary concentration of *ortho*-toluidine increased from 18 µg/L (SD, 27 µg/L) to 104 µg/L (SD, 111 µg/L) during the work shift (Teass et al., 1993). [In the latter study, the concentration of *ortho*-toluidine in the working atmosphere was not measured.] Co-exposure to nitrobenzene (in 1990) in the rubber-chemicals department was reported to range between 0.067 and 0.076 ppm [0.34 and 0.39 mg/m^3] for the antioxidant process but was not detected in the recycle process or the accelerant process (Hanley et al., 2012).

(c) Manufacturing of rubber and rubber goods

Workers manufacturing rubber and rubber goods are potentially exposed to *ortho*-toluidine. Non-smoking workers manufacturing rubber products for the automobile industry (three plants, all jobs combined) had a median *ortho*-toluidine exposure of 26.3 µg/m^3 (range, 0.1–524.0 µg/m^3) in the breathing zone, and a median *ortho*-toluidine concentration in urine post-shift of 6 µg/L (range, < LOD to 294.4 µg/L) (Korinth et al., 2007; Table 1.6). No quantitative exposure data were found for other co-exposures for this scenario.

Table 1.6 Studies on environmental and biological monitoring of occupational co-exposure to other bladder carcinogens among workers with aniline exposure

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Production of aniline								
Riffelmann et al. (1995)	Germany, NR	Primarily synthesis and processing of aniline and 4-chloroaniline	22 smokers	NR	Mean, 0.6 (SD, 1.0); median, 0.0 (range, 0.0–2.8)	Mean, 1.6 (SD, 3.1); median, 0.4 (range, 0.0–14.2)	Mean, 3.9 (SD, 2.2); median, 3.9(range, 0.0–9.8)	Mean, 0.3 (SD, 0.6); median, 0.0 (range, 0.0–2.2)
			21 non-smokers		Mean, 0.4 (SD, 1.1); median, 0.0 (range, 0.0–4.2)	Mean, 2.6 (SD, 3.5); median, 0.0 (range, 0.0–10.4)	Mean, 2.1 (SD, 2.8); median, 1.7 (range, 0.0–11.6)	Mean, 0.1 (SD, 0.5); range, 0.0–0.0
Manufacture of rubber chemicals								
Hanley et al. (2012) [Same plant as in Ruder et al. (1992); Stettler et al. (1992); Teass et al. (1993); Ward et al. (1996)]	USA, 1990	Rubber-chemical manufacturing plant Data collected by NIOSH			NR	NR	NR	NR
		All jobs combined	45	GM, 0.070 (SD, 1.84) ppm [0.41 (10.8) mg/m³] Range, 0.020–0.37 ppm [0.12–2.17 mg/m³]				
		Antioxidant process	17	GM, 0.096 (SD, 1.74) ppm [0.56 (10.2) mg/m³] Range, 0.035–0.35 ppm [0.21–2.05 mg/m³]				
		Maintenance	7	GM, 0.086 (SD, 2.02) ppm [0.50 (11.9) mg/m³] Range, 0.054–0.37 ppm [0.32–2.17 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Hanley et al. (2012) (cont.)		Recycle process	3	GM, 0.052 (SD, 2.35) ppm [0.31 (13.8) mg/m³] Range, 0.020–0.10 ppm [0.12–0.59 mg/m³]				
		Accelerant process	18	GM, 0.051 (SD, 1.50) ppm [0.30 (8.80) mg/m³] Range, 0.029–0.099 ppm [0.17–0.58 mg/m³]				
		Rubber-chemical manufacturing plant, USA Data collected by company			NR	NR	NR	NR
	1976–1979	Rubber chemicals	30	GM, 0.10 (GSD, 5.9) ppm [0.59 (34.6) mg/m³] Range, ND (< 0.023) to 1.8 ppm [ND (< 0.14) to 10.6 mg/m³]				
	1980–1994		200	GM, 0.015 (GSD, 3.2) ppm [0.09 (18.78) mg/m³] Range, 0.0025–1.5 ppm [0.015–8.80 mg/m³]				
	1995–2004		127	GM, 0.0028 (GSD, 3.8) ppm [0.016 (22.31) mg/m³] Range, 0.00021–0.22 ppm [0.001–1.29 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Hanley et al. (2012) (cont.)	1980–1994	Maintenance	43	GM, 0.0049 (GSD, 4.4) ppm [0.03 (25.8) mg/m³] Range, 0.00051–0.12 ppm [0.0030–0.70 mg/m³]				
	1995–2004		63	GM, 0.0014 (GSD, 5.6) ppm [0.008 (32.9) mg/m³] Range, ND (< 0.0001) to 0.24 ppm [< 0.0006–1.41 mg/m³]				
	1980–1994	Laboratory	4	NR Range, 0.0018–0.002 ppm [0.011–0.012 mg/m³]				
	1995–2004		1	NR Range, ND (< 0.0020 ppm) [ND (< 0.012 mg/m³)]				
	1980–1994	Warehouse	2	NR ND (< 0.008 ppm) [0.047 mg/m³]				
	1995–2004		11	NA Range, 0.00020–0.0020 ppm [< 0.0012–0.012 mg/m³]				
	1980–1994	Vinyl chemicals	39	NA Range, < 0.0004–0.056 ppm [< 0.002–0.33 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Ward et al. (1996)	USA, 1990	Manufacture of rubber chemicals	43 exposed	Measurements on a subset of the workers during the week before sampling: I. AM, 0.412 (SD, 0.366) II. AM, 0.516 (SD, 0.513)	Pre-shift: AM, 15.4 (SD, 27.1) Post-shift: AM, 98.7 (SD, 119.4)	NR	NR	NR
			Non-smokers (*n* = 28)		Pre-shift: AM, 16.1 (SD, 33.0) Post-shift: AM, 80.1 (SD, 94.0)			
			Smokers (*n* = 15)		Pre-shift: AM, 14.3 (SD, 10.2) Post-shift: AM, 132.1 (SD, 153.1)			
Teass et al. (1993) [Same data set and results in Stettler et al. (1992)]	USA, 1990	Manufacture of rubber additives (rubber antioxidant and rubber accelerator)	Unexposed (*n* = 31)	NR	Pre-shift: 1.1 (SD, 1.0) Post-shift: 2.7 (SD, 1.4)	NR	NR	NR
			Exposed (*n* = 46)		Pre-shift: 18 (SD, 27) Post-shift: 104 (SD, 111)			
Ruder et al. (1992) [Same dataset as in Teass et al. (1993) and Stettler et al. (1992)]			Exposed non-smokers (*n* = 29) Exposed smokers (*n* = 19)		Pre-shift: 17.5 Post-shift: 83.9 Pre-shift: 20.0 Post-shift: 135.6	NR	NR	NR

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Manufacturing of rubber and rubber goods								
Korinth et al. (2007)	Germany, NR	Supplier for the automobile industry Manufacturing of rubber products (mixing raw materials, semi-finishing/ assembling, curing, deburring and final inspection of the products) 51 workers	Non-smokers (n = 15)	Range, 0.0001–0.524 Median, 0.0263 Mean, 0.0614 95th percentile, 0.524	Range, < LOD to 292.4 Median, 6.0 Mean, 38.6 95th percentile, 292.4	NR	NR	NR
			Smokers (n = 36)	Range, < LOD to 0.0939 Median, 0.0004 Mean, 0.011 95th percentile, 0.0725	Range, < LOD to 242.9 Median, 0.6 Mean, 14.5 95th percentile, 100.0			

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; ND, not detected; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; ppm, parts per million; SD, standard deviation; TWA, time-weighted average.

(d) Manufacturing of dye and pigments

In addition to aniline, workers in the manufacturing of dye and pigment intermediates are potentially exposed to bladder carcinogens including 2-naphthylamine, 4-aminobiphenyl, auramine, benzidine, magenta, and *ortho*-toluidine. These workers may also be exposed to *ortho*-anisidine, 2,4-xylidine, *ortho*-chloroaniline, 1-naphthylamine, and acenaphthene. No study on aniline-exposed workers reported any quantitative exposure data for these agents.

(e) Foundries

In foundry workers there is a potential for exposure to soot, which (in the setting of exposures among chimney sweeps) shows *limited* evidence of bladder carcinogenicity (IARC, 1984).

1.4.4 Exposure of the general population

The general population may be exposed to aniline via the environmental release of industrial effluents to air (Käfferlein et al., 2014), water, land, or groundwater (US EPA, 1994; ATSDR, 2002). Available quantitative information on concentrations of aniline in drinking-water in Canada was restricted to the results of a survey conducted in Québec in which this substance was not detected (i.e. concentrations were < 0.5 µg/L) in samples from 17 municipalities (Government Canada, 1994). The estimated aniline concentrations in drinking-water in England and Wales, UK, using the most effective and the least effective removal technique, were 0.0224 µg/L and 0.2245 µg/L, respectively (Rockett et al., 2014). A detailed study on 24 amines in a drinking-water treatment plant in Spain reported average aniline concentrations of 9.2 ng/L [0.009 µg/L] in the distribution system. These levels were subject to seasonal variation, increasing during high rainfall or cold temperature events to 13 ng/L [0.013 µg/L] and 11 ng/L [0.011 µg/L] on average, respectively (Jurado-Sanchez et al., 2012). Data from 15 dug wells in a coal industry area in Burnpur, West Bengal, India, showed that the average aniline concentration in groundwater of the study area was 0.242 mg/L [242 µg/L] (Mohanta & Mishra, 2020).

Cigarette smoking is one of the main contributors to aniline exposure in non-occupational environments. Most measurements showed considerable contamination with aromatic amines derived from sidestream tobacco smoke, which was detected also in parts of the buildings in which tobacco smoking was not allowed (Luceri et al. 1993). Air from aniline emissions from materials and products attains a high concentration in heavily contaminated indoor environments, due to tobacco smoking and poor ventilation. High concentrations of aniline in outdoor air (120–340 ng/m^3) were also measured in the centre of Florence and in the Brindizi industrial zone, in Italy (Palmiotto et al., 2001).

Aniline is a component of tobacco smoke. Smoking or inhaling sidestream smoke (environmental tobacco smoke) will therefore lead to aniline exposures (Luceri et al., 1993; Goniewicz & Czogała, 2005; Xie et al., 2013). A few studies have reported that the concentration of urinary aniline in smokers (3.1 µg/24-hour urine sample) is higher than in non-smokers (2.8 µg/24-hour urine sample) (el-Bayoumy et al., 1986). Among workers employed in manufacture of rubber additives but not exposed to aniline, urinary aniline concentrations were statistically significantly higher in smokers – pre-shift, 4.2 µg/L (SD, 3.1 µg/L); post-shift, 6.2 µg/L (SD, 2.9 µg/L) – than in non-smokers – pre-shift, 1.6 µg/L (SD, 1.1 µg/L); post-shift, 2.6 µg/L (SD, 1.8 µg/L) (Ward et al., 1996). Urinary concentrations of aniline in the two studies were approximately equivalent. [The Working Group noted that the study by Ward et al. (1996) was performed in the same plant in which a cohort study (Carreón et al., 2014) of incident cases of bladder cancer was conducted, as described in Section 1.6.1 and Section 2.1.]

Aniline can be released to the environment from products (described below) and building materials.

Indoor use of products includes, for example, automotive care products, paints and coatings or adhesives, and fragrances and air fresheners. Aniline was shown to migrate from polyamide cooking utensils (Brede & Skjevrak, 2004), and shoe polish (Zhu & Aikawa, 2004; Health Canada, 2011). In one study in Canada, air samples were collected during a shoe-polishing activity at home, and indoor aniline concentration increased sharply from 0.016 μg/m^3 to 0.53 μg/m^3 (Zhu & Aikawa, 2004). Although information from the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers provided by registrants indicates the presence of aniline in a broad range of articles and consumer products, including fabrics, detergents, diapers, and feminine hygiene products (ECHA, 2020b), the Working Group was not able to identify data that would characterize and quantify these exposures.

Indoor use in long-life materials with low release rate includes, for example, flooring, furniture, toys, construction materials, curtains, footwear, leather products, paper and cardboard products, and electronic equipment. A residual aniline migration concentration of 0.4 μg/g was determined in a polyurethane toy (Abe et al., 2016).

Outdoor use in long-life materials with low release rate includes, for example, metal, wooden, and plastic construction and building materials.

Aniline content in tattoo inks ranges from 5 to 61 mg/kg (ECHA, 2019b). The European Chemicals Agency (ECHA) estimated that 12% of European citizens are tattooed and that this prevalence may be doubled in the younger generations (age, 18–35 years). [The Working Group noted that, as aniline is a constituent in cigarettes and tattoo colourants, individuals smoking tobacco and having their bodies tattooed will have a greater aniline exposure than others in the general population.]

Biomonitoring of aniline exposures has been reported (Dierkes et al., 2014). In 1986 in New York, USA, among healthy men aged 25–45 years, the urinary aniline concentration range was from 3.1 μg/24-hour urine sample in smokers to 2.8 μg/24-hour urine sample in non-smokers (el-Bayoumy et al., 1986). In a cross-sectional population-based survey in Germany, in which 93.9% of 1004 individuals had detectable urinary aniline concentrations, the mean urinary aniline concentration was 5.44 μg/L (range, 0.1–384.04 μg/L) (Kütting et al., 2009). Breast milk samples from smokers and non-smokers had quantifiable concentrations of aniline (0.05–5.2 μg/L). There was no statistically significant difference in the mean concentration of aniline in breast milk between smokers and non-smokers (DeBruin et al., 1999).

1.5 Regulations and guidelines

ECHA has classified aniline as carcinogenic (Category 2), mutagenic (Category 2), skin sensitizing (Category 1), damaging to eyes (Category 1), and causing acute toxicity (Category 3). Aniline and its salts are banned from use in any cosmetic products marketed for sale or use in the European Union. Workers who are under age 18 years, pregnant, or breastfeeding, may not be exposed to aniline. Employers are obliged to minimize worker exposure to aniline as far as possible, and must arrange for medical surveillance of exposed workers (ECHA, 2020c).

The United States Environmental Protection Agency (US EPA) has listed aniline as a hazardous air pollutant (HAP) (US EPA, 2018); its emissions are subject to regulation under the Clean Air Act Amendments. Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the reportable quantity for aniline is 5000 pounds [2.27 tonnes]. Releases of CERCLA hazardous substances, in quantities

equal to or greater than their reportable quantity, are subject to reporting to the National Response Center under CERCLA. Under the Emergency Planning and Community Right-To-Know Act (Section 313), or EPA's Toxics Release Inventory (TRI), emissions, transfers, and waste management data must be reported annually. Discarded commercial-grade aniline or container and spill residues are listed as a toxic waste under the U012 code. Additionally, aniline has a threshold planning quantity (TPQ) of 1000 pounds [0.45 tonnes] which triggers development of emergency response plans within communities and localities (US EPA, 2019). As a chemical that is known or anticipated to occur in public water systems and which may require regulation under the United States Safe Drinking-water Act, aniline is listed on the Contaminant Candidate List 3 (US EPA, 2009); however, it is currently not subject to any proposed or promulgated national primary drinking-water standard. As of 28 September 1979, notification must be given to the US EPA of any discharge into waterways of mixtures containing 454 kg or more of aniline (US EPA, 1979). As of 6 February 2020, the reportable quantity was 2270 kg (Office of the Federal Register, 2019b).

In the state of Washington, USA, aniline was added to the Children's Safe Product Act Reporting Rule as a chemical of high concern to children (CHCC) (Washington State Department of Ecology, 2018) which must be reported in children's products (Washington State Chemicals of High Concern Reporting Rule; Department of Ecology (2020)).

In the USA, the colour additives in tattoo pigments are subject to premarket approval under the United States Federal Food, Drug, and Cosmetic Act. However, the Food and Drug Administration (FDA) traditionally has not exercised regulatory authority for colour additives on the pigments used in tattoo inks (FDA, 2019).

In the European Union, tattoo inks are covered primarily by the General Product Safety Directive. Seven European Union Member States have developed specific tattoo legislation, three Member States have drafts in place, and others are developing various legislative instruments (Piccinini et al., 2016; ECHA, 2019b). These are informed by concentration limits proposed by ECHA in 2017; the limit for aniline is 0.0005% w/w (ECHA, 2017).

Under the Association of Southeast Asian Nations (ASEAN) Harmonised Cosmetic Regulatory Scheme, aniline, its salts, and its halogenated and sulfonated derivatives are on the list of substances which must not form part of the composition of cosmetic products. The colouring agent Solvent Red 23 (Colour Index number 26100), allowed for use exclusively in cosmetic products intended not to come into contact with the mucous membranes may contain ≤ 0.2% aniline (ASEAN, 2019). [The Working Group noted that these colouring agents would include tattoo ink.]

1.5.1 Occupational exposure limits

Occupational exposure limits for aniline exist in several countries, as shown in Table 1.7 (European Commission, 2016; ILO, 2011).

NIOSH considers aniline to be a potential occupational carcinogen as defined by the Occupational Safety and Health Administration (OSHA) carcinogen policy (NIOSH, 2012). No recommended exposure limit (REL) has been established (NIOSH, 2018).

Since the US EPA has identified aniline as a toxic waste, as of 19 November 1980, persons who generate, transport, treat, store, or dispose of aniline must comply with the regulations of the federal hazardous waste management programme. Included in the list of hazardous wastes are: distillation bottoms from aniline production, process residues from aniline extraction from the production of aniline, and combined wastewater streams generated from

Table 1.7 Occupational 8-hour exposure limits for aniline

Country	Year[a]	Limit value, 8 h			Status
		(mg/m³)	(ppm)	Interpretation	
Australia	2020	7.6	2	TWA	Safe Work Australia guideline
Austria	2011	8	2	TWA	Regulation
Belgium	2018	7.7	2	TWA	Royal decree
Canada, Ontario	2016		2	TWA	Ontario Ministry of Labour regulation
Canada, Québec	2020	7.6	2	TWA	Regulation
China	2019	3			
Denmark	2016	4	1	TWA	
European Union	2019	7.74	2	TWA	SCOEL Recommendation, Commission Directive
Finland	2019	1.9	0.5	TWA	Regulation
France[b]	2016	10	2	TWA	
Germany	2019	7.7	2	MAK	DFG Recommendation
Hungary	2000	8			Regulation
Ireland	2011	3.8	1		
Japan	2018	3.8	1	TWA	Guideline
Latvia	2019	0.1	1	TWA	WES
Mexico		10	2		
New Zealand	2020	4	1	TWA	Regulation
Poland	2011	1.9	0.8	TWA	
Romania	2018	3	0.8	TWA	
Singapore[c]	2020	7.6	2	PEL (long term)	Regulation
South Africa		10	2		
Republic of Korea		10			
Spain	2018	7.7	2		
Sweden	2020	4	1	TWA	Regulation
Switzerland	2020	8	2	TWA	
United Kingdom	2020	4	1	TWA	Regulation
USA	2020	19	5	PEL (TWA)	OSHA Regulation
		7.6	2	TLV	ACGIH recommendation

ACGIH, American Conference of Governmental Industrial Hygienists; DFG, Deutsche Forschungsgemeinschaft, German Research Foundation; MAK, maximale Arbeitsplatz-Konzentration, maximum workplace concentration; PEL, permissible exposure limit; OSHA, Occupational Safety and Health Administration; SCOEL, Scientific Committee on Occupational Exposure Limits; TLV, threshold limit value; TWA, time-weighted average; WES, workplace exposure standard.

[a] Year is either the year of latest update or if this is not given, the year is 2020 when this was accessed.

[b] In France, an 8-hour occupational exposure limit value of 7.6 mg/m³ has been established for aniline salts.

[c] In Singapore, an 8-hour occupational exposure limit value of 0.5 mg/m³ has been established for aniline salts.

From IRSST (2010), ILO (2011); IFA (2019).

nitrobenzene/aniline production (Office of the Federal Register, 2019c).

Additionally, as of 20 November 1980, shipments of aniline in the USA are subject to a variety of labelling, packaging, quantity, and shipping restrictions consistent with the designation of aniline as a hazardous material (IARC, 1982).

1.5.2 Reference values for biological monitoring of exposure

Nine Member States of the European Union (Croatia, Germany, Hungary, Ireland, Poland, Romania, Slovakia, Slovenia, and Spain) have adopted biological limit values (BLV) for aniline. In Romania and Slovenia, they are statutory or obligatory, whereas in the other countries they are facultative. In blood, BLVs vary between 0.015 and 0.05 mol methaemoglobin/mol haemoglobin (1.5–5%, at the end of the work shift), or < 100 µg/L of aniline in the erythrocyte fraction. The statutory urine BLVs are: *para*-aminophenol [4-aminophenol], 10 mg/g creatinine; or *para*-aminophenol-methaemoglobin, 10 µg/L, at end of shift (European Commission, 2019).

Table 1.8 provides reference values for biomarkers of exposure established by ACGIH (2018) and DFG (2018).

1.6 Quality of exposure assessment in key epidemiological studies

1.6.1 Exposure assessment in cohort studies

Occupational cohorts with potential exposure to aniline have been studied in aromatic amine dye-manufacturing plants in the United Kingdom (Case et al., 1954; Case & Pearson, 1954), and the USA (Ott & Langner, 1983), and in rubber-chemical manufacturing plants in the United Kingdom (Sorahan et al., 2000; Sorahan, 2008) and the USA (Hanley et al., 2012; Carreón et al., 2014). A review and critique of the exposure assessments conducted in these studies is provided in Table S1.9 (Annex 1, Supplementary material for Section 1, web only; available from: https://publications.iarc.fr/599).

(a) Exposure assessment methods used in cohort studies

(i) Manufacture of aromatic amine dyes

In an early study by the Association of British Chemical Manufacturers in the dye-manufacturing industry, "firms participating in the scheme were asked to provide a nominal roll of all workers known to have had any contact with aniline, benzidine, α-naphthylamine [1-naphthylamine] or β-naphthylamine [2-naphthylamine]" (Case et al., 1954). Exposure to aniline was not clearly defined. No exposure measurements were taken, and potential exposure to aniline (as "ever/never") was estimated from process records and work history. It was possible to identify aniline-exposed workers who were not also exposed to magenta, auramine, benzidine, or α- or β-naphthylamine (Case & Pearson, 1954). The reference group for statistical analyses was the general population of England and Wales, on the reasonable assumption that the general population during this period was not exposed to these substances.

A company conducted a small cohort study of mortality in employees who had worked at an aromatic amine dye-manufacturing plant in the USA (Ott & Langner, 1983). Employment history and process records were used to determine potential exposure to aniline and derivatives of *ortho*-toluidine, with aniline exposure considered to have been present in the indigo- and acetanilide-production processes. Other exposures in the indigo-production process were to chloroacetic acid, phenyl glycine, aniline tars, and indoxyl. Workers in the acetanilide-production process were also potentially exposed to acetic acid, acetic anhydride, and acetanilide. Reference was made to a 1950 industrial hygiene survey that measured levels of aniline exposure in the indigo-production area. This survey found that operators in the area were exposed to aniline concentrations of 2.0–8.4 ppm [7.6–32.0 mg/m^3]; however, this information was not used to assess

Table 1.8 Reference values for biomarkers of exposure to aniline[a]

Organization	Biomarker	Sampling time	Biological value	Value
ACGIH	Aniline in urine	End of shift	BEI	–
	Aniline released from haemoglobin in blood	End of shift	BEI	–
	para-Aminophenol in urine	End of shift	BEI	50 mg/L
Germany (DFG)	Aniline in urine after hydrolysis	End of shift	BAT	500 µg/L
	Aniline released from aniline–haemoglobin conjugate in the erythrocyte fraction of whole blood	After exposure for at least 3 months	BLW	100 µg/L
Switzerland (SUVA)	Aniline in urine after hydrolysis	End of shift	VBT	1 mg/L urine
	Aniline released from aniline–haemoglobin conjugate in the erythrocyte fraction of whole blood	After exposure for at least 3 months	VBT	100 µg/L
	para-Aminophenol in urine	End of shift	VBT	50 mg/g creatinine

ACGIH, American Conference of Governmental Industrial Hygienists; BAT, Biologische Arbeitsstoff-Toleranzwerte, biological tolerance value; BEI, biological exposure index; BLW, Biologische Leit-Werte, biological guidance value; DFG, Deutsche Forschungsgemeinschaft, German Research Foundation; SUVA, Schweizerische Unfallversicherung, Swiss National Accident Insurance Fund; VBT, valeurs biologiques tolérables, biologically tolerable values.

[a] Established by ACGIH (2018) and DFG (2018).

intensity of exposure, and assessment was limited to "ever/never" and duration of exposure. Workers with potential exposure to arsenic, vinyl chloride, or asbestos were not included in the analyses of risk associated with aniline exposure. The general population was used as the comparison group.

(ii) Rubber-chemical manufacturing

Sorahan et al. (2000) investigated mortality and cancer incidence in a cohort of 2160 male production workers from a manufacturer of vulcanization inhibitors and accelerators, antioxidants, and other proprietary products for the rubber industry, in Wales. Employment history and process records, reviewed by a former occupational hygienist employed in this factory, were used to estimate the potential for exposure to aniline as well as to *ortho*-toluidine, phenyl-β-naphthylamine [*N*-phenyl-2-naphthylamine] (PBN), and MBT. Aniline exposure was judged initially (Sorahan et al., 2000) to occur in four departments, but in the later update (Sorahan, 2008) aniline exposure was found in six departments, including a department that manufactured PBN. Quantitative estimates of exposure by different time periods were used to develop a detailed job-exposure matrix (JEM) for MBT; however, for aniline it was not possible to derive estimates more specific than duration of employment by department with potential for exposure. Periods of employment in the aniline department were classified separately from period of employment in the department in which there was potential exposure to *ortho*-toluidine; however, a significant proportion of workers had been exposed to more than one of the four agents of interest (Sorahan, 2008). [The Working Group noted that although 44% (266/611) of workers were members of more than one of the four subcohorts, it was impossible to calculate how many of the aniline-exposed workers had also been exposed to one of the other agents of interest.] In the updated analysis of mortality and cancer incidence in this cohort with revised exposure estimates, the analyses were performed using "ever/never" exposure status and duration of employment (0.1–4.9 years, ≥ 5 years),

adjusting for exposure to the other three agents of interest.

Carreón et al. (2014) conducted an updated investigation of the incidence of bladder cancer at a rubber-chemical manufacturing plant in the USA, using a comprehensive retrospective exposure-assessment methodology (Hanley et al., 2012). The exposure assessment incorporated reviews of historical process records, as well as company breathing-zone exposure-monitoring data for aniline, *ortho*-toluidine, and nitrobenzene covering the period 1976–2004, and exposure-monitoring data from a survey by NIOSH in 1990. The investigators described conducting a site visit and plant walk-through, and interviews with current and former employees, company management, and union representatives. While this comprehensive retrospective exposure assessment methodology was used to gain insight into exposures in the plant, (Hanley et al., 2012), subsequent analyses classified job title and department into one of four ordinal categories of exposure to an amalgamated exposure factor consisting of aniline, *ortho*-toluidine, and nitrobenzene combined (probably not exposed, probably exposed low and irregularly/occasionally, probably exposed low and regularly, definitely exposed moderate/high) (Carreón et al., 2014). Each department and job-title combination was assigned a relative rank (0–10) for these exposures within period of employment. The relative ranks were used to estimate the cumulative exposure rank defined as the product of the number of days in each department/job-title and the assigned rank, summed over all jobs worked. The study could not differentiate the effect of aniline alone or aniline adjusted for other exposures.

(b) Quality of exposure assessment methods in cohort studies

The exposure assessment methods of the key cohort studies cited in this monograph are evaluated according to five principal considerations: exposure opportunity, carcinogenic co-exposures, completeness of exposure history data, accuracy of exposure intensity measurement, and appropriateness of exposure metrics used in the epidemiological models of risk of cancer.

(i) Exposure opportunity

In an ideal cohort study of the carcinogenicity of aniline, workers with known exposure to aniline would be identified and differentiated from those who are clearly not exposed. Aniline exposure was specifically identified on an individual worker level by employers in Case et al. (1954), and by detailed process review in Sorahan (2008). In Ott & Langner (1983), the aniline exposure was measured only on a department level, and in Carreón et al. (2014), aniline exposure was not specifically identified.

(ii) Carcinogenic co-exposures

As reviewed in Section 1.4.3, there are several agents other than aniline used in both the dye and rubber industries (see Table 1.5 and Table 1.6). Some of these have been previously evaluated by the *IARC Monographs* programme as having *sufficient* or *limited* evidence for bladder carcinogenicity in humans: MBT, 2-naphthylamine, 4-aminobiphenyl, 4-chloro-*ortho*-toluidine, auramine production, benzidine, magenta production, rubber production, and *ortho*-toluidine. Others are similar chemicals for which the *IARC Monographs* programme has previously determined there is *inadequate* evidence for bladder carcinogenicity in humans: magenta, nitrobenzene, PBN, *ortho*-anisidine, 2,4-xylidine, *ortho*-chloroaniline, 1-naphthylamine, acenaphthene, and 2-amino benzoic acid.

Each of the cohort studies evaluated above identified co-exposure to some carcinogenic aromatic amines; however, only Sorahan (2008) was able to adjust for exposure to other carcinogens (*ortho*-toluidine, PBN, and MBT). That study also excluded workers with potential exposure to arsenic, vinyl chloride or asbestos. Case et al.

(1954) was able to divide the aniline-exposed subjects into those with or without exposure to magenta, leaving the number with exclusive exposure to aniline too small to observe a statistically significant effect if one were there. Neither of the other studies (Ott & Langner, 1983; Carreón et al., 2014) could conclusively differentiate the effects of the different substances.

(iii) Completeness of exposure histories

In each of the cohort studies evaluated, the work history records by job and department were provided by the employers. It is not possible to confirm the completeness of these records. The minimum period of employment required for an individual to be included in each cohort was not stated (Case & Pearson, 1954) not clear (Ott & Langner, 1983) or varied from 1 day (Carreón et al., 2014) to 6 months (Sorahan et al., 2000).

(iv) Accuracy of exposure intensity measurement

None of the cohort studies reviewed included quantitative estimates of aniline exposure. The chemical manufacturing study in the USA (Ott & Langner, 1983) and the rubber-chemical manufacturing study in the United Kingdom (Sorahan et al., 2000) both used the duration of employment in departments that used or produced aniline as a proxy for exposure intensity. Although the rubber-chemical manufacturing industry study in the USA included an intensity ranking, this was for aniline, *ortho*-toluidine, and nitrobenzene combined.

(c) Overall summary of exposure assessment in key cohort studies

The Working Group noted that the cohort studies reviewed all suffer from limitations in exposure assessment and/or in their ability to differentiate aniline-related effects from the effects of co-exposures. As described above, the exposure classifications are either: (i) ever/never employed in departments using aniline, with cumulative exposure assessment based on duration of employment; or (ii) ranked exposure to a combination of agents. The Working Group considered that in none of the studies was the exposure assessment of a sufficient standard to provide clear evidence that aniline was the responsible agent for the bladder cancer excesses observed.

1.6.2 Exposure assessment in case–control studies

See Table S1.10 (Annex 1, Supplementary material for Section 1, web only; available from: https://publications.iarc.fr/599).

(a) Exposure assessment methods used in case–control studies

Preti et al. (1988) conducted a hospital-based case–control study of bronchogenic carcinoma and volatile organic compounds in lung air in Pennsylvania, USA. [The Working Group noted that the selection criteria for controls included that they did not have chronic or acute lung diseases, had no industrial dust exposure, and had normal chest X-rays. These selection criteria resulted in the probability of exposure to aniline among the controls being less than that of the cases and thus may have biased the study.] Cases and controls were asked to exhale end-expiratory air into a Tedlar bag. The bags were immediately returned to the laboratory and the contents transferred to an adsorbent tube using a vacuum pump. The tubes were frozen until analysis, which was within 1 week of detection. Analyses were performed by desorbing the volatiles from the tubes onto the capillary column of the GC-MS system. [The Working Group noted that detection of aniline in exhaled breath at the time of diagnosis of lung cancer is not likely to be a good measure of exposures in the past that are likely to be more etiologically relevant given the long latency (i.e. > 15–20 years) of most occupational

and environmental carcinogens, and the short half-life of aniline in the body.]

Nizamova (1991) reported findings from a case–control study on bladder cancer in the Tambov manufacturing region of the Russian Federation. This region is described as having an advanced chemical industry, including aniline dyes. No details were provided on how exposure to aniline dyes and other chemicals was identified.

Feingold et al. (1992) conducted a population-based case–control study on childhood cancer in Denver, Colorado, USA. Parental work history information was obtained from a questionnaire that was administered mostly at home by a trained interviewer (not blinded to case status) to the parents (mostly mothers). The work histories included job title, industry, and dates of employment for each job held for at least 6 months between the year before the child's birth and the year of diagnosis. Occupational histories were coded and linked with a JEM that was previously developed by the National Cancer Institute (Hoar et al., 1980). The JEM allowed for assignment of exposures based on the combination of job title and industry that was derived from previous industrial hygiene surveys and knowledge of industrial processes. The JEM also provided an estimate as high, medium, low, or unknown. All maternal and paternal jobs held for a period of 6 months or longer during the year before birth were linked to the JEM to identify chemicals associated with a particular job. All exposures estimated using the JEM were included for individuals who had had two jobs during the year before the child's birth.

To reduce the large number of chemicals identified by the JEM, the researchers limited the study to 13 parental occupations that had been previously associated with childhood cancer: carpenter, dyer, electrician, lumberman, machine repairman, machinist, miner, motor vehicle driver, motor vehicle mechanic, painter, printer, service station attendant, and welder. The JEM identified 220 chemicals associated with one or more of these occupations. Further restriction was made to include only those chemicals associated with four or more of the occupations (45 chemicals). Exposure was assigned to the highest level for groups of exposures (e.g. solvents). Exposed (yes/no) was assigned when the specific chemical exposure was high or medium. Only 10 cases of childhood cancer (acute lymphocytic leukaemia, 5 cases; brain cancer, 2 cases; and other cancers, 3 cases) were determined to be exposed to aniline (Feingold et al., 1992).

Alguacil et al. (2000) conducted a case–control study on pancreatic cancer in Spain. Occupational histories were obtained by direct interviews and were available for approximately 90% of the cases and controls. Most interviews (88%) were performed with the patient, 6% involved interviewing a relative, and the interviews for the remaining 6% were not described. A sample of the relatives was concurrently and separately interviewed and agreement between the responses was compared. [The Working Group noted that the results of this comparison were not presented; the authors refer to another publication by Gavaldà et al. (1995), which also does not present the results of this comparison of occupational exposures.]

Participants were asked if they had ever worked in any of 10 activities or industries believed to be potentially associated with risk of pancreatic or biliary cancer based on a literature review. These activities/industries included pesticide use, handling of petroleum derivatives, and working in the chemical industry, metal industry, rubber industry, graphic arts, jewellery, manufacture or repair of automobiles, leather tanning, or textile industry. Individuals who reported having worked in one of these activities were asked about the duration of time worked, specific activities, and products to which they were exposed. Two additional questionnaire sections were reserved for reporting any other job activities performed for at least 6 years. Two

industrial hygienists evaluated the potential for exposure to 22 suspected carcinogens based on a review of the work histories. [The Working Group noted that the authors do not describe how these carcinogens were chosen.] Cases and controls were classified as being exposed, unexposed, or unknown for each of these agents. Only 6 cases and 5 controls were determined to have exposure to aniline derivatives. The intensity of exposure was also coded as high, low, unknown, or none, and analyses were performed with and without a 10-year lag (Alguacil et al., 2000). [The Working Group noted that the authors do not mention whether the industrial hygienists were blinded as to the case status or whether the exposures changed over calendar time.] In addition, analyses were performed using a JEM called FINJEM (Kauppinen et al., 1998), but aniline was not on the list of chemicals evaluated using FINJEM.

(b) Quality of exposure assessment methods in case–control studies

The exposure assessment of the key epidemiological studies cited in this monograph are evaluated according to five principal considerations: exposure opportunity, carcinogenic co-exposures, completeness of exposure history data, accuracy of exposure intensity measurement, and appropriateness of exposure metrics used in the epidemiological models of risk of cancer.

(i) Exposure opportunity

An ideal epidemiological study for investigating the carcinogenicity of aniline would evaluate a population exposed to a high concentration over a long period of time. The Working Group evaluated each study against this ideal. This first consideration of quality does not strictly concern the exposure assessment, but rather the exposure to the chemical of interest and its distribution across the population and over time.

In general, the available case–control studies do not provide much information on the intensity and duration of exposures to aniline. The case–control study on bronchogenic lung cancer by Preti et al. (1988), which was based on measurements of exhaled breath, did not present any data on level or duration of exposure to aniline. Nizamova (1991) did not provide any information on duration or intensity of exposure. The case–control study by Feingold et al. (1992) on childhood cancer did not present any analyses stratified by aniline intensity or duration of exposure. The case–control study on pancreatic cancer by Alguacil et al. (2000) only examined the risk of those "highly" exposed to aniline compared with those non-exposed, and separate analyses were conducted for those exposed for at least 10 years and those exposed for only 6 months.

(ii) Carcinogenic co-exposures

Although the case–control studies (Preti et al., 1988; Nizamova, 1991; Feingold et al., 1992; Alguacil et al., 2000) examined other potentially carcinogenic exposures, none of the studies provided information or controls for other exposures when examining the association between aniline and cancer. [The Working Group noted that these publications do not report whether there was co-exposure to aniline and these carcinogenic exposures, but there most probably was co-exposure for industries such as the rubber and dye industries.]

(iii) Completeness of exposure histories

Preti et al. (1988) did not consider occupational or environmental exposure histories in their bronchogenic lung cancer case–control study in which they measured aniline in expired air. Nizamova (1991) did not provide any information on how work histories were obtained, except that "sometimes" it was possible to contact workers and conduct detailed interviews. In the study on childhood cancer by Feingold et al. (1992), one parent was interviewed (usually the mother), who might not have been aware of all

details of the other parent's job. Work histories in the case–control study on pancreatic cancer by Alguacil et al. (2000) were based on interviews of patients and their controls or of a relative. It is difficult to know how complete these histories were, but of particular concern is the completeness of the information obtained from the relatives. Alguacil et al. (2000) mention a comparison of responses from a sample of relatives who were separately interviewed but did not provide the results of this comparison.

(iv) Accuracy of exposure intensity measurement

None of the case–control studies reported information on the intensity of exposure to aniline.

(v) Appropriateness of the exposure metrics

The use of analysis of lung air for aniline in the bronchogenic cancer case–control study by Preti et al. (1988) is not an appropriate measure given that aniline is a highly volatile chemical and measurements in breath do not reflect past exposures, and that occupational and environmental causes of lung cancer are generally related to exposures that occurred at least 10–20 years before diagnosis. Nizamova (1991) did not provide any information on how exposure was assessed and thus it is not possible to judge whether the method used was appropriate. The case–control study on childhood cancer developed estimates of exposure (yes/no) by linkage of the self-reported work histories with a JEM (Feingold et al., 1992). It is well-recognized that these qualitative methods for estimating exposures have a high degree of exposure misclassification. The exposure measure in the case–control study on pancreatic cancer was based on a review of the occupational histories by two industrial hygienists who coded the exposure as exposed, unexposed, or unknown (Alguacil et al., 2000).

(c) Overall summary of exposure assessment in key case–control studies

The Working Group noted that the case–control studies reviewed in this monograph suffer from several limitations in their exposure assessment for aniline. The studies do not provide information on duration, intensity, or cumulative exposures to aniline. They are fundamentally based on work histories derived from interviews of the study participants or a family member who may have an incomplete recollection, with potential for recall bias. One study is based on analysis of aniline in lung air after a diagnosis of lung cancer, which is a poor measure of past exposures. These studies do not account for the potential for confounding by co-exposures. Considering these limitations, the Working Group considered that these case–control studies should be given low weight in the review of the epidemiological evidence of cancer in humans.

2. Cancer in Humans

This section comprises a review of the evidence from studies of cancer in humans. The epidemiological database for the evaluation of aniline is quite limited, comprising only four cohort studies and four case–control studies. It is noteworthy that in the present review the Working Group also included consideration of case reports and case series (hereafter described as "case reports"). In many instances, case reports may not greatly contribute to our understanding of causality since no information is provided on the number of expected cases. However, there are some important exceptions, such as the discovery of unusually high numbers of cases of specific cancer types among workers in some occupations, for example, mesothelioma among South African asbestos miners (Wagner et al., 1960) and angiosarcoma among workers

exposed to vinyl chloride monomer (Creech & Johnson, 1974). In these examples, the fact that these are very rare cancers with few known risk factors made these initial case reports credible, and the association between these exposures and cancer was confirmed in subsequent formal epidemiological investigations.

Bladder cancer is the 10th most common cancer in the world (Bray et al., 2018). Although far more common than mesothelioma or angiosarcoma, bladder cancer is still sufficiently rare that a few cases occurring in a small industrial facility may be suggestive of an occupational etiology. The interpretation of clusters of cases of bladder cancer is complicated by the fact that there are many known risk factors with *sufficient* evidence in humans, including tobacco smoking, aluminium production, 4-aminobiphenyl, arsenic and inorganic arsenic compounds, auramine production, benzidine, chlornaphazine, cyclophosphamide, magenta production, 2-naphthylamine, painting, the rubber manufacturing industry, *Schistosoma haematobium*, *ortho*-toluidine, and X- and gamma-radiation (see IARC, 2020). Case reports have, however, played an important role in identifying the carcinogenic hazards associated with occupational exposures to aromatic amines. Rehn (1895) was the first to report an unusually large number of incident bladder cancer cases among workers exposed to aromatic amines in the aniline dye industry in Germany.

Some studies included in this review are based on bladder cancer incidence, whereas others are based on bladder cancer mortality; the two approaches may yield different results. For bladder cancer, survival rates are relatively high. For example, the 5-year survival rate for bladder cancer between 2009 and 2015 in the USA was 77% for all stages combined (American Cancer Society, 2020), and the 5-year survival rate in three regions of China during the 1990s ranged from 43% to 75% (Sankaranarayanan et al., 2011). Thus studies that rely on mortality from bladder cancer may omit the large proportion of cases that are not fatal, which would reduce the statistical power of these studies to detect an effect of exposure, if one exists. Furthermore, studies that examine mortality may tend to overrepresent more aggressive tumours, which could have a different etiology to that of less aggressive forms. Although incidence is generally preferable to mortality for epidemiological investigations of cancer, there may be a potential for bias in some studies in which routine occupational screening for bladder cancer has been conducted. This bias may result in inflated rates of cancer in the workplace when compared with national or local rates in the general population.

2.1 Cohort studies

See Table 2.1.

Studies of exposure to aniline were carried out in aromatic amine dye-manufacturing plants and in rubber-chemical manufacturing plants. Four cohort studies were identified that investigated cancer risks in workers occupationally exposed to aniline. Two of the cohorts were followed-up repeatedly, and the earlier follow-up publications (e.g. Ward et al., 1991; Sorahan et al., 2000; Carreón et al., 2010) are not described in detail below. One series of additional studies of workers in the aniline-dye industry within the Russian Federation was excluded because it did not mention exposure to aniline specifically (Bul'bulian & Goldfarb, 1991; Bul'bulian, 1991; Bulbulyan et al., 1995).

Tumours of the urinary bladder in workers engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry have been examined (Case et al., 1954). The firms participating in the study were asked to provide a "nominal roll" of all workers known to have had any contact with aniline, benzidine, α-naphthylamine [1-naphthylamine] or β-naphthylamine [2-naphthylamine]. The authors judged the roll to be reasonably complete from

Table 2.1 Cohort studies on cancer and exposure to aniline

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Case et al. (1954) UK 1921–1949	4622 male workers employed ≥ 6 mo in the British chemical industry and known to have had contact with aniline, benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine] Exposure assessment method: records; ever/never exposure assessment, based on reports from companies as to workers' exposure to each chemical	Urinary bladder	Exposed to aniline, but without any of the following contacts: magenta, benzidine, α-naphthylamine [1-naphthylamine], β-naphthylamine [2-naphthylamine]	1	–	Age, sex, year	*Exposure assessment critique*: Poorly defined exposure and poorly characterized exposure assessment with no details on total cohort, only of cases Other comments: 4 cases (2 alive; 2 dead) exposed to aniline, but not magenta (or benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine]), who may also have been exposed to auramine. The single case reported here is for mortality. Strengths: exhaustive search for deaths Limitations: the exhaustive search for deaths may have biased the SIRs upwards

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ott & Langner (1983) USA 1940–1975	275 men identified from workplace census lists who were working as of (or hired after) 1940 in one of three dye-production areas of a chemical manufacturer, without high exposure to arsenicals, vinyl chloride, or asbestos. Exposure assessment method: records; exposure was based on years working in an area with exposure to aniline; no other details of exposure levels; some workers worked in other areas with potential exposure to *ortho*-toluidine	All cancers combined, mortality	Indigo- and potassium phenyl glycine-production area (SMR)	10	[1.16 (0.56–2.14)]	Age, sex, race, calendar period	*Exposure assessment critique*: Moderately well-defined exposure, but poorly characterized exposure assessment. Not adjusted for other potential exposures, no information on intensity of exposure. Strengths: occupational study of workers with known aniline exposure Limitations: small cohort; inadequate description of mortality outcome assessment; mortality study inadequate for bladder cancer; prevalent hire bias; inadequately described; short follow-up
			Acetanilide production area (SMR)	4	[1.29 (0.35–3.30)]		
		Colon and rectum (includes all digestive organs and peritoneum), mortality	All production areas combined (SMR)	10	[1.75 (0.84–3.23)]		
		Lung (includes all respiratory system), mortality	All production areas combined (SMR)	6	[1.18 (0.43–2.56)]		
		Urinary bladder (includes all urinary tract), mortality	All production areas combined (SMR)	0	[0 (0–3.07)]		
		Lymphatic and haematopoietic system, mortality	All production areas combined (SMR)	1	[1 (0.03–5.57)]		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2008) Wales, UK 1955–2005 (mortality), 1971–2005 (cancer incidence)	2160 male production workers, with ≥ 6 mo of employment at the chemical factory from 1955 to 1984 Exposure assessment method: questionnaire; cohort-specific JEM of ever/never worked in department with potential exposure to aniline applied to job history; exposure assigned as years with potential exposure to aniline with no further details	All causes of death (ICD 001–999)	Overall cohort (SMR)	1334	1.02 (0.97–1.08)	Age, sex, year	*Exposure assessment critique*: Moderately well-defined exposure, but poorly characterized exposure assessment. Adjusted for other potential exposures, but no information on intensity of exposure. Strengths: availability of job histories Limitations: aniline-exposed subcohort quite small, exposures not assessed
		Lung, mortality	Overall cohort (SMR)	120	0.91 (0.75–1.09)		
		Urinary bladder, mortality	Combined exposed subcohort (potential exposure to one or more of MBT, aniline, *ortho*-toluidine, or PBN) (SMR)	11	2.78 (1.39–4.97)		
			Remainder of cohort (SMR)	11	1.05 (0.52–1.88)		
			Overall cohort (SMR)	22	1.52 (0.96–2.31)		
		Urinary bladder, mortality	Aniline-exposed subcohort (SMR)	8	2.77 (1.19–5.45)		
		Urinary bladder, incidence	Combined exposed subcohort (potential exposure to one or more of MBT, aniline, *ortho*-toluidine, or PBN) (SIR)	18	2.14 (1.27–3.37)		
			Remainder of cohort (SIR)	21	1.06 (0.65–1.62)		
		Urinary bladder, incidence	Aniline-exposed subcohort (SIR)	15	2.45 (1.37–4.05)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2008) (cont.)		Urinary bladder, incidence and mortality	Duration of employment in aniline-exposed department (RR):			Age, sex, year, exposure to MBT, PBN, and *ortho*-toluidine	
			None	37	1		
			0.1–4.9 yr	8	1.24 (0.52–3.00)		
			≥ 5 yr	11	1.35 (0.56–3.25)		
			Trend-test *P* value, 0.44				
Carreón et al. (2014) USA Follow-up, 1976–2007	1875 workers at a rubber-chemical manufacturing plant that used *ortho*-toluidine, aniline, and nitrobenzene combined; employed for ≥ 1 day in 1946–2006 Exposure assessment method: records; cohort-specific JEM to combined exposures was based on measurements and detailed understanding of process; applied to job history and multiplied by no. of days in that job	Urinary bladder, incidence	Category of *ortho*-toluidine, aniline, and nitrobenzene exposure (SIR):			Age, sex, race, year	*Exposure assessment critique*: Intensive work to develop JEM for combined exposure but impossible to determine exposure to aniline alone Strengths: large exposure contrast Limitations: small number of cases; despite the adequate exposure assessment, exposures to aniline, *ortho*-toluidine, and nitrobenzene could not be separately discerned
			Probably not exposed	< 5	0.59 (0.07–2.12)		
			Probably exposed (low, irregularly/ occasionally)	< 5	2.47 (0.67–6.33)		
			Probably exposed (low, regularly)	< 5	4.21 (1.15–10.8)		
			Definitely exposed (moderate/high)	27	3.90 (2.57–5.68)		
			Overall	37	2.87 (2.02–3.96)		
		Urinary bladder, incidence	Duration of exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SIR):				
			< 5 yr	< 10	1.98 (0.80–4.08)		
			5 to < 10 yr	< 5	4.52 (0.93–13.2)		
			≥ 10 yr	17	6.24 (3.63–9.99)		
		Urinary bladder, incidence	Duration of exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SRR):				
			< 5 yr	< 10	1		
			5 to < 10 yr	< 5	2.56 (0.60–10.9)		
			≥ 10 yr	17	4.50 (1.74–11.6)		
			Trend-test *P* value, < 0.001				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2014) (cont.)		Urinary bladder, incidence	Time since first exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SIR):			Age, sex, race, year	
			< 10 yr	< 5	1.74 (0.04–9.68)		
			10 to < 20 yr	< 5	3.41 (0.93–8.72)		
			20 to < 30 yr	9	4.75 (2.17–9.02)		
			≥ 30 yr	13	3.97 (2.11–6.79)		
		Urinary bladder, incidence	Time since first exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SRR):				
			< 10 yr	< 5	1		
			10 to < 20 yr	< 5	7.09 (0.76–66.2)		
			20 to < 30 yr	9	13.4 (1.59–[113[a]])		
			≥ 30 yr	13	8.61 (1.11–67.0)		
			Trend-test *P* value, < 0.001				
		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (SIR):				
			< 11 000 unit-days	9	1.32 (0.61–2.51)		
			11 000 to < 27 000 unit-days	10	3.37 (1.62–6.20)		
			27 000 to < 48 000 unit-days	9	5.44 (2.49–10.3)		
			≥ 48 000 unit-days	9	6.13 (2.80–11.6)		
		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (SRR):				
			< 11 000 unit-days	9	1		
			11 000 to < 27 000 unit-days	10	3.05 (1.13–8.22)		
			27 000 to < 48 000 unit-days	9	6.37 (2.30–17.7)		
			≥ 48 000 unit-days	9	7.34 (2.44–22.1)		
			Trend-test *P* value, < 0.001				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2014) (cont.)		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	9	1		
			11 000 to < 27 000 unit-days	10	3.76 (1.37–10.7)		
			27 000 to < 48 000 unit-days	9	4.65 (1.55–14.0)		
			≥ 48 000 unit-days	9	8.94 (3.57–24.6)		
		Urinary bladder, incidence	Cumulative rank quartile, 20-yr lag (SRR):			Age, sex, race, year	
			< 2800 unit-days	9	1		
			2800 to < 11 000 unit-days	10	2.95 (1.00–8.70)		
			11 000 to < 28 000 unit-days	9	2.22 (0.78–6.37)		
			≥ 28 000 unit-days	9	6.70 (2.09–21.5)		
			Trend-test *P* value, 0.037				
		Urinary bladder, incidence	Cumulative rank quartile, age < 60 yr, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	< 5	1		
			11 000 to < 27 000 unit-days	< 5	6.07 (1.33–31.0)		
			27 000 to < 48 000 unit-days	7	10.2 (2.16–54.3)		
			≥ 48 000 unit-days	5	23.6 (6.66–113)		
		Urinary bladder, incidence	Cumulative rank quartile, age ≥ 60 yr, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	5	1		
			11 000 to < 27 000 unit-days	6	1.73 (0.45–7.09)		
			27 000 to < 48 000 unit-days	< 5	1.63 (0.32–7.45)		
			≥ 48 000 unit-days	< 5	2.46 (0.64–10.0)		

CI, confidence interval; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; MBT, 2-mercaptobenzothiazole; mo, month; PBN, *N*-phenyl-2-naphthylamine; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; SRR, standardized rate ratio; yr, year.

[a] This value was incorrectly reported in the original publication as 11.3, but was verified by the Secretariat with the authors (Carreón et al., 2014).

1920 onwards. A total of 4622 male workers were included in the cohort who had worked in the industry for at least 6 months. Cases of bladder cancer were identified from several sources: reported by firms; hospital records confirmed by firms, or patients, or relatives of patients; death certificates with mentions of occupation in the chemical industry; and coroners' records. As reported in Section 1.6.1, in the absence of exposure measurements, potential exposure to aniline (as "ever/never") was estimated from process records and work histories. Follow-up was from 1921 to early 1952 (1921–1949 for deaths) during which time 341 cases of bladder cancer were identified, 298 (87.4%) of which were in workers deemed to have had contact with benzidine, α-naphthylamine, or β-naphthylamine, and 32 (9.4%) in workers who had not had contact with any of these agents. In total there were 13 cases of bladder cancer in workers exposed to aniline: 9 workers who had possible contact with magenta and 4 who did not. There was a single death among those exposed to aniline but not magenta, for which "bladder tumour" was mentioned on the death certificate, with 0.54 deaths expected. A companion study examined bladder cancer risk in 1223 workers involved in the manufacture of magenta (which included exposure to aniline) and in the manufacture of auramine (with no exposure to aniline) (Case & Pearson, 1954). Follow-up for this analysis was to the end of 1952. Among those men who had contact with aniline but not with magenta, auramine, benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine], 3 cases of bladder tumour were found, and there was a single death, with 0.83 expected. [The Working Group noted that although this was a well-conducted study for its time, case ascertainment is likely to have been incomplete and the reference rates are likely to be subject to error. There was only one death in the cohort exposed to aniline and not to other known or suspected occupational bladder carcinogens and this, together with the lack of control for tobacco smoking, means that this study is fairly uninformative in terms of bladder cancer risk after aniline exposure.]

Ott & Langner examined mortality among 342 white male workers assigned to three aromatic amine dye-production areas at a facility in the USA (Ott & Langner, 1983). Exposure to aniline occurred during the production of indigo dye, the operation dating back to approximately 1914 and discontinued by 1958. The plant had four production areas for: (i) acetanilide; (ii) indigo; (iii) bromindigo; and (iv) thioindigo. Indigo production comprised two steps, both with exposure to aniline: (i) the manufacture of potassium phenyl glycine from aniline and chloroacetic acid; and (ii) the manufacture of indigo from potassium phenyl glycine. The acetanilide production area involved exposure to aniline, whereas the bromindigo and thioindigo production areas did not involve exposure to aniline. The acetanilide process was operated from 1934 to 1958. The cohort was identified from yearly census lists available from the mid-1920s onward. All employees working for the company as of 1 January 1940 or hired after this date were included. The authors state that "nearly all employees who worked for at least one year would have been identified; however, some employees who worked for less than one year may not have been included in the cohort." As indicated in Section 1.6.1, employment history and process records were used to determine the potential for exposure to aniline and other known or suspected occupational bladder carcinogens. Work histories were obtained for all identified employees. Employees lost to direct company follow-up (n = 124) were followed-up via the social security administration. Deaths were coded according to the seventh or eighth revision of the International Classification of Diseases and were followed-up until 31 December 1975. Standardized mortality ratios (SMRs) were computed based on mortality data for USA white males in five-year age groups and five-year calendar year groups. Of the 342

employees, analyses were done excluding 56 workers who had worked in the past at an arsenicals-formulating plant and 11 who had worked with vinyl chloride or asbestos. The standardized mortality ratio for all causes for the 275 dye employees without high exposure to arsenicals, vinyl chloride, or asbestos was 0.98 [95% CI, 0.80–1.20], based on 98 observed deaths. For all malignant neoplasms for the indigo- and potassium phenyl glycine-production area, the standardized mortality ratio was 1.16 [95% CI, 0.56–2.14], and for the acetanilide production area the standardized mortality ratio was 1.29 [95% CI, 0.35–3.30]. For the indigo- and potassium phenyl glycine-production area, only a single death occurred from respiratory cancer with a latency of 15 years or more and for a duration of employment of 5 years or more, and for the acetanilide production area there was a single death from respiratory cancer with a latency of 15 years or more but for a duration of employment of less than 1 year. There were no observed deaths from cancer of the urinary tract, 1.2 were expected. There were no data on tobacco smoking in this study. [The Working Group noted that this study was relatively small and therefore limited in its informativeness in relation to bladder cancer risk.]

The bladder cancer risk in workers from a factory in Wales, UK, manufacturing chemicals for the rubber industry was examined (Sorahan, 2008). Mortality follow-up was from 1955 to 2005 and cancer incidence follow-up was from 1971 to 2005. The cohort consisted of 2160 male production workers with at least 6 months employment at the factory between 1955 and 1984. Job histories were available for the period 1930–1988. Altogether 611 exposed workers were categorized into overlapping subcohorts with exposure to aniline (n = 442), PBN [*N*-phenyl-2-naphthylamine] (n = 94), *ortho*-toluidine (n = 53), and/or MBT (n = 363) (an agent with limited evidence of bladder carcinogenicity in humans; see Section 1.4.3). As noted in Section 1.6.1, quantitative estimates of exposure were used to develop a detailed JEM for MBT and its derivatives, but for aniline, PBN, and *ortho*-toluidine, it was not possible to derive estimates more specific than duration of employment by department with potential for exposure. Mortality from all causes combined in the overall cohort was close to expected, as was mortality from lung cancer. In the overall cohort, for bladder cancer, the standardized mortality ratio was 1.52 (95% CI, 0.96–2.31) based on 22 observed deaths. In the combined exposed subcohort with potential exposure to one or more agents among MBT, aniline, *ortho*-toluidine, or PBN, the bladder cancer standardized mortality ratio was 2.78 (95% CI, 1.39–4.97; 11 deaths). In the remainder of the cohort, the bladder cancer standardized mortality ratio was 1.05 (95% CI, 0.52–1.88; 11 deaths). In the subcohort exposed to aniline and potentially other exposures listed above, the standardized mortality ratio was 2.77 (95% CI, 1.19–5.45; 8 deaths). The standardized incidence ratio for bladder cancer in the subcohort with potential exposure to MBT, aniline, *ortho*-toluidine, or PBN was 2.14 (95% CI, 1.27–3.37; 18 cases), and in the remainder of the cohort the standardized incidence ratio was 1.06 (95% CI, 0.65–1.62; 21 cases). In the aniline-exposed subcohort, the standardized incidence ratio was 2.45 (95% CI, 1.37–4.05; 15 cases). A Poisson regression analysis examined duration of employment (in years) in aniline-exposed and *ortho*-toluidine-exposed departments. Without adjustment for other chemicals, a significant positive trend ($P < 0.05$) was found for aniline exposure and a highly significant positive trend ($P < 0.001$) was found for *ortho*-toluidine; however, when adjusted for other chemical exposures, the trend for duration of aniline exposure was not significant ($P = 0.44$), although a significantly increasing trend with duration of *ortho*-toluidine exposure ($P < 0.05$) remained. No adjustment was made for tobacco smoking. [The Working Group noted that lung cancer standardized

mortality ratios were not elevated compared with the general population, suggesting that tobacco smoking confounding was unlikely in the standardized mortality ratio estimates for bladder cancer. The Working Group also noted that since the subcohorts analysed are overlapping, a considerable proportion of the aniline-exposed workers were also exposed to *ortho*-toluidine and other potentially carcinogenic occupational chemicals, possibly resulting in overadjustment in models with multiple occupational exposures included, which may have reduced the precision of the estimates.]

A study in the USA examined bladder cancer incidence among workers at a rubber-chemical manufacturing plant where *ortho*-toluidine, aniline, and nitrobenzene were used (Ward et al., 1991; Carreón et al., 2010, 2014; Hanley et al., 2012). Among other chemicals, aniline was used in antioxidant production until 1992. This cohort has been analysed using three different exposure assessment methods. First, the study by Ward et al. (1991) evaluated exposure based on department. Second, Carreón et al. (2010) presented a reanalysis of the Ward et al. (1991) study data that reclassified exposure for some departments. Third, Carreón et al. (2014) extended follow-up and used a revised exposure metric, as described in Section 1.6.1, in which work history records were assigned to one of four exposure categories (probably not exposed; probably exposed low and irregularly/occasionally; probably exposed low and regularly; definitely exposed moderate/high and regularly) representing combined exposures to *ortho*-toluidine, aniline, and nitrobenzene, and a relative rank based on job, department, and era was used to estimate a cumulative exposure rank. None of the three studies, therefore, could estimate the effect of aniline alone or aniline adjusted for other occupational exposures (Hanley et al., 2012). The third cohort included 1875 workers ever employed at the plant between 1946 and 2006 (Carreón et al., 2014). Incident cancers were identified from state cancer registries. Standardized incidence ratios and standardized rate ratios (SRRs) for bladder cancer were derived by exposure category and cumulative rank quartiles for different lag periods. Cox regression was used to model bladder cancer incidence by estimated cumulative exposure rank, adjusting for confounders. Indirect methods were used to adjust for tobacco smoking (Steenland & Greenland, 2004). Relative risks were not estimated separately for aniline or *ortho*-toluidine. Overall, the standardized incidence ratio for bladder cancer was 2.87 (95% CI, 2.02–3.96; 37 observed cases). For those probably exposed to low levels and regularly, the standardized incidence ratio was 4.21 (95% CI, 1.15–10.80; fewer than 5 cases). For those definitely exposed to moderate/high levels, the standardized incidence ratio was 3.90 (95% CI, 2.57–5.68; 27 cases). Examination of duration of exposure among those definitely exposed to moderate/high levels revealed a trend towards increasing risk with increasing duration of exposure (P for trend, < 0.001). A trend with increasing time since first exposure in this same group of workers was also observed (P for trend, < 0.001). A significant trend was also observed for cumulative rank across all exposed workers for an unlagged analysis and for a lag of 10 or 20 years, but not 30 years. Cox regression, where the lag of 10 years was deemed to provide the best fit, was used to fit a variety of models. Cumulative rank was significantly associated with bladder cancer hazard rate in categorical models using quartiles, quintiles, and deciles, based on the exposure distribution among all cases and in continuous models including log–linear, log–square root and log–log as well as restricted cubic spline models. The bias factor for tobacco smoking was 1.08 and so made little difference to the inferences. [The Working Group noted that in this study it was not possible to separate any aniline-specific effect due to the limited available records on worker exposure to the different chemicals used.]

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

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Preti et al. (1988) USA NR	Cases: 10 hospital-based cases confirmed by X-ray, bronchoscopy, and biopsy Controls: 16, in two groups; 8 controls were younger (age, 22–41 yr) and possibly had non-cancer lung diseases; 8 controls were healthy hospital employees matched by age (age, 57–66 yr) Exposure assessment method: quantitative measurements; aniline and other compounds measured by GC-MS in exhaled air	Lung (ICD10 C34), incidence	Aniline detected above detection limit (0.1 ng per 20 L lung air) in exhaled air (OR): No Yes	 5 5	 1 [7.0 (1.0–48.3)]	None	*Exposure assessment critique*: Exposure not well-defined nor well assessed. Current exposure unsuitable for cancers due to long latency. Mass spectrometry in 1988 may not have identified aniline correctly. Exposure assessed after disease for cases. Controls selected to not have industrial exposure. Other comments: aniline was detected in exhaled air of 5 of the cases, none of the older controls, and 2 of the younger controls; measures of association were not reported, but crude ORs were calculated using information from table, but could not be stratified or adjusted due to small study size Strengths: case diagnoses were medically confirmed. Limitations: small study size; one group of controls was hospital-based; all cases were heavy smokers; no adjustment for confounders was conducted; although exposure was measured quantitatively, exhaled air is not an indicator of long-term exposure

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Nizamova (1991) the Russian Federation NR	Cases: 258 bladder cancer cases; no other information provided. Controls: 454 healthy controls, matched by sex, place of residence, and age in a 1:2 ratio Exposure assessment method: exposure assessment by interview but not otherwise described	Urinary bladder (ICD10 C67), incidence	Aniline dye production (OR): Never exposed Ever exposed	 3 4	 1 2.4 (0.1–69.5)	Sex, residence, age	*Exposure assessment critique*: Exposure not well-defined. Quality is therefore uncertain and probably poor. Other comments: chance, bias or confounding could explain the findings Limitations: no information on case or control selection; no information on exposure assessment; small sample size; no adjustment for confounders
Feingold et al. (1992) USA 1976–1983	Cases: 252 cases of childhood cancer (age, 0–14 yr), ascertained through cancer registry, supplemented by hospital and pathology records, and reviewed by paediatric oncologists Controls: 222 controls identified through random-digit dialling and matched individually by age (± 3 yr), sex, and telephone exchange area Exposure assessment method: expert judgement; parental occupation obtained by interview and exposure assigned on the basis of job title and industry with a JEM; exposure was defined as those whose parent had aniline (and other chemical) exposure at a medium or high level for at least 6 mo in the year before the birth of the child	All cancers combined, incidence Leukaemia (acute lymphoblastic/ lymphocytic leukaemia; ICD10 C91), incidence Brain (childhood cancer, ICD10 C71), incidence	Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed	 NR 10 NR 3 NR 2	 1 1.8 (0.6–6.0) 1 2.1 (0.4–10.5) 1 1.4 (0.2–10.9)	Age, sex, area, father's education	*Exposure assessment critique*: Exposure was defined but not well-assessed. Overall quality assessed as poor. Other comments: response rate was approximately 70%; high degree of correlation among exposures and small study size prevented adjustment for other exposures; interviewers assigned occupation and were not blinded to case ascertainment, but were trained to be objective and were not aware of the study hypotheses Strengths: diagnostic confirmation of case status; adjustment for paternal education Limitations: small study size; one parent usually reported both parents' occupation, which could lead to exposure misclassification

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Alguacil et al. (2000) Spain 1992–1995	Cases: 164 hospital-based incident cases Controls: 238 hospital-based controls free of pancreatic cancer, admitted under suspicion of pancreatic cancer, biliary cancer, or chronic pancreatitis. Exposure assessment method: expert judgement; interviews of subjects or next of kin about employment in different industries, with industrial hygiene expert review about potential exposure to 22 agents, including aniline, and exposure categorized as high, low, unknown, or none	Pancreas (ICD10 C25), incidence	Exposure to aniline (OR):			Sex, age, hospital, smoking (status and pack-years), alcohol consumption	*Exposure assessment critique*: Exposure was defined but not well assessed. Overall quality assessed as poor. Strengths: diagnostic confirmation of cases; high percentage (90%) of subjects with occupational histories; occupational exposures were assessed by expert judgement Limitations: small study size; potential for non-differential misclassification of exposure; potential for selection bias since controls had chronic pancreatitis and other cancers, but sensitivity analyses found only small decreases in risk estimates
			Never	NR	1		
			Ever exposed	6	1.35 (0.36–5.11)		
		Pancreas (ICD10 C25), incidence	High exposure to aniline for ≥ 6 mo (OR):				
			Never	158	1		
			Ever exposed	5	1.77 (0.36–8.57)		
		Pancreas (ICD10 C25), incidence	High exposure to aniline for ≥ 10 yr, 10 yr before diagnosis (OR):				
			Never	158	1		
			Ever exposed	5	2.58 (0.43–15.3)		

CI, confidence interval; GC-MS, gas chromatography-mass spectrometry; ICD10, International Classification of Diseases, 10th edition; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; yr, year.

2.2 Case–control studies

See Table 2.2.

Four case–control studies investigated the association between exposure to aniline and various cancers. Each study focused on a different cancer: bladder, lung, pancreatic, or childhood cancer.

A case–control study by Preti et al. (1988) used exhaled air measurements of volatile compounds among newly diagnosed cases of lung cancer and two groups of controls in Pennsylvania, USA. The study included 10 hospital-based cases (seven men, three women; age range, 59–77 years). The diagnosis of lung cancer (squamous cell carcinoma, 6 cases; undifferentiated large cell cancer, 2 cases; and adenocarcinoma, 2 cases) was confirmed by X-ray, bronchoscopy, and biopsy. One control group included eight individuals who were younger than the cases (range, 22–41 years) and who were recruited from a lung disease programme at the same hospital as the cases and were free of lung cancer. [The Working Group noted that the article did not mention whether these individuals had another health condition apart from lung cancer for which they were receiving treatment.] The second group of controls included eight healthy hospital employees of similar age as the cases (range, 57–66 years). The selection criteria for these controls were: (i) absence of chronic or acute lung disease; (ii) no industrial dust exposure; (iii) normal chest X-ray; and (iv) no medication use at the time of the study. [The Working Group noted that the first three control selection criteria likely reduced the probability of exposure to aniline among the controls to be less than that of cases and thus may have biased the study.] Tobacco smoking and occupational histories were obtained from all study participants. All cases were in patients who were or had been smokers (consumed more than a half pack of cigarettes per day).

No measures of association were reported in the study; however, based on data from a table, the Working Group calculated a crude odds ratio (OR) for aniline in exhaled air above the limit of detection versus none-detected of 7.0 (95% CI, 1.0–48.3) using all controls. [The Working Group considered this study uninformative for several reasons: that aniline was measured after disease occurrence, that aniline in exhaled breath likely reflects only very recent exposure because of its demonstrated short half-life (see Section 4.1), the very small study size, and the potential bias due to control selection criteria.]

Nizamova (1991) conducted a case–control study on bladder cancer among the population of the industrial region of Tambov in the Russian Federation. The study included 258 patients with bladder cancer and 454 controls matched by sex, place of residence, and age. [The Working Group noted that no case definition or method of case ascertainment was provided, and the source of controls and whether they were free of disease were not reported. The time frame in which the study was conducted was not reported. No details were provided on how the exposure assessment was conducted.] Overall, people with any contact with aromatic amines showed an increased risk of bladder cancer (OR, 4.7; 95% CI, 1.2–19.2). For exposure in aniline dye production, the odds ratio was 2.4 (95% CI, 0.1–69.5) (Nizamova, 1991). [The Working Group did not find the study informative; as noted above, the study did not provide information on case or control ascertainment, aniline exposure, or occupational co-exposures. The study size was underpowered, hence the imprecise confidence intervals, and the risk estimates were not adjusted for any confounders.]

A case–control study on parental occupation and childhood cancer was conducted in the USA by Feingold et al. (1992). The study included 252 cases of childhood cancer diagnosed between 1976 and 1983 that were ascertained through the Colorado Cancer Registry and supplemented by

record review at area hospitals, and that were confirmed microscopically (95%) or through direct visualization or radiography (3%). In addition, case records were reviewed by paediatric oncologists for diagnostic accuracy. Controls (n = 222) were identified through random-digit dialling and were individually matched to cases by age (± 3 years), sex, and telephone exchange area.

As described in Section 1.6.2, information on parental work history was obtained from a questionnaire that was administered mostly to the mothers. Occupational histories were coded and linked with a JEM that was previously developed by the National Cancer Institute (Hoar et al., 1980). The JEM contained information on 220 chemicals associated with one or more parental occupations that had been previously associated with childhood cancer. Only 10 childhood cancer cases were in children whose fathers were considered to have any history of occupational exposure to aniline.

Since only a small number of mothers reported an occupational history with any exposure to hydrocarbons (which included aniline), no results were reported for mothers. For fathers' exposure to aniline, the odds ratio, adjusted for father's education, was 1.8 (95% CI, 0.6–6.0; 10 exposed cases) for all childhood cancers, 2.1 (95% CI, 0.4–10.5; 3 exposed cases) for acute lymphocytic leukaemia, and 1.4 (95% CI, 0.2–10.9; 2 exposed cases) for brain tumours. Although maternal tobacco smoking during pregnancy confounded the association in stratified analyses, it was no longer a confounder in unconditional logistic regression models when paternal education was included (Feingold et al., 1992).

[The Working Group noted that although analyses were adjusted by father's education, the high degree of correlation between exposures and the small number of cases prevented adjustment for other exposures. In addition, one parent usually reported both parents' occupation, which could lead to exposure misclassification.]

The PANKRAS II study, a case–control study of pancreatic cancer associated with occupational exposures, was conducted between 1992 and 1995 at five hospitals in eastern Spain (Alguacil et al., 2000). The study included 164 hospital-based incident cases of pancreatic cancer for which diagnoses were reviewed by a panel of experts. Controls (n = 238) were admitted to the same hospitals as cases and were free of pancreatic cancer, but had chronic pancreatitis (n = 93), acute pancreatitis (n = 34), other benign pathologies (n = 70), or other cancers (n = 41). Trained interviewers administered a questionnaire that obtained clinical history, symptoms before admission, occupational history, and lifestyle information. [The Working Group noted that information regarding whether the interviewers were blinded as to case status was not provided but it seems unlikely that they were.]

As described in Section 1.6.2, occupational histories were obtained by direct interviews and were available for approximately 90% of cases and controls. Participants were asked if they had ever performed any of 10 workplace activities that were believed to be potentially associated with pancreas and biliary cancer risk. Odds ratios were adjusted for sex, age, hospital, tobacco smoking (status and pack-years), and alcohol consumption using unconditional logistic-regression models. The risk of pancreatic cancer associated with ever exposure to aniline derivatives was 1.35 (95% CI, 0.36–5.11; 6 exposed cases). For high exposure to aniline derivatives for at least 6 months, the odds ratio was 1.77 (95% CI, 0.36–8.57; 5 exposed cases), and for high exposure to aniline derivatives for at least 10 years, 10 years before diagnosis, the odds ratio was 2.58 (95% CI, 0.43–15.3; 5 exposed cases) (Alguacil et al., 2000).

[The Working Group considered that the study had strengths, including diagnostic confirmation of cases, a high percentage (> 90%) of participants with occupational histories, and occupational exposures assessed by expert

judgement. Limitations of the study included the small numbers of exposed cases. Although the authors reported that the potential for misclassification of exposure is non-differential since cases were interviewed in the same way as controls, differential misclassification of exposure is possible since the interviewers were most likely not blinded as to case status. There is also potential for selection bias since controls had chronic pancreatitis and other cancers; however, sensitivity analyses excluding controls with pancreatitis (chronic or acute) found only small changes in risk estimates.]

2.3 Case reports and case series

See Table S2.3 (Annex 2, Supplementary material for Section 2, web only; available from: https://publications.iarc.fr/599).

Altogether 17 case reports or series on aniline exposure and cancer risk are reported in this section, 16 on bladder tumours and one on lung cancer. Several other potentially relevant case reports on cancers of the bladder or other organ sites were identified from a literature search but that were considered ineligible because the title or abstract did not indicate likely aniline exposure, they were not actual case reports, or they were replicated elsewhere. The Working Group, in reviewing these case reports and case series, also noted when the study mentioned co-exposures to other chemicals for which there is *sufficient* or *limited* evidence that they cause bladder cancer in humans (see Section 1.4.3; and also IARC, 2020).

(a) Bladder cancer

Ludwig Rehn was the first to report on the occurrence of bladder tumours in workers exposed to aniline who were engaged in fuchsine dye production (Rehn, 1895). He noted 3 cases among 45 workers who had all worked in the same room of a factory in Frankfurt, Germany, where toluidine, aniline, nitrobenzene, and iron chloride were mixed and heated to produce "raw" fuchsine. Case No. 1 was a worker diagnosed with papillary fibroma of the bladder at age 40 years, after 15 years of employment at the factory. Case No. 2 was a worker aged 29 years when diagnosed with a papillary fibroma of the bladder; the duration of employment was not known. Case No. 3 was a worker aged 49 years who had worked at the plant for 20 years when he was diagnosed with what appeared to be a bladder carcinoma, by cystoscopy, but histologically was classified as a bladder sarcoma. [The Working Group noted that workers were exposed not only to aniline, but also to other known occupational bladder carcinogens such as toluidine (isomer not specified).]

At a surgical congress in Berlin, Germany, in 1904, a series of 23 cases of bladder tumours was reported that had been observed in factories (in Germany and England) where aniline and "its homologues and allied substances" were produced (Anon., 1904). There were five papillomas (of which two became malignant), one sarcoma, and 17 carcinomas. The shortest latency period observed was 9 years. [The Working Group noted that there was no information on individual cases, specific exposures, duration of exposure, or observation period, and that co-exposure to known occupational bladder carcinogens (e.g. 2-naphthylamine, benzidine, *ortho*-toluidine), is probable in the aniline production industry.]

In 1901–1910, 6 deaths resulting from bladder tumours were reported among 840 workers employed in aniline factories in Basel, Switzerland (Anon., 1921). During the same period, 12 additional deaths from bladder tumours occurred among 56 500 male workers in the city. In 1861–1900, before the chemical industry was established in the city, 6 cases of bladder tumours were seen at the surgical clinic; while during the following 10 years (1901–1910), 16 cases appeared at the clinic, 10 of which were

in workers exposed to aniline, 2 were involved in dye manufacturing. A large proportion of the bladder tumours were malignant and were diagnosed mainly in men older than 40 years and employed for "many years" in the aniline industry. Benzidine and β-naphthylamine [2-naphthylamine] are mentioned as suspected co-exposure bladder carcinogens in these factories (Anon., 1921). [The Working Group noted that no information on individual cases is given and co-exposure to other occupational bladder carcinogens was reported.]

In a review discussing several aspects of the "aniline tumours", Berenblum (1932) referred to three case reports (Curshmann, 1920; Schwerin, 1920; Oppenheimer, 1927) on bladder tumours in which 8, 5, and 24 cases, respectively, were in workers who had been exposed to aniline only.

Gehrmann (1934) reported findings from two plants within the dye industry in the USA where workers had been systematically screened for bladder tumours by cystoscopy since 1931. In a group of 577 workers, 27 bladder tumours were observed. Of these, 13 workers had been exposed to benzidine and β-naphthylamine [2-naphthylamine], 10 to β-naphthylamine only, 2 to benzidine only, and 2 to α-naphthylamine [1-naphthylamine] only. In a group of workers [number not stated] exposed to aniline only, also subject to regular cystoscopic examinations, no bladder tumours were observed. [The Working Group noted that no bladder tumours occurred in the aniline-only exposed group. The absence of information on the size of this group, however, renders this finding uninformative.]

In the USA, aniline production started in 1915; the first bladder tumour among workers was diagnosed in 1931. Routine cystoscopy among workers at one plant identified 63 bladder tumours, of which 24 were carcinomas and 39 were papillomas (Gehrmann, 1936). The average duration of exposure to aniline was 13.2 years for the group diagnosed with carcinoma. In addition to aniline, the workers were exposed to the bladder carcinogens β-naphthylamine [2-naphthylamine], and benzidine, as well as α-naphthylamine [1-naphthylamine]. [The Working Group noted that no information on individual cases was given and that co-exposure to occupational bladder carcinogens was reported.]

By 1938, approximately 550 so-called "aniline tumours" had been reported in the literature, according to a review by Hueper (1938). Of these, more than 300 cases were reported in Germany, more than 80 (since 1905) in Switzerland, and about 40 in England. In the USA, approximately 100 cases were reported, all in the same company. In the former Soviet Union, three cases were reported in 1926, and more cases [numbers not stated] in 1932. In Austria, cases [no numbers given] were reported in 1926, 1927, and 1932. Reports from Italy in 1936 and 1937 stated that 12 aniline tumours had occurred during the past few years among 86 workers in one dye factory. The review notes that the occurrence of bladder tumours is often associated with prolonged occupational contact with phenylamine [aniline), its isomers and homologues (toluidine, isomer not specified, and xylidine) and other amino derivatives; diphenylamine (benzidine), its isomers and derivatives (e.g. toluidine, isomer not specified, or dianisidine); and naphthylamines and related compounds (e.g. naphthylene diamines) (Hueper, 1938). [The Working Group noted that no data on individual cases were given and occupational co-exposure to known bladder carcinogens occurred.]

Orts (1948) reported on 2 cases of bladder cancer in Galicia, Spain, where aniline was widely used as a colourant in red wine. The author describes how wine was served in big cups, which remained stained a strong red colour after emptying. Both cases were in men who were heavy drinkers, and who were diagnosed at ages 60 and 75 years, respectively. The author suspected that the cancers were caused by aniline exposure. No occupational or other source of aniline exposure is mentioned. [The Working

Group considered the study uninformative, as exposure information is very limited.]

Goldblatt (1949) reported details of 99 cases of bladder tumour diagnosed between 1934 and 1947 in some 4000 men employed in two chemical factories in the United Kingdom. Three papillomas arose in a subgroup of men exposed only to aniline. Larger numbers of tumours, comprising more carcinomas than papillomas, arose in men exposed only to α-naphthylamine [1-naphthylamine] (n = 11 tumours), to β-naphthylamine [2-naphthylamine] (n = 22 tumours), or to benzidine (n = 6 tumours), or to the combination of two or more of these substances (n = 34 tumours). [The Working Group noted that no information was given on the number of workers exposed to aniline only.]

Pujol (1950) reported one case of a bladder tumour in a worker employed for 26 years in a hat-manufacturing factory where several aniline-based dyes were used, in Spain. Exposure through dermal contact and inhalation had been nearly continuous. No protective equipment was used. One additional worker at the plant had unspecified bladder issues but was not examined. The other workers had been at the plant for shorter periods and were asymptomatic. [The Working Group noted that no data on specific exposures or tumour characteristics were given and co-exposure to other occupational bladder carcinogens is probable.]

Aboulker & Smagghe (1953) reported on 21 cases of bladder tumour occurring in workers in the dye industry in France between 1941 and 1952. Three of the cases were exposed to aniline. [The Working Group noted that for all three cases, no occupational co-exposure to any bladder carcinogen was reported.] Case No. 5 of the series was diagnosed in 1947 at the age of 57 years, had worked for 17 years in a dye-production factory [The Working Group noted that no further exposure details were provided]; latency was unknown; tumour histology was unknown. Case No. 6 was diagnosed in 1947 at the age of 47 years, had worked for 24 years in a dye-production factory, and had been exposed to aniline and α-naphthylamine [1-naphthylamine] for 13 years [the Working Group noted that no further exposure details were provided]; latency was 5 years; histology was "benign tumour". Case No. 9 was diagnosed in 1949 at the age of 69 years, had worked for 38 years in a dye-production factory, and had been exposed to aniline and α-naphthylamine [1-naphthylamine] for 26 years [the Working Group noted that no further exposure details were provided]; latency duration was unknown; histology was "malignant tumour". The 18 other cases were exposed to α-naphthylamine, β-naphthylamine, benzidine, *ortho*-toluidine (these latter three are bladder carcinogens) and/or α-aminoanthraquinone. [Information on tobacco smoking was not given.]

Pujol (1954) reported on a second bladder tumour in Spain. The case was in a worker aged 52 years employed for 30 years in the preparation of tints who had used synthetic dyes made of water/alcohol- and oil-based anilines, potassium bichromate, and other series of tints. The worker never used personal protective equipment. The tumour was described as an infiltrating epithelioma; the patient had local recurrence, spreading to the pelvic wall, 1 year after operation. [The Working Group noted that co-exposure to other occupational bladder carcinogens could not be excluded.]

In an autopsy study on multiple primary cancers, Link (1961) noted one case of bladder cancer in a man who had worked in dye production, where he had been specifically exposed to aniline and toluidine. [The Working Group noted that this bladder cancer case was also exposed to toluidine, a known bladder carcinogen.]

Vigliani & Barsotti (1961) reported on bladder tumours in workers in six dyestuff factories in Italy in 1931–1960. Among the 42 cases of carcinoma and 36 cases of papilloma reported, only 1 case of papilloma occurred in a worker exposed to aniline. No co-exposure to bladder carcinogens

was reported for this case. The remaining cases were tumours diagnosed in workers exposed to benzidine only (31 carcinomas, 16 papillomas), β-naphthylamine [2-naphthylamine] only (7 carcinomas, 12 papillomas), benzidine and β-naphthylamine [2-naphthylamine] in combination (2 carcinomas, 1 papilloma), α-naphthylamine [1-naphthylamine] only (1 carcinoma, 3 papillomas), and to azo-dyes in production [the Working Group noted that no information on specific exposure is given for this group] (1 carcinoma, 3 papillomas). [The Working Group noted that no information on the number of workers in each exposed group, age at diagnosis, duration of exposure, or latency was given.]

Temkin (1963) reported on 208 cases of bladder disease related to occupational exposure observed during 25 years in the aniline dye industry in the former Soviet Union. Among workers who had undergone cystoscopy once or twice each year, 125 cases of bladder disease occurred; of these, 5 were malignant tumours. In a group of workers not systematically examined by cystoscopy, 83 workers presented with bladder tumours, of which 32 were malignant. [The Working Group noted that there was no information on specific exposures, duration of exposure, or number of workers in the screened and unscreened groups; the numbers of malignant/benign and examined/not examined cases did not add up correctly; and co-exposure to occupational bladder carcinogens other than aniline is probable.]

Nakano et al. (2018) reported on 10 cases of bladder cancer in male Japanese workers exposed primarily to *ortho*-toluidine and employed at two plants producing organic dye and pigment intermediates. No exposure measurements were available, but four jobs or production processes were identified, and levels of exposure to *ortho*-toluidine and other aromatic amines were estimated based on number of years and proportion of time spent on each of the four processes each month. In most of the 10 cases, there was a higher level of exposure to *ortho*-toluidine than to the other amines. Co-exposure to aniline occurred in 9 out of 10 cases. The 10 identified cases were in workers hired between 1987 and 1997. Duration of exposure to aniline ranged from 3 to 21 years (mean, 13.6 years), estimated latency from the initial exposure to aniline to diagnosis ranged from 15 to 27 years (mean, 21.7 years). Eight out of the nine cases were tobacco smokers and the mean number of pack-years for the smokers was 29.9 (range, 10–45). All the affected workers had been primarily engaged in drying and packing the product made from *ortho*-toluidine, a known bladder carcinogen. [The Working Group noted that all aniline workers were co-exposed to *ortho*-toluidine. Eight of the nine cases were also tobacco smokers.]

(b) Lung cancer

Thiess et al. (1969) reported on a series of lung cancer cases diagnosed between 1957 and 1967 at BASF (Badische Anilin und Soda Fabrik) plants in Germany. In the group of workers belonging to the company's dye warehouses, including the aniline book-binding department, the department for tri-colouring, the indigo, aniline, and alizarin departments, and the engineering department, 2 cases of lung cancer occurred among 185 workers. Both cases were in workers aged 53 years at diagnosis, and their smoking status was unknown. [The Working Group concluded that the study is uninformative.]

2.4 Evidence synthesis for cancer in humans

Epidemiological studies available for the evaluation of the carcinogenicity of aniline are scarce. Since the publication of *IARC Monographs* Volumes 4 and 27 and Supplement 7 (IARC, 1974, 1982, 1987), only four case–control studies and two new cohort studies have been published. Cohort studies examined bladder

cancer incidence and mortality, as well as mortality from cancers of the digestive organs, lung, and haematopoietic malignancies. Each case–control study examined a different cancer site in association with aniline: lung, bladder, and pancreas in adults, and leukaemia and brain cancer in children. Seventeen case reports and case series were reviewed; 16 of these reported on bladder tumour cases and one reported on lung cancer cases. These case series and case reports provided historical perspective but were mostly uninformative.

2.4.1 Exposure assessment and misclassification of exposure

Quality of exposure assessment was a major consideration in the evaluation of the studies by the Working Group. Detailed reports on the strengths and limitations of exposure evaluations in cohort and case–control studies are provided in Sections 1.6.1 and 1.6.2, respectively.

In the three cohort studies with moderate to well-defined exposure assessments (Ott & Langner, 1983; Sorahan, 2008; Carreón et al., 2014), the information was collected retrospectively. This could have resulted in non-differential misclassification of exposure (bias due to inaccurate reporting independent of disease status) that may result in an underestimate of the true strength of an association between exposure and disease. The Working Group also noted that none of the cohort studies could provide clear evidence that aniline was the agent responsible for any bladder cancer excesses observed.

The Working Group noted that the case–control studies did not provide information on duration, intensity, or cumulative exposure to aniline. These studies were fundamentally based on work histories derived from interviews of the study participants or a family member who may have had an incomplete recollection, and thus have the potential for recall bias. One study was based on analysis of aniline in exhaled breath after a diagnosis of lung cancer, which is a poor measure of past exposures.

2.4.2 Co-exposures to other agents with sufficient or limited evidence of bladder carcinogenicity in humans

The main factor that affected most studies was their inability to control for concurrent exposures to other agents for which there is *sufficient* (e.g. *ortho*-toluidine, 2-naphthylamine) or *limited* (e.g. MBT) evidence of bladder carcinogenicity in humans and therefore to differentiate aniline-related effects from the effects of co-exposures. Only two case series (Aboulker & Smagghe, 1953; Vigliani & Barsotti, 1961) reported 3 cases and 1 case, respectively, that may not have had exposure to other occupational bladder carcinogens; however, no information was given on tobacco smoking history for these cases. None of the case–control studies accounted for the potential for confounding by co-exposures. Each of the cohort studies identified concurrent occupational exposure to some carcinogenic aromatic amines, but only Sorahan (2008) was able to estimate risk of bladder cancer in aniline-exposed workers while adjusting for duration of work in a department where *ortho*-toluidine was present, work in a department where PBN was present, and cumulative exposure to MBT.

2.4.3 Tobacco smoking

Tobacco smoking is the most important risk factor for bladder cancer, and smokers are at least three times more likely to develop bladder cancer than are never-smokers (Cumberbatch et al., 2018). In addition, aniline is a component of tobacco smoke, but the contribution of tobacco smoking to aniline exposure among aniline workers is considered to be negligible to minimal (see Section 1.4.1). Only one case series (Nakano et al., 2018) informed on tobacco smoking status; 8 out of 9 aniline-exposed cases were smokers.

Only one case–control study (of pancreatic cancer) controlled for potential cofounding by tobacco smoking (Alguacil et al., 2000). Tobacco smoking was indirectly adjusted for in one of the cohort studies (Carreón et al. 2014); in this study, the estimated bias factor was small, compared with the risk estimate due to exposure to aniline, *ortho*-toluidine, and nitrobenzene combined. The Working Group considered that studies that did not evaluate the contribution of tobacco smoking to their risk estimates were of low quality.

2.4.4 Bladder cancer

In total, four cohort studies (Case et al., 1954; Case & Pearson, 1954; Ott & Langner, 1983; Sorahan, 2008; Carreón et al., 2014) and one case–control study (Nizamova, 1991) evaluated the association between aniline exposure and bladder cancer risk. The case–control study is not considered informative and therefore will not be further discussed. Of the four cohort studies, two were considered to be potentially informative for the evaluation. Sorahan (2008) found significant excesses of both bladder cancer mortality and incidence in aniline-exposed workers, in analyses that did not control for co-exposure to other known or suspected bladder carcinogens. They also found a non-significant increased risk by duration of employment in the aniline-exposed department, after adjusting for other known or suspected occupational bladder carcinogens. Carreón et al. (2014) found increased bladder cancer incidence associated with exposure to aniline, *ortho*-toluidine, and nitrobenzene combined. Statistically significant trends were also observed by duration of exposure, time since first exposure, and cumulative rank exposure lagged 10 and 20 years; these trends are unlikely to have occurred by chance. Both studies were methodologically sound, and both had reasonable power; however, the study by Carreón et al. (2014) could not separate any aniline-specific effect from the effects of *ortho*-toluidine and nitrobenzene. Overall, the Working Group considered that these available studies in humans are of good quality, but they are not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between aniline and bladder cancer.

2.4.5 Other cancers

Other cancer sites (lung and pancreas, and brain and leukaemia in children), were evaluated each in a different case–control study (Preti et al., 1988; Feingold et al., 1992; Alguacil et al., 2000). Ott & Langner (1983) also reported findings for mortality of cancer of the digestive organs, lung, and haematopoietic system in their cohort study. Sorahan (2008) reported a small non-significant deficit in lung cancer mortality compared with the national population in its cohort study. Some positive associations were observed in other studies. Overall, exposures were not well assessed, and there was a small number of exposed cases among other limitations, therefore these studies are of low to moderate quality and informativeness.

3. Cancer in Experimental Animals

Aniline was evaluated previously by the *IARC Monographs* programme in 1981 and 1987 (IARC, 1982, 1987). In its evaluation in 1987 (IARC, 1987), the Working Group concluded that there was *limited evidence* in experimental animals for the carcinogenicity of aniline.

See Table 3.1.

Table 3.1 Studies of carcinogenicity with aniline and aniline hydrochloride in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 107 wk (lower and higher dose) or 109 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.6%, 1.2% in feed for 103 wk 50, 50, 50 33, 43, 41	*Thyroid gland*: follicular-cell adenoma or carcinoma (combined) 0/38, 3/43, 1/43	NS	Principal strengths: appropriate statistics; sufficient number of animals and survival; use of males and females; adequate durations of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. Seven control males died and were found autolysed in wk 11–13. On the basis of survival data, adequate numbers of male mice were at risk for late-developing tumours (82% at the higher dose, 86% at the lower dose, and 66% of the control group survived until the end of the study). All mice were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on mice that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on mice that survived at least as long as the animal in which the first tumour was found
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 107 wk (lower and higher dose) or 109 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.6, 1.2% for 103 wk 50, 50, 49 30, 37, 41	*Liver*: hepatocellular carcinoma 1/46, 5/48, 5/48	NS	Principal strengths: appropriate statistics; sufficient number of animals and survival; use of males and females; adequate durations of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of female mice were at risk for late-developing tumours (84% at the higher dose, 74% at the lower dose, and 60% of the control mice survived until the end of the study). All mice were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on mice that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on mice that survived at least as long as the animal in which the first tumour was found

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 12 days 9.5 mo Delclos et al. (1984)	Intraperitoneal injection Aniline hydrochloride, NR Sodium acetate buffer 0 (control), 0.15 µmol/g bw Single injection of 10 µL of a 50 mM sodium acetate buffer solution (pH, 5.0), ± aniline hydrochloride 33, 26 NR	*Liver*: hepatoma Tumour incidence, 3/33 (9%), 2/26 (8%) Tumour multiplicity (mean ± standard deviation), 0.1 ± 0.3, 0.1 ± 0.3	 NS NS	Principal limitations: no body-weight data; not a long-term carcinogenicity study No. of animals at start is the number of weaned animals (at 28 days)
Co-carcinogenicity Mouse, CBA × C57/Bl6 (F) NR [weight, 10–12 g] 26 or 39 wk Litvinov et al. (1984)	Oral Aniline, NR Drinking-water 0 (for 26 wk), 1.0 (for 26 wk), 0 (for 39 wk), 1.0 (for 39 wk) mg/L + NDMA (10 mg/L) 50, 50, 50, 50 30, 15, 25, 32	*Liver* Tumours (epithelial) 0/30, 0/15, 2/25, 4/32 Tumours (endothelial [vascular]) 0/30, 1/15, 11/25, 5/32 Tumours (all) 0/30, 1/15, 13/25, 9/32 *Lung*: tumours 16/30, 12/15, 20/25, 14/32	 [NS] [NS] [NS] [NS]	Principal limitations: no body-weight data. Total doses/animal: NDMA (26 wk), 5.050 mg; NDMA (39 wk), 6.825 mg; aniline (26 wk), 0.505 mg; aniline (39 wk), 0.683 mg. Histopathological examination of liver, lung, kidney, spleen, and all gross lesions. Pairwise comparison between NDMA + aniline-treated group versus NDMA-only group at 26 wk and at 39 wk. No results were provided for an aniline-only and an untreated group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Co-carcinogenicity Mouse, CBA × C57/Bl6 (F) NR [weight, 10–12 g] 12 mo Litvinov et al. (1986)	Oral Aniline, NR Drinking-water 0, 1.0 mg/L + NDEA (10.0 mg/L) 100, 100 NR	*Liver* Haemangioma 4/79, 19/88* Angiosarcoma 1/79, 0/88 Adenoma 1/79, 2/88 Carcinoma 3/79, 2/88 Tumours (all) 9/79, 23/88* *Lung*: adenoma 22/79, 55/88*	 *[$P = 0.003$; 2-tail Fisher exact test] [NS] [NS] [NS] *[$P = 0.02$; 2-tail Fisher exact test] *[$P < 0.0001$; 2-tail Fisher exact test]	Principal strengths: high number of mice per group Principal limitations: no body-weight data, no survival data, lack of aniline-only or untreated control groups Total doses: aniline, 1.09 mg; NDEA, 10.95 mg. No group treated with aniline only was available. Histopathological examination of liver, lung, kidney, oesophagus, stomach, spleen, and all gross lesions. The effective number of mice was the number of animals surviving after identification of the first tumour. No haemangiosarcoma of the liver was observed in any group. Potential differences between the current histopathological classification and that used when the study was performed

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 107 wk (lower dose), 108 wk (higher dose), or 110 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.3, 0.6% for 103 wk 25, 50, 50 17, 34, 27	*Spleen* Fibrosarcoma or sarcoma (NOS) (combined) 0/25, 7/50, 9/46*	*P* = 0.020, Cochran–Armitage trend-test; **P* = 0.015, Fisher exact test	Principal strengths: appropriate statistics; use of males and females; sufficient survival; adequate duration of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of male rats were at risk for late-developing tumours (54% at the higher dose, 68% at the lower dose, and 68% of the control rats survived until the end of the study). All rats were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on rats that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on rats that survived at least as long as the animal in which the first tumour was found
		Haemangiosarcoma 0/25, 19/50*, 20/46*	*P* = 0.001, Cochran–Armitage trend-test; **P* < 0.001, Fisher exact test	
		Body cavities, multiple organs (other than spleen): fibrosarcoma or sarcoma (NOS) (combined) 0/25, 2/50, 9/48*	*P* = 0.004, Cochran–Armitage trend-test; **P* = 0.017, Fisher exact test	
		Spleen or body cavities, multiple organs (other than spleen) (combined) Fibrosarcoma or sarcoma (NOS) (combined) 0/25, 5/50, 18/48*	*P* < 0.001, Cochran–Armitage trend-test; **P* < 0.001, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
NCI (1978) (cont.)		Haemangiosarcoma		
		0/25, 19/50*, 21/48*	$P = 0.001$, Cochran–Armitage trend-test; *$P < 0.001$, Fisher exact test	
		Spleen: fibroma		
		0/25, 7/50, 6/46	NS	
		Adrenal gland: benign or malignant (combined) pheochromocytoma		
		2/24, 6/50, 12/44	$P = 0.022$, Cochran–Armitage trend-test	
Full carcinogenicity Rat, F344 (F) 6 wk 107 wk (lower dose), 108 wk (higher dose), or 110 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.3, 0.6% for 103 wk 25, 50, 50 16, 44, 41	*Spleen or body cavities, multiple organs (other than spleen) (combined)*: fibrosarcoma or sarcoma (NOS) (combined)		Principal strengths: appropriate statistics; use of males and females; sufficient survival; adequate duration of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of female rats were at risk for late-developing tumours (82% at the higher dose, 88% at the lower dose, and 64% of the control rats survived until the end of the study). All rats were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on animals that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before week 52; when such an early tumour was found, comparisons were based on rats that survived at least as long as the animal in which the first tumour was found
		0/24, 1/50, 7/50	$P = 0.009$, Cochran–Armitage trend-test	
		Spleen: sarcoma (NOS)		
		0/23, 0/50, 3/50	NS	
		Uterus: endometrial stromal polyps		
		2/24, 15/48*, 7/50	*$P = 0.027$, Fisher exact test	
		Body cavities, multiple organs (other than spleen): fibrosarcoma or sarcoma (NOS) (combined)		
		0/24, 1/50, 4/50	NS	
		Adrenal gland: benign or malignant (combined) pheochromocytoma		
		1/24, 0/50, 5/48	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) NR [weight, ~150 g] 80 wk Hagiwara et al. (1980)	Oral (feed) Aniline, NR Feed 0, 0.03, 0.06, 0.12% 28, 28, 28, 28 NR	*Forestomach*: papilloma 0/15, 1/10, 1/18, 0/16 *Pituitary gland*: adenoma 0/15, 1/10, 0/18, 0/16	NR, [NS] NR, [NS]	Principal strengths: multiple-dose study Principal limitations: limited reporting of survival data; not a long-term carcinogenicity study
Full carcinogenicity Rat, F344 albino (CD-F) (M) 4–5 wk 104 wk US EPA (1982)	Oral Aniline hydrochloride, assumed to be 100% pure Feed 0, 10, 30, 100 mg/kg bw per day for 104 wk 130, 130, 130, 130 114, 116, 115, 104	*Spleen* Stromal sarcoma 0/123, 0/129, 1/128, 21/130* Haemangiosarcoma 0/123, 0/129, 0/128, 6/130* Fibrosarcoma 0/123, 0/129, 0/128, 3/130 Osteogenic sarcoma	 NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P < 0.0001$, Fisher exact test] NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P = 0.03$, Fisher exact test] NR [NS]	Principal strengths: adequate duration of exposure and observation; multiple-dose study; use of males and females; high number of rats per group; sufficient survival Principal limitations: data are not clearly presented throughout the study report, no statistics reported in the study report This report is difficult to follow especially in terms of clarity of incidence numbers. All major tissues and organs from the killed animals in the control and highest-dose groups, and only the spleen and any unusual lesions from the lowest- and intermediate-dose groups, were examined microscopically. There was a significant lower survival rate in high-dose males compared with controls; however, adequate numbers of male rats were at risk for late-developing tumours (80% at the highest dose, 88% at the intermediate dose, 89% at the lowest dose, and 88% of the control male rats survived until the end of the study). Food consumption during the first 50 wk was lower for treated male rats than for controls; however, the authors stated that "these differences were not considered to be treatment-related and were within the range of normal biological variability"

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
US EPA (1982) (cont.)		0/123, 0/129, 0/128, 3/130	NR [NS]	
		Mesothelioma, metastatic		
		0/123, 2/129, 4/128, 2/130	NR [NS]	
		Stromal hyperplasia		
		1/123, 2/129, 0/128, 31/130*	NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P < 0.0001$, Fisher exact test]	
		Testes: mesothelioma, tunica vaginalis		
		1/114, 4/130, 9/130*, 1/104	NR [$P = 0.019$, Cochran–Armitage trend-test]; *[$P = 0.022$, Fisher exact test]	
Full carcinogenicity Rat, F344 albino (CD-F) (F) 4–5 wk 104 wk US EPA (1982)	Oral Aniline hydrochloride, assumed to be 100% pure Feed 0, 10, 30, 100 mg/kg bw per day for 104 wk 130, 130, 130, 130 110, 109, 116, 117	*Spleen*: stromal hyperplasia		Principal strengths: adequate duration of exposure and observation; multiple-dose study; use of males and females; high number of rats per group; sufficient survival Principal limitations: data are not clearly presented throughout the study report; no statistics reported in the study report This report is difficult to follow especially in terms of clarity of incidence numbers. All major tissues and organs from the killed animals in the control and highest-dose groups, and only the spleen and any unusual lesions from the lowest- and intermediate-dose groups, were examined microscopically. Adequate numbers of female rats were at risk for late-developing tumours (90% at the highest-dose, 89% at the intermediate dose, 84% at the lowest-dose, and 85% of the control female rats survived until the end of the study). No significantly increased incidence of tumours was observed in treated female rats
		0/129, 0/129, 0/130, 9/130*	NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P = 0.003$, Fisher exact test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Co-carcinogenicity Rat, albino outbred (M) NR [weight, 150–200 g] 19 mo Litvinov et al. (1982)	Oral Aniline, NR Drinking-water 0, 0.5 mg/L + NDMA (10 mg/L, 0.5 mg/kg bw per day) 50, 50 42, 44	*Liver*: tumours [mainly hepatocellular] 15/42, 34/44*	*[$P < 0.0002$; 2-tail Fisher exact test]	Principal limitations: strain NR; no body-weight data. Histopathological examination of the liver, lung, kidney, spleen, and all gross lesions. No liver, kidney, or lung tumours occurred in 38 untreated rats (number at start, 50). No group treated with aniline alone was available. The effective number of animals was the number of surviving animals
Full carcinogenicity Hamster, Syrian golden (M) 8 wk 87 wk Hecht et al. (1983)	Subcutaneous injection Aniline, NR Peanut oil 0, 99 mmol/kg bw total dose 52 weekly injections of 1.9 mmol/kg bw 15, 15 NR	*Any tumour type* 0/15, 0/15	NA	Principal limitations: limited reporting of survival and body-weight data; only gross lesions and representative samples of all major organs were processed for microscopic evaluation Mean survival time was shorter in the treated group: 67.7 wk for treated males (compared with 75.5 wk for male controls). Unconventional route of exposure
Full carcinogenicity Hamster, Syrian golden (F) 8 wk 87 wk Hecht et al. (1983)	Subcutaneous injection Aniline, NR Peanut oil 0, 99 mmol/kg bw total dose 52 weekly injections of 1.9 mmol/kg bw 15, 15 NR	*Any tumour type* 0/15, 0/15	NA	Principal limitations: limited reporting of survival and body-weight data; only gross lesions and representative samples of all major organs were processed for microscopic evaluation Mean survival time was shorter in the treated group: 62.1 wk for treated females (compared with 68.7 wk for female controls). Unconventional route of exposure
Full carcinogenicity Rabbit, NR (M) NR (weight, ~2 kg) Lifetime (up to 362 days) Yamazaki & Sato (1937)	Urinary bladder instillation Aniline, NR Water 0.6 g/wk 10 mL of 1% aniline (0.1 g) given by daily instillation (0.6 g/wk) 30 0	*Urinary bladder*: papillomas 12/30	NA	Principal strengths: high quality of gross descriptions and microscopic examinations Principal limitations: lack of adequate control group A control group of 20 rabbits was instilled with water only or olive oil only, but the number of animals instilled with water only was unspecified. No rabbits developed urinary bladder tumours in this control group. Unconventional route of exposure

bw, body weight; F, female; M, male; mo, month; NA, not applicable; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NOS, not otherwise specified; NR, not reported; NS, not significant; wk, week.

3.1 Mouse

3.1.1 Oral administration (feed)

Groups of 50 male and 49–50 female B6C3F$_1$ mice (age, 6 weeks) were given feed containing aniline hydrochloride [purity not reported; chemical analysis performed by the authors suggested a compound of high purity] at 0% (controls), 0.6% (lower dose) or 1.2% (higher dose) for 103 weeks (NCI, 1978). Mice at the higher dose, lower dose, and in the control group were killed at 107, 107, and 109 weeks, respectively. There was no significant effect on survival in groups of treated male and female mice. Seven control males died and were found autolysed in weeks 11–13. Adequate numbers of mice were at risk of late-developing tumours: for males, 82% (41/50) at the higher dose, 86% (43/50) at the lower dose, and 66% (33/50) of the controls survived until the end of the study; for females, 84% (41/49) at the higher dose, 74% (37/50) at the lower dose, and 60% (30/50) of the controls survived until the end of the study. Significant mean body-weight decreases occurred in both groups of treated males. An anatomopathological study with histopathological examination was performed on gross lesions and all major organs and tissues. There was no significant increase in the incidence of tumours in the groups of treated male and female mice compared with their respective controls (NCI, 1978). [The Working Group noted the principal strengths of the study: there was a sufficient number of mice at start and adequate survival, appropriate statistics were performed, males and females were used, and the durations of exposure and observation were adequate.]

3.1.2 Intraperitoneal injection

Two groups of male B6C3F$_1$ mice [number of mice per group at start unspecified] (age, 12 days) were given a single intraperitoneal injection of aniline hydrochloride [purity not reported] at a dose of 0 (vehicle control) or 0.15 μmol/g body weight (bw) in 10 μL/g bw of 50 mM sodium acetate buffer solution (pH, 5.0) (Delclos et al., 1984). Mice were weaned at age 28 days (26 treated mice and 33 controls), and the study was terminated when mice were aged 10 months. On death or at the termination of the study, gross routine autopsies were performed for all mice; this included inspection of the skin, subcutaneous tissues, and the organs of the abdominal and thoracic cavities. In the liver, hepatic nodules were enumerated to determine the incidence and multiplicity of hepatoma. Representative liver tumours from each mouse were evaluated by microscopic examination. The hepatic tumours were diagnosed histologically as type A hepatoma, type B hepatoma, or as mixed type A–type B hepatoma. In addition, all gross tumours in other tissues were examined histologically. [The Working Group noted the high quality of the gross description and microscopic examination, with meticulous care for detail.] Neither the incidence of hepatoma nor the average number of hepatomas per mouse was significantly higher in the treated group compared with vehicle controls. [The Working Group noted that this was not a long-term carcinogenicity study, and that body-weight data were lacking.]

3.1.3 Subcutaneous injection

In a 24-month study, two groups of 10 Stock female mice and one group of 10 strain D female mice were given repeated [no further details provided] subcutaneous injections of 0.25 mL of lard containing 0.5% aniline [purity not reported]. Five mice per group survived at the end of the study. No tumours were detected at necropsy (Shear & Stewart, 1941). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

One group of 20 strain C female mice was given eight subcutaneous injections of 5 mg of aniline [purity not reported] in olive oil in a

15-month experiment, and one group of 11 strain C female mice was given 13 subcutaneous injections of 4 mg of aniline hydrochloride [purity not reported] in aqueous solution in a 12-month experiment. No tumours were detected at necropsy (Hartwell & Andervont, 1951). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

3.1.4 Co-carcinogenicity

In a study by Litvinov et al. (1984), four groups of 50 female CBA × C57/Bl6 mice [age not reported] (body weight, 10–12 g) were given drinking-water containing *N*-nitrosodimethylamine (NDMA) at a concentration of 10 mg/L plus aniline [purity not reported] at a concentration of 0 (control group) or 1.0 mg/L for either 26 weeks (total dose, aniline, 0.505 mg; NDMA, 5.050 mg) or 39 weeks (total dose, aniline, 0.683 mg; NDMA, 6.825 mg). Two additional groups of 50 female mice received drinking-water only or aniline only (total dose, 0.683 mg) for 39 weeks. Necropsy was performed on surviving mice, and the liver, lung, kidney, spleen, and all gross lesions were examined histologically. The effective number of animals was the number of surviving mice. There was no increase in the incidence of tumours of the liver, kidney, or lung after 26 or 39 weeks in the groups treated with NDMA plus aniline compared with their respective NDMA-only controls. [The Working Group noted the lack of body-weight data. No results were provided for the aniline-only treated group and the untreated group.]

In a study by Litvinov et al. (1986), two groups of 100 female CBA × C57/Bl6 mice [age not reported], (body weight, 10–12 g), were given drinking-water containing *N*-nitrosodiethylamine (NDEA) at a concentration of 10.0 mg/L (total dose, 10.95 mg) plus aniline [purity not reported] at a concentration of 0 (control group) or 1.0 mg/L (total dose, 1.09 mg). There was no group treated with aniline alone. The study lasted 12 months. Necropsy was performed on all mice, and the liver, lung, kidney, oesophagus, stomach, spleen, and all gross lesions were examined histologically. The effective number of animals was the number of mice surviving after identification of the first tumour. The incidence of liver tumours was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (23/88 versus 9/79; [$P = 0.02$]). The incidence of liver haemangioma was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (19/88 versus 4/79; [$P = 0.003$]). The incidence of lung adenoma was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (55/88 versus 22/79; [$P < 0.0001$]). There was no increase in the incidence of tumours of the oesophagus, kidney, or forestomach, or other types of tumours in the group treated with NDEA plus aniline when compared with NDEA-only controls. [The Working Group noted the high number of mice per group, the lack of body-weight and survival data, the absence of aniline-only or untreated control groups, and the potential differences between the current histopathological classification and that used when the study was performed.]

3.2 Rat

3.2.1 Oral administration (feed)

In a lifetime study, a group of 43 male and female Osborne-Mendel rats [age not reported; body weight, 75–85 g] were given feed containing aniline hydrochloride [purity not reported] at a concentration of 0.033% for 420–1032 (average, 654) days (White et al., 1948). Hepatomas in the liver (4/43) and fibrosarcomas of the spleen (3/43) were reported. [These tumours were considered by the authors not to be spontaneous because hepatomas had never been seen before in this

strain of rats, including in rats aged 2 years or older, and all tumours were present before day 700.] [The Working Group noted the principal limitations of the study: the use of only one dose group; the lack of controls; and that it was not clear whether the number of animals per group (43) was the starting, effective, or surviving number. The Working Group considered the study inadequate for the evaluation due to the absence of controls.]

Groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing aniline hydrochloride [purity not reported; chemical analysis performed by the authors suggested a compound of high purity] at a concentration of 0.3% (lower dose) or 0.6% (higher dose) for 103 weeks (NCI, 1978). Groups of 25 male and 25 female Fischer 344 rats (control groups) were given feed only for 103 weeks. Rats at the higher dose, lower dose, and in the control groups were killed at 108, 107, and 110 weeks, respectively. There was no significant effect on survival and body weight in groups of treated male and female rats. Adequate numbers of rats were at risk of late-developing tumours: for males, 54% (27/50) at the higher dose, 68% (34/50) at the lower dose, and 68% (17/25) of the controls survived until the end of the study; for females, 82% (41/50) at the higher dose, 88% (44/50) at the lower dose, and 64% (16/25) of the controls survived until the end of the study. An anatomopathological study with histopathological examination was performed on gross lesions and all major organs and tissues. Reference was made to neoplasms of "multiple organs within the body cavities"; such a neoplasm was observed in more than one of the organs [other than spleen] located in the pleural or the abdominal cavity (or both).

The incidence of fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) of the spleen was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 7/50; and higher dose, 9/46) with a significant positive trend ($P = 0.020$, trend test; $P = 0.015$ for the group at the higher dose compared with controls). The incidence of haemangiosarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 19/50; and higher dose, 20/46), with a significant positive trend ($P = 0.001$, trend test; $P < 0.001$ for the groups at the lower and higher doses compared with controls). The incidence of fibrosarcoma or sarcoma NOS (combined) in multiple organs other than spleen within the body cavities was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 2/50; and higher dose, 9/48) with a significant positive trend ($P = 0.004$, trend test; $P = 0.017$ for the group at the higher dose compared with controls). The incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 5/50; and higher dose, 18/48), with a significant positive trend ($P < 0.001$, trend test; $P < 0.001$ for the group at the higher dose compared with controls). The incidence of haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 19/50; and higher dose, 21/48), with a significant positive trend ($P = 0.001$, trend test; $P < 0.001$ for the groups at the lower and higher doses compared with controls). There was a significant positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland in treated male rats compared with controls (control group, 2/24; lower dose, 6/50; and higher dose, 12/44; $P = 0.022$, trend test).

There was a significant positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) in treated female rats compared with controls (control group, 0/24; lower dose, 1/50;

and higher dose, 7/50; $P = 0.009$, trend test). The incidence of endometrial stromal polyp of the uterus was significantly higher in treated female rats than in controls (control group, 2/24; lower dose, 15/48; and higher dose, 7/50; $P = 0.027$ for the group at the lower dose compared with controls).

Regarding non-neoplastic lesions, only treated male and female rats developed fibrosis of the splenic capsule and trabeculae, with the presence of scattered large fat cells in the splenic parenchyma. Many treated male and female rats also developed papillary hyperplasia of the splenic capsule surface (NCI, 1978). [The Working Group noted the principal strengths of the study: there was adequate survival, appropriate statistical analyses were performed, males and females were used, and the durations of exposure and observation were adequate.]

Four groups of 28 male Wistar rats [age not reported; weight, approximately 150 g] received feed containing aniline [purity not reported] at a concentration of 0 (controls), 0.03%, 0.06%, or 0.12% for 80 weeks (Hagiwara et al., 1980). No marked adverse effects on body weight were observed. A complete analysis was conducted on all rats found dead or killed when moribund, as well as on rats surviving up to 80 weeks. The effective numbers of rats were 15, 10, 18, and 16 in the control group and at the lowest, intermediate, and highest dose, respectively. Papilloma of the forestomach was observed in 0/15, 1/10, 1/18, and 0/16 rats in the four groups, respectively; pituitary adenoma was observed only in 1/10 rats at the lowest dose. No tumours were observed in the urinary bladder and subcutaneous tissues. [The Working Group noted the multiple doses tested, limited reporting of survival data, and that this was not a long-term carcinogenicity study.]

Groups of 130 male and 130 female Fischer 344 albino (CD-F) rats (age, 4–5 weeks) were given feed containing aniline hydrochloride [assumed by the authors to be 100% pure] for 104 weeks at a dose of 0 (controls), 10, 30, or 100 mg/kg bw per day (US EPA, 1982). There was no significant effect on body weight in the treated groups. Adequate numbers of male rats were at risk of late-developing tumours, although the survival rate was significantly lower in males at the highest dose than in controls: 104/130 (80%) at the highest dose, 115/130 (88%) at the intermediate dose, 116/130 (89%) at the lowest dose, and 114/130 (88%) in the control group survived until the end of the study. Adequate numbers of female rats were at risk of late-developing tumours: 117/130 (90%) at the highest dose, 116/130 (89%) at the intermediate dose, 109/130 (84%) at the lowest dose, and 110/130 (85%) in the control group survived until the end of the study. Food consumption during the first 50 weeks was lower for treated male rats than for controls; however, these differences were not considered to be treatment-related and were within the range of normal biological variability. All major tissues and organs from the rats in the control group and at the highest dose, and only the spleen and any unusual lesions from rats at the lowest and intermediate doses, were examined microscopically.

The incidence of stromal sarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/123; lowest dose, 0/129; intermediate dose, 1/128; and highest dose, 21/130), with a significant positive trend [$P < 0.001$, trend test; $P < 0.0001$ for the group at the highest dose compared with controls]. The incidence of haemangiosarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/123; lowest dose, 0/129; intermediate dose, 0/128; and highest dose, 6/130), with a significant positive trend [$P < 0.001$, trend test; $P = 0.03$ for the group at the highest dose compared with controls]. The incidence of mesothelioma of the tunica vaginalis of the testis was significantly higher in treated male rats than in controls (control group, 1/114; lowest dose, 4/130; intermediate dose, 9/130; and highest dose, 1/104), with a significant positive trend [$P = 0.019$, trend test; $P = 0.022$ for the

group at the intermediate dose compared with controls].

Regarding non-neoplastic lesions, the incidence of stromal hyperplasia of the spleen was significantly higher in treated male rats than in controls (control group, 1/123; lowest dose, 2/129; intermediate dose, 0/128; and highest dose, 31/130), with a significant positive trend [$P < 0.001$, trend test; $P < 0.0001$ for the group at the highest dose compared with controls]. [The presence of stromal hyperplasia lends further support to the evidence that stromal sarcomas of the spleen were induced by aniline hydrochloride. The authors noted that the stromal hyperplasias in the spleen "often appeared similar in cell type and morphology to the stromal sarcomas, but lacked invasion", so stromal hyperplasia of the spleen probably represents a precursor (pre-neoplastic lesion) to the stromal sarcoma.]

The incidence of tumours in treated female rats was not significantly higher than in controls; however, the incidence of stromal hyperplasia of the spleen was significantly higher in treated female rats than in controls (control group, 0/129; lowest dose, 0/129; intermediate dose, 0/130; and highest dose, 9/130), with a significant positive trend [$P < 0.001$, trend test; $P = 0.003$ for the group at the highest dose compared with controls] (US EPA, 1982). [The Working Group noted the principal strengths of the study: the high number of animals at the start and adequate survival, the use of males and females, the adequate durations of exposure and observation, and multiple doses tested. The principal limitations were: no statistics were reported in the study report, and the data in the report were not clearly presented.]

[The Working Group noted that the aromatic amine *ortho*-toluidine, which is *carcinogenic to humans* (IARC Group 1) also causes malignant tumours of the spleen and mesothelioma of the tunica vaginalis of the testis when administered to male Fischer 344 rats (IARC, 2012).]

3.2.2 Oral administration (drinking-water)

In a lifetime study, 50 rats [strain and sex unspecified] (age, 100 days) were given drinking-water containing aniline hydrochloride [purity not reported] to provide an intake of 22 mg/day. Half of the rats lived for more than 425 days, the last rat surviving up to day 750. The total doses administered were between 14 g and 16.5 g/rat. Necropsy was performed, but only the urinary bladder, liver, spleen, and kidneys were examined in all rats. No tumours were observed (Druckrey, 1950). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

3.2.3 Co-carcinogenicity

Litvinov et al. (1982) gave two groups of 50 albino outbred male rats [strain and age unspecified] (body weight, 150–200 g) drinking-water containing NDMA at a concentration of 10 mg/L (0.5 mg/kg bw per day) alone or with aniline [purity not reported] at a concentration of 0.5 mg/L. A third group of 50 untreated control rats was given drinking-water only; no group treated with aniline alone was available. The experiment lasted 19 months. Necropsy was performed on all rats, and the liver, lung, kidney, and spleen, and all gross lesions were examined histologically. The effective number of animals was the number of surviving rats. The incidence of liver tumours [mainly hepatocellular tumours, NOS] was significantly higher in rats receiving NDMA plus aniline than in rats receiving NDMA alone [34/44 versus 15/42; $P < 0.0002$]. There were no liver tumours in 38 untreated control rats. There was no significant increase in the incidence of kidney or lung tumours in rats receiving NDMA plus aniline compared with rats receiving NDMA alone. No kidney or lung tumours occurred in 38 untreated control rats. [The Working Group noted the lack

of body-weight data and absence of an aniline-only treated group.]

3.3 Hamster

Subcutaneous injection

Two groups of 15 male and 15 female Syrian golden hamsters (age, 8 weeks) were given aniline [purity not reported] at a dose of 1.9 mmol/kg bw in peanut oil by weekly subcutaneous injection for 52 weeks. Two vehicle-control groups of 15 male and 15 female hamsters were injected with peanut oil only (Hecht et al., 1983). After the injections were complete, the hamsters were observed until moribund. The experiment was terminated after 87 weeks. Mean survival times were shorter in treated groups of males and females than in controls: treated males, 67.7 weeks; treated females, 62.1 weeks; male controls, 75.5 weeks; and female controls, 68.7 weeks. There was a decrease in body weight in treated males and females compared with males and females in the vehicle-control groups. Upon necropsy, gross lesions and representative samples of all major organs were processed for microscopic evaluation. Aniline at a total dose of 99 mmol/kg bw did not induce any tumours in male and female hamsters. No tumours were observed in the controls. [The Working Group noted the limited data on survival and body weight, and the incomplete histopathological examination. The outcome of the study may reflect the low dose used, the unconventional route of administration, or a possible species-specific difference.]

3.4 Rabbit

3.4.1 Subcutaneous injection

A group of 12 male rabbits [age not reported; weight, approximately 2 kg] received daily subcutaneous injections of 1 mL of 1% aniline [purity not reported] (10 mg) in water (60 mg/week) for up to 216 days. The rabbits did not develop any urinary bladder tumours (Yamazaki & Sato, 1937). [The Working Group noted the lack of vehicle-control group, and the unconventional route of administration. The study was considered inadequate for the evaluation.]

3.4.2 Bladder instillation

In a lifetime study, a group of 30 male rabbits [age not reported; weight, approximately 2 kg] was given 10 mL of 1% aniline [purity not reported] (0.1 g) in water by daily instillation (0.6 g/week) into the urinary bladder. An additional group of 4 male rabbits received 10 mL of 5% aniline (0.5 g) in water by daily instillation (3.0 g/week). A further group of 3 male rabbits received 10 mL of 10% aniline (1.0 g) in water by daily instillation (6.0 g/week). A group of 20 controls received water only or olive oil only by daily instillation. In the group receiving 1% aniline for up to 362 days, 12/30 rabbits developed urinary bladder papillomas after 13–307 days. The rabbits receiving 5% or 10% aniline died within 4–15 days from urinary bladder necrosis and did not develop any urinary bladder tumours. No urinary bladder tumours were observed in the controls (Yamazaki & Sato, 1937). [The Working Group noted the high quality of gross descriptions and microscopic examinations, and the unconventional route of administration. The Working Group also noted that 12/30 treated rabbits developed urinary bladder papillomas, and there were no tumours in 20 controls that had been instilled with water only or olive oil only. However, the number of rabbits instilled with water only in the control group was not reported, and thus this study was considered limited by the Working Group because of the incomplete reporting regarding vehicle controls.]

3.5 Synthesis

In one independent study in male and female Fischer 344 rats treated by oral administration (in feed), aniline hydrochloride caused a significant increase, with a significant positive trend, in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen; haemangiosarcoma of the spleen; fibrosarcoma or sarcoma NOS (combined) of multiple organs other than spleen within the body cavities; fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined); and haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) in male rats. Aniline hydrochloride also caused a significant positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland in male rats. Aniline hydrochloride caused a significant positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined), and a significant increase in the incidence of endometrial stromal polyps of the uterus in female rats (NCI, 1978).

In another independent study in male and female Fischer 344 rats treated by oral administration (in feed), aniline hydrochloride caused a significant increase, with a significant positive trend, in the incidence of stromal sarcoma of the spleen, haemangiosarcoma of the spleen, and mesothelioma of the tunica vaginalis of the testis in male rats. There was no significant increase in the incidence of tumours in treated female rats (US EPA, 1982).

In one co-carcinogenicity study in female CBA × C57/Bl6 mice treated by oral administration (in drinking-water), in which NDEA was administered in the presence or absence of aniline, there was a significant increase in the incidence of liver tumours, haemangioma of the liver, and lung adenoma in mice treated with NDEA plus aniline, compared with mice treated with NDEA alone (Litvinov et al., 1986). In another co-carcinogenicity study in male albino outbred rats treated by oral administration (in drinking-water), in which NDMA was administered in the presence or absence of aniline, there was a significant increase in the incidence of liver tumours in mice treated with NDMA plus aniline, compared with mice treated with NDMA alone (Litvinov et al., 1982).

In one study in male rabbits treated by urinary bladder instillation, aniline induced papillomas of the urinary bladder in 12 out of the 30 treated animals; however, this study was considered limited by the incomplete reporting regarding vehicle controls (Yamazaki & Sato, 1937).

In one oral administration (in feed) study in male and female B6C3F$_1$ mice (NCI, 1978), one oral administration (in feed) study in male Wistar rats (Hagiwara et al., 1980), one intraperitoneal injection study in male B6C3F$_1$ mice (Delclos et al., 1984), one subcutaneous injection study in male and female Syrian golden hamsters (Hecht et al., 1983), and one co-carcinogenicity study in female CBA × C57/Bl6 mice (Litvinov et al., 1984), aniline or aniline hydrochloride did not induce any tumours or did not cause a significant increase in the incidence of tumours.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Exposed humans

(a) Absorption

Aniline is absorbed via dermal, inhalation, or oral routes. Depending on the task performed and the aniline content of the product handled, reports from controlled studies indicate that the dermal route may contribute significantly

to total uptake (Piotrowski, 1957; Dutkiewicz & Piotrowski, 1961; Baranowska-Dutkeiwicz, 1982; Korinth et al., 2007). A study of occupationally exposed workers for whom urinary aniline and haemoglobin adducts were used as biomarkers of exposure demonstrated that skin lesions (such as erythema and scaling) on the hands facilitated the dermal absorption of aniline (Korinth et al., 2007). The use of barrier creams also increased exposure, a phenomenon known as "penetration enhancement" (Korinth et al., 2008). Shi & Ma (2009) reported a case of fatal acute aniline poisoning by dermal exposure.

Regarding inhalation exposure, Käfferlein et al. (2014) performed a controlled study on 19 non-smoking subjects; they demonstrated that environmental exposure to aniline at 2 ppm [7.6 mg/m^3] for 6 hours (including 1 hour with exercise) resulted in a mean urinary concentration of aniline of 168.0 µg/L (SD, 51.8 µg/L). The corresponding methaemoglobinaemia level was 1.21% (SD, 0.29%) (Table 1.4).

Facile oral absorption of aniline was also demonstrated by a more recent study with isotope-labelled aniline (Modick et al., 2016; described below). Most studies of aniline metabolism in humans have used oral administration.

Studies of methaemoglobinaemia, long-established as the principal clinical manifestation of aniline poisoning, indicate that aniline is absorbed through the skin and by the oral route. As early as 1885, aniline ink (applied to mark diapers) was reported to cause "cyanosis" in infants (Rayner, 1886); reports of similar incidents continued for many years afterwards (Ramsay & Harvey, 1959). Methaemoglobinaemia was also reported after accidental (usually, dermal) (Phillips et al., 1990; Lee et al., 2013; Shatila et al., 2017) or deliberate (criminal) (Iwersen-Bergmann & Schmoldt, 2000) acute oral aniline poisonings and after oral administration of aniline (up to 65 mg) to human subjects (Jenkins et al., 1972).

[The Working Group noted that aniline is a small lipophilic molecule that is expected to be readily absorbed by all routes of exposure, based on its chemical properties.]

(b) Distribution, metabolism, and excretion

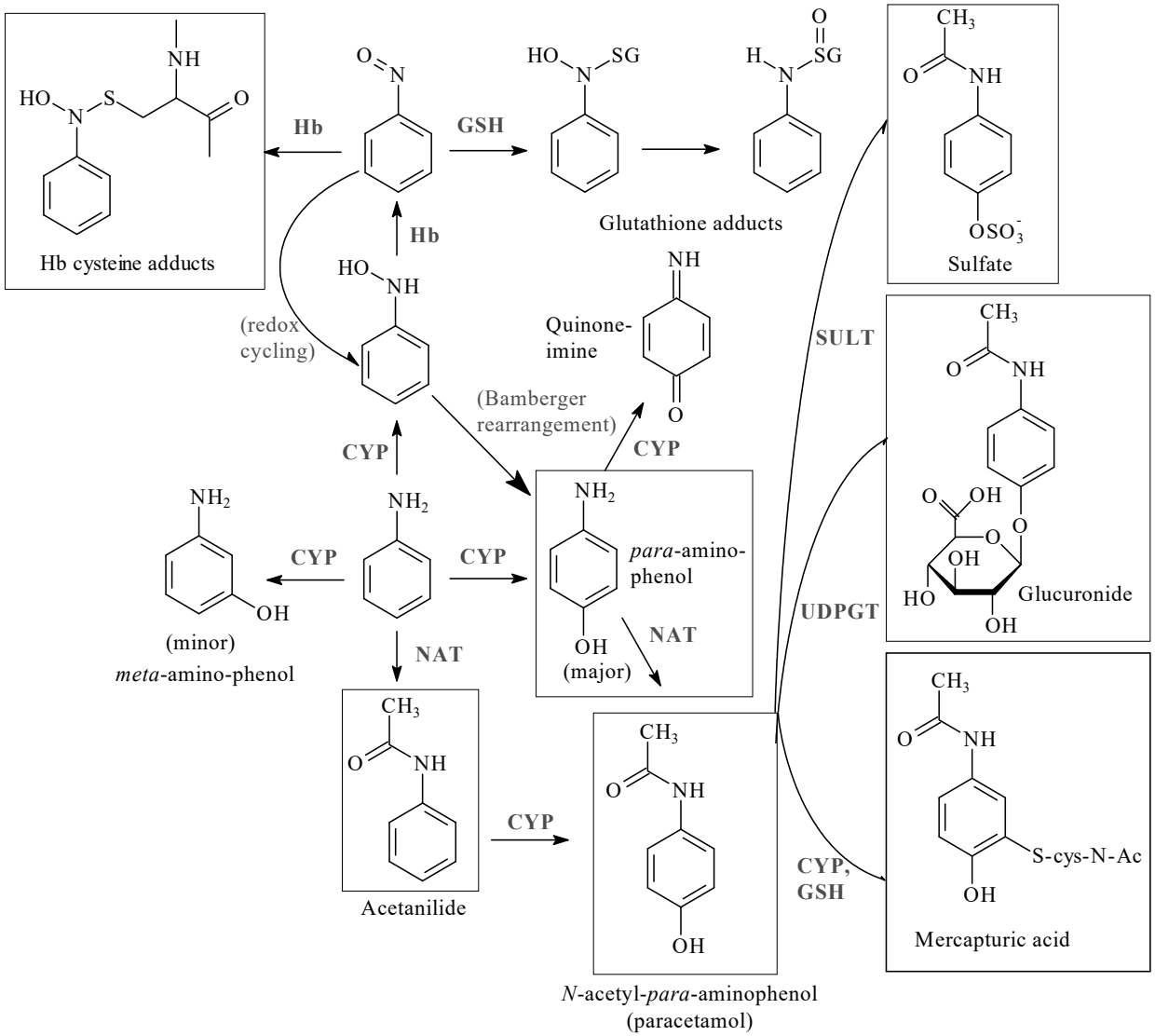
Identified human urinary metabolites and haemoglobin adducts are shown in boxes in Fig. 4.1. See Table 1.4 for a summary of levels of haemoglobin adducts reported in workers occupationally exposed to aniline (Riffelmann et al., 1995; Ward et al., 1996; Thier et al., 2001; Beyerbach et al., 2006; Korinth et al., 2007).

Two metabolic steps, namely cytochrome P450 (CYP)-dependent hydroxylation to *para*-aminophenol [4-aminophenol] and *N*-acetyltransferase (NAT)-dependent N-acetylation (in either sequence), convert aniline into *N*-acetyl-*para*-aminophenol [the chemical name for paracetamol, also known as acetaminophen or *para*-acetyl-aminophenol], which is a widely used analgesic drug. After acute oral exposure of an adult female to aniline, the parent compound and the metabolites acetanilide and *N*-acetyl-*para*-aminophenol were detected in the plasma. The same compounds, as well as *N*-acetyl-*para*-aminophenol conjugates (released by glucuronidase/arylsulfatase treatment), were detected in the urine (GC-MS analysis). Traces of *para*-aminophenol were detected in the urine, but *ortho*-aminophenol was not detected (Iwersen-Bergmann & Schmoldt, 2000).

N-Acetyl-*para*-aminophenol was detected in the urine samples of 21 subjects (not occupationally exposed to aniline) (Modick et al., 2013). This metabolite was detected in all urine samples tested, including samples from the general population, from individuals exposed to aniline in an occupational setting, and from paracetamol (*N*-acetyl-*para*-aminophenol) users (Dierkes et al., 2014).

Modick et al. (2016) dosed four healthy male subjects (two NAT2 "fast" and two NAT2 "slow" acetylators) orally with 5 mg of isotope-labelled

Fig. 4.1 Major pathways in the metabolism of aniline



CYP, cytochrome P450; GSH, glutathione; Hb, haemoglobin; NAT, *N*-acetyl transferase; SULT, sulfotransferase; UDPGT, uridine diphosphoglucuronyltransferase.
Human urinary metabolites and haemoglobin adducts are shown in boxes.
Created by the Working Group.

aniline and urine samples were collected over 2 days. After enzymatic hydrolysis, *N*-acetyl-*para*-aminophenol was the predominant urinary metabolite of aniline (55.7–68.9% of the oral dose). The mercapturic acid conjugate of *N*-acetyl-*para*-aminophenol represented 2.5–6.1% of the administered dose. Acetanilide and free aniline were found in small amounts, 0.14–0.36%. Combined, these metabolites (urine, 48 hours post-dose) accounted for 62.4–72.1% of the oral aniline dose. The elimination half-lives were: *N*-acetyl-*para*-aminophenol, 3.4–4.3 hours; mer-

capturic acid conjugate, 4.1–5.5 hours; acetanilide, 1.3–1.6 hours; aniline, 0.6–1.2 hours.

The metabolism of aniline in humans in vivo to give protein (haemoglobin and serum albumin) adducts is discussed in Section 4.2.1(a).

4.1.2 Human cells in vitro

(a) CYP-dependent metabolism of aniline

Recombinant human CYP2E1 catalyses the hydroxylation of aniline to *para*-aminophenol [4-aminophenol] (Dai et al., 1993; Yamazaki et al., 1996) and CYP2E1 is thought to be the major CYP enzyme carrying out aniline hydroxylation. [The Working Group noted that, although not demonstrated directly with human CYP2E1, on the basis of rodent metabolism studies it is likely that the other isomers, 2-aminophenol [*ortho*-aminophenol] and 3-aminophenol [*meta*-aminophenol], are also formed, albeit in much smaller amounts.]

Hartman and colleagues studied the metabolism of aniline to *para*-aminophenol [4-aminophenol] catalysed by pooled human liver microsomes. The contributions of specific hepatic CYP isoforms were assessed by examining the effects of enzyme-specific inhibitors. The major contributors to aniline 4-hydroxylation were found to be CYP2E1, CYP2A6, and CYP2C9 (Hartman et al., 2014). Both endoplasmic reticulum-localized and mitochondrion-localized forms of CYP2E1 contributed (Hartman et al., 2015). [The Working Group noted that the N-hydroxylation of aniline is presumably catalysed by CYP in the human liver.]

No data were available to the Working Group on CYP-dependent N-oxidation of aniline. CYP-dependent N-oxidation of the aniline derivative, 2,6-dimethylaniline, has been studied in vitro with human liver microsomes and recombinant human CYPs (Gan et al., 2001; Skipper et al., 2010). Like aniline, 2,6-dimethylaniline can undergo *para*-hydroxylation to the *para*-aminophenol [4-aminophenol], or N-hydroxylation to the hydroxylamine. It was observed that, at micromolar concentrations, only *para*-hydroxylation was detectable, but at nanomolar concentrations, N-hydroxylation was a major pathway. Using CYP-specific inhibitors and inhibitory mouse monoclonal antibodies, CYP2A6 was identified as the major form responsible for N-hydroxylation. Also, recombinant human cytochrome CYP2E1 and human liver microsomes catalysed the NADPH-independent rearrangement of *N*-(2,6-dimethylphenyl) hydroxylamine to 4-amino-3,5-dimethylphenol (the "Bamberger rearrangement") (Gan et al., 2001). [The Working Group noted the relevance of these findings on 2,6-dimethylaniline to aniline metabolism.]

(b) N-acetylation of aniline

N-Acetylation of aniline has been demonstrated in vitro with recombinant forms of both human acetyl coenzyme A (CoA):aromatic amine *N*-acetyltransferase enzymes, NAT1 and NAT2 (Liu et al., 2007). [The Working Group noted that it is likely that aniline and all its metabolites with free arylamine NH_2 groups undergo acetylation, at least to some extent, in humans.]

4.1.3 Experimental systems

Aniline is rapidly absorbed by experimental animals after oral administration, application to the skin, or inhalation (Carpenter et al., 1949; Roudabush et al., 1965; Kiese, 1966). After intravenous administration to male Fischer 344 rats, [^{14}C]-labelled aniline was distributed throughout the body, as observed by whole-body autoradiography (Irons et al., 1980). Aniline was shown to readily pass the placental barrier in pregnant Sprague-Dawley rats (Maickel & Snodgrass, 1973).

Plasma clearance of aniline administered subcutaneously to male albino rats was increased after pretreatment with phenobarbital or benzo[*a*]

pyrene (Wiśniewska-Knypl & Jabłońska, 1975). When [^{14}C]-labelled aniline was administered to dairy cattle, residue was detected in the edible tissues and in the milk (Eisele et al., 1985).

After oral administration of aniline to dogs and acid hydrolysis of conjugates, *para*-aminophenol [4-aminophenol] was identified in the urine (Schmiedeberg, 1877). Smith & Williams (1949) reported that about 28% of aniline, orally administered to rabbits, was excreted as sulfate conjugates of *ortho*-aminophenol [2-aminophenol], *para*-aminophenol [4-aminophenol], and 4-aminoresorcinol (the product of both *ortho*- and *para*-hydroxylation of aniline). About 70% of the administered dose was excreted as glucuronides, including *para*-acetamido- and *para*-aminophenyl glucuronides. Acetylated products were also found.

All species tested (rabbit, rat, mouse, guinea-pig, gerbil, hamster, cat, dog, pig, sheep) hydroxylate aniline to *ortho*- and *para*-aminophenol, which are excreted in the urine as conjugates (Williams, 1959; Parke, 1960; Kao et al., 1978). The ratio of the isomers differs among species. *meta*-Aminophenol was also detected, in trace quantities, in the urine of dogs and rabbits (Parke, 1960).

Small amounts of free aniline, phenylsulfamic acid, and aniline *N*-glucuronide are found in the urine of some species after administration of aniline (Boyland et al., 1957; Parke, 1960). The mercapturic acids of *ortho*- and *para*-aminophenol and *N*-acetyl-*para*-aminophenol are excreted in rats. *N*-acetyl-*para*-aminophenol and its mercapturic acid are excreted in rabbits (Boyland et al., 1963). Acetanilide was found in the urine of rabbits, but not in the urine of dogs (Williams, 1959); the absence of acetylated metabolites in the dog is consistent with the absence of aromatic amine *N*-acetyltransferase genes and enzymes in that species (Trepanier et al., 1997).

Phenylhydroxylamine (*N*-hydroxyaniline) has not been detected in the urine of experimental animals given aniline; however, phenylhydroxylamine and nitrosobenzene are found in the blood (see Section 4.1.4). The N-hydroxylation of aniline by hepatic microsomal preparations from pre-treated rats and mice has been observed in vitro (McCarthy et al., 1985).

In male Sprague-Dawley rats given [^{14}C]-labelled aniline by gavage and killed after 24 hours, dose-dependent binding and accumulation of radiolabel were seen in erythrocytes and in the spleen (Khan et al., 1995).

4.1.4 Methaemoglobinaemia

Methaemoglobinaemia has been demonstrated in humans and in various species of experimental animal, including rats, and is attributed to phenylhydroxylamine formation (Kiese & Taeger, 1976; Harrison & Jollow, 1987). N-Hydroxylation of aniline to give phenylhydroxylamine has been demonstrated after intravenous administration of aniline to dogs (Kiese, 1959), intraperitoneal administration of aniline to rats (Harrison & Jollow, 1987), in liver microsomal preparations of rat and mouse (McCarthy et al., 1985), and rabbit (Burstyn et al., 1991), and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the metabolites by binding to haemoglobin (Eyer et al., 1980).

The methaemoglobin-inducing species are the oxidized (hydroxylamine and nitroso) metabolites rather than aniline itself. Phenylhydroxylamine is a potent inducer of methaemoglobin (Kiese & Taeger, 1976).

[The Working Group noted that, although the conversion of haemoglobin (Hb) to methaemoglobin is an oxidation, it can be induced by reducing agents (such as nitrite and phenylhydroxylamine), which "unleash" the oxidizing power of bound O_2, via "co-oxidation" processes such as:

$$Hb^{2+}\,O_2 + X_{red} \rightarrow Hb^{3+} + HOOH + X_{ox}$$

The detailed chemical mechanisms of methaemoglobin formation are very complex and are still not fully understood (Gladwin et al., 2009). There are several reasons for this, most or all of which apply in the case of aniline:

(i) Haemoglobin is a macromolecule with four subunits/four iron atoms, and these subunits interact strongly.

(ii) There are many distinct steps in the pertinent chemical processes.

(iii) Haemoglobin has catalytic (peroxidase) activity, in addition to carrying oxygen (Vlasova, 2018).

(iv) There are competing reactions, such as free-radical reactions with the protein's cysteine residues (Maples et al., 1990). These reactions generate covalent addition products (adducts) which can serve as biomarkers of exposure (Pathak et al., 2016).

(v) Redox-cycling can occur, so that the agents inducing methaemoglobinaemia may act as catalysts, with one molecule of toxicant causing multiple haemoglobin oxidations (Vásquez-Vivar & Augusto, 1994).

(vi) The agents inducing methaemoglobinaemia may be metabolites of the toxicant rather than the parent compound.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) including whether aniline (and aniline hydrochloride) is electrophilic or can be metabolically activated to an electrophile; is genotoxic; induces oxidative stress; or alters cell proliferation, cell death, or nutrient supply. For the evaluation of other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

This section covers covalent binding to proteins and to DNA.

(a) Protein adducts

See Fig. 4.2.

(i) Haemoglobin adducts in humans

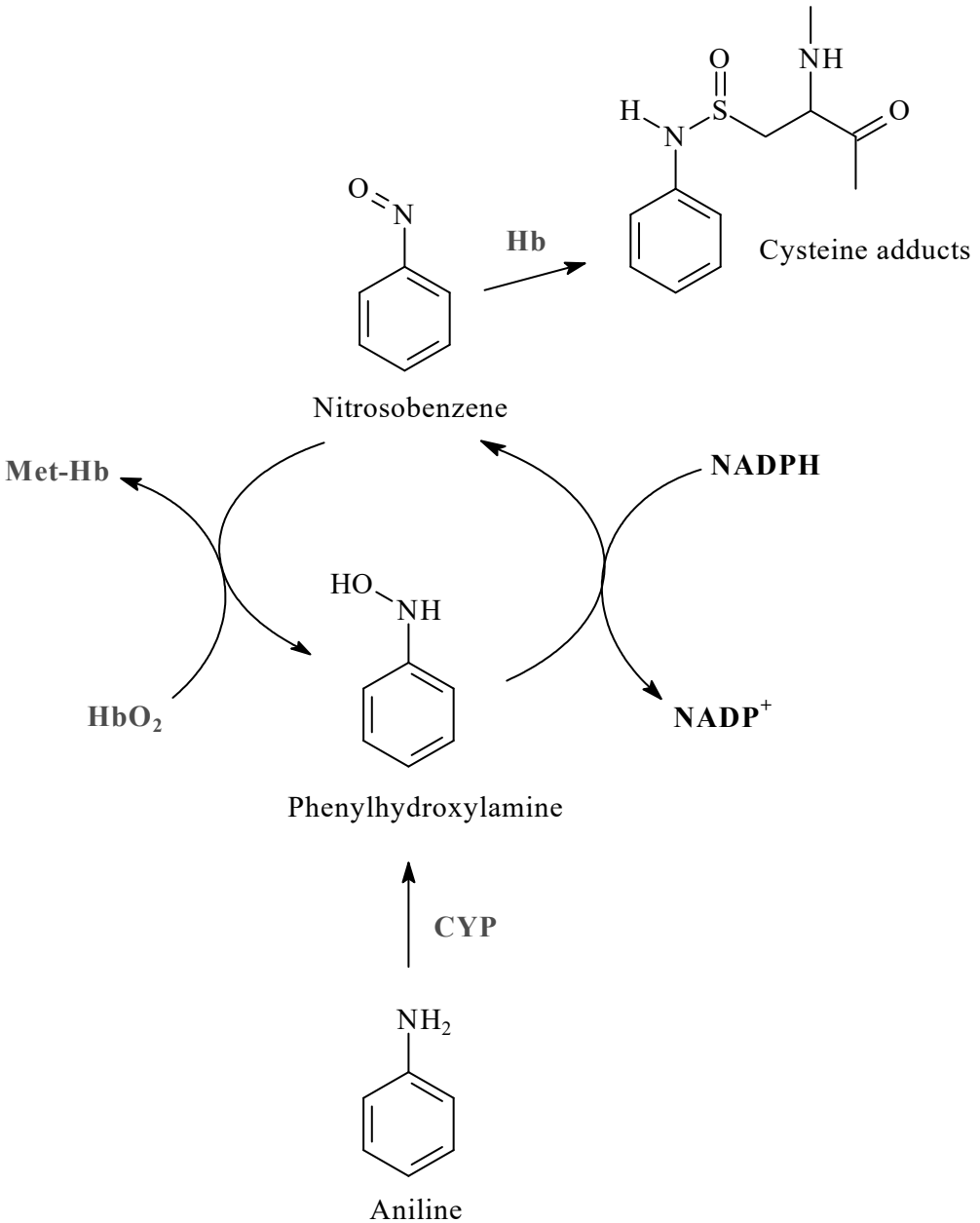
In exposed humans, haemoglobin adducts are commonly used as biomarkers of exposure to aniline. An analytical technique has been applied in which blood haemoglobin is purified and hydrolysed in acid or alkali; bound aniline released by hydrolysis is detected by GC-MS. The bound aniline was used as a biomarker of exposure to aniline, for example, in a study in chemical-plant workers engaged in synthesis and processing of aniline and 4-chloroaniline [*para*-chloroaniline] (Riffelmann et al., 1995), and in a study of workers engaged in the manufacture of rubber additives (Ward et al., 1996) (see also Section 1.4.2). Adducts are also formed with serum albumin, as demonstrated in studies of exposed male workers in a nitrobenzene-reduction plant (Thier et al., 2001; see also Section 1.4.2).

(ii) Protein adducts in experimental systems

Neumann and colleagues (Albrecht & Neumann, 1985; Birner & Neumann, 1988) gave aniline orally to female Wistar rats and showed that aniline bound to haemoglobin, using an analytical technique similar to that used in humans (in which blood haemoglobin was purified and hydrolysed in acid or alkali, and the aniline released by hydrolysis was detected by GC-MS); see also a study in male Fischer 344 rats (Zwirner-Baier et al., 2003).

Adducts with serum albumin were shown to be formed in a study in which beagle dogs were exposed to aniline vapour (Pauluhn, 2002).

Fig. 4.2 Formation of aniline–haemoglobin cysteine adducts



CYP, cytochrome P450; Hb, haemoglobin; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form

Aniline is oxidized to phenylhydroxylamine (*N*-hydroxyaniline) in a cytochrome P450-catalysed reaction. Phenylhydroxylamine is co-oxidized by oxyhaemoglobin (HbO_2) in a complex process that yields nitrosobenzene and methaemoglobin (Met-Hb); the illustration is not to be read as displaying the reaction stoichiometry. Nitrosobenzene can react with cysteine–SH groups on the haemoglobin protein to form covalent adducts. The cysteine adducts can undergo further S-oxidation (not shown).

Adapted with permission from Pathak et al. (2016), Methemoglobin formation and characterization of hemoglobin adducts of carcinogenic aromatic amines and heterocyclic aromatic amines, Chemical Research in Toxicology, Volume 29, issue 3, pp. 255–269. Copyright (2016) American Chemical Society.

Roberts & Warwick (1966) reported the binding of [^{3}H]-labelled aniline to protein (as well as to DNA and ribosomal RNA, rRNA) in the liver, spleen, and kidney of male albino rats (Chester Beatty stock), after intraperitoneal administration. In Fischer 344 rats and C57BL/6 × C3H F_1 mice given [^{14}C]-labelled aniline intraperitoneally, protein and RNA of the kidney, large intestine, and spleen were the major macromolecular targets, with low but significant binding to DNA (McCarthy et al., 1985).

In a study of a rat microsomal preparation incubated with [^{14}C]-labelled aniline and an NADPH-generating system, covalent binding to microsomal protein was observed, but adducts were not characterized. In experiments in which the rats were pretreated with either benzene or phenobarbital for CYP induction, the formation of both water-soluble metabolites and protein-bound material was increased (Gut et al., 1996).

(iii) Aniline metabolites involved in haemoglobin adduct formation

The formation of covalent adducts with haemoglobin is attributed to N-oxidation of aniline via formation of the aniline metabolite phenylhydroxylamine. Phenylhydroxylamine is co-oxidized with haemoglobin in the erythrocyte to form nitrosobenzene. Nitrosobenzene binds to the haem iron centre of haemoglobin even more tightly than does oxygen (O_2) (Eyer & Ascherl, 1987). It is also an electrophilic compound that reacts with the sulfhydryl groups of glutathione or of haemoglobin cysteine residues, forming sulfinamide adducts that can be further oxidized to sulfonamides (Kiese & Taeger, 1976; Eyer et al., 1980; Maples et al., 1990; Sabbioni & Beyerbach, 1995; Möller et al., 2017).

(b) DNA adducts

See Fig. 4.3.

(i) DNA adducts in humans

No data were available to the Working Group.

(ii) DNA adducts in experimental systems

Roberts & Warwick (1966) reported the binding of [^{3}H]-labelled aniline to DNA, as well as to rRNA and to protein, in the liver, spleen, and kidney of rats exposed in vivo. In Fischer 344 rats given [^{14}C]-labelled aniline intraperitoneally, binding of the radiolabel was predominantly to protein and RNA. Binding to DNA was detected in various tissues, with the highest levels detected in the kidney, large intestine, and spleen; however, complete purification of the adducted DNA was not attempted and the adducts were not identified (McCarthy et al., 1985). [The Working Group noted that the level of binding was described as "low but significant".] The covalent binding index values for all tissues were less than 15, in contrast to a covalent binding index of 17 000 for aflatoxin B_1 and 560 for 2-acetylaminofluorene (Lutz, 1979, 1981).

[The Working Group noted that, although aniline itself was not examined, alkyl derivatives (2,6-dimethylaniline, 3,5-dimethylaniline, and 3-ethylaniline) were found to bind to DNA (Skipper et al., 2006). Wildtype C57/BL6 mice were given [^{14}C]-labelled compounds intraperitoneally, and tissue DNA was analysed for labelling by sensitive accelerator mass spectrometry. All the alkylanilines produced detectable labelling of DNA, up to about one modified base per 10^7 bases (for 3,5-dimethylaniline, in the liver) (Skipper et al., 2006); (see also the ^{32}P-post-labelling studies in Section 4.2.2)].

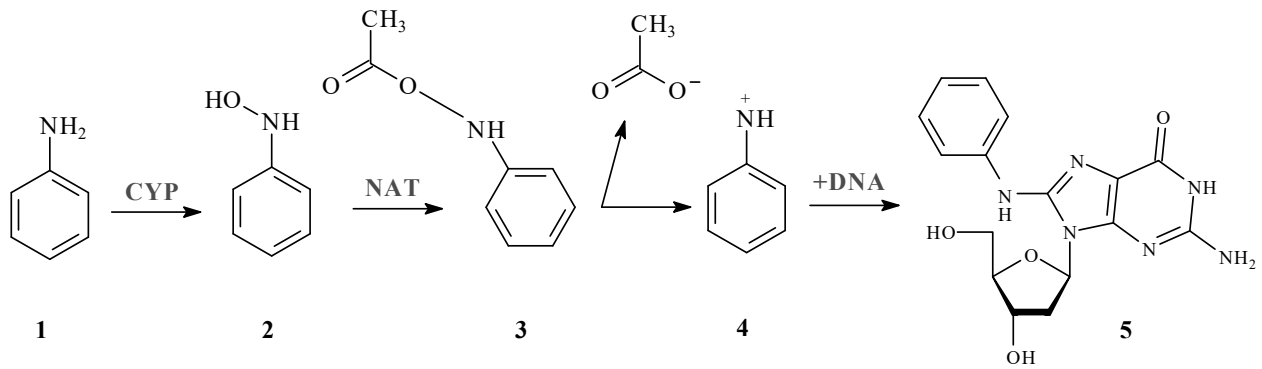
(iii) Aniline metabolites involved in DNA adduction

See Fig. 4.3.

As noted above, no aniline–DNA adducts have been identified in vivo.

N-Acetoxyaniline (the putative metabolic precursor of the phenylnitrenium ion), prepared synthetically, reacts in an acellular system with the DNA nucleoside deoxyguanosine to give an aniline–C8 adduct (Famulok & Boche, 1989). In a subsequent study using a similar chemical

Fig. 4.3 Postulated route to formation of aniline–DNA adducts



CYP, cytochrome P450; NAT, *N*-acetyl transferase.
Aniline–DNA adducts have not been isolated and characterized from cells or tissues, but they have been synthesized chemically by the reactions of *N*-acetoxyaniline (a presumed phenylnitrenium ion precursor) with deoxynucleosides or DNA (Famulok & Boche, 1989; Králík et al., 2015). The scheme shows: the cytochrome P450 (CYP)-catalysed N-oxidation of aniline (1) to phenylhydroxylamine (*N*-hydroxyaniline) (2); O-acetylation (possibly catalysed by *N*-acetyltransferase + acetyl-coenzyme A) to give *N*-acetoxyaniline (3); heterolysis of *N*-acetoxyaniline to give phenylnitrenium ion (4) and a typical DNA adduct (deoxyguanosine C adduct) (5). Králík et al. (2015) isolated adenine adducts at positions C2, C8, *N*7, and N^6, and guanine adducts at positions C8, *N*7, and N^2.
Created by the Working Group.

approach, Králík et al. (2015) separated and identified multiple adducts formed in DNA treated with *N*-acetoxyaniline, characterizing adenine C2, C8, *N*7, and N^6 aniline adducts and guanine C8, *N*7, and N^2 aniline adducts. [The Working Group noted that a similar spectrum of DNA adducts is seen with other aromatic amines such as 4-aminobiphenyl (*para*-phenylaniline), which is classified as *carcinogenic to humans* (IARC Group 1) by the *IARC Monographs* programme (IARC, 2012).]

Adducted aniline is predicted (on the basis of molecular mechanics calculations) to distort the DNA double-helix structure less than do larger aromatic amine ring systems (Shapiro et al., 1998).

As shown in Fig. 4.3, a possible route to the formation of these DNA adducts begins with CYP-catalysed N-hydroxylation of aniline (see Section 4.1), a process that is known to occur, as discussed in Section 4.2.1(a), and that is an obligate step in the pathway leading to haemoglobin adducts. The hydroxylamine metabolite can be further activated, for example, by O-acetylation catalysed by the *N*-acetyltransferase. [The Working Group noted that further activation of the *N*-hydroxy compound by acetylation has not been directly demonstrated in an in vitro system.] The resulting *N*-acetoxyaniline, mentioned above, undergoes spontaneous heterolysis (loss of acetate anion) to give a reactive electrophilic nitrenium ion (Shamovsky et al., 2012). As discussed above, N-hydroxylation of aniline to phenylhydroxylamine has been shown in dogs and rats; in liver microsomal preparations from rat, mouse, and rabbit; and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the oxidized metabolites by binding to haemoglobin. [The Working Group noted that this pathway of DNA adduct formation parallels an established paradigm for aromatic amines, including 4-aminobiphenyl (*para*-phenylaniline), 2-naphthylamine, and *ortho*-toluidine (*ortho*-methylaniline), which have been classified as *carcinogenic to humans* (IARC Group 1)

Table 4.1 Genetic and related effects of aniline, aniline hydrochloride, and aniline metabolites in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aniline						
DNA strand breaks (comet assay)	Human MCL-5 cells	+	NT	2.44 mM [227 µg/mL]		Martin et al. (1999)
DNA damage (γ-H2AX induction)	Human urothelial cell line 1T1	+	NT	7.5 mM [697.5 µg/mL]		Qi et al. (2020)
Sister-chromatid exchange	Human lymphoblastoid cells, NL3 (cell line)	–	–	0.1 mM [9.3 µg/mL]	Only one concentration studied	Tohda et al. (1983)
Aniline hydrochloride						
Unscheduled DNA synthesis	Primary human hepatocytes	–	–	1 mM [129 µg/mL]	Test for 6 cases	Butterworth et al. (1989)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	5 or 10 mM [645 or 1290 µg/mL]	Minimal although statistically significant increases [reported as negative in Suppl. 6 of the *IARC Monographs* (IARC (1987)]	Wilmer et al. (1981)
Sister-chromatid exchange	Human lymphocytes	–	NT	1 mM [129 µg/mL]		Wilmer et al. (1984)
Sister-chromatid exchange	Human whole blood	+	NT	1 mM [129 µg/mL]		Wilmer et al. (1984)
Sister-chromatid exchange	Human lymphocytes, peripheral blood (primary culture)	–	–	1 mM [129 µg/mL]		Takehisa & Kanaya (1982)
Aniline metabolites						
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	Acetanilide 10 mM [1351.7 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	2-Aminophenol 0.1 mM [10.91 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	4-Aminophenol 0.2 mM [21.83 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	2-Hydroxyacetanilide 10 mM [1511.63 µg/mL]		Wilmer et al. (1981)

Table 4.1 (continued)

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	4-Hydroxyacetanilide [paracetamol] 10 mM [1511.65 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	Phenylhydroxylamine 0.5 mM [54.56 µg/mL]		Wilmer et al. (1981)

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

[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of aniline and aniline hydrochloride in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline							
Covalent DNA binding	Rat, F344, (NR)	Kidney, large intestine, and spleen	+	100 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 50 and 100 mg/kg bw [^{14}C]-labelled aniline	At high dose, CBI of 14.2, 4.3, 3.7 µmol/mol nucleotides	McCarthy et al. (1985)
Covalent DNA binding	Rat, F344 (NR)	Liver and small intestine	–	100 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 50 and 100 mg/kg bw [^{14}C]-labelled aniline	CBI, < 2 µmol/mol nucleotides	McCarthy et al. (1985)
Covalent DNA binding	Mice, B6C3F$_1$ (NR)	Liver, kidney, spleen, small intestine, and large intestine	–	500 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 250 and 500 mg/kg bw [^{14}C]-labelled aniline	CBI, < 2.6 µmol/mol nucleotides	McCarthy et al. (1985)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver, bladder, colon, lung, and kidney	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, brain, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, bladder, kidney, and lung	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver, colon, brain, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, kidney, and lung,	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Colon, brain, bladder, liver, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1981)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	105 mg/kg bw	i.p.; 1×; sampled after 4 h; 53, 105, 210, 420, and 840 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 210 and 840 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	–	210 mg/kg bw	i.p.; 1×; sampled after 48, 72, and 120 h; single dose		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Kidney	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; 105, 210, and 420 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Spleen	–	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1982)
DNA strand breaks (viscometric assay)	Rat, Sprague-Dawley (M)	Liver	–	210 mg/kg bw	i.p.; 1×; sampled after 4, 12, and 24 h; single dose		Brambilla et al. (1985)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, lung, brain, and bone marrow	+	1000 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, kidney, and bladder	–	1000 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, bladder, lung, brain, and bone marrow	+	1000 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, and kidney	–	1000 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, lung, brain, and bone marrow	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, kidney, and bladder	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, bladder, lung, brain, and bone marrow	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, and kidney	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Colon, bladder, lung, and brain	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, stomach, kidney, and bone marrow	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Liver	–	300 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Cesarone et al. (1982)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Kidney	+	300 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Cesarone et al. (1982)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Liver, kidney, and bone marrow	–	420 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose for liver and kidney assay; 210 and 420 mg/kg bw for bone marrow assay		Parodi et al. (1982)
Gene mutation	Rat, Big Blue F344 (M)	Liver, spleen, and bone marrow	–	100 mg/kg bw	Oral, vehicle NR; gavage daily for 28 days; sample collected at day 31; single dose		Koenig et al. (2018)
Gene mutation	Rat, Sprague-Dawley (M)	Urine (Host-mediated activation using *Salmonella typhimurium* TA98)	+	300 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h; single dose		Tanaka et al. (1980)
Micronucleus formation	Rat, Big Blue F344 (M)	Peripheral blood	+	100 mg/kg bw	Oral, vehicle NR; gavage daily for 28 days; sampled at day 4 and 29; single dose		Koenig et al. (2018)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, SJL Swiss (M, F)	Bone marrow	+	50 mg/kg bw	i.p.; 1×; sampled after 24 h; 5, 50, 100, and 200 mg/kg bw		Sicardi et al. (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	–	300 mg/kg bw	i.p.; 2×; sampled after 6 h; 100, 200, 250, and 300 mg/kg bw		Ashby et al. (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	+	380 mg/kg bw	i.p.; 2×; sampled after 24 h; 237.5 and 380 mg/kg bw		Ashby et al. (1991); Tinwell & Ashby (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	–	380 mg/kg bw	i.p.; 2×; sampled after 48 h; 237.5 and 380 mg/kg bw		Ashby et al. (1991)
Micronucleus formation	Mouse, ICR Swiss (M)	Bone marrow	–	250 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h; 125 and 250 mg/kg bw;		Harper et al. (1984)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow	+	23 mg/kg bw	Oral, in corn oil; 2×; sampled after 24 h; 12, 23, 47, 120, and 470 mg/kg bw		Ress et al. (2002)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 61, 123, 210, and 420 mg/kg bw	Commercial aniline	Parodi et al. (1982)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; single dose	Distilled (purified) aniline	Parodi et al. (1982)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 210 and 420 mg/kg bw		Parodi et al. (1983)
Aniline hydrochloride							
DNA damage (γ-H2AX induction)	Rat, F344/DuCrl-Crlj (M)	Bladder	–	0.6% in the feed	Oral, in feed; for 4 weeks		Toyoda et al. (2019)
Chromosomal aberration	Rat, PVG (M)	Bone marrow	+	500 mg/kg bw	Oral, in water; 1×; sampled after 18 h; 300, 400, and 500 mg/kg bw		Bomhard (2003)
Chromosomal aberration	Rat, PVG (M)	Bone marrow	–	500 mg/kg bw	Oral, in water; 1×; sampled after 30 h; single dose		Bomhard (2003)
Chromosomal aberration	Mouse, CBA (M)	Bone marrow	–	380 mg/kg bw	i.p.; 2×; sampled after 16, 20 or 24 h; 220, 300, and 380 mg/kg bw		Jones and Fox (2003)
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	287 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 215, 287, 400, and 500 mg/kg bw		George et al. (1990)
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	400 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 215, 287, 400, and 500 mg/kg bw		George et al. (1990)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	300 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 300, 400, and 500 mg/kg bw		Bomhard (2003)
Micronucleus formation	Rat, PVG (M)	Bone marrow	–	300 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 300, 400, and 500 mg/kg bw		Bomhard. (2003)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	+	1000 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 400, 500, and 1000 mg/kg bw		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	–	1000 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 400, 500, and 1000 mg/kg bw		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	+	380 mg/kg bw	i.p.; 1×; sampled after 24 h; single dose		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, $B6C3F_1$ (M, F)	Peripheral blood	+	65 mg/kg bw	Oral, in feed; daily for 90 days; 500, 1000, and 2000 mg/kg in feed, equivalent to 65, 130, and 260 mg/kg bw/day		Witt et al. (2000)

bw, body weight; CBI, covalent binding index; F, female; h, hour; γ-H2AX, phosphorylated histone 2AX; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male; NR, not reported.

[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of aniline and aniline hydrochloride in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aniline						
DNA strand breaks (comet assay)	Rat, Sprague-Dawley, primary hepatocytes	+	NT	2.5 µg/mL		Wang et al. (2016)
DNA strand breaks (alkaline elution assay)	Mouse, lymphoma, L5178Y	–	(+)	21.5 mM [2000 µg/mL]		Garberg et al. (1988)
DNA strand breaks (alkaline elution assay)	Chinese hamster, lung, V79	–	–	3 mM [279 µg/mL]		Swenberg et al. (1976); Swenberg (1981)
Unscheduled DNA synthesis (DNA repair assay)	Rat, primary hepatocytes	–	NT	1 mM [93 µg/mL]		Williams (1980; 1981)
Unscheduled DNA synthesis (DNA repair assay)	Rat, F344, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Unscheduled DNA synthesis (DNA repair assay)	Mice, CD-1, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Unscheduled DNA synthesis (DNA repair assay)	Hamster, Syrian golden, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	3.7 mM [344 µg/mL]		Amacher et al. (1980)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	2.5 mM [581 µg/mL]		Wangenheim & Bolcsfoldi (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	0.5 mM [46.5 µg/mL]		Wangenheim & Bolcsfoldi (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	+	1 µL/mL [1000 µg/mL]		Myhr & Caspary (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	0.8 µL/mL [800 µg/mL]		Mitchell et al. (1988); Caspary et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	0.41 µL/mL [410 µg/mL]		Mitchell et al. (1988); Caspary et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	1600 µg/mL		McGregor et al. (1991)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	500 µg/mL		McGregor et al. (1991)
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79	–	NT	20 mM [1860 µg/mL]		Fassina et al. (1990)
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79	NT	+	60 mM [5580 µg/mL]		Fassina et al. (1990)

Table 4.3 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79 (co-cultured with rat hepatocytes)	–	NT	10 mM [930 µg/mL]		Fassina et al. (1990)
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	+	5 mg/mL		Galloway et al. (1987)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	444 µg/mL		Chung et al. (1995; 1996)
Micronucleus formation	Syrian hamster, embryo, SHE	–	NT	NR		Fritzenschaf et al. (1993)
Micronucleus formation	Chinese hamster, lung, CHL/IU	–	NT	2 mg/mL		Matsushima et al. (1999)
Micronucleus formation	Chinese hamster, lung, CHL/IU	NT	+	250 µg/mL		Matsushima et al. (1999)
Sister-chromatid exchange	Rat, liver epithelial, RL_4	+	NT	0.5 mM [46.5 µg/mL]		Cunningham & Ringrose (1983)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	+	5 mg/mL	Different treatment periods: 2 h (+S9) vs about 26 h (–S9)	Galloway et al. (1987)
Aniline hydrochloride						
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	1 mM [129 µg/mL]		Yoshimi et al. (1988)
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	1 mM [129 µg/mL]		Butterworth et al. (1989)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	1 mM [129 µg/mL]		Takehisa et al. (1988)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	NT	–	1 mM [129 µg/mL]	Negative in the presence of rat liver S9 or *Vicia* root S10	Takehisa et al. (1988)
Chromosomal aberrations	Chinese hamster, Don	–	NT	5 mM [645 µg/mL]		Abe & Sasaki (1977)
Chromosomal aberrations	Chinese hamster, lung fibroblasts, CHL	–	NT	1 mg/mL		Ishidate (1983)
Chromosomal aberrations	Chinese hamster, lung fibroblasts, CHL	NT	–	2 mg/mL		Ishidate (1983)

Table 4.3 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	NT	4 mM [516 µg/mL]		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	NT	+	1 mM [129 µg/mL]	With *Vicia* S10 mix	Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	NT	4 mM [516 µg/mL]	With *Pisum* or *Lactuca* S10 mix	Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	–	–	4 mM [516 µg/mL]	With *Vicia*, *Pisum*, or *Lactuca* S10 mix	Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, Don	+	NT	10^{-6} M [0.13 µg/mL]		Abe & Sasaki (1977)
Aniline metabolites (2-AP, 3-AP, 4-AP)						
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	0.1 mM 2-AP		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	2 mM 3-AP		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	0.05 mM 4-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	0.1 mM 2-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	2 mM 3-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	0.1 mM 4-AP		Kanaya (1996)

2-AP, 2-aminophenol; 3-AP, 3-aminophenol; 4-AP, 4-aminophenol; h, hour; Hprt, hypoxanthine-guanine phosphoribosyltransferase; HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; S10, 10 000 × *g* supernatant; *Tk*, thymidine kinase.

[a] +, positive; (+), weakly positive; –, negative.

Table 4.4 Genetic and related effects of aniline and aniline hydrochloride 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			
Aniline						
Embryo-fetal chicken livers	DNA adducts, nucleotide ^{32}P-post-labelling	–	NA	20 mg/egg (injection)		Kobets et al. (2019)
Embryo-fetal chicken livers	DNA strand breaks, comet assay	–	NA	20 mg/egg (injection)		Kobets et al. (2019)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	400 mg/kg (injection)		Yoon et al. (1985)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	600 mg/kg (feeding)		Yoon et al. (1985)
Drosophila melanogaster	Interchromosomal mitotic recombination (white/white$^+$) eye mosaic assay	–	NA	600 mg/kg (feeding)		Vogel & Nivard (1993)
Wheat seeds	Micronucleus formation	+	NA	5 mg/L		Tao et al. 2017
Aspergillus nidulans	Reverse mutation	–	NA	200 µg/mL		Prasad (1970)
Saccharomyces cerevisiae D3	Homozygosis, mitotic recombination	–	–	0.5% v/v [10 000 µg/mL]	One concentration only	Simmon (1979)
Saccharomyces cerevisiae, RS112	DEL recombination	+	NA	4 mg/mL		Schiestl (1989); Schiestl et al. (1989)
Saccharomyces cerevisiae, RS112	DEL recombination	+	NA	5 mg/mL		Brennan & Schiestl (1997)
Saccharomyces cerevisiae, HAN	Mutation or small deletion	+	NA	10 µL/mL cell suspension		Schafer et al. (2008)
Saccharomyces cerevisiae, DAN	Recombination	+	NA	10 µL/mL cell suspension		Schafer et al. (2008)
Salmonella typhimurium TA98	DNA adducts, nucleotide ^{32}P-post-labelling	–	–	4 mg/4 mL cell culture		Mori et al. (1996)
Salmonella typhimurium TA1357/pSK1002	DNA damage, *umu* test	–	–	4000 µg/mL		Sakagami et al. (1988)
Bacillus subtilis H17 rec$^+$ and M45 rec$^-$	DNA damage, rec assay	–	NT	3 µg/well	MIC, 3 µg/well in H17 rec$^+$; and 0.2 µg/well in M45 rec$^-$	McCarroll et al. (1981)
Escherichia coli KWP2, WP100	DNA damage, rec assay	NT	–	2000 µg/mL		Mamber et al. (1983)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Escherichia coli K12	DNA damage, prophage induction	NT	–	2000 µg/plate	Only dose reported	Mamber et al. (1984)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	+	+	25 µL/plate		Fluck et al. (1976)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	+	+	25 µL/plate	5 days after distillation	Fluck et al. (1976)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	–	–	25 µL/plate	Freshly distilled	Fluck et al. (1976)
Vibrio fischeri	DNA damage	+	–	2760 µM	EC_{50}	Osano et al. (2002)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	2500 µg/plate		Parodi et al. (1981)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	1000 µg/plate	Co-incubated with plant cells	Gentile et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	10 000 µg/plate	Rat liver S9	Gentile et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	–	+	10 000 µg/plate	Pea apical bud S9; concentration-related increase but significant only at the highest concentration	Gentile et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	1000 µg/plate	Rat liver S9	Rashid et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	698 µg/plate	Rat S9	Nohmi et al. (1984)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	0.05 µmol/100 µL per plate	Positive after nitrite treatment	Kato et al. (1991)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	5000 µg/plate		Assmann et al. (1997)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	3000 µg/plate	Rat liver S9	Chung et al. (1995)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	5000 µg/plate	Ethanol-induced rat liver S9	Burke et al. (1994)
Salmonella typhimurium TA98, TA98NR, TA100, TA100NR	Reverse mutation	–	–	3000 µg/plate	Rat liver S9	Chung et al. (1996)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100, TA97	Reverse mutation	–	–	2000 µg/plate		Brams et al. (1987)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	NT	–	2500 µg/plate		Ashby et al. (1981)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	3333 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537	Reverse mutation	NT	–	5000 µg/plate	Pyrazole-induced rat liver S9	Burke et al. (1994)
Salmonella typhimurium TA102	Reverse mutation	–	–	5000 µg/plate	Rat liver S9	Jung et al. (1992)
Salmonella typhimurium TA98, TA100, TA1538	Reverse mutation	–	–	5000 µg/plate	Rat liver S9	Chung et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	NT	500 µg/plate	Concentrations, 0.8, 4, 20, 100, and 500 µg/plate	Ashby et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	NT	–	200 µg/plate		Nagao et al. (1977)
Salmonella typhimurium TA98	Reverse mutation	NT	–	1000 µg/plate		Ho et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 µg/plate	Pea apical bud S9	Gentile et al. (1987)
Salmonella typhimurium TA1538	Reverse mutation	–	–	100 µg/plate	Rat liver S9	Garner & Nutman (1977)
Escherichia coli (WP2 *uvra*)	Reverse mutation	–	NT	2 mM [186 µg/mL]		Pai et al. (1985)
Aniline hydrochloride						
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	10% solution for feeding	Increases in nondisjunction observed	Muñoz & Barnett (1998)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	5% (0.4 µL) for injection		Muñoz & Barnett (1998)
Vicia faba	Chromosomal aberrations	–	NA	1 mM [129 µg/mL]	At 1 mM, positive response seen at 20 h recovery time but not longer	Kanaya (1990)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Vicia faba	Sister-chromatid exchange	–	NA	4 mM [4386 µg/mL]	Positive responses from metabolites 2-, 3-, and 4-aminophenol	Kanaya (1990)
Salmonella typhimurium TA100	Reverse mutation	NT	–	2000 µg/plate		Imamura et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	5000 µg/plate	S9 from rat liver, rat spleen, hamster liver and hamster spleen	Shahin (1989)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	10 000 µg/plate	S9 from livers of rat, mouse or hamster	Dunkel et al. (1985)
Escherichia coli (WP2 *uvrA*)	Reverse mutation	–	–	10 000 µg/plate	S9 from livers of rat, mouse or hamster	Dunkel et al. (1985)
Escherichia coli (WP2 *uvrA*), IC188, IC203	Reverse mutation	–	NT	1000 µg/plate	Single dose tested only	Martínez et al. (2000)

DEL, deletion; EC_{50}, half maximal effective concentration; h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; MIC, minimal inhibitory concentration; NA, not applicable; NT, not tested; TA98NR, TA98 nitroreductase-deficient mutant strain; TA100NR, TA100 nitroreductase-deficient mutant strain.

[a] +, positive; –, negative.

by the *IARC Monographs* programme (IARC, 2010, 2012).]

4.2.2 Is genotoxic

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the available studies of the genetic and related effects of aniline and its hydrochloride.

(a) Humans

(i) Exposed humans

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

(ii) Human cells in vitro

See Table 4.1.

DNA damage

Significantly increased DNA damage measured by comet assay was seen in metabolically competent human MCL-5 cells treated with aniline at a concentration of 2.44 mM and higher. DNA repair inhibitors (hydroxyurea and cytosine arabinoside) markedly enhanced the effects (Martin et al., 1999). A significant induction of γ-H2AX (a biomarker of DNA damage) was observed in the human urothelial 1T1 cell line exposed to aniline at a concentration of 7.5 mM and the response was concentration-related (Qi et al., 2020). However, a test for unscheduled DNA synthesis in primary human hepatocytes was reported to give negative results with aniline hydrochloride at concentrations up to 1 mM (Butterworth et al., 1989).

Chromosomal damage

At a low concentration, exposure of human lymphoblast NL3 cells to aniline (0.1 mM) did not result in induction of sister-chromatid exchange in the presence of absence of endogenous metabolic activation (Tohda et al., 1983). Wilmer et al. (1981) reported marginal but significant increases in the frequency of sister-chromatid exchange in human fibroblasts exposed to aniline hydrochloride at higher concentrations (5 mM). Further study confirmed that aniline hydrochloride at a lower concentration (1 mM) also induced a significant concentration-related increase in the frequency of sister-chromatid exchange in human whole blood cell cultures, but not in mononuclear leukocytes (Wilmer et al., 1984). Furthermore, Takehisa & Kanaya (1982) showed that aniline hydrochloride at a concentration of 1 mM was unable to induce an increase in sister-chromatid exchange in human peripheral blood lymphocytes in the presence or absence of endogenous metabolic activation. [The Working Group noted that cell type and concentration of aniline were the two main factors that influenced the outcomes of the exposure.] Some hydroxylated metabolites of aniline, e.g. 2-aminophenol and phenylhydroxylamine, were more potent than aniline in sister-chromatid exchange induction; others (acetanilide, 4-aminophenol, and 2- and 4-hydroxyacetanilide) gave negative results (Wilmer et al., 1981).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

Several studies investigated the genotoxic effects of exposure to aniline or aniline hydrochloride in experimental animals in vivo. The end-points include DNA binding, DNA damage, gene mutation, chromosomal aberration, micronucleus formation, and sister-chromatid exchange.

DNA damage

Significantly increased values for the covalent binding index were reported in the kidney, spleen and large intestine of Fischer 344 rats after intraperitoneal injection of [^{14}C]-labelled aniline at a dose of 100 mg/kg bw (McCarthy et al., 1985).

No increases in phosphorylated histone H2AX (γ-H2AX) formation (a biomarker of DNA damage) were observed in the bladder urothelium of Fischer 344/DuCrl-Crlj rats given feed

containing 0.6% aniline hydrochloride (Toyoda et al. 2019).

DNA strand breaks measured by comet assay were investigated by several techniques in various tissues of rats and mice after oral or intraperitoneal exposure to aniline or aniline hydrochloride. Significant increases in the frequency of single-strand breaks were observed in the liver, bladder, lung, colon, and kidney; but not in the stomach, brain, or bone marrow in Wistar rats after a single oral dose of aniline of 150 mg/kg bw at the 3-hour sampling time. At two other sampling times (8 hours or 24 hours after treatment), the pattern of organs testing positive for DNA damage was slightly different. For example, at the 24-hour time point, DNA damage was seen only in stomach, kidney, and lung (Sekihashi et al., 2002). [The Working Group noted that spleen was not investigated in these studies with the comet assay in rats.]

In a preliminary study in which aniline was administered by intraperitoneal injection, DNA fragmentation (measured by alkaline elution assay) was observed in liver samples from male Sprague-Dawley rats 4 hours and 24 hours after a single dose of aniline at 210 mg/kg bw (half of the median lethal dose, LD_{50}) (Parodi et al., 1981). In a more in-depth study, male Sprague-Dawley rats were given a single intraperitoneal injection of aniline at a dose ranging from 53 to 840 mg/kg bw, with sampling at 4 hours after treatment, or at a dose of 210 mg/kg bw with sampling times at 4–120 hours after treatment. In the 4-hour dose–response experiment, DNA fragmentation (alkaline elution assay) was significantly increased in the liver (at 105 mg/kg bw and above) and in the kidneys (at 210 and 420 mg/kg bw), but not in the spleen (at up to 210 mg/kg bw). In the time-course experiment, no DNA fragmentation was observed in the liver samples collected at 48 hours or after (Parodi et al., 1982). [The Working Group noted that sampling time seems to be a critical factor for observation of DNA damage.] Moreover, a purified aniline sample after distillation was found to behave very similarly to the commercial grade, at the test dose of 210 mg/kg bw (Parodi et al., 1982). Brambilla et al. (1985) reported no DNA damage (by viscometric assay) in liver samples (24 hours after treatment) in male Sprague-Dawley rats that received a single intraperitoneal injection of aniline at 210 mg/kg bw.

In male ddY mice given a single oral dose of aniline at 1000 mg/kg bw, significantly increased DNA damage, as measured by the comet assay, was observed in liver, lung, brain, and bone marrow, but not in stomach, colon, kidney, or bladder at the sampling time of 3 hours after treatment. At a sampling time of 8 hours after treatment, DNA damage also occurred in the bladder (Sasaki et al., 1999). Following a similar protocol, Sekihashi et al. (2002) obtained similar DNA damage results in varied organs in male ddY mice given much lower single oral doses (100 mg/kg bw) at three sampling times, 3, 8, and 24 hours after treatment. [The Working Group noted that the patterns of the positive results for DNA damage slightly varied at the different sampling time-points (see Table 4.2), and that the spleen was not investigated in these studies with the comet assay in mice.]

Significant induction of single-strand breaks as measured by alkaline elution assay was observed in male Swiss CD1 mouse kidney 4 hours after a single intraperitoneal injection of aniline (300 mg/kg bw), whereas a negative finding was obtained in liver (Cesarone et al., 1982). No induction of DNA fragmentation as measured by alkaline elution assay was seen in the liver, kidney, or bone marrow of male Swiss mice after a single intraperitoneal injection of aniline at a dose of up to 420 mg/kg bw (Parodi et al., 1982).

Gene mutation

One study on gene mutation in vivo in Big Blue rats and one host-mediated assay were available. No significant increases in mutant

frequency at the *cII* gene were observed in the liver, spleen, or bone marrow after a daily exposure at 100 mg/kg bw by gavage for 28 consecutive days (Koenig et al., 2018). Aniline was mutagenic in a host-mediated assay for mutation in *Salmonella*. Urine samples were collected for 24 hours from rats given aniline orally at a dose of 300 mg/kg bw. Ether extracts of the urine samples were tested for mutagenicity in *Salmonella typhimurium* TA98 and TA100. A clear concentration-dependent increase in gene mutation frequency was obtained with TA98 in the presence of endogenous metabolic activation (Tanaka et al., 1980).

Chromosomal aberration

There are few studies in vivo on chromosomal aberration with aniline or aniline hydrochloride. A slight but significant increase in the frequency of chromosomal aberration was observed in the bone marrow of male PVG rats given aniline hydrochloride as a single oral dose at 500 mg/kg bw (the highest dose tested) with a sampling time of 18 hours after treatment; however, no increased effect on chromosomal aberration was seen at the sampling time of 30 hours (Bomhard, 2003). Similarly, no clastogenic effect was observed in the bone marrow of male CBA mice treated with up to two intraperitoneal injections of aniline hydrochloride (380 mg/kg bw) and with sampling times of 16, 20, and 24 hours after the second treatment (Jones & Fox, 2003).

Micronucleus formation

Several studies have investigated micronucleus induction by aniline or aniline hydrochloride in rats. Significant increases in the frequency of micronucleus formation were seen in peripheral blood of Big Blue F344 rats treated with aniline at dose of 100 mg/kg bw by gavage daily for 4 days or 28 days (Koenig et al., 2018). A dose-related increase in the frequency of micronucleus formation was observed in the bone marrow of male PVG rats that received aniline hydrochloride as a single oral dose at 0, 215, 287, 400, or 500 mg/kg bw (as aniline base) (George et al., 1990). The lowest effective dose was 287 mg/kg bw when the bone marrow was collected at 24 hours after exposure. In a similar study carried out by Bomhard (2003), a small but statistically significant and dose-related induction of micronuclei was observed 24 hours but not 48 hours after a single oral dose of aniline hydrochloride at 0, 300, 400, or 500 mg/kg bw in male PVG rats.

Micronucleus induction by aniline or aniline hydrochloride has also been investigated in mice dosed via feed, gavage, or intraperitoneal injection.

In a 90-day study, an increased frequency of micronucleus formation was observed in peripheral blood in male and female B6C3F$_1$ mice given feed containing aniline hydrochloride at a concentration of 500, 1000, or 2000 mg/kg (equivalent to 65, 130, and 260 mg/kg bw per day) for 90 days (Witt et al., 2000).

Harper et al. (1984) reported a negative result for micronucleus induction in male ICR mice given a single oral dose of aniline at up to 250 mg/kg bw. [The Working Group noted that aniline enhanced the effect of benzene in the micronucleus test in a dose-related manner.] In a study on micronucleus formation in male B6C3F$_1$ mice (treated with aniline at 12, 23, 47, 120, or 470 mg/kg bw for 24 hours, orally), a significant induction of micronucleus was only observed at doses of 23 and 470 mg/kg bw. No dose–response relationship was seen (Ress et al., 2002). Aniline hydrochloride significantly induced micronucleus formation in the bone marrow of male CRH mice only at the highest dose tested (1000 mg/kg bw) at the 24-hour but not the 48-hour time point (Westmoreland & Gatehouse, 1991).

A dose-related increase in the frequency of micronucleated polychromatic erythrocytes was observed in bone marrow cells of Swiss mice given a single intraperitoneal injection of

purified aniline (5, 50, 100, or 200 mg/kg bw for 24 hours) (Sicardi et al., 1991). After intraperitoneal injections of aniline (up to 300 mg/kg bw), male CBA mice failed to show a significant increase in micronucleus frequency 6 hours after treatment (Ashby et al., 1991). A significant increase (about 7-fold over the control) was seen at a dose of 380 mg/kg bw 24 hours after treatment (Ashby et al., 1991; Tinwell & Ashby, 1991); the effect was not significant at 48 hours (Ashby et al., 1991). Westmoreland & Gatehouse (1991) confirmed the positive response for micronucleus induction in bone marrow of male CHR mice 24 hours after administration of aniline hydrochloride as a single intraperitoneal dose at 380 mg/kg bw.

[The Working Group noted that single oral dose studies seemed to be less sensitive than intraperitoneal injection studies.]

Sister-chromatid exchange

Only two in vivo studies were available for the induction of sister-chromatid exchange, both in Swiss mice given aniline intraperitoneally. A clear dose-dependent induction of sister-chromatid exchange in bone marrow cells of male Swiss mice was reported 24 hours after a single injection of aniline at a dose of 61–420 mg/kg bw. The lowest effective dose was 210 mg/kg bw, and raw chemical and purified aniline showed similar activities (Parodi et al., 1982). In addition, a significant dose-related induction of sister-chromatid exchange in male Swiss mice was seen after a single intraperitoneal injection of 210 or 420 mg/kg bw with sampling time of 24 hours after treatment (Parodi et al., 1983).

Sperm head abnormalities

In (CBA × BALB/c)F_1 male mice that received five daily intraperitoneal injections of aniline hydrochloride at doses ranging from 17 to 200 mg/kg bw, no increase in the frequency of sperm head abnormalities was observed for 5 weeks after the last dose (Topham, 1980).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

Several studies investigated the genotoxic effects of exposure to aniline or aniline hydrochloride in non-human mammalian cells in vitro. The end-points included DNA damage, gene mutation, chromosomal aberration, sister-chromatid exchange, and unscheduled DNA synthesis.

DNA damage

Aniline (1.25–10 µg/mL for 24 hours) caused a concentration-related increase in the frequency of DNA strand breaks (comet assay) in cultured primary hepatocytes isolated from Sprague-Dawley rats. Addition of *N*-acetyl-L-cysteine reduced the effects (Wang et al., 2016).

No DNA damage (measured by alkaline elution assay) was seen in mouse lymphoma L5178Y cells in the absence of endogenous metabolic activation, whereas positive results were seen in the presence of endogenous metabolic activation, at the higher dose (Garberg et al., 1988).

Exposure of Chinese hamster lung fibroblasts (V79) to aniline (up to 3 mM for 4 hours) with or without endogenous metabolic activation did not affect the rate of elution of DNA (Swenberg et al., 1976; Swenberg, 1981).

Unscheduled DNA synthesis tests with aniline or aniline hydrochloride in primary rat or mouse hepatocytes all gave negative results (Williams, 1980, 1981; McQueen et al., 1981; Yoshimi et al., 1988; Butterworth et al., 1989).

Gene mutation

Aniline or aniline hydrochloride gave positive results for gene mutation in the mouse lymphoma L5178Y *Tk*$^{+/-}$ assay consistently in several independent studies, even though most positive results occurred at high concentrations. In the presence of endogenous metabolic activation, the mutation frequency in the *Tk*$^{+/-}$ mutation assay increased in a concentration-related manner up

to 4.98 mM and doubled at a concentration of 3.7 mM (Amacher et al., 1980). Wangenheim & Bolcsfoldi (1988) reported a concentration-related increase in aniline-induced mutation frequency in the $Tk^{+/-}$ mutation assay in both the presence (aniline, 0.5–5 mM) and absence (aniline, 2.5–15 mM) of endogenous metabolic activation. The lowest effective dose was 0.5 mM and the presence of endogenous metabolic activation enhanced the sensitivity of aniline-induced mutation. Similar results for aniline concentration-related increases in frequency of gene mutation were also obtained in several other studies (Caspary et al., 1988; Mitchell et al., 1988; Myhr & Caspary, 1988; McGregor et al., 1991). [The Working Group noted that aniline or aniline hydrochloride has the potential to cause mutations at the thymidine kinase (*Tk*) locus in mouse lymphoma L5178Y cells. The $Tk^{+/-}$ mutation assay can detect a wide range of genetic events, including point mutations, deletions, chromosomal rearrangements, mitotic recombination, and nondisjunction. Generally, the induction of small colonies of mutants is associated with chemicals that induce gross chromosomal aberrations, whereas the induction of large colonies of mutants is associated with chemicals that induce point mutations; however, in the available studies, the colony sizes were not reported.]

In addition, Fassina et al. (1990) reported a marginal but significant increase in mutation frequency in the *Hprt* mutation assay in Chinese hamster V79 cells exposed to aniline at up to 60 mM in the presence of endogenous metabolic activation from rat S9; however, no induction of mutation was seen with aniline in the absence of S9 in the cell culture or when co-cultured with rat hepatocytes. [The Working Group noted that aniline caused mutations at the *Hprt* locus only at a high concentration in the presence of endogenous metabolic activation.]

Chromosomal aberrations

Clastogenic activity with aniline or aniline hydrochloride has also been studied in mammalian cells. The studies from Galloway et al. (1987) showed negative results in Chinese hamster ovary cells exposed to aniline in the absence of endogenous metabolic activation, whereas a significantly increased frequency of chromosomal aberrations was seen at the highest test concentration of aniline (5 mg/mL) in the presence of endogenous metabolic activation. [The Working Group noted that the test gave weakly positive results at the highest dose; and that a dose–response relationship with endogenous metabolic activation was observed.] Aniline induced a concentration-related increase in the frequency of chromosomal aberrations in Chinese hamster embryo cells in the absence of endogenous metabolic activation. The lowest effective concentration was 444 µg/mL and the main aberrations were dicentric chromosomes and breaks (Chung et al., 1995, 1996).

Early studies showed that aniline hydrochloride was not clastogenic in Chinese hamster Don cells at concentrations of up to 5 mM [0.645 mg/mL] (Abe & Sasaki, 1977) or in Chinese hamster lung cells at up to 1 mg/mL in the absence of endogenous metabolic activation (Ishidate & Odashima, 1977; Ishidate, 1983). Moreover, Kanaya (1996) reported that *Vicia faba* extract could activate aniline to induce chromosomal damage in Chinese hamster ovary cells, whereas extracts from *Pisum sativum* and *Lactuca sativa* did not. Furthermore, aniline metabolites *ortho*-, *meta*-, and *para*-aminophenol [2-, 3-, and 4-aminophenol] induced an increased frequency of chromosomal aberrations in Chinese hamster ovary cells in a concentration-related manner (Kanaya 1996).

Micronucleus formation

Fritzenschaf et al. (1993) reported that aniline [dose not reported] did not induce micronucleus formation in Syrian hamster embryo cells in the

absence of endogenous metabolic activation, whereas Matsushima et al. (1999) reported a significant increase in the frequency of micronucleated cells in the Chinese hamster lung cell line exposed to aniline at a concentration of 125–2000 µg/mL with endogenous metabolic activation. No induction of micronuclei was seen in the absence of endogenous metabolic activation in the test system (Matsushima et al., 1999).

Sister-chromatid exchange

A concentration-related increase in sister-chromatid exchange frequency was seen in RL4 rat liver epithelial cells treated with aniline (0.1, 0.2, 0.5, 1 mM for 24 hours) in the absence of endogenous metabolic activation. The doubling effect occurred at concentration of 0.5 mM (Cunningham & Ringrose, 1983). Galloway et al. (1987) reported a slight increase in the frequency of sister-chromatid exchange in Chinese hamster ovary W-B1 cells treated with aniline (50–500 µg/mL for 26 hours) without endogenous metabolic activation; and at concentrations of 4 mg/mL and 5 mg/mL in an experiment with incubation for 2 hours with endogenous metabolic activation.

Takehisa et al. (1988) also reported that aniline hydrochloride (0.01, 0.1, 1 mM, for 24 hours) alone could induce sister-chromatid exchange in Chinese hamster ovary cells, although the response was weak; however, the presence of endogenous metabolic activation with *Vicia* root S10 or rat liver S9 did not induce an increase in sister-chromatid exchange frequency above that of the controls. An early study showed that aniline hydrochloride caused a concentration-related effect on sister-chromatid exchange induction in Chinese hamster Don cells (Abe & Sasaki, 1977). Kanaya (1996) reported that none of the plant extracts tested (*Vicia*, *Pisum*, or *Lactuca* S10 mix) could activate aniline to induce sister-chromatid exchange in Chinese hamster ovary cells; however, the aniline metabolites *ortho*-, *meta*-, and *para*-aminophenol [2-, 3-, and 4-aminophenol] increased the frequency of sister-chromatid exchange in Chinese hamster ovary cells in a concentration-related manner.

(iii) Non-mammalian experimental systems in vivo and in vitro

See Table 4.4.

Aniline did not induce DNA adduct formation (measured by nucleotide ^{32}P-post-labelling) or cause DNA strand breaks when assessed in embryo-fetal chicken livers (Kobets et al., 2019).

No DNA adducts were found (by ^{32}P-post-labelling) in *S. typhimurium* TA98 after treatment with aniline alone at a concentration of 1 mg/mL with or without endogenous metabolic activation; however, DNA adducts were detected in the presence of norharman and endogenous metabolic activation (Mori et al., 1996).

Yoon et al. (1985) reported that aniline was not mutagenic in the test for sex-linked recessive lethal mutations in *Drosophila melanogaster* exposed to aniline at 400 mg/kg by injection or at 600 mg/kg via feed. Moreover, in an eye mosaic (white/white$^+$) assay, aniline induced a modest increase in the frequency of interchromosomal mitotic recombination after treatment of larvae at 2 mM (Vogel & Nivard, 1993). Muñoz & Barnett (1998) showed that aniline hydrochloride was not mutagenic in the test for sex-linked recessive lethal mutations in *Drosophila* after intra-abdominal injection (0.4 µL) or feeding (10% solution); however, significant increases in the frequency of nondisjunction were observed in *Drosophila* in the feeding study.

In a plant study, aniline increased the frequency of micronucleus formation in wheat (*Triticum aestivum*) root tip cells, in a dose-dependent manner at concentrations as low as 5 mg/L in the culture solution (Tao et al., 2017). Exposure of *Vicia faba* seeds to aniline hydrochloride resulted in significant increases in the frequency of chromosomal aberrations but not sister-chromatid exchange in the root cells (Kanaya, 1990).

Previous studies indicated that aniline did not cause mutation in *Aspergillus nidulans* (Prasad, 1970) and gave negative results for recombinogenic activity in an assay in vitro with *Saccharomyces cerevisiae* D3 (Simmon, 1979). It was subsequently reported that aniline (1, 2, 4, or 7 mg/mL, for 17 hours) induced intrachromosomal recombination in yeast *S. cerevisiae* RS112 at a higher concentration (7.9-fold at 7 mg/mL) (Schiestl, 1989; Schiestl et al., 1989). Furthermore, Brennan & Schiestl (1997) reported that aniline (5, 10, or 12 mg/mL, for 17 hours) and its metabolites 2- and 4-aminophenol induced intrachromosomal (DEL) recombination in *S. cerevisiae* strain RS112. Schafer et al. (2008) showed that aniline was mutagenic and a recombinogen in the eukaryotic organisms *S. cerevisiae* strains HAN and DAN.

Sakagami et al. (1988) reported that aniline did not caused DNA damage in *S. typhimurium* as measured by the SOS/*umu* genotoxicity assay at concentrations of up to 4000 µg/mL with or without metabolic activation. Aniline did not cause DNA damage in *Escherichia coli* KWP2, WP100, or K12 (Mamber et al., 1983, 1984) or in the rec assay with *Bacillus subtilis* strains H17 rec^+ and M45 rec^- (McCarroll et al., 1981). Stock aniline or aniline that was not freshly distilled (5 days after distillation) was reported to cause DNA damage in the *E. coli* pol A^+/pol A^- assay with or without metabolic activation; but the freshly distilled aniline gave negative results (Fluck et al., 1976). Moreover, aniline induced DNA damage in the Mutatox genotoxicity test with *Vibrio fischeri* (dark variant) without endogenous metabolic activation; but was not genotoxic in the presence of endogenous metabolic activation (Osano et al., 2002).

Negative results were obtained in most of the *S. typhimurium* assays with aniline or aniline hydrochloride in tester strains TA98, TA100, TA1535, TA1537, TA1358, TA98NR, or TA100NR in the presence or absence of metabolic activation from varied types of S9 (from rat, mouse, hamster, pig, or plant) (Garner & Nutman, 1977; Nagao et al., 1977; Ashby et al., 1981, 1983; Chung et al., 1981, 1995, 1996; Ho et al., 1981; Parodi et al., 1981; Haworth et al., 1983; Imamura et al., 1983; Nohmi et al., 1984; Dunkel et al., 1985; Brams et al., 1987; Gentile et al., 1987; Rashid et al., 1987; Shahin, 1989; Kato et al., 1991; Jung et al., 1992; Burke et al., 1994; Assmann et al., 1997). Only one positive result was seen in strain TA100 at the highest test concentration, 10 000 µg/plate, in the presence of metabolic activation from pea apical bud S9 (Gentile et al., 1987). Notably, Shahin (1989) re-evaluated the mutagenicity of aniline hydrochloride in TA98, TA100, TA1535, TA1537, and TA1538 with metabolic activation from S9 prepared from rat liver or spleen, or hamster liver or spleen, and the results confirmed that aniline was not mutagenic in *S. typhimurium* at concentrations up to 5 mg/plate.

Similarly, aniline (Pai et al., 1985) and aniline hydrochloride (Dunkel et al., 1984, 1985; Martínez et al., 2000) were also not mutagenic in forward mutation assays in *E. coli* with or without metabolic activation from S9 from various animal livers.

4.2.3 Induces oxidative stress

Table 4.5, Table 4.6, and Table 4.7 summarize the available studies of effects related to oxidative stress after exposure to aniline and aniline hydrochloride.

(a) Humans

No data were available to the Working Group on oxidative stress related to aniline or aniline hydrochloride in exposed humans.

In human cells in vitro, Horinouchi et al. (2015) reported that aniline did not cause membrane damage or free radical generation (measured by immuno-spin trapping using in-cell Western experiments and confocal microscopy) in HepG2 cells at a concentration of 100 µM (the only concentration tested).

Table 4.5 Oxidative damage to DNA in experimental animals

End-point/biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Aniline hydrochloride						
8-OHdG	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day [129 mg/kg bw]	Gavage, 7 days	Wu et al. (2005)
8-OHdG, Ogg1 mRNA, protein levels and enzyme activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day [64.8 mg/kg bw]	Drinking-water, 30 days	Ma et al. (2008)
Base excision repair enzymes Neil1/2, Nth1, and Ape-1 mRNA, protein levels and enzyme activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day [64.8 mg/kg bw]	Drinking-water, 30 days	Ma et al. (2011, 2013)

Ape-1, apurinic/apyrimidinic endonuclease 1; bw, body weight; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HID, highest ineffective dose; LED, lowest effective dose; M, male; Neil1/2, nei-like DNA glycosylase; Nth1, endonuclease III homologue 1; Ogg1, 8-oxoguanine glycosylase 1.
[a] +, positive.

(b) Experimental systems

(i) Oxidative damage to DNA

See Table 4.5.

Aniline treatment of male Sprague-Dawley rats resulted in a significant increase of 200% in splenic iron content and an 83% increase in 8-hydroxy-2′-deoxyguanosine (8-OHdG) in spleen compared with controls (Wu et al., 2005). 8-Oxoguanine glycosylase (Ogg1) is a specific DNA glycosylase enzyme and plays an important role in the removal of 8-OHdG adducts during base excision repair (BER). Subchronic exposure of male Sprague-Dawley rats to aniline for 30 days resulted in increases of 2.8-fold in 8-OHdG levels, 2-fold in *Ogg1* messenger RNA (mRNA) expression, and 1.3-fold in Ogg1 BER activity (Ma et al., 2008). [The Working Group noted that the results suggest that aniline-induced oxidative stress is associated with increased oxidative damage to DNA.]

Like Ogg1, nei-like DNA glycosylase (Neil1/2) is also a BER enzyme; distinct from Ogg1, it is able to excise oxidized base lesions from regions of single-stranded DNA. Ma et al. (2011) reported that male Sprague-Dawley rats exposed to aniline had increased levels of Neil1/2 activity, mRNA and protein, and Neil immunoreactivity. [The Working Group noted that NEIL1/2 may play a unique role in maintaining the functional integrity of mammalian genomes. This study confirmed that aniline-induced oxidative stress and related DNA damage could also be removed by another BER enzyme, Neil1/2, in addition to Ogg1.] Ma et al. (2013) further reported that aniline exposure led to increased levels of mRNA, protein, protein-associated BER activity, and immunoreactivity for the BER enzymes endonuclease III homologue 1 (Nth1) [nth like glycosylase 1, Nthl1] and apurinic/apyrimidinic endonuclease 1 (Ape1) [apurinic/apyrimidinic endodeoxyribonuclease 1, Apex1]. [The Working Group noted that these data consistently revealed that aniline exposure caused reactive oxygen species (ROS)-mediated DNA damage in rats, as demonstrated by the increased splenic level of 8-OHdG (Wu et al., 2005) and increased expression and activity of BER proteins Ogg1 (Ma et al., 2008), Neil1/2, Nth1, and Ape1 (Ma et al., 2011).]

(ii) Other oxidative stress markers

See Table 4.6.

In studies in experimental animals, a single acute exposure to aniline vapour induced superoxide dismutase (SOD) isozymes in rabbit lung (Kakkar & Viswanathan, 1987) and increased

Table 4.6 Oxidative stress in experimental animals

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline							
SOD activity	Rabbit, albino (M)	Lung	+	15 302 ppm [58 300 mg/m^3]	Chamber exposure, 30 min, 15 302 ppm aniline vapours		Kakkar & Viswanathan. (1987)
SOD activity and MDA content	Rat, Wistar (M)	Brain	+	15 302 ppm [58 300 mg/m^3]	Chamber exposure, 10 min, 15 302 ppm aniline vapours		Kakkar et al. (1992)
Aniline hydrochloride							
MDA content	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Gavage; 0.25, 0.5, 1, 2 mmol/kg bw per day for 4 days		Khan et al. (1997)
MDA, protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 4, 7 days		Khan et al. (1997)
MDA, protein carbonyl content, and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	65 mg/kg bw per day	Drinking-water; 30, 60, 90 days	Negative results for protein carbonyl content at day 30; stronger response in longer exposure	Khan et al. (1999a)
MDA protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day [129.5 mg/kg bw per day]	Gavage; 7 days		Khan et al. (2003a)
MDA protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	65 mg/kg bw per day	Drinking-water; 30 days		Khan et al. (2003b)
MDA, GSH, NO content	Rat, Wistar (M)	Spleen and liver	+	100 ppm [381 mg/m^3]	Drinking-water; 30 days, ± *Dioscorea alata L* extract		Khan et al. (2014)
MDA, GSH, NO content	Rat, Wistar (M)	Spleen	+	100 ppm [381 mg/m^3]	Drinking-water; 28 days, ± protocatechuic acid or ascorbic acid		Khairnar et al. (2016)
Free iron level, total iron or ferritin level	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 1, 4, 7 days	Significant increased free iron content at day 7 Significant increased total iron or ferritin level at day 4 and 7	Wang et al. (2010)
Formation of nitrated protein, iNOS mRNA and protein expression	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Fan et al. (2011)

Table 4.6 (continued)

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Phenylhydroxylamine, an oxidized metabolite of aniline							
MDA, protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	0.025 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, 0.2 mmol/kg bw per day for 4 days		Khan et al. (1998)
Nitrosobenzene, an N-oxidized metabolite of aniline							
MDA content and MDA-protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	0.025 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, and 0.2 mmol/kg bw per day for 4 days		Khan et al. (2000)
Protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	0.1 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, and 0.2 mmol/kg bw per day for 4 days		Khan et al. (2000)

bw, body weight; GSH, glutathione; HID, highest ineffective dose; iNOS, inducible nitric oxide synthase; LED, lowest effective dose; M, male; MDA, malondialdehyde; min, minute; NO, nitric oxide; ppm, parts per million; SOD, superoxide dismutase.

[a] +, positive.

Table 4.7 Gene expression responses to aniline and aniline hydrochloride related to the key characteristics of carcinogens

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline hydrochloride							
TGF-β1 mRNA and protein	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Khan et al. (2003b)
IL1α, IL6, and TNFα mRNA and protein; NF-κB binding activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Wang et al. (2005)
IL1α, IL6, TNFα mRNA and protein; NF-κB and AP-1 binding activity	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Wang et al. (2008)
HO-1 mRNA and protein	Rat, Sprague-Dawley (M)	Spleen	(+)	1 mmol/kg bw per day	Gavage; 1, 4, 7 days	Purity, NR	Wang et al. (2010)
Cyclins and cyclin-dependent kinases	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Wang et al. (2011)
Cell cycle regulatory proteins	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days	Significant increase in the expression of cyclins, Cdk1 and aberrant regulation of miRNAs	Wang et al. (2015)
Cell cycle regulatory proteins	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Wang et al. (2017)
Aniline							
Gene expression of *Gst D2* and *Gst D5*	*Drosophila melanogaster*	NA	+	NR; < 2.0 µL/tube	On filter paper; 1.2, 1.4, 1.6, 1.8, and 2.0 µL/tube		Chan et al. (2015)
Gene expression of *Gst D6*	*Drosophila melanogaster*	NA	+	2.0 µL/tube	On filter paper; 1.2, 1.4, 1.6, 1.8, and 2.0 µL/tube		Chan et al. (2015)

AP-1, activator protein-1; bw, body weight; Cdk1, cyclin-dependent kinase 1; *Gst D*, glutathione *S*-transferase delta; HO-1, haem oxygenase-1; HID, highest ineffective dose; IL, interleukin; LED, lowest effective dose; M, male; miRNA, microRNA; NA, not available; NR, not reported; TGF-β1, tumour growth factor beta 1; TNFα, tumour necrosis factor alpha, NF-κB, nuclear factor-kappa B.

[a] +, positive; (+), weakly positive.

lipid peroxidation (measured by malondialdehyde formation) and SOD activity in rat brain (Kakkar et al., 1992). Using male Sprague-Dawley rats as an experimental model, Khan and colleagues investigated the early biological effects of aniline-induced short-term or subchronic splenic toxicity after oral exposure. Dose-related increases in splenic iron concentration, malondialdehyde, and protein carbonyl content were reported in the spleen (Khan et al., 1997; Wang et al., 2010). Further studies demonstrated that the aniline metabolites phenylhydroxylamine and nitrosobenzene also produced these biological effects, but at much lower concentrations than did aniline (Khan et al., 1998, 2000). Khan et al. (2014) showed that an ethanol extract of *Dioscorea alata L.* (an antioxidant) significantly reduced aniline-induced effects on iron deposition, lipid peroxidation, reduced glutathione, and nitric oxide levels in the spleen in Wistar rats. Moreover, subchronic supplementation with protocatechuic acid (a natural phenolic compound) or with the combination of protocatechuic acid and ascorbic acid significantly ameliorated the effect on glutathione depletion by aniline, and reduced aniline-induced iron deposition, lipid peroxidation, and nitric oxide generation in the spleen of Wistar rats (Khairnar et al., 2016). [The Working Group noted that the protective effects of the antioxidants support the tenet that aniline-induced splenic effects occur through oxidative stress.]

Using proteomic approaches, Fan et al. (2011) showed that aniline exposure resulted in significantly increased formation of nitrated proteins in the spleen of rats. Furthermore, aniline exposure also led to significantly increased expression of inducible nitric oxide synthase mRNA and protein in the spleen. [The Working Group noted that aniline exposure induced nitrosative stress by generating reactive nitrogen species, which contributed to the increase in nitrated proteins.]

Early studies in vitro suggested that aniline depleted the cellular glutathione pool in liver microsomes (Aikawa et al., 1978). In a study in cultured primary rat hepatocytes in vitro, Wang et al. (2016) demonstrated that aniline exposure significantly increased the levels of ROS and malondialdehyde; and significantly decreased the levels of glutathione, catalase, SOD activity, and mitochondrial membrane potential, and caused DNA damage. The addition of *N*-acetyl-l-cysteine, an ROS scavenger, significantly reduced the adverse effects.

(iii) Gene expression responses related to the key characteristics of carcinogens

See Table 4.7.

Acute exposure to aniline induces methaemoglobinaemia, haemolytic anaemia, and haemolysis. The damaged erythrocytes may be scavenged by the spleen and the resulting splenotoxicity may be associated with the release of iron, oxidative and nitrosative stress, and induction of oxidative stress-related gene expression. In male Sprague-Dawley rats, short-term (7 days) exposure to aniline led to upregulation of transforming growth factor-beta 1 (TGF-β1 gene) (Khan et al., 2003b, 2006); activation of transcription factor activator protein-1 (AP-1) and mitogen-activated protein kinases (Khan et al., 2006). The short-term exposure also activated both redox-sensitive transcription factors, nuclear factors NF-κB and AP-1; upregulated fibrogenic cytokines (interleukins IL1 and IL6, and tumour necrosis factor alpha, TNFα) (Wang et al., 2008); and significantly increased levels of free iron, total iron, or ferritin, haem oxygenase mRNA and protein levels in rat spleen (Wang et al., 2010). Subsequently, 30-day studies in male rats confirmed that aniline exposure caused the increased expression of IL1α, IL6, and TNFα at both mRNA and protein levels; and activation of NF-κB (Wang et al., 2005). Such exposure also enhanced expression of cyclins (D1, D2, D3, E) and cyclin-dependent kinases (CDKs), and overexpression of cell proliferation marker proteins nuclear Ki67 and mini-chromosome

maintenance MCM2 protein (Wang et al., 2011). Moreover, subchronic exposure of rats to aniline resulted in a significant increase in the expression of cyclins and CDK1, and aberrant regulation of microRNAs (miRNAs), which led to accelerated G2/M transition of the splenocytes, and potentially to a tumorigenic response (Wang et al., 2015, 2017). Thus, oxidative stress leads to transcriptional upregulation of fibrogenic/inflammatory factors (cytokines IL1, IL6, and TNFα) through the activation of NF-κB, AP-1, and other redox-sensitive transcription factors. In addition, Chan et al. (2015) reported that aniline exposure increased glutathione transferase delta gene expression in *Drosophila melanogaster* in a dose-related manner (see also Section 4.2.5).

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Male Wistar rats given feed containing aniline (0.03–0.12%) for 80 weeks did not present hyperplasia in the urinary bladder (Hagiwara et al., 1980). Male and female Fischer 344 rats given feed containing aniline hydrochloride at 0.3% or 0.6% for 103 weeks showed fibrosis and papillary hyperplasia of the spleen, as well as endometrial stromal polyps (NCI, 1978). Stromal hyperplasia and fibrosis of the splenic red pulp, which may represent a precursor lesion of sarcoma, was also observed in male Fischer 344 rats treated with aniline at 100 mg/kg bw and, to a lesser degree, in female rats (US EPA, 1982).

Male Sprague-Dawley rats receiving drinking-water containing aniline hydrochloride at a dose of 65 mg/kg bw per day for 1, 2, or 3 months presented splenomegaly accompanied by morphological changes, including marked red pulp expansion due to prominently dilated splenic sinusoids and fibroblasts, vascular congestion, and splenic hyperplasia. Mitotic activity was not prominent (Khan et al., 1999).

In male Sprague-Dawley rats receiving drinking-water containing aniline hydrochloride (0.5 mmol/kg bw per day for 30 days) (dose that caused upregulation of pro-fibrogenic cytokines) resulted in increased spleen weight and increased splenocyte population (Wang et al., 2011). These findings were confirmed by a significant increase in the expression of splenic proteins that are considered markers of cell proliferation: proliferating cell nuclear antigen (PCNA), nuclear Ki67 protein and minichromosome maintenance 2 (MCM2) protein (Wang et al., 2011). The associated molecular mechanisms were: (i) the increased protein expression of cell cycle regulators, such as cyclins (A, B1, D1, D2, D3, and E), cyclin-dependent kinases (CDK1, CDK2, CDK4, and CDK6), and phosphorylated retinoblastoma protein (pRb-p) (Wang et al., 2011, 2015); and (ii) downregulation of CDK inhibitors p21 and p27 at protein and mRNA levels (Wang et al., 2015). In experimental conditions that precede fibrogenic responses in rats (given drinking-water containing aniline at 1 mmol/kg bw per day, for 7 days), activation of mitogen-activated protein kinases (MAPKs) (Wang et al., 2008) and increased protein expression of cyclins (A, B1, D3, and E) and CDKs (CDK1, CDK2, CDK4, and CDK6) (Wang et al., 2017) were observed.

4.2.5 Evidence relevant to other key characteristics of carcinogens

Experimental evidence related to other key characteristics of carcinogens is described below, including whether aniline or aniline hydrochloride: alters DNA repair; induces epigenetic alterations; induces chronic inflammation; is immunosuppressive; modulates receptor-mediated effects; and causes immortalization.

As noted above (see Section 4.2.3(b), rats given drinking-water containing aniline hydrochloride at a dose of 0.5 mmol/kg bw per day

for 30 days presented increased expression and activity of BER enzymes in the spleen, including Ogg1, Neil1/2, Nth1, and Ape1 (Ma et al., 2008, 2011, 2013).

Decreased expression of miRNAs that may regulate the expression of cyclins and increased expression of miRNAs that may regulate the expression of CDKs were also observed in rats exposed to aniline hydrochloride (Wang et al., 2015, 2017).

In male Fischer rats given feed containing aniline hydrochloride (100 mg/kg bw per day) for 4 weeks there was induction of focal perisplenitis (Mellert et al., 2004). In Sprague-Dawley rats given aniline hydrochloride at a dose that elicits a fibrogenic response (in drinking-water, 0.5 mmol/kg bw per day, for 30 days) there was overexpression (both at mRNA and protein levels) of three cytokines (interleukin IL1α, IL6, and TNFα) through the activation of NF-κB (Wang et al., 2005). Administration of aniline under experimental conditions that precede fibrogenic responses in rats (in drinking-water, 1 mmol/kg bw per day, for 7 days) resulted in activation of NF-κB and AP-1, phosphorylation of IκB kinase (IKK) and upregulation of pro-inflammatory and pro-fibrogenic cytokines in the spleen (Wang et al., 2008). Aniline hydrochloride (0.1–10 μM) inhibited α/β interferon induction in mouse embryo fibroblast cell cultures (Sonnenfeld, 1983). There was a decrease in interferon induction when 3.25 mg of aniline hydrochloride were injected intraperitoneally into mice 24 hours after interferon induction (Sonnenfeld & Hudgens, 1983). [The Working Group noted that the effects of aniline hydrochloride on interferon inhibition were more modest than the effects of 4-aminobiphenyl.]

Increased levels of progesterone, 17α-hydroxyprogesterone, and testosterone were reported in human adrenocortical carcinoma cell lines exposed for 48 hours with different concentration of aniline (0.0001–1000 μM) (Holm et al., 2015). Repeated daily subcutaneous administration of aniline to rats caused significant adrenal enlargement and increase in lipid accumulation in corpora lutea and adrenal cortex (Kovacs et al., 1970, 1971; Hatakeyama et al., 1971; Horvath et al., 1971). [The Working Group noted that these studies are in general descriptive.] Plasma corticosterone levels were decreased 24 hours after a single subcutaneous dose of aniline (30 mg) (Toth et al., 1971).

Aniline induced cell transformation in rodent cells in some studies. Aniline at doses of 485, 544, and 908 μg/mL gave positive results in the Syrian hamster embryo cell transformation assay (Plöttner et al., 2013). Aniline at 0.8 μg/mL induced cell transformation in mouse BALB/C3T3 cells (Dunkel et al., 1981). The results were negative in Fischer rat embryo cells with aniline at 0.001 μg/mL (Price & Mishra, 1980) and in Syrian hamster embryo cells at 5 μg/mL (Dunkel et al., 1981), and at up to 10 μg/mL (Pienta, 1980), and in Syrian hamster kidney cells (BHK21) at up to 250 μg/mL (Purchase et al., 1978). [The Working Group noted that Fischer rat embryo cells are not metabolically competent.]

4.3 Data relevant to comparisons across agents and end-points

The analysis of the in vitro bioactivity of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018).

Aniline and aniline hydrochloride were among thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 26 April 2020. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). [The Working Group noted that the metabolic

capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 235 assays in which aniline was tested, it was found to be inactive in all assays related to key characteristics of carcinogens (US EPA, 2019a). Among the 432 assays in which aniline hydrochloride was tested (at concentrations up to 100 µM), it was found to be active in 9 assays (US EPA, 2019b).

An effect on upregulation of nuclear receptor subfamily 1, group H, member 4 (NR1H4) was reported in the human kidney cell line, HEK293T, at a half-maximal activity concentration (AC_{50}) of 39.2 µM. Thyroid peroxidase inhibition was observed in a rat thyroid gland cell line at an AC_{50} of 0.592 µM and in a porcine thyroid gland cell line at an AC_{50} of 15.3 µM.

Borderline activity was reported for several assays. These included the activation of the receptors estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2), retinoic acid receptor α (RARA), androgen receptor (AR), and peroxisome proliferator activated receptor α (PPARα) in different cell lines and increase in NaCl co-transporter (NCCT) assay in *E. coli*. [The Working Group considered the relevance of these end-points to the key characteristics of carcinogens to be unclear.]

4.4 Other relevant evidence

4.4.1 Humans

No data were available to the Working Group, other than on methaemoglobinaemia (see Section 4.1).

4.4.2 Experimental systems

A histopathological review of rat spleens from a bioassay with aniline hydrochloride from the National Cancer Institute (NCI, 1978) reported an increased incidence of splenotoxic changes including fatty metamorphosis, fibrosis, capsule hyperplasia, and haemorrhage in male and female Fischer 344 rats treated with aniline hydrochloride (0.6% in feed, for 103 weeks) (Weinberger et al., 1985). In another bioassay, fatty metamorphosis of splenic red pulp and haematological alterations were observed in male and female Fischer 344 rats given feed containing aniline hydrochloride, as was an increase in the spleen weight, associated with vascular congestion (US EPA, 1982).

In several short-term studies in rats, a correlation was observed between haematopoietic toxicity of aniline (leading to methaemoglobinaemia and iron deposition in spleen) and splenotoxicity (Khan et al., 1997, 1999; Mellert et al., 2004; see also Section 4.2.3(b)).

Aniline hydrochloride induced an increase in spleen weight, associated with vascular congestion, when fed to male Fischer rats for 1 or 4 weeks at a dietary dose of 30 or 100 mg/kg bw (actual intake, ≥ 17 and ≥ 57 mg/kg bw, respectively), and induced hypercellularity of the bone marrow, predominantly of the erythropoietic cell line, at 100 mg/kg bw after 4 weeks. Other observations included regenerative toxic haemolytic anaemia (lower erythrocyte, reduced haemoglobin, and reduced haematocrit values) and alteration in erythrocyte and leukocyte parameters such as: polychromasia, hyperchromasia, normoblasts, higher leukocyte values, anisocytosis, and higher polymorphonuclear neutrophil values. In addition, increased transferrin concentration and total iron binding capacity and haemosiderin deposition in Kupffer cells of the liver were observed (Mellert et al., 2004).

5. Summary of Data Reported

5.1 Exposure characterization

Aniline, the parent compound of aniline hydrochloride, is a basic compound and will undergo acid–base reactions. Aniline and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

Aniline is primarily produced by catalytic reduction of nitrobenzene. Aniline hydrochloride is prepared by reacting aniline vapour and hydrogen chloride gas.

Almost no information was found on occurrence, use, and exposure to aniline hydrochloride.

Aniline is a High Production Volume chemical. In the chemical industry, aniline is used for the synthesis of many compounds, including isocyanates, dyes and pigments, antioxidants and accelerators in rubber processing, pharmaceuticals, varnishes, perfumes, photographic chemicals, herbicides, and fungicides. Northeastern Asia was the largest producer of aniline during 2013–2018, accounting for more than half of the world's production of aniline, followed by western Europe and the USA.

The general population may be exposed to aniline from the release of industrial effluents in the environment to air, water, land, or groundwater. Aniline has been detected in drinking-water in several well-conducted studies in North America and Europe. On the basis of limited recent data, food does not appear to be a significant source of aniline at present. Aniline is used as an intermediate for the production of pharmaceuticals and in many consumer products, such as fabrics, textiles and apparel, leather, paper, plastic, and colourants, including tattoo ink. Cigarette smoking is one of the main contributors of aniline in non-occupational environments. Most measurements showed that the levels of aniline in sidestream tobacco smoke were considerably higher than those in mainstream tobacco smoke.

The main scenarios for occupational exposure to aniline are during production and distribution of aniline, production of other chemicals and products for which aniline is used as a chemical intermediate, and by handling and using products containing residual aniline. Production of 4,4-methylene diphenyl diisocyanate (mainly used in the production of rigid polyurethane) accounts for more than 90% of aniline use, followed by production of rubber-processing chemicals, and to a minor extent, dyes prepared from aniline derivatives. In many occupational settings, exposure to aniline co-occurs with exposure to other chemicals that are known bladder carcinogens. Despite the widespread use of aniline, exposure data is scarce for all industries and scenarios where there is a significant potential for aniline exposure. Time-weighted average occupational exposure limits for aniline have been established in several countries.

Although inhalation and skin exposures to aniline occur, only limited biomonitoring data for aniline have been reported for either occupational settings (rubber-chemical manufacturing workers and aniline-production workers) or the general population (mostly analysed separately for smokers and non-smokers).

5.2 Cancer in humans

The body of research available related to cancers in humans was sparse and was limited to four cohort studies in aromatic amine dye- and rubber-chemical manufacturing plants, four population-based case–control studies, and several case reports and case series of bladder cancer in occupational settings. All cohort studies evaluated bladder cancer outcomes, but only two were considered to be potentially informative for the evaluation. Both studies were of good quality, but for one it was not possible to separate any aniline-specific effect from the effect of other co-exposures such as *ortho*-toluidine. The other cohort study evaluated the association

between aniline exposure and bladder cancer while controlling for concurrent exposures; however, the small sample size and strong correlations between the exposures resulted in statistically unstable estimates of the effect for aniline and confounding from co-exposures to other agents with *sufficient* and *limited* evidence of bladder carcinogenicity in humans (*ortho*-toluidine and 2-mercaptobenzothiazole, respectively) could not be ruled out. One case–control study on bladder cancer did not provide information on case or control ascertainment, aniline exposure, or occupational co-exposures. Among the case reports and case series, confounding from co-exposures to occupational bladder carcinogens and/or tobacco smoking could not be ruled out. Overall, the available studies do not permit a conclusion to be drawn about the presence of a causal association between aniline exposure and bladder cancer.

There was no convincing or consistent evidence reported for any other cancer in humans.

5.3 Cancer in experimental animals

Aniline hydrochloride caused an increase in the incidence of malignant neoplasms in two independent studies in one species.

In one independent study in male and female Fischer 344 rats, aniline hydrochloride administered orally (in feed) caused an increase in the incidence of fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) of the spleen; haemangiosarcoma of the spleen; fibrosarcoma or sarcoma NOS (combined) of multiple organs other than spleen within the body cavities; fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined); and haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) in male rats. A positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland was also observed in male rats receiving aniline hydrochloride. A positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) was observed in female rats receiving aniline hydrochloride.

In another independent study in male Fischer 344 rats, aniline hydrochloride administered orally (in feed) caused an increase in the incidence of stromal sarcoma of the spleen, haemangiosarcoma of the spleen, and mesothelioma of the tunica vaginalis of the testis.

5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of aniline, data are available from studies in humans, and from experimental systems. In humans, aniline is readily absorbed by the dermal, oral and inhalation routes. Studies of aniline-induced methaemoglobinaemia, dating back to the 1800s, indicate skin absorption. Facile oral absorption of aniline was demonstrated in a recent thorough study of aniline metabolism in human subjects. Cytochrome P450 (CYP)-dependent hydroxylation to *para*-aminophenol [4-aminophenol] followed by N-acetylation (or the reverse sequence) converts aniline into *N*-acetyl-*para*-aminophenol (the chemical name for the analgesic drug paracetamol, also known as acetaminophen). *N*-Acetyl-*para*-aminophenol, acetanilide, and the parent compound were detected in the plasma and urine. More than half of an orally administered dose of aniline is excreted in the urine as *N*-acetyl-*para*-aminophenol and its conjugates (glucuronide, sulfate, mercapturic acid). The elimination half-lives of these urinary metabolites range from less than 1 hour to a few hours. *N*-Acetyl-*para*-aminophenol is found in urine samples from the general population, from individuals exposed to aniline in an occupational setting, and from paracetamol users. Multiple

studies of aniline metabolism in experimental animals, including rat, mouse, rabbit, guinea-pig, gerbil, hamster, cat, dog, pig, and sheep, are consistent with the evidence in humans.

There is consistent and coherent evidence in experimental systems that aniline exhibits multiple key characteristics of carcinogens. Aniline is metabolically activated to electrophiles. In exposed humans, aniline forms haemoglobin adducts, which are commonly used as biomarkers of aniline exposure. No data on DNA adducts in humans were available. In experimental systems, binding of aniline to DNA has been observed, including in the liver, spleen, and kidney of rats treated with aniline. Although not directly demonstrated at every step, there is a plausible pathway for formation of aniline–DNA adducts that parallels an established paradigm for aromatic amines. This bioactivation pathway begins with CYP-catalysed N-hydroxylation to phenylhydroxylamine; further activation by O-acetylation; and spontaneous heterolysis of the *N*-acetoxy metabolite to give a DNA-reactive electrophilic nitrenium ion. The presence of this pathway is supported by evidence showing N-hydroxylation of aniline to phenylhydroxylamine in dogs and rats; in liver microsomal preparations from rat, mouse, and rabbit; and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the oxidized metabolites by binding to haemoglobin. The *N*-acetoxy derivative of aniline has been prepared synthetically; it reacts with DNA to give a guanine C8 adduct as well as guanine *N*7 and N^2, and adenine C2, C8, *N*7, and N^6 adducts.

Aniline is also genotoxic. No data were available in exposed humans, and only one study, with positive results for sister-chromatid exchange, was conducted in human primary cells. DNA damage was seen in human cell lines in vitro, and in both rats and mice. Aniline was consistently clastogenic, with dose-dependent increases in the frequency of micronucleus formation in orally exposed rats and mice, and the induction of chromosomal aberrations, micronucleus formation, and sister-chromatid exchange in mammalian cells. Aniline was mutagenic in the mouse lymphoma L5178Y cell $Tk^{+/-}$ assay and in the Chinese hamster V79 *Hprt* assay. In experiments with bacteria, aniline neither formed DNA adducts nor caused DNA damage, and it did not cause gene mutations in standard *Salmonella typhimurium* or *Escherichia coli* assays.

In addition, aniline induces oxidative stress. Aniline induces 8-hydroxy-2′-deoxyguanosine and upregulates DNA base-excision repair proteins in rats. In rodent studies in vivo and in vitro, aniline exposure variously increased reactive oxygen and reactive nitrogen species, depleted glutathione, and increased malondialdehyde and protein carbonyl contents.

Aniline alters cell proliferation, cell death, or nutrient supply. Hyperplasia of the spleen was seen in male and female Fischer 344 rats after chronic exposure and in male Sprague-Dawley rats after short-term exposure. After short-term exposure, aniline increased markers of cell proliferation, including proliferating cell nuclear antigen and nuclear Ki67 protein in rats.

Aniline or its hydrochloride form was mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

Overall, the evidence is consistent and coherent that aniline belongs, on the basis of mechanistic considerations, to a class of aromatic amines. Members of this class, including 4-aminobiphenyl (*para*-phenylaniline), 2-naphthylamine, and *ortho*-toluidine (*ortho*-methylaniline) have been classified as *carcinogenic to humans* (IARC Group 1) by the *IARC Monographs* programme. Aniline is structurally similar to these aromatic amines. It also bears similarity with respect to the mechanism of bioactivation to electrophiles, its genotoxicity, and the target organs of carcinogenicity in chronic animal bioassays. For instance, both aniline and *ortho*-toluidine cause malignant

tumours of the spleen and mesothelioma of the tunica vaginalis of the testis when administered orally to male Fischer 344 rats. Therefore, these mechanistic considerations go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of aniline and aniline hydrochloride.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that aniline belongs, based on mechanistic considerations, to a class of aromatic amines for which several members have been classified as carcinogenic to humans. There is also *strong evidence* that aniline exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

Aniline and aniline hydrochloride are *probably carcinogenic to humans (Group 2A).*

6.5 Rationale

The *Group 2A* evaluation is based on *strong* mechanistic evidence that aniline, on the basis of mechanistic considerations, belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans. Aniline is concordant with other agents in this class with respect to its bioactivation mechanism to electrophiles, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays.

There was also *sufficient evidence* of carcinogenicity in experimental animals, on the basis of increased incidence of malignant neoplasms in two independent studies in one species. In addition, there is *strong evidence* that aniline exhibits key characteristics of carcinogens in experimental systems. Aniline is metabolically activated to electrophiles, it is genotoxic, it induces oxidative stress, and it alters cell proliferation, cell death, or nutrient supply.

The evidence for cancer in humans was *inadequate* because the effects of aniline in workers could not be distinguished from those of co-exposures to other occupational bladder carcinogens in the two available high-quality cohort studies.

Aniline hydrochloride exists in equilibrium with aniline; therefore, the classification of carcinogenic hazard applies to both aniline and its hydrochloride form.

References

Abe S, Sasaki M (1977). Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J Natl Cancer Inst.* 58(6):1635–41. doi:10.1093/jnci/58.6.1635 PMID:864744

Abe Y, Yamaguchi M, Mutsuga M, Akiyama H, Kawamura Y (2016). Survey of primary aromatic amines and colorants in polyurethane, nylon and textile toys. *Shokuhin Eiseigaku Zasshi.* 57(2):23–31. doi:10.3358/shokueishi.57.23 PMID:27211915

Aboulker P, Smagghe G (1953). [Tumours of the bladder in workers exposed to dyes; report of 21 cases and note on choice of method for screening]. *Arch Mal Prof.* 14(4):380–6. [French] PMID:13115009

ACGIH (2018). American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA:

American Conference of Governmental Industrial Hygienists.

Aikawa K, Satoh T, Kobayashi K, Kitagawa H (1978). Glutathione depletion by aniline analogs in vitro associated with liver microsomal cytochrome P-450. *Jpn J Pharmacol*. 28(5):699–705. doi:10.1254/jjp.28.699 PMID:722999

Albrecht W, Neumann HG (1985). Biomonitoring of aniline and nitrobenzene. Hemoglobin binding in rats and analysis of adducts. *Arch Toxicol*. 57(1):1–5. doi:10.1007/BF00286566 PMID:4015392

Alguacil J, Kauppinen T, Porta M, Partanen T, Malats N, Kogevinas M, et al.; PANKRAS II Study Group (2000). Risk of pancreatic cancer and occupational exposures in Spain. *Ann Occup Hyg*. 44(5):391–403. doi:10.1016/S0003-4878(99)00119-2 PMID:10930502

Amacher DE, Paillet SC, Turner GN, Ray VA, Salsburg DS (1980). Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. *Mutat Res*. 72(3):447–74. doi:10.1016/0027-5107(80)90118-9 PMID:7453708

American Cancer Society (2020). Cancer facts and figure 2020. Atlanta (GA), USA: American Cancer Society. Available from: https://www.cancer.org/cancer/bladder-cancer/detection-diagnosis-staging/survival-rates.html.

American Chemistry Council (2020). Introduction to polyurethanes: flexible polyurethane foam. Available from: https://polyurethane.americanchemistry.com/Flexible-Polyurethane-Foam/, accessed 4 March 2021.

Amini B, Lowenkron S (2003). Aniline and its derivatives. In: Kirk-Othmer encyclopaedia of chemical technology. Wiley; p. 783.

Anon. (1904). Aniline dyes and tumours of the bladder. *Hospital (Lond 1886)*. 36(936):390. PMID:29817271

Anon. (1921). Tumour of the bladder among workers in aniline factories. *BMJ*. 1(3148):645–6. doi:10.1136/bmj.1.3148.645-a PMID:20770280

ASEAN (2019). Cosmetic Documents, Annexes II Part 1 and IV Part 1. Annexes of the ASEAN Cosmetic Directive, Version No.: 2019-01 15 July 2019. Available from: https://www.hsa.gov.sg/docs/default-source/hprg-cosmetics/annexes-of-the-asean-cosmetic-directive-(updated-july-2019).pdf, accessed 4 March 2021.

Ashby J, Lefevre PA, Callander RD (1983). The possible role of azoreduction in the bacterial mutagenicity of 4-dimethylaminoazobenzene (DAB) and 2 of its analogues (6BT and 5I). *Mutat Res*. 116(3–4):271–9. doi:10.1016/0165-1218(83)90064-2 PMID:6403852

Ashby J, Trueman RW, Styles J, Penman MG, Paton D (1981). 4-Chloromethylbiphenyl (4CMB): a novel mutagen and potential carcinogen. *Carcinogenesis*. 2(1):33–8. doi:10.1093/carcin/2.1.33 PMID:7023721

Ashby J, Vlachos DA, Tinwell H (1991). Activity of aniline in the mouse bone marrow micronucleus assay. *Mutat Res*. 263(2):115–7. doi:10.1016/0165-7992(91)90068-F PMID:2046703

Assmann N, Emmrich M, Kampf G, Kaiser M (1997). Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship. *Mutat Res*. 395(2–3):139–44. doi:10.1016/S1383-5718(97)00158-7 PMID:9465924

ATSDR (2002). Aniline. Division of Toxicology ToxFAQsTM. Atlanta (GA), USA: Agency for Toxic Substances and Disease Registry, United States Department of Health and Human Services. Available from: https://www.atsdr.cdc.gov/toxfaqs/tfacts171.pdf, accessed 4 March 2021.

Baranowska-Dutkeiwicz B (1982). Skin absorption of aniline from aqueous solutions in man. *Toxicol Lett*. 10(4):367–72. doi:10.1016/0378-4274(82)90231-4 PMID:7089990

Berenblum I (1932). Aniline cancer. *The Cancer Review*. 7(7–8):337–55.

Beyerbach A, Rothman N, Bhatnagar VK, Kashyap R, Sabbioni G (2006). Hemoglobin adducts in workers exposed to benzidine and azo dyes. *Carcinogenesis*. 27(8):1600–6. doi:10.1093/carcin/bgi362 PMID:16497705

Birner G, Neumann HG (1988). Biomonitoring of aromatic amines II: Hemoglobin binding of some monocyclic aromatic amines. *Arch Toxicol*. 62(2–3):110–5. doi:10.1007/BF00570128 PMID:3196145

Bomhard EM (2003). High-dose clastogenic activity of aniline in the rat bone marrow and its relationship to the carcinogenicity in the spleen of rats. *Arch Toxicol*. 77(5):291–7. doi:10.1007/s00204-003-0443-1 PMID:12734644

Boyland E, Manson D, Nery R (1963). The biochemistry of aromatic amines. 9. Mercapturic acids as metabolites of aniline and 2-naphthylamine. *Biochem J*. 86(2):263–71. doi:10.1042/bj0860263 PMID:14014729

Boyland E, Manson D, Orr SF (1957). The biochemistry of aromatic amines. II. The conversion of arylamines into arylsulphamic acids and arylamine-*N*-glucosiduronic acids. *Biochem J*. 65(3):417–23. doi:10.1042/bj0650417a PMID:13412641

Brambilla G, Carlo P, Finollo R, Ledda A (1985). Viscometric detection of liver DNA fragmentation in rats treated with ten aromatic amines. Discrepancies with results provided by the alkaline elution technique. *Carcinogenesis*. 6(9):1285–8. doi:10.1093/carcin/6.9.1285 PMID:4028326

Brams A, Buchet JP, Crutzen-Fayt MC, De Meester C, Lauwerys R, Léonard A (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). *Toxicol Lett*. 38(1–2):123–33. doi:10.1016/0378-4274(87)90120-2 PMID:3307023

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality

worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 68(6):394–424. doi:10.3322/caac.21492 PMID:30207593

Brede C, Skjevrak I (2004). Migration of aniline from polyamide cooking utensils into food simulants. *Food Addit Contam*. 21(11):1115–24. doi:10.1080/02652030400019349 PMID:15764341

Brennan RJ, Schiestl RH (1997). Aniline and its metabolites generate free radicals in yeast. *Mutagenesis*. 12(4):215–20. doi:10.1093/mutage/12.4.215 PMID:9237764

Bul'bulian MA (1991). [An epidemiological study of the cancer morbidity in persons having industrial contact with carcinogenic amino compounds]. *Vopr Onkol*. 37(3):275–9. [Russian] PMID:2031321

Bul'bulian MA, Goldfarb AN (1991). [Retrospective analysis of carcinogenic hazards of beta naphthylamine and benzidine production as a stage of an epidemiological study of tumors in workers engaged in aniline dye industry]. *Gig Tr Prof Zabol*. (7):39–41. [Russian] PMID:1916415

Bulbulyan MA, Figgs LW, Zahm SH, Savitskaya T, Goldfarb A, Astashevsky S, et al. (1995). Cancer incidence and mortality among beta-naphthylamine and benzidine dye workers in Moscow. *Int J Epidemiol*. 24(2):266–75. doi:10.1093/ije/24.2.266 PMID:7635585

Burke DA, Wedd DJ, Herriott D, Bayliss MK, Spalding DJM, Wilcox P (1994). Evaluation of pyrazole and ethanol induced S9 fraction in bacterial mutagenicity testing. *Mutagenesis*. 9(1):23–9. doi:10.1093/mutage/9.1.23 PMID:8208127

Burstyn JN, Iskandar M, Brady JF, Fukuto JM, Cho AK (1991). Comparative studies of *N*-hydroxylation and *N*-demethylation by microsomal cytochrome P-450. *Chem Res Toxicol*. 4(1):70–6. doi:10.1021/tx00019a009 PMID:1912302

Butterworth BE, Smith-Oliver T, Earle L, Loury DJ, White RD, Doolittle DJ, et al. (1989). Use of primary cultures of human hepatocytes in toxicology studies. *Cancer Res*. 49(5):1075–84. PMID:2917345

Cao XL, Zhu J, Macdonald S, Lalonde K, Dabeka B, Cisse M (2009). Aniline in vegetable and fruit samples from the Canadian Total Diet Study. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 26(6):808–13. doi:10.1080/02652030902759046 PMID:19680954

Carpenter CP, Smyth HF Jr, Pozzani UC (1949). The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. *J Ind Hyg Toxicol*. 31(6):343–6. PMID:15407114

Carreón T, Hein MJ, Hanley KW, Viet SM, Ruder AM (2014). Bladder cancer incidence among workers exposed to *o*-toluidine, aniline and nitrobenzene at a rubber chemical manufacturing plant. *Occup Environ Med*. 71(3):175–82. doi:10.1136/oemed-2013-101873 PMID:24368697

Carreón T, Hein MJ, Viet SM, Hanley KW, Ruder AM, Ward EM (2010). Increased bladder cancer risk among workers exposed to *o*-toluidine and aniline: a reanalysis. *Occup Environ Med*. 67(5):348–50. PMID:19884651

Case RA, Hosker ME, McDonald DB, Pearson JT (1954). Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. I. The role of aniline, benzidine, alpha-naphthylamine, and beta-naphthylamine. *Br J Ind Med*. 11(2):75–104. doi:10.1136/oem.11.2.75 PMID:13149741

Case RA, Pearson JT (1954). Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. II. Further consideration of the role of aniline and of the manufacture of auramine and magenta (fuchsine) as possible causative agents. *Br J Ind Med*. 11(3):213–6. doi:10.1136/oem.11.3.213 PMID:13182161

Caspary WJ, Daston DS, Myhr BC, Mitchell AD, Rudd CJ, Lee PS (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: interlaboratory reproducibility and assessment. *Environ Mol Mutagen*. 12(Suppl 13):195–229. doi:10.1002/em.2860120506 PMID:3416840

CDC (2011). Aniline. Estimated numbers of employees potentially exposed to specific agents by occupation. National Occupational Exposure Survey (1981–1983). National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Available from: https://web.archive.org/web/20111028105325/http:/www.cdc.gov/noes/noes2/06580occ.html, accessed 4 March 2021.

Cesarone CF, Bolognesi C, Santi L (1982). Evaluation of damage to DNA after in vivo exposure to different classes of chemicals. *Arch Toxicol Suppl*. 5:355–9. doi:10.1007/978-3-642-68511-8_66

Cha MG, Kim H-M, Kang Y-L, Lee M, Kang H, Kim J, et al. (2017). Thin silica shell coated Ag assembled nanostructures for expanding generality of SERS analytes. *PLoS One*. 12(6):e0178651. doi:10.1371/journal.pone.0178651 PMID:28570633

Chan WC, Chien YC, Chien CI (2015). Aniline exposure associated with up-regulated transcriptional responses of three glutathione *S*-transferase delta genes in *Drosophila melanogaster*. *Environ Toxicol Pharmacol*. 39(2):622–7. doi:10.1016/j.etap.2014.12.023 PMID:25682008

ChemView (2016). Benzenamine. National aggregate production volumes. Chemical Data Reporting [online database]. Chemical Safety and Pollution Prevention. United States Environmental Protection Agency. Available from: https://chemview.epa.gov/chemview.

Chen X, Zhao Y, Jin M (2015). [Determination of aniline in whole blood by using solid-phase extraction and high-performance liquid chromatography with fluorescence detector]. *Wei Sheng Yan Jiu*. 44(2):299–302. [Chinese] PMID:25997238

Chung KT, Fulk GE, Andrews AW (1981). Mutagenicity testing of some commonly used dyes. *Appl Environ Microbiol*. 42(4):641–8. doi:10.1128/AEM.42.4.641-648.1981 PMID:7039509

Chung KT, Murdock CA, Stevens SE Jr, Li YS, Wei CI, Huang TS, et al. (1995). Mutagenicity and toxicity studies of *p*-phenylenediamine and its derivatives. *Toxicol Lett*. 81(1):23–32. doi:10.1016/0378-4274(95)03404-8 PMID:8525495

Chung KT, Murdock CA, Zhou Y, Stevens SE Jr, Li YS, Wei CI, et al. (1996). Effects of the nitro-group on the mutagenicity and toxicity of some benzamines. *Environ Mol Mutagen*. 27(1):67–74. doi:10.1002/(SICI)1098-2280(1996)27:1<67::AID-EM9>3.0.CO;2-B PMID:8625950

Creech JL Jr, Johnson MN (1974). Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J Occup Med*. 16(3):150–1. PMID:4856325

Cumberbatch MGK, Jubber I, Black PC, Esperto F, Figueroa JD, Kamat AM, et al. (2018). Epidemiology of bladder cancer: a systematic review and contemporary update of risk factors in 2018. *Eur Urol*. 74(6):784–95. doi:10.1016/j.eururo.2018.09.001 PMID:30268659

Cunningham ML, Ringrose PS (1983). Benzo(a)pyrene and aniline increase sister chromatid exchanges in cultured rat liver fibroblasts without addition of activating enzymes. *Toxicol Lett*. 16(3–4):235–9. doi:10.1016/0378-4274(83)90182-0 PMID:6304944

Curshmann F (1920). *Z Gew-Hyg*. 8:145.

Dai Y, Rashba-Step J, Cederbaum AI (1993). Stable expression of human cytochrome P4502E1 in HepG2 cells: characterization of catalytic activities and production of reactive oxygen intermediates. *Biochemistry*. 32(27):6928–37. doi:10.1021/bi00078a017 PMID:7687464

DeBruin LS, Pawliszyn JB, Josephy PD (1999). Detection of monocyclic aromatic amines, possible mammary carcinogens, in human milk. *Chem Res Toxicol*. 12(1):78–82. doi:10.1021/tx980168m PMID:9894021

Delclos KB, Tarpley WG, Miller EC, Miller JA (1984). 4-aminoazobenzene and *N*,*N*-dimethyl-4-aminoazobenzene as equipotent hepatic carcinogens in male C57BL/6 X C3H/He F_1 mice and characterization of *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene as the major persistent hepatic DNA-bound dye in these mice. *Cancer Res*. 44(6):2540–50. PMID:6426782

Department of Ecology (2018). Children's safe products reporting rule. Rationale for reporting list of chemicals of high concern to children 2011–2017. November 2018. Publication 18-04-025. Washington (DC), USA: Washington State Department of Ecology. Available from: https://fortress.wa.gov/ecy/publications/documents/1804025.pdf#page=12, accessed 4 March 2021.

Department of Ecology (2020). Children's safe products reporting rule guidance. Washington (DC), USA: Washington State Department of Ecology. Available from: https://ecology.wa.gov/Regulations-Permits/Reporting-requirements/Reporting-for-Childrens-Safe-Products-Act, accessed 4 March 2021.

DFG (2018). List of MAK and BAT values 2018: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 54. Deutsche Forschungsgemeinschaft. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9783527818402, accessed January 2020.

Dierkes G, Weiss T, Modick H, Käfferlein HU, Brüning T, Koch HM (2014). *N*-Acetyl-4-aminophenol (paracetamol), *N*-acetyl-2-aminophenol and acetanilide in urine samples from the general population, individuals exposed to aniline and paracetamol users. *Int J Hyg Environ Health*. 217(4–5):592–9. doi:10.1016/j.ijheh.2013.11.005 PMID:24370547

Druckrey H (1950). Beiträge zur Pharmakologie cancerogener Substanzen. Versuche mit Anilin. Arch. exper. Path. *Pharmakol*. 210:137–158. [German]

Duke J (2004). Aniline (FDB003571). Dr. Duke's phytochemical and ethnobotanical databases. United States Department of Agriculture. Agricultural Research Service. Available from: https://foodb.ca/compounds/FDB003571, accessed 19 October 2020.

Dunkel VC, Pienta RJ, Sivak A, Traul KA (1981). Comparative neoplastic transformation responses of Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical compounds. *J Natl Cancer Inst*. 67(6):1303–12. PMID:6947113

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. (1984). Reproducibility of microbial mutagenicity assays: I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ Mutagen*. 6(Suppl 2):1–251. doi:10.1002/em.2860060702 PMID:6394312

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. (1985). Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen*. 7(Suppl 5):1–248. doi:10.1002/em.2860070902 PMID:3905369

Dutkiewicz T, Piotrowski J (1961). Experimental investigations on the quantitative estimation of aniline absorption in man. *Pure Appl Chem*. 3(1–2):319–24. doi:10.1351/pac196103010319

ECB (2004). Aniline. European Union Risk Assessment Report. First priority list, Vol. 50. Report No. EUR 21092 EN. European Chemicals Bureau, Joint Research Centre, European Commission. Available from: http://

echa.europa.eu/documents/10162/0abd36ad-53de-4b0f-b258-10cf90f90493.

ECHA (2017). Annex XV restriction report. Proposal for a restriction on substances in tattoo inks and permanent make up. Version 1.2, October 2017. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/documents/10162/0/restriction_axvrep_tattoo_inks_sps-012420-16_en.pdf/f8c09d52-1f42-9b9c-4a54-90e8c843d205.

ECHA (2019a). Aniline. Registered substance [online database]. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15333/5/2/4.

ECHA (2019b). Background document to the Opinion on the Annex XV dossier proposing restrictions on substances in tattoo inks and permanent make up. Available from: https://echa.europa.eu/documents/10162/23665416/rest_tattoo_inks_bd_final_12674_en.pdf.

ECHA (2020a). Aniline. Manufacture, use and exposure. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/15333.

ECHA (2020b). Substance information: benzenamine, reaction products with aniline hydrochloride and nitrobenzene. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.099.853.

ECHA (2020c). Aniline. Substance regulatory obligations. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/legislation-obligation/-/obligations/100.000.491. [Last updated 11 October 2020.]

EFSA (2015). Conclusion on the peer review of the pesticide risk assessment for the active substance buprofezin in light of confirmatory data. *EFSA J*. 13(8):4207. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4207doi:10.2903/j.efsa.2015.4207.

EFSA (2018). Conclusion on the peer review of the pesticide risk assessment of the active substance desmedipham. *EFSA J*. 16(1):5150. Available from: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2018.5150.

Eisele GR, Tsai SC, Payne J, Kyle B, Urso I (1985). The distribution of orally administered ^{14}C-aniline HCl in tissues of dairy cattle, swine and laying pullets. *J Anim Sci*. 61(6):1492–7. doi:10.2527/jas1985.6161492x PMID:4086399

el-Bayoumy K, Donahue JM, Hecht SS, Hoffmann D (1986). Identification and quantitative determination of aniline and toluidines in human urine. *Cancer Res*. 46(12 Pt. 1):6064–7. Available from: https://cancerres.aacrjournals.org/content/canres/46/12_Part_1/6064.full.pdf PMID:3779628

European Commission (2004). Aniline. CAS No: 62-53-3. EINECS No: 200-539-3. Summary risk assessment report. Special publication I.04.67. Available from: https://echa.europa.eu/documents/10162/462b7066-c639-4883-b384-3daf4ec88ded, accessed 4 March 2021.

European Commission (2016). SCOEL/REC/153 Aniline. Recommendation from the Scientific Committee on Occupational Exposure Limits. Scientific Committee on Occupational Exposure Limits. Available from: https://publications.europa.eu.

European Commission (2019). Second study to collect updated information for a limited number of chemical agents with a view to analyse the health, socio-economic and environmental impacts in connection with possible amendments of Directive 2004/37/EC. Final report. Luxembourg: Publications Office of the European Union, 2019. Available from: https://publications.europa.eu.

Eyer P, Ascherl M (1987). Reactions of *para*-substituted nitrosobenzenes with human hemoglobin. *Biol Chem Hoppe Seyler*. 368(3):285–94. doi:10.1515/bchm3.1987.368.1.285 PMID:3593541

Eyer P, Kampffmeyer H, Maister H, Rösch-Oehme E (1980). Biotransformation of nitrosobenzene, phenylhydroxylamine, and aniline in the isolated perfused rat liver. *Xenobiotica*. 10(7–8):499–516. doi:10.3109/00498258009033785 PMID:6893777

Famulok MBG, Boche G (1989). Formation of *N*-(deoxyguanosin-8-yl)aniline in the in vitro reaction of *N*-acetoxyaniline with deoxyguanosine and DNA. *Angew Chem Int Ed Engl*. 28(4):468–9. doi:10.1002/anie.198904681

Fan X, Wang J, Soman KV, Ansari GA, Khan MF (2011). Aniline-induced nitrosative stress in rat spleen: proteomic identification of nitrated proteins. *Toxicol Appl Pharmacol*. 255(1):103–12. doi:10.1016/j.taap.2011.06.005 PMID:21708182

Fassina G, Abbondandolo A, Mariani L, Taningher M, Parodi S (1990). Mutagenicity in V79 cells does not correlate with carcinogenity in small rodents for 12 aromatic amines. *J Toxicol Environ Health*. 29(1):109–30. doi:10.1080/15287399009531376 PMID:2299684

FDA (2019). Tattoos & permanent makeup: fact sheet. United States Food and Drug Administration. Available from: https://www.fda.gov/cosmetics/cosmetic-products/tattoos-permanent-makeup-fact-sheet, accessed 4 March 2021.

Feingold L, Savitz DA, John EM (1992). Use of a job-exposure matrix to evaluate parental occupation and childhood cancer. *Cancer Causes Control*. 3(2):161–9. doi:10.1007/BF00051656 PMID:1562706

Fluck ER, Poirier LA, Ruelius HW (1976). Evaluation of a DNA polymerase-deficient mutant of *E. coli* for the rapid detection of carcinogens. *Chem Biol Interact*. 15(3):219–31. doi:10.1016/0009-2797(76)90148-4 PMID:793736

Fritzenschaf H, Kohlpoth M, Rusche B, Schiffmann D (1993). Testing of known carcinogens and noncarcinogens in the Syrian hamster embryo (SHE) micronucleus test in vitro; correlations with in vivo micronucleus formation and cell transformation. *MutatRes.*319(1):47–53.doi:10.1016/0165-1218(93)90029-D PMID:7690458

Furukawa K, Hashimoto M, Kaneco S (2017). Optimization of analytical conditions for a rapid determination of aniline in environmental water by liquid chromatography/tandem mass spectrometry. *Anal Sci*. 33(10):1189–91. doi:10.2116/analsci.33.1189 PMID:28993595

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*. 10(Suppl 10):1–175. doi:10.1002/em.2850100502 PMID:3319609

Gan J, Skipper PL, Tannenbaum SR (2001). Oxidation of 2,6-dimethylaniline by recombinant human cytochrome P450s and human liver microsomes. *Chem Res Toxicol*. 14(6):672–7. doi:10.1021/tx000181i PMID:11409937

Garberg P, Akerblom E-L, Bolcsfoldi G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. *Mutat Res*. 203(3):155–76. doi:10.1016/0165-1161(88)90101-X PMID:2836728

Garner RC, Nutman CA (1977). Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA 1538. *Mutat Res*. 44(1):9–19. doi:10.1016/0027-5107(77)90110-5 PMID:331098

Gavaldà L, Porta M, Malats N, Piñol JL, Fernández E, Maguire A, et al. (1995). [Agreement between information supplied by the patient and a family member on medical history, consumption of tobacco, alcohol and coffee and diet in cancer of the exocrine pancreas and extrahepatic biliary tract]. *Gac Sanit*. 9(51):334–42. [Spanish] PMID:8666512

Gehrmann GH (1934). The carcinogenetic agent—chemistry and industrial aspects. *J Urol*. 31(2):126–137. doi:10.1016/s0022-5347(17)72399-3

Gehrmann GH (1936). Papilloma and carcinoma of the bladder in dye workers. *JAMA*. 107(18):1436–9. doi:10.1001/jama.1936.02770440012003

Gentile JM, Gentile GJ, Plewa MJ (1987). Mutagenicity of selected aniline derivatives to Salmonella following plant activation and mammalian hepatic activation. *Mutat Res*. 188(3):185–96. doi:10.1016/0165-1218(87)90088-7 PMID:3600686

George E, Andrews M, Westmoreland C (1990). Effects of azobenzene and aniline in the rodent bone marrow micronucleus test. *Carcinogenesis*. 11(9):1551–6. doi:10.1093/carcin/11.9.1551 PMID:2401044

Gladwin MT, Grubina R, Doyle MP (2009). The new chemical biology of nitrite reactions with hemoglobin: R-state catalysis, oxidative denitrosylation, and nitrite reductase/anhydrase. *Acc Chem Res*. 42(1):157–67. doi:10.1021/ar800089j PMID:18783254

GlobeNewswire (2020). Aniline market to reach USD 14.57 billion by 2027. Reports and data; 16 January 2020. GlobalNewswire. Available from: https://www.globenewswire.com/fr/news-release/2020/01/16/1971498/0/en/Aniline-Market-To-Reach-USD-14-57-Billion-By-2027-Reports-And-Data.html.

Goldblatt MW (1949). Vesical tumours induced by chemical compounds. *Br J Ind Med*. 6(2):65–81. PMID:18119364

Goniewicz ML, Czogała J (2005). Exposure of active and passive smokers to aromatic amines present in tobacco smoke. *Toxicol Mech Methods*. 15(3):235–45. doi:10.1080/15376520590945676 PMID:20021088

Government Canada (1994). Canadian Environmental Protection Act, Priority substances list assessment report: Aniline. Available from: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/aniline/aniline-eng.pdf.

Greve PA, Wegman RC (1975). [Determination and occurrence of aromatic amines and their derivatives in Dutch surface waterways]. *Schriftenr Ver Wasser Boden Lufthyg*. (46):59–80. [German] PMID:1233728

Gut I, Nedelcheva V, Soucek P, Stopka P, Vodicka P, Gelboin HV, et al. (1996). The role of CYP2E1 and 2B1 in metabolic activation of benzene derivatives. *Arch Toxicol*. 71(1–2):45–56. doi:10.1007/s002040050357 PMID:9010585

Hagiwara A, Arai M, Hirose M, Nakanowatari JI, Tsuda H, Ito N (1980). Chronic effects of norharman in rats treated with aniline. *Toxicol Lett*. 6(2):71–5. doi:10.1016/0378-4274(80)90170-8 PMID:7414622

Hanley KW, Viet SM, Hein MJ, Carreón T, Ruder AM (2012). Exposure to *o*-toluidine, aniline, and nitrobenzene in a rubber chemical manufacturing plant: a retrospective exposure assessment update. *J Occup Environ Hyg*. 9(8):478–90. doi:10.1080/15459624.2012.693836 PMID:22708702

Hansen PL, Tønning K, Malmgren-Hansen B, Jacobsen E (2008). Survey and health assessment of chemical substances in hobby products for children. Survey of Chemical Substances in Consumer Products, No. 93. Danish Technological Institute, Danish Environmental Protection Agency. Available from: https://www2.mst.dk/udgiv/publications/2008/978-87-7052-763-7/pdf/978-87-7052-764-4.pdf.

Harper BL, Ramanujam VMS, Gad-El-Karim MM, Legator MS (1984). The influence of simple aromatics on benzene clastogenicity. *Mutat Res*. 128(2):105–14. doi:10.1016/0027-5107(84)90097-6 PMID:6472309

Harrison JH Jr, Jollow DJ (1987). Contribution of aniline metabolites to aniline-induced methemoglobinemia. *Mol Pharmacol*. 32(3):423–31. PMID:3670278

Hartman JH, Knott K, Miller GP (2014). CYP2E1 hydroxylation of aniline involves negative cooperativity. *Biochem Pharmacol*. 87(3):523–33. doi:10.1016/j.bcp.2013.12.003 PMID:24345333

Hartman JH, Martin HC, Caro AA, Pearce AR, Miller GP (2015). Subcellular localization of rat CYP2E1 impacts metabolic efficiency toward common substrates. *Toxicology*. 338:47–58. doi:10.1016/j.tox.2015.10.001 PMID:26463279

Hartwell & Andervont (1951). Aniline and aniline hydrochloride. In: Shubik P, Hartwell JL, editors. Survey of compounds which have been tested for carcinogenic activity. United States Public Health Service Publication No. 149. Washington (DC), USA: Government Printing Office; p. 50.

Hatakeyama S, Kovacs K, Yeghiayan E, Blascheck JA (1971). Aniline-induced changes in the corpora lutea of rats. *Am J Obstet Gynecol*. 109(3):469–76. doi:10.1016/0002-9378(71)90347-4 PMID:5103248

Hauri U, Lutolf B, Schlegel U, et al. (2005). Determination of carcinogenic aromatic amines in dyes, cosmetics, finger paints and inks for pens and tattoos with LC/MS. *Mitt Lebensm Hyg*. 96:321–35.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*. 5(Suppl 1):1–142. doi:10.1002/em.2860050703 PMID:6365529

He D, Zhao B, Tang C, Xu Z, Zhang S, Han J (2014). [Determination of aniline in water and fish by liquid chromatography-tandem mass spectrometry]. *Se Pu*. 32(9):926–9. [Chinese] doi:10.3724/SP.J.1123.2014.05025 PMID:25757229

Health Canada (2011). Canadian Environmental Protection Act, 1999. Follow-up report on a PSL substance for aniline Chemical Abstract Registry Number 62-53-3 Health Canada, December 2011. Available from: http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=CCDA73CC-1.

Hecht SS, El-Bayoumy K, Rivenson A, Fiala ES (1983). Bioassay for carcinogenicity of 3,2′-dimethyl-4-nitrosobiphenyl, O-nitrosotoluene, nitrosobenzene and the corresponding amines in Syrian golden hamsters. *Cancer Lett*. 20(3):349–54. doi:10.1016/0304-3835(83)90034-4 PMID:6627231

Herzel F, Schmidt G (1977). [Aniline and urea derivatives in soil and water samples]. *Gesund Ing*. 98(9):221–6. [German] PMID:902991

Ho CH, Clark BR, Guerin MR, Barkenbus BD, Rao TK, Epler JL (1981). Analytical and biological analyses of test materials from the synthetic fuel technologies. IV. Studies of chemical structure-mutagenic activity relationships of aromatic nitrogen compounds relevant to synfuels. *Mutat Res*. 85(5):335–45. doi:10.1016/0165-1161(81)90224-7 PMID:7029261

Hoar SK, Morrison AS, Cole P, Silverman DT (1980). An occupation and exposure linkage system for the study of occupational carcinogenesis. *J Occup Med*. 22(11):722–6. PMID:7441390

Holm JB, Chalmey C, Modick H, Jensen LS, Dierkes G, Weiss T, et al. (2015). Aniline is rapidly converted into paracetamol impairing male reproductive development. *Toxicol Sci*. 148(1):288–98. doi:10.1093/toxsci/kfv179 PMID:26259604

Holt LC, Daudt HW, inventors; DuPont, assignee (1935). Aniline hydrohalide preparation. United States Patent Office. United States patent 20071992. Available from: http://www.freepatentsonline.com/2007199.html, accessed 7 October 2020.

Horinouchi Y, Summers FA, Ehrenshaft M, Mason RP (2015). Free radical generation from an aniline derivative in HepG2 cells: a possible captodative effect. *Free Radic Biol Med*. 78:111–7. doi:10.1016/j.freeradbiomed.2014.10.577 PMID:25450331

Horowitz MR, Hallock MF (2019). Table 1.5: Common flux materials. In: Mansdorf SZ, editor. Handbook of occupational safety and health. 3rd ed.; p. 25. Available from: https://www.wiley.com/en-us/Handbook+of+Occupational+Safety+and+Health%2C+3rd+Edition-p-9781119581468.

Horvath E, Kovacs K, Yeghiayan E (1971). Histochemical study of the "adrenocortical lipid hyperplasia" induced in rats by aniline. *Acta Histochem*. 39(1):154–61. PMID:4996949

HSE (1997). Aniline. Risk Assessment Document. No. EH72/8. Suffolk, UK: Health & Safety Executive. Available from: https://products.ihs.com/Ohsis-SEO/106459.html.

Hsu TM, Welner DH, Russ ZN, Cervantes B, Prathuri RL, Adams PD, et al. (2018). Employing a biochemical protecting group for a sustainable indigo dyeing strategy. *Nat Chem Biol*. 14(3):256–61. doi:10.1038/nchembio.2552 PMID:29309053

Hueper W (1938). Aniline tumors' of the bladder. *Arch Pathol (Chic)*. 25:856–99.

Hushon J, Clerman R, Small R, Sood S, Taylor A, Thoman D (1980). An assessment of potentially carcinogenic, energy-related contaminants in water. Prepared for the United States Department of Energy and National Cancer Institute. McLean (VA), USA: MITRE Corporation; p. 102.

IARC (1974). Some aromatic amines, hydrazine and related substances, *N*-nitroso compounds and miscellaneous alkylating agents. *IARC Monogr Eval Carcinog Risk Chem Man*, 4:1–286. Available from: https://publications.iarc.fr/22.

IARC (1978). Some aromatic amines and related nitro compounds - hair dyes, colouring agents and miscellaneous industrial chemicals. *IARC Monogr Eval*

Carcinog Risk Chem Man, 16:1–400. Available from: http://publications.iarc.fr/34.

IARC (1980). Some pharmaceutical drugs. *IARC Monogr Eval Carcinog Risk Chem Hum*, 24:1–337. Available from: http://publications.iarc.fr/42 PMID:6937434

IARC (1982). Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking-water and dental preparations. *IARC Monogr Eval Carcinog Risk Chem Hum*, 27:1–341. Available from: http://publications.iarc.fr/45 PMID:6955259

IARC (1984). Polynuclear aromatic compounds, Part 3: Industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding. *IARC Monogr Eval Carcinog Risk Chem Hum*, 34:1–219. Available from: http://publications.iarc.fr/52.

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs Volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7:1–440. Available from: https://publications.iarc.fr/139 PMID: 3504843

IARC (2010). Some aromatic amines, organic dyes, and related exposures. *IARC Monogr Eval Carcinog Risks Hum*, 99:1–658. Available from: http://publications.iarc.fr/117 PMID:21528837

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: https://publications.iarc.fr/123 PMID: 23189753

IARC (2020). List of classifications by cancer sites with sufficient or limited evidence in humans, IARC Monographs Volumes 1–129. Lyon, France: Available from: https://monographs.iarc.fr/wp-content/uploads/2019/07/Classifications_by_cancer_site.pdf, accessed 5 April 2021.

ICIS (2008). Chemical profile: aniline. Independent Commodity Intelligence Service. Available from: https://www.icis.com/explore/resources/news/2008/01/07/9089893/chemical-profile-aniline.

IFA (2019). Aniline. GESTIS substance database. Information system on hazardous substances of the German Social Accident Insurance. Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA). Available from: https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp.

IHS Markit (2019). Aniline. Chemical economics handbook. Available from: https://ihsmarkit.com/products/aniline-chemical-economics-handbook.html,accessed before website was updated in September 2020.

IHS Markit (2020). Nitrobenzene and aniline. Chemical economics handbook. Available from: https://ihsmarkit.com/products/nitrobenzene-and-Aniline-chemical-economics-handbook.html.

ILO (2011). Chemical exposure limits. Geneva, Switzerland: International Labour organisation (ILO). Available from: https://www.ilo.org/safework/info/publications/WCMS_151534/lang--en/index.htm#P45_1147.

Imamura A, Kurumi Y, Danzuka T, Kodama M, Kawachi T, Nagao M (1983). Classification of compounds by cluster analysis of Ames test data. *Gan*. 74(2):196–204. PMID:6345254

Intratec Solutions (2016). Aniline production from nitrobenzene. 1 March 2016. Chemical Engineering. Available from: https://www.chemengonline.com/aniline-production-nitrobenzene-liquid-phase-intratec-solutions/.

Irons RD, Gross EA, White EL (1980). Aniline: evidence for an enterogastric cycle in the rat. *Food Cosmet Toxicol*. 18(4):393–7. doi:10.1016/0015-6264(80)90196-0 PMID:7461519

IRSST (2010). Aniline. Available from: https://www.irsst.qc.ca/laboratoires/analyses/contaminants-air/substance/i/449.

Ishidate M, Jr (1983). The data book of chromosomal aberration tests in vitro on 587 chemical substances using Chinese hamster fibroblast cell line (CHL cells). Tokyo, Japan: The Realize Inc.; p. 26.

Ishidate M Jr, Odashima S (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro–a screening for chemical carcinogens. *Mutat Res*. 48(3–4):337–53. doi:10.1016/0027-5107(77)90177-4 PMID:876270

Iwersen-Bergmann S, Schmoldt A (2000). Acute intoxication with aniline: detection of acetaminophen as aniline metabolite. *Int J Legal Med*. 113(3):171–4. doi:10.1007/s004140050292 PMID:10876991

Jenkins FP, Robinson JA, Gellatly JB, Salmond GW (1972). The no-effect dose of aniline in human subjects and a comparison of aniline toxicity in man and the rat. *Food Cosmet Toxicol*. 10(5):671–9. doi:10.1016/S0015-6264(72)80147-0 PMID:5084408

Jones E, Fox V (2003). Lack of clastogenicity activity of aniline in the mouse bone marrow. *Mutagenesis*. 18:283–6. doi:10.1093/mutage/18.3.283 PMID:12714695

Jung R, Engelhart G, Herbolt B, Jäckh R, Müller W (1992). Collaborative study of mutagenicity with *Salmonella typhimurium* TA102. *Mutat Res*. 278(4):265–70. doi:10.1016/S0165-1218(10)80006-0 PMID:1373863

Jurado-Sanchez B, Ballesteros E, Gallego M (2012). Occurrence of aromatic amines and *N*-nitrosamines in the different steps of a drinking water treatment plant. *Water Research*. 46(14):4543-4555. doi:10.1016/j.watres.2012.05.039 PMID:22703862

Käfferlein HU, Broding HC, Bünger J, Jettkant B, Koslitz S, Lehnert M, et al. (2014). Human exposure to airborne aniline and formation of methemoglobin: a contribution to occupational exposure limits. *Arch*

Toxicol. 88(7):1419–26. doi:10.1007/s00204-014-1266-y PMID:24899222

Kakkar P, Awasthi S, Viswanathan PN (1992). Oxidative changes in brain of aniline-exposed rats. *Arch Environ Contam Toxicol*. 23(3):307–9. doi:10.1007/BF00216238 PMID:1456776

Kakkar P, Viswanathan PN (1987). Induction of superoxide dismutase isozymes in rabbit lung due to aniline exposure. *J Appl Toxicol*. 7(1):7–13. doi:10.1002/jat.2550070103 PMID:3611601

Kanaya N (1990). Induction of chromosome damage by aniline and its C-hydroxylated metabolites in the root cells of Vicia faba. *Mutat Res*. 245(4):311–5. doi:10.1016/0165-7992(90)90162-D PMID:2266983

Kanaya N (1996). Activation of aniline by extracts from plants and induction of chromosomal damages in Chinese hamster ovary cells. *Genes Genet Syst*. 71(5):319–22. doi:10.1266/ggs.71.319 PMID:9037777

Kao J, Faulkner J, Bridges JW (1978). Metabolism of aniline in rats, pigs and sheep. *Drug Metab Dispos*. 6(5):549–55. PMID:30604

Kato T, Tadokoro N, Tsutsui M, Kikugawa K (1991). Transformation of arylamines into direct-acting mutagens by reaction with nitrite. *Mutat Res*. 249(1):243–54. doi:10.1016/0027-5107(91)90151-D PMID:2067537

Kauppinen T, Toikkanen J, Pukkala E (1998). From cross-tabulations to multipurpose exposure information systems: a new job-exposure matrix. *Am J Ind Med*. 33(4):409–17. doi:10.1002/(SICI)1097-0274(199804)33:4<409::AID-AJIM12>3.0.CO;2-2 PMID:9513649

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, et al. (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*. 25(7):1287–302. doi:10.1021/mp3004385 PMID:23323940

Khairnar U, Upaganlawar A, Upasani C (2016). Ameliorative effect of chronic supplementation of protocatechuic acid alone and in combination with ascorbic acid in aniline hydrochloride induced spleen toxicity in rats. *Scientifica (Cairo)*. 2016:4306984. doi:10.1155/2016/4306984 PMID:27418998

Khan MF, Boor PJ, Gu Y, Alcock NW, Ansari GA (1997). Oxidative stress in the splenotoxicity of aniline. *Fundam Appl Toxicol*. 35(1):22–30. doi:10.1006/faat.1996.2259 PMID:9024670

Khan MF, Green SM, Ansari GAS, Boor PJ (1998). Phenylhydroxylamine: role in aniline-associated splenic oxidative stress and induction of subendocardial necrosis. *Toxicol Sci*. 42(1):64–71. doi:10.1093/toxsci/42.1.64 PMID:9538049

Khan MF, Kannan S, Wang J (2006). Activation of transcription factor AP-1 and mitogen-activated protein kinases in aniline-induced splenic toxicity. *Toxicol Appl Pharmacol*. 210(1–2):86–93. doi:10.1016/j.taap.2005.08.006 PMID:16169568

Khan MF, Kaphalia BS, Ansari GA (1995). Erythrocyte-aniline interaction leads to their accumulation and iron deposition in rat spleen. *J Toxicol Environ Health*. 44(4):415–21. doi:10.1080/15287399509531970 PMID:7723074

Khan MF, Wu X, Alcock NW, Boor PJ, Ansari GA (1999). Iron exacerbates aniline-associated splenic toxicity. *J Toxicol Environ Health A*. 57(3):173–84. doi:10.1080/009841099157746 PMID:10376884

Khan MF, Wu X, Ansari GA (2000). Contribution of nitrosobenzene to splenic toxicity of aniline. *J Toxicol Environ Health A*. 60(4):263–73. doi:10.1080/00984100050027815 PMID:10914691

Khan MF, Wu X, Ansari GAS, Boor PJ (2003b). Malondialdehyde-protein adducts in the spleens of aniline-treated rats: immunochemical detection and localization. *J Toxicol Environ Health A*. 66(1):93–102. doi:10.1080/15287390306464 PMID:12587293

Khan MF, Wu X, Boor PJ, Ansari GAS (1999a). Oxidative modification of lipids and proteins in aniline-induced splenic toxicity. *Toxicol Sci*. 48(1):134–40. doi:10.1093/toxsci/48.1.134 PMID:10330693

Khan MF, Wu X, Wang J (2003a). Up-regulation of transforming growth factor-beta 1 in the spleen of aniline-treated rats. *Toxicol Appl Pharmacol*. 187(1):22–8. doi:10.1016/S0041-008X(02)00041-8 PMID:12628581

Khan R, Upaganlawar AB, Upasani C (2014). Protective effects of *dioscorea alata L*. in aniline exposure-induced spleen toxicity in rats: a biochemical study. *Toxicol Int*. 21(3):294–9. doi:10.4103/0971-6580.155371 PMID:25948969

Kiese M (1959). [Oxidation of aniline to nitrosobenzene in dogs]. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol*. 235(4):354–9. [German] PMID:13657190

Kiese M (1966). The biochemical production of ferrihemoglobin-forming derivatives from aromatic amines, and mechanisms of ferrihemoglobin formation. *Pharmacol Rev*. 18(3):1091–161. PMID:5343079

Kiese M, Taeger K (1976). The fate of phenylhydroxylamine in human red cells. *Naunyn Schmiedebergs Arch Pharmacol*. 292(1):59–66. doi:10.1007/BF00506490 PMID:934354

Kobets T, Cartus AT, Fuhlbrueck JA, Brengel A, Stegmüller S, Duan JD, et al. (2019). Assessment and characterization of DNA adducts produced by alkenylbenzenes in fetal turkey and chicken livers. *Food Chem Toxicol*. 129:424–33. doi:10.1016/j.fct.2019.05.010 PMID:31077736

Koenig CM, Beevers C, Pant K, Young RR (2018). Assessment of the mutagenic potential of *para*-chloroaniline and aniline in the liver, spleen, and bone marrow of Big Blue® rats with micronuclei analysis in peripheral blood. *Environ Mol Mutagen*. 59(9):785–97. doi:10.1002/em.22241 PMID:30216547

Korinth G, Lüersen L, Schaller KH, Angerer J, Drexler H (2008). Enhancement of percutaneous penetration of aniline and *o*-toluidine in vitro using skin barrier creams. *Toxicol In Vitro*. 22(3):812–8. doi:10.1016/j.tiv.2007.11.006 PMID:18155878

Korinth G, Weiss T, Penkert S, Schaller KH, Angerer J, Drexler H (2007). Percutaneous absorption of aromatic amines in rubber industry workers: impact of impaired skin and skin barrier creams. *Occup Environ Med*. 64(6):366–72. doi:10.1136/oem.2006.027755 PMID:17182646

Kouris C, Northcott J (1963). Aniline and its derivatives. In: Kirk RE, Othmer DF, editors. Encyclopedia of chemical technology. Vol. 2. 2nd ed. New York (NY), USA: John Wiley & Sons; pp. 411–9.

Kovacs K, Blascheck JA, Yeghiayan E, Hatakeyama S, Gardell C (1971). Adrenocortical lipid hyperplasia induced in rats by aniline. A histologic and electron microscopic study. *Am J Pathol*. 62(1):17–34. PMID:4922953

Kovacs K, Yeghiayan E, Hatakeyama S, Selye H (1970). Adrenocortical lipoid hyperplasia induced in rats by aniline. *Experientia*. 26(9):1014–5. doi:10.1007/BF02114167 PMID:4320239

Králík A, Linhart I, Váňa L, Moulisová A (2015). Identification of new DNA adducts of phenylnitrenium. *Chem Res Toxicol*. 28(6):1317–25. doi:10.1021/acs.chemrestox.5b00120 PMID:25992791

Kütting B, Göen T, Schwegler U, Fromme H, Uter W, Angerer J, et al. (2009). Monoarylamines in the general population–a cross-sectional population-based study including 1004 Bavarian subjects. *Int J Hyg Environ Health*. 212(3):298–309. doi:10.1016/j.ijheh.2008.07.004 PMID:18789761

Lee CH, Kim SH, Kwon DH, Jang KH, Chung YH, Moon JD (2013). Two cases of methemoglobinemia induced by the exposure to nitrobenzene and aniline. *Ann Occup Environ Med*. 25(1):31. doi:10.1186/2052-4374-25-31 PMID:24475977

Lewalter J, Angerer J, Sabbioni G (2012). Haemoglobin adducts of aromatic amines: aniline, *o*-, *m*- and *p*-toluidine, *o*-anisidine, *p*-chloroaniline, α-and β-naphthylamine, 4-aminodiphenyl, benzidine, 4,4′-diaminodiphenylmethane, 3,3′-dichlorobenzidine [Biomonitoring Methods, 2000]. The MAK-Collection for Occupational Health and Safety; pp. 191–219.

Link R (1961). [On the multiplicity of primary malignant tumors in occupational malignant bladder tumour]. *Arch Gewerbepathol Gewerbehyg*. 18:394–409. [German] PMID:13762453

Litvinov NN, Voronin VM, Kazachkov VI (1982). [Experimental study of aniline, lead nitrate and sodium alkylsulfate as modifiers of chemical blastomogenesis]. *Vopr Onkol*. 28(7):56–9. [Russian] PMID:7101830

Litvinov NN, Voronin VM, Kazachkov VI (1984). [Characteristics of aniline, lead nitrate, carbon tetrachloride and formaldehyde as modifiers of chemical carcinogenesis]. *Vopr Onkol*. 30(4):56–60. [Russian] PMID:6730407

Litvinov NN, Voronin VM, Kazachkov VI (1986). [Carcinogenesis-modifying properties of aniline, carbon tetrachloride, benzene, and ethanol]. *Eksp Onkol*. 8(2):21–3. [Russian] PMID:3698878

Liu L, Von Vett A, Zhang N, Walters KJ, Wagner CR, Hanna PE (2007). Arylamine *N*-acetyltransferases: characterization of the substrate specificities and molecular interactions of environmental arylamines with human NAT1 and NAT2. *Chem Res Toxicol*. 20(9):1300–8. doi:10.1021/tx7001614 PMID:17672512

Liu S, Wang W, Chen J, Sun J (2012). Determination of aniline and its derivatives in environmental water by capillary electrophoresis with on-line concentration. *Int J Mol Sci*. 13(6):6863–72. doi:10.3390/ijms13066863 PMID:22837668

Luceri F, Pieraccini G, Moneti G, Dolara P (1993). Primary aromatic amines from side-stream cigarette smoke are common contaminants of indoor air. *Toxicol Ind Health*. 9(3):405–13. doi:10.1177/074823379300900302 PMID:8367883

Lutz WK (1979). In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. *Mutat Res*. 65(4):289–356. doi:10.1016/0165-1110(79)90006-X PMID:390383

Lutz WK (1981). Constitutive and carcinogen-derived DNA binding as a basis for the assessment of potency of chemical carcinogens. *Adv Exp Med Biol*. 136(Pt B):1349–65. PMID:7344514

Lynch MK, Ryan LP (2012). Nitrobenzene/aniline/MDI. PERP 2011-4 Chemsystems PERP Program. Report Abstract, May 2012. Available from: https://thinking.nexant.com/sites/default/files/report/field_attachment_abstract/201205/2011_4_abs.pdf.

Ma H, Wang J, Abdel-Rahman SZ, Boor PJ, Khan MF (2008). Oxidative DNA damage and its repair in rat spleen following subchronic exposure to aniline. *Toxicol Appl Pharmacol*. 233(2):247–53. doi:10.1016/j.taap.2008.08.010 PMID:18793663

Ma H, Wang J, Abdel-Rahman SZ, Boor PJ, Khan MF (2013). Induction of base excision repair enzymes NTH1 and APE1 in rat spleen following aniline exposure. *Toxicol Appl Pharmacol*. 267(3):276–83. doi:10.1016/j.taap.2013.01.005 PMID:23352893

Ma H, Wang J, Abdel-Rahman SZ, Hazra TK, Boor PJ, Khan MF (2011). Induction of NEIL1 and NEIL2 DNA glycosylases in aniline-induced splenic toxicity. *Toxicol Appl Pharmacol*. 251(1):1–7. doi:10.1016/j.taap.2010.12.001 PMID:21145906

Maickel RP, Snodgrass WR (1973). Physicochemical factors in maternal-fetal distribution of drugs. *Toxicol Appl Pharmacol*. 26(2):218–30. doi:10.1016/0041-008X(73)90255-X PMID:4751100

Mamber SW, Bryson V, Katz SE (1983). The *Escherichia coli* WP2/WP100 rec assay for detection of potential chemical carcinogens. *Mutat Res*. 119(2):135–44. doi:10.1016/0165-7992(83)90121-5 PMID:6338367

Mamber SW, Bryson V, Katz SE (1984). Evaluation of the I K12 inductest for detection of potential chemical carcinogens. *Mutat Res*. 130(3):141–51. doi:10.1016/0165-1161(84)90116-X PMID:6374442

Maples KR, Eyer P, Mason RP (1990). Aniline-, phenylhydroxylamine-, nitrosobenzene-, and nitrobenzene-induced hemoglobin thiyl free radical formation in vivo and in vitro. *Mol Pharmacol*. 37(2):311–8. PMID:2154677

Martin FL, Cole KJ, Orme MH, Grover PL, Phillips DH, Venitt S (1999). The DNA repair inhibitors hydroxyurea and cytosine arabinoside enhance the sensitivity of the alkaline single-cell gel electrophoresis ('comet') assay in metabolically-competent MCL-5 cells. *Mutat Res*. 445(1):21–43. doi:10.1016/S1383-5718(99)00116-3 PMID:10521689

Martínez A, Urios A, Blanco M (2000). Mutagenicity of 80 chemicals in *Escherichia coli* tester strains IC203, deficient in OxyR, and its oxyR(+) parent WP2 uvrA/pKM101: detection of 31 oxidative mutagens. *Mutat Res*. 467(1):41–53. doi:10.1016/S1383-5718(00)00020-6 PMID:10771270

Matsushima T, Hayashi M, Matsuoka A, Ishidate M Jr, Miura KF, Shimizu H, et al. (1999). Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU). *Mutagenesis*. 14(6):569–80. doi:10.1093/mutage/14.6.569 PMID:10567032

McCarroll NE, Keech BH, Piper CE (1981). Amicrosuspension adaptation of the *Bacillus subtilis* "rec" assay. *Environ Mutagen*. 3(6):607–16. doi:10.1002/em.2860030603

McCarthy DJ, Waud WR, Struck RF, Hill DL (1985). Disposition and metabolism of aniline in Fischer 344 rats and C57BL/6 X C3H F_1 mice. *Cancer Res*. 45(1):174–80. PMID:2578091

McGregor DB, Brown AG, Howgate S, McBride D, Riach C, Caspary WJ, et al. (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals. *Environ Mol Mutagen*. 17(3):196–219. doi:10.1002/em.2850170309 PMID:1902415

McQueen CA, Maslansky CJ, Crescenzi SB, Williams GM (1981). The genotoxicity of 4,4′-methylenebis-2-chloroaniline in rat, mouse, and hamster hepatocytes. *Toxicol Appl Pharmacol*. 58(2):231–5. doi:10.1016/0041-008X(81)90427-0 PMID:7245198

Meijers A, van der Leer R (1976). The occurrence of organic micropollutants in the river Rhine and the river Maas in 1974. *Water Res*. 10(7):597–604. doi:10.1016/0043-1354(76)90140-8

Mellert W, Deckardt K, Gembardt C, Zwirner-Baier I, Jäckh R, van Ravenzwaay B (2004). Aniline: early indicators of toxicity in male rats and their relevance to spleen carcinogenicity. *Hum Exp Toxicol*. 23(8):379–89. doi:10.1191/0960327104ht466oa PMID:15346719

Menichini E, Boniforti L, di Marzio S (1989). Occupational exposure to airborne aromatic amines in rubber manufacturing: Determination by gas chromatography-mass spectrometry. *Toxicol Environ Chem*. 22(1–4):9–16. doi:10.1080/02772248909357419

Mitchell AD, Rudd CJ, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. *Environ Mol Mutagen*. 12(Suppl 13):37–101. doi:10.1002/em.2860120504 PMID:3416841

Modick H, Schütze A, Pälmke C, Weiss T, Brüning T, Koch HM (2013). Rapid determination of *N*-acetyl-4-aminophenol (paracetamol) in urine by tandem mass spectrometry coupled with on-line clean-up by two dimensional turbulent flow/reversed phase liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci*. 925:33–9. doi:10.1016/j.jchromb.2013.02.023 PMID:23518644

Modick H, Weiss T, Dierkes G, Koslitz S, Käfferlein HU, Brüning T, et al. (2016). Human metabolism and excretion kinetics of aniline after a single oral dose. *Arch Toxicol*. 90(6):1325–33. doi:10.1007/s00204-015-1566-x PMID:26233686

Mohanta VL, Mishra BK (2020). Integration of cancer and non-cancer human health risk assessment for aniline enriched groundwater: a fuzzy inference system-based approach. *Environ Geochem Health*. doi:10.1007/s10653-020-00590-7 PMID:32419090

Möller C, Davis WC, Thompson VR, Marí F, DeCaprio AP (2017). Proteomic analysis of thiol modifications and assessment of structural changes in hemoglobin induced by the aniline metabolites *N*-phenylhydroxylamine and nitrosobenzene. *Sci Rep*. 7(1):14794. doi:10.1038/s41598-017-14653-w PMID:29093547

Mori M, Totsuka Y, Fukutome K, Yoshida T, Sugimura T, Wakabayashi K (1996). Formation of DNA adducts by the co-mutagen norharman with aromatic amines. *Carcinogenesis*. 17(7):1499–503. doi:10.1093/carcin/17.7.1499 PMID:8706255

Muñoz ER, Barnett B (1998). Evaluation of the genotoxicity of aniline.HCl in *Drosophila melanogaster*. *Mutat Res*. 413(1):15–22. doi:10.1016/S1383-5718(98)00007-2 PMID:9602854

Myhr BC, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. *Environ Mol Mutagen*. 12(Suppl 13):103–94. doi:10.1002/em.2860120505 PMID:3416838

Nagao M, Yahagi T, Honda M, Seino Y, Matsushima T, Sugimura T (1977). Demonstration of mutagenicity of aniline and *o*-toluidine by norharman. *Proc Jpn Acad*. 53(1):34–7. doi:10.2183/pjab.53.34

Nakano M, Omae K, Takebayashi T, Tanaka S, Koda S (2018). An epidemic of bladder cancer: ten cases of bladder cancer in male Japanese workers exposed to *ortho*-toluidine. *J Occup Health*. 60(4):307–11. doi:10.1539/joh.2017-0220-OA PMID:29743389

Nature (2018). Chemists go green to make better blue jeans. Editorial. *Nature*. 553(7687):128. doi:10.1038/d41586-018-00103-8 PMID:29323300

NCBI (2020a). Aniline. Compound summary. PubChem CID 6115. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Aniline, accessed 1 October 2020.

NCBI (2020b). Aniline hydrochloride. Compound summary. PubChem CID 8870. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Aniline-hydrochloride, accessed 1 October 2020.

NCI (1978). Bioassay of aniline hydrochloride for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser*. 130:1–115. PMID:12799662

NEMI (1998). Semivolatile organic compounds by GC/MS. Method EPA-RCA: 8270D. National Environmental Methods Index. Available from: https://www.nemi.gov/methods/method_summary/4726/.

NEMI (2001). Semivolatile organic compounds by isotope dilution GCMS. Method EPA-EAD: 1625. National Environmental Methods Index. Available from: https://www.nemi.gov/methods/method_summary/4686/.

Neurath GB, Dünger M, Pein FG, Ambrosius D, Schreiber O (1977). Primary and secondary amines in the human environment. *Food Cosmet Toxicol*. 15(4):275–82. doi:10.1016/S0015-6264(77)80197-1 PMID:590888

NIOSH (1981). Health hazard evaluation report. HHE-80-215-911. McGlothlin JD, Cone JE, investigators. Wilson Sporting Goods Company, Ironton (OH), USA: National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/hhe/reports/pdfs/80-215-911.pdf, accessed May 2020.

NIOSH (1998). Aniline, *o*-toluidine, and nitrobenzene. Method 2017. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/docs/2003-154/pdfs/2017.pdf.

NIOSH (2003). Aniline and *o*-toluidine in urine. Method 8317. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/docs/2003-154/pdfs/8317.pdf.

NIOSH (2012). Occupational cancer. Carcinogen list. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/niosh/topics/cancer/npotocca.html.

NIOSH (2018). Appendix A - NIOSH potential occupational carcinogens. New policy. NIOSH pocket guide to chemical hazards. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/npg/nengapdxa.html, accessed 4 March 2021.

NIOSH (2019). Aniline (and homologs). NIOSH pocket guide to chemical hazards. National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/npg/npgd0033.html, accessed 2 October 2020.

Nizamova RS (1991). [Occupational hazards and bladder cancer]. *Urol Nefrol (Mosk)*. (5):35–8. [Russian] PMID:1836689

Nohmi T, Yoshikawa K, Nakadate M, Miyata R, Ishidate M Jr (1984). Mutations *in Salmonella typhimurium* and inactivation of *Bacillus subtilis* transforming DNA induced by phenylhydroxylamine derivatives. *Mutat Res*. 136(3):159–68. doi:10.1016/0165-1218(84)90049-1 PMID:6429526

Northcott J (1978). Amines, aromatic (aniline). In: Kirk RE, Othmer DF, editors. Encyclopedia of chemical technology. Vol. 2. 3rd ed. New York (NY), USA: John Wiley & Sons; pp. 309–21.

NTP (2019). NTP research report on the chemical and physical characterization of recycled tire crumb rubber. Research Triangle Park (NC), USA: National Toxicology Program. Research Report 11. doi:10.22427/NTP-RR-11.

OECD (2009). The 2007 OECD list of high production volume chemicals. OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 112. ENV/JM/MONO(2009)40. Paris, France: Organisation for Economic Co-operation and Development. Available from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2009)40&doclanguage=en.

Office of the Federal Register (2019b). 40 CFR Part 117 Water programs; determination of reportable quantities for hazardous substances. United States Code of Federal Regulations. Washington (DC), USA: National Archives and Records Administration. Available from: https://www.govinfo.gov/content/pkg/CFR-2019-title40-vol24/pdf/CFR-2019-title40-vol24-part117.pdf, accessed 11 February 2020.

Office of the Federal Register (2019c). 40 CFR Part 261 Hazardous waste management system: identification and listing of hazardous wastes. United States Code of Federal Regulations. Washington (DC), USA: National Archives and Records Administration. Available from: https://www.govinfo.gov/content/pkg/CFR-2019-title40-vol28/pdf/CFR-2019-title40-vol28-part261.pdf, accessed 11 February 2020.

Oppenheimer R (1927). [On diseases of the urinary tract seen in workers of the chemical industry]. *Z Urol Chir.* 21:336. [German]

Orts G (1948). Tumores vesicales y anilinas [Bladder and aniline tumours]. *Intercamb Urol Corresp.* 3:2. [Spanish] PMID:18104281

Osano O, Admiraal W, Klamer HJC, Pastor D, Bleeker EAJ (2002). Comparative toxic and genotoxic effects of chloroacetanilides, formamidines and their degradation products on *Vibrio fischeri* and *Chironomus riparius*. *Environ Pollut.* 119(2):195–202. doi:10.1016/S0269-7491(01)00334-7 PMID:12152826

OSHA (1994). Aniline. Method PV2079. Washington (DC), USA: United States Department of Labor. Available from: https://www.osha.gov/dts/sltc/methods/partial/t-pv2079-01-9406-ch/t-pv2079-01-9406-ch.html, accessed 4 March 2021.

Ott MG, Langner RR (1983). A mortality survey of men engaged in the manufacture of organic dyes. *J Occup Med.* 25(10):763–8. doi:10.1097/00043764-198310000-00018 PMID:6631562

Pai V, Bloomfield SF, Gorrod JW (1985). Mutagenicity of *N*-hydroxylamines and *N*-hydroxycarbamates towards strains of *Escherichia coli* and *Salmonella typhimurium*. *Mutat Res.* 151(2):201–7. doi:10.1016/0027-5107(85)90072-7 PMID:3897848

Palmiotto G, Pieraccini G, Moneti G, Dolara P (2001). Determination of the levels of aromatic amines in indoor and outdoor air in Italy. *Chemosphere.* 43(3):355–61. doi:10.1016/S0045-6535(00)00109-0 PMID:11302581

Parke DV (1960). Studies in detoxication. 84. The metabolism of [C]aniline in the rabbit and other animals. *Biochem J.* 77(3):493–503. doi:10.1042/bj0770493 PMID:16748856

Parodi S, Pala M, Russo P, Zunino A, Balbi C, Albini A, et al. (1982). DNA damage in liver, kidney, bone marrow, and spleen of rats and mice treated with commercial and purified aniline as determined by alkaline elution assay and sister chromatid exchange induction. *Cancer Res.* 42(6):2277–83. PMID:7074610

Parodi S, Taningher M, Russo P, Pala M, Tamaro M, Monti-Bragadin C (1981). DNA-damaging activity in vivo and bacterial mutagenicity of sixteen aromatic amines and azo-derivatives, as related quantitatively to their carcinogenicity. *Carcinogenesis.* 2(12):1317–26. doi:10.1093/carcin/2.12.1317 PMID:7034987

Parodi S, Zunino A, Ottaggio L, De Ferrari M, Santi L (1983). Lack of correlation between the capability of inducing sister-chromatid exchanges in vivo and carcinogenic potency, for 16 aromatic amines and azo derivatives. *Mutat Res.* 108(1–3):225–38. doi:10.1016/0027-5107(83)90122-7 PMID:6835220

Pathak KV, Chiu TL, Amin EA, Turesky RJ (2016). Methemoglobin formation and characterization of hemoglobin adducts of carcinogenic aromatic amines and heterocyclic aromatic amines. *Chem Res Toxicol.* 29(3):255–69. doi:10.1021/acs.chemrestox.5b00418 PMID:26824300

Pauluhn J (2002). Aniline-induced methemoglobinemia in dogs: pitfalls of route-to-route extrapolations. *Inhal Toxicol.* 14(9):959–73. doi:10.1080/08958370290084719 PMID:12396405

Phillips D, Gradisek R, Heiselman DE (1990). Methemoglobinemia secondary to aniline exposure. *Ann Emerg Med.* 19(4):425–9. doi:10.1016/S0196-0644(05)82352-2 PMID:2321830

Piccinini P, Contor L, Pakalin S, Raemaekers T, Senaldi C (2015). Safety of tattoos and permanent make-up. State of play and trends in tattoo practices. JRC Technical Reports No. EUR 27528 EN, European Commission.

Piccinini P, Pakalin S, Contor L, Bianchi I, Senaldi C (2016). Safety of tattoos and permanent make-up: final report. No. EUR 27947 EN. Available from: https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/safety-tattoos-and-permanent-make-final-report.

Pienta RJ (1980). Transformation of Syrian hamster embryo cells by diverse chemicals and correlation with their reported carcinogenic and mutagenic activities. In: de Serres FJ, Hollaender A, editors. Chemical mutagens. Vol. 6. New York (NY), USA: Plenum Press; pp. 175–202. doi:10.1007/978-1-4613-3072-1_7

Piotrowski J (1957). Quantitative estimation of aniline absorption through the skin in man. *J Hyg Epidemiol Microbiol Immunol.* 1(1):23–32. PMID:13475789

Plöttner S, Käfferlein HU, Brüning T (2013). Miniaturization of cytotoxicity tests for concentration range-finding studies prior to conducting the pH 6.7 Syrian hamster embryo cell-transformation assay. *Mutat Res.* 755(2):108–14. doi:10.1016/j.mrgentox.2013.04.021 PMID:23830925

Prasad I (1970). Mutagenic effects of the herbicide 3′,4′-dichloropropionanilide and its degradation products. *Can J Microbiol.* 16(5):369–72. doi:10.1139/m70-064 PMID:5419803

Preti G, Labows JN, Kostelc JG, Aldinger S, Daniele R (1988). Analysis of lung air from patients with bronchogenic carcinoma and controls using gas chromatography-mass spectrometry. *J Chromatogr A.* 432:1–11. doi:10.1016/S0378-4347(00)80627-1 PMID:3220881

Price PJ, Mishra NK (1980). The use of Fischer rat embryo cells as a screen for chemical carcinogens and the role of the nontransforming type 'c' RNA tumor viruses in the assay. *Adv. Mod. Environ. Toxicol.* 1:213–39.

Pujol A (1950). [Vesical tumor, probably caused by aniline]. *Rev Argent Urol.* 19(1–6):39–44. [Spanish] PMID:14786562

Pujol A (1954). [Vesical cancer due possibly to aniline dyes]. *Rev Argent Urol.* 23(3–4):101–2. [Spanish] PMID:13186209

Purchase IF, Longstaff E, Ashby J, Styles JA, Anderson D, Lefevre PA, et al. (1978). An evaluation of 6 short-term tests for detecting organic chemical carcinogens. *Br J Cancer.* 37(6):873–903. doi:10.1038/bjc.1978.132 PMID:354672

Qi Y, Toyooka T, Nie J, Ohta H, Koda S, Wang RS (2020). Comparative γ-H2AX analysis for assessment of the genotoxicity of six aromatic amines implicated in bladder cancer in human urothelial cell line. *Toxicol In Vitro.* 66:104880. doi:10.1016/j.tiv.2020.104880 PMID:32387221

Ramsay DH, Harvey CC (1959). Markingink poisoning: an outbreak of methaemoglobin cyanosis in newborn babies. *Lancet.* 1(7079):910–2. doi:10.1016/S0140-6736(59)91310-8 PMID:13655626

Rashid KA, Arjmand M, Sandermann H, Mumma RO (1987). Mutagenicity of chloroaniline/lignin metabolites in the Salmonella/microsome assay. *J Environ Sci Health B.* 22(6):721–9. doi:10.1080/03601238709372581 PMID:3323289

Rayner W (1886). Cyanosis in newly born children caused by aniline marking ink. *Br Med J.* 1(1311):294. doi:10.1136/bmj.1.1311.294 PMID:20751466

Rehn L (1895). Blasengeschwülste bei Fuchsin-Arbeitern [Bladder tumors among fuchsin workers]. *Arch Klin Chir.* 50:588–600. [German]

Renman L, Sangö C, Skarping G (1986). Determination of isocyanate and aromatic amine emissions from thermally degraded polyurethanes in foundries. *Am Ind Hyg Assoc J.* 47(10):621–8. doi:10.1080/15298668691390340

Ress NB, Witt KL, Xu J, Haseman JK, Bucher JR (2002). Micronucleus induction in mice exposed to diazoaminobenzene or its metabolites, benzene and aniline: implications for diazoaminobenzene carcinogenicity. *Mutat Res.* 521(1–2):201–8. doi:10.1016/S1383-5718(02)00241-3 PMID:12438016

Riffelmann M, Müller G, Schmieding W, Popp W, Norpoth K (1995). Biomonitoring of urinary aromatic amines and arylamine hemoglobin adducts in exposed workers and nonexposed control persons. *Int Arch Occup Environ Health.* 68(1):36–43. doi:10.1007/BF01831631 PMID:8847111

Roberts JJ, Warwick GP (1966). The covalent binding of metabolites of dimethylaminoazobenzene, beta-naphthylamine and aniline to nucleic acids in vivo. *Int J Cancer.* 1(2):179–96. doi:10.1002/ijc.2910010207

Rockett L, Aldous A, Benson V, Brandon Y, Briere B, Dee T, et al. (2014). Volatile organic compounds - understanding the risks to drinking water. National Centre for Environmental Toxicology. Report DWI9611.04, October 2014. Drinking Water Inspectorate. Available from: http://dwi.defra.gov.uk/research/completed-research/reports/DWI70-2-292.pdf.

Roudabush RL, Terhaar CJ, Fassett DW, Dziuba SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs. *Toxicol Appl Pharmacol.* 7(4):559–65. doi:10.1016/0041-008X(65)90041-4 PMID:5839391

Ruder AM, Ward EM, Roberts DR, Teass AW, Brown KK, Fingerhut MA, et al. (1992). Response of the National Institute for Occupational Safety and Health to an occupational health risk from exposure to *ortho*-toluidine and aniline. *Scand J Work Environ Health.* 18(Suppl 2):82–4. PMID:1514097

Saalo A, Soosaar A, Mikkola J (2016). ASA 2014. Syopasairauden vaaraa aiheuttaville aineille ja menetelmille ammatissaan altistuneiksi ilmoitetut Suomessa. Tyoterveyslaitos, Helsinki. Available from: http://urn.fi/URN:ISBN 978-952-261-658-6 (PDF) [Swedish].

Sabbioni G, Beyerbach A (1995). Determination of hemoglobin adducts of arylamines in humans. *J Chromatogr B Biomed Appl.* 667(1):75–83. doi:10.1016/0378-4347(94)00599-Z PMID:7663688

Sakagami Y, Yamazaki H, Ogasawara N, Yokoyama H, Ose Y, Sato T (1988). The evaluation of genotoxic activities of disinfectants and their metabolites by umu test. *Mutat Res.* 209(3–4):155–60. doi:10.1016/0165-7992(88)90034-6 PMID:3143068

Sankaranarayanan R, Swaminathan R, Lucas E (2011). Cancer survival in Africa, Asia, the Caribbean and Central America (SurvCan). IARC Scientific Publications. Volume 162. Lyon, France: International Agency for Research on Cancer.

Sasaki YF, Fujikawa K, Ishida K, Kawamura N, Nishikawa Y, Ohta S, et al. (1999). The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and US NTP. *Mutat Res.* 440(1):1–18. doi:10.1016/S1383-5718(99)00006-6 PMID:10095124

Schafer B, Neffgen A, Klinner U (2008). A novel yeast-based tool to detect mutagenic and recombinogenic effects simultaneously. *Mutat Res.* 652(1):20–9. doi:10.1016/j.mrgentox.2007.11.007 PMID:18243774

Schiestl RH (1989). Nonmutagenic carcinogens induce intrachromosomal recombination in yeast. *Nature.* 337(6204):285–8. doi:10.1038/337285a0 PMID:2643057

Schiestl RH, Gietz RD, Mehta RD, Hastings PJ (1989). Carcinogens induce intrachromosomal recombination in yeast. *Carcinogenesis.* 10(8):1445–55. doi:10.1093/carcin/10.8.1445 PMID:2665967

Schmiedeberg O (1877). Ueber das Verhältniss des Ammoniaks und der primären Monaminbasen zur Harnstoffbildung im Thierkörper. *Archiv f. experiment. Pathol. u. Pharmakol.* 8(1–2):1–14. [German] doi:10.1007/BF01831350

Schubert J, Kappenstein O, Luch A, Schulz TG (2011). Analysis of primary aromatic amines in the mainstream waterpipe smoke using liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr A*. 1218(33):5628–37. doi:10.1016/j.chroma.2011.06.072 PMID:21752381

Schwerin H (1920). Blasengeschwulst e bei anilinarbeitern in chemishen betrieben. *Zntrbl Gewerberbehyg.* 8:64–69. [German]

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, et al. (2002). Comparative investigation of multiple organs of mice and rats in the comet assay. *Mutat Res*. 517(1–2):53–75. doi:10.1016/S1383-5718(02)00034-7 PMID:12034309

Shackelford WM, Keith LH (1976). Frequency of organic compounds identified in water. Report EPA-600/4-76-062. Athens (GA), USA: Environmental Research Laboratory, Analytical Chemistry Branch, United States Environmental Protection Agency; pp. 9, 58, 59.

Shahin MM (1989). Studies on the mutagenicity of aniline in association with norharman in the Salmonella/mammalian microscome assay. *Int J Cosmet Sci*. 11(3):129–40. doi:10.1111/j.1467-2494.1989.tb00502.x PMID:19456945

Shamovsky I, Ripa L, Blomberg N, Eriksson LA, Hansen P, Mee C, et al. (2012). Theoretical studies of chemical reactivity of metabolically activated forms of aromatic amines toward DNA. *Chem Res Toxicol*. 25(10):2236–52. doi:10.1021/tx300313b PMID:22946514

Shapiro R, Ellis S, Hingerty BE, Broyde S (1998). Effect of ring size on conformations of aromatic amine-DNA adducts: the aniline-C8 guanine adduct resides in the B-DNA major groove. *Chem Res Toxicol*. 11(4):335–41. doi:10.1021/tx970211q PMID:9548804

Shatila W, Verma A, Adam S (2017). Plasmapheresis in severe methemoglobinemia following occupational exposure. *Transfus Apher Sci*. 56(3):341–4. doi:10.1016/j.transci.2017.02.003 PMID:28259531

Shear MJ, Stewart HL (1941). In: Shubik P, Hartwell JL, editors. Survey of compounds which have been tested for carcinogenic activity. Washington (DC), USA: Government Printing Office, United States Public Health Service Publication No. 149; p. 50.

Shi J, Ma DY (2009). [One case of acute aniline poisoning induced abnormal increase of white blood cell number]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 27(12):774. [Chinese] [no abstract available] PMID:21141147

Sicardi SM, Martiarena JL, Iglesias MT (1991). Mutagenic and analgesic activities of aniline derivatives. *J Pharm Sci*. 80(8):761–4. doi:10.1002/jps.2600800811 PMID:1791537

Simmon VF (1979). In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J Natl Cancer Inst*. 62(4):901–9. PMID:372658

Skipper PL, Kim MY, Sun HL, Wogan GN, Tannenbaum SR (2010). Monocyclic aromatic amines as potential human carcinogens: old is new again. *Carcinogenesis*. 31(1):50–8. doi:10.1093/carcin/bgp267 PMID:19887514

Skipper PL, Trudel LJ, Kensler TW, Groopman JD, Egner PA, Liberman RG, et al. (2006). DNA adduct formation by 2,6-dimethyl-, 3,5-dimethyl-, and 3-ethylaniline in vivo in mice. *Chem Res Toxicol*. 19(8):1086–90. doi:10.1021/tx060082q PMID:16918249

Smith JN, Williams RT (1949). Studies in detoxication. 23. The fate of aniline in the rabbit. *Biochem J*. 44(2):242–50. doi:10.1042/bj0440242 PMID:16748506

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Sonnenfeld G (1983). Effect of sidestream tobacco smoke components on alpha/beta interferon production. *Oncology*. 40(1):52–6. doi:10.1159/000225691 PMID:6823405

Sonnenfeld G, Hudgens RW (1983). Effect of carcinogenic components of cigarette smoke on in vivo production of murine interferon. *Cancer Res*. 43(10):4720–2. PMID:6883330

Sorahan T (2008). Bladder cancer risks in workers manufacturing chemicals for the rubber industry. *Occup Med (Lond)*. 58(7):496–501. doi:10.1093/occmed/kqn104 PMID:18725381

Sorahan T, Hamilton L, Jackson JR (2000). A further cohort study of workers employed at a factory manufacturing chemicals for the rubber industry, with special reference to the chemicals 2-mercaptobenzothiazole (MBT), aniline, phenyl-beta-naphthylamine and *o*-toluidine. *Occup Environ Med*. 57(2):106–15. doi:10.1136/oem.57.2.106 PMID:10711278

Statista (2020a). Aniline production in the United States from 1990 to 2019. Available from: https://www.statista.com/statistics/974679/us-aniline-production-volume/, accessed 4 March 2021.

Statista (2020b). Production volume of aniline in India from financial year 2013 to FY 2019. Available from: https://www.statista.com/statistics/727869/india-aniline-production-volume/, accessed 4 March 2021.

Steenland K, Greenland S (2004). Monte Carlo sensitivity analysis and Bayesian analysis of smoking as an unmeasured confounder in a study of silica and lung

cancer. *Am J Epidemiol*. 160(4):384–92. doi:10.1093/aje/kwh211 PMID:15286024

Stettler LE, Savage RE, Brown KK, Cheever KL, Weigel WW, DeBord DG, et al. (1992). Biological monitoring for occupational exposures to ortho-toluidine and aniline. *Scand J Work Environ Health*. 18(Suppl 2):78–81. PMID:1514095

Swenberg JA (1981). Utilization of the alkaline elution assay as a short-term test for chemical carcinogens. In: Stich HF, San RHC, editors. Short-term tests for chemical carcinogens. New York, USA; p. 48. doi:10.1007/978-1-4612-5847-6_5

Swenberg JA, Petzold GL, Harbach PR (1976). In vitro DNA damage/alkaline elution assay for predicting carcinogenic potential. *Biochem Biophys Res Commun*. 72(2):732–8. doi:10.1016/S0006-291X(76)80100-3 PMID:985507

Takehisa S, Kanaya N (1982). SCE induction in human lymphocytes by combined treatment with aniline and norharman. *Mutat Res*. 101(2):165–72. doi:10.1016/0165-1218(82)90006-4 PMID:7099184

Takehisa S, Kanaya N, Rieger R (1988). Promutagen activation by *Vicia faba*: an assay based on the induction of sister-chromatid exchanges in Chinese hamster ovary cells. *Mutat Res*. 197(2):195–205. doi:10.1016/0027-5107(88)90093-0 PMID:3340085

Tanaka KI, Marui S, Mii T (1980). Mutagenicity of extracts of urine from rats treated with aromatic amines. *Mutat Res*. 79(2):173–6. doi:10.1016/0165-1218(80)90086-5 PMID:7001226

Tao N, Liu G, Bai L, Tang L, Guo C (2017). Genotoxicity and growth inhibition effects of aniline on wheat. *Chemosphere*. 169:467–73. doi:10.1016/j.chemosphere.2016.11.063 PMID:27889512

Teass AW, DeBord DG, Brown KK, Cheever KL, Stettler LE, Savage RE, et al. (1993). Biological monitoring for occupational exposures to *o*-toluidine and aniline. *Int Arch Occup Environ Health*. 65(1 Suppl):S115–8. doi:10.1007/BF00381320 PMID:8406905

Temkin IS (1963). The clinical prophylaxis of the urinary bladder cancer in the workers of the aniline dyeing industry. *Acta Unio Int Contra Cancrum*. 19:465–6. PMID:14050581

Thier R, Lewalter J, Selinski S, Bolt HM (2001). Biological monitoring in workers in a nitrobenzene reduction plant: haemoglobin versus serum albumin adducts. *Int Arch Occup Environ Health*. 74(7):483–8. doi:10.1007/s004200100250 PMID:11697451

Thiess AM, Oettel H, Uhl C (1969). [Contribution to the problems of lung cancer due to occupation. Long term observations from the Badischen Anilin- und Soda-Fabrik AG in Ludwigshafen on the Rhine]. *Zentralbl Arbeitsmed*. 19(4):97–113. [German] PMID:5616789

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The United States Federal Tox21 Program: a strategic and operational plan for continued leadership. *ALTEX*. 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

Tinwell H, Ashby J (1991). Micronucleus morphology as a means to distinguish aneugens and clastogens in the mouse bone marrow micronucleus assay. *Mutagenesis*. 6(3):193–8. doi:10.1093/mutage/6.3.193 PMID:1881350

TMR (2020). Acetanilide market - global industry analysis, size, share, growth, trends, and forecast 2018–2026. Transparency Market Research. Available from: https://www.transparencymarketresearch.com/acetanilide-market.html, accessed 4 March 2021.

Tohda H, Tada M, Sugawara R, Oikawa A (1983). Actions of amino-β-carbolines on induction of sister-chromatid exchanges. *Mutat Res*. 116(2):137–47. doi:10.1016/0165-1218(83)90104-0 PMID:6828050

Topham JC (1980). The detection of carcinogen-induced sperm head abnormalities in mice. *Mutat Res*. 69(1):149–55. doi:10.1016/0027-5107(80)90184-0 PMID:6987494

Toth S, Yeghiayan E, Kovacs K (1971). Effect of aniline on plasma corticosterone in rats. *Can J Physiol Pharmacol*. 49(5):433–5. doi:10.1139/y71-052 PMID:4330432

Toyoda T, Matsushita K, Morikawa T, Yamada T, Miyoshi N, Ogawa K (2019). Distinct differences in the mechanisms of mucosal damage and γ-H2AX formation in the rat urinary bladder treated with *o*-toluidine and *o*-anisidine. *Arch Toxicol*. 93(3):753–62. doi:10.1007/s00204-019-02396-8 PMID:30656379

Trepanier LA, Ray K, Winand NJ, Spielberg SP, Cribb AE (1997). Cytosolic arylamine *N*-acetyltransferase (NAT) deficiency in the dog and other canids due to an absence of NAT genes. *Biochem Pharmacol*. 54(1):73–80. doi:10.1016/S0006-2952(97)00140-8 PMID:9296352

Trier X, Okholm B, Foverskov A, Binderup ML, Petersen JH (2010). Primary aromatic amines (PAAs) in black nylon and other food-contact materials, 2004-2009. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 27(9):1325–35. doi:10.1080/19440049.2010.487500 PMID:20640962

US EPA (1979). Water programs; determination of reportable quantities for hazardous substances. United States Code Fed. Regul. J. Title 40, Part 117. *Fed Regist*. 44(169):50766–79.

US EPA (1982). CIIT aniline hydrochloride study. 104-Week chronic toxicity study in rats: Aniline hydrochloride. Final report. Vols I and II. Prepared by Haskell Laboratory Submitted to the United States Environmental Protection Agency. National Technical Reports Library-National Technical Information Service (NTIS). Accession No. OTS00000721. Available from: https://ntrl.ntis.gov/NTRL/, accessed 6 October 2020.

US EPA (1994). Aniline fact sheet. OPTT chemical fact sheets. EPA 749-F-95-002. United States Environmental Protection Agency. Available from:

https://nepis.epa.gov/Exe/ZyPDF.cgi/P1000IC7.PDF?Dockey=P1000IC7.PDF, accessed 4 March 2021.

US EPA (2009). Contaminant Candidate List 3 – (CCL 3). United States Environmental Protection Agency. Available from: https://www.epa.gov/ccl/contaminant-candidate-list-3-ccl-3, accessed 4 March 2021.

US EPA (2018). Hazardous air pollutants. United States Environmental Protection Agency. Available from: https://www.epa.gov/haps, accessed 4 March 2021.

US EPA (2019). List of lists. Consolidated List of Chemicals Subject to the Emergency Planning and Community Right To-Know Act (EPCRA), Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and Section 112(r) of the Clean Air Act. United States Environmental Protection Agency, Office of Land and Emergency Management. EPA 550-B-19-003. June 2019. Available from: https://www.epa.gov/sites/production/files/2015-03/documents/list_of_lists.pdf, accessed 4 March 2021.

US EPA (2019a). Aniline. DSSTox Substance. Distributed Structure-Searchable Toxicity (DSSTox) database. Available from https://comptox.epa.gov/dashboard/dsstoxdb/results?search=aniline, accessed 4 March 2021.

USEPA (2019b). Aniline hydrochloride. DSSTox Substance. Distributed Structure-Searchable Toxicity (DSSTox) database. Available from: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=aniline%20hydrochloride, accessed 4 March 2021.

US EPA (2020a). Aniline. Chemistry Dashboard. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/DTXSID8020090, accessed 3 June 2020.

US EPA (2020b). Aniline hydrochloride. Chemistry Dashboard. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/DTXSID3020091, accessed 3 June 2020.

US EPA (2020c). EPA: high production volume list. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/chemical_lists/EPAHPV#:~:text=Chemicals%20considered%20to%20be%20HPV,one%20million%20pounds%20per%20year.&text=However%2C%20EPA%20will%20mandate%20testing,Substance%20Control%20Act%20(TSCA), accessed 4 March 2021.

US EPA (2021). Aniline. Chemical activity summary. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=aniline#bioactivity, accessed 3 March 2021.

US International Trade Commission (1980a). United States production and sales 1979 (USITC Publication 1099). Washington (DC), USA: United States Government Printing Office; pp. 25, 31, 48, 65–66, 68, 71, 74, 77,80, 94.

Van Wagenen HD (1985). In-depth survey report: occupational hazard control options for chemical process unit operations at US Steel Corporation, USS Chemicals, Division Site, Haverhill, Ohio. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. OH.ECTB, 101-15bPB85-234359. Retrieved from: https://www.cdc.gov/niosh/surveyreports/pdfs/101-15b.pdf.

Vásquez-Vivar J, Augusto O (1994). Oxidative activity of primaquine metabolites on rat erythrocytes in vitro and in vivo. *Biochem Pharmacol*. 47(2):309–16. doi:10.1016/0006-2952(94)90022-1 PMID:8304975

Vigliani EC, Barsotti M (1961). Environmental tumors of the bladder in some Italian dye-stuff factories. *Med Lav*. 52:241–50. PMID:13781259

Vitzthum OG, Werkhoff P, Hubert P (1975). New volatile constituents of black tea aroma. *J Agric Food Chem*. 23(5):999–1003. doi:10.1021/jf60201a032

Vlasova II (2018). Peroxidase activity of human hemoproteins: keeping the fire under control. *Molecules*. 23(10):E2561. doi:10.3390/molecules23102561 PMID:30297621

Vogel EW, Nivard MJ (1993). Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. *Mutagenesis*. 8(1):57–81. doi:10.1093/mutage/8.1.57 PMID:8450769

Wagner JC, Sleggs CA, Marchand P (1960). Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Br J Ind Med*. 17:260–71. PMID:13782506

Wang G, Wang J, Khan MF (2017). Altered miRNA expression in aniline-mediated cell cycle progression in rat spleen. *Toxicol Mech Methods*. 27(7):511–7. doi:10.1080/15376516.2017.1324932 PMID:28463034

Wang J, Kannan S, Li H, Khan MF (2005). Cytokine gene expression and activation of NF-kappa B in aniline-induced splenic toxicity. *Toxicol Appl Pharmacol*. 203(1):36–44. doi:10.1016/j.taap.2004.07.012 PMID:15694462

Wang J, Ma H, Boor PJ, Ramanujam VM, Ansari GA, Khan MF (2010). Up-regulation of heme oxygenase-1 in rat spleen after aniline exposure. *Free Radic Biol Med*. 48(4):513–8. doi:10.1016/j.freeradbiomed.2009.11.027 PMID:19969074

Wang J, Wang G, Ansari GA, Khan MF (2008). Activation of oxidative stress-responsive signalling pathways in early splenotoxic response of aniline. *Toxicol Appl Pharmacol*. 230(2):227–34. doi:10.1016/j.taap.2008.02.022 PMID:18420242

Wang J, Wang G, Khan MF (2015). Disorder of G2-M checkpoint control in aniline-induced cell proliferation in rat spleen. *PLoS One*. 10(7):e0131457. doi:10.1371/journal.pone.0131457 PMID:26192324

Wang J, Wang G, Ma H, Khan MF (2011). Enhanced expression of cyclins and cyclin-dependent kinases in aniline-induced cell proliferation in rat spleen. *Toxicol Appl Pharmacol*. 250(2):213–20. doi:10.1016/j.taap.2010.10.026 PMID:21070798

Wang Y, Gao H, Na XL, Dong SY, Dong HW, Yu J, et al. (2016). Aniline induces oxidative stress and apoptosis of primary cultured hepatocytes. *Int J Environ Res Public Health*. 13(12):E1188. doi:10.3390/ijerph13121188 PMID:27916916

Wangenheim J, Bolcsfoldi G (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis*. 3(3):193–205. doi:10.1093/mutage/3.3.193 PMID:3045481

Ward E, Carpenter A, Markowitz S, Roberts D, Halperin W (1991). Excess number of bladder cancers in workers exposed to *ortho*-toluidine and aniline. *J Natl Cancer Inst*. 83(7):501–6. doi:10.1093/jnci/83.7.501 PMID:2005633

Ward EM, Sabbioni G, DeBord DG, Teass AW, Brown KK, Talaska GG, et al. (1996). Monitoring of aromatic amine exposures in workers at a chemical plant with a known bladder cancer excess. *J Natl Cancer Inst*. 88(15):1046–52. doi:10.1093/jnci/88.15.1046 PMID:8683635

Weinberger MA, Albert RH, Montgomery SB (1985). Splenotoxicity associated with splenic sarcomas in rats fed high doses of D & C Red No. 9 or aniline hydrochloride. *J Natl Cancer Inst*. 75(4):681–90. PMID:3862900

Westberg HB, Seldén AI, Bellander T (2001). Exposure to chemical agents in Swedish aluminum foundries and aluminum remelting plants–a comprehensive survey. *Appl Occup Environ Hyg*. 16(1):66–77. doi:10.1080/104732201456140 PMID:11202030

Westmoreland C, Gatehouse DG (1991). Effects of aniline hydrochloride in the mouse bone marrow micronucleus test after oral administration. *Carcinogenesis*. 12(6):1057–9. doi:10.1093/carcin/12.6.1057 PMID:2044184

White FR, Eschenbrenner AD, White J (1948). Oral administration of *p*-amino-dimethylaniline, aniline and *p*-aminoazobenzene and the development of tumors in rats. *Unio int contra cancrum Acta*. 6:75-78.

Williams GM (1980). The detection of chemical mutagens/carcinogens by DNA repair and mutagenesis in liver cultures. In: de Serres FJ, Hollaender A, editors. Chemical mutagens: principles and methods for their detection. Vol. 6. Boston (MA), USA: Springer US; pp. 61–79. doi:10.1007/978-1-4613-3072-1_3

Williams GM (1981). Mammalian culture systems for the study of genetic effects of N-substituted aryl compounds. *Natl Cancer Inst Monogr*. (58):237–42. PMID:7341980

Williams RT (1959). Detoxication mechanisms. New York (NY), USA: John Wiley & Sons.

Wilmer JL, Erexson GL, Kligerman AD (1984). The effect of erythrocytes and hemoglobin on sister chromatid exchange induction in cultured human lymphocytes exposed to aniline HCI. *Basic Life Sci*. 29(Pt B):561–7. PMID:6529416

Wilmer JL, Kligerman AD, Erexson GL (1981). Sister chromatid exchange induction and cell cycle inhibition by aniline and its metabolites in human fibroblasts. *Environ Mutagen*. 3(6):627–38. doi:10.1002/em.2860030605

Wiśniewska-Knypl JM, Jabłońska JK (1975). The rate of aniline metabolism in vivo in rats exposed to aniline and drugs. *Xenobiotica*. 5(8):511–9. doi:10.3109/00498257509056121 PMID:1166665

Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, et al. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. *Environ Mol Mutagen*. 36(3):163–94. doi:10.1002/1098-2280(2000)36:3<163::AID-EM1>3.0.CO;2-P PMID:11044899

Wu X, Kannan S, Ramanujam VM, Khan MF (2005). Iron release and oxidative DNA damage in splenic toxicity of aniline. *J Toxicol Environ Health A*. 68(8):657–66. doi:10.1080/15287390590921757 PMID:15901093

Xie F, Yu J, Wang S, Zhao G, Xia Q, Zhang X, et al. (2013). Rapid and simultaneous analysis of ten aromatic amines in mainstream cigarette smoke by liquid chromatography/electrospray ionization tandem mass spectrometry under ISO and "Health Canada intensive" machine smoking regimens. *Talanta*. 115:435–41. doi:10.1016/j.talanta.2013.05.048 PMID:24054615

Yamazaki H, Nakano M, Gillam EM, Bell LC, Guengerich FP, Shimada T (1996). Requirements for cytochrome b_5 in the oxidation of 7-ethoxycoumarin, chlorzoxazone, aniline, and *N*-nitrosodimethylamine by recombinant cytochrome P450 2E1 and by human liver microsomes. *Biochem Pharmacol*. 52(2):301–9. doi:10.1016/0006-2952(96)00208-0 PMID:8694855

Yamazaki J, Sato S (1937). [Experimentelle Erzeugung von Blasengeschwülsten durch Anilin und o-Amidoazotoluol bei Kaninchen]. *Japan Zeitschr Dermatol Urol*. 42:332–42. [German]

Yoon JS, Mason JM, Valencia R, Woodruff RC, Zimmering S (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen*. 7(3):349–67. doi:10.1002/em.2860070310 PMID:3930235

Yoshimi N, Sugie S, Iwata H, Niwa K, Mori H, Hashida C, et al. (1988). The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. *Mutat Res*. 206(2):183–91. doi:10.1016/0165-1218(88)90159-0 PMID:3173389

Zhu J, Aikawa B (2004). Determination of aniline and related mono-aromatic amines in indoor air in selected Canadian residences by a modified thermal desorption GC/MS method. *Environ Int*. 30(2):135–43. doi:10.1016/S0160-4120(03)00168-5 PMID:14749101

Zinin N (1842). [Beschreibung einiger neuer organischer Basen, dargestellt durch die Einwirkung des Schwefelwasserstoffes auf Verbindungen der Kohlenwasserstoffe mit Untersalpetersäure]. *J Prakt Chem*. 27(1):140–53. [German] doi:10.1002/prac.18420270125

Zwirner-Baier I, Deckart K, Jäckh R, Neumann HG (2003). Biomonitoring of aromatic amines VI: determination of hemoglobin adducts after feeding aniline hydrochloride in the diet of rats for 4 weeks. *Arch Toxicol*. 77(12):672–7. doi:10.1007/s00204-003-0473-8 PMID:14557919

CUPFERRON

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

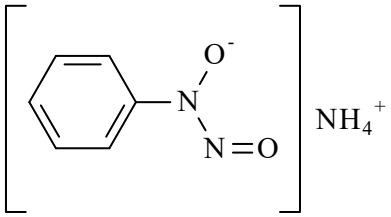
Chem. Abstr. Serv. Reg. No.: 135-20-6

EC No.: 201-183-2

IUPAC systematic name: ammonium 2-oxo-1-phenylhydrazinolate

Synonyms and abbreviations: cupferron; hydrogen cupferron; *N*-nitroso-*N*-phenylhydroxylamine; *N*-nitroso-*N*-phenylhydroxylamine ammonium salt; azanium *N*-oxido-*N*-phenylnitrous amide; ammonium *N*-nitroso-*N*-oxidoaniline; tongtielin; nitrosophenylhydroxylamine azamethane.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_9N_3O_2$

Relative molecular mass: 155.15 (NTP, 2016; ChemSpider, 2020; NCBI, 2020).

1.1.3 Chemical and physical properties of the pure substance

Description: cupferron appears as light yellow or cream-coloured crystals or a brown crystalline solid (NCBI, 2020)

Boiling point: 278.9 °C (Chemical Book, 2017)

Melting point: 163.5 °C (NCBI, 2020)

Flash point: not available; probably combustible (NCBI, 2020)

Density: 1.3092 g/cm^3 (Chemical Book, 2017)

Vapour pressure: 6.29×10^{-5} mm Hg [0.0084 Pa] at 25 °C (NTP, 2016)

Solubility: soluble in water, alcohol, and ether (NTP, 2016)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, –1.73 (NTP, 2016).

1.2 Production and use

1.2.1 Production process

Cupferron, the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine, is prepared by the reaction of phenylhydroxylamine with a nitrite source (NCBI, 2020).

1.2.2 Production volume

Cupferron is listed as an existing substance by international substance registries such as the European Chemicals Agency, the

National Industrial Chemicals Notification and Assessment Scheme, and the United States Environmental Protection Agency (US EPA), but no additional information was available to the Working Group (New Jersey Department of Health and Senior Services, 2001; ECHA, 2019; NICNAS, 2019; OECD, 2019; US EPA, 2019a).

Cupferron is currently not on the United States Toxic Substances Control Act (TSCA) Chemical Data Reporting (CDR) inventory. This information is collected every 4 years from manufacturers and importers when production volumes for the chemical are 25 000 pounds [11 300 kg] or greater in any reporting year. In 2020, cupferron was available from approximately 30 suppliers, mainly based in China and the USA (Chemical Register, 2020). The NTP Report on Carcinogens (NTP, 2016) provides additional historical context, indicating that cupferron was produced by one manufacturer in east Asia and four manufacturers in India, and was available from 28 suppliers, including 17 suppliers in the USA in 2009. Reports submitted to the US EPA under the TSCA inventory requirements from 1986 to 2002 (except in 1994) indicated that USA production plus imports of cupferron totalled about 10 000–500 000 pounds [4500–230 000 kg] (NCBI, 2020).

1.2.3 Uses

Cupferron is soluble in water and alcohol, and as a common reagent can be used to separate metals such as copper, iron, tin, vanadium, and thorium from other metals (NTP, 2016; NCBI, 2020). In analytical laboratories, cupferron is a reagent used for quantitative determination of vanadates and titanium, and the colorimetric determination of aluminium (NTP, 2016; Grabarczyk & Adamczyk, 2017; NCBI, 2020). [The Working Group noted that, despite the sizable global production volume of cupferron, little to no information was available on the distribution and levels of use across different industries and occupations.]

1.3 Measurement and analysis

There are no reported methods for chemical analysis of cupferron in exposure-relevant matrices. The purity of cupferron reagent is quantified by chemical manufacturers using high-performance liquid chromatography coupled to ultraviolet detection (HPLC-UV) at λ_{max} = 282 nm (VWR, 2020).

1.4 Occurrence and exposure

No Toxic Release Inventory (TRI) data required by the TSCA were available after 2001 for cupferron reported to be disposed of or otherwise released on-site and off-site in the USA. No recent data on occupational or general population exposures to cupferron were available. The primary routes of potential human exposure to cupferron are ingestion and inhalation of the dust of the dry salt. Dermal and eye contact are secondary routes of potential exposure (NTP, 2016; NCBI, 2020). An earlier report by the National Cancer Institute (NCI) noted that the potential for exposure appeared to be greatest among individuals engaged in analytical or research studies involving the use of cupferron. Workers may also potentially be exposed during manufacturing processes (NCI, 1978). [The Working Group noted that original references for these statements could not be identified.]

The United States National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that 136 chemical technicians in the primary metal industries were potentially exposed to cupferron (NOES, 1990).

1.5 Regulations and guidelines

Cupferron is reportable under the Emergency Planning and Community Right-To-Know Act (Section 313), or the US EPA's TRI (US EPA, 2019a), with a "de minimus" concentration of 0.1%. This "de minimus" concentration refers to the presence of cupferron in a mixture of chemicals in a regulated facility at a concentration below 0.1% of the mixture (US EPA, 2017).

The California Office of Environmental Health Hazard Assessment (OEHHA) "safe harbour" level (no-significant-risk level) is 3 µg/day (OEHHA, 1992).

No occupational exposure limits have been established for cupferron. In Germany, the first general administrative regulation for the Federal Emissions Control Act of 2002 against harmful effects from air pollution sets the maximum allowed mass concentration in exhaust gas at 20 mg/m^3. No other exposure limits, guidelines, or reference values were identified internationally (NTP, 2016; IFA, 2020).

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 (feed)

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks), received feed containing cupferron (purity, approximately 93%) at time-weighted average concentrations of 0.2% (lower dose) or 0.4% (higher dose), 7 days per week, for 78 weeks, followed by an additional observation period of 17–18 weeks. [Lower-dose and higher-dose mice were treated with 0.3% or 0.6%, respectively, for 35 weeks, then 0.1% or 0.2%, respectively, for 43 weeks, due to high mortality and excess decrease in mean body weight.] Groups of 50 male and 50 female control mice (age, 11 weeks) received feed alone for 98 weeks (NCI, 1978). There was a positive dose-related trend in mortality that was significant for both male and female mice. In males, survival to the end of the study was 42/50 (controls), 29/50 (lower dose), and 20/50 (higher dose); however, survival for at least 75 weeks was 49/50, 44/50, and 31/50, respectively. In females, survival to the end of the study was 40/50, 34/50, and 29/50, respectively. [Sufficient numbers of male and female mice survived long enough to evaluate the risk of late-developing tumours.] There was a significant dose-related decrease in mean body weight in treated male and female mice compared with controls over the course of the study. Full histopathology was performed on major tissues, organs, and gross lesions taken from mice that were killed and from mice found dead.

In male B6C3F$_1$ mice at the higher dose, there was a significant increase ($P = 0.013$) in the incidence of haemangiosarcoma of the circulatory system [not otherwise specified (NOS), mostly originating in the spleen] compared with male controls, with a significant positive trend ($P = 0.008$). There was also a significant positive trend ($P = 0.028$) and increase ($P < 0.036$) in the incidence of adenoma (NOS) of the Harderian gland at the higher dose compared with male controls. In female mice, there was a significant positive trend [$P < 0.05$] and significant increase in the incidence of haemangioma or haemangiosarcoma (combined) of the circulatory system at the lower ($P = 0.003$) and higher dose ($P = 0.044$) compared with female controls. There was also a significant positive trend ($P = 0.038$) and significant increase ($P = 0.044$) in the incidence of haemangiosarcoma of the circulatory system in females at the higher dose compared

Table 3.1 Studies of carcinogenicity with cupferron in mice and rats

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, $B6C3F_1$ (M) 6 wk (controls, 11 wk) 95–98 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.2%, 0.4%, 7 days/wk for 78 wk 50, 50, 50 42, 29, 20	*Circulatory system*: haemangiosarcoma 1/50, 3/45, 7/40*	$P = 0.008$, Cochran–Armitage trend test; *$P = 0.013$, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate. Principal limitations: TWA doses; mice were treated with 0.3% (lower dose) or 0.6% (higher dose) for 35 wk, then 0.1% (lower dose) or 0.2% (higher dose) for 43 wk, due to high mortality and excess mean body-weight decrease. A positive dose-related trend in mortality was significant for males ($P < 0.001$). Five mice in the control group were killed at wk 80 and five mice at the higher dose were killed at wk 78. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found.
		Harderian gland: adenoma, NOS 0/50, 3/45, 4/40*	$P = 0.028$, Cochran–Armitage trend test; *$P < 0.036$, 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, B6C3F$_1$ (F) 6 wk (controls, 11 wk) 95–98 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.2%, 0.4%, 7 days/wk, for 78 wk 50, 50, 50 40, 34, 29	*Circulatory system* Haemangiosarcoma 1/50, 5/47, 6/46*	$P = 0.038$, Cochran–Armitage trend test; *$P = 0.044$, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate. Principal limitations: TWA doses; mice were treated with 0.3% (lower dose) or 0.6% (higher dose) for 35 wk, then 0.1% (lower dose) or 0.2% (higher dose) for 43 wk, due to high mortality and excess mean body-weight decrease. A positive dose-related trend in mortality was significant for females ($P = 0.009$). Five mice in the control group were killed at wk 80 and five mice at the higher dose were killed at wk 78. Incidence of Zymbal gland squamous cell carcinoma or sebaceous adenocarcinoma in historical controls was 0/275. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found.
		Haemangioma or haemangiosarcoma (combined) 1/50, 10/47*, 6/46**	[$P < 0.05$, Cochran–Armitage trend test]; *$P = 0.003$, Fisher exact test; **$P = 0.044$, Fisher exact test	
		Lung Bronchioloalveolar adenoma 1/50, 8/45*, 8/46**	[$P = 0.022$, Cochran–Armitage trend test]; *[$P = 0.012$, Fisher exact test]; **[$P = 0.013$, Fisher exact test]	
		Bronchioloalveolar carcinoma 3/50, 3/45, 1/46	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 4/50, 11/45*, 9/46	*$P = 0.027$, Fisher exact test	
		Liver Hepatocellular carcinoma 2/49, 9/46*, 13/45**	$P = 0.001$, Cochran–Armitage trend test; *$P = 0.019$, Fisher exact test; **$P = 0.001$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined) 2/49, 12/46*, 16/45**	$P < 0.001$, Cochran–Armitage trend test; *$P = 0.002$, Fisher exact test; **$P < 0.001$, 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, B6C3F$_1$ (F) 6 wk (controls, 11 wk) 95–98 wk NCI (1978) (cont.)		*Harderian gland*: adenoma, NOS 0/50, 2/47, 6/46*	*P* = 0.006, Cochran–Armitage trend test; **P* = 0.010, Fisher exact test	
		Zymbal gland: squamous cell carcinoma or sebaceous adenocarcinoma (combined) 0/50, 0/47, 3/46	*P* = 0.033, Cochran–Armitage trend test	
Rat, F344 (M) 6 wk (controls, 16 wk) 97–110 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.15%, 0.30%, 7 days/wk, for 78 wk 50, 50, 49 32, 0, 0	*Circulatory system*: haemangiosarcoma 0/50, 38/49*, 35/44*	*P* < 0.001, Cochran–Armitage trend test; **P* < 0.001, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate; the schedule of exposure was adequate. Principal limitations: one rat was removed (wrong sex) from the group of males at the higher dose. There was a significant (*P* < 0.001) positive association between dosage and mortality in males. In historical controls, 11/250 (4%) of the untreated male F344 rats had subcutaneous fibroma. Five males in the control group were killed at wk 78. Statistical analyses were performed on rats that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on animals that survived at least as long as the animal in which the tumour was found. Further subtyping of liver neoplastic nodules was not possible due to the lack of original data for review.
		Liver Hepatocellular carcinoma 0/49, 8/48*, 4/43**	[*P* = 0.011, Cochran–Armitage trend test]; **P* = 0.003, Fisher exact test; ***P* = 0.044, Fisher exact test	
		Hepatocellular carcinoma or neoplastic nodules (combined) 0/49, 12/48*, 5/43**	*P* = 0.048, Cochran–Armitage trend test; **P* < 0.001, Fisher exact test; ***P* = 0.020, Fisher exact test	
		Forestomach Squamous cell carcinoma		

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 (M) 6 wk (controls, 16 wk) 97–110 wk NCI (1978) (cont.)		0/49, 19/48*, 17/38*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined)		
		0/49, 32/48*, 24/38*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Body cavities: malignant mesothelioma or mesothelioma (NOS) (combined)		
		0/50, 5/49*, 1/44	*$P = 0.027$, Fisher exact test	
		Subcutaneous tissue: fibroma		
		1/50, 15/49*, 5/44	[$P = 0.04$, Cochran–Armitage trend-test]; *$P < 0.001$, 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 (F) 6 wk (controls, 16 wk) 106–110 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.15%, 0.30%, 7 days/wk, for 78 wk 50, 50, 50 36, 8, 2	*Circulatory system*: haemangiosarcoma 0/49, 28/45*, 37/47*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	Principal strengths: adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate; the schedule of exposure was adequate; use of males and females. There was a significant ($P < 0.001$) positive association between dosage and mortality in females. Five female controls were killed at wk 78. Statistical analyses were performed on animals that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found. Further subtyping of liver neoplastic nodules was not possible due to the lack of original data for review.
		Liver Hepatocellular carcinoma 1/48, 24/44*, 10/44**	$P = 0.012$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test; **$P = 0.002$, Fisher exact test	
		Hepatocellular carcinoma or neoplastic nodules (combined) 1/48, 26/44*, 12/44*	$P = 0.004$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Forestomach Squamous cell carcinoma 0/49, 14/43*, 22/43*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined) 0/49, 19/43*, 24/43*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	

F, female; M, male; NOS, not otherwise specified; NS, not significant; TWA, time-weighted average; wk, week.

with controls. There was a significant positive trend [$P = 0.022$] and significant increase in the incidence of bronchioloalveolar adenoma in females at the lower [$P = 0.012$] and higher dose [$P = 0.013$] compared with controls (controls, 1/50; lower dose, 8/45; higher dose, 8/46). There was a small but significant increase ($P = 0.027$) in the incidence of bronchioloalveolar adenoma or carcinoma (combined) in females at the lower dose compared with controls (controls, 4/50; lower dose, 11/45, higher dose, 9/46); however, the increase in the group at the higher dose and the trend were not significant, and the incidence of bronchioloalveolar carcinoma was not significantly increased in treated female mice (controls, 3/50; lower dose, 3/45; higher dose, 1/46) [The Working Group considered that the increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) was not related to the treatment]. There was a significant increase ($P \leq 0.019$) in the incidence of hepatocellular carcinoma in females at the lower and higher dose, with a significant positive trend ($P = 0.001$). There was also a significant increase ($P \leq 0.002$) in the incidence of hepatocellular adenoma or carcinoma (combined) in females at the lower and higher dose, with a significant positive trend ($P < 0.001$), compared with controls. In female mice, a significant increase ($P = 0.010$) in the incidence of Harderian gland adenoma (NOS), with a significant positive trend ($P = 0.006$), was observed at the higher dose compared with controls. In females, there was a significant positive trend ($P = 0.033$) in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland compared with controls (NCI, 1978).

[The Working Group noted the adequate number of animals used, random allocation in groups, and the use of males and females. The duration of exposure and observation was adequate.]

3.2 Rat

Oral administration (feed)

Groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing cupferron (purity, approximately 93%) at a concentration of 0.15% (lower dose) or 0.30% (higher dose), 7 days per week, for 78 weeks and then untreated for an additional observation period of 19–28 weeks. Control groups of 50 males and 50 females (age, 16 weeks) received feed alone for 110 weeks (NCI, 1978). One rat was removed (wrong sex) from the group of males at the higher dose. There was a significant positive trend in mortality in treated male and female rats. The median survival of male rats was 63 weeks in the group at the higher dose (all rats had died by week 98), and 84 weeks in the group at the lower dose (all rats had died by week 105). In the control group, 64% (32/50) of the males survived until the end of the study. The median survival of female rats at the higher dose was 68 weeks, with two rats surviving until the end of the study. The median survival of female rats at the lower dose was 91 weeks, with eight rats surviving until the end of the study. In the control group, 72% (36/50) of the female rats survived until the end of the study. [The early mortality in treated males and females may have resulted from an increased incidence of haemangiosarcoma as early as week 42 in males and week 43 in females.] Full histopathology was performed on major tissues, organs, and gross lesions taken from rats that were killed and rats that were found dead.

In male and female rats, there was a significant increase ($P < 0.001$), with a significant positive trend ($P < 0.001$), in the incidence of haemangiosarcoma of the circulatory system (mostly originating in the spleen) at the lower and higher dose compared with controls. In male rats, the incidence of hepatocellular carcinoma or neoplastic nodules (combined) of the liver [further subtyping of the liver neoplastic

nodules was not possible due to the lack of original data for review] was significantly increased, with a significant positive trend ($P = 0.048$), at the lower dose ($P < 0.001$) and at the higher dose ($P = 0.020$); in female rats, the incidence was significantly increased, with a significant positive trend ($P = 0.004$), at the lower dose ($P < 0.001$) and at the higher dose ($P < 0.001$), compared with controls. There was also a significant positive trend [$P \leq 0.012$] and significant increase in the incidence of hepatocellular carcinoma at the lower ($P \leq 0.003$) and higher ($P \leq 0.044$) doses in male and female rats. In males and in females, there was a significant positive trend ($P < 0.001$) and a significant increase ($P < 0.001$) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach, and of squamous cell carcinoma of the forestomach at the lower and higher doses compared with controls. There was a significant increase ($P = 0.027$) in the incidence of malignant mesothelioma or mesothelioma (NOS) (combined) of the body cavities in male rats at the lower dose compared with controls. In male rats, the incidence of fibroma of the subcutaneous tissue was significantly increased ($P < 0.001$) at the lower dose compared with controls, with a significant positive trend [$P = 0.04$].

Regarding non-neoplastic lesions, basophilic foci and clear cell foci of the liver were observed only in treated male and female rats, and the incidence of focal and diffuse basal cell hyperplasia of the forestomach was significantly increased in treated male and female rats (NCI, 1978). [Hyperplasia of the forestomach may be a pre-neoplastic lesion, based on the presence of forestomach tumours.]

[The Working Group noted the adequate number of animals used, random allocation in groups, and the use of males and females. The duration of exposure and observation was adequate, and the schedule of exposure was adequate.]

3.3 Synthesis

In one study in male and female B6C3F$_1$ mice treated by oral administration (in feed), cupferron caused a significant increase, with a significant positive trend, in the incidence of haemangiosarcoma of the circulatory system and adenoma (NOS) of the Harderian gland in males; and of haemangiosarcoma and haemangioma or haemangiosarcoma (combined) of the circulatory system, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined), bronchioloalveolar adenoma, and adenoma (NOS) of the Harderian gland in females. There was also a significant positive trend in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland in females (NCI, 1978).

In one study in male and female Fischer 344 rats treated by oral administration (in feed), cupferron caused a significant increase, with a significant positive trend, in the incidence of haemangiosarcoma of the circulatory system, hepatocellular carcinoma and hepatocellular carcinoma or neoplastic liver nodules (combined), and squamous cell carcinoma and squamous cell papilloma or carcinoma (combined) of the forestomach in males and females; and of fibroma of the subcutaneous tissue in males. There was also a significant increase in the incidence of malignant mesothelioma or mesothelioma (NOS) (combined) of the body cavities in male rats (NCI, 1978).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

No information on the absorption, distribution, metabolism, or excretion of cupferron in biological systems was available to the Working Group.

4.2 Evidence relevant to the key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether cupferron is genotoxic, and alters cell proliferation, cell death or nutrient supply. For the evaluation of the other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is genotoxic

(a) Humans

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

In a test for inhibition of DNA synthesis in cultured HeLa cells (a human cervical carcinoma cell line), cupferron (in the absence of exogenous metabolic activation) inhibited replicative DNA synthesis (Heil & Reifferscheid, 1992).

(b) Experimental systems

See Table 4.1.

(i) Cytogenetic effects in mammalian cells

In tests for cytogenetic effects in Chinese hamster ovary (CHO) cells, cupferron caused significant increases in the frequency of chromosomal aberrations in two studies and sister-chromatid exchange in two studies in a concentration-related manner in the presence, but not in the absence, of rat liver S9 (NTP, 1989a, b). [The Working Group noted that the highest concentrations tested in the absence of S9 were 10 times lower than the lowest concentrations tested in the presence of S9.]

*(ii) Gene mutation and SOS/*umu *genotoxicity in bacteria*

Cupferron gave generally positive results in *Salmonella typhimurium* strain TA1538 (in the absence of S9, and in the presence of mouse, rat, or hamster liver S9) and in strain TA98 (in the absence of S9 and in the presence of rat or hamster liver S9) (Dunkel et al., 1985; NTP, 1989c; Zeiger et al., 1992). Positive mutagenic responses were obtained in *Escherichia coli* in the presence of either rat or hamster S9 (Dunkel et al., 1985). Cupferron gave negative results in tests in various other *Salmonella typhimurium* strains including TA97, TA98, TA100, TA1535, and TA1537 (Dunkel et al., 1985; NTP, 1989c; Zeiger et al., 1992).

Cupferron gave positive results in the SOS/*umu* genotoxicity assay in *Salmonella typhimurium* TA1535/pSK1002 and NM2009 strains (Reifferscheid & Heil, 1996).

Contrera et al. (2005) used electrotopological E-state indices and MDL quantitative structure–activity relationship (QSAR) software to predict the mutagenic potential of cupferron. The probability that cupferron is a member of the high-risk class was predicted to be 1 (from a range of 0 to 1).

4.2.2 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Male and female Fischer 344 rats receiving feed containing cupferron at a concentration of 0.15% or 0.3% for 78 weeks exhibited an increased incidence of focal and diffuse basal cell hyperplasia in the forestomach, a site at which treatment-related increases in tumour incidence were also observed (NCI, 1978).

4.3 Data relevant to comparisons across agents and end-points

The analysis of the in vitro bioactivity of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity

Table 4.1 Genetic and related effects of cupferron in experimental systems in vitro

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
CHO cells	Chromosomal aberrations		+	541 µg/mL	Purity, > 98 RLI; LEC was the lowest concentration tested	NTP (1989a)
CHO cells	Chromosomal aberrations		+	2000 µg/mL	Purity, > 98%; RLI; LEC was the lowest concentration tested	NTP (1989a)
CHO cells	Chromosomal aberrations	–		54 µg/mL	Purity, > 98%	NTP (1989a)
CHO cells	Sister-chromatid exchange		+	833 µg/mL	Purity, > 98%; RLI	NTP (1989b)
CHO cells	Sister-chromatid exchange		+	1500 µg/mL	Purity, > 98%; RLI; LEC was the lowest concentration tested	NTP (1989b)
CHO cells	Sister-chromatid exchange	–		83 µg/mL	Purity, > 98%	NTP (1989b)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; three positive findings with RLN, MLN, and MLI	Dunkel et al. (1985)
Salmonella typhimurium TA1538	Reverse mutation	±		1000 µg/plate	Purity, > 98%	Dunkel et al. (1985)
Salmonella typhimurium TA98	Reverse mutation	±	+	1000 µg/plate, –S9; 333 µg/plate, +S9	Purity, > 98%; one positive with HLN	Dunkel et al. (1985)
Salmonella typhimurium TA100, TA1535, TA1537	Reverse mutation	–	–	1000 µg/plate	Purity, > 98%; all negative	Dunkel et al. (1985)
Salmonella typhimurium TA1538	Reverse mutation	+		100 µg/plate	Purity, > 98%	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	166 µg/plate	Purity, > 98%; 5% HLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; 10% or 30% HLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	100 µg/plate	Purity, > 98%; 5% RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; 10% RLI	NTP (1989c); Zeiger et al. (1992)

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 TA1538	Reverse mutation		–	666 µg/plate	Purity, > 98%; 30% RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA97	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA98	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 10% HLI or RLI; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA98	Reverse mutation		+	333 µg/plate	Purity, > 98%; 5% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA100, TA1535	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1535/pSK1002 and NM2009 (SOS/*umu* test)	DNA damage	+	+	NR	Purity, NR; RLI	Reifferscheid & Heil (1996)
Escherichia coli WP2*uvrA*	Reverse mutation		+	333 µg/mL	Purity, > 98%; two positive findings with RLI, HLI	Dunkel et al. (1985)
Escherichia coli WP2*uvr*A	Reverse mutation	–	–	1000 µg/mL	Purity, > 98%; with RLN, MLN, MLI, HLN	Dunkel et al. (1985)

CHO, Chinese hamster ovary; HIC, highest ineffective concentration; HLI, hamster liver induced S9; HLN, hamster liver S9; LEC, lowest effective concentration; MLI, mouse liver induced S9; MLN, mouse liver S9; NR, not reported; RLI, rat liver induced S9; RLN, rat liver S9; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study).

Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Of the 31 ToxCast/Tox21 tests in which cupferron was active, many assay end-points are indicative of or associated with nuclear receptor binding or activation, or DNA binding. The half-maximal activity concentration (AC_{50}) values for cupferron in these assays typically ranged from 27 to 70 μM and were orders of magnitude higher than the cupferron concentration identified as the cytotoxic limit (US EPA, 2019b). The Tox21 programme reported quality control (QC) grades for the two cupferron samples tested. The QC grade for sample Tox21_201493 was "F: Caution, incorrect MW [molecular weight]; Biological activity unreliable" at T0 (the beginning of testing) and "Still under analysis" at T4 (the end of the four-month testing period). The QC grade for the other sample (Tox21_302952) was "F: Caution, incorrect MW; Biological activity unreliable" at T0 and "Fns: No sample detected; Biological activity unreliable" at T4 (NCATS, 2021). [The Working Group considered that the Tox21 tests of cupferron were non-informative, based on QC information on the substance tested in those assays. Interpretation of the ToxCast results was considered highly uncertain, based on quality control concerns regarding the substance tested, as well as observations that assay AC_{50} values exceeded the cytotoxic limit by orders of magnitude.]

5. Summary of Data Reported

5.1 Exposure characterization

Cupferron is the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine. It is a reagent used to separate metals such as copper, iron, tin, vanadium, and thorium from other metals. In 2020, cupferron was available from approximately 30 suppliers, mainly based in China and the USA. No information on uses outside the USA was identified. While there are limited data available publicly, cupferron is not listed under the requirements of the United States Environmental Protection Agency Toxic Substances Control Act Chemical Data Reporting rule, which indicates that it is produced or imported at levels below 11 300 kg/year in the USA at a single site. Occupational exposures to cupferron may occur through ingestion and inhalation of the dust of the dry salt. No recent data on occupational or general population exposures to cupferron were identified. No specific occupational or other exposure limits, guidelines, or reference values were found internationally.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Cupferron caused an increased incidence of malignant neoplasms in two species.

In $B6C3F_1$ mice, cupferron administered orally (in feed) in one study caused an increase in the incidence of haemangiosarcoma of the circulatory system in males and females, and of hepatocellular carcinoma in females. A positive trend in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland was also observed in female mice.

In Fischer 344 rats, cupferron administered orally (in feed) in one study caused an increase in the incidence of haemangiosarcoma of the circulatory system, hepatocellular carcinoma, and squamous cell carcinoma of the forestomach in males and females, and of malignant mesothelioma or mesothelioma (not otherwise specified) (combined) of the body cavities in males.

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, or excretion in humans or experimental animal systems in vivo were available.

There is consistent and coherent evidence that cupferron exhibits key characteristics of carcinogens in experimental systems. No data in humans or in experimental animals in vivo were available; however, consistent findings were seen across several test systems from different species. Cupferron is genotoxic. In the one available study in cultured human cells, cupferron inhibited DNA synthesis, an indirect indication of DNA damage, in human cervical carcinoma (HeLa) cells. In other mammalian systems in vitro, it is clastogenic in Chinese hamster ovary cells, inducing chromosomal aberrations and sister-chromatid exchanges in the presence of S9. In bacteria, cupferron causes DNA damage as shown by positive responses in the SOS/*umu* assay for DNA damage with or without rat liver S9. It is also a gene mutagen in bacteria, notably when metabolically activated by S9 derived from different rodent species. Cupferron was mutagenic in the frameshift *Salmonella typhimurium* strain TA1538 and its derivative TA98 with or without S9 derived from rat, mouse, and hamster. It was also mutagenic in *Escherichia coli* WP2 *uvrA* with S9 derived from rat and from hamster (but not from mouse). Quantitative structure-activity relationship modelling analyses predicted the mutagenic potential of cupferron.

Cupferron increased the incidence of focal and diffuse basal cell hyperplasia in the forestomach in male and female rats exposed chronically via the feed. No data on hyperplasia were available in mice exposed chronically.

Tests of cupferron in the assay battery of the Tox21 and ToxCast research programmes were uninformative.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of cupferron.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that cupferron exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

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

6.5 Rationale

The Group 2B evaluation for cupferron is based on *sufficient evidence* of cancer in experimental animals, and on *strong* mechanistic evidence. The evidence on cancer in humans was *inadequate* as no data were available. The *sufficient evidence* of carcinogenicity in experimental animals is based on an increased incidence of malignant neoplasms in two species. There is also *strong evidence* in experimental systems that cupferron exhibits key characteristics of carcinogens. Cupferron is genotoxic.

References

ChemicalBook (2017). ChemicalBook. CAS Database list. Cupferron. Available from: https://www.chemicalbook.com/ChemicalProductProperty_EN_cb5679513.htm, accessed 2 October 2020.

Chemical Register (2020). Cupferron. Suppliers by chemical/CAS number. The online chemical buyer's guide. Available from: https://www.chemicalregister.com/find/Find.asp?SearchTy=Product&SearchSu=135-20-6&SearchKe=AllKey&SearchLo=ALL&x=15&y=14, accessed 25 January 2021.

ChemSpider (2020). Cupferron profile. Royal Society of Chemistry. Available from: http://www.chemspider.com/Chemical-Structure.2006262.html?rid=6f076ac4-a946-42a4-897b-04fd4af5dbcf, accessed 25 January 2021.

Contrera JF, Matthews EJ, Kruhlak NL, Benz RD (2005). In silico screening of chemicals for bacterial mutagenicity using electrotopological E-state indices and MDL QSAR software. *Regul Toxicol Pharmacol*. 43(3):313–23. doi:10.1016/j.yrtph.2005.09.001 PMID:16242226

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. (1985). Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen*. 7(Suppl 5):1–248. doi:10.1002/em.2860070902 PMID:3905369

ECHA (2019). *N*-nitroso-*N*-phenylhydroxylamine, ammonium salt. Substance infocard. Available from: https://echa.europa.eu/substance-information/-/substanceinfo/100.004.713, accessed 25 January 2021.

Grabarczyk M, Adamczyk M (2017). A simple, fast, and inexpensive simultaneous determination of trace bismuth(III) and lead(II) in water samples by adsorptive stripping voltammetry. *J Anal Methods Chem*. 2017:1486497. doi:10.1155/2017/1486497 PMID:28932618

Heil J, Reifferscheid G (1992). Detection of mammalian carcinogens with an immunological DNA synthesis-inhibition test. *Carcinogenesis*. 13(12):2389–94. doi:10.1093/carcin/13.12.2389 PMID:1473248

IFA (2020). Cupferron. GESTIS substance database. Germany: Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance). Available from: https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp, access 25 January 2021.

NCBI (2020). Cupferron. Compound summary for CID 135465076. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Cupferron, accessed 1 October 2020.

NCI (1978). Bioassay of cupferron for possible carcinogenicity (CAS No. 135-20-6). *Natl Cancer Inst Carcinog Tech Rep Ser*. 100:1–131. PMID:12806391

NCATS (2021). Query: cupferron. Tox21 samples. Tox21 data browser. Bethesda (MD), USA: National Center for Advancing Translational Sciences. Available from: https://tripod.nih.gov/tox21/search?q=cupferron&kind=, accessed 5 February 2021.

New Jersey Department of Health and Senior Services (2001). Cupferron. Hazardous substance factsheet. Trenton (NJ), USA: New Jersey Department of Health and Senior Services. Available from: https://nj.gov/health/eoh/rtkweb/documents/fs/0545.pdf, accessed 25 January 2021.

NICNAS (2019). Benzenamine, *N*-hydroxy-*N*-nitroso-, ammonium salt (cupferron). Sydney, Australia: Australian Government, National Industrial Chemicals Notifications and Assessment Scheme. Available from: https://www.industrialchemicals.gov.au/chemicals/benzenamine-n-hydroxy-n-nitroso-ammonium-salt.

NOES (1990). National Occupational Exposure Survey 1981–83. Cincinnati (OH), USA: National Occupational Exposure Survey, National Institute for Occupational Safety and Health. [Database has not been updated since 1 July 1990]. Available from: https://web.archive.org/web/20110716084755/http:/www.cdc.gov/noes/, accessed 25 January 2021.

NTP (1989a). Cytogenetic study of cupferron in Chinese hamster ovary cell chromosome aberrations test. Chemical effects in biological systems (CEBS), under cupferron (135-20-6). Study 705717_CA. National Toxicological Program, United States Department of Health and Human Services. Available from: https://manticore.niehs.nih.gov/cebssearch/genetox/002-01916-0002-0000-1/, accessed 3 June 2020.

NTP (1989b). Cytogenetic study of cupferron in Chinese hamster ovary cell sister chromatid exchange test. Chemical effects in biological systems (CEBS), under cupferron (135-20-6). Study 705717_SCE. National Toxicological Program, United States Department of Health and Human Services. Available from: https://manticore.niehs.nih.gov/cebssearch/genetox/002-01916-0001-0000-0/, accessed 3 June 2020.

NTP (1989c). Genetic toxicity evaluation of cupferron in Salmonella/*E. coli* mutagenicity test or Ames test. Chemical effects in biological systems (CEBS), under cupferron (135-20-6). Study 353732. National Toxicological Program, United States Department of Health and Human Services. Available from: https://manticore.niehs.nih.gov/cebssearch/study/002-01916-0003-0000-2, accessed 3 June 2020.

NTP (2016). Cupferron. Report on Carcinogens, 14th Ed. Research Triangle Park (NC), USA: National Toxicological Program, United States Department of Health and Human Services. Available from: https://

ntp.niehs.nih.gov/ntp/roc/content/profiles/cupferron.pdf, accessed 25 January 2021.

OECD (2019). Cupferron. e-chem Portal. Organisation for Economic Co-operation and Development. Available from: https://www.echemportal.org/echemportal/substance-search, accessed 25 January 2021.

OEHHA (1992). Cupferron. Sacramento (CA), USA: Office of Environmental Health Hazard Assessment. Available from: https://oehha.ca.gov/chemicals/cupferron.

Reifferscheid G, Heil J (1996). Validation of the SOS/*umu* test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. *Mutat Res*. 369(3–4):129–45. doi:10.1016/S0165-1218(96)90021-X PMID:8792833

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: A strategic and operational plan for continued leadership. *ALTEX*. 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

US EPA (2017). Table II. EPCRA Section 313 Chemical list for reporting year 2017 (including toxic chemical categories). Toxics Release Inventory reporting forms and instructions. United States Environmental Protection Agency. Available from: https://www.epa.gov/sites/production/files/2018-04/documents/ry_2017_tri_chemical_list_4_24_2018.pdf, accessed 25 January 2025.

US EPA (2019a). Cupferron. DTXSID1020352. CompTox chemicals dashboard. United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1020352#details, accessed 3 June 2020.

US EPA (2019b). Cupferron. Substance registry services. United States Environmental Protection Agency. Available from: https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do?details=displayDetails&selectedSubstanceId=46607#, accessed 25 January 2021.

VWR (2020). Cupferron ≥ 98.0% (by HPLC, titration analysis). Avantor™, VWR International, LLC. Available from: https://us.vwr.com/store/product/9984131/cupferron-98-0-by-hplc-titration-analysis, accessed 25 January 2021.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ Mol Mutagen*. 19(Suppl 21):2–141. doi:10.1002/em.2850190603 PMID:1541260

LIST OF ABBREVIATIONS

4-ABP	4-aminobiphenyl
8-OHdG	8-hydroxy-2′-deoxyguanosine
AC_{50}	half-maximal activity concentration
AHR	aryl hydrocarbon receptor
ASEAN	Association of Southeast Asian Nations
BER	base excision repair
BLV	biological limit value
bw	body weight
CDK	cyclin-dependent kinases
CDR	Chemical Data Reporting
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CoA	acetyl coenzyme A
CYP	cytochrome P450
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties
EC	European Community
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
GC-ECD	gas chromatography with electron-capture detection
GC-FID	gas chromatography and flame ionization detection
GC-MS	gas chromatography-mass spectrometry
GLP	good laboratory practice
GM	geometric mean
hOGG1	human 8-oxoguanine DNA-glycosylase 1
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
IUPAC	International Union of Pure and Applied Chemistry
JEM	job-exposure matrix
LD_{50}	median lethal dose
LOQ	limit of quantification
MBT	2-mercaptobenzothiazole
MDI	4,4-methylene diphenyl diisocyanate
miRNA	microRNA
mRNA	messenger RNA
NAT	*N*-acetyltransferase

NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NIOSH	National Institute for Occupational Safety and Health
NOS	not otherwise specified
NSRL	no-significant-risk level
OEL	occupational exposure limit
OGG1	8-oxoguanine glycosylase
OSHA	Occupational Safety and Health Administration
PBN	phenyl-β-naphthylamine
PEL	permitted exposure limit
PXR	pregnane X receptor
QSAR	quantitative structure–activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	reactive oxygen species
RQ	reportable quantity
RR	relative risk
rRNA	ribosomal RNA
SCE	sister-chromatid exchange
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SRR	standardized rate ratio
TLV	threshold limit value
TRI	Toxics Release Inventory
TWA	time-weighted average
US EPA	United States Environmental Protection Agency
w/w	weight per weight

ANNEX 1. SUPPLEMENTARY MATERIAL FOR SECTION 1, EXPOSURE CHARACTERIZATION

The supplementary web-only tables presented in Annex 1 (available from: https://publications.iarc.fr/599, and listed below) were produced in draft form by the Working Group and were subsequently fact-checked and edited. Please report any errors to imo@iarc.fr.

Table S1.9 Review of exposure assessment quality in cohort studies on exposure to aniline

Table S1.10 Review of exposure assessment quality in case–control studies on exposure to aniline

ANNEX 2. SUPPLEMENTARY MATERIAL FOR SECTION 2, CANCER IN HUMANS

The supplementary web-only table presented in Annex 2 (available from: https://publications.iarc.fr/599, and listed below) was produced in draft form by the Working Group and subsequently fact-checked and edited. Please report any errors to imo@iarc.fr.

Table S2.3 Individual case reports and case series on cancer and exposure to aniline

SUMMARY OF FINAL EVALUATIONS

Summary of final evaluations for Volume 127

Agent	Evidence stream			Overall evaluation
	Cancer in humans	Cancer in experimental animals	Mechanistic evidence	
ortho-Anisidine	*Inadequate*		*Strong*[a,b]	Group 2A[c]
ortho-Anisidine hydrochloride	*Inadequate*	*Sufficient*		Group 2A[c]
ortho-Nitroanisole	*Inadequate*	*Sufficient*	*Strong*[a,b]	Group 2A
Aniline	*Inadequate*		*Strong*[a,b]	Group 2A[d]
Aniline hydrochloride	*Inadequate*	*Sufficient*		Group 2A[d]
Cupferron	*Inadequate*	*Sufficient*	*Strong*[b]	Group 2B

[a] There is *strong evidence* that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified as *carcinogenic to humans*.
[b] There is *strong evidence* that the agent exhibits key characteristics of carcinogens in experimental systems.
[c] *ortho*-Anisidine hydrochloride exists in equilibrium with *ortho*-anisidine; therefore, the classification of carcinogenic hazard applies to both *ortho*-anisidine and its hydrochloride form.
[d] Aniline hydrochloride exists in equilibrium with aniline; therefore, the classification of carcinogenic hazard applies to both aniline and its hydrochloride form.

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of six chemicals: *ortho*-anisidine and *ortho*-anisidine hydrochloride, *ortho*-nitroanisole, aniline and aniline hydrochloride, and cupferron.

ortho-Anisidine, and its salt, *ortho*-anisidine hydrochloride, are mainly used as chemical intermediates in the synthesis of azo pigments and dyes for consumer products, textiles, paper, and cardboard.

ortho-Nitroanisole is used primarily as a precursor for the manufacture of *ortho*-anisidine.

Aniline, the parent compound of aniline hydrochloride, is a High Production Volume chemical used in the synthesis of isocyanates, dyes and pigments, and rubber-processing chemicals, and in the production of pharmaceuticals, herbicides, fungicides, and of many consumer goods, including textiles, leather, and colourants, including tattoo ink. Tobacco smoke is a main source of exposure to aniline in the general population.

Cupferron is a reagent used to separate metals such as copper, iron, tin, vanadium, and thorium from other metals.

For all agents, data were sparse regarding exposure levels, but indicated that exposures are higher in occupational situations than in the general population.

An *IARC Monographs* Working Group reviewed evidence from cancer studies in humans, cancer bioassays in experimental animals, and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- *ortho*-anisidine and *ortho*-anisidine hydrochloride, *ortho*-nitroanisole, and aniline and aniline hydrochloride are *probably carcinogenic to humans (Group 2A)*
- cupferron is *possibly carcinogenic to humans (Group 2B)*.

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