IARC MONOGRAPHS

COBALT, ANTIMONY COMPOUNDS, AND WEAPONS-GRADE TUNGSTEN ALLOY

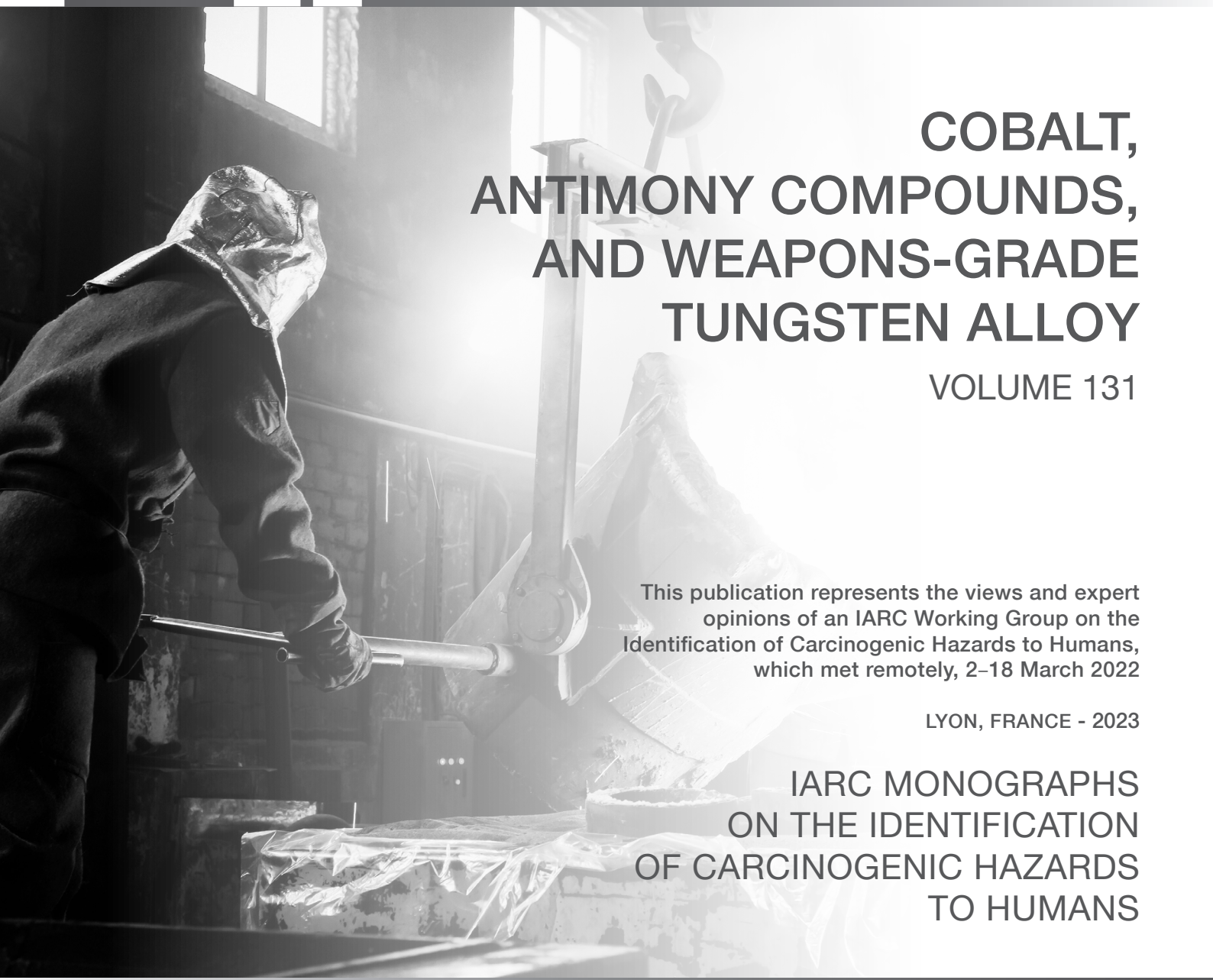
VOLUME 131

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



COBALT, ANTIMONY COMPOUNDS, AND WEAPONS-GRADE TUNGSTEN ALLOY

VOLUME 131

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 2–18 March 2022

LYON, FRANCE - 2023

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.who.int/.

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

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About the cover: Workers involved in smelting processes are potentially exposed to cobalt metal and cobalt compounds, and to antimony.

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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, 25 avenue Tony Garnier, CS 90627, 69366 Lyon CEDEX 07, France, or via email at imo@iarc.who.int, 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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[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. Invited Specialists do not serve as Meeting Chair or Subgroup Chair, draft text that pertains to the description or interpretation of cancer or mechanistic data, or participate in the evaluations. Each participant was asked to declare potentially relevant research, employment, and financial interests that are current or that have occurred during the past 4 years. Minimal interests are not disclosed here, and include stock valued at no more than US$ 1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All other non-publicly funded grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are disclosed as potentially significant conflicts of interest.

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[3] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.

[4] Craig John Boreiko attended as an Observer for the International Antimony Association (IAA), Belgium. He is employed by CJB Risk Analysis as a consultant for the IAA and the Nickel Institute (amount not disclosed) on cancer, genotoxicity, and related issues. On behalf of these organizations, he provides expert opinions on regulatory policy related to antimony to numerous governmental organizations, including the European Chemicals Agency and the United States Environmental Protection Agency (US EPA). He notes that IARC classifications may have an adverse impact on his close professional colleagues in industry sectors that produce and use metals.

[5] Samuel Buxton is a salaried employee of, and attended as an Observer for, NiPERA Inc., Nickel Institute, USA.

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[6] Ranulfo Lemus Olalde is a salaried employee of, and attended as an Observer for, the International Tungsten Industry Association (ITIA), UK. He has provided numerous public statements and positions regarding carcinogenicity classifications on behalf of ITIA for tungsten metal and compounds (including hard metals containing cobalt with tungsten carbide) to governmental bodies in Australia, the European Union, Japan, the Republic of Korea, and the USA. He consults for an unnamed tobacco company (amount not disclosed) on the registration of tobacco products in the USA.
[7] Vanessa Viegas is a salaried employee of, and attended as an Observer for, the Cobalt Institute Ltd (a trade association for the cobalt industry) and Cobalt REACH Consortium Ltd. She receives substantial research funding and monetary support from these organizations for travel to meetings with regulatory authorities. She has provided expert opinions on REACH restrictions proposed for five cobalt salts, on a cobalt data submission to the Texas Commission on Environmental Quality, and on the US EPA's draft Contaminant Candidate List 5.

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.who.int/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.who.int/).

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 effects", are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by

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).

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.

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GENERAL REMARKS

This one-hundred-and-thirty-first volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of cobalt metal (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, cobalt(II) oxide, cobalt(II,III) oxide, cobalt(II) sulfide, other cobalt(II) compounds, trivalent antimony, pentavalent antimony, and weapons-grade tungsten (with nickel and cobalt) alloy. For cobalt metal and the cobalt oxides, particles of all sizes were included in the evaluation. Due to the coronavirus disease (COVID-19) pandemic, this meeting was held remotely.

Cobalt and cobalt compounds were most recently evaluated by the *IARC Monographs* programme in 2006 (IARC, 2006). Cobalt metal without tungsten carbide, as well as cobalt sulfate and other soluble cobalt(II) salts were classified as *possibly carcinogenic to humans (Group 2B)*. Metallic cobalt with tungsten carbide (used in the hard-metal industry) was classified as *probably carcinogenic to humans (Group 2A)*. Different cobalt-based alloys were evaluated by the *IARC Monographs* programme in 1999 (IARC, 1999). Implanted foreign bodies of cobalt-based alloys were evaluated as *not classifiable as to their carcinogenicity to humans (Group 3)* (IARC, 1999). Antimony trioxide (Sb_2O_3) was previously evaluated as *possibly carcinogenic to humans (Group 2B)* (IARC, 1989a). Two of these agents – pentavalent antimony and weapons-grade tungsten (with nickel and cobalt) alloy – were evaluated by the Working Group for the first time.

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that cobalt and cobalt compounds, and weapons-grade tungsten (with nickel and cobalt) alloy be evaluated with high priority; and antimony trioxide with medium priority (IARC, 2019a; Marques et al., 2019). A summary of the findings of this volume appears in *The Lancet Oncology* (Karagas et al., 2022).

The history of cobalt classification by the *IARC Monographs* programme

The *IARC Monographs* programme was originally due to consider cobalt at the meeting for Volume 49 (Chromium, nickel, and welding) in 1989; however, during the preparation of that meeting, it was decided the time available was insufficient to consider this topic adequately. In 1991, for Volume 52, the Working Group considered metallic cobalt, cobalt alloys (including cobalt-containing medical implants), and cobalt compounds (but not organic cobalt-containing agents such as vitamin B_{12}) (IARC, 1991). At this time, the evidence for the carcinogenicity of cobalt and cobalt compounds in humans was found to be *inadequate*, and the evaluation

was based mainly on the results of studies in experimental animals, with *sufficient* evidence for the carcinogenicity of cobalt metal powder and cobalt(II) oxide, and *limited* evidence for cobalt(II) chloride and metal alloys containing cobalt, chromium, and molybdenum. The overall evaluation was that cobalt and cobalt compounds were *possibly carcinogenic to humans (Group 2B)*.

Cobalt was subsequently evaluated as a component of metallic implants and foreign bodies for Volume 74 (Surgical implants and other foreign bodies) in 1999 (IARC, 1999). At that time there was *inadequate* evidence for the carcinogenicity of metallic implants and metallic foreign bodies in humans. In experimental animals, there was *sufficient* evidence for the carcinogenicity of implants of metallic cobalt, metallic nickel, and nickel alloy powder containing approximately 66–67% nickel, 13–16% chromium, and 7% iron (which were classified as Group 2B, *possibly carcinogenic to humans*), and *limited* evidence for the carcinogenicity of implants of alloys containing cobalt and alloys containing nickel, other than the specific aforementioned alloy. Overall, orthopaedic implants of complex composition and cardiac pacemakers were each evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)*.

By Volume 86 (Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide, and vanadium pentoxide) (IARC, 2006), the *IARC Monographs* programme was able to evaluate cobalt in hard metals (with or without tungsten carbide) because of the availability of new epidemiology studies on workers in the hard-metal production industry. The evaluation of cobalt sulfate was updated because a new inhalation bioassay on cobalt sulfate heptahydrate had become available. The Working Group found that the evidence for cancer in humans was *limited* for cobalt metal with tungsten carbide and *inadequate* for cobalt metal without tungsten carbide. In experimental animals, there was *sufficient* evidence for cobalt sulfate and cobalt metal powder, and *limited* evidence for metal alloys containing cobalt. Overall, cobalt metal with tungsten carbide was evaluated as *probably carcinogenic to humans (Group 2A)*, whereas cobalt metal without tungsten carbide was *possibly carcinogenic to humans (Group 2B)*. Cobalt sulfate and other soluble cobalt(II) salts were *possibly carcinogenic to humans (Group 2B)*.

For the present volume, new evidence on cobalt was available in the form of bioassay and mechanistic data and new epidemiological studies of cobalt exposure without tungsten carbide in occupational cohorts and the general population. The Working Group elected not to re-evaluate cobalt metal with tungsten carbide because of a lack of new informative studies that could have changed the classification (IARC, 2019a).

Evaluation of trivalent and pentavalent antimony

In the present volume, the Working Group evaluated trivalent antimony and pentavalent antimony separately. There were some data suggesting that there is interconversion of pentavalent antimony and trivalent antimony, but this was overall considered to be an evidence gap. In humans given a single intramuscular injection, about 23.3% of the administered pentavalent antimony compound was reduced to trivalent antimony in the blood (Vásquez et al., 2006). In monkeys given repeated intramuscular injections of a pentavalent antimony compound, the proportion of trivalent antimony in the plasma increased from 5% to 50% between day 1 and day 9 (Friedrich et al., 2012). In experimental systems, in vitro reduction occurred in the presence of glutathione, cysteine, or cysteinyl-glycine (Frézard et al., 2001). The valence state of

antimony also affects its distribution and excretion (NTP, 2018).

The Working Group noted differences in the genotoxic effects induced by the two valent forms. There was consistent and coherent evidence in human primary cells in multiple studies and in human cell lines that trivalent antimony induces DNA damage, chromosomal aberrations, micronucleus formation, and/or increased frequency of sister-chromatid exchange (Paton & Allison, 1972; Gebel et al., 1997; Elliott et al., 1998); however, the findings for genotoxicity were mixed in mice and rats. For pentavalent antimony, the results were mixed in human primary cells (Migliore et al., 1999; Lima et al., 2010), but consistent in mice. Pentavalent antimony induced DNA damage and micronucleus formation in mice exposed by intraperitoneal injection in several studies (Lima et al., 2010; Cantanhêde et al., 2015; Moreira et al., 2017; de Jesus et al., 2018).

Considering that the standard treatment for leishmaniasis in humans involves the administration of pentavalent antimony compounds and that there is suggestive evidence that these pentavalent forms exhibit key characteristics of carcinogens, the Working Group concluded that it was important to evaluate pentavalent antimony and trivalent antimony separately.

Exposure data for cobalt and antimony

Identification of the agent

While chemical names may be reported as a "synonym" of a particular agent, such reporting may not always be accurate, and relevant papers may be silent on characterizations, such as speciation, particle size, or trace element impurities that may be critical to informing understanding of the reactivity or toxicity of the agent within the product or of the product overall (Gaultieri, 2017). In studies of human exposure, many of the analytical methods used quantified total cobalt, and did not allow for the identification of different species or types of compound. This is particularly relevant to occupational settings and where exposures are mainly through inhalation of airborne particles. Similarly, the Working Group noted that most of the available data on antimony exposure of the general population consisted of measurements of total antimony concentrations (e.g. in urine), which reflect exposure to any of the individual compounds within the scope of this evaluation. For population-based studies, without information on the exposure source, it is difficult to attribute total antimony concentrations to a particular agent.

Paucity and representativeness of the data

The data on exposure reported in this volume may not be fully comprehensive of industries and exposure contexts in which cobalt and antimony are currently being used. While several publications reported antimony concentrations in the environment (soil, sediment, water including drinking-water, and food), few quantitative data were available on occupational exposure in mining. There has been increasing use of cobalt in battery manufacture in recent years. However, there is lack of data on cobalt exposure in the battery-manufacturing or electroplating industries.

There are data indicating that mining and refining of both cobalt and antimony are widespread throughout the world, but few exposure or epidemiology studies were available in these contexts.

Few data were available on exposure to nanoparticles. Many studies in humans did not provide details on the size distribution of particulate exposures. The use of biological samples,

as was the case in many studies, cannot provide insight into this.

There was sparse information available to the Working Group on the role of ingestion resulting from hand-to-mouth contact as an exposure route in the workplace.

Geographical and sociodemographic aspects

People with low incomes are more likely to live in polluted areas and be exposed to unsafe food, drinking-water, and consumer products (Vaccarella et al., 2019). However, few data were available on cobalt exposure in different socioeconomic strata. There is a particularly notable gap in exposure data among children and elderly people, who may be at higher risk of health problems related to pollutants.

Exposure data for weapons-grade tungsten (with nickel and cobalt) alloy

Few exposure data for this alloy were available to the Working Group. The information on current and historical use of the alloy within munitions around the world is also sparse. This leads to challenges in identifying groups of people who may potentially be exposed during production, combat, or training. Exposure assessment data, where available, are often limited to measuring the concentrations of each individual element contained in the alloy.

The Working Group also noted that there was little information available on the relative concentrations of the tungsten, nickel, and cobalt metals and metallic species involved in exposure to the alloy.

Lack of epidemiological studies in low- and middle-income countries

The Working Group noted a lack of epidemiological studies available from low- and middle-income countries, where personal safety measures and working environments may be different from those in high-income countries.

There was also a lack of epidemiological studies of workers involved in mining activities and of those environmentally exposed from mining operations. In certain parts of the world, such as in the African Copperbelt, there are a few studies suggesting that people may be occupationally and environmentally exposed to elevated levels of cobalt. However, although a few biomonitoring surveys have indicated elevated cobalt concentrations in biological specimens collected from workers at some mining sites, the Working Group was unable to identify cancer epidemiology studies or mechanistic studies in humans in these places.

Other issues with epidemiological studies

The epidemiological studies evaluated in the present volume were largely occupational studies of workers exposed to cobalt metal and to trivalent antimony. Studies of the general population reported on concentrations of biomarkers of exposure to cobalt and antimony, including in the urine, plasma, and toenail clippings, without specification of chemical form. Levels in urine and plasma represent relatively short-term biomarkers, whereas levels in toenails generally reflect exposure in the previous 3–12 months. The reliability of these markers (i.e. in comparison with other measures) has been studied to a limited extent, and scant data were available on the reproducibility of these measures over time.

Two studies included in this volume suggest that toenail clipping measurements in women are correlated with exposure to cobalt over a period of several years (Garland et al., 1993; O'Brien et al., 2019).

Some of the available population-based studies had limited statistical power due to the narrow (and vastly lower) range of exposure compared with that in occupational studies. It was not possible to assess the role of dietary exposures in any of the cancer epidemiology studies.

For epidemiological studies of cobalt metal in the hard-metal industry, the main challenge was intractable confounding by co-exposure to the composite material, cobalt with tungsten carbide.

Role of nanoparticles in cancer pathology

Regular exposure to airborne particles is known to cause cancer (e.g. IARC, 2015). Most of the effects of nanoparticles are long-term and difficult to discern from those of microparticles. There is an arbitrary and loose definition of nano-dimensionality, which is usually that one dimension of the particle should measure less than 100 nm (or 0.1 μm). Microparticles are particles with a size ranging from about 1 to 1000 μm. Microparticles and nanoparticles may be either natural or man-made. For any given particle exposure setting, determining which is the dominant mechanism of tissue damage that could potentially lead to carcinogenesis may be challenging. The inhalation of microparticles and nanoparticles is associated with reactive changes (i.e. inflammation) in the epithelium of the upper respiratory tract (microparticles) and alveolar changes in the epithelium of the lower respiratory tract (nanoparticles and respirable microparticles). Pro-inflammatory signalling, inflammasome activation, and modulation of programmed cell death are topics of intense research at the present time. It has been proved that inert particles in a large range of sizes (with a diameter of more than 50 nm to micron-sized particles) can convey therapeutic antigens and become ensnared in the airways (Morris et al., 2017; Jalikis & Reyes Gil, 2019; Mishra & Singh, 2020; Singhvi et al., 2020). In conclusion, nanoparticle size-dependent effects will need to be addressed in detail in future monographs on particles, as far as the available studies on nanoparticles will allow, as noted in the Report of the Advisory Group to Recommend Priorities for the *IARC Monographs* during 2020–2024 (IARC, 2019a).

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of the evaluated agents 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 compound cobalt(II) sulfate heptahydrate was considered active in small numbers of assay end-points mapped to two key characteristics of carcinogens (Smith et al., 2016): "induces oxidative stress" (two assays), and "modulates receptor-mediated effects" (one assay). The four antimony(III) compounds, including acetic acid, antimony(III) salt; antimony(III) potassium tartrate trihydrate; antimony(III) trichloride; and antimony(III) potassium tartrate hydrate, were tested in assays mapped to the key characteristics "is genotoxic", "induces oxidative stress", "modulates receptor-mediated effects", and "alters cell proliferation, cell death, or nutrient supply". The results were considered uninformative, except for the key characteristic "modulates

receptor-mediated effects". The antimony(IV) compounds and antimony(V) sulfide were inactive for any key characteristic, while the results for antimony(V) compounds were uninformative for all key characteristics.

The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits; Reisfeld et al., 2022). The mapping of assay end-points to each key characteristic follows that described in *IARC Monographs* Volume 123 (IARC, 2019b). All ToxCast/Tox21 data were obtained from the United States Environmental Protection Agency CompTox Chemicals Dashboard 10th Release (US EPA, 2022) at the time of the evaluations performed for the *IARC Monographs* Volume 131 in March 2022. These programmes are constantly being improved and new assays are added over time. However, at present, the general lack of metabolic activation and the small number of genotoxicity assays in these high-throughput screening programmes restrict their value in determining whether a chemical is genotoxic as part of an assessment of carcinogenicity.

Scope of systematic review

Standardized searches of the PubMed database (NCBI, 2021) 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 for the agent, including the full set of search terms for the agent name and each outcome type, are available online.[1]

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[1] The literature trees for the present volume are available at: https://hawcproject.iarc.who.int/assessment/678/ (cobalt and cobalt compounds), https://hawcproject.iarc.who.int/assessment/673/ (trivalent and pentavalent antimony), https://hawcproject.iarc.who.int/assessment/677/ (weapons-grade tungsten (with nickel and cobalt) alloy).

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COBALT METAL (WITHOUT TUNGSTEN CARBIDE) AND SOME COBALT COMPOUNDS

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature, synonyms, trade names, molecular formulae, and relative molecular mass

The agents considered in the present monograph include metallic cobalt (Co) (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, and the relatively insoluble compounds cobalt(II) and cobalt(II,III) oxides (CoO and Co_3O_4, respectively) and cobalt(II) sulfide (CoS). Metallic cobalt in the composite material cobalt with tungsten carbide (WC-Co), used in the hard-metal industry, has been described previously in *IARC Monographs* Volume 86 (IARC, 2006) and is not within the scope of this volume. Metallic implants containing cobalt alloys are beyond the scope of this volume and are described in *IARC Monographs* Volume 74 (IARC, 1999). Vitamin B_{12}, non-ionic organometalloid compounds containing cobalt, and radioisotopes of cobalt are also beyond the scope of this volume.

Cobalt is a naturally occurring metallic element. It is one of the first-row transition metal members of group VIII of the periodic table, members of which include iron (Fe) and nickel (Ni). The relative atomic mass of cobalt is 58.93. Only one of its isotopes, ^{59}Co, is stable and occurs naturally. There are approximately 26 known radioactive cobalt isotopes, among which only two are of commercial importance, i.e. ^{60}Co and ^{57}Co. The former provides a widely used source of radioactivity for applications in food sterilization, radiography, and radiotherapy. The latter is the most widely used isotope in γ-resonance spectroscopy (Donaldson & Beyersmann, 2011). These radioactive forms of cobalt are outside the scope of this monograph but are classified in IARC Group 1 (*carcinogenic to humans*), as described in *IARC Monographs* Volume 100D (IARC, 2012).

Pure metallic cobalt has limited uses, but it is a strategically important metal because of its use as an alloying element and as a source in chemical production (Donaldson & Beyersmann, 2011). Nomenclature, synonyms and trade names, molecular formulae, and the relative molecular masses for these cobalt compounds and salts are presented in Table 1.1 (IARC, 1991; NCBI, 2021a). The cobalt compounds and salts given in Table 1.1 are not an exhaustive list, nor are they necessarily the most commercially important cobalt-containing substances.

Table 1.1 Registry numbers, synonyms and trade names, molecular formulae, and relative molecular masses for cobalt metal and cobalt(II) salts and compounds

Chemical name	CAS No.[a]	Synonyms and trade names	Formulae[b]	Relative molecular mass[c]
Cobalt metal				
Cobalt (NCBI, 2021b)	7440-48-4 [177256-35-8; 184637-91-0; 195161-79-6; 1245817-40-6; 1262528-32-4; 132965-60-7; 335349-43-4]	CI 77320; cobalto; cobalt moncation; cobalt, elemental; cobalt-59; cobaltum; cobalt powder; cobalt metal powder; cobalt fume; cobalt foil; fine cobalt powder; cobalt nanoparticles; cobalt powder; cobalt nanofoil; cobalt nanorods; cobalt nanopowder; cobalt nanowires; cobalt rod; cobalt wire, kobalt	Co	58.93
Soluble cobalt(II) salts				
Cobalt(II) acetate (NCBI, 2021j)	71-48-7 [33327-32-1; 68279-06-1; 73005-84-2; 256431-41-1; 2649269-82-7]	Acetic acid, cobalt(2+) salt; acetic acid, cobalt salt; bis(acetato)cobalt; cobalt acetate; cobalt(2+) acetate; cobalt diacetate; cobalt(2+) diacetate; cobalt di(acetate); cobaltous acetate; cobaltous diacetate	$Co(CH_3CO_2)_2$	177.02
Cobalt(II) acetate tetrahydrate (NCBI, 2021k)	6147-53-1	Cobaltous acetate tetrahydrate; cobalt acetate tetrahydrate; cobalt diacetate tetrahydrate; cobalt(2+);diacetate;tetrahydrate; cobalt(cento) acetate tetrahydrate; bis(acetato)tetraquacobalt; acetic acid, cobalt(2+) salt, tetrahydrate; cobalt diacetate-tetrahydrate	$Co(CH_3CO_2)_2{\cdot}4H_2O$	249.08
Cobalt(II) chloride (NCBI, 2021l)	7646-79-9	Cobalt chloride ($CoCl_2$); cobalt dichloride; cobaltous chloride; cobalt chloride anhydrous; cobalt(II) chloride hydrate	$CoCl_2$	129.84
Cobalt(II) chloride hexahydrate (NCBI, 2021m)	7791-13-1	Cobalt chloride hexahydrate; cobalt dichloride hexahydrate; cobalt chloride hydrate; cobalt(II) chloride hexahydrate; cobaltous chloride, hexahydrate; cobalt chloride, hexahydrate; cobalt(2+) dichloride hexahydrate	$CoCl_2{\cdot}6H_2O$	237.93
Cobalt(II) nitrate (NCBI, 2021n)	10141-05-6 [14216-74-1]	Cobalt bis(nitrate); cobalt dinitrate; cobalt(2+) dinitrate; cobalt(2+) nitrate; cobalt nitrate salt; cobaltous nitrate; nitric acid, cobalt(2+) salt; nitric acid, cobalt salt	$Co(NO_3)_2$	182.94
Cobalt(II) nitrate hexahydrate (NCBI, 2021o)	10026-22-9	Cobaltous nitrate hexahydrate; cobalt nitrate hexahydrate; cobalt dinitrate hexahydrate; cobalt(2+) nitrate hexahydrate; cobalt(II) nitrate hexahydrate; dinitrate cobalt hexahydrate; cobalt(2+), hexaaqua-, dinitrate; cobaltous nitrate 6-hydrate	$Co(NO_3)_2{\cdot}6H_2O$	291.04
Cobalt(II) sulfate (NCBI, 2021p)	10124-43-3 [139939-65-4]	Cobalt monosulfate; cobaltous sulfate; cobalt sulfate (1:1); cobalt(2 +) sulfate; cobalt sulfate; cobalt sulfate; cobalt(II) sulfate; sulfuric acid, cobalt(2+) salt (1:1); cobalt(II) sulfate, anhydrous; cobalt brown	$CoSO_4$	155.00
Cobalt(II) sulfate heptahydrate (NCBI, 2021q)	10026-24-1	Cobalt monosulfate heptahydrate; cobalt sulfate heptahydrate; cobalt(II) sulfate (1:1), heptahydrate; cobalt(2+) sulfate heptahydrate; cobaltous sulfate heptahydrate; sulfuric acid, cobalt(2+) salt, hydrate (1:1:7); cobalt sulfate heptahydrate; sulfuric acid, cobalt(2+) salt (1:1), heptahydrate	$CoSO_4{\cdot}7H_2O$	281.11

Table 1.1 (continued)

Chemical name	CAS No.[a]	Synonyms and trade names	Formulae[b]	Relative molecular mass[c]
Cobalt(II) octanoate (NCBI, 2021r; NCBI, 2021s)	1588-79-0; 6700-85-2 [13595-74-9; 16971-12-3; 20161-50-6]	Cobalt octanoate; cobalt(2+);octanoate; cobalt dioctanoate; cobaltous octanoate; octanoic acid cobalt salt; bisoctanoic acid cobalt(II) salt	$C_{16}H_{30}CoO_4$	345.34
Cobalt(II) 2-ethylhexanoate (NCBI, 2021t)	136-52-7 [221315-92-0, 25360-65-0, 87947-13-5]	Cobalt octoate; cobaltous octoate; cobalt bis(2-ethylhexanoate); cobaltous 2-ethylhexanoate; cobalt 2-ethylhexanoate; cobalt 2-ethylhexanoate; hexanoic acid, 2-ethyl-, cobalt(2+) salt; 2-ethylhexanoic acid cobalt salt; cobalt(2+); 2-ethylhexanoate; hexanoic acid, 2-ethyl-, cobalt(2+) salt (2:1); cobalt 2-ethylcaproate; 2-ethylhexanoic acid cobalt(2+) salt; cobalt(II) bis(2-ethylhexanoate); cobalt(2+) bis(2-ethylhexanoate)	$C_{16}H_{30}CoO_4$	345.34
Insoluble cobalt(II or II,III) compounds				
Cobalt(II) oxide (NCBI, 2021u)	1307-96-6 [185461-93-2; 186373-01-3]	CI 77322; CI Pigment Black 13; cobalt black; cobalt monoxide; cobalt monooxide; cobaltous oxide; cobalt oxide (CoO); cobalt(2+) oxide; monocobalt oxide; zaffre	CoO	74.93
Cobalt(II,III) oxide (NCBI, 2021v)	1308-06-1	Cobaltic-cobaltous oxide; cobalto-cobaltic oxide; cobalto-cobaltic tetroxide; cobaltosic oxide; cobalt oxide (Co_3O_4); cobalt tetraoxide; tricobalt tetraoxide; tricobalt tetroxide (IARC, 1991)	Co_3O_4	240.80
Cobalt(II) hydroxide (NCBI, 2021w)	21041-93-0 [12672-51-4]	Cobalt(2+)hydroxide; cobalt dihydroxide; cobalt hydroxide ($Co(OH)_2$); cobalthydroxide; cefamandolenafate	CoH_2O_2	92.95
Cobalt(II) sulfide (NCBI, 2021x)	1317-42-6	Cobalt monosulfide; cobaltous sulfide; cobalt(2+) sulfide; sulfanylidenecobalt; cobalt sulphide	CoS	91.00
Organic cobalt(II) compounds				
Cobalt(II) resinate (NCBI, 2021y)	68956-82-1 (ECHA, 2021)	Cobaltous resinate; 1,4a-dimethyl-7-propan-2-yl-2,3,4,4b,5,6,10,10a-octahydrophenanthrene-1-carboxylate; cobalt(2+)	$C_{40}H_{58}CoO_4$	661.8
Cobalt(II) acetyl acetonate (NCBI, 2023)	123334-29-2	Cobalt(II) 2,4-pentanedionate; bis(2,4-pentanedionato)cobalt(II)	$C_{10}H_{14}CoO_4$	257.15
Cobalt(II) oxalate (NCBI, 2021z)	814-89-1	Cobalt; oxalic acid; ethanedioic acid	$C_2H_2CoO_4$	146.95

CAS No., Chemical Abstracts Services Registry number; CI, Colour Index.

[a] Deleted CAS Nos are shown in square brackets.

[b] Chemical formulae of cobalt alloys are shown as a list of constituent elements using square brackets.

[c] Relative molecular masses of cobalt alloys are shown as the sum of atomic masses of constituent elements calculated by the Working Group.

1.1.2 Chemical and physical properties of metallic cobalt and cobalt compounds

Cobalt is a silver-grey, shiny, hard, and ductile metal element. Cobalt commonly occurs in the 0, +2, and +3 valence states. Metallic cobalt that is commonly used in various metal alloys is in the 0 valence state, whereas cobalt in compounds occurs in two predominant oxidation states (+2 and +3 valence states). The +2 valence state (Co^{2+}) is the most common valence state in commercially available cobalt compounds and in the environment (Patnaik, 2003). Cobalt is a metal component of vitamin B_{12}, also known as cyanocobalamin, which is required for the production of erythrocytes. Other cobalt compounds are described as toxic for the environment and the human body after excessive exposure (Leyssens et al., 2017). Cobalt(II) is far more stable in normal aqueous conditions, although cobalt(III) may be stabilized by changing the ligand environment. Water-soluble cobalt compounds release cobalt(II) ions into solution that can, in turn, form various complexes with organic or inorganic anions, with equilibrium conditions depending on redox potential (E_h), pH, and the presence of anions (Krupka & Serne, 2002).

Cobalt(II) forms a wide range of simple and hydrated salts that comprise all the common anions, such as acetate, bromide, carbonate, chloride, fluoride, nitrate, perchlorate, and sulfate. Several of the hydrated salts and their solutions contain the pink octahedral $[Co(H_2O)_6]^{2+}$ ion (Donaldson & Beyersmann, 2011). When heated, cobalt is first oxidized to cobalt(II,III) oxide, then forms cobalt(III) oxide at temperatures above 900 °C. Cobalt(II) sulfide is formed by heating cobalt metal with hydrogen sulfide at 700 °C. Cobalt(II) hydroxide ($Co(OH)_2$) is a product of the hydrolysis of solutions containing Co^{2+} ions. These compounds are insoluble in water (see Table 1.2, which presents selected chemical and physical properties of metallic cobalt and cobalt compounds covered in this monograph) (Donaldson & Beyersmann, 2011).

1.1.3 Technical grade and impurities

(a) Cobalt metal

Metallic cobalt powders are widely produced in high-purity form for use in the hard-metal industry, the manufacturing of superalloys, and in other applications. Commercial metallic cobalt powders are available in purities ranging from 99% to ≥ 99.999% in many grades, particle size ranges, and forms (IARC, 2006). The agents considered in this monograph exclude WC-Co. In these hard metals, metallic cobalt powders are used as a binder, and relevant information on the hard-metal industry related to metallic cobalt powders is available in a previous monograph in *IARC Monographs* Volume 86 (IARC, 2006).

(b) Cobalt compounds

Cobalt compounds are produced in forms with purities greater than 99.9% for cobalt(II) acetate ($Co(CH_3CO_2)_2$) (Sigma-Aldrich, 2021a), cobalt(II) chloride ($CoCl_2$) (Sigma-Aldrich, 2021b), cobalt(II) hydroxide (Alfa Aesar, 2022), cobalt(II) nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$) (Sigma-Aldrich, 2021c), cobalt(II) oxide (Sigma-Aldrich, 2021d), cobalt(II,III) oxide (Sigma-Aldrich, 2021e), and cobalt(II) sulfide (Sigma-Aldrich, 2022a); 99% for cobalt(II) acetate tetrahydrate ($Co(CH_3CO_2)_2{\cdot}4H_2O$) (Sigma-Aldrich, 2022b), and cobalt(II) sulfate heptahydrate ($CoSO_4{\cdot}7H_2O$) (Sigma-Aldrich, 2022c); and 98% for cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) (Fisher Scientific, 2022). [The Working Group noted that this list is not exhaustive.]

Table 1.2 Chemical and physical properties of the pure substances containing cobalt

Chemical name	Typical physical description	Melting point (°C)	Boiling point (°C)	Density (g/cm^3)	Solubility
Cobalt metal					
Cobalt (NCBI, 2021a)	Silvery bluish-white, grey, shining, hard, magnetic, ductile, somewhat malleable metal	1495	2927	8.9 at 20 °C	Soluble in dilute acids; readily soluble in dilute nitric acid; insoluble in water
Soluble cobalt(II) salts					
Cobalt(II) acetate (NCBI, 2021j)	Light pink crystals, pink crystals	298 (dec)	NA	1.71 at 68 °F [20 °C]	Readily soluble in water; soluble in alcohol and dilute acids
Cobalt(II) acetate tetrahydrate (NCBI, 2021k)	Red crystals	140	NA	1.7	Soluble in water
Cobalt(II) chloride (NCBI, 2021l)	Pale-blue hygroscopic leaflets, colourless in very thin layers, blue hexagonal leaflets	735	1049	3.36 at 25 °C/4 °C	Soluble in water, alcohols, acetone, ether, glycerol, and pyridine
Cobalt(II) chloride hexahydrate (IARC, 1991)	Pink to red, slightly deliquescent, monoclinic, prismatic	86; loss of four H_2O at 52–56, an additional H_2O by 100, and another H_2O at 110	NA	1.92 (Nielsen et al., 2013)	Soluble in ethanol, water, acetone, diethyl ether, and glycerol
Cobalt(II) nitrate (NCBI, 2021n)	Pale red powder, red crystals	100–105 (dec)	NA	2.49	Soluble in water
Cobalt(II) nitrate hexahydrate (NCBI, 2021o)	Red crystals	55	NA	1.88	Soluble in water
Cobalt(II) sulfate (NCBI, 2021p)	Red to lavender dimorphic, orthorhombic crystals, red powder, rose-pink solid	735	NA	3.71 at 25 °C/4 °C	Dissolves slowly in boiling water; 38.3 g/100 g water at 25 °C
Cobalt(II) sulfate heptahydrate (NCBI, 2021q)	Pink to red monoclinic, prismatic crystals (O'Neil, 2001)	96.8	420	1.95	Soluble in water; slightly soluble in ethanol and methanol (O'Neil, 2001)
Cobalt(II) octanoate (NCBI, 2021r; NCBI, 2021s)	NA	NA	NA	NA	[Soluble in water]
Cobalt(II) 2-ethylhexanoate (NCBI, 2021t)	Blue liquid, blue-violet mass, purple to dark blue waxy solid at 20 °C	53–58 if heated in an aluminium crucible under nitrogen; 64–84 if heated in a glass capillary under air	Dec at approximately 90; dec before boiling if heated in an aluminium crucible under nitrogen	1.25 at 21.6 °C	Soluble in water

Table 1.2 (continued)

Chemical name	Typical physical description	Melting point (°C)	Boiling point (°C)	Density (g/cm^3)	Solubility
Insoluble cobalt(II or II,III) compounds					
Cobalt(II) oxide (NCBI, 2021u)	Powder or cubic or hexagonal crystals; colour varies from olive green to red depending on particle size, but the commercial material is usually dark grey	1935	NA	5.7–6.7	Insoluble in water and ammonium hydroxide; soluble in acids or alkalis
Cobalt(II,III) oxide (IARC, 1991)	Black or grey crystals	895; transition point to CoO is 900–950	NA	6.07 (ATSDR, 2004)	Practically insoluble in water, aqua regia, and hydrochloric or nitric acid; soluble in sulfuric acid and fused sodium hydroxide
Cobalt(II) hydroxide (Patnaik, 2003)	Rose-red powder (more stable) and bluish-green powder less stable than the red form; rhombohedral crystals	NA	NA	3.597	Insoluble in water; soluble in acids and ammonia; insoluble in dilute alkalis
Cobalt(II) sulfide (NCBI, 2021x)	Black amorphous powder	1117	NA	5.45	Insoluble in water; soluble in acid
Organic cobalt(II) compounds					
Cobalt(II) resinate (NCBI, 2021y)	NA	NA	NA	NA	NA

dec, decomposes; NA, not available.

1.2 Production and use

1.2.1 Production

(a) Cobalt metal

The main ores of cobalt are cobaltite, erythrite, glaucodot, and skutterudite. However, cobalt is produced mainly as a by-product of the mining and processing of the ores of other metals, particularly those of copper, nickel, and silver, and also gold, lead, and zinc. The first stages of the production of cobalt from its ores involve the separation of cobalt-bearing minerals – including arsenide, sulfoarsenide, sulfide, arsenic-free cobalt–copper, lateritic, and oxide ores from the gangue (the portion of the ore composed of minerals that are not of commercial value) – and from minerals that include other metals. The concentrates gained with the application of physical separation methods to ores, such as gravity separation or froth flotation, can increase the cobalt contents of cobalt-rich ores by 10–15%. Using the more common ores less rich in cobalt, these processes only increase the levels of cobalt from 0.1–0.6% to a few per cent. Subsequently, cobalt is extracted from concentrates and occasionally directly from the ore itself by hydrometallurgical, pyrometallurgical, and electrometallurgical processes (Donaldson & Beyersmann, 2011; Afolabi et al., 2021).

Global mine and refinery production figures for cobalt from 1960 to 2019 are shown in Fig. 1.1 (USGS, 2021a). Global production from cobalt mining and production of refined cobalt has increased steadily over the past two decades, reaching 144 000 and 132 000 tonnes, respectively, in 2019. Data representing global cobalt mine production and refinery production (including the production of cobalt compounds) from 2015 to 2019 are presented in Table S1.3 and Table S1.4, respectively (USGS, 2021b; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The largest site of cobalt mine production is in Kinshasa, the Democratic Republic of the Congo (100 000 tonnes in 2019), followed by the Russian Federation (6300 tonnes), Australia (5742 tonnes), and the Philippines (5100 tonnes). The largest producer of refined cobalt can be found in China (90 000 tonnes in 2019), followed by Finland (12 526 tonnes), Canada (6075 tonnes), and Norway (4354 tonnes).

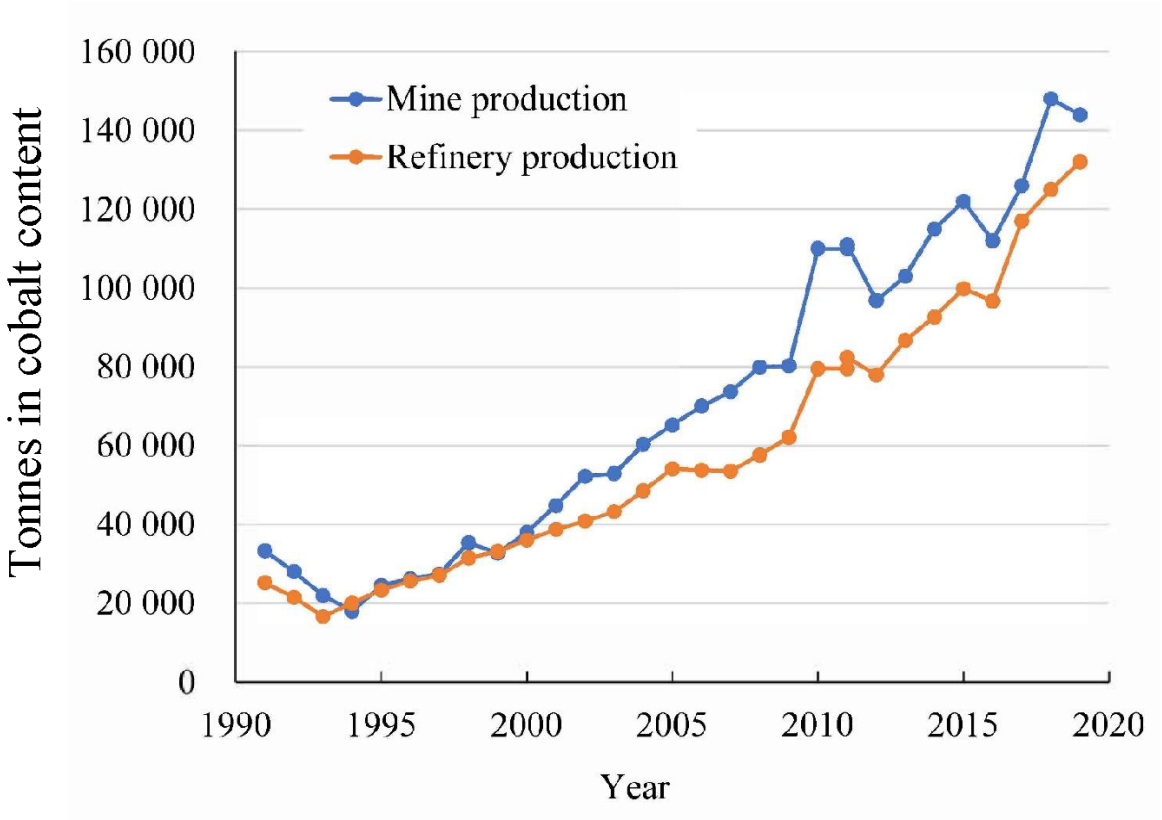
(b) Cobalt compounds

The processes used in the production of cobalt(II) salts are described in this section. Cobalt(II) acetate is prepared by dissolving cobalt(II) carbonate ($CoCO_3$) or hydroxide ($Co(OH)_2$) in dilute acetic acid, followed by crystallization. It can also be prepared by oxidation of dicobalt octacarbonyl in the presence of acetic acid (Patnaik, 2003). Cobalt(II) acetate tetrahydrate is prepared by concentrating solutions of cobalt(II) hydroxide or carbonate in acetic acid (Donaldson & Beyersmann, 2011).

Cobalt(II) chloride is prepared by the reaction of cobalt metal or its oxide, hydroxide, or carbonate with hydrochloric acid. Upon concentration and cooling, crystals of hexahydrate form from solution that, on heating with thionyl chloride, dehydrate to anhydrous cobalt(II) chloride. Alternatively, the hexahydrate may be converted to anhydrous cobalt(II) chloride by dehydration in a stream of hydrogen chloride and dried in a vacuum at 100–150 °C. The anhydrous compound also may be obtained by passing chlorine over cobalt powder (Patnaik, 2003). Cobalt(II) chloride hexahydrate is prepared by concentrating a hydrochloric acid solution of cobalt(II) oxide or carbonate (Donaldson & Beyersmann, 2011).

Cobalt(II) nitrate hexahydrate is prepared by concentrating a nitric acid solution of cobalt(II) oxide or carbonate. The hexahydrate loses water rapidly at 55 °C to yield the trihydrate; the monohydrate can also be prepared using higher temperatures (Donaldson & Beyersmann, 2011).

Fig. 1.1 Global production of cobalt in mines and refineries, 1991–2019



Data for refinery production before 1967 were not available.
Created by the Working Group with data from USGS (2021a, b).

Cobalt(II) oxide is typically prepared by the controlled oxidation of the metal at temperatures > 900 °C, followed by cooling in a protective atmosphere to prevent partial oxidation to cobalt(II,III) oxide. Cobalt(II,III) oxide can be prepared by the controlled oxidation of cobalt metal or cobalt(II) oxide, or by the thermal decomposition of cobalt(II) salts at temperatures < 900 °C (Donaldson & Beyersmann, 2011). Cobalt(II) hydroxide is obtained as a precipitate when an alkaline hydroxide is added to an aqueous solution of cobalt(II) salt (Patnaik 2003).

Cobalt(II) sulfate is prepared by dissolving cobalt(II) oxide, hydroxide, or carbonate in dilute sulfuric acid, followed by crystallization. Crystallization yields the commercial product pink heptahydrate (Patnaik, 2003).

Cobalt(II) sulfide exists in nature as the mineral sycoporite. It can also be readily prepared in the laboratory. A black precipitate of cobalt(II) sulfide is obtained by passing hydrogen sulfide through an alkaline solution of a cobalt(II) salt such as cobalt(II) chloride. Cobalt(II) sulfide can also be produced by heating cobalt metal with hydrogen sulfide at 700 °C (Patnaik, 2003).

Cobalt(II) resinate can be made by the direct reaction of cobalt powder, oxide, or hydroxide with resin acid, or by precipitation reactions involving the addition of sodium resinate to an aqueous solution of a cobalt(II) salt, such as the sulfate (Donaldson & Beyersmann, 2011).

Cobalt(II) 2-ethylhexanoate can be produced by metathesis reaction of cobalt(II) salt solutions and sodium 2-ethylhexanoate, by oxidation of cobalt metal in the presence of 2-ethylhexanoic acid, and by neutralization of 2-ethylhexanoic acid using cobalt carbonate or cobalt hydroxide (NCBI, 2021t). [Information concerning production of cobalt(II) octanoate was not available to the Working Group.]

1.2.2 Use

From a historical perspective, although very little cobalt metal was used until the 20th century, its ores have been used for thousands of years as blue colouring agents for glass and pottery. The brilliant blue pigment used for these purposes was probably produced by fusing an ore containing cobalt oxide with potash and silica to produce a vitreous material called smalt, which was powdered to produce the pigment. In the 16th century, a blue pigment called zaffre was produced from silver–cobalt–bismuth–nickel–arsenate ores found in Saxony, Germany (IARC, 2006). The next development in the use of cobalt-containing materials was the discovery, early in the 18th century, that solutions containing bismuth and cobalt could be used as sympathetic inks (Donaldson & Beyersmann, 2011). In 1923, the discovery that mixing cobalt with tungsten carbide produced hard metal initiated its use in a variety of industrial applications. Cobalt was used as a constituent in the first permanent magnetic alloy in 1933 (IARC, 2006).

Cobalt is used in many industries, including in the manufacture of cutting and grinding tools, pigments and paints, coloured glass, surgical implants, batteries, and some types of electroplating. Global consumption of cobalt has increased markedly over the past decade, as has its production. Consumption has increased at an annual rate of approximately > 5% since 2013. Much of this consumption increase is a result of the demand for cobalt in the production of lithium-ion batteries (Cobalt Institute, 2021). All lithium-ion batteries use a *p*-type semiconductor, lithium cobalt oxide ($CoLiO_2$), as the active material for the positive electrode (Tukamoto & West, 1997; Donaldson & Beyersmann, 2011). Demand for cobalt in lithium-ion batteries, used chiefly in portable electronics and electric vehicles, increased at an annual rate of 10% between 2013 and 2020. Batteries accounted for 57% of total global cobalt consumption in 2020, followed by nickel-based alloys, which accounted for 13%; other uses included tool materials, pigments, catalysts, magnets, and soaps and dryers (Cobalt Institute, 2021). In a projected growth scenario using a multiregional input–output analysis model – assuming region- and sector-specific gross domestic product growth as projected by the Organisation for Economic Co-operation and Development, constant technology, and constant background import shares – global annual cobalt demand was estimated as likely to increase from 50 000 tonnes in 2007 to 110 000 tonnes in 2030, and to 190 000 tonnes in 2050, which is an increase of roughly threefold over the given period (Tisserant & Pauliuk, 2016).

Geographically, Asia is the region that consumes the largest proportion of cobalt, accounting for around one half of total global cobalt consumption in 2020. This reflects the high production volumes of battery materials in Asia, particularly in China, Japan, and the Republic of Korea. China is the greatest cobalt consumer, accounting for 32% of global consumption in 2020. China is also a major producer of tool materials, magnets, and pigments. Europe and North America also consume large amounts of cobalt, together accounting for 40% of global consumption in 2020. In these regions, cobalt is mostly used in batteries, nickel-based alloys, and tool materials (Cobalt Institute, 2021).

(a) Cobalt metal

Pure metallic cobalt has limited applications. It is used as a source of chemical production and as an alloying element. Cobalt is not the most abundant of the elements added to steels but is an important component of high-strength steel. Maraging steels, used as ultrahigh-strength alloys, contain 5–20% cobalt, which is alloyed with nickel and molybdenum (Donaldson & Beyersmann, 2011). In electrodeposited alloys, protective coatings of electroplated nickel–cobalt alloys have been used. A 25% cobalt alloy, deposited from sulfate electrolytes, is approximately three times as hard as electroplated nickel and has almost the same hardness as pure cobalt (Donaldson & Beyersmann, 2011).

End-uses of cobalt-containing alloys include magnetic alloys for powerful permanent magnets, hard-metal alloys for cutting-tool materials, superalloys for aircraft engines, cemented carbides, corrosion-resistant alloys, wear-resistant alloys, and electrodeposited alloys to deliver wear and corrosion-resistant metal coatings (Donaldson & Beyersmann, 2011).

Several cobalt-containing alloys have special applications. For example, cobalt–chromium alloys are used as dental materials. These alloys are used for casting denture bases, complex partial dentures, and selected types of bridgework. The most widely used alloy is vitallium, which is a cobalt-based alloy that contains 64.5% cobalt, 30% chromium, 5% molybdenum, and 0.5% each of carbon and silicon. Vitallium is also used for surgical implants and bone replacement and repair (Donaldson & Beyersmann, 2011).

Metallic cobalt nanoparticles (NPs, < 10 nm in size) are used because there exists an efficient means for manipulating or modifying their optical, mechanical, magnetic, chemical, and electronic properties, which can be readily used in a wide variety of technological applications. Cobalt NPs provide excellent magnetic, electrical, and catalytic properties that are of scientific and technological interest in various fields, including recording media, magnetic sensors, magnetic memories, magnetic fluids, magnetic composites, and catalysis. Cobalt NPs are also used in biomedical-related fields, e.g. drug delivery and magnetic resonance imaging (Ansari et al., 2017). Additionally, cobalt-based NPs are produced as cobalt oxide, organometallic compounds, or biopolymers. In biomedical applications, cobalt-based NPs are used as starting materials for the formation of magnetic polymer microspheres and dextran coating (Magaye et al., 2012).

(b) Cobalt compounds

Cobalt salts are mainly used as pigments in the glass, ceramics, and paint industries, as catalysts in the petroleum industry, as paint driers, and as trace metal additives for agricultural and medical use.

Cobalt(II) acetate and cobalt(II) acetate tetrahydrate are used in the manufacturing of drying agents for lacquers and varnishes, sympathetic inks, catalysts for oxidation, pigments for oilcloth, mineral supplements, and aluminium anodizing solutions; as stabilizers for malt beverages; and in agricultural industries, for example, as treatments of soils or ruminant animals to reduce cobalt deficiencies (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Anhydrous cobalt(II) chloride is blue and the change to hexahydrate red is used as a humidity indicator in silica gel desiccants. It is also used in the electroplating, ceramics, glass, malt beverage stabilizer, mineral supplement, chemical, agricultural, and pharmaceutical industries (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) chloride is also used to prepare several other cobalt salts and in the manufacture of synthetic vitamin B_{12} (Patnaik, 2003). Cobalt(II) chloride hexahydrate is used in sympathetic inks, hydrometers, plating baths, metal refining, pigments, and catalysts (Donaldson & Beyersmann, 2011).

Cobalt(II) nitrate is an important source of high-purity cobalt for use in the electronics and related industries, and the compound also has uses in the chemical and ceramics industries. Cobalt(II) nitrate hexahydrate is used in pigments, chemicals, ceramics, feed supplements, and catalysts (Patnaik, 2003; EFSA, 2009a; Donaldson & Beyersmann, 2011).

Cobalt(II) oxide is used as a starting material for the manufacture of other chemicals and catalysts, in pigments such as colour reagents and ceramics, gas sensors, and thermistors. Cobalt(II,III) oxide is used in the production of enamels and semiconductors (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) hydroxide is used as a drier for paints and varnishes, and is added to lithographic printing inks to enhance their drying properties. Other applications are in the preparation of cobalt salts, as a catalyst, and in storage battery electrodes (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Cobalt(II) sulfate is widely used as a source of cobalt(II) in solution for the manufacturing of chemicals and the electroplating industries. The sulfates are also used in the ceramics, linoleum, and agricultural industries. Cobalt(II) sulfate heptahydrate is used for the manufacturing of other chemicals and pigments in ceramics. Cobalt(II) sulfide is used as a catalyst for the hydrogenation or hydrodesulfurization of organic compounds in petroleum refining (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Cobalt(II) resinate is used as a paint and varnish drier, and as a catalyst (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) octanoate is used in home maintenance, e.g. in oil-based polyurethane varnish for wood (NCBI, 2021w). Cobalt(II) 2-ethylhexanoate is used in paints and coatings, process regulators, surface-active agents, bonding agents, catalysts, building and construction materials (e.g. flooring, tiles, sinks, bathtubs, mirrors, wall materials/drywalls, wall-to-wall carpets, insulation, and playground surfaces), and detergent alcohol (NCBI, 2021t).

1.3 Detection and quantification

1.3.1 Air

Analytical methods used until the early 21st century to measure cobalt concentrations in airborne particulate matter have been reviewed in previous *IARC Monographs* Volume 52 and Volume 86 (IARC, 1991, 2006). Spectrometric analytical procedures, electrochemical analysis methods, and radioactivation processes continue to be employed for the analysis of cobalt and its compounds (Table 1.5). Atomic absorption spectrometry (AAS), using either flame AAS (FAAS) or electrothermal AAS (ETAAS) atomization, has been used extensively and remains the most widely employed technique for the elemental analysis of airborne particles (NIOSH, 1994; US EPA, 1999a). Other standard methods for measuring cobalt in air samples are the use of inductively coupled plasma (ICP)-atomic emission spectroscopy (ICP-AES) or ICP mass spectrometry (ICP-MS) detection systems (US EPA, 1999b; ATSDR, 2004). As shown in Table 1.5, reported sample limits of detection (LODs) for cobalt range from 0.01 ng/m^3 using ICP-MS (US EPA, 1999b) to 2.2 ng/m^3 using FAAS (US EPA, 1999a). Where available, other methods such as instrumental neutron activation analysis (INAA) and X-ray fluorescence analysis can provide quantitative data for cobalt concentrations in airborne particulate matter (Hamilton, 1994).

The advantages and shortcomings of these and other less frequently used techniques have been extensively examined (IARC, 1991, 2006; Ram et al., 2003; Pitzke et al., 2020). The more frequently reported ones are chosen on the basis of cost and efficiency. For instance, AAS instrumentation usually provides low LODs, but only one element can be analysed at a time (Schroeder

et al., 1987). Multi-element analysis techniques, such as ICP and INAA, require significant investments in equipment and are not available in all laboratories. Although more affordable, X-ray fluorescence analysis lacks sensitivity for most environmental concentrations of cobalt unless used in combination with electron microprobes (Hamilton, 1994).

1.3.2 Water

Various methodologies are currently available for the measurement of cobalt, even at low levels, in water and other environmental samples. Instrumental techniques such as electroanalytical techniques (Zhang et al., 2016), FAAS (Büyükpınar et al., 2019; Tekin et al., 2020), ETAAS (Amjadi et al., 2010), ICP-AES, ICP-MS, and ICP with optical emission spectrometry (Thomassen et al., 2004; Ndilila et al., 2014; Cheng et al., 2019; Bi et al., 2020) have been extensively reported (Table 1.5). However, low analyte concentrations and matrix effects can cause difficulties in the direct measurement of cobalt concentrations in water or other environmental samples (Aggarwal et al., 1992; Amjadi et al., 2010). Consequently, numerous preconcentration and separation techniques have been developed to extract cobalt from sample matrices. Pre-treatment procedures include ion exchange (Jiang et al., 2005), solid-phase extraction (Duran et al., 2007; Ghaedi et al., 2007; Talio et al., 2014), liquid-phase extraction (Amjadi et al., 2010; Tekin et al., 2020), liquid–liquid extraction (Todorovska et al., 2003; Reza Jamali et al., 2007), dispersive liquid–liquid microextraction (Berton et al., 2012), coprecipitation, and cloud point extraction (Ghaedi et al., 2008; Citak & Tuzen, 2010; Temel et al., 2018). The United States Environmental Protection Agency (US EPA) recommends ICP-MS for measuring the concentrations of metals in water due to its high analytical sensitivity (Table 1.5) (US EPA, 2014). ICP-MS typically requires filtration and acidification of the sample before analyses and offers LODs in the sub or low parts-per-billion range for most metals (US EPA, 2014).

New research trends include the development of chemosensors for Co^{2+} ions, characterized by high sensitivity and easy operation (Kuwar et al., 2014; Dogaheh et al., 2017). A few chemosensors using ion-selective membranes or chemical probes for fluorescence or colorimetric cobalt detection have been reported. The sensors have been applied to estimation of the concentrations of Co^{2+} ions in water, food products, and pharmaceutical formulations (Na et al., 2016; Khalil & El-Sharnouby, 2021). Colorimetric methods further allow easy monitoring of target ions with the naked eye (Na et al., 2016). [The Working Group noted that the need for cost-effective and increasingly sensitive analytical methods to quantify cobalt and cobalt speciation possibly explains the current interest in this new line of research.]

1.3.3 Other environmental samples, food and feed, consumer products, and cosmetics

Concentrations of cobalt in foodstuffs are usually very low and, despite the sensitive analytical instruments currently available (Table 1.5), pre-treatment techniques are often required (Citak & Tuzen, 2010; Tekin et al., 2020). The use of affordable and non-toxic solvents is becoming the main goal of extraction methods. Deep eutectic solvents and ionic liquids share characteristics that make them attractive as potential substitutes for traditional organic solvents. Another alternative to the traditional chemical decomposition of food samples with common acid mixtures is ultrasonic extraction (Yebra-Biurrun & Cancela-Pérez, 2007; Temel et al., 2018). For the measurement of concentrations of cobalt ions in soil samples, the US EPA (2014) recommends microwave-assisted acid digestion followed by ICP-MS analysis.

Table 1.5 Analytical methods for the measurement of cobalt in environmental samples, food and feedstuffs, consumer products, and cosmetics

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Environmental samples				
Air	Collection on glass fibre filters using high-volume sampler; extraction by hot acid procedure or microwave extraction; microwave extraction preferred (EPA Method IO-3.1)	AAS (EPA Method IO-3.2)	FAAS, 2.2 ng/m^3; ETAAS, 0.02 ng/m^3	US EPA (1999a)
Air	Collection on glass or quartz fibre filter; microwave or hot acid digestion (EPA Method IO-3.1)	ICP-MS (EPA Method IO-3.5)	0.01 ng/m^3	US EPA (1999b)
Air	Sample filter digested by wet acid ashing	FAAS (NIOSH Method 7027)	0.4 µg/m^3	ATSDR (2004)
Air	Sample filter digested by wet acid ashing	ICP-AES (NIOSH Method 7300)	0.5 µg/m^3	ATSDR (2004)
Inhalable aerosols	Uses ammonium citrate leachate to obtain the water-soluble fraction before the acid digestion	ICP-OES	0.1 µg/m^3	Thomassen et al. (2004)
Air: $PM_{2.5}$	High-volume sampler; microwave-assisted acid digestion	ICP-OES	NR	Bi et al. (2020)
Water	Filtration and acid digestion	ICP-MS (EPA Method 6020B)	NR	US EPA (2014)
Water	Complexation with BSOPD	UV-vis spectrophotometry	0.015 mg/L	Ahmed & Uddin (2007)
Water	Ionic liquid-based microextraction with $[C_6MIM][PF_6]$–APDC complex	ETAAS	0.04 µg/L	Amjadi et al. (2010)
Water	Online flow injection pre-concentration by ion-pair adsorption in a knotted reactor	ETAAS	5 ng/L	Benkhedda et al. (2000)
Seawater	Pre-concentration using PAR and a Dowex-1-chloride anion exchange resin	EDXRF	[1.53 µg/L][a]	Jiang et al. (2005)
Soil	Microwave-assisted acid digestion and filtration (EPA SW-846 Method 3051A)	ICP-MS (EPA Method 6020B)	NR	US EPA (2014)
Soil	Uses a photochemical vapour generation system equipped with a batch-type gas–liquid separator system to separate cobalt from the sample matrix	FAAS	8.7 µg/L	Büyükpınar et al. (2019)
Soil, house dust, and drinking-water	Metal extraction by acid digestion	ICP-AES (Method 3050B Revision 2, US EPA (1996))	NR	Ndilila et al. (2014)
Marine sediment	Slurry extraction	ETAAS	0.43 µg/g	Barciela-Alonso et al. (2003)
Wild fish	Microwave-assisted acid digestion	ICP-MS	1 µg/kg	Cheng et al. (2019)

Table 1.5 (continued)

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Feedstuffs, foodstuffs, consumer products, and cosmetics				
Consumer products: fashion and piercing jewellery	Sample parts were immersed in artificial sweat	ICP-OES; ICP-MS; FAAS (EN 1811:2011/AC:2012)	NR	Uter & Wolter (2018)
Cosmetics	Microwave-assisted acid digestion	ICP-OES	NR	Bruzzoniti et al. (2017)
Cosmetics (toy make-up)	Microwave-assisted acid digestion	ETAAS	0.2 µg/g	Corazza et al. (2009)
Nutrient supplements	Liquid–liquid microextraction with 1N2N–$[C_6MIM][PF_6]$ complex	ETAAS	5.4 ng/L	Berton et al. (2012)
Feed grains and forages	Extraction with 1N2N2 in glacial acetic acid	ETAAS	1 ng/g	Blanchflower et al. (1990)
Feedstuffs (grass and cereals)	Microwave partial vapour-phase acid digestion	ETAAS	NR	Araújo et al. (2000)
Foodstuffs (canned fish, black tea, green tea, tomato sauce, and honey)	Cloud point extraction with the 1-PTSC–Triton X-114 complex	FAAS	1 µg/L	Citak & Tuzen (2010)
Foodstuffs (tea leaves)	Deep eutectic solvent–liquid-phase microextraction with (*Z*)-3-bromo-5-((*p*-tolylimino)methyl)phenol	FAAS	2 µg/L	Tekin et al. (2020)

AAS, atomic absorption spectrometry; 1N2N, 1-nitroso-2-naphthol; 1-PTSC: 1-phenylthiosemicarbazide; APDC, ammonium pyrroldinedithiocarbamate; BSOPD, bis(salicylaldehyde) orthophenylenediamine; $[C_6MIM][PF_6]$, 1-hexyl-3-methylimidazolium hexafluorophosphate; EDXRF, energy-dispersive X-ray fluorescence spectrometry; EPA, Environmental Protection Agency; ETAAS, electrothermal atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma with optical emission spectrometry; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health; PAR, 4-(2-pyridylazo)resorcinol; $PM_{2.5}$, particulate matter with aerodynamic diameter < 2.5 µm; Triton X-114, octylphenoxypolyethoxyethanol; UV-vis, ultraviolet-visible light.

[a] Working Group conversion to International System of Units.

In recent years, increasing attention has been paid to issues concerning the exposure of skin to chemicals such as cobalt. Several studies have used methods that specifically quantify concentrations of cobalt on the skin, which has led to a better understanding of this exposure route (Lidén et al., 2008; Julander et al., 2010; Erfani et al., 2017; Kettelarij et al., 2018a; Uter & Wolter, 2018). The cobalt spot test is a simple colorimetric method that is commonly used for screening purposes (Thyssen et al., 2012; Hamann et al., 2013). Despite their reported limitations regarding the adequate quantification of exposure (Uter & Wolter, 2018), these screening tests have the advantage of helping co-allergic patients to avoid exposure to this trace metal.

1.3.4 Human biomarkers

A wide variety of techniques have been used for the determination of cobalt in human biological samples, which are roughly summarized in Table 1.6. Over the last three decades, these techniques have increased in sensitivity. Techniques such as ICP, gas chromatography–mass spectrometry, and INAA are increasingly used for multi-elemental analysis of biological samples (Goullé et al., 2005; Rocha et al., 2016; Chellini et al., 2017; Nisse et al., 2017; Capiau et al., 2020). However, ICP and INAA instruments are not available in all laboratories, and suitable alternatives are necessary for the accurate measurement of cobalt at concentrations as low as 0.5–1 µg/L. ETAAS is probably the most frequently used technique due to its sensitivity and relatively low cost of instrumentation (Todorovska et al., 2003; Berton & Wuilloud, 2010). Analytical methods recommended for determining the levels of cobalt in the blood and urine of environmentally and occupationally exposed people are ICP-MS, ICP-AES, and ETAAS (NIOSH, 1994; WHO, 1996; CDC, 2017, 2019). Since biological cobalt often occurs in complex matrices at very low levels, pre-treatment is commonly required before samples are analysed. Preconcentration techniques for cobalt quantification in biological samples are, in essence, similar to those discussed earlier (Table 1.5).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Cobalt is ubiquitous in the environment, generally occurring at low levels in rocks, soil and sediments, groundwater and surface water, and air (Hamilton, 1994; ATSDR, 2004; WHO, 2006). The upper continental crust has an average cobalt abundance of 17 mg/kg (Rudnick & Gao, 2014). Cobalt is not usually detected in drinking-water, but levels of a few micrograms per litre have been measured in samples from lakes, groundwater, and spring and well water (Hamilton, 1994). Cobalt concentrations in soil, where it usually occurs as cobalt(II), are generally within the range of 1–50 mg/kg, with an average concentration of 7 mg/kg (Hamilton, 1994; ATSDR, 2004). In ambient air, cobalt is mainly associated with the resuspension of soil particles. Atmospheric cobalt levels at unpolluted sites are generally < 2.0 ng/m^3 (Hamilton, 1994; Leyssens et al., 2017). However, anthropogenic activities such as mining, smelting and other related industrial processes, coal combustion, and vehicular traffic result in elevated levels of cobalt and cobalt compounds in the environment (Barciela-Alonso et al., 2003; Banza et al., 2009; Guéguen et al., 2012; Boev et al., 2013; Bari et al., 2015; Kamunda et al., 2016; Leyssens et al., 2017; Pan et al., 2017; Kravchenko and Lyerly, 2018; Mwaanga et al., 2019). Table 1.7 illustrates the occurrence of cobalt in environmental matrices such as ambient air, household dust, soil, and various water sources.

Because of the widespread occurrence of cobalt, the primary routes of human exposure are by inhaling ambient air (Rivas et al., 2014, Bari et al., 2015; Mohmand et al., 2015), ingesting

Table 1.6 Analytical methods for the measurement of cobalt in biological samples

Sample matrix	Sample preparation	Analytical technique (method)	LOD	Reference
Urine	Urine dilution and acidification	ICP-MS with DRC technology (CDC Method 3018.6–06)	0.023 µg/L	CDC (2019)
Urine	Urine chelation and concentration, and acidification	ETAAS	0.1–0.3 µg/L	WHO (1996)
Urine	Complexation with lithium bis(trifluoroethyl) dithiocarbamate)	GC-MS	1 µg/L	Aggarwal et al. (1992)
Urine	Extraction with HMADTC–xylene in diisopropylketone	ETAAS	6 µg/L	Bouman et al. (1986)
Urine	Urine dilution and acidification	ICP-MS with DRC technology (Method developed according to the Polish/European norm PN-EN ISO/IEC 17 025:2005)	0.004 µg/L	Brodzka et al. (2013)
Urine	Pre-concentration using 5-Br-PADAP and Amberlite XAD-7 resin	ICP-AES	25 ng/L	Farias et al. (2002)
Urine and saliva	Liquid–liquid microextraction with 1N2N–$[C_6MIM][PF_6]$ complex	ETAAS	3.8 ng/L	Berton & Wuilloud (2010)
Serum and urine	Liquid–liquid extraction with the APDC–IMBK complex	ETAAS (Method derived from the IUPAC reference method for nickel determination)	Serum, 1.93 nmol/L; urine, 1.89 nmol/L	Baruthio & Pierre (1993)
Serum	Complexation with DMG	AdSV	0.007 µg/L	Kajič et al. (2003)
Blood and urine	Ion exchange extraction	ETAAS	2–3 nmol/L	Alexandersson (1988)
Blood	Extraction with an alkaline extraction mixture	ICP-MS	NR	Capiau et al. (2020)
Whole blood	Collection in tube with anticoagulant, mixing and dilution of sample	ICP-MS (CDC Method 3030.1–03)	0.06 µg/L	CDC (2017)
Blood or tissue	Acid digestion	ICP-AES (NIOSH Method 8005)	Blood, 1 µg/100 g; tissue, 0.2 µg/g	NIOSH (1994)
Placenta and cord blood	Microwave-assisted acid digestion	ICP-MS	0.002 ng/g	Fagerstedt et al. (2015)
Hair	Microwave-assisted acid digestion	ICP-MS	0.001 µg/g	Elenge et al. (2011)

1N2N, 1-nitroso-2-naphthol; 5-Br-PADAP, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; AdSV, adsorptive stripping voltammetry; APDC, ammonium pyrroldinedithiocarbamate; $[C_6MIM][PF_6]$, 1-hexyl-3-methylimidazolium hexafluorophosphate; CDC, Centers for Disease Control and Prevention; DMG, dimethylglyoxime; DRC, dynamic reaction cell; ETAAS, electrothermal atomic absorption spectrometry; GC-MS, gas chromatography-mass spectrometry; HMADTC, *N*,*N*-hexamethyleneammonium-hexa-methylenedithiocarbamic acid; IBMK, 4-methyl-pentant-2-one (isobutyl methyl ketone); ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ISO/IEC, International Organization for Standardization/International Electrotechnical Commission; IUPAC, International Union of Pure and Applied Chemistry; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health.

drinking-water (Amer et al., 1990; Iqbal et al., 2012; Ndilila et al., 2014; Mwesigye et al., 2016), and consuming food grown at contaminated sites (Gál et al., 2008; Mwesigye et al., 2016; Sharma et al., 2018; Cheng et al., 2019). Airborne particulate matter is a complex mixture of solids or liquids with different masses, numbers, sizes, shapes, surface areas, chemical compositions, acidities, and solubilities (Fortoul et al., 2015). The origins of the particles have critical effects on their size (or aerodynamic diameter), composition, transport in the atmosphere, and their ability to be inhaled into the respiratory system and cause health effects (Potter et al., 2021; Alghamdi et al., 2022). It is generally assumed that particulate matter with an aerodynamic diameter of 0.1 μm or less – $PM_{0.1}$ (ultrafine) particles – have highly toxic properties because they have large surface areas that can absorb a variety of toxic substances, such as cobalt (Slezakova et al., 2013). [The Working Group noted that the $PM_{0.1}$ particles described here and the NPs mentioned in Section 4 of the present monograph are generally equivalent in terms of aerodynamic diameter.] After inhalation, the ultrafine particulates can penetrate deep into the circulatory system via the respiratory tract and are translocated into various organs in the body (Kwon et al., 2020; Schraufnagel 2020; Phairuang et al., 2021; Potter et al., 2021).

Numerous studies of contaminated soils from areas where mining or smelting takes place have documented trace metal uptake by food crops, vegetables and fruits, and wild fish (Table 1.7). The results suggest that the consumption of these foodstuffs may be a significant contributor to cobalt intake (Cheyns et al., 2014; Mwesigye et al., 2016; Cheng et al., 2019). In addition, a substantial body of evidence suggests that unintentional ingestion of contaminated soil and dust, even at low cobalt concentrations, is a significant environmental exposure pathway to humans, because of the potential for long-term exposure (Cheyns et al., 2014; Ndilila et al., 2014). In soil, cobalt remains distributed between highly soluble and exchangeable fractions and relatively unreactive residual mineral phases. The distribution of cobalt in the solid phase influences the mobility and bioavailability of the element. Bioavailability based on the form of exposure (soluble versus insoluble) has been identified as an important factor influencing exposure to cobalt metal and its alloys and compounds (Behl et al., 2015). Indoor settled dust that contains cobalt may represent an important source of exposure, because people spend up to 90% of their time indoors in places such as homes, workplaces, and schools (Rivas et al., 2014). Important indoor sources of cobalt include decorative paints, cleaning products, and combustion products that result from cooking, heating, and smoking (Vilaplana et al., 1988; Bocca et al., 2014; Pinto et al., 2017). Although the process by which cobalt permeates human skin is not well documented (Larese Filon et al., 2004; Leyssens et al., 2017), skin contact with contaminated soil or water may also increase the potential for exposure (Ngole-Jeme & Fantke, 2017).

1.4.2 Occupational exposure

(a) Overview of occupational exposure scenarios

The main route of occupational exposure to cobalt is expected to be via the respiratory tract because of the inhalation of dust, fumes, or mists containing cobalt (IARC, 1991). Dermal exposure to cobalt (e.g. resulting from the deposition of particles and dust, handling of hard-metal items during production, and touching production equipment and other work material) is also a concern because of the potential for direct absorption through the skin and/or hand-to-mouth contact (Scansetti et al., 1994; Kettelarij et al., 2018a). Occupational exposure mainly occurs in industries involved in hard-metal production, processing, and use; during the production of cobalt powder; in the use of

Table 1.7 Occurrence of cobalt species in environmental matrices, foodstuffs, tobacco, and cosmetics

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Contaminated air, water, sediment, dust, and soil							
Outdoor ambient air: $PM_{2.5}$ samples	Beijing city, China, 2008–2012	$n_{suburban}$ = 59 n_{urban1} = 66 n_{urban2} = 63	Suburban: 0.38 ng/m³ (NR) Urban1: 0.36 ng/m³ (NR) Urban2: 0.35 ng/m³ (NR)	0.37 ng/m³ (0.28 ng/m³); percentiles calculated for the entire data set	ICP-MS (NR)		Wu et al. (2012b)
Indoor ambient air: PM_1 samples	Edmonton city, Canada, 2010	Winter: n = 173 Summer: n = 329	Winter: NR (0.007–1.6 ng/m³) Summer: NR (0.0–0.9 ng/m³)	Winter: 0.03 ng/m³ (NR) Summer: 0.02 ng/m³ (NR)	ICP-MS (NR)		Bari et al. (2015)
Indoor ambient air: $PM_{2.5}$ samples	Barcelona city, Spain, 2012–2013	n = 77	0.21 ng/m³ (< 0.1–0.81)	0.16 ng/m³ (NR)	ICP-MS (NR)		Rivas et al. (2014)
Household settled dust	Lahore and Sargodha cities, Pakistan, date of sample collection NR	n_{rural} = 10 n_{urban} = 10 $n_{industrial}$ = 10	Rural: [1.7 µg/g (1–2)][a] Urban: [3 µg/g (1–6)][a] Industrial: [2 µg/g (1–3)][a]	Rural: [1.6 µg/g][a] (NR) Urban: [2 µg/g][a] (NR) Industrial: [2 µg/g][a] (NR)	ICP-MS (0.001 µg/L)		Mohmand et al. (2015)
Soil and household settled dust	Copperbelt region of Zambia, date of sample collection NR	n_{soil} = 36; n_{dust} = 31	Soil: 4.63[b] mg/kg (< LOD–18.1) Dust: 1.80[b] mg/kg (< LOD–227)	NR	ICP-AES (NR)	EPA Method 3050B Revision 2, US EPA (1996)	Ndilila et al. (2014)
Household settled dust: indoor and paired outdoor samples	Katanga Copperbelt, Democratic Republic of the Congo, 2009–2011	n = 26	Indoor dust: 490 µg/g (NR) Outdoor dust: 330 µg/g (NR)	NR	ICP-OES (0.07 µg/g)		Cheyns et al. (2014)
Road dust	Xi'an city, China, 2015	n = 90	30.9 mg/kg (15.2–54.8)	29.4 mg/kg (NR)	XRF (0.5–1.0 mg/kg)		Pan et al. (2017)
Bottom ash from informal e-waste recycling	Agbogbloshie, Accra city, Ghana, 2012–2013	n = 210 (collected from 3 different sites)	Site 1: 123 mg/kg (NR) Site 2: 87 mg/kg (NR) Site 3: 96 mg/kg (NR)	NR	FAAS (NR)		Obiri et al. (2016)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Mine soil: tailings and topsoil	Witwatersrand Gold Mining Basin, South Africa, date of sample collection NR	$n = 73$	25.6 mg/kg (11.8–33.7)	NR	ICP-MS (0.001 µg/L)		Kamunda et al. (2016)
Geophagic soil samples	Democratic Republic of the Congo, Togo, South Africa, and Eswatini, date of sample collection NR	$n_{total} = 57$; $n_{DRC} = 14$; $n_{South\ Africa} = 27$; $n_{Swaziland} = 12$; $n_{Togo} = 4$	Democratic Republic of the Congo: 6.57 mg/kg (NR) South Africa: 14.30 mg/kg (NR) Eswatini: 10.86 mg/kg (NR) Togo: 4.95 mg/kg (NR)	NR	ICP-MS (NR)		Ngole-Jeme et al. (2018)
Kindergarten soil	Yerevan city, Armenia, 2012	$n = 111$	15.2 mg/kg (10.6–20.0)	15 mg/kg (NR)	XRF (5 mg/kg)	EPA Standard Method 6200	Tepanosyan et al. (2017)
Paddy field topsoil	Xiangtan city, China, 2017	$n = 63$	18.75 mg/kg (16.5–21.7)	NR	ICP-AES (NR)		Deng et al. (2019)
Lake water	Haro River, Khanpur, Pakistan, 2009	$n = 50$	0.303 mg/L (0.081–0.848)	NR	FAAS (NR)		Iqbal et al. (2012)
Groundwater	Najran city, Saudi Arabia, 2012	$n = 11$	0.02 µg/L (0.01–0.09)	NR	ICP-MS (0.002 µg/L)		Brima (2017)
Domestic water	Kilembe copper mine, Uganda, 2014	$n = 12$	20 µg/L (0.03–66)	NR	ICP-MS (NR)		Mwesigye et al. (2016)
Marine sediment	Ria of Ferrol, Spain, date of sample collection NR	$n = 35$	10 µg/g (5.0–21.0)	NR	ETAAS (0.43 µg/g)		Barciela-Alonso et al. (2003)
Mangrove sediments	Shenzhen, China, date of sample collection NR	$n = 27$	129 mg/kg (15.0–502)	NR	ICP-AES (1 mg/kg)		Xu et al. (2015)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Food, beverages, and wild fish							
Food crops: Wheat grains Mustard seeds Rice grains Maize grains	Ropar wetland, Punjab, India, 2013	n_{total} = 36 n_{wheat} = 9 $n_{mustard}$ = 9 n_{rice} = 13 n_{maize} = 5	Units, mg/kg dw: Wheat: 14.0 (12.8–14.7) Mustard: 13.5 (12.4–14.8) Rice: 15.2 (13.5–16.8) Maize: 15.1 (14.0–16.1)	NR	FAAS (NR)		Sharma et al. (2018)
Food crops: *Amaranthus tricolour* Maize Bananas	Kilembe copper mine, Uganda, 2014	31 4 5	Units, mg/kg dw: 4.20 (0.01–81) 0.16 (0.01–0.47) 0.17 (0.01–0.50)	NR	ICP-MS (NR)		Mwesigye et al. (2016)
Cassava		2	0.78 (0.15–1.41)				
Mangoes		2	0.26 (0.26–0.41)				
Rice	Gonbad county, Iran (Islamic Republic of), 2018	n = 90 (total), n = 8 (Gonbad)	NR (0–0.110 625 mg/kg)	NR	NR		Kiani et al. (2021)
Fish	Gaotang Lake, Huainan, China, date of sample collection NR	n = 28 Crucian carp: n = 5 Bighead carp: n = 5 Silver carp: n = 4 Tilapia: n = 5 Common carp: n = 5 Grass carp: n = 4	Units, mg/kg fw: Crucian carp: 0.074 Bighead carp: 0.101 Silver carp: 0.076 Tilapia: 0.089 Common carp: 0.060 Grass carp: 0.149	NR	ICP-MS (1 µg/kg)		Cheng et al. (2019)
Vegetables (excluding potatoes)	France, 2007–2009	n = 269	0.0061 mg/kg fw (NR)	NR	ICP-MS (0.002 mg/kg)	French Total Diet Study	Arnich et al. (2012)
Potatoes	Lebanon, 2008	n = 15	37.53 µg/kg fw (34.50–44.10)	NR	ICP-MS (0.0005 mg/kg)	Lebanese Total Diet Study	Nasreddine et al. (2010)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Beverages	France, 2006–2007	n = 143	0.007 mg/kg fw (0.001–0.032)	NR	ICP-MS (NR)	French Total Diet Study	Noël et al. (2012)
Infant formulas (RTU, CL, or P formula)	Canada, 1986–1987	n_{RTU} = 49	RTU: 1.22 ng/g (0.21–5.2)	RTU: 0.53 ng/g	GFAAS (0.04–0.21 µg/kg)		Dabeka (1989a, b)
		n_{CL} = 50	CL: 2.89 ng/g (0.25–11.8)	CL: 2.27 ng/g			
		n_{P} = 64	P: 12.7 ng/g (2.6–53)	P: 9.54 ng/g			
Tobacco and cosmetics							
Tobacco	Oporto city, Portugal, 2014	n = 40	0.84 µg/g dw (0.61–1.08)	NR	ICP-MS (0.006 µg/g)		Pinto et al. (2017)
Henna dye	Republic of Korea, date of sample collection NR	n = 15	NR (LOD–3.54 mg/kg)	NR	FAAS (1.25 mg/L)		Kang & Lee (2006)
Hair dyes	Kashan city, Iran (Islamic Republic of), 2019	n = 36	0.475 µg/g (NR)	NR	ICP-OES (0.007 µg/g)		Mostafaii et al. (2022)
Eye liner	Seoul city, Republic of Korea, date of sample collection NR	n = 13	11.80 µg/g (0.11–41.08)	NR	ICP-MS (NR)		Lim et al. (2018)

CL, concentrated liquid; DRC, Democratic Republic of the Congo; dw, dry weight; EPA, Environmental Protection Agency; ETAAS, electrothermal atomic absorption spectrometry; e-waste, electronic and/or electrical waste; FAAS, flame atomic absorption spectrometry; fw, fresh weight; GFAAS, graphite furnace atomic absorption spectrometry; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma with optical emission spectrometry; IQR, interquartile range; LOD, limit of detection; NR, not reported; P, powdered; PM_1, particulate matter with aerodynamic diameter < 1.0 µm; $PM_{2.5}$, particulate matter with aerodynamic diameter < 2.5 µm; RTU, ready to use; XRF, X-ray fluorescence spectrometry.

[a] Working Group conversion to International System of Units.

[b] Geometric mean.

[c] Mean values are expressed as the arithmetic mean unless stated otherwise.

cobalt-containing pigments and driers; battery production; and electronics recycling. The largest occupational groups exposed are likely to include welders and related machine operators; dental technologists, technicians, and laboratory assistants; and machinists and machining and tooling inspectors. Other occupations with potential for exposure to cobalt include workers involved in smelting and refining, the mining of ores containing cobalt and other metals, cobalt dye painting, cobalt chemical production, diamond polishing, glassware or porcelain work, offset printing, goldsmithing, and rockwool insulation (IARC, 1991, 2006; Donaldson & Beyersmann, 2011; CAREX Canada, 2022).

The National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), conducted between 1981 and 1983, estimated the number of workers exposed to chemical, physical, and biological agents, including cobalt compounds, on the basis of site visits to approximately 4500 workplaces, and representing roughly 1.8 million workers across the USA. Using industrial classifications, the survey estimated that metallic cobalt exposure was most common among workers in primary metal industries (19.2% of all exposed workers). Exposure to cobalt(II) compounds was most common in the chemicals and applied products industries, representing 47.0%, 40%, 55.1%, and 71.2% of all workers exposed to cobalt(II) chloride, cobalt(II) acetate, cobalt(II) oxide, and cobalt(II,III) oxide, respectively (NIOSH, 1990). From an occupational perspective, welders and cutters, chemical technicians, and clinical laboratory technologists and technicians were groups estimated to be most likely to be exposed to metallic cobalt (13.8% of exposed workers), cobalt(II) chloride (38.7% of exposed workers), and cobalt(II) acetate (15.4% of exposed workers), respectively (NIOSH, 1990).

Table 1.8 summarizes the data on occupational exposure to cobalt, as measured by analysis of concentrations in air, or via biological monitoring of cobalt concentrations in blood or urine samples among workers, in various types of industries and at different production stages. Occupational exposure to cobalt occurs predominantly during refining of cobalt, production of cobalt metals and cobalt compounds, use of diamond–cobalt tools, production of dental materials, manufacture of nickel–hydrogen batteries, and plate painting with cobalt pigments. Workers may be exposed to a mixture of various cobalt compounds and cobalt metal powders. In addition, the potential for co-exposure to nickel and other known or suspected human carcinogens has been reported in various occupational studies (see Table 1.8 and Table 1.9).

Scarselli et al. (2020) reported the ranges of exposure levels of cobalt and cobalt(II) compounds from an occupational exposure registry in Italy between 1996 and 2016. Most exposures occurred during the manufacture of fabricated metal products (50%) and among metal finishing, plating, and coating machine operators (42%). The manufacture of basic metals was the industrial sector in which exposure to cobalt, as a metal, was most frequently reported (mainly for metal smelters, casters, and rolling-mill operators), whereas exposure to cobalt nitrate was principally reported in the manufacture of fabricated metal products (mainly for metal finishing, plating, and coating machine operators). Exposure to cobalt sulfate was widespread in all sectors, except for the manufacture of basic metals. Overall, cobalt sulfate heptahydrate was the compound with the highest mean level of exposure (GM, 1.09 μg/m^3), whereas cobalt nitrate had the lowest level (GM, 0.11 μg/m^3) (Scarselli et al., 2020).

Table S1.10 summarizes the distribution of air concentrations of cobalt across the industrial sectors recorded by the Italian occupational exposure registry (see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The manufacture

Table 1.8 Occupational exposure to cobalt as measured among workers in various types of industries and production stages

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
General industrial settings						
Industrial settings obtained from Italian occupational exposure registry, Italy, 1996–2016	Personal or stationary air of Co and Co compounds	459	Co: 0.33 µg/m³ [a]		Over whole 8-h work shift sampling.	Scarselli et al. (2020)
		109	Co sulfate heptahydrate: Co 1.09 µg/m³ [a]			
		50	Co acetate: Co 0.81 µg/m³ [a]			
		92	Co chloride: Co 0.76 µg/m³ [a]			
		112	Co nitrate hexahydrate: Co 0.61 µg/m³ [a]			
		67	Co acetate tetrahydrate: Co 0.50 µg/m³ [a]			
		325	Co sulfate: Co 0.44 µg/m³ [a]			
		76	Co chloride hexahydrate: Co 0.42 µg/m³ [a]			
		369	Co nitrate: Co 0.11 µg/m³ [a]			
Occupational exposure in different Co industries, United Kingdom, 1988–1991	Urinary Co	780		93.0 (90th, 486) nmol/mmol creatinine	End of working week at end-of-shift sampling; workers of chemical manufacture (manufacturing and handling of Co powders, Co salts, and pigments).	White & Dyne (1994)
	Urinary Co			19.0 (90th, 107) nmol/mmol creatinine	Same sampling; workers of hard-metal manufacture (pre-sinter operations such as mixing, pressing, and furnace operation, and post-sinter grinding operations).	
	Urinary Co			17.0 (90th, 80) nmol/mmol creatinine	Same sampling; workers of hard-metal finishing (grinding and sharpening of hard-metal tools).	
	Urinary Co			< 3.0 (90th, 42) nmol/mmol creatinine	Same sampling; workers of other metal working (welding and prosthesis manufacture using Co-containing metals and alloys).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Cobalt refinery and production of cobalt metal and salts						
Co refinery workers, Belgium, 1993	Personal air of Co dusts	82	127.5 (2–7700) μg/m³ on Monday 120.9 (1–7772) μg/m³ on Friday	84.5 μg/m³ on Monday 110.0 μg/m³ on Friday	Workers exposed to a mixture of various Co salts, oxides, and fine Co metal powders; 6 h sampling.	Swennen et al. (1993)
	Blood Co	82	1.10 (0.2–12.0) μg/dL	1.10 μg/dL	Monday end-of-shift sampling.	
	Blood Co	82	1.27 (0.2–12.0) μg/dL	1.20 μg/dL	Friday end-of-shift sampling.	
	Urinary Co	82	52.9 (2.66–2245) μg/g creatinine	44.1 μg/g creatinine	Monday end-of-shift sampling.	
	Urinary Co	82	69.8 (1.56–2038) μg/g creatinine	72.4 μg/g creatinine	Friday end-of-shift sampling.	
Co refinery workers, Belgium, 2008–2009	Personal air of Co dusts	249	NR (1–108 μg/m³) in 2007	15.0 (1.0–108.0) μg/m³ in 2007	Workers exposed to a mixture of various Co salts, oxides, and fine Co metal powders; 6 h sampling; Follow-up in engineering improvements from Swennen et al. (1993).	Lantin et al. (2011, 2013)
	Blood Co	249		0.10 (< 0.05–3.20) μg/100 mL		
	Urinary Co	249		3.9 (0.3–204.3) μg/g creatinine		
Co plant that produces fine Co metal powders, Co oxides, and Co salts, Belgium, 1988–2001	Urinary Co	122	Dry-stage area (approx. 70–250 μg/g creatinine) was the highest, followed by wet-stage area (approx. 15–55 μg/g creatinine) and mixed-exposure area (135–35 μg/g creatinine)		End of working week end-of-shift sampling; Three types of exposure: (1) production of Co metal powder, Co oxides, or salts in the dry-stage area; (2) wet-stage area, and (3) mixed exposure for maintenance workers and foremen involved at different steps of the process.	Verougstraete et al. (2004)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Production of Co metal and Co salts, Kokkola, Finland, 1967–2000	Stationary and personal air of Co dusts	110 to 93	Range, 0.05–0.25 mg/m³		Reduction and powder production.	Linna et al. (2003, 2004)
	Stationary and personal air of Co sulfates		Range, 0.02–1.0 mg/m³		Sulfatizing roasting process.	
	Stationary and personal air of Co sulfates		Range, 0.01–0.05 mg/m³		Leaching and solution purification process.	
	Stationary and personal air of mixtures of Co sulfates, carbonates, oxides, and hydroxides		Range, 0.01–0.20 mg/m³		Chemical processing department.	
	Urinary Co		Maximum, 16 000 nmol/L		Reduction and powder production.	
	Urinary Co		Range, 300–2000 nmol/L		Purification and chemical department.	
Production of Co metal and Co salts, Kokkola, Finland, 1999–2006	Urinary Co	29		240 (range, 11–4107) nmol/L	Reduction and powder production 1999–2000.	Linna et al. (2020)
	Urinary Co	113		230 (range, 6–6278) nmol/L	Reduction and powder production 2005–2006.	
Cobalt containing diamond tooling						
Diamond polisher in workshops, Belgium	Stationary air of Co dusts	8	17.9 (0.1–45.0) µg/m³		No "hard metals" have been found; 1 m sampling from the middle of the rotating disc in the worker's breathing zone.	van den Oever et al. (1990)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Diamond polisher in workshops, Belgium (date of sample collection NR)	Personal air of Co dusts	92	15.1 (0.7–42.8) µg/m³		Polishing discs showed no presence of tungsten (i.e. no "hard metal"); higher exposure group; sampling began 2 h after starting work and lasted until 1 h before the end of the working day.	Nemery et al. (1992)
	Stationary air of Co dusts	92	10.2 (3.8–19.8) µg/m³			
	Personal air of Co dusts	102	5.3 (0.2–11.2) µg/m³		Lower exposure group.	
	Stationary air of Co dusts	102	1.6 (0.5–4.3) µg/m³			
	Urinary Co	92	20.5 (2.3–75.0) µg/g creatinine		Higher exposure group.	
	Urinary Co	102	7.0 (0.7–26.5) µg/g creatinine		Lower exposure group.	
Alloys or plating containing cobalt						
Manufacturers of permanent magnets, USA, 1988	Personal air of Co dusts	100	17.5[a] (1–466) µg/m³		Whole-work-shift sampling; Ni: 4.4 (ND–368) µg/m³; Nd: 2.6 (ND–52) µg/m³. Sm: 3.9[a] (ND–528) µg/m³.	Deng et al. (1991)
Electroplaters in bright plating factory (date of sample collection NR)	Stationary air of Co dusts	42	Below LOD (< 0.12 ng/m³)		Cr: 0.0002 ± 0.0001 mg/m³.	Wultsch et al. (2017)
	Blood Co	42	0.85 ± 0.32 µg/L		Cr: 0.44 ± 0.24 (µg/L).	
Dental procedures						
Dental technicians, Sweden (date of sample collection NR)	Stationary air of Co dusts	8	Maximum value of 1.6 mg/m³		Whole-work-shift sampling; CoCrMo alloys (60–66%, 25–32%, 4–6% content, respectively); without local exhaust ventilation.	Seldén et al. (1995)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Dental technicians of metal prostheses, Ankara, Türkiye (date of sample collection NR)	Urinary Co	23	24.8 (0.4–111.5) µg/g creatinine		End-of-shift sampling after four consecutive exposure periods; Ni: 7.7 (4.2–12.6) µg/g creatinine; Cr: 4.4 (0.5–11.9) µg/g creatinine.	Burgaz et al. (2002)
Dental technicians of metal prostheses, Indonesia (date of sample collection NR)	Blood Co	40	26.8 (95% CI, 14.8–38.7) µg/L		Morning (07:00–09:00) sampling; Ni: 36.8 (95% CI, 22.0–51.6) µg/L; Cr: 346.4 (95% CI, 303.8–388.9) µg/L.	Berniyanti et al. (2020)
Cobalt pigments						
Porcelain plate painters exposed to Co blue dye in porcelain factory, Denmark, 1981	Personal air of Co dusts	19		0.80 (0.068–8.61) Co mg/m^3	3 h sampling; Co blue underglaze colour is made by melting together a mixture of Co-Zn-silicate, Zn oxide, and silicon oxide; there was no detectable concentration of silica.	Raffn et al. (1988)
Porcelain plate painters exposed to Co blue dye in porcelain factory, Denmark, 1982	Blood Co	46	36.7 (3.40–407) nmol/L		Working 4 wk.	Raffn et al. (1988)
	Blood Co	46	8.05 (1.70–22.1) nmol/L		Off work for 6 wk.	
	Urinary Co	46	141.8 (4.04–2776) nmol/mmol creatinine		Working 4 wk.	
	Urinary Co	46	8.82 (< 1.70–65.2) nmol/mmol creatinine		Off work for 6 wk.	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Pottery plate painters, Copenhagen, Denmark, 1982 and 1984	Personal air of soluble Co-Zn-silicate	46	Range, 0.07–8.61 mg/m^3		No correlation: air/blood and air/urine.	Christensen & Mikelsen (1986)
	Personal air of insoluble Co aluminate dyes	15	Range, 0.05–0.25 mg/m^3			
	Blood Co of soluble Co-Zn-silicate	46	2.16 (0.2–24) µg/L	1.00 µg/L	Significant correlation: blood/urine (r = 0.82).	
	Blood Co of insoluble Co aluminate dyes	15	0.63 (0.37–1.58) µg/L	0.60 µg/L	Significant correlation: blood/urine (r = 0.88).	
	Urinary Co of soluble Co-Zn-silicate	46	8.35 (0.24–163) µg/mmol creatinine	2.67 µg/mmol creatinine		
	Urinary Co of insoluble Co aluminate dyes	15	0.13 (0.02–0.37) µg/mmol creatinine	0.11 µg/mmol creatinine		
Pottery plate painters, Copenhagen, Denmark, 1982–1991	Personal air of soluble Co-Zn-silicate	8–100	Mean range 1982–1991; 1356–454 nmol/m^3		Follow-up from Christensen & Mikelsen (1986).	Christensen & Poulsen (1994)
	Urinary Co of soluble Co-Zn-silicate	27–145	Mean range 1982–1991; 133.4–18.8 nmol/mmol creatinine		Follow-up from Christensen & Mikelsen (1986).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Electrical industry						
Battery plant manufacturing Ni–hydrogen batteries, Japan (date of sample collection NR)	Personal air of Co dusts	30	0.067 (0.004–0.330) mg/m³		Over 9 h sampling; Ni: 0.481 (0.018–2.376) mg/m³; significant correlation: Co/Ni (r = 0.96).	Yokota et al. (2007)
	Urinary Co	128	38.6 (1.0–76.8) µg/L corrected for specific gravity		End of working week end-of-shift sampling. Ni: 21.5 (5.0–67.5) µg/L corrected for specific gravity. Significant correlation: Co/Ni (r = 0.70).	
Digital video cassette manufacturing plant, Japan (date of sample collection NR)	Personal air of Co oxide dusts, including both cobalt(II) oxide and cobalt(II,III) oxide	20	Range, below LOD < 1 to approx. 22 µg/m³		8 h sampling; significant correlation: air/urine (r = 0.76).	Fujio et al. (2009)
	Urinary Co	20	Range, below LOD < 1 to approx. 27.5 µg/g creatinine		End-of-shift sampling.	
E-waste recycling industry						
Recycling workers, Sweden, 2007–2009	Personal air of Co dusts	77	0.066[a] (0.0017–3.3) µg/m³		Inhalable fraction according to EN 481; 10 h work shift sampling; Hg: 0.011[a] (0.00 031–0.21) µg/m³; Pb: 7.0[a] (0.011–130) µg/m³; Cd: 0.18[a] (0.0011–11) µg/m³; Ni: 0.49[a] (0.0089–15) µg/m³; Cr: 0.45[a] (0.0050–6.9) µg/m³; As: 0.04[a] (0.001–0.730) µg/m³; Sb: 0.21[a] (0.0041–1.1) µg/m³.	Julander et al. (2014)
	Blood Co	50		0.081 (0.050–0.67) µg/L	Hg: 1.4 (0.28–18) µg/L; Pb: 32 (9.5–230) µg/L; Cr: 1.4 (0.34–5.0) µg/L.	
	Urinary Co	52		0.25 (0.12–1.3) µg/L	Hg: 1.4 (0.35–4.4) µg/L; Pb: 1.8 (0.19–17) µg/L; Cd: 0.37 (0.12–2.4) µg/L; Cr: 0.74 (0.0097–5.29) µg/L; As: 13 (2.4–410).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Recycling workers, Germany, 2017–2018	Personal air of Co dusts	40	0.041[a] (0.018–0.31) μg/m^3	0.035 μg/m^3	Inhalable fraction; during disassembly work; Ni: 0.27[a] (0.058–1.9) μg/m^3; Sb: 0.091[a] (0.051–0.34) μg/m^3; Cr: 0.20[a] (0.081–1.4) μg/m^3; As: 0.033[a] (0.021–0.069) μg/m^3; Cd: 0.017[a] (0.005–0.23) μg/m^3; Hg; 0.47[a] (0.14–3.3) μg/m^3.	Gerding et al. (2021)
	Stationary air of Co dust	21	0.035[a] (0.015–0.18) μg/m^3	0.033 μg/m^3	Inhalable fraction; during disassembly work; Ni: 0.11[a] (0.052–0.69) μg/m^3; Sb: 0.067[a] (0.047–0.17) μg/m^3; Cr: 0.12[a] (0.063–0.64) μg/m^3; As: 0.031[a] (0.018–0.130) μg/m^3; Cd: 0.009[a] (0.006–0.044) μg/m^3; Hg; 0.46[a] (0.16–6.6) μg/m^3.	
	Personal air of Co dusts	4	0.021[a] (0.018–0.024) μg/m^3	0.022 μg/m^3	Respirable fraction; during disassembly work; Ni: 0.076[a] (0.064–0.088) μg/m^3; Sb: 0.076[a] (0.064–0.088) μg/m^3; Cr: 0.077[a] (0.065–0.09) μg/m^3; As: 0.025[a] (0.022–0.029) μg/m^3; Cd: 0.008[a] (0.007–0.009) μg/m^3.	
	Stationary air of Co dust	12	0.034[a] (0.018–0.062) μg/m^3	0.034 μg/m^3	Respirable fraction; during disassembly work; Ni: 0.08[a] (0.047–0.20) μg/m^3; Sb: 0.064[a] (0.047–0.14) μg/m^3; Cr: 0.11[a] (0.065–0.24) μg/m^3; As: 0.033[a] (0.021–0.077) μg/m^3; Cd: 0.007[a] (0.005–0.015) μg/m^3.	
	Urinary Co	51	0.32[a] (0.15–1.6) μg/L	0.50 μg/L	End-of-shift sampling; Ni: 0.74[a] (0.15–3.8) μg/L; Sb: 0.26[a] (0.15–2.4) μg/L; Cr: 0.10[a] (0.08–1.1;) μg/L; As: 1.96[a] (1.0–8.6) μg/L; Cd: 0.16[a] (0.08–1.8) μg/L; Hg; 0.38[a] (0.1–4.6) μg/L.	

As, arsenic; Cd, cadmium; CI, confidence interval; Co, cobalt; Cr, chromium; e-waste, electronic and/or electrical waste; Hg, mercury; LOD, limit of detection; Mo, molybdenum; Nd, neodymium; ND, not detected; Ni, nickel; NR, not reported; Pb, lead; Sb, antimony; SD, standard deviation; Sm, samarium; W, tungsten; wk, week; Zn, zinc.

[a] Geometric mean ± geometric SD.

Table 1.9 Exposures to IARC Group 1 and Group 2A agents potentially co-occurring with cobalt in epidemiological studies considered by the Working Group[a]

Agent (CAS No.)	Occupational settings[c]											IARC Group (Vol., year)	Study design and reference	
	A	B	C	D	E	F	G	H	I	J	K		Cohort	Case–control
Arsenic and inorganic arsenic compounds (7440-38-2)				✓		✓	✓	✓				1 Vols 23, Suppl. 7, 100C (2012)	Marsh et al. (2009); Moulin et al. (1993)	Grimsrud et al. (2005); Rodrigues et al. (2020)
Asbestos (all forms) and other fibres[b] (1332-21-4, 12172-73-5, 12001-29-5, 12001-28-4)			✓	✓			✓	✓		✓		1 Vols 14 Suppl. 7, 100C (2012)	Moulin et al. (1993, 1998); Tüchsen et al. (1996); Wild et al. (2000)	Grimsrud et al. (2005); Rodrigues et al. (2020)
Benzene (71-43-2)								✓				1 Vols 29, Suppl. 7, 100F, 120 (2018)		Rodrigues et al. (2020)
Beryllium and beryllium compounds (7440-41-7)								✓				1 Vols 58, 100C (2012)		Rodrigues et al. (2020)
Cadmium and cadmium compounds (7440-43-9)	✓					✓		✓				1 Vols 58, 100C (2012)	Marsh et al. (2009); Li et al. (2021a)	Rodrigues et al. (2020); Bai et al. (2019)
Chromium(VI) compounds (18540-29-9)	✓							✓			✓	1 Suppl. 7, Vols 49, 100C (2012)	Hogstedt & Alexandersson (1990); Moulin et al. (1998, 2000); Wild et al. (2000); Li et al. (2021a) (chromium species not specified)	Bai et al. (2019) (chromium species not specified); Rodrigues et al. (2020)
Crystalline silica[b] (14808-60-7)								✓			✓	1 Suppl. 7, Vols 68, 100C (2012)	Wild et al. (2000)	Rodrigues et al. (2020)
Epichlorohydrin (106-89-8)								✓				2A Vols 11, Suppl. 7, 71 (1999)		Rodrigues et al. (2020)
Formaldehyde (50-00-0)								✓				1 Suppl. 7, Vols 62, 88, 100F (2012)		Rodrigues et al. (2020)
Lead (7439-92-1)												2A Suppl. 7, Vol. 87 (2006)		Rodrigues et al. (2020)
Methylene chloride (75-09-2)								✓				2A Suppl. 7, Vols 71, 110 (2017)		Rodrigues et al. (2020)

Table 1.9 (continued)

Agent (CAS No.)	Occupational settings[c]											IARC Group (Vol., year)	Study design and reference	
	A	B	C	D	E	F	G	H	I	J	K		Cohort	Case–control
Nickel (7440-02-0)	✓	✓		✓	✓		✓	✓	✓	✓	✓	1 (Nickel compounds; and metallic) Suppl. 7, Vols 49, 100C (2012)	Cuckle et al. (1980); Hogstedt & Alexandersson (1990); Moulin et al. (1993, 1998, 2000); Sauni et al. (2017); Tüchsen et al. (1996); Wild et al. (2000); Li et al. (2021a); (nickel species not specified)	Bai et al. (2019); Grimsrud et al. (2005); Rodrigues et al. (2020); (nickel species not specified)
Perchloroethylene [tetrachloroethylene] (127-18-4)								✓				2A Suppl. 7, Vols 63, 106 (2014)		Rodrigues et al. (2020)
Sulfuric acid (7664-93-9) Included in acid mists, strong inorganic				✓				✓				1 Vols 54, 100F (2012)		Grimsrud et al. (2005); Rodrigues et al. (2020)
Trichloroethylene (79-01-6)								✓				1 Suppl. 7, Vols 63, 106 (2014)		Rodrigues et al. (2020)
Vinyl chloride (75-01-4)								✓				1 Suppl. 7, Vols 97, 100F (2012)		Rodrigues et al. (2020)

CAS No., Chemical Abstracts Service Registry number; IARC, International Agency for Research on Cancer; Suppl., supplement; Vol., volume.

[a] Not comprehensive of all possible co-exposures in the listed study populations. Cobalt metal with tungsten carbide was not included in this table as a co-exposure.

[b] A case series study was also available, Dufresne et al. (1996).

[c] Occupational settings: A, automobile manufacturing; B, manufacturing of cobalt and nickel salts; C, aluminium smelting; D, nickel refining E, metal plants; F, copper smelting; G, production of cobalt and sodium; H, semi-conductor and electronic storage-device manufacturing; I, production of cobalt powder; J, porcelain factories; K, production of stainless and alloyed steel.

of cutlery, tools, and general hardware showed the highest value (GM, 3.69 μg/m³, men; arithmetic mean, AM, 7.32 μg/m³) (Scarselli et al., 2020). Table S1.11 shows the distribution of air concentrations of cobalt across occupational groups in the industrial sectors recorded by the Italian occupational exposure registry (Scarselli et al., 2020; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The occupational groups with the highest measured exposures were machine-tool setters and setter operators (GM, 5.32 μg/m³, men; AM, 5.98 μg/m³), although this was based on a relatively low number of measurements (n = 50). Regarding the distributions of cobalt exposure levels according to the workforce size, microfirms (1–9 workers) had the highest value for cobalt exposure (GM, 1.06 μg/m³), whereas medium-sized firms (50–99 workers) had the lowest (GM, 0.07 μg/m³) (Scarselli et al., 2020).

The French National Institute for Research and Occupational Safety (INRS) has reported data for two periods of measurement of cobalt concentrations in air relating to industrial sectors and job titles, recorded between 1987 and 1999, and 2000 and 2020 (INRS, 2022). Table 1.12 and Table 1.13 summarize the distributions across industrial sectors and job titles, respectively, between 2000 and 2020. The manufacture of food products had the highest arithmetic mean value for personal air sampling (105 μg/m³), followed by human health and social work activities (76 μg/m³), manufacture of fabricated metal products (69 μg/m³), and mining and quarrying (49 μg/m³) (Table 1.12). Across the different job titles, clerical support workers (production clerks) had the highest arithmetic mean value (816 μg/m³), followed by farming, forestry, and fisheries advisers (616 μg/m³) (Table 1.13).

In a study in different industries in the UK in which urinary cobalt levels were collected during the period 1988–1991, it was reported that workers who manufactured and handled cobalt powders, cobalt salts, and pigments in chemical industries had the highest median concentration of cobalt in urine (93 nmol/mmol creatinine [48 μg/g creatinine]), followed by workers involved in hard-metal manufacturing (19 nmol/mmol creatinine [10 μg/g creatinine]) and hard-metal finishing (17 nmol/mmol creatinine [9 μg/g creatinine]), and workers involved in other metalworking industries (< 3.0 nmol/mmol creatinine [< 1.6 μg/g creatinine]) including welding and prosthesis manufacturing, where several cobalt-containing metals and alloys were employed (see Table 1.8) (White & Dyne, 1994).

Several scenarios or situations involving exposure to cobalt and cobalt compounds in occupational settings are summarized in the following sections.

(b) Skin exposure in occupational settings

Many cobalt alloys and platings can release significant amounts of cobalt upon contact with skin. Occupational skin exposure to cobalt has been studied in different settings (Julander et al., 2010). Skin exposure to metallic cobalt has been reported in several industrial settings as follows: in the manufacture of components for gas turbines and space propulsion using metal alloys, stainless steels, hard-metal items, and metal powders for thermal application (Julander et al., 2010); contact with raw and sintered materials and contaminated surfaces in hard-metal production (Kettelarij et al., 2018b); the handling of metal tools in the hairdressing trade, including hair clips, tweezers, sectioning clips, and straight razors (Symanzik et al., 2021); and hand-held work tools, including cutting tools, handsaws, and paint-scraping tools (Thyssen et al., 2011). Skin exposure can also occur in the construction industry. Several studies have reported hypersensitivity to cobalt chloride among construction workers (Bock et al., 2003; Uter et al., 2004; Lazzarini et al., 2012). Cases of on-the-skin exposure to cobalt have been reported for ceramics decorators (Kargar et al., 2013).

Table 1.12 Distribution of air concentrations of cobalt in industrial sectors, 2000–2020, France[a]

Industrial sector	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Mining and quarrying	11	49	12	9–29	34	23	10	5–15
Manufacture of food products	176	105	2	< LOQ–5	265	54	1	< LOQ–3
Manufacture of leather and related products	7	2	NC	NC	NC	NC	NC	NC
Manufacture of wood and of products of wood and cork, except furniture; manufacture of articles of straw and plaiting materials	45	37	11	4–42	31	17	3	< LOQ–16
Printing and reproduction of recorded media	5	< LOQ	NC	NC	NC	NC	NC	NC
Manufacture of coke and refined petroleum products	15	< LOQ	< LOQ	< LOQ to < LOQ	NC	NC	NC	NC
Manufacture of chemicals and chemical products	56	29	< LOQ	< LOQ–1	16	4	0	< LOQ–3
Manufacture of rubber and plastics products	26	8	1	0–3	7	19	NC	NC
Manufacture of other non-metallic mineral products	44	18	< LOQ	< LOQ–3	21	5	< LOQ	< LOQ to < LOQ
Manufacture of basic metals	170	20	2	< LOQ–9	100	12	4	< LOQ–8
Manufacture of fabricated metal products, except machinery and equipment	1095	69	4	< LOQ–23	371	97	2	< LOQ–9
Manufacture of computer, electronic, and optical products	35	9	< LOQ	< LOQ–4	19	1	< LOQ	< LOQ to < LOQ
Manufacture of electrical equipment	70	4	< LOQ	< LOQ–2	58	39	1	< LOQ–10
Manufacture of machinery and equipment	200	4	0	< LOQ–2	34	1	< LOQ	< LOQ to < LOQ
Manufacture of motor vehicles, trailers, and semi-trailers	46	1	< LOQ	< LOQ–0	28	1	< LOQ	< LOQ to < LOQ
Manufacture of other transport equipment	94	46	2	< LOQ–19	53	11	< LOQ	< LOQ–2
Manufacture of furniture	11	13	5	< LOQ–19	4	NC	NC	NC
Other manufacturing	334	21	2	< LOQ–10	134	6	< LOQ	< LOQ–3
Repair and installation of machinery and equipment	145	31	< LOQ	< LOQ–6	59	3	< LOQ	< LOQ to < LOQ
Electricity, gas, steam, and air-conditioning supply	9	1	NC	NC	21	< LOQ	< LOQ	< LOQ to < LOQ
Water supply; sewerage, waste management, and remediation activities	103	3	< LOQ	< LOQ–1	76	1	< LOQ	< LOQ to < LOQ
Construction	88	6	< LOQ	< LOQ–2	51	2	< LOQ	< LOQ to < LOQ
Wholesale and retail trade; repair of motor vehicles and motorcycles	105	45	1	< LOQ–12	66	17	< LOQ	< LOQ–3
Transportation and storage	24	1	< LOQ	< LOQ to < LOQ	31	< LOQ	< LOQ	< LOQ to < LOQ
Financial and insurance activities	9	3	NC	NC	58	< LOQ	< LOQ	< LOQ to < LOQ
Real estate activities	30	11	1	< LOQ–5	33	2	< LOQ	< LOQ to < LOQ

Table 1.12 (continued)

Industrial sector	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Professional, scientific, and technical activities	19	5	< LOQ	< LOQ to < LOQ	5	< LOQ	NC	NC
Administrative and support service activities	6	0	NC	NC	NC	NC	NC	NC
Public administration and defence; compulsory social security	35	< LOQ	< LOQ	< LOQ to < LOQ	5	< LOQ	NC	NC
Education	7	< LOQ	NC	NC	NC	NC	NC	NC
Human health and social work activities	25	76	2	< LOQ–25	11	< LOQ	< LOQ	< LOQ to < LOQ
Other service activities	6	1	NC	NC	29	2	< LOQ	< LOQ to< LOQ

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements; NC, not calculated.

[a] Data from the French National Institute for Research and Occupational Safety, INRS (2022).

Table 1.13 Distribution of air concentrations of cobalt according to job title, 2000–2020, France[a]

Job titles	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Professionals	50	358	5	< LOQ–50	46	297	12	2–59
Physical and earth science professionals	6	< LOQ	NC	NC	NC	NC	NC	NC
Farming, forestry, and fisheries advisers	29	616	34	11–94	40	341	16	5–61
Environmental protection professionals	12	2	1	< LOQ–2	6	< LOQ	NC	NC
Technicians and associate professionals	335	27	< LOQ	< LOQ–6	169	6	< LOQ	< LOQ–4
Clerical support workers	20	372	3	0–81	14	0	< LOQ	< LOQ to < LOQ
Stock clerks	8	11	NC	NC	NC	NC	NC	NC
Production clerks	9	816	NC	NC	NC	NC	NC	NC
Craft and related trades workers	1641	23	2	< LOQ–7	449	10	< LOQ	< LOQ–2
Metal, machinery, and related trades workers	1530	24	2	< LOQ–8	376	11	< LOQ	< LOQ–2
Handicraft and printing workers	48	2	< LOQ	< LOQ–2	49	1	< LOQ	< LOQ to < LOQ
Electrical and electronic trades workers	5	26	NC	NC	NC	NC	NC	NC
Food processing, wood working, garment and other craft, and related trades workers	29	26	< LOQ	< LOQ–2	16	2	< LOQ	< LOQ to < LOQ
Plant and machine operators, and assemblers	454	19	1	< LOQ–5	291	9	< LOQ	< LOQ–3
Stationary plant and machine operators	429	20	2	< LOQ–6	275	9	< LOQ	< LOQ–3
Assemblers	7	1	NC	NC	8	< LOQ	NC	NC
Drivers and mobile plant operators	18	1	< LOQ	< LOQ to < LOQ	8	< LOQ	NC	NC
Elementary occupations	122	12	< LOQ	< LOQ–2	52	1	< LOQ	< LOQ to < LOQ

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements.

[a] Data from the French National Institute for Research and Occupational Safety, INRS (2022).

One study reported that the excretion of cobalt in urine was elevated after skin exposure to a coolant solution containing cobalt. The amount of cobalt excreted by five people during the 24-hour period before the skin exposure averaged 18.1 nmol (range, 6.8–34.3 nmol). After the skin exposure to the coolant solution, the average amount of cobalt excreted over 24 hours increased to 38.5 nmol (range, 14.2–61.4 nmol). This increase was statistically significant (P = 0.012) (Linnainmaa & Kiilunen, 1997). The amount of cobalt on the skin has also been shown to be significantly correlated with concentration of cobalt in samples of urine (Kettelarij et al., 2018a) and blood (Klasson et al., 2017; Wahlqvist et al., 2020) obtained from workers in factories involved in hard-metal manufacturing.

Further potential for occupational exposure because of inadvertent ingestion by workers, resulting from contact between contaminated hands or objects and mouths, has been suggested (Cherrie et al., 2006). As such inadvertent ingestion exposure is anticipated to arise mainly as a result of hand-to-mouth contact, it is closely linked to dermal exposure and is potentially a significant source of occupational exposure to metals (Gorman Ng et al., 2012). Although a direct significant association between hand exposure to metals and increased metal concentrations measured by biomonitoring, such as concentrations in the urine or blood, has not been demonstrated, hand exposure to cobalt was reported to be significantly positively correlated with perioral exposure among workers in engine repair facilities (involved in painting, metal spraying, metal processing, and brazing) (Gorman Ng et al., 2017). Direct uptake of cobalt through skin has also been reported. In a simple experiment in volunteers exposed to hard-metal cobalt powder via the skin, the concentration of cobalt in urine samples increased tenfold after exposure (Scansetti et al., 1994). [The Working Group noted that a lack of data limits understanding of the role of ingestion due to hand-to-mouth contact in relation to overall occupational exposure to cobalt.]

(c) Cobalt refining and production of cobalt metal and cobalt compounds

Exposure to cobalt has been reported among workers involved in cobalt refining and the production of cobalt metal and cobalt compounds. In a cobalt refinery in Belgium that consumed a wide variety of starting raw materials (mainly cobalt metal cathodes, intermediate products, and residues), workers were exposed to a mixture of cobalt salts, oxides, and fine cobalt metal powders without being exposed to tungsten, titanium, iron or silica (or their carbides), or diamond (Swennen et al., 1993; Verougstraete et al., 2004; Lantin et al., 2011; Lantin et al., 2013). Exposure levels were high in 1993 when the median concentrations of cobalt in personal air and end-of-shift urine samples were reported to be 110.0 μg/m^3 and 72.4 μg/g creatinine, respectively (Swennen et al., 1993). Between 1992 and 2001, cobalt exposure declined sharply, as did urinary cobalt concentrations, a pattern attributed to improvements in working conditions, including the implementation of hygiene controls [details not provided in the paper] (Verougstraete et al., 2004). From 2002, all workers at the refinery were required to wear protective masks, which may have further reduced their exposure to cobalt. In 2007, the median concentration of cobalt in personal air was reported to be 15.0 μg/m^3, and mean urinary cobalt concentration was reported to be 3.9 μg/g creatinine for 2008–2009 (Lantin et al., 2011; Lantin et al., 2013). [The Working Group noted that the decrease in cobalt exposure over time reported by these studies may not be representative of trends in the industry as a whole.]

In a cobalt-producing plant in Kokkola, Finland, cobalt powder was produced from a pyrite ore concentrate between 1966 and 1987. Thereafter, cobalt powder, inorganic cobalt,

and nickel compounds were produced using by-products of the metallurgic industry as raw materials. Workers were potentially exposed to metallic cobalt and cobalt sulfates, carbonates, oxides, and hydroxides via the reduction and powder production, sulfatizing roasting, and leaching and solution purification processes, and in the chemical processing department (Linna et al., 2003, 2004, 2020). According to biological monitoring data, exposure to cobalt was highest among workers in the reduction department. The highest urinary concentration of cobalt was reported to be approximately 16 000 nmol/L (943 μg/L), compared with an average concentration of < 40 nmol/L (2.4 μg/L) among unexposed individuals (Linna et al., 2003, 2004).

(d) Cobalt-containing diamond tooling

Diamond tools are used to cut stone, marble, glass, wood, and other materials, and to grind or polish various materials (IARC, 2006). These tools do not contain hard metals and do not include tungsten carbide (van den Oever et al., 1990; Nemery et al., 1992; IARC, 2006).

Diamond polishers often use high-speed polishing discs, which have surfaces composed of micro diamonds cemented in ultrafine cobalt metal powder. During polishing activities, cobalt dust is formed and may be inhaled by the diamond polisher (Barceloux, 1999). As an example, the fashioning of a diamond involves several steps, starting with the inspection and marking of the stone, followed by cleaving, sawing, and rough-cutting processes. Finally, the stone is polished with cobalt-containing polishing discs. One study reported that the mean air concentrations of cobalt in the breathing zones of workers during such processes were 15.1 μg/m^3 and 5.3 μg/m^3 in workshops with high and low exposures to cobalt, respectively, and 0.4 μg/m^3 in a control group that was not occupationally exposed to cobalt. The same trend was reported for the mean urinary cobalt concentrations of the groups (Nemery et al., 1992).

(e) Alloys or plating containing cobalt

In a magnet-manufacturing plant located in the mid-western USA, sintered permanent magnets were produced from cobalt, nickel, and aluminium metal powders and various rare-earth metals. Exposure to cobalt, nickel, neodymium, samarium, and other metal constituents occurred during the preparation of raw materials, which included processes such as pressing, casting, break-out, blasting, and grinding (Deng et al., 1991). Exposure to cobalt was reported to be greater than to other metals (Deng et al., 1991).

In bright electroplating, materials are coated with thin layers of metal to achieve a shiny decorative surface; during the process, there is the potential for worker exposure to cobalt and chromium. Compared with hard electroplating, in which a thicker layer of metal is applied, bright electroplating has been reported to be associated with lower levels of exposure to some metals (Guillemin & Berode, 1978). In one bright-electroplating factory, the mean air concentration of cobalt in the workplace did not exceed the LOD of 0.12 ng/m^3. No significant differences ($P > 0.05$) were reported between mean concentrations of cobalt and chromium in the plasma in blood samples obtained from exposed workers (cobalt, 0.85 μg/L; chromium, 0.44 μg/L) and unexposed controls (cobalt, 0.80 μg/L; chromium, 0.41 μg/L) (Wultsch et al., 2017).

(f) Dental procedures

Dental laboratory technicians are potentially exposed to metal alloys that are used in the production of crowns, bridges, and removable partial dentures, in which alloys are frequently used for the fusion of metal frames for sectional prostheses. These alloys comprise 35–65% cobalt, 20–30% chromium, 0–30% nickel, and small amounts of molybdenum, silica, beryllium, boron, and carbon (Burgaz et al., 2002). Among

dental technicians in Türkiye and Indonesia, who worked on the production of dental prostheses, the mean cobalt levels were 24.8 µg/g creatinine in urine (Burgaz et al., 2002) and 26.8 µg/L in blood (Berniyanti et al., 2020).

(g) Cobalt pigments

Cobalt is used as a pigment in the ceramics, glass, and paint industries. Cobalt is often present in paints or inks as a siccative to facilitate the drying process, and in cobalt blue dyes for the painting of porcelain pottery. In the pigment production and paint industry, the primary exposure routes are skin contact and inhalation of paint fumes and dust (Leyssens et al., 2017). Combinations of cobalt oxides and oxides of aluminium, magnesium, zinc, and silicon are constituents of blue and green ceramic glazes and pigments (Donaldson & Beyersmann, 2011). Cobalt blue pigment is produced by calcining cobalt(II) oxide with aluminium(III) oxide (Bolt et al., 1998; Karmaoui et al., 2013). Cobalt-zinc-silicate is used in a blue underglaze paint for pieces of porcelain; the pigment was specially developed to withstand intense heat (Raffn et al., 1988).

In a porcelain factory in Denmark, porcelain plates were produced by underglazing with a blue cobalt colour. This colour is made by melting together a mixture of cobalt-zinc-silicate, zinc oxide, and silicon oxide. Among workers in the factory, mean blood cobalt concentrations approximately 4 weeks after resuming work after 6 weeks off work were 36.7 nmol/L and 4.04 nmol/L for plate painters who used this cobalt underglaze and unexposed controls, respectively. Mean urinary cobalt levels were 141.8 nmol/mmol creatinine (73.9 µg/g creatinine) for underglaze users and 1.53 nmol/mmol creatinine (0.80 µg/g creatinine) for the controls (Raffn et al., 1988).

In pottery factories producing porcelain plates in Denmark, plate painters were exposed to two different types of cobalt dyes, one type containing an insoluble cobalt aluminate compound and the other containing a soluble cobalt-zinc-silicate compound, the latter type being used most frequently. Concentrations of cobalt in personal air, blood, and urine measured in 1982 and 1984 were greater in workers exposed to cobalt-zinc-silicate compounds than in those exposed to cobalt aluminate compounds. Significant correlations between blood and urine concentrations were observed. Nevertheless, there were no significant correlations between concentrations in air and blood, or air and urine (Christensen & Mikelsen, 1986). After follow-up during the period 1982–1992, exposure of the plate painters to cobalt was reported to have decreased as a result of improvement of the working environment. From 1982 until 1991, concentrations of cobalt in personal air had decreased from 1356 to 454 nmol/m^3 in 1991 (80 to 26 µg/m^3, respectively), and in urine from 133.4 to 18.8 nmol/mmol creatinine (69.5 to 9.8 µg/g creatinine, respectively) (Christensen & Poulsen, 1994).

(h) Electrical industry

In nickel–hydrogen battery-manufacturing plants in Japan, workers were engaged in anode plate-making, which comprised mixing, filling, drying, and rolling processes, as well as board processing. A master batch for anode materials contained 200 kg by weight of nickel hydroxide, 18 kg of recycled nickel powder, 10 kg of metallic cobalt, and 10 kg of cobalt oxyhydroxide (CoO(OH)). Commercial nickel(II) hydroxide ($Ni(OH)_2$) powder of specific particle diameter (9–12 µm) included 97.2% nickel hydroxide and 2.8% cobalt hydroxide. The level of exposure to cobalt in personal air was approximately one-seventh that for nickel. A significant correlation between cobalt and nickel concentrations was reported in post-shift urine samples from workers (r = 0.833, P < 0.0001); however, the mean urinary level of cobalt was higher than that of nickel (Yokota et al., 2007).

In a digital video cassette-manufacturing plant in Japan, almost all processes were automated to avoid exposure of the workers to cobalt oxide dust. However, some of the workers involved in the cobalt vapour deposition process cleaned and removed cobalt oxides that adhered to the insides of the cobalt vapour deposition machines under dry conditions several times during their work shifts. According to personal and biomonitoring data, the air concentrations of cobalt in the personal air samples of 16 workers ranged from below the LOD to approximately 22 μg/m^3. Urinary cobalt concentrations ranged from below the LOD to approximately 27.5 μg/g creatinine in end-of-shift samples (Fujio et al., 2009).

(i) *Electronic and/or electrical waste recycling industry*

Electronic and/or electrical waste (e-waste) contains multiple metals, including cobalt. The hazardous components in e-waste include cathode ray tubes, liquid crystal display screens, light-emitting diode lights, batteries, circuit boards, mercury-containing equipment, and plastic with brominated flame retardants (Julander et al., 2014).

Julander et al. (2014) analysed exposure concentrations of 20 metals, including cobalt, among e-waste recycling workers in Sweden. The workers recycled similar types of goods, such as television sets and computers, electronic tools, toys, and small and large household appliances, and their main tasks were the dismantling, handling, inspection, and transportation of goods. The level of exposure to cobalt in personal air was approximately 19 times as high for the recycling workers as for unexposed office workers; however, no significant differences ($P = 0.05$) in blood or urine cobalt concentrations were found between the two groups.

At sheltered workshops for workers with disabilities recycling e-waste in Germany, where the workers mainly disassembled cathode ray tubes and liquid crystal displays, and performed sorting related to the recycling of small electronic devices such as consumer electronics, urinary cobalt concentrations did not differ significantly ($P < 0.05$) between recycling workers and unexposed controls (Gerding et al., 2021).

1.4.3 *Exposure of the general population*

(a) *Dietary exposure*

For the general population in non-polluted areas, dietary intake of cobalt (estimated as ranging between 3 and 40 μg per day) represents the primary source of cobalt exposure (Hokin et al., 2004; Grübl et al., 2007; Turconi et al., 2009; Nasreddine et al., 2010; Arnich et al., 2012; Domingo et al., 2012; Noël et al., 2012; Cheyns et al., 2014; Tvermoes et al., 2014; Monnot et al., 2021). Cobalt has no known nutritional function, except as a component of vitamin B_{12}. Microorganisms such as bacteria and algae synthesize vitamin B_{12} and constitute the only source of this vitamin. The vitamin B_{12} produced by microorganisms enters the human food chain through incorporation into foods of animal origin. Gastrointestinal fermentation by animals promotes the growth of these microorganisms, and vitamin B_{12} is subsequently absorbed and incorporated into animal tissues, especially the liver (WHO, 2005; González-Montaña et al., 2020). Biomagnification of cobalt up the food chain does not occur (WHO, 2006). Humans derive their required dietary vitamin B_{12} from animal tissues or products (i.e. milk, butter, cheese, eggs, meat, poultry, etc.), unless the region in which the animal is reared is geochemically deficient in cobalt. No significant amount of the vitamin B_{12} required by humans is derived from microflora (FAO & WHO, 1998). Vitamin B_{12} represents only a small fraction of total cobalt intake (Lison, 2015). The cobalt content in vitamin B_{12} varies between 4.4% and 5.8% (González-Montaña et al., 2020). Trace elements may be present in food naturally (e.g.

minerals), or may originate from either environmental contamination derived from agricultural practices (e.g. pesticide residues) or food processing and packaging (Arnich et al., 2012). In the last two decades, several countries have carried out total diet studies, which were national surveys that estimated exposure to metals (Turconi et al., 2009; Nasreddine et al., 2010; Arnich et al., 2012; Domingo et al., 2012; Noël et al., 2012). Considering data on food prepared and consumed by the populations studied, the highest mean concentrations were found in chocolate, butter, coffee, shellfish, nuts, vegetables (e.g. spinach, sweet potato leaves), and ice cream (Ghaedi et al., 2008; Noël et al., 2012; Nemery & Banza Lubaba Nkulu, 2018). In addition to traditional dietary sources, some people (e.g. athletes) may deliberately ingest cobalt in the form of cobalt-containing supplements with the aim of stimulating endogenous erythropoietin (EPO) biosynthesis, and fat and carbohydrate metabolism (Simonsen et al., 2012; Unice et al., 2012; Finley et al., 2013; Tvermoes et al., 2014; Leyssens et al., 2017). The deliberate ingestion of high concentrations of vitamin B_{12} via dietary supplements (600 µg daily for 3 months) has also been reported (Pongcharoensuk & Thaiwat, 2021). [The Working Group noted that the data reviewed for the exposure of the general population consisted of measurements of total cobalt concentrations in biological specimens, which may reflect exposure to one or several of the individual agents evaluated in this monograph.]

(b) Exposure from medical devices

Medical devices such as orthopaedic implants, prosthetics, and stents composed of cobalt-containing alloys are potential sources of cobalt exposure (van Lingen et al., 2017). Metal-on-metal implants are predominantly composed of cobalt (> 34%) and chromium (20–28%), with small amounts of other metals such as molybdenum and nickel (Leyssens et al., 2017). Metal wear or corrosion of these medical devices may lead to the dissemination of metal debris and ions throughout the body (Williams et al., 2011; Polyzois et al., 2012; Blackburn & Whitehouse, 2014; Scharf et al., 2014; Lombardi et al., 2016). Increases in concentrations of cobalt ions in serum, urine, and erythrocyte samples from patients with metal-on-metal devices have been widely reported (Dumbleton & Manley, 2005; Cobb & Schmalzreid, 2006; Witzleb et al., 2006; Imanishi et al., 2010; Friesenbichler et al., 2014), see also Table 1.14. However, it is unclear from the available studies whether increased cobalt concentrations in biological samples correlate with implant wear (Blackburn & Whitehouse, 2014; Somers et al., 2016; van Lingen et al., 2017).

(c) Cosmetics, jewellery, and other consumer products

A smaller number of studies delivered evidence for cobalt release from electronic devices, leather goods, jewellery items and coins (Hamann et al., 2013; Leyssens et al., 2017; Uter & Wolter, 2018; Alinaghi et al., 2020), household products (Basketter et al., 2003), and cosmetics, in which the trace element can be present as an impurity (Bocca et al., 2014; Bruzzoniti et al., 2017; Mostafaii et al., 2022).

Both intentional and unintentional inhalation of tobacco smoke is another potentially significant source of exposure to cobalt (Table 1.7). Although other trace elements in tobacco smoke are more commonly associated with health effects (IARC, 1987, 1990, 1993), the presence of cobalt may also contribute to the harmful effects of smoking (Pinto et al., 2017; Kaplan et al., 2019; Mansouri et al., 2020).

(d) Biomonitoring levels

Risks for toxicity in humans have been estimated mainly on the basis of measurements of cobalt content and its release from various environmental matrices (Iqbal et al., 2012; Kurt-Karakus, 2012; Obiri et al., 2016; Ngole-Jeme &

Table 1.14 Measurement of total cobalt concentrations in human biological specimens

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Exposure from medical devices								
Total cobalt in blood	Patients who had MoM articulations in situ for > 30 yr, United Kingdom, date of sample collection NR	MoM radiologically stable: $n = 3$ MoM radiologically loose: $n = 2$	MoM radiologically stable: 1.97 ng/g MoM radiologically loose: 35.5 ng/g (range values NR)	NR (NR)	NR	ICP-MS (0.07 ng/g)		Dunstan et al. (2005)
Total cobalt in serum	Patients with a hip replacement in place for > 10 yr, Vienna, Austria, 2003–2004	$n = 22$	NR (0.3–50.1 µg/L)	0.75 µg/L (NR)	NR	FAAS (0.3 µg/L)		Grübl et al. (2007)
Total cobalt in serum	Patients who received MoM hinged revision knee prostheses, Marburg, Germany, 2018	$n = 23$	NR [(1.0–47.5 µg/g)][a]	[10.5 µg/g][a] (NR)	NR	ICP-MS (NR)		Klasan et al. (2019)
Total cobalt in serum	Patients with MoM total hip arthroplasty Vancouver, Canada, 2004–2007	$n = 31$	NR (0.54–58.78 µg/L)	4.50 µg/L (NR)	NR	ICP-MS (NR)		Williams et al. (2011)
Environmental exposure								
Total cobalt in urine (µg/g creatinine)	Adults and children living close to the mines or smelting plants, Katanga, Democratic Republic of the Congo, 2009–2011	$n_{adults} = 79$; $n_{children} = 32$	Adults: 11.7[b] µg/g Children: 27.9[b] µg/g (range values NR)	Adults: (7.1–22.0 µg/g) Children: (13.4–62.7 µg/g) (median values NR)	NR	ICP-MS (0.018 µg/L)	Spot urine samples.	Cheyns et al. (2014)
Total cobalt in urine (µg/L × 1000)	Residents exposed to emissions from a metal recycling plant, Italy, 2011–2013	$n = 153$	0.43[b] µg/L (0.07–2.95)	0.45 µg/L (NR)	NR	ICP-MS (NR)	24 h urine samples.	Chellini et al. (2017)

Table 1.14 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Total cobalt in urine	People who smoked > 10 cigarettes/day for ≥ 5 yr, Iran (Islamic Republic of), 2018	$n_{\text{non-smokers}} = 35$ $n_{\text{smokers}} = 64$	NR (NR)	Non-smokers: 0.6 µg/L (0.32–0.9) Smokers: 1.22 µg/L (0.72–1.65)	NR	ETAAS (0.1 µg/L)	First morning void urine samples.	Mansouri et al. (2020)
Total cobalt in placenta and cord blood	Non-smoking mothers with anthroposophic lifestyle, Sweden, 2004–2007	$n_{\text{placenta}} = 40$ $n_{\text{cord blood}} = 40$	Placenta: (1.29–8.01 µg/kg) Cord blood: (0.04–0.51 µg/kg) (mean values NR)	Placenta: 3.13 µg/kg (NR) Cord blood: 0.08 µg/kg (NR)	NR	ICP-MS (0.002 ng/g)		Fagerstedt et al. (2015)
Total cobalt in blood	Non-occupationally exposed individuals, Canada, date of sample collection NR	$n = 100$	NR (NR)	0.25 µg/L (NR)	0.64 µg/L	ICP-MS (0.017 µg/L)		Goullé et al. (2005)
Dietary exposure								
Total cobalt in serum	Non-smoking oyster growers, British Columbia, Canada (date of sample collection NR)	$n = 39$	8.69 nmol/L[b] (6.28–106.9) [0.512 (0.370–6.30) µg/L]	7.98 nmol/L (NR) [0.470 µg/L]	12.05 nmol/L [0.710 µg/L]	ICP-MS (NR)		Clark et al. (2007)
General population, background concentrations								
Total cobalt in urine	General population, Taiwan, China, 2005–2008	$n = 780$	1.07[b] µg/L (0.05–22.43)	1.05 µg/L (0.70–1.65)	3.43 µg/L	ICP-MS (0.003 µg/L)	First morning void spot urine samples.	Liao et al. (2019)
Total cobalt in urine	Women from the general population, Japan, 2000–2005	$n = 1000$	0.68[b] µg/L (< LOD–281)	0.70 µg/L (NR)	NR	GFAAS (0.1 µg/L)		Ohashi et al. (2006)
Total cobalt in urine	The US population from the National Health and Nutrition Examination Survey, 2015–2016	$n = 3061$	0.414[b] µg/L (NR)	0.434 µg/L (NR)	1.53 µg/L	ICP-MS (0.023 µg/L)		CDC (2021)
Total cobalt in blood	General population, Canada, 2009–2011	$n = 6009$	NR (NR)	NR (NR)	0.38 µg/L	ICP-MS (0.04 µg/L)		Saravanabhavan et al. (2017)

Table 1.14 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Total cobalt in blood	General population, France, 2008–2010	*n* = 1992	0.30 µg/L (NR)	0.29 µg/L (0.24–0.37)	0.54 µg/L	ICP-MS (0.0016 µg/L)		Nisse et al. (2017)
Total cobalt in blood	The US population from the National Health and Nutrition Examination Survey 2015–2016	*n* = 3454	0.151[b] µg/L (NR)	0.130 µg/L (NR)	0.400 µg/L	ICP-MS (0.06 µg/L)		CDC (2021)
Total cobalt in hair	General population, Katanga province, Democratic Republic of the Congo, date of sample collection NR	*n* = 109	1.16 mg/kg (NR)	NR (NR)	4.20 mg/kg	ICP-MS (0.001 µg/g)	Hair samples were taken from the neck.	Elenge et al. (2011)

ETAAS, electrothermal atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; IQR, interquartile range; LOD, limit of detection; MoM, metal-on-metal; NR, not reported; US, United States; yr, year.

[a] Working Group conversion to International System of Units.

[b] Geometric mean.

Fantke, 2017; Kiani et al., 2021; Mostafaii et al., 2022). Blood, serum, and urinary concentrations of cobalt have long been considered suitable indicators of cobalt exposure (Dunstan et al., 2005; Grübl et al., 2007; Finley et al., 2013; Chellini et al., 2017; Nisse et al., 2017; Liao et al., 2019). Of note, studies of the general population have shown wide variations in urinary cobalt concentrations (Cheyns et al., 2014; Chellini et al., 2017; Mansouri et al., 2020). This variability has been attributed to either oral or dermal absorption (Scansetti et al., 1994; Linnainmaa & Kiilunen,1997). Nevertheless, there is a general understanding that data available from the literature are sufficient for the monitoring of urinary cobalt as a biomarker of exposure to this chemical to be recommended (ANSES, 2013). Details on the absorption, distribution, metabolism, and excretion of cobalt are described in Section 4.1. Biomonitoring data obtained from a selection of studies are briefly summarized in Table 1.14. Other biological specimens have been used to estimate human exposure, either by attempting to measure cobalt concentrations at the target-organ level or because they provide information on different exposure windows. These include nails (Rogers et al., 1993; Ndilila et al., 2014; Gutiérrez-González et al., 2019), hair (Vienna et al., 1995; Elenge et al., 2011), exhaled breath condensate (Goldoni et al., 2004; Mutti & Corradi, 2006; Broding et al., 2009), saliva (Berton & Wuilloud, 2010), lacrimal fluid and sweat (Bruzzoniti et al., 2017), and placenta (Fagerstedt et al., 2015).

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

To reduce the risk of adverse health effects, several organizations have proposed occupational exposure limits for many hazardous substances. In particular, exposure limits are set for inhalable dust fractions in workplace air. The United States Occupational Safety and Health Administration permissible exposure limit for cobalt and cobalt inorganic compounds is 0.1 mg/m^3 as a time-weighted average (TWA) over an 8-hour (OSHA, 2017) or up to 10-hour working shift (NIOSH, 2007). The American Conference of Governmental Industrial Hygienists recommends a threshold limit value of 0.02 mg/m^3, to which nearly all workers may be exposed for a working lifetime without adverse health effects (ACGIH, 2019). In Europe, exposure limits vary between countries, ranging from 10 to 100 µg/m^3 (Kettelarij, 2018). Table S1.15 shows some examples of occupational exposure limits in different countries (see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). It is of note that cobalt compounds are readily absorbed through the skin and that unintentional ingestion of cobalt can occur from mucociliary clearance after air exposure, when contaminated hands or objects come in contact with the mouth, or when cobalt is deposited around the mouth or in the oral cavity (Kettelarij, 2018). Hence, the monitoring of airborne concentrations alone may not be a reliable proxy of actual exposure (Julander et al., 2010; Klasson et al., 2016; Gorman Ng et al., 2017; Kettelarij et al., 2018a).

(b) Industrial emissions, air, water, soil, consumer products, food, and feed

In addition to occupational limit values, regulations and guidelines exist for different environmental matrices that aim to reduce exposure of the general population to cobalt. The European Union (EU) has established emission limit values for cobalt in waste gases produced by specific industrial activities (European Commission, 2010). The Environment Agency of the UK recommends a soil screening value for total cobalt of 4.2 mg/kg dry weight, based on soil ecotoxicity (Environment Agency, 2022).

British Columbia, Canada, and the USA have use-dependent soil standards and screening levels, respectively, for the protection of human health (as listed in Table S1.16, which presents a few examples of national and international guideline values for cobalt in environmental matrices, consumer products, and foodstuffs; see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). A safe recommended dietary allowance for cobalt intake has not been set yet. For guidance purposes only, the UK Expert Group on Vitamins and Minerals has expressed the opinion that a cobalt intake of 0.023 mg/kg body weight (bw) per day would not be expected to result in any adverse effects (FSA, 2003). Although safety data on cobalt-containing alloys, such as those used in medical devices, were specifically excluded from a European Chemicals Agency evaluation (Eichenbaum et al., 2021), European Medical Device Regulations require that medical devices containing > 0.1% w/w of cobalt are appropriately labelled with a justification for the use of the substance (European Union, 2017). The UK recommends a threshold concentration of 7 mg/L of cobalt in blood for specific medical implants, which is greater than the 3 mg/L value recommended in the USA (Matharu et al., 2017). The recommendation further indicates the need for complementary diagnostic tools for the detection of adverse reactions (AAOS, 2012). [The Working Group noted that the literature shows that guidelines for follow-up of these medical implants differ considerably between regulatory authorities worldwide, which indicates that consensus regarding acceptably safe levels has not yet been reached.] Norway has established a guidance value of 0.023 mg/kg bw per day for cobalt exposure from ceramic articles (Norwegian Scientific Committee for Food and Environment, 2007), while the EU has derived threshold values for cobalt in toys (European Commission, 2009). A few agencies have prohibited the use of specific forms of cobalt for certain purposes. For instance, the World Anti-Doping Agency has prohibited the use of cobalt at all times because it is a hypoxia-inducible factor (HIF)-activating agent (Schmidt et al., 2019). The European Food Safety Authority (EFSA, 2009b) has considered the use of cobalt(II) chloride hexahydrate for nutritional purposes as a source of cobalt in food supplements to be a safety concern. The underlying reason was the greater bioavailability of cobalt from cobalt(II) chloride than from other inorganic cobalt compounds (i.e. cobalt oxide).

1.5.2 Guidance and reference values for biological monitoring

Biological monitoring of exposure to chemicals in the workplace is used to assess exposure and reduce the risk of adverse health effects. Analysis of cobalt in biological matrices has been recommended for monitoring occupational exposure (Simonsen et al., 2012; Klasson et al., 2016). Several institutions have proposed biomonitoring guidance and reference values. As an example, for a TWA exposure threshold of 0.02 mg/m^3, the American Conference of Governmental Industrial Hygienists has defined a biological exposure index of 15 µg/L as total cobalt concentration in urine collected at the end of the shift at the end of the last day of the work week (ACGIH, 2019; see Table S1.15, Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

Biomonitoring studies to assess exposure in the general population have been conducted at the national level in Canada (Saravanabhavan et al., 2017), France (Fréry et al., 2011), and Japan (Ohashi et al., 2006). Reference values (RV95s) have been derived statistically from these studies to indicate background exposure to chemical substances in reference populations. Because these values are influenced by environmental and lifestyle factors and may differ between regions,

it has been suggested that they should be established at national/regional levels (Hoet et al., 2013). Biomonitoring guidance and reference values derived for environmental and occupational exposures are summarized in Table S1.17 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 *Epidemiological studies of cancer in humans*

For each study on cancer in humans, the reviews and critiques undertaken in relation to different aspects of exposure assessment are tabulated in Table S1.18 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618), and are summarized in the following sections.

(a) Exposure assessment methods

The Working Group identified 31 key studies of cancer in humans for which a critical appraisal of exposure assessment methods was undertaken. These comprised 21 industry-based studies: 18 of workers producing cobalt or other metals and products (Cuckle et al., 1980; Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 2000; Grimsrud et al., 2005; Marsh et al., 2009; Sauni et al., 2017), including hard metals (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994; Dufresne et al., 1996; Moulin et al., 1998; Wild et al., 2000; Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017), and three of other worker populations (Bai et al., 2019; Rodrigues et al., 2020; Li et al., 2021a); eight general-population studies (Rogers et al., 1993; O'Rorke et al., 2012; Kresovich et al., 2019; White et al., 2019; Duan et al., 2020; Niehoff et al., 2021; Pan et al., 2021; Mérida-Ortega et al., 2022); and two meta-analyses including both cobalt in industrial settings and populations with orthopaedic implants (Holy et al., 2022; Zhang et al., 2021a). In addition, Marsh et al. (2017b) reported a pooled analysis of hard-metal workers, as described in Marsh et al. (2017a), McElvenny et al. (2017), Morfeld et al. (2017), Svartengren et al. (2017), Wallner et al. (2017), and Westberg et al. (2017).

(i) Industry-based studies: workers producing cobalt or other products

A retrospective cohort study by Moulin et al. (1993) used company administrative records of a French electrochemical plant specializing in cobalt and sodium production to assess exposure (defined as employment for at least 1 year between 1950 and 1980) against various cancer mortality end-points. Analyses examined ever/never employed as well as a metric to apply four subgroups defined according to work areas (cobalt production, sodium production, maintenance, and administration).

Tüchsen et al. (1996) conducted a retrospective cohort study on exposure to cobalt aluminate spinel (assessed via employment history) of workers in departments responsible for plate underglazing at two porcelain factories in Copenhagen, Denmark, and assessed data against various end-points of cancer incidence. The authors noted that from 1907 to 1972 only cobalt aluminate spinel was used. Factory 1 changed from cobalt aluminate spinel to cobalt silicate in 1972. Ever/never employment categories were applied in standardized mortality ratio analyses, capturing employment periods between 1943 and 1987 at factory 1, and 1962 and 1987 at factory 2. Co-exposure to other agents, such as quartz (used in glazing until 1952 in factory 1 and for an unknown period in factory 2) and

nickel (representing less than 0.5% of cobalt dyes used) was possible.

Retrospective cohort and nested case–control studies by Moulin et al. (2000) examined workers employed for at least 1 year between 1 January 1968 and 31 December 1991 in a French factory producing stainless and alloyed steel (with employment potentially dating back to the 1920s). Company records were used to assess employment history, and a job-exposure matrix (JEM) was developed based on expert knowledge, interviews with co-workers, previous measurements in French factories, and a literature review. This information was used to assign semiquantitative estimates of exposure to metals (iron, chromium and/or nickel, and cobalt) and/or their compounds, acid mists, polycyclic aromatic hydrocarbons, silica, and asbestos. As no on-site air measurements were available for the employment period considered, an attempt was made to construct the JEM with consideration of changes in exposure over time according to periods doing different jobs on the basis of the information obtained (e.g. through workplace interviews). Categorical metrics including ever/never employed (for general-population comparison), duration of exposure, cumulative exposure, and frequency of exposure were applied in the analyses.

Grimsrud et al. (2005) conducted a nested case–control study examining lung cancer incidence among a cohort of individuals with minimum employment of 1 year at a Norwegian nickel refinery treating sulfidic nickel copper concentrate (consisting of approximately 45% nickel, 25% copper, 23% sulfur, and 2% cobalt, with < 2% iron and precious metals). Company administrative records were used to assess job histories; quantitative measurements of cobalt were used to produce cobalt:nickel ratios using a previously developed nickel JEM. Nearly 3500 personal samples, obtained and analysed for cobalt between 1982 and 1994 as part of routine sampling, were used to calculate arithmetic 8-hour TWAs for the departments in question. The cobalt:nickel ratios in air were computed for departments and periods with measured values (cobalt amounted to approximately 4–15% of total nickel except in the department where cobalt electrolysis was undertaken, where the amount of cobalt was triple that of nickel). Departments with no measurements used a ratio of 7.1% (average for all departments exclusive of cobalt electrolysis). [The Working Group highlighted the study authors' observation that cobalt would probably always be present with nickel in raw materials and intermediates at the refinery.] Exposure metrics applied in analyses included cumulative exposure, duration of employment (overall and divided between three major department groups at the refinery), and time of first employment.

A retrospective study by Sauni et al. (2017) examined incident cancer (multiple end-points) in a cohort employed for at least 1 year between 1968 and 2004 at a cobalt plant in Finland. This plant produced cobalt powder from pyrite ore concentrate between 1966 and 1987, and produced cobalt powder, inorganic cobalt, and nickel compounds using by-products of metallurgic industry as raw materials between 1987 and 1999. Company administrative records were used to assess job histories. Subcohorts categorized by exposure level were developed according to workers' first department of employment at the plant, and assessed against industrial hygiene measurements collected since 1966 (area and personal samples) and biological monitoring; sampling details are available in other publications (Linna et al., 2003, 2004, as cited in Sauni et al., 2017). Exposure metrics included categorical duration of employment as well as categorical exposure groupings based on department ("variable exposure with peaks" corresponds to factory maintenance; "low exposure" corresponds to leaching and solution purification; "moderate exposure" corresponds to chemical department, test plant; and "high

exposure" corresponds to sulfatizing, roasting, reduction, and powder production).

Studies deemed less informative to the Working Group's analysis included Cuckle et al. (1980) and Marsh et al. (2009). Marsh et al. (2009) published a retrospective cohort study of workers with employment of at least 3 years at a facility conducting copper smelter, mill, or sulfur operations at Copperhill, Tennessee, USA, between 1946 and 1996, and cancer mortality with various end-points. A JEM based on relative exposure intensities over time was used to estimate job- and time-specific exposures to six agents: lead, sulfur dioxide, arsenic, cadmium, dust, and cobalt, assessed using a modification of the "process-based projection of exposure measurements". Analyses of the cobalt exposure group (compared with an unexposed group) were based on employment. The retrospective cohort analysis of lung cancer mortality conducted by Cuckle et al. (1980) assessed historical employment (at least 12 months between 1933 and 1960) at a company manufacturing nickel and cobalt compounds in the UK. Exposure was indirectly assessed via duration of employment at the company. Various categorical metrics were applied in analyses (e.g. ever/never employed for at least 12 months, employment duration, and years since first employment). Exposure metrics were not specific to cobalt exposure for either study.

(ii) Industry-based studies: workers producing hard metals

In most studies of hard-metal workers reviewed by the Working Group, exposure to cobalt alone could not be separated from exposure to WC-Co. Two studies, Moulin et al. (1998) and Wild et al. (2000), contained additional analyses of exposure to cobalt with potential separation from WC-Co.

A retrospective cohort with nested case–control study by Moulin et al. (1998) examined lung cancer mortality in workers employed for at least 3 months in 10 French hard-metal factories, from the date the factory opened (which varied by factory) to 31 December 1991. Other production activities performed in some of the factories studied included powder metallurgy to produce equipment made of single metals (iron or nickel) or alloys (containing iron, copper, and tin), as well as foundry processes to produce cobalt superalloys and magnets (with cobalt, tungsten, nickel, chromium, and carbon). The qualitative definition of cobalt exposure was simultaneous exposure to WC-Co specific to hard-metal manufacture and other cobalt exposure resulting from other production activities. Exposure was assessed using company administrative records to assess job histories, with a JEM for exposure to cobalt and to tungsten carbide developed using expert knowledge and interviews with co-workers, which was validated with previously recorded measurements of cobalt concentrations in air (744 measurements were recorded in three factories: 382 short-duration (15–20 minutes) samples gathered between 1971 and 1983, and 362 long-duration (4–8 hours) samples (of which 264 were of personal air) gathered between 1982 and 1984). Linear regression analysis indicated significantly increasing trends between cobalt concentrations in air (excluding the cobalt powder-manufacturing workshop) and those assigned to the JEM. For simultaneous cobalt and tungsten carbide exposure, exposure metrics included ever/never employment, maximum intensity score coded according to job history, duration of exposure (years) at or above defined exposure intensity score, and estimated cumulative exposure divided into quartiles.

In their retrospective cohort study, Wild et al. (2000) examined workers employed for at least 3 months in a French factory producing hard metals as well as other metallurgical products containing cobalt (this included foundries producing magnets with cobalt and other metals; equipment made from other sintered allows of iron, nickel, copper, and tin; and production

of cobalt and tungsten carbide powders). The authors used company administrative records to categorize 14 workshop groups on the basis of the work conducted within them: powder production, hard-metal production before sintering, hard-metal production after sintering, foundries (three workshops), production of other sintered alloys (six workshops), maintenance, and other non-exposed workshops. The JEM used by Moulin et al. (1998) was used to assign exposure intensities, durations, and cumulative exposures using previous exposure measurements to validate JEM coding. Time spent at past and present workplaces was divided into up to three consecutive periods with varying exposure levels. Metrics included ever/never exposed, workshop-based categories (e.g. "ever employed in" as well as "only employed in"), highest exposure score experienced in work history, duration at or above defined exposure score, and cumulative exposure categories.

Other studies of hard-metal workers deemed uninformative by the Working Group, because of high potential for simultaneous exposure to cobalt and WC-Co, are summarized below.

Lasfargues et al. (1994) examined multiple cancer mortality end-points in a retrospective cohort study of workers employed at a plant producing hard-metal tools for at least 1 year between 1 January 1956 and 31 December 1989 in France. Categorical assignment to various "degrees of exposure" (unknown, low, medium, and high exposure) was based on job histories/work locations obtained from company administrative records, as well as previous air and biological measurements obtained from an earlier epidemiological study conducted in 1983 (Meyer-Bisch et al., 1989). Exposure metrics with categories of duration of employment (years) and years since first employment were developed for the medium- and high-exposure categories.

Dufresne et al. (1996) reported on a case series of five aluminium smelter workers, four of whom died from lung cancer and one from mesothelioma. In addition to analysis of the occupational history of each deceased worker, fibrous and non-fibrous particles in lung tissue were visualized using phase-contrast microscopy and transmission electron microscopy. The composition of particles was determined using an energy-dispersive spectrometer and spectra were compared with those in a database of known minerals. The authors reported the concentration of "metal-rich" particles (millions of particles of > 0.1 μm in lung tissue, dry weight) for each case. Cobalt in lung tissue was reported to have been observed (qualitatively) in two of the five cases (both of whom were welders).

A retrospective cohort study examined cancer mortality (lung cancer and other end-points) of workers who had been employed at three Swedish hard-metal plants for at least 1 year (Hogstedt & Alexandersson, 1990). Production at the plants had started in the late 1930s, early 1940s, and 1950s. Company administrative records and expert opinion were used to describe job roles and work locations, which were combined with air concentration data (average levels, $\mu g/m^3$) collected between 1940 and 1982 to develop five exposure categories that were ultimately collapsed into "high-exposure" and "low-exposure" categories for the analyses.

A series of companion papers examined cancer (primarily mortality) retrospectively among hard-metal production workers in five countries (Sweden, Austria, Germany, the UK, and the USA) (Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). A pooled analysis by Marsh et al. (2017b) included all these countries. In Marsh et al. (2017a, b) and McElvenny et al. (2017), exposure assessment was based on a quantitative JEM that had been constructed for cobalt, tungsten, and nickel over the period 1952 to 2014 by Kennedy et al. (2017). [The Working Group noted that none of these studies were able to separate exposure to cobalt alone from exposure to WC-Co.]

(iii) Industry-based studies: other worker populations

A nested case–control study by Rodrigues et al. (2020) examined incidence and mortality related to brain and other central nervous system (CNS) cancers of employees at three facilities engaged in the manufacture of semiconductors and electronic storage devices in the USA. Administrative records were used to assess work histories from 1965, the first year for which detailed job information was available, or the date of hire at a study facility (whichever was later). Ten primary exposure groups (PEGs) were defined on the basis of the type of production taking place, tasks performed, work environment, and the potential for chemical and physical agents to be present within that environment. Cases were assigned to PEGs on the basis of the division and department in which the individual had worked and their job title (details available in Rodrigues et al., 2019). Mean concentrations for each exposure matrix cell (chemical/PEG/manufacturing era) were linked to participants' work history.

The studies of Bai et al. (2019) and Li et al. (2021b) focused on subsets of the Dongfeng-Tongji cohort, an ongoing prospective study of 27 009 retired workers who had been employed in the automotive industry. The prospective cohort study performed by Li et al. (2021b) examined incident cancers in patients with type 2 diabetes. Fasting blood samples were collected at enrolment baseline to assess plasma levels of nine essential metals (cobalt, iron, copper, zinc, selenium, chromium, manganese, molybdenum, and nickel) and three heavy metals (arsenic, cadmium, and lead). Bai et al. (2019) conducted a nested case–control study including 440 incident lung cancer cases and 1320 controls. The authors analysed baseline plasma concentrations of 11 essential metals, including cobalt, using single- and multiple-metal models.

(iv) General-population studies

The studies of White et al. (2019) and Niehoff et al. (2021) were both based on the nationwide Sister Study (which focused on breast cancer) conducted in the USA. White et al. (2019) assessed environmental exposure to airborne cobalt and other metals by linking census tract-level concentrations obtained from the National Air Toxics Assessment (NATA) database of the US EPA for 2005 to participants' residential addresses at the time of enrolment. Niehoff et al. (2021) determined concentrations of cobalt and other metals in toenail cuttings (all toes) from each participant at the time of enrolment in their case–cohort study.

Kresovich et al. (2019) reported a population-based analysis of 696 women enrolled in the Breast Cancer Care in Chicago study who had received a breast cancer diagnosis between 2005 and 2008. The residential addresses of participants 3–6 years before diagnosis were used to assign ambient exposure to cobalt at the census tract-level using US EPA NATA data, as done in White et al. (2019).

In a population-based case–control study by Mérida-Ortega et al. (2022), concentrations of cobalt and other metals in spot urine samples (obtained during first morning void) were used to examine environmental factors associated with breast cancer among women (452 cases and 439 controls) in some states of northern Mexico between 2007 and 2011. Principal component analysis (PCA) was used to examine patterns of exposure to a variety of metals in breast cancer cases and controls.

Duan et al. (2020) analysed concentrations of urinary cobalt and other metals in urine and/or blood to assess cancer mortality in a sample of 26 056 participants drawn from the United States National Health and Nutrition Examination Survey (NHANES) 1999–2014 (a total of 82 091 participants). The authors used statistical modelling to assess whether associations

might be driven by single contaminants versus mixtures. Pan et al. (2021) described a case–control study on oesophageal precancerous lesions in study participants identified through The Early Diagnosis and Early Treatment Project of Esophageal Cancer (EDETPEC), a surveillance programme in China's Huai'an District, which is considered a high-risk area for oesophageal cancer. Exposure to cobalt was assessed with a single blood sample at enrolment and with 3-day duplicate diet samples collected on consecutive days (two work days and one weekend day); no other metals were assessed.

O'Rorke et al. (2012) assessed exposure to cobalt using toenail cuttings (big toe only) in a population-based case–control study on oesophageal adenocarcinoma and Barrett oesophagus in Ireland. Sampling took place at recruitment, and concentrations of other metals were measured alongside cobalt but were considered separately. Rogers et al. (1993) reported on a case–control study on oral cavity cancer in Washington State, USA. Exposure to cobalt was assessed in toenail cuttings at recruitment. Exposure to other metals was assessed but also considered separately.

(v) Meta-analyses

Companion meta-analyses by Zhang et al. (2021a) and Holy et al. (2022) reported a systematic review and meta-analysis of epidemiological studies to examine overall cancer risk (Zhang et al., 2021a) and multiple cancer end-points (Holy et al., 2022) in relation to exposure to cobalt in occupational cohorts, as well as in total joint-replacement patient populations.

(b) Critical review of exposure assessment

(i) Industry-based studies

An important limitation of most studies assessed was the inability to rule out the effects of other potentially carcinogenic exposures (see Table 1.9 in Section 1.4.2 for a summary of other potentially co-occurring agents classified in IARC Group 1, *carcinogenic to humans*, or Group 2A, *probably carcinogenic to humans*, reported in the epidemiological studies assessed). This was particularly apparent in studies of hard-metal workers (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994; Moulin et al., 1998; Wild et al., 2000; Marsh et al., 2017a, b; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017), which shared similar limitations with respect to simultaneous exposure to cobalt and tungsten carbide specific to hard-metal manufacture.

Two studies of hard-metal workers, by Moulin et al. (1998) and Wild et al. (2000), reported analyses of exposure to cobalt with potential for separation from exposure to WC-Co. However, in Moulin et al. (1998) it is difficult to ascertain whether cobalt was the main driver of effects observed in the "other cobalt exposure" category, which included a number of production activities besides hard-metal manufacture, since exposure to other metals may have also occurred in these settings. With their workshop-based groupings, Wild et al. (2000) attempted to separate exposure to "hard metal dust of cobalt and tungsten carbide combined" from other processes with potential for cobalt exposure. However, similarly to Moulin et al. (1998), it is not clear that cobalt exposure occurred independently via these other processes (e.g. co-exposure to tungsten carbide powders may have occurred in the powder-production workshop; the foundry grouping may have included exposure to other metals that are potentially carcinogenic, such as nickel and chromium).

In the study by Marsh et al. (2009), which was based on a cobalt semiquantitative JEM, the authors noted that exposure to lead, arsenic, cadmium, and cobalt never occurred in isolation during any of the jobs studied. Moulin et al. (2000) observed that cobalt was moderately correlated with chromium and/or nickel (r = 0.67) in their semiquantitative JEM. Moulin et al. (1993) constructed mutually exclusive subgroups (e.g. workers only employed in cobalt production) but

could not account for potential co-exposure to arsenic and nickel (Mur et al., 1987). Sauni et al. (2017) acknowledged the potential for co-exposure to nickel, while pointing out that measured levels of nickel were relatively low in the work environments studied (see Table 1.9). Tüchsen et al. (1996) identified the potential for co-exposure to other carcinogenic agents, such as quartz (used in glazing until 1952 in factory 1 and for an unknown period in factory 2) and nickel (representing less than 0.5% of cobalt dyes used), which was not accounted for in the analyses. In the case series reported by Dufresne et al. (1996), all workers had at least one type of asbestos fibre identified in their lungs and concentrations of "metal-rich" particles were reported that were not specific to cobalt.

The studies that used JEMs for exposure characterization all shared a common strength of systematic assessment but a common limitation of non-differential exposure misclassification because of broad exposure categories; these include Moulin et al. (1998, 2000), Wild et al. (2000), Grimsrud et al. (2005), Marsh et al. (2009, 2017a, b), McElvenny et al. (2017), and Morfeld et al. (2017). JEMs, typically consisting of job and exposure axes, are retrospective exposure-assessment tools that are commonly used in occupational epidemiology because they permit the translation of job histories into exposures in a systematic and unbiased way (Peters, 2020). However, the informativeness and quality of JEMs vary depending on the specificities of job titles, the extent of quantitative exposure information available, and whether the exposure data cover the entire study period. Because a JEM assigns the same exposure estimates to all workers with the same job title (or other grouping), it may not reflect variability between workers in the same group (Kromhout et al., 1993). Any misclassification introduced by a JEM is expected to be non-differential with respect to the outcome, attenuating the risk estimates.

The JEM described by Kennedy et al. (2017) was used in the international pooled analysis reported by Marsh et al. (2017b), as well as in the UK- and USA-based studies by Marsh et al. (2017a, b) and McElvenny et al. (2017). The quantitative nature of this JEM and the extensive nature of the underlying data (site visits, industrial hygiene record reviews, and quantitative measurements) were strengths compared with other studies. However, a key limitation of these studies was that independent exposure estimates of cobalt versus WC-Co could not be generated due to their co-occurrence at some level for all job classes within the JEM. Furthermore, measurements were not available for all facilities and countries studied or for earlier periods, which challenges the validity of extrapolation across facilities and time periods.

Several studies reported the use of statistical modelling (linear models with the outcome being the natural logarithm of exposure measurements because of the usually skewed distribution of environmental exposures) of available exposure measurements, arguably the most efficient way to extract information from exposure databases while accounting for the combined influence of several exposure determinants (Burstyn & Teschke, 2010). In the study by Wallner et al. (2017) concerning an Austrian plant, the models were based on a relatively small number of measurements (~150 measurements of cobalt in air) although trends were reported to correlate well with ~250 urinary cobalt measurements. High correlation was observed between cobalt dust and tungsten. Westberg et al. (2017) used statistical models, based on ~2700 measurements collected from the early 1970s to 2012, to estimate airborne cobalt exposure across three plants. Sensitivity analyses were run to assess different back-extrapolation methods before 1970. Exposure to nickel and tungsten was dichotomized into two intensity groups because of the low number of measurements. A similar approach was reported by Svartengren et al. (2017). A study of hard-metal

workers employed at three production sites in Germany (Morfeld et al., 2017) also reported using statistical modelling based on ~1500 measurements, using two exposure groups (low versus high), production site, and calendar time as the main predictors.

A limitation of multiple studies reviewed was the use of the qualitative exposure metric of "employed versus never employed" for external comparison in retrospective occupational cohort studies conducted on workers exposed to cobalt (Cuckle et al., 1980; Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 1998; Moulin et al., 2000; Wild et al., 2000; Marsh et al., 2009; Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). This metric suggests that exposure occurred in all employed individuals, with high potential for non-differential exposure misclassification. The use of "no" versus "low" versus "high" exposure in another study (Hogstedt & Alexandersson, 1990) has a similar likelihood of exposure misclassification because of broadly defined exposure groups.

In a similar vein, the subcohort analyses of some studies exclusively applied time-dependent exposure metrics (e.g. duration of employment, years since first employment) to serve as a proxy of exposure. A key limitation of these metrics was that they are not specific to a particular contaminant and do not account for differences in exposure according to which tasks were performed and where. Therefore, the studies relying on employment duration or related metrics had similar limitations and are likely to have high potential for non-differential exposure misclassification due to the grouping of workers across exposure conditions that may have been very different. Moulin et al. (1993) attempted to address some of these concerns by defining exposure groupings where workers were employed exclusively in one department (e.g. cobalt production or sodium production) for some analyses. Similar classification was performed by Sauni et al. (2017) and Wild et al. (2000).

(ii) General-population studies

A common limitation in the population-based cohort study of adults participating in NHANES by Duan et al. (2020) and the assessment of urinary cobalt among women in northern Mexico by Mérida-Ortega et al. (2022) is the measurement of metal concentrations in spot urine samples. Urinary levels of cobalt have relatively short half-lives; hence, the measured concentrations may have reflected recent rather than long-term exposure. In addition, Mérida-Ortega et al. (2022) assessed exposure after participants had received a cancer diagnosis, which is a limitation because the relevant exposure window may not have been reflected in the exposure estimates. In the study by Duan et al. (2020), metals were measured in NHANES 1999–2002 and mortality between 1999 and 2015 was assessed, potentially resulting in a short time period between any measured exposure to cobalt and the outcome under study. A strength of both studies was their use of statistical approaches that accounted for co-exposure to multiple metals.

Three studies conducted among women in the USA (Kresovich et al., 2019; White et al., 2019; Niehoff et al., 2021) assessed metals singly and as mixtures; White et al. (2019) and Niehoff et al. (2021) both reported on analyses from the Sister Study cohort. The exposure metrics in the studies by White et al. (2019) and Kresovich et al. (2019) are similar; modelled census tract-level estimates of outdoor air levels of cobalt and antimony were linked to residential addresses. In White et al. (2019), this was done for a single year and for participants' addresses at enrolment; in Kresovich et al. (2019), participants' addresses 3–6 years before diagnosis were used for exposure assessment. In both cases, the exposure assessment did not capture temporal trends in outdoor air concentrations of metals, nor did it account for variation in levels within census

tracts. Furthermore, there was no consideration of residential mobility. In White et al. (2019), a sensitivity analysis was conducted that was restricted to women who did not move during the follow-up period. Another investigation of the Sister Study cohort used toenail cuttings (all toes) to assess exposures to cobalt and antimony (together with 15 other metals) (Niehoff et al., 2021). Two additional case–control studies also used toenail cuttings (big toes) (Rogers et al., 1993; O'Rorke et al., 2012). As a biomarker of exposure, toenail cuttings are advantageous because collection is simple and non-invasive. As with other biomarkers, toenails reflect exposure from all routes. A strength of the study by Niehoff et al. (2021) was that toenail cuttings were collected at enrolment in the cohort study and before the outcome diagnosis, whereas toenail cuttings were collected at enrolment, but after the outcome had been diagnosed among cases, in the case–control studies by Rogers et al. (1993) and O'Rorke et al. (2012), which is a limitation and probably contributed to non-differential exposure misclassification. An important consideration, and potential limitation, is the period of exposure reflected in samples of toenail cuttings and whether it is aligned with the relevant at-risk period for the outcome under study. Metal concentrations in toenails have been shown in a recent review to represent exposures 3–12 months before sampling (Gutiérrez-González et al., 2019). In occupational studies of metals, the correlation between metal concentrations in toenails and exposure is stronger for the 7–12-month period before sampling (Laohaudomchok et al., 2011; Grashow et al., 2014). In a general-population study by Garland et al. (1993) and a breast cancer case–control study by O'Brien et al. (2019), correlations between repeated measures of metals in toenail clippings were reported for participants with no identified occupational exposures to metals. Garland et al. (1993) found that correlations between metals in toenails collected ~6 years apart ranged from $r = 0.26$ for copper to $r = 0.58$ for zinc; the correlation for cobalt was $r = 0.35$. O'Brien et al. (2019) reported correlations between metal concentrations in two toenail samples collected ~8 years apart in the Sister Study cohort in the USA that ranged from $r = 0.18$ for antimony to $r = 0.71$ for mercury, among cases and controls; the correlation for cobalt was $r = 0.34$ overall ($r = 0.46$ for controls and $r = 0.24$ for cases).

The serum samples used in the case–control study by Pan et al. (2021) integrate exposure from all routes of exposure. The exposure measures are limited by the fact that they rely on one blood sample collected after the diagnosis of the outcome, and thus may not have captured the relevant exposure window, leading to potential non-differential misclassification of exposure. Pan et al. (2021) also collected dietary samples, representing ingestion (intentional) exposure. Although dietary samples were collected in triplicate, they were collected on consecutive days and may not reflect variability in dietary patterns.

(iii) Meta-analyses

A major limitation of the meta-analyses by Zhang et al. (2021a) and Holy et al. (2022) is the lack of comparability between study populations with respect to exposure contexts, routes, and metallic speciation for occupationally exposed workers compared with patients implanted with artificial joints. In occupational analyses, meta-estimates included studies on both hard and non-hard metals, resulting in a lack of interpretability for exposure to cobalt without tungsten carbide. In addition, only broad exposure metrics were extracted from the occupational cohort studies (e.g. employment in cobalt-related work for at least 1 year), which further limits their informativeness.

1.6.2 Mechanistic studies in humans

The Working Group identified 27 mechanistic studies in which a critical appraisal of exposure assessment methods was undertaken. The critiques undertaken for each study in relation to different aspects of exposure assessment are tabulated in Table S1.19 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

(a) Exposure assessment methods

The studies identified included seven observational studies of the general population (Arslan et al., 2011; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Bibi et al., 2016; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021), 16 industry-based studies (Bencko et al., 1983, 1986a; Nemery et al., 1990; Gennart et al., 1993; Swennen et al., 1993; Rizzato et al., 1994; Shirakawa & Morimoto, 1997; De Boeck et al., 2000; Hengstler et al., 2003; Krakowiak et al., 2005; Mateuca et al., 2005; Walters et al., 2012; Tilakaratne & Sidhu, 2015; Princivalle et al., 2017; Wultsch et al., 2017; Andersson et al., 2021), and three experimental studies (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008).

(i) General-population studies

Most of the general-population studies were cross-sectional (Calderón-Garcidueñas et al., 2013; Bibi et al., 2016; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021) with two being case–control studies (Arslan et al., 2011; Johnstone et al., 2014). Five studies used biological samples to quantitatively assess exposure. Xue et al. (2021) and Li et al. (2021b) assessed concentrations of cobalt and other metals in blood samples from individuals living near e-waste–recycling sites and individuals living elsewhere. Calderón-Garcidueñas et al. (2013) assessed cobalt quantitatively in the frontal cortex tissues of 59 decedents living in cities with differing levels of air pollution. Howe et al. (2021) used spot urine samples from women in the first trimester of pregnancy to quantitatively assess concentrations of cobalt and eight other metals (cross-sectional analysis within a prospective cohort study). Bibi et al. (2016) assessed concentrations of cobalt in the urine, blood, and nails, although the analysis of mechanistic end-points only used data relating to the blood samples.

In the case–control studies, Arslan et al. (2011) used biological samples (blood) in their study of oxidative stress in those with and without malignant glial tumours, while Johnstone et al. (2014) assessed urinary concentrations of cobalt (along with several other metals and trace elements) among 495 women participating in the Endometriosis Natural History, Diagnosis and Outcomes study.

(ii) Experimental studies

Three experimental studies were also identified for review (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008). Amirtharaj et al. (2008) examined the role of oxidative stress and fatty acids in modulating cobalt binding to albumin in patients with fatty liver disease by applying a known amount of cobalt chloride (0.232 mmol/L) to serum samples (ex vivo) to assess cobalt binding. In a larger study of 180 cement workers who participated in repeated patch testing, Katsarou et al. (1997) examined the T-cell responses of 20 people: 10 who had consistently positive patch-test results and 10 who had previously had a positive patch test and now had a negative patch test. In this study, cobalt chloride was applied to primary T-cells isolated from the individuals, and responses were assessed (ex vivo), but neither the amount nor the concentration of cobalt applied was reported. L'vova et al. (1990) applied cobalt chloride to human primary cell cultures (ex vivo) to assess mutagenicity.

(iii) Industry-based studies

Four publications reported on case studies or a case series. Tilakaratne & Sidhu (2015) described the work histories and patch test results for two workers with histories of work in home renovations, one with a diagnosis of cutaneous T-cell lymphoma (the other was a suspected case). Nemery et al. (1990) measured the concentration of cobalt (as well as iron, nickel, and chromium) in postmortem lung tissue collected from a 52-year-old man who had worked as a diamond polisher using polishing discs containing cobalt, and who died from interstitial fibrosis (Nemery et al., 1990). Rizzato et al. (1994) assessed cobalt concentrations in various biological specimens (e.g. blood, urine, pubic hair, nails, and sperm) from three individuals with sarcoidosis who all had an occupational history of exposure to cobalt. Krakowiak et al. (2005) described the occupational history of a diamond polishing-disc former with asthma. The patient's response to controlled exposure to cobalt chloride was assessed using patch testing and nasal-provocation testing.

There were 12 cross-sectional studies focused on different occupational groups, many of which relied on biological samples for the exposure assessment. Cobalt exposure was prospectively assessed in urine and blood samples collected at multiple time points from 34 workers in a hard-metal manufacturing plant (Principalle et al., 2017). Although there was potential exposure to WC-Co, no data on WC-Co exposure were reported. Hengstler et al. (2003) assessed cobalt exposure using air and urine samples (spot samples given the same day as air sampling) from 78 workers at 10 facilities engaged in either the production of cadmium-containing pigments or batteries, or the recycling of electric tools; exposures to lead and cadmium were additionally assessed. Walters et al. (2012) reported on 62 workers employed at an aerospace manufacturing company. Urinary cobalt was assessed using spot samples (as was urinary chromium). In addition, semiquantitative exposure groups were constructed on the basis of exposure to metal working fluids. Wultsch et al. (2017) measured cobalt concentrations in blood samples collected from 42 workers at a bright electroplating factory (comparison group, 43 jail wardens). In addition to the concentration of cobalt in blood, duration of exposure (years) was considered as a semiquantitative (categorical) exposure metric. De Boeck et al. (2000) and Mateuca et al. (2005) reported on the same study population, which included workers exposed to cobalt, workers exposed to WC-Co, and workers with neither exposure. Although urinary cobalt concentration was assessed with a single end-of-week sample collection, qualitative groups were used in the analysis (cobalt-exposed, hard-metal (WC-Co) exposed, and unexposed controls). Gennart et al. (1993) assessed cobalt concentrations in urine samples from 24 men employed at a metal-powder production factory and compared them with those of 23 clerical workers. Results from the biological samples suggested higher levels of exposure among the production workers, but mechanistic outcomes were limited to comparisons between the two groups (exposed/unexposed) and by duration of exposure categories (0 years, less than 5 years, and at least 5 years). Andersson et al. (2021) assessed cobalt exposure among 72 Swedish hard-metal workers via quantitative measures of inhalation exposure, using personal and stationary samples, that described multiple size fractions for mass concentrations (total, inhalable, and respirable) as well as particle surface areas and numbers. Andersson et al. (2021) also used biological monitoring (blood and urine) to assess internal cobalt concentrations.

In the other studies, exposure was defined in a qualitative manner (Swennen et al., 1993; Shirakawa & Morimoto, 1997). Swennen et al. (1993) compared 82 workers at a cobalt plant with 82 unexposed workers. Employment at the plant (yes/no) was used as the exposure measure

in the analysis of mechanistic end-points; air and biological samples demonstrated low exposure among the comparison group (Swennen et al., 1993). Shirakawa & Morimoto (1997) qualitatively assessed hard-metal exposure (yes/no) among workers at hard-metal plants. Workers included in the analysis were engaged in the production of hard metals, a process that included other metals (e.g. tungsten, nickel, and molybdenum), as described by Kusaka et al. (1986). Two studies did not provide information on the occupational settings in which the exposed workers were employed (Bencko et al., 1983, 1986a), only describing the exposure group as "occupationally exposed" to cobalt.

(b) Critical review of exposure assessment

Many studies used biological measures to assess exposure to cobalt (Nemery et al., 1990; Gennart et al., 1993; Hengstler et al., 2003; Arslan et al., 2011; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Wultsch et al., 2017; Andersson et al., 2021; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). The timing of biological samples is important because it determines the period of exposure reflected in sample results. Different types of biological sample (e.g. urine, blood, hair) are likely to represent different time periods of exposure. The exposure-only study from Princivalle et al. (2017) reported that cobalt in urine has a shorter half-life (5.3 days) than that of cobalt in blood (12.3 days), and the authors concluded that measurement of cobalt concentrations in blood is preferable to measurement in urine because the results are more reliable; this would apply to studies of the general population and to groups of workers. Two studies used data from deceased individuals (Nemery et al., 1990; Calderón-Garcidueñas et al., 2013) and thus were limited to available samples. Many studies relied on a single biological sample to assess exposure (Gennart et al., 1993; Hengstler et al., 2003; Arslan et al., 2011; Walters et al., 2012; Johnstone et al., 2014; Wultsch et al., 2017; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021), which was a limitation because single samples do not capture exposure variability and introduce non-differential exposure misclassification. A strength of the study by Andersson et al. (2021) was the collection of two biological samples in the same week, which allowed for comparison between the two time points.

A strength of several studies was the inclusion of air monitoring data that quantitatively described the external exposure conditions in the workplaces. Results from air sampling were reported in five studies, including those concerning workers involved in cobalt refining (Swennen et al., 1993), hard-metal production (Shirakawa & Morimoto, 1997), cadmium pigment/battery production and electric tool recycling (Hengstler et al., 2003), bright electroplating (Wultsch et al., 2017), and metal-powder production (Gennart et al., 1993). Only in the cases of Shirakawa & Morimoto (1997) and Hengstler et al. (2003) were these external exposure measures considered in the statistical analyses reported.

Several studies examined working populations for which co-exposure to other metals was probable, including those involved in hard-metal production (Shirakawa & Morimoto, 1997; Princivalle et al., 2017; Andersson et al., 2021), the production of cadmium-containing pigments (Hengstler et al., 2003), battery manufacture (Hengstler et al., 2003), recycling of electric tools (Hengstler et al., 2003), bright electroplating (Wultsch et al., 2017), aerospace manufacturing (Walters et al., 2012), and metal-powder production (Gennart et al., 1993). As noted in Section 1.6.1(b)(i), a limitation of these studies was the inability to rule out the effects of co-exposures to other metals, some of which are known carcinogens (e.g. chromium(VI), nickel, and cadmium). A summary of other potentially co-occurring IARC Group 1 and Group 2A agents in selected occupational settings is presented

in Table 1.9 A key limitation of the studies of hard-metal workers was the probable co-exposure to WC-Co (Shirakawa & Morimoto, 1997; Princivalle et al., 2017; Andersson et al., 2021). The study by Shirakawa & Morimoto (1997) was limited because the authors did not assess exposure to cobalt specifically, instead using exposure to hard metal (yes/no) as the exposure metric. Princivalle et al. (2017) prospectively assessed cobalt concentrations in urine and blood samples collected from hard-metal workers, and while the potential for co-exposure to WC-Co was a limitation, the mechanistic outcomes under study were specific to cobalt. Andersson et al. (2021) characterized cobalt exposure and particulate matter exposure in detail among a population of Swedish hard-metal workers (and the cobalt exposures were relatively low, as noted by the authors), but the reporting of tungsten exposure in all job groups under study was a limitation.

A strength of the studies by Hengstler et al. (2003), Princivalle et al. (2017), and Wultsch et al. (2017) was the use of biological samples (urine or blood) to quantify exposure to cobalt. Gennart et al. (1993) used biological samples to assess exposure among production and clerical workers, but analysis of mechanistic end-points was limited to qualitative and semiquantitative exposure metrics (e.g. exposed/unexposed, duration of exposure) that were not specific to cobalt exposure. Shirakawa & Morimoto (1997) and Walters et al. (2012) both employed qualitative exposure definitions that were not specific to cobalt (e.g. exposed/unexposed, work areas). Exposure assessment was probably more specific to cobalt in Swennen et al. (1993), where cobalt production workers were under study and a qualitative metric of exposure was used (exposed/unexposed). A strength of De Boeck et al. (2000) and Mateuca et al. (2005), two studies based on the same population, was the identification of a group of workers with only cobalt exposure, which was compared with a group of workers who had WC-Co exposure.

A strength of many population-based studies and studies of workers was the inclusion of exposure assessment for co-exposure to metals other than cobalt (Arslan et al., 2011; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Bibi et al., 2016; Wultsch et al., 2017; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). One exception was the study of hard-metal workers by Shirakawa & Morimoto (1997) that only considered cobalt exposure (qualitatively), which was a limitation of this study. Despite the large number of studies that measured exposure to other metals, only Hengstler et al. (2003) accounted for exposure to other metals (cadmium, nickel, and lead) in their analysis. All case reports and case series (Nemery et al., 1990; Rizzato et al., 1994; Krakowiak et al., 2005; Tilakaratne & Sidhu, 2015) noted other potential exposures. Nemery et al. (1990) measured concentrations of cobalt, iron, nickel, and chromium in lung tissue. Tilakaratne & Sidhu (2015) discussed nickel and chromium as potential co-exposures, but only qualitatively, and did not confirm exposure to cobalt. There was potential exposure to WC-Co and other metals in the case reported by Krakowiak et al. (2005), but challenge testing was completed only for cobalt. Rizzato et al. (1994) quantified cobalt, tungsten, and tantalum in all biological samples.

By definition, cross-sectional studies are limited because exposure and outcome are assessed at the same point in time and thus cannot provide evidence of temporality (Bencko et al., 1983, 1986a; Gennart et al., 1993; Swennen et al., 1993; Shirakawa & Morimoto, 1997; De Boeck et al., 2000; Hengstler et al., 2003; Mateuca et al., 2005; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Bibi et al., 2016; Wultsch et al., 2017; Andersson et al., 2021; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). Two studies reporting on the same population did not provide sufficient information for the exposure assessment to be assessed, which was a major limitation because no information on the industry or occupational

exposure of workers, or the potential for co-exposure, could be ascertained (Bencko et al., 1983, 1986a).

A strength of the experimental studies was that the exposure is a known, and controlled, element of the experiment (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008) and thus the exposure is well characterized. The potential limitations of these studies pertained to the relevance of the experimental exposure level to real-world exposure levels and the lack of variability in the experimental exposure levels, as would be expected among exposed humans.

2. Cancer in Humans

In this section, a review of the evidence from studies of cancer in humans exposed to cobalt metal (without tungsten carbide) and cobalt compounds is presented. Cobalt metal without tungsten carbide was evaluated previously in 2003 and is described in *IARC Monographs* Volume 86 (IARC, 2006). In that volume, the evidence relating to cancer in humans consisted of a series of studies of the French and Swedish hard-metal industries (e.g. Hogstedt & Alexandersson 1990; Moulin et al., 1998; Wild et al., 2000), the French cobalt-production industry (Moulin et al., 1993), and a Danish study of porcelain painters (Tüchsen et al., 1996). Cobalt metal without tungsten carbide was evaluated as IARC Group 2B, *possibly carcinogenic to humans*, with a determination that the evidence in humans was *inadequate*. *IARC Monographs* Volume 86 also found that the evidence regarding cancer in humans was *inadequate* for other forms of cobalt besides WC-Co. Since then, several additional studies of occupational cohorts and the general population have been published. In considering occupational cohort studies with multiple publications, only those with the most recent cancer follow-up were reviewed in detail.

The Working Group identified 12 occupational studies on the risk of lung and other cancers at hard-metal production sites (see Table 2.1), but the majority were considered uninformative because there were no specific analyses related to cobalt without simultaneous co-exposure to tungsten carbide. Most workers in the hard-metal industry are exposed to a composite of WC-Co, unsintered or sintered. The production of WC-Co hard metals involves the mixing and granulation of tungsten carbide and cobalt powders. After mixing, the material is pressed and shaped by cutting and drilling. These steps give rise to unsintered hard-metal dust that results in simultaneous exposure to cobalt and tungsten carbide. The pieces are then sintered at a high temperature in an oxygen-free furnace and are finally finished by grinding and drilling, giving rise to simultaneous exposure to sintered WC-Co hard-metal dust. Exposure to WC-Co was evaluated in *IARC Monographs* Volume 86 as having *limited evidence* for cancer in humans on the basis of positive associations with lung cancer in cohorts of workers involved in hard-metal production. Because of the nature of the hard-metal production process, it is difficult to evaluate risk of exposure to cobalt alone (i.e. in the absence of the WC-Co composite material) in most studies of hard-metal workers. The Working Group reviewed the 12 hard-metal production studies to determine whether any presented analyses for cobalt exposure might be relevant for this evaluation. Two such studies were identified (Moulin et al., 1998; Wild et al., 2000).

Five occupational studies of other industries that reported results for lung cancer in relation to cobalt exposure were reviewed (Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 2000; Grimsrud et al., 2005; Sauni et al., 2017). Two additional studies on lung cancer among workers at a nickel refinery and copper smelter were not considered informative for this evaluation (Cuckle et al., 1980; Marsh et al., 2009). One other

Table 2.1 Epidemiological studies of cancer of the lung in workers producing hard metals

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort	7459 workers (5777 men, 1682 women) with ≥ 3 mo experience in hard-metal production (10 facilities); for 61 lung cancer cases with job histories, exposure–response analyses used a nested case–control design in which 3 controls were sampled from each case's risk set, matching on sex and date of birth (± 3 mo) (180 controls) Exposure assessment method: Cohort: exposure via all routes (indirectly) assessed qualitatively using company administrative records; exposure metrics: ever employment (≥ 3 mo) Nested case–control: JEM used to assign cumulative exposure based on maximum intensity score and duration (unweighted or additionally weighted by frequency)	Lung, mortality	SMR:			Age, sex, and calendar period	*Exposure assessment critique*: Key strengths include: JEM validated with atmospheric cobalt measurements. Key limitations include: Non-differential exposure misclassification likely (broad exposure categories for cohort and JEM for case–control study). Cobalt exposure not assessed independently (all analyses included co-exposure to tungsten carbide or other agents). *Other strengths:* A large study involving nearly 7500 workers, 684 deaths (any cause), 63 from lung cancer. In the nested case–control study, smoking status was known for about 80% of the cases and controls, and there was a suggestion of slight negative confounding. *Other limitations*: No adjustment for occupational exposures other than for WC-Co and other cobalt exposure. *General comments*: Exposures lagged 10 yr.
			Whole cohort (men and women, any exposure)	63	1.30 (1.00–1.66)		
		Lung, mortality	Level of WC-Co exposure, smoking habits known (OR):			Age, sex, year of birth	
			0–1	NR	1		
			2–9	NR	2.29 (1.08–4.88)		

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort (cont.)		Lung, mortality	Level of WC-Co exposure, smoking habits known (OR):			Age, sex, year of birth, smoking	
			0–1	NR	1		
			2–9	NR	2.60 (1.16–5.82)		
		Lung, mortality	Level of WC-Co exposure, and other cobalt exposure (ever/never) (OR):			Age, sex, year of birth, exposure to WC-Co, and "other cobalt exposure" mutually adjusted	
			0–1	26	1		
			2–9	35	1.93 (1.03–3.62)		
			Other cobalt exposure	15	2.21 (0.99–4.90)		
		Lung, mortality	Level of WC-Co exposure, and other cobalt exposure (ever/never) (OR):				
			0–1	26	1		
			2–3	8	3.37 (1.19–9.56)		
			4–5	19	1.54 (0.76–3.12)		
			6–9	8	2.79 (0.96–8.10)		
			Other cobalt exposure	15	2.05 (0.94–4.45)		
			Trend-test *P*-value, 0.08 (for WC-Co)				
		Lung, mortality	Duration of WC-Co exposure at level ≥ 2, and other cobalt exposure (ever/never) (OR):				
			Non-exposed	26	1		
			≤ 10 yr	19	1.61 (0.78–3.34)		
			10–20 yr	12	2.77 (1.12–6.82)		
			> 20 yr	4	2.03 (0.49–8.51)		
			Other cobalt exposure	15	2.20 (0.99–4.87)		
			Trend-test *P*-value, 0.03 (for WC-Co)				

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort (cont.)		Lung, mortality	Unweighted cumulative dose of WC-Co (months × levels), and other cobalt exposure (ever/ never) (OR):			Age, sex, year of birth, exposure to WC-Co, and "other cobalt exposure" mutually adjusted	
			< 32	6	1		
			32–142	16	2.64 (0.93–7.47)		
			143–299	16	2.59 (0.88–7.60)		
			> 299	23	4.13 (1.49–11.47)		
			Other cobalt exposure	15	1.83 (0.86–3.91)		
			Trend-test *P*-value, 0.01 (for WC-Co)				

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wild et al. (2000) France 1950–1992/ 1968–1992 Cohort	2860 workers (2216 men, 644 women); ≥ 3 mo experience in the largest hard-metal facility in France Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed qualitatively using company administrative records; exposure metric: ever employment, workshop-based categories; JEM used to assign semiquantitative exposure intensity, duration, and cumulative exposure (1, sum of score by duration; 2, score weighted by frequency code)	Lung, mortality	Employed ≥ 3 mo (SMR):			Age, calendar period	*Exposure assessment critique*: Key strengths include: low job turnover noted at this site (most with same job for entire work history) and large exposure gradient noted for hard-metal dust across work areas. Key limitations include: non-differential exposure misclassification likely (broad exposure categories). High likelihood of simultaneous exposures, particularly in maintenance (where asbestos exposure estimated in JEM). *Other strengths*: Prospective follow-up. Nearly full work history (at the plant). *Other limitations*: Smoking habits collected retrospectively from former workers. Simultaneous exposure to powders containing known or potentially carcinogenic metals or metal compounds. *General comments*: Follow-up ended at age 85 yr, death, or end of 1992. Exposures lagged 10 yr.
			Men	46	1.70 (1.24–2.26)		
			Women	1	1.19 (0.02–6.62)		
		Lung, mortality	Men only employed in powder production (SMR):	2	1.39 (0.17–5.02)		
		Lung, mortality	Men ever employed in powder production (SMR):	5	1.92 (0.62–4.49)		
		Lung, mortality	Men ever exposed to cobalt except in combination with tungsten carbide (SMR):	15	1.95 (1.09–3.22)		

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wild et al. (2000) France 1950–1992/ 1968–1992 Cohort (cont.)		Lung, mortality	Exposed to IARC carcinogens, men (SMR):			Age, calendar period	
			Asbestos	13	1.95 (1.04–3.33)		
			PAH	13	1.99 (1.09–3.40)		
			Silica	9	1.73 (0.79–3.29)		
			Nickel compounds	15	1.76 (0.99–2.91)		
			Chromium compounds	2	2.08 (0.23–7.52)		
			Any of the above	26	2.05 (1.34–3.00)		
		Lung, mortality	Exposed to any IARC carcinogen (asbestos, PAH, silica, nickel, chromium compounds), men (RR):			Age, calendar period, smoking, exposure to dust from sintered hard metal (yes/ no), duration of exposure to dust from unsintered hard metal (unexposed, 3 levels of duration)	
			Never	NR	1		
			Ever	NR	1.48 (0.81–2.68)		

CI, confidence interval; IARC, International Agency for Research on Cancer; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; SIR, standardized incidence ratio; SMR, standardized mortality ratio; RR, relative risk; WC-Co, cobalt with tungsten carbide; yr, year.

study examined the incidence of lung cancer in relation to plasma levels of metals, including cobalt, in a population of retired workers who had been employed in the automotive industry (Bai et al., 2019). Only one occupational study was identified that did not report results for lung cancer. Rodrigues et al. (2020) analysed the risk of malignant CNS cancers in workers employed in the semiconductor and electronic storage device-manufacturing industry.

There were also several studies that examined associations between general-population exposures to cobalt and various cancer types. White et al. (2019) examined the relationship between estimated air concentrations of cobalt in the participants' census tract of residence at the time of enrolment and breast cancer incidence. Studies have also examined associations of cobalt with various cancers using biomarkers of exposure, including studies investigating cobalt concentrations measured in toenail cuttings in relation to breast, larynx, oral cavity, and oesophageal cancers (including oesophageal precursor lesions) (Rogers et al., 1993; O'Rorke et al., 2012; Niehoff et al., 2021), as well as studies of cobalt concentrations measured in plasma, serum, or urine (Bai et al., 2019; Duan et al., 2020; Li et al., 2021a; Pan et al., 2021; Mérida-Ortega et al., 2022). The types and routes of cobalt exposure assessed via biological samples represented all routes of exposures, including ingestion through the diet as well as inhalation of ambient air.

Workers in smelting processes and other industries studied were potentially exposed to cobalt metal and cobalt compounds. Co-exposure to substances evaluated by IARC as *carcinogenic to humans (Group 1)* with evidence for lung cancer was present in most occupational studies and was a key consideration of the Working Group in evaluating studies.

The outcomes examined in most occupational studies were based on cancer mortality rather than incidence. For lung cancer, which tends to have a shorter survival time, this is a reasonable approximation of lung cancer incidence. However, this is not the case for many other types of cancers and is particularly problematic when evaluating all other types of cancers combined, which comprises a heterogeneous group of outcomes. Furthermore, the case definitions for the incidence studies tended to be more valid and based on histological confirmation.

The Working Group noted that the meta-analysis studies of Zhang et al. (2021a) and Holy et al. (2022) included studies of hard-metal production workers. While the meta-analyses also included some studies that were considered to be informative, the reported meta-estimates could not be adequately interpreted for exposure specific to cobalt and were therefore considered uninformative for this evaluation.

2.1 Lung cancer

2.1.1 Studies of workers producing hard metals

See Table 2.1.

Particles of metal carbides cemented in a metal binder constitute the most common composite hard metals used for cutting tools and other industrial purposes. The carcinogenic potential of exposure to WC-Co was evaluated by the *IARC Monographs* programme in 2003 (Volume 86; IARC, 2006) and is not within the scope of this monograph, in which exposure to cobalt metal alone or with co-exposure to substances other than WC-Co are evaluated. Nonetheless, the Working Group reviewed studies of hard-metal workers to identify analyses that may contribute to the present evaluation, for example, where exposure to cobalt could potentially be assessed independently of exposure to WC-Co. Studies without data relevant for this purpose are only briefly mentioned and are not shown in Table 2.1.

There were four studies of lung cancer mortality among hard-metal workers from the 20th century: three conducted in France and

one in Sweden. Two of them covered only exposure to WC-Co (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994). They were evaluated by *IARC Monographs* Volume 86 (IARC, 2006) and are not considered here. Moulin et al. (1998) assessed lung cancer mortality among 7459 workers at 10 hard-metal production facilities in France, who were employed for at least 3 months, with follow-up from 1968 to 1991. The study included the largest production site that was investigated separately by Wild et al. (2000), described below. The study by Moulin et al. (1998) reported that, across the 10 cohorts assessed, the overall risk of lung cancer mortality was elevated compared with national mortality rates, with a standardized mortality ratio (SMR) of 1.30 (95% confidence interval, CI, 1.00–1.66; 63 deaths). Exposures during the 10 years before death were disregarded in nested case–control analyses. In the case–control analyses, risk was estimated in models including two types of cobalt exposure: cobalt in dust from hard metal (WC-Co) with different exposure metrics (categories of duration, intensity, and cumulative measures) and ever exposure to cobalt in other situations with no WC-Co, denoted "other cobalt exposure" (dichotomous variable). For workers exposed to WC-Co, there were signs of increasing risk with duration of exposure and with increasing cumulative exposure, adjusted for "other cobalt exposure". The risk of lung cancer mortality in workers ever exposed to "other cobalt exposure" ranged between odds ratios (ORs) of 1.83 and 2.21 in different models (15 exposed cases), with lower bounds of the 95% confidence intervals varying between 0.86 and 0.99. However, these models were not adjusted for exposure to other agents classified by IARC in Group 1, *carcinogenic to humans*, with *sufficient* evidence in the lung, such as asbestos, arsenic, and chromium, nickel, and cadmium compounds, which were known to be present at the facilities under study. Analyses that included smoking habits, which involved 80% of the nested case–control sample, suggested a slight negative confounding effect of smoking on exposure to WC-Co. [The Working Group considered subanalyses of "other cobalt exposure" potentially informative because the models controlled for exposure to WC-Co. However, there were no exposure–response analyses presented for other cobalt exposures. Another concern is that there may be residual confounding from exposure to WC-Co, the predominant form of cobalt exposure at the facilities, which may not have been fully captured by the models. Importantly, this study did not account for exposures to other lung carcinogens present at the plants and had no information about the extent or level of exposure to other lung carcinogens, which makes it difficult to rule out their contribution to the observed excess of lung cancer.]

Wild et al. (2000) analysed lung cancer mortality data from 1968 to 1992 among 2860 workers employed at the largest of the production sites studied by Moulin et al. (1998) (see above). Complete work histories were available for most workers, and the workers were followed for 1 year longer than in the study by Moulin et al. (1998). The overall risk of lung cancer in the cohort was higher among men than among the local population (SMR, 1.70; 95% CI, 1.24–2.26). A subgroup of men was engaged in the production of cobalt powder by reducing cobalt hydroxide. Powder production also included the manufacture of tungsten carbide powder from wolframite ore, but risk estimates were not reported specifically for workers exposed only to cobalt metal powders. In analyses in which exposure groups were categorized by production department (workshop), men ever employed in departments of powder production (i.e. exposed to cobalt powder only, or separately to tungsten carbide powder and to cobalt powder) had a lung cancer standardized mortality ratio of 1.92 (95% CI, 0.62–4.49; 5 deaths). For men only employed in powder production, the standardized mortality ratio for lung cancer was 1.39 (95% CI, 0.17–5.02; 2 deaths). When exposure

was defined according to a JEM, men exposed to cobalt excluding WC-Co had a standardized mortality ratio for lung cancer of 1.95 (95% CI, 1.09–3.22; 15 deaths). In further analyses of exposures to specific agents classified by IARC as "lung carcinogens", including asbestos and metal compounds, standardized mortality ratios were in the range of 1.73–2.08. [The Working Group noted that the authors selected five Group 1 agents with *sufficient* evidence for lung cancer in humans, although there is no *IARC Monographs* evaluation of "PAHs" as a class of compounds.] The standardized mortality ratio for men with exposure to any "IARC carcinogen" was 2.05. In an internal analysis with Poisson regression, the relative risk associated with exposure to agents classified by IARC as lung carcinogens among men was 1.48 (95% CI, 0.81–2.68), adjusted for age, calendar period, smoking, and exposure to dust from WC-Co. [The Working Group considered the subanalyses of "powder production workers" and workers exposed to "cobalt except hard metal" as potentially informative. However, it was not possible to estimate the risk from cobalt exposure adjusted for occupational exposures other than for those exposed to dust from WC-Co. According to the JEM, exposure to IARC-classified lung carcinogens was common at the plant and positively associated with an increased risk of lung cancer among the workers.]

Additional cohort studies published in 2017 reported lung cancer mortality or incidence among hard-metal workers in Sweden, Germany, Austria, the UK, and the USA, together with a pooled analysis (Marsh et al., 2017a, b; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). None of these studies reported risk in workers exposed to cobalt without concomitant exposure to WC-Co. [The Working Group considered these studies uninformative for the purpose of the present monograph.]

2.1.2 *Studies of workers with other occupational exposure to cobalt*

See Table 2.2.

Seven unique study populations were identified that constituted non-hard-metal cobalt production workers.

A cohort of 1143 workers with a minimum of 1 year of employment at a factory producing cobalt and sodium in south-eastern France was followed up for mortality from 1950 to 1980 by Mur et al. (1987). Some analyses were conducted only among workers born in France because of high loss to follow-up among workers born outside of France. The cohort ($n = 1148$) mortality and job histories were updated by Moulin et al. (1993) until 1988. In the second follow-up (Moulin et al., 1993), a small excess of lung cancer was reported (Moulin et al., 1993), with a standardized mortality ratio of 1.16 (95% CI, 0.24–3.40) compared with French national rates (SMR, 1.16; 95% CI, 0.24–3.40), based on three lung cancer deaths among French-born workers employed exclusively in cobalt production. [The Working Group noted that interpretation of this study was limited given the very small number of expected cases in the cobalt production workshop, resulting in statistical imprecision, and the possible confounding with asbestos exposure.]

A population of Danish women working in two porcelain factories in Copenhagen, comprising 874 workers exposed to a dye containing cobalt and 520 who were not exposed, was followed up for mortality (from 1968 until the end of 1992) and cancer incidence (from 1943 or 1962 to 1992) by Tüchsen et al. (1996). The dye used was reported to contain 25% cobalt and < 0.5% nickel. Cobalt aluminate spinel dye was replaced by cobalt silicate dye in factory 1 in 1972 and in factory 2 in 1989. Industrial hygiene measurements of dust (possibly quartz) and ambient air cobalt silicate concentrations were measured on an annual basis between 1982 and 1988 in factory 1 and between 1981 and 1988

Table 2.2 Epidemiological studies of cancer of the lung in workers with other occupational exposure to cobalt

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1993) France Enrolment, 1950–1980/ follow-up, 1988 Cohort	1148 men employed at an electrochemical plant producing cobalt and sodium for ≥ 12 mo between 1950 and 1980 (same population as in Mur et al., 1987) Exposure assessment method: exposure to cobalt via all routes (indirectly) was assessed qualitatively based on production area using company job history records	Lung, mortality	Employed in cobalt production, French-born workers (SMR): Exclusively in cobalt production Ever in cobalt production	 3 4	 1.16 (0.24–3.40) 1.18 (0.32–3.03)	Age, calendar period	*Exposure assessment critique*: Key strengths include: clearly defined exposure groups. Key limitations include: non-differential exposure misclassification likely (broad exposure categories). Possible co-exposures identified could not be fully accounted for in analyses. *Other strengths*: Analyses in subgroup without loss to follow-up. *Other limitations*: causes of death before 1968 assessed by physicians. Incomplete follow-up among non-French-born. No smoking data. *General comments*: results from several analyses could not be adequately interpreted for exposure to cobalt alone.

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tüchsen et al. (1996) Denmark Enrolment, factory 1, 1943–1987; enrolment, factory 2, 1962–1987/ follow-up, 1992 Cohort	874 exposed/520 unexposed; all women working in one of two porcelain factories employed in the plate underglazing departments (exposed to cobalt) and a referent population (unexposed) working in cobalt-free departments in the same factories Exposure assessment method: exposure (indirectly) to cobalt aluminate spinel via all routes was assessed qualitatively using company administrative records; exposure metrics: ever/never employed	Lung, incidence	Exposure group (SIR):			Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Possible co-exposure to dusts (including quartz) and nickel at "insignificant" levels not accounted for in analyses. *Other strengths*: a long follow-up period and few lost to follow-up. Cancer incidence obtained through the national registers. *Other limitations*: a limited population with corresponding low expected numbers of cancers.
			All exposed	8	2.35 (1.01–4.62)		
			Factory 1, exposed	3	[1.60 (0.41–4.37)]		
			Factory 2, exposed	5	[3.25 (1.19–7.20)]		
			Referents	7	1.99 (0.80–4.11)		
		Lung, incidence	Exposure group (RR):				
			Referents	7	1		
			All exposed	8	1.2 (0.4–3.8)		

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (2000) France Enrolment, 1968–1991/ follow-up, 1992 Nested case–control	Cases: 54; death from lung cancer in a cohort of workers in a stainless steel-producing factory, employed for ≥ 1 yr between 1968 and 1991 Controls: 162; 3 workers per case sampled from the case's risk set and matched on sex and date of birth (± 6 mo) Exposure assessment method: Cohort: employment of ≥ 1 yr in steel-producing factory assessed qualitatively using company administrative records; exposure metric: ever employment of ≥ 1 yr between 1968 and 1991 Case–control: semiquantitative JEM used to assign exposures examining duration, intensity, frequency of exposure by job period with 10 yr lag period applied; employment by work area also examined	Lung, mortality	Cobalt exposure, 10 yr lag, smoking history known (OR):			Age, sex, year of birth, PAH, silica, smoking status	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely (broad exposure categories for cohort and JEM for case–control). Co-exposure to chromium and/or nickel likely. *Other strengths*: a relatively large cohort with complete administrative records of job histories. *Other limitations*: incomplete smoking histories (only known for 67% of cases and 73% of controls). No exposure measurements. Co-exposure to other metals not accounted for in analyses.
			None	NR	1		
			Ever	12	0.44 (0.17–1.16)		
		Lung, mortality	Exposure level (OR):			Age, sex, year of birth, smoking status	
			Per 1 unit increase in level (0, none; 1, low; 2, medium; 3, high)	12	0.54 (NR)		
			Trend-test *P*-value, 0.09				
		Lung, mortality	Duration of exposure (OR):				
			Per one-category increase (1, < 10 yr; 2, 10–19 yr; 3, 20–29 yr; 4, ≥ 30 yr)	12	0.56 (NR)		
			Trend-test *P*-value, 0.04				
		Lung, mortality	Frequency unweighted cumulative dose quartile (OR):				
			Per one-quartile increase (quartiles among controls, coded 1–4)	12	0.55 (NR)		
			Trend-test *P*-value, 0.02				
		Lung, mortality	Frequency-weighted cumulative dose quartile (OR):				
			Per one-quartile increase (quartiles among controls, coded 1–4)	12	0.54 (NR)		
			Trend-test *P*-value, 0.02				

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Grimsrud et al. (2005) Norway 1952–1995 Nested case–control	Cases: 213; lung cancer cases among employees with employment ≥ 1 yr in a nickel refinery Controls: 525; controls matched to cases by sex and year of birth, free from lung cancer at the time of diagnosis Exposure assessment method: exposure to cobalt (all routes) assessed qualitatively and quantitatively in a cohort of nickel refinery workers based on company records, using job categories and quantitative exposure ratios with nickel	Lung, incidence	Cumulative cobalt exposure (OR):			Age, sex, year of birth, smoking in 5 categories (never, former, current: 1–10, 11–20, > 20 g/day)	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Exposure to nickel and cobalt highly correlated. *Other strengths*: available historical personnel files. Smoking information obtained from subjects or next of kin. A large study with corresponding large power. *Other limitations*: inability to evaluate cobalt independently from nickel.
			Unexposed	9	1		
			Low: 0.31–29.5 μg/m³-years	49	1.5 (0.6–3.8)		
			Medium 29.7–142 μg/m³-years	73	2.4 (1.0–5.6)		
			High 144–3100 μg/m³-years	82	2.9 (1.2–6.8)		
		Lung, incidence	Cumulative cobalt exposure (OR):				
			Per 1 unit increase [(mg/m³) × yr]	303	1.3 (0.9–1.8)		
		Lung, incidence	Cumulative cobalt exposure (OR):			Age, sex, year of birth, smoking in 5 categories (never, former, current: 1–10, 11–20, > 20 g/day); cumulative exposure to nickel, arsenic, asbestos, sulfuric acid, carcinogenic work outside the refinery (year)	
			Per 1 unit increase [(mg/m³) × yr]	303	0.7 (0.3–1.4)		

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sauni et al. (2017) Finland Enrolment, 1968–2004/ follow-up, 2013 Cohort	995; men employed for ≥ 1 yr at a Finnish cobalt plant between 1968 and 2004 Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed semiquantitatively using company administrative records; exposure metrics: duration and departmental exposure groupings	Lung, incidence Lung, incidence Lung, incidence	Incidence (SIR): Total Exposure group (SIR): Variable exposure Low Moderate High Duration of employment (SIR): > 1 yr > 5 yr	 6 0 2 0 4 6 5	 0.50 (0.18–1.08) 0 (0–6.68) 0.41 (0.05–1.47) 0 (0–5.56) 0.67 (0.18–1.72) 0.50 (0.18–1.08) 0.52 (0.17–1.22)	Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential misclassification likely. Possible co-exposure to nickel. *Other strengths*: complete work histories from personnel records. Precise exposure characterization. Cancer incidence rather than mortality. Some smoking data suggested that the low lung cancer risk was not due to lower smoking rates. *Other limitations*: relatively small number of expected cases. Possible co-exposure to nickel not accounted for in analyses.

CI, confidence interval; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; SIR, standardized incidence ratio; SMR, standardized mortality ratio; RR, relative risk; yr, year.

in factory 2. Cobalt concentrations in air in these factories were occasionally noted to be high (sometimes > 1000 μg/m^3) (Christensen & Poulsen, 1994). Both exposed women (standardized incidence ratio, SIR, 2.35; 95% CI, 1.01–4.62; n = 8) and the reference group (SIR, 1.99; 95% CI, 0.80–4.11; n = 7) had greater risk of lung cancer than the national reference rate. However, the exposed group had a relative risk ratio of 1.2 (95% CI, 0.4–3.8) when compared with the reference group. [The Working Group noted that the low number of lung cancer cases limited the statistical precision of the results. Inclusion of an internal unexposed referent group is an important strength of the study, and elevated standardized incidence ratios in both the exposed and referent groups suggest that the increased risk of lung cancer was not related to cobalt exposure.]

Workers at a factory producing stainless and alloyed steel (n = 4897) were followed up for mortality between 1968 and 1992 (Moulin et al., 2000). A JEM was developed to assign semiquantitative estimates of exposure to cobalt, as well as other metals and agents. Cobalt exposure was correlated with nickel and chromium (r = 0.67) as classified by the JEM. Within this factory, a metal powder production process for cobalt, nickel, and iron was initiated in 1972. No information was available regarding the number of workers in this workshop or expected numbers of lung cancer cases, and no lung cancer cases were observed. In a nested case–control study including 54 lung cancer cases and 162 controls, only 1 control and no cases had been involved in metal powder production, indicating that the expected number of cases was very small in this workshop. Based on 12 exposed cases, the odds ratio for ever versus never cobalt exposure, lagged by 10 years and adjusted for smoking and exposure to polycyclic aromatic hydrocarbons and silica, was equal to 0.44 (95% CI, 0.17–1.16). Inverse trends were observed for increasing exposure-level categories, duration of exposure, and cumulative exposure indices (based on exposure level and duration), adjusted for smoking. [The Working Group noted that this study provides no information on lung cancer risk associated with cobalt given the very small number of expected cases in the cobalt production workshop, and the likelihood of confounding by nickel or chromium in the stainless-steel production process.]

Grimsrud et al. (2005) conducted a nested case–control study within a Norwegian cohort of refinery workers in which there were 213 cases of lung cancer and 525 controls matched on age, sex, and year of birth. A positive trend in smoking-adjusted odds ratios was observed for cobalt exposure assessed using a JEM based on quantitative exposure measurements. However, associations with cobalt exposure were confounded by nickel exposure, as all individuals who were exposed to cobalt were also exposed to nickel, generally at much higher levels than to cobalt. The positive trend with cobalt was reversed after further adjusting for other occupational exposures (nickel, arsenic, asbestos, sulfuric acid mist, and carcinogenic exposure in work outside the refinery). [The Working Group noted that no dose-related positive trend was seen for cobalt exposure after adjustment for exposure to known lung carcinogens.]

A cohort of 995 workers at a metal refinery – who were potentially exposed to metallic cobalt powder, cobalt salts, oxides, sulfides, and nickel compounds – in Kokkola, Finland, was followed up for cancer incidence through 2013 (Sauni et al., 2017). The cohort was further divided into subcohorts by exposure levels, according to the departments in which employees had first worked at the plant. The exposures in different departments were based on regular industrial hygiene measurements taken between 1986 and 2014. The mean concentrations of cobalt exposure varied between < 0.02 mg/m^3 and 0.10 mg/m^3 depending on when the measurement was taken and the workshop. The standardized incidence ratio for lung cancer among men employed for more than 1 year was 0.50 (95% CI, 0.18–1.08;

6 cases) (the rates used for comparison were local rates from Central Ostrobothnia, Finland). An exposure subgroup analysis (variable, low, moderate, or high) showed no statistically significant excesses in any subgroup nor any positive trends. Smoking rates among workers towards the end of the follow-up period were higher in the study population than in the region from which comparative lung cancer rates were available. Thus, the lower-than-expected lung cancer incidence cannot be explained by lower smoking rates. The cohort was relatively young, with person-years above age 60 years accounting only for 15% of the total person-years. [The Working Group noted that the small number of cases and the relatively young age of the cohort limited the potential for a meaningful exposure–response analysis to be conducted.]

[Two additional occupational studies of lung cancer in industries with potential cobalt exposures were considered uninformative by the Working Group. A cohort study of workers at a company manufacturing nickel and cobalt salts in Clydach, south Wales, UK (Cuckle et al., 1980), was considered uninformative because of co-exposure to nickel, an IARC Group 1 lung carcinogen, and a lack of analyses specifically related to cobalt. A study by Marsh et al. (2009) of workers employed at a facility conducting smelter, mill, or sulfur operations between 1946 and 1996 in Copperhill, Tennessee, USA, was considered uninformative because the prevalence of cobalt exposure in the study population was unclear and exposure to other metals was common, making it difficult to interpret findings for the evaluation of cobalt.]

2.1.3 Studies of other populations

See Table 2.3.

One nested case–control study from China (Bai et al., 2019) examined the association between plasma concentrations of selenium and other metals [most of them essential elements] and the risk of lung cancer among a cohort of retired workers who had been employed at a car production facility. [The Working Group noted that the occupational cohort was not selected on the basis of high cobalt exposure, and it was unclear whether the study participants had any specific exposure to cobalt in their work.] The study was based on the Dongfeng-Tongji cohort and included 27 009 retired workers from an automotive manufacturing company who were recruited to the study between 2008 and 2010. Between April and October 2013, an additional 14 120 new retirees were also enrolled. A total of 452 lung cancer cases were identified with follow-up to the end of 2016 (440 who had available fasting peripheral venous blood samples were included). The self-reported new lung cancer cases were confirmed from medical records or death certificates. The diagnosis of lung cancer for surgery patients was based on histopathological analysis of surgical pathology archives from the Tongji Hospital. Healthy controls (n = 1320) were frequency matched on age and sex. Baseline plasma concentrations of 11 elements including cobalt were measured. The main focus of the study was the potential protective effect of zinc exposure on lung cancer risk. A one-unit increase in the natural log-transformed concentration of cobalt in plasma (μg/L) was weakly associated with increased risk of incident lung cancer in all participants (OR, 1.07; 95% CI, 0.86–1.32), men only (OR, 1.10; 95% CI, 0.84–1.45), and women only (OR, 1.07; 95% CI, 0.76–1.51) in single-metal models, which were adjusted for potentially confounding factors, but the associations were imprecise. [The Working Group noted that the plasma concentration of cobalt was measured only once, at the start of the study, and may not reflect long-term exposures that are likely to be associated with lung cancer. The time period between the measurement of metal concentrations and outcome was small (≤ 8 years).]

Table 2.3 Epidemiological studies of cancer of the lung in other populations

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bai et al. (2019) China Enrolment, 2008–2010 and 2013/ follow-up, 2016 Nested case–control	Cases: 440 incident cases of lung cancer in the Dongfeng-Tongji cohort; cohort consisted of retired (n = 27 009, 87% of invited participants agreeing) and newly retired (n = 14 120) employees of an automotive-manufacturing company; participants provided baseline blood samples and questionnaire information between September 2008 and June 2010; the newly retired workers were recruited between April and October 2013. Controls: 1320 healthy controls frequency-matched (1:3) to cases by age (± 3 yr) and sex – who were free of cancer, diabetes, and cardiovascular disease at baseline and during the follow-up period – were selected from the Dongfeng-Tongji cohort. Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using blood samples (focus on "essential metals" in an occupational cohort)	Lung, incidence	Plasma cobalt level (μg/L) (OR): Median, 0.14 μg/L; IQR, 0.09–0.27 μg/L; per 1 unit increase (natural log-transformed) Plasma cobalt level (μg/L), men (OR): Per 1 unit increase (natural log-transformed) Plasma cobalt level (μg/L), women (OR): Per 1 unit increase (natural log-transformed)	 440 275 165	 1.07 (0.86–1.32) 1.10 (0.84–1.45) 1.07 (0.76–1.51)	Age, sex, BMI, education level, smoking status, alcohol-drinking status, regular physical activity status, pack-year smoking, family history of cancer	*Exposure assessment critique*: Key limitations include: differential misclassification possible, non-differential likely. Possible co-exposure to other carcinogens in workplace not discussed. Time period between metal measurement and outcome potentially very small. *Other strengths*: Large cohort. Results adjusted for covariates. *Other limitations*: Plasma concentrations of cobalt measured only once at baseline, which does not consider the possible variability over time.

BMI, body mass index; CI, confidence interval; IQR, interquartile range; OR, odds ratio; yr, year.

2.2 Breast cancer

See Table 2.4.

Three cohort studies, one case series, and one case–control study investigated the association between cobalt exposure and breast cancer.

The study by Tüchsen et al. (1996), described in Section 2.1.2 above, estimated the risk of breast cancer among women occupationally exposed to cobalt aluminate and cobalt silicate spinel dye at two porcelain factories in Copenhagen, Denmark. The cohort consisted of 874 women exposed occupationally to cobalt and 520 women employed at the factories but not exposed to cobalt (referents). Cancer cases were identified from the Danish Cancer Registry, and there was a long follow-up period (1943–1992). When compared with national reference rates, standardized incidence ratios for breast cancer were not notably increased among exposed workers overall (SIR, 0.93; 95% CI, 0.53–1.52), among workers in factory 1 (SIR, 0.76; 95% CI, 0.31–1.57), or among workers in factory 2 (SIR, 1.12; 95% CI, 0.52–2.13). The standardized incidence ratio among the unexposed workers was 0.81 (95% CI, 0.44–1.38). [The Working Group noted that the results were not adjusted for reproductive risk factors like parity and age at first birth. The number of cancer cases was also rather low and risk estimates were imprecise.]

The study population analysed in the studies by White et al. (2019) and Niehoff et al. (2021) was based on the nationwide Sister Study in the USA, a cohort of 50 884 women (age, 35–74 years) who had a sister who had received a diagnosis of breast cancer. However, in the latter study, the researchers selected a race-stratified case–cohort consisting of non-Hispanic Black and non-Hispanic White participants.

The study by White et al. (2019) identified a total of 2587 breast cancer cases during follow-up (mean, 7.4 years). Exposure to cobalt was estimated on the basis of the 2005 US EPA's NATA census tract estimates of metal concentrations in outdoor air at participants' places of residence at the time of enrolment. The results were reported according to the quintiles of air concentrations of cobalt. The adjusted hazard ratios for quintiles 2 and 3 were 1.2 (95% CI, 1.0–1.3) and 1.2 (95% CI, 1.0–1.3), respectively. Quintiles 4 and 5 did not indicate an increased risk of breast cancer. The adjusted hazard ratios for postmenopausal breast cancer in quintiles 2 and 3 were 1.2 (95% CI, 1.0–1.3) and 1.2 (95% CI, 1.0–1.4), respectively. In the premenopausal group, there was no evidence of an association between exposure to cobalt and breast cancer. In analyses stratified by estrogen receptor (ER) status, positive associations with no clear exposure gradient were noted among the ER-positive cases versus non-cases, but not among ER-negative cases. [The Working Group noted that census tract-level concentrations used in the study are very broad proxies for personal exposures. In addition, the evaluation of cobalt concentration in air represented only one time point and the baseline cobalt air concentration in the Sister Study was very low. Exposure may vary locally, and people may move during the follow-up period. However, the hazard ratio for breast cancer according to cobalt exposure was not increased among those who never moved during follow-up.]

In the other case–cohort study nested within the Sister Study cohort (Niehoff et al., 2021), exposure to metals, including cobalt, was based on analyses of toenail cuttings. The toenail cuttings were collected at baseline before breast cancer diagnosis. The cohort consisted of 1499 patients with breast cancer and a subcohort of 1607 women in the USA and Puerto Rico who were selected at random. The period of follow-up was 7.5 years on average. The study did not identify increased hazard ratios for breast cancer in any of the cobalt concentration tertiles. Relative to tertile 1, the hazard ratio for tertile 2 was 1.00 (95% CI, 0.82–1.21) and for tertile 3 was 1.01 (95% CI, 0.83–1.22). The results were further stratified by race/ethnicity, ER status (ER-positive

Table 2.4 Epidemiological studies of cancer of the breast and exposure to cobalt

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tüchsen et al. (1996) Denmark Enrolment, factory 1: 1943–1987; factory 2: 1962–1987/ follow-up, 1992 Cohort	874 exposed/520 unexposed; all women working in one of two porcelain factories employed in the plate underglazing departments (exposed to Co) and a referent population (unexposed) working in Co-free departments in the same factories Exposure assessment method: exposure to Co aluminate spinel via all routes (indirectly) was assessed qualitatively using company administrative records	Breast, incidence	Exposure group (SIR): All exposed Factory 1, exposed Factory 2, exposed Referents	 14 6 8 12	 [0.93 (0.53–1.52)] [0.76 (0.31–1.57)] [1.12 (0.52–2.13)] [0.81 (0.44–1.38)]	Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Possible co-exposure to dusts (e.g. quartz) and Ni at "insignificant" levels. *Other strengths*: Long follow-up period. Exposure measurements from the two factories from several years. Linkage of the cohort with national cancer register. *Other limitations*: The results were not adjusted for confounders including co-exposures. High number of emigrated workers. Information bias possible.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort	2587 breast cancer cases; prospective cohort study of 50 884 women (age 35–74 yr) who had a sister diagnosed with breast cancer but no prior breast cancer at enrolment (Sister Study), followed through July 2015 Exposure assessment method: assessment of Co metal exposure was made for a single year in time quantitatively based on address at enrolment before the development of the outcome; annual census-tract estimates of metal concentrations in air (μg/m³) for Co – along with As, Cd, Cr, Pb, Mn, Hg, Ni, Sb, and Se from the US EPA 2005 NATA – were linked to participants' geocoded residences at baseline and categorized into quintiles for analysis	Breast, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	*Exposure assessment critique*: Key strengths include: weighted quantile sum regression was used to assess metal mixtures. Key limitations include: non-differential exposure misclassification likely, as neither temporal trends in outdoor metal levels nor residential mobility was accounted for in the exposure assessment, and potential for within census-tract variability in outdoor air levels of metals also probably introduced error in the exposure assessment. *Other strengths*: Large prospective cohort study. Address ascertained at baseline. Extensive covariate information.
			Quintile 1	472	1		
			Quintile 2	549	1.2 (1.0–1.3)		
			Quintile 3	552	1.2 (1.0–1.3)		
			Quintile 4	508	1.1 (0.94–1.2)		
			Quintile 5	487	1.0 (0.91–1.2)		
			Trend-test *P*-value, 0.9				
		Breast, incidence	Quintiles of residential airborne Co concentration, premenopausal women (HR):				
			Quintile 1	88	1		
			Quintile 2	114	1.1 (0.86–1.5)		
			Quintile 3	109	1.1 (0.79–1.4)		
			Quintile 4	104	0.97 (0.72–1.3)		
			Quintile 5	119	1.1 (0.83–1.5)		
			Trend-test *P*-value, 0.9				
		Breast, incidence	Quintiles of residential airborne Co concentration, postmenopausal women (HR):				
			Quintile 1	383	1		
			Quintile 2	435	1.2 (1.0–1.3)		
			Quintile 3	442	1.2 (1.0–1.4)		
			Quintile 4	403	1.1 (0.95–1.3)		
			Quintile 5	367	1.0 (0.87–1.2)		
			Trend-test *P*-value, 0.9				
		Breast, ER+ vs non-cases, incidence	Quintiles of residential airborne Co concentration (HR):				
			Quintile 1	269	1		
			Quintile 2	333	1.26 (1.07–1.48)		
			Quintile 3	313	1.19 (1.01–1.40)		
			Quintile 4	298	1.14 (0.96–1.36)		
			Quintile 5	274	1.07 (0.89–1.27)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort (cont.)		Breast, ER– vs non-cases, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race (non-Hispanic White, other), education, annual household income, marital status, parity (continuous), census-tract median income, geographical region	An independent validation study in California found good agreement between monitored data and certain air toxics in the 2005 NATA data release. *Other limitations*: For exposure analysis, only the exposure levels at the enrolment residence were considered.
			Quintile 1	61	1		
			Quintile 2	48	0.79 (0.54–1.15)		
			Quintile 3	58	0.92 (0.63–1.34)		
			Quintile 4	47	0.72 (0.48–1.08)		
			Quintile 5	47	0.72 (0.48–1.08)		
		Breast: ER+ vs ER– cases, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	
			Quintile 1	NR	1		
			Quintile 2	NR	1.59 (1.05–2.41)		
			Quintile 3	NR	1.29 (0.85–1.95)		
			Quintile 4	NR	1.58 (1.02–2.45)		
			Quintile 5	NR	1.49 (0.95–2.32)		
		Breast, incidence	Quintiles of residential airborne Co concentration, BMI < 25 (HR):				
			Quintile 1	158	1		
			Quintile 2	204	1.25 (1.01–1.54)		
			Quintile 3	216	1.35 (1.10–1.67)		
			Quintile 4	178	1.08 (0.87–1.35)		
			Quintile 5	173	1.11 (0.88–1.39)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort (cont.)		Breast, incidence	Quintiles of residential airborne Co concentration, BMI ≥ 25 (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	
			Quintile 1	314	1		
			Quintile 2	345	1.10 (0.94–1.28)		
			Quintile 3	336	1.04 (0.89–1.22)		
			Quintile 4	330	1.06 (0.91–1.25)		
			Quintile 5	314	0.99 (0.84–1.17)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2017 Cohort	50 884 women (age 35–74 yr), who had a sister diagnosed with breast cancer but no prior breast cancer at enrolment (Sister Study); case–cohort study design evaluated 1495 incident breast cancers (all non-Hispanic Black cases and a random sample of non-Hispanic White cases) and 1605 women randomly sampled from the cohort, stratified by race/ethnicity Exposure assessment method: concentrations of 15 metals, including Co, were measured in toenail clippings collected at baseline and categorized into tertiles for analysis.	Breast, incidence	Co level (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	*Exposure assessment critique*: Key strengths include: exposures were assessed before the development of the outcome and analyses considered the metal mixture. Key limitations include: non-differential exposure misclassification was likely, and metals in toenails typically represent exposures 3–12 mo before sampling (Gutiérrez-González et al., 2019); hence, a single toenail specimen may not have represented average exposure during the follow-up period. *Other strengths*: Cases and controls were drawn from a large, national prospective study population.
			Tertile 1 (< 5.2 ng/g)	524	1		
			Tertile 2 (5.2–10.5 ng/g)	488	1.00 (0.82–1.21)		
			Tertile 3 (> 10.5 ng/g)	483	1.01 (0.83–1.22)		
			Trend-test *P*-value, 1.0				
		Breast, ER+	Co level (HR):				
			Tertile 1 (< 5.2 ng/g)	366	1		
			Tertile 2 (5.2–10.5 ng/g)	355	1.01 (0.82–1.24)		
			Tertile 3 (> 10.5 ng/g)	372	1.07 (0.87–1.32)		
		Breast, ER–	Co level (HR):				
			Tertile 1 (< 5.2 ng/g	67	1		
			Tertile 2 (5.2–10.5 ng/g)	64	1.07 (0.72–1.58)		
			Tertile 3 (> 10.5 ng/g)	55	0.92 (0.61–1.37)		
		Breast	Co level, non-Hispanic White (HR):				
			Tertile 1 (< 5.2 ng/g)	402	1		
			Tertile 2 (5.2–10.5 ng/g)	418	1.03 (0.84–1.27)		
			Tertile 3 (> 10.5 ng/g)	432	1.02 (0.84–1.26)		
		Breast	Co level, non-Hispanic Black (HR):				
			Tertile 1 (< 5.2 ng/g)	122	1		
			Tertile 2 (5.2–10.5 ng/g)	70	0.71 (0.49–1.01)		
			Tertile 3 (> 10.5 ng/g)	51	0.78 (0.52–1.18)		
		Breast (ductal carcinoma in situ)	Co level (HR):				
			Tertile 1 (< 5.2 ng/g)	121	1		
			Tertile 2 (5.2–10.5 ng/g)	116	1.06 (0.78–1.43)		
			Tertile 3 (> 10.5 ng/g)	104	0.95 (0.70–1.29)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2017 Cohort (cont.)		Breast (invasive breast cancer)	Co level (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	The case–control study had a large sample size and extensive covariate information *Other limitations*: Unclear how informative the exposure contrast in the study was, as there are no agreed toxic or harmful levels of the metals measured in the toenails.
			Tertile 1 (< 5.2 ng/g)	403	1		
			Tertile 2 (5.2–10.5 ng/g)	372	0.98 (0.80–1.20)		
			Tertile 3 (> 10.5 ng/g)	379	1.02 (0.83–1.25)		
		Breast	Co level, postmenopausal women (HR):				
			Tertile 1 (< 5.2 ng/g)	449	1		
			Tertile 2 (5.2–10.5 ng/g)	396	0.92 (0.75–1.13)		
			Tertile 3 (> 10.5 ng/g)	398	0.95 (0.77–1.16)		
		Breast	Co level, premenopausal women (HR):				
			Tertile 1 (< 5.2 ng/g)	75	1		
			Tertile 2 (5.2–10.5 ng/g)	92	1.04 (0.67–1.62)		
			Tertile 3 (> 10.5 ng/g)	85	0.90 (0.91–1.10)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kresovich et al. (2019) USA Enrolment, 2005–2008 Case series [case–case comparison study]	Cases: 696; incident breast cancers among women (age 30–79 yr) diagnosed with a first primary in situ or invasive breast cancer, self-identified as non-Latina White, non-Latina Black, or Latina living in the metropolitan Chicago area at time of diagnosis, enrolled in the Breast Cancer Care in Chicago study and evaluated for ER/PR status (ER/PR-negative if both ER-negative and PR-negative (n = 147); ER/PR-positive, otherwise (n = 549)) Exposure assessment method: total ambient inhalation exposure to Co was quantitatively estimated at the census-tract level using the US EPA NATA data that account for mobile and stationary sources of exposure, but do not include indoor sources or other occupational exposures	Breast, incidence	Quintiles of residential airborne cobalt in ER/PR-negative vs ER/PR-positive cases (OR): Quintile 1 (< 0.010 ng/m^3) Quintile 2 (0.010–0.014 ng/m^3) Quintile 3 (0.014–0.017 ng/m^3) Quintile 4 (0.017–0.024 ng/m^3) Quintile 5 (> 0.024 ng/m^3) Trend-test P-value, 0.04	 NR NR NR NR	 1 1.4 (0.7–2.8) 2.2 (1.2–4.2) 1.8 (0.9–3.4) 2.0 (0.9–4.4)	Age, race/ethnicity, education, BMI, income, census-tract affluence and disadvantage, reproductive factors	*Exposure assessment critique*: Key strengths include: evaluation of co-exposure to other metals in ambient air. Key limitations include: non-differential misclassification likely. Timing of exposure measurement may be outside the relevant time window of exposure for cancer outcome under study. Census-tract level concentrations are broad proxies for personal exposures. The reliance on residential address at a single point in time may have introduced non-differential misclassification.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kresovich et al. (2019) USA Enrolment, 2005–2008 Case series [case–case comparison study] (cont.)							*Other strengths*: High proportion of ER/PR-negative cases (21%) giving enough power to detect etiological heterogeneity. Co-exposures considered in analyses. *Other limitations*: 11% of participants were excluded due to missing residential history and 20% were missing information on tumour ER/PR status.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mérida-Ortega et al. (2022) Northern Mexico Enrolment, 2007–2011 Case–control	Cases: 452 histopathologically confirmed breast cancer cases from main public and academic hospitals (age ≥ 18 yr), no personal history of other type of cancer, ≥ 1 year residence in study area, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations Controls: 439 women with ≥ 1 yr residence in study area with no personal history of cancer, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations, matched by age to cases (± 5 yr) Exposure assessment method: this study quantitatively assessed Co exposure (all routes) in urine samples collected at a single point in time; in addition, exposure to other metals and trace elements was assessed	Breast	Co quartile (μg/g creatinine): Quartile 1 Quartile 2 Quartile 3 Quartile 4	 NR NR NR Trend-test *P*-value, 0.000	 1 1.20 (0.82–1.75) 0.79 (0.54–1.18) 0.45 (0.28–0.70)	Age, schooling, estrogenic index, alcohol consumption, BMI	*Exposure assessment critique*: Key strengths include: consideration of co-exposure to other metals and trace elements. Key limitations include: reliance on a single spot urine sample. The use of a single sample may not reflect the relevant exposure window, particularly as the sample was collected after the outcome. *Other strengths*: Population-based case–control study design; area studied has natural contamination by metals in water and the largest non-ferrous metal processing site worldwide. Exposure to other metals accounted for in the statistical analysis.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mérida-Ortega et al. (2022) Northern Mexico Enrolment, 2007–2011 Case–control (cont.)							*Other limitations*: Urine samples collected after diagnosis, leading to potential for reverse causality. Spot urine sample may not reflect exposures during biologically relevant time period.

As, arsenic; BMI, body mass index; Cd, cadmium; CI, confidence interval; Co, cobalt; Cr, chromium; ER, estrogen receptor; Hg, mercury; HR, hazard ratio; LOD, limit of detection; Mn, manganese; Mo, molybdenum; mo, month; NATA, National Air Toxics Assessment; ND, not detected; Ni, nickel; OR, odds ratio; Pb, lead; PR, progesterone receptor; Sb, antimony; SD, standard deviation; Se, selenium; US EPA, United States Environmental Protection Agency; W, tungsten; wk, week; yr, year; Zn, zinc.

versus ER-negative), menopausal status, and invasiveness. Cobalt concentration was not associated with risk of breast cancer in any of these additional analyses. [The Working Group noted the strengths of the study with respect to design and sample size. Assessment of exposure at a single point in time was a limitation, and there was only a twofold difference in cut-off points used to define the lowest (< 5.2 ng/g) and highest (> 10.5 ng/g) exposure groups. Sample sizes were small for some subgroup analyses, resulting in wide confidence intervals for ER-negative breast cancers and cancers among non-Hispanic Black women.]

The association between the ER and progesterone receptor (PR) status of breast cancer and cobalt exposure was also examined by Kresovich et al. (2019) in a case series [case–case comparison] study. Altogether, 989 participants with diagnoses of incident cases of breast cancer were enrolled into the Breast Cancer Care in Chicago study between 2005 and 2008; ER/PR status was known for 696 cases. The 2002 US EPA's NATA census tract estimates of metal concentrations including cobalt were matched to participants' places of residence in the same year. When the highest and lowest quintiles were compared, elevated odds of ER/PR-negative tumours were identified for cobalt (OR, 2.0; 95% CI, 0.9–4.4; *P* for trend = 0.04). [The Working Group noted that while the authors describe this as a case series study, it might more accurately be described as a case–case comparison study. This design is of limited usefulness because it does not directly estimate the risk of breast cancer associated with cobalt exposure. It is included here because the associations between cobalt exposure and ER/PR-negative and ER/PR-positive breast cancer in women can be compared with the findings by White et al. (2019).]

A population-based case–control study in Mexico examined associations of certain metals or metalloids with incident breast cancer in a region that has naturally high levels of metals in water, and which houses the world's fourth largest non-ferrous metal processing facility (Mérida-Ortega et al., 2022). Women with histopathologically confirmed breast cancer (*n* = 499) were identified from public hospitals in several states in northern Mexico and were age-matched (± 5 years) with controls. [The Working Group noted that the authors did not mention how the case group was selected from the larger case pool of 1045 histopathologically confirmed cases.] Interviews were conducted to obtain covariate information, and height and weight were measured. Metal concentrations were measured in urine samples (first morning void) near the time of interview and for the women with breast cancer before any treatment had begun (on average, 2 months after diagnosis). After excluding cases and controls with exceptionally low or high creatinine concentrations, 452 cases and 439 controls were analysed. Odds ratios were calculated for creatinine-adjusted metal concentrations, both individually in models and grouped together using PCA to assess mixture patterns. [The Working Group noted the somewhat imprecise age matching as a limitation and the high response rates (> 90%) of both cases and controls, as well as good control for confounding factors – such as body mass index (BMI), endogenous estrogen exposure, and alcohol consumption – as strengths.] The median cobalt concentration in urine samples from women with breast cancer was significantly lower than that of controls. An inverse association was noted between cobalt urine concentration and breast cancer overall (highest quartile OR, 0.45; 95% CI, 0.28–0.70). A positive interaction was reported between cobalt and molybdenum regarding their association with breast cancer. [The Working Group noted that the interaction results were not shown and that the reported interaction was hard to interpret, because both cobalt and molybdenum were strongly inversely associated with breast cancer. Case exposure was measured near the time of diagnosis. The study was conducted in

a population with suspected exposure to metals that is greater than that of the general population because of its geographical location.]

2.3 Cancer of the oral cavity, pharynx, larynx, and oesophagus

See Table 2.5.

In total, five epidemiological studies reported on the association between cobalt exposure and the risk of cancer of the oral cavity, larynx, and oesophagus. Data on Barrett oesophagus and oesophageal precancerous lesions were included because these lesions were considered precursors of oesophageal cancer.

Moulin et al. (1993) followed a cohort of 1148 men employed at a cobalt- and sodium-producing plant for at least 1 year between 1950 and 1980 in France. The study is an update of a previous study by Mur et al. (1987), which is not described here. Exposure was assessed by records of work areas: cobalt production, sodium production, production of other chemicals, maintenance, and general service. One quarter of the workforce was born outside of France, and the vital status of these workers could not be assessed completely. Causes of death were ascertained from 1950 to 1967 based on medical records, and from 1968 to 1988 based on death certificates retrieved from the national register at INSERM (the French National Institute of Health and Medical Research). Results were reported as standardized mortality ratios using French national death rates as standards. In the cohort as a whole, 12 workers died from cancer of the buccal cavity and pharynx (SMR, 1.47; 95% CI, 0.76–2.57); of these 9 were born in France (SMR, 1.56; 95% CI, 0.71–2.96). Four workers died from oesophageal cancer (SMR, 0.51; 95% CI, 0.14–1.31); of these three were born in France (SMR, 0.55; 95% CI, 0.11–1.61). Six workers died from laryngeal cancer (SMR, 0.91; 95% CI, 0.34–1.99); all of these were born in France (SMR, 1.31; 95% CI, 0.48–2.85). Data were not reported by work area for these cancer sites. [The Working Group noted that workers were identified from company records only. For these uncommon cancers, results were not presented separately for exposure subgroups.]

Rogers et al. (1993) undertook a case–control study in three counties in Washington State, USA, in which 960 patients who had received diagnoses of cancer of the larynx, oesophagus, or oral cavity, in 1983–1987 were identified. Controls were identified via random-digit dialling. For 3798 households that were willing to participate, 625 eligible controls (frequency-matched to cases on sex and 5-year age intervals) were identified. Toenail cuttings collected at recruitment to the study (available for 354 cases and 434 controls) were used to assess exposure to cobalt. Concentrations of iron, calcium, zinc, chromium, and cobalt were measured in the toenail cuttings and used to stratify the participants into three exposure groups. Unconditional logistic regression was used to assess the association between cobalt exposure groups and risk of cancer of the larynx, oesophagus, or oral cavity. For cancer of the oral cavity, odds ratios were 1.5 (95% CI, 0.9–2.6) and 1.9 (95% CI, 1.0–3.6) in individuals with cobalt concentrations of 0.05–0.17 ppm and > 0.17 ppm, respectively, using individuals with concentrations of < 0.05 ppm as the referent. For oesophageal cancer, the odds ratio for individuals with cobalt concentrations of 0.05–0.17 ppm was 2.4 (95% CI, 0.8–7.2), and there was an increased odds ratio of 9.0 (95% CI, 2.7–30.0) for those with concentrations of > 0.17 ppm, using individuals with concentrations of < 0.05 ppm as the referent.No association was found between cobalt concentration and laryngeal cancer. [The Working Group noted that toenail cuttings were collected some length of time after diagnosis, and some eligible cases died between the time of diagnosis and the request for toenail cuttings (Rogers et al., 1991). Therefore,

Table 2.5 Epidemiological studies of cancer of the oral cavity, larynx, pharynx, and oesophagus and exposure to cobalt

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1993) France Enrolment, 1950–1980/ follow-up, 1988 Cohort	1148 men employed for ≥ 1 yr at a plant producing cobalt and sodium Exposure assessment method: exposure to cobalt via all routes (indirectly) was assessed qualitatively and semiquantitatively using company job history records; exposure metrics: employed ≥ 12 mo between 1950 and 1980, occupational categories, time since first employment (man-years), and duration of employment	Buccal cavity and pharynx, mortality	Employed in cobalt production (SMR):			Age, calendar period	*Exposure assessment critique*: Non-differential exposure misclassification likely (broad exposure categories). Possible co-exposures identified could not be fully accounted for in analyses. *Strengths*: Cohort study. *Limitations*: Identification of cohort based on company records only. Incomplete vital status assessment for foreigner workers. Cause of death 1950–1967 based on medical records only.
			All workers	12	1.47 (0.76–2.57)		
			French-born workers	9	1.56 (0.71–2.96)		
		Oesophagus, mortality	Employed in cobalt production (SMR)				
			All workers	4	0.51 (0.14–1.31)		
			French-born workers	3	0.55 (0.11–1.61)		
		Larynx, mortality	Employed in cobalt production (SMR)				
			All workers	6	0.91 (0.34–1.99)		
			French-born workers	6	1.31 (0.48–2.85)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rogers et al. (1993) Washington State, USA, 3 counties 1983–1987 Case–control	Cases: 960 individuals aged 20–70 yr diagnosed with cancers of the oral cavity (516), oesophagus (203), or larynx (241) through the local SEER cancer registry; toenail samples available for only 354 cases (oral cavity, 281; oesophagus, 73; larynx, 153) Controls: 625 controls identified using random-digit dialling, frequency-matched to oral cancer cases by sex and 5 yr age intervals, but toenail samples available for only 434 Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using toenail samples	Oral cavity, incidence	Cobalt level in nail tissue (OR):			Age, sex, pack-year of cigarette use, drink-years of alcohol, energy intake (kcal/day), β-carotene intake (mg/day), ascorbic acid intake (mg/day)	*Exposure assessment critique*: Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of the cancer outcomes under study (oral, oesophageal, and laryngeal) potentially leading to non-differential misclassification of exposure. *Other strengths*: Population-based case–control study. Measurement of metal in toenail samples. *Other limitations*: Toenail samples available for only some of eligible cases and controls. [sum of case numbers reported by level of exposure does not match total number].
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	1.5 (0.9–2.6)		
			> 0.17 ppm	NR	1.9 (1.0–3.6)		
		Oesophagus, incidence	Cobalt level in nail tissue (OR):				
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	2.4 (0.8–7.2)		
			> 0.17 ppm	NR	9.0 (2.7–30.0)		
		Larynx, incidence	Cobalt level in nail tissue (OR):				
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	2.0 (1.0–3.8)		
			> 0.17 ppm	NR	1.0 (0.4–2.6)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
O'Rorke et al. (2012) Ireland Enrolment, 2002–2004 Case–control	Cases: 451 participants aged ≤ 85 yr diagnosed with adenocarcinoma of the oesophagus (227) or Barrett oesophagus (224); toenail clippings available for 137 and 182 cases, respectively Controls: 260 population controls aged 35–84 yr with no prior history of oesophageal/ gastrointestinal malignancy selected at random from general practitioner lists in Ireland, frequency-matched to cases on sex and age (5 yr bands); toenail clippings available for 221 controls Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using toenail samples	Oesophagus (adenocarcinoma), incidence	Natural log-transformed cobalt level in nail tissue (μg/g) (OR):			Age at interview, sex, smoking status, gastro-oesophageal reflux symptoms, education, location, *H. pylori* infection	*Exposure assessment critique*: Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of oesophageal cancer or Barrett oesophagus, potentially leading to non-differential misclassification of exposure. *Other strengths*: Population-based measurements in toenail clippings. *Other limitations*: Toenail clippings only available for part of eligible cases and controls.
			< −5.4824	34	1		
			−5.4824 to < −4.4705	39	1.06 (0.57–1.98)		
			≥ −4.4705	52	1.54 (0.84–2.85)		
			Trend-test *P*-value, 0.16				
		Oesophagus (Barrett oesophagus), incidence	Natural log-transformed cobalt level in nail tissue (μg/g) (OR):				
			< −5.4824	55	1		
			−5.4824 to < −4.4705	54	1.08 (0.55–2.10)		
			≥ −4.4705	64	1.97 (1.01–3.85)		
			Trend-test *P*-value, 0.05				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sauni et al. (2017) Finland Enrolment, 1968–2004/ follow-up, 2013 Cohort	995 men employed for ≥ 1 yr at a Finnish cobalt plant Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed semiquantitatively using company administrative records Exposure metrics: duration and departmental exposure groupings	Tongue, incidence	Exposure group (SIR):			Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential misclassification likely. Possible co-exposure to nickel not accounted for in analyses. *Other strengths*: Identification of cohort members and follow-up for deaths and emigration were complete. *Other limitations*: Identification of cobalt from company records only. Concomitant exposure to iron, zinc, and nickel.
			Variable exposure	1	26.4 (0.67–14.0)		
			Low	1	6.48 (0.16–36.1)		
			Moderate	0	0 (NR)		
			High	1	6.12 (0.15–34.1)		
		Tongue, incidence	Duration of employment (SIR):				
			> 1 yr	3	7.39 (1.52–21.6)		
			> 5 yr	3	10.0 (2.06–29.2)		
		Oesophagus, incidence	Duration of employment (SIR):				
			> 1 yr	2	1.74 (0.21–6.28)		
			> 5 yr	2	2.24 (0.27–8.08)		
		Larynx, incidence	Duration of employment (SIR):				
			> 1 yr	2	2.45 (0.30–8.86)		
			> 5 yr	2	3.09 (0.37–11.2)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control	Cases: 100 participants identified as having mild/moderate OPL from a cohort of 1731 residents, aged 35–75 yr, who underwent endoscopic examination; 144 mild/moderate OPL identified; 100 randomly selected for study Controls: 100 healthy controls, matched to cases on sex, age (± 2 yr), and village Exposure assessment method: exposure to cobalt was assessed quantitatively in two ways: (1) repeated dietary samples (triplicate) to assess ingestion; (2) single blood sample (plasma) to assess exposure through all routes (indirectly)	Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day) (OR):			Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	*Exposure assessment critique*: Key strengths include: measurements in serum and duplicate diet portions. Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of OPL, potentially leading to non-differential misclassification of exposure. Results for dietary cobalt do not consider other routes of exposure that may be significant for some participants (e.g. inhalation). *Other limitations*: Many comparisons and inconsistency between pattern in men and women.
			Quartile 1 (4.67–20.92)	32	1		
			Quartile 2 (20.92–32.14)	28	0.84 (0.35–2.04)		
			Quartile 3 (32.14–58.05)	24	0.37 (0.15–0.93)		
			Quartile 4 (58.05–1915.77)	16	0.34 (0.12–0.96)		
			Trend-test *P*-value, 0.034				
		Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day), men (OR):			Age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (4.67–20.92)	12	1		
			Quartile 2 (20.92–32.14)	16	1.43 (0.39–5.22)		
			Quartile 3 (32.14–58.05)	10	1.20 (0.29–5.04)		
			Quartile 4 (58.05–1915.77)	14	0.88 (0.25–3.03)		
			Trend-test *P*-value, 0.537				
		Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day), women (OR):				
			Quartile 1 (4.67–20.92)	20	1		
			Quartile 2 (20.92–32.14)	12	0.58 (0.16–2.09)		
			Quartile 3 (32.14–58.05)	14	0.40 (0.12–1.37)		
			Quartile 4 (58.05–1915.77)	2	0.12 (0.02–0.80)		
			Trend-test *P*-value, 0.025				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control (cont.)		Oesophagus (OPL), incidence	Plasma cobalt (μg/L) (OR):			Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (0.00–0.58)	26	1		
			Quartile 2 (0.58–1.19)	25	0.53 (0.21–1.32)		
			Quartile 3 (1.19–1.78)	26	0.65 (0.24–1.75)		
			Quartile 4 (1.78–20.82)	23	0.49 (0.17–1.45)		
			Trend-test *P*-value, 0.253				
		Oesophagus (OPL), incidence	Plasma cobalt (μg/L), men (OR):			Age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (0.00–0.58)	15	1		
			Quartile 2 (0.58–1.19)	11	0.62 (0.18–2.16)		
			Quartile 3 (1.19–1.78)	10	0.27 (0.07–1.04)		
			Quartile 4 (1.78–20.82)	11	0.70 (0.20–2.46)		
			Trend-test *P*-value, 0.611				
		Oesophagus (OPL), incidence	Plasma cobalt (μg/L), women (OR):				
			Quartile 1 (0.00–0.58)	11	1		
			Quartile 2 (0.58–1.19)	14	1.31 (0.31–5.49)		
			Quartile 3 (1.19–1.78)	16	1.35 (0.34–5.41)		
			Quartile 4 (1.78–20.82)	7	0.14 (0.02–0.80)		
			Trend-test *P*-value, 0.020				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control (cont.)		Oesophagus (OPL), incidence	Serum Tc II level (ng/L) (OR): Quartile 1 (49.36–73.21) Quartile 2 (73.21–86.30) Quartile 3 (86.30–116.24) Quartile 4 (116.24–675.39) Trend-test *P*-value, < 0.001	 37 33 17 13	 1 0.85 (0.29–2.49) 0.22 (0.08–0.64) 0.19 (0.07–0.51)	Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	

BMI, body mass index; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; mo, month; NR, not reported; OPL, oesophageal precancerous lesion; OR, odds ratio; SEER, Surveillance, Epidemiology, and End Results; SIR, standardized incidence ratio; SMR, standardized mortality ratio; Tc II, transcobalamin II; yr, year.

the survivors may not be representative of the recruited cases. The numbers of cases included in the analyses with cobalt concentrations in toenail cuttings were not clearly reported.]

O'Rorke et al. (2012) undertook an all-Ireland case–control study to assess the association between toenail concentrations of cobalt and incidence of oesophageal adenocarcinoma and Barrett oesophagus. In total, 227 patients with oesophageal adenocarcinoma and 224 with Barrett oesophagus who received diagnoses between 2002 and 2004 were recruited along with 260 population-based controls. Toenail cuttings were obtained from participants (oesophageal adenocarcinoma, n = 137; Barrett oesophagus, n = 182; controls, n = 221) and analysed for iron, selenium, zinc, cobalt, chromium, cerium, and mercury concentrations. The mean concentration of cobalt in toenail cuttings was 0.02 µg/g for oesophageal adenocarcinoma, Barrett oesophagus, and controls. Exposure levels were stratified into tertiles on the basis of levels in controls. Using the lowest tertile as baseline, the adjusted odds ratios for oesophageal adenocarcinoma with medium and high cobalt exposure were 1.06 (95% CI, 0.57–1.98) and 1.54 (95% CI, 0.84–2.85), respectively (P for trend = 0.16). For Barrett oesophagus, the adjusted odds ratios for medium and high exposure were 1.08 (95% CI, 0.55–2.10) and 1.97 (95% CI, 1.01–3.85), respectively (P for trend = 0.05). [The Working Group noted the low exposure level in the general population.]

Sauni et al. (2017) identified and followed 995 men, employed for at least 1 year during the period 1968–2004, at the Kokkola cobalt plant, Finland. From 1966 to 1987, cobalt was produced from pyrite ore concentrate, and after 1987 from by-products of the metallurgical industry. Cohort members were followed up for cancer incidence by linkage to the Finnish Cancer Register for the period 1968–2013, and standardized incidence ratios were calculated using cancer incidence rates from the local area around the Kokkola plant. The study reported a substantially increased risk of cancer of the tongue across the entire cohort (SIR, 7.39; 95% CI, 1.52–21.6; 3 cases) and for workers employed > 5 years (SIR, 10.0; 95% CI, 2.06–29.2; 3 cases), although there were few cases. There were 2 cases of cancer of the larynx (SIR, 2.45; 95% CI, 0.30–8.86), 2 cases of oesophageal cancer (SIR, 1.74; 95% CI, 0.21–6.28), and fewer than 2 cases of cancer of the pharynx with no results reported for this cancer. [The Working Group noted the small size of the cohort as a limitation of this study. Overall smoking prevalence in the cohort was fairly similar to that in the local area.]

Pan et al. (2021) undertook a case–control study nested in a screening programme for detection of oesophageal precancerous lesions in Huai'an District, China, a region that has high rates of oesophageal squamous cell carcinoma. In the period 2015–2017, 1731 residents aged 35–75 years underwent endoscopic examination. Of these, 144 received diagnoses of mild/moderate oesophageal precancerous lesion; 100 were randomly selected as cases, and controls were 100 sex-, age-, and village-matched screen-negative individuals. Among other exposures, plasma cobalt concentration and dietary cobalt intake were measured using fasting blood samples and 3-day duplicate dietary samples. Levels of cobalt were divided into quartiles, and associations between exposure and risk of oesophageal precancerous lesions were assessed with conditional logistic regression. For dietary cobalt intake there was a decreasing risk of oesophageal precancerous lesions with increasing exposure level (P = 0.034; adjusted OR for highest versus lowest, 0.34; 95% CI, 0.12–0.96). There was no association between plasma cobalt concentration and risk of oesophageal precancerous lesion (P = 0.253). [The Working Group noted that the results were not consistent between men and women, and that data were not presented for oesophageal cancer.]

2.4 Other cancers and all cancers combined

See Table S2.6 (Annex 2, Supplementary material for Section 2, Cancer in Humans, web only, available from: https://publications.iarc.fr/618).

Five cohort studies (three industrial cohorts and two other cohorts) and one nested case–control study were available for the Working Group to review.

The study of Moulin et al. (1993) was an extension of a previous study published by the same research group (Mur et al., 1987). Both studies were based on a cohort of workers in an electrochemical factory that produced cobalt and sodium in France. The mortality data reported in the first study were based on medical records from the years 1950 to 1980, but Moulin et al. (1993) extended the study to 1981–1988 and used data from death certificates. In the study by Moulin et al. (1993), the analyses were performed in two separate cohorts: one overall cohort including all workers and the other including only workers born in France, since many foreign-born workers were lost to follow-up. The overall cohort comprised 1148 people. Neither of the studies identified increased mortality for all cancers combined. In the study by Moulin et al. (1993), the standardized mortality ratio was 0.83 (95% CI, 0.66–1.03) in the overall cohort and 1.00 (95% CI, 0.78–1.26) for workers born in France. In total, there were 5 deaths from brain cancer (SMR, 3.57; 95% CI, 1.16–8.32), of which 4 were among French-born workers (SMR, 3.98; 95% CI, 1.08–10.19). The workers who died had been involved in maintenance and administration. The study did not find increased mortality from cancers of the stomach, intestine, rectum, pancreas, urinary bladder, or prostate, or from lymphoma, leukaemia, or bone sarcoma. [The Working Group noted that non-differential exposure misclassification in the study was probable, as broad exposure categories were used. Possible co-exposure to other carcinogens (e.g. asbestos) was also identified. Follow-up was incomplete among those born outside of France.]

In the cohort study by Tüchsen et al. (1996), which concerned two Danish porcelain factories, the cancer risk of several organ sites was examined. The cohort consisted of 874 women exposed occupationally to cobalt and 520 women who were employed at the same factories but who had not been exposed to cobalt. Standardized incidence ratios using national reference rates were reported separately for all exposed workers, those exposed in factory 1, those exposed in factory 2, and the unexposed workers. Standardized incidence ratios were increased for cancer of the uterine cervix among all exposed workers (SIR, 2.31; 95% CI, 1.19–4.03) and for cancer of the uterine corpus among the unexposed workers (SIR, 3.02; 95% CI, 1.38–5.73). There were 4 cases of brain cancer: 1 case in a cobalt-exposed worker (SIR, 0.50 [95% CI, 0.03–2.48]) and 3 in non-exposed workers (SIR, 1.68 [95% CI, 0.43–4.59]). The standardized incidence ratio for all cancers combined was 1.20 (95% CI, 0.94–1.52) for all exposed workers, 1.12 [95% CI, 0.79–1.55] for workers exposed in factory 1, 1.29 [95% CI, 0.90–1.79] for those exposed in factory 2, and 0.99 [95% CI, 0.76–1.27] for unexposed workers. [The Working Group noted that the number of observed and expected cases for less common types of cancer was quite low, resulting in imprecise risk estimates. Inclusion of an internal unexposed referent group was an important strength of the study.]

A Finnish cohort study of 995 men, all cobalt-production workers, investigated risk of cancers of the stomach, colon, rectum, pancreas, prostate, kidney, and urinary bladder, and of melanoma, non-melanoma skin cancer, basal cell carcinoma, non-Hodgkin lymphoma, and leukaemia (Sauni et al., 2017). The cohort was further divided into subcohorts by exposure levels, according to the departments in which employees had first worked at the plant.

Exposures in different departments were based on regular industrial hygiene measurements recorded between 1986 and 2014. There were no notable increases in risk for any of the specific cancer types assessed. The standardized incidence ratios of all cancers combined were 1.00 (95% CI, 0.81–1.22) for those employed for at least 1 year and 1.08 (95% CI, 0.85–1.34) for those employed for at least 5 years. [The Working Group noted that the number of observed and expected cases for less common types of cancer was quite low, resulting in imprecise risk estimates.]

Rodrigues et al. (2020) undertook a case–control study nested in a cohort of workers from three facilities engaged in the manufacture of semiconductor and electronic storage devices, located in New York, Vermont, and California, USA. In total, 126 836 workers had been included in a 1965–1999 mortality study, and 89 054 of them in a 1976–1999 cancer incidence study. Fatal cases of malignant CNS neoplasms were identified from the National Death Index. Incident cases were identified from linkage with state cancer registries. For each case, 10 cohort members were selected as controls using incidence density sampling and further matched on year of birth, facility, sex, and race. On the basis of information about the workers' roles in production, 10 PEGs were constructed. For each case and control, work history was mapped on the basis of combinations of division, department, and job title, and these combinations were classified by PEG. Manufacturing periods were divided into eras, and a chemical/PEG/manufacturing era matrix was constructed for 31 chemicals. Odds ratios were calculated for the associations between tertiles of cumulative exposure to cobalt and risk of CNS cancer, stratified by facility. In total, 120 cases and 1028 controls were identified. There was little evidence of positive associations between cumulative exposure to cobalt and CNS cancer in any of the three facilities, and estimates were statistically imprecise. [The Working Group noted that workers could have been exposed to more than one chemical in a given PEG, and that half of the cases and controls had worked in more than one PEG. Numerous exposures were assessed in this study, and positive associations were found for the highest tertile of estimated cumulative exposure for several chemicals, including 2-butoxyethanol, cyclohexanone, *ortho*-dichlorobenzene, cadmium, molybdenum, trichloroethylene, and vinyl chloride.]

The study by Duan et al. (2020) was based on a sample (n = 26 056) drawn from the NHANES 1999–2014 cohort, and mortality was followed until the end of 2015. The study investigated the association between heavy metal concentrations in urine (barium, cadmium, cobalt, caesium, molybdenum, lead, antimony, titanium, tungsten, and uranium) at the time of the enrolment and cancer mortality. Urinary cobalt concentration (median, 0.35 µg/L) was not associated with increased overall cancer mortality (relative risk, 1.05; 95% CI, 0.85–1.30) when cobalt was analysed with covariate adjustment for other metals in the multiple-metal analysis. When the association between single urinary metals and cancer mortality was estimated, cobalt was associated with all cancers combined in a model adjusted for sex, age, age^2, ethnicity, and urine creatinine concentration (relative risk, 1.23; 95% CI, 1.03–1.46). The association remained similar but was slightly attenuated in two other models adjusted for additional medical and socioeconomic risk factors. [The Working Group noted that the metals were measured in NHANES 1999–2014, and mortality was assessed between 1999 and 2015; therefore, there was a short time period between exposure and outcome.]

Li et al. (2021a) studied the associations between plasma concentrations of 12 metals (iron, copper, zinc, selenium, chromium, manganese, molybdenum, cobalt, nickel, arsenic, cadmium, and lead) at baseline and cancer risk in 4573 patients with type 2 diabetes. The participants were from the Dongfeng-Tongji cohort,

which comprises 27 009 retired workers who had been employed by an automotive manufacturing corporation. [The Working Group noted that the occupational cohort was not selected on the basis of high cobalt exposure, and that it was unclear whether the study participants had any specific exposure to cobalt in their work.] Enrolment took place between 2008 and 2010 and follow-up through 2018. The results were reported according to the quartiles of plasma concentrations of cobalt. The hazard ratios for all types of cancer combined were not associated with the plasma concentrations of cobalt in any of the quartiles. When compared with the lowest exposure quartile, the hazard ratios were 0.96 (95% CI, 0.76–1.20), 0.79 (95% CI, 0.62–1.00), and 0.80 (95% CI, 0.63–1.02) for quartiles 2, 3, and 4, respectively. [The Working Group noted that the plasma level of cobalt, natural log-transformed for skewness, was lower in cancer cases (median [presumed], 0.24 μg/L; interquartile range, 0.19–0.31) than in non-cases (median [presumed], 0.26 μg/L; interquartile range, 0.20–0.32). The timing of exposure measurement may be outside the relevant time window of exposure for the cancer outcome under study.]

2.5 Evidence synthesis for cancer in humans

The studies considered by the Working Group to be most relevant to the evaluation of cancer in humans resulting from exposure to cobalt metal (without tungsten carbide or other metal alloys) and cobalt compounds included occupational studies of cobalt-exposed workers, one study in an occupational group not specifically exposed to cobalt, and several studies in the general population. Among the occupational studies considered relevant for this evaluation, two concerned hard-metal industries and six investigated other industries. All these studies analysed lung cancer, and some analysed cancers at other organ sites. Among the non-occupational studies, one analysed risk of breast cancer in relation to baseline residential air pollution levels, and several studies of other cancer types used biomarkers from one-time samples of toenail cuttings, blood, or urine to estimate individual exposures. No informative studies were found that permitted the separation of the effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II) oxide or cobalt(II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

2.5.1 Quality of exposure assessment for cobalt and co-exposures

Quality of the exposure assessment was an important factor in evaluating the informativeness of studies by the Working Group. Detailed reports on the strengths and limitations of exposure evaluations in cohort and case–control studies are provided in Section 1.6.1.

The most informative occupational studies assessed potential associations between cobalt exposure and lung cancer. Weaknesses in the exposure assessments in many of these studies included use of qualitative rather than quantitative exposure metrics for cobalt, lack of control for co-exposures to the WC-Co composite (an agent excluded from this evaluation) in studies of the hard-metal industry, and lack of control for carcinogenic co-exposures that may have been present in many of the facilities studied, including recognized lung carcinogens such as asbestos, arsenic, and chromium, nickel, and cadmium compounds. The study by Moulin et al. (1998) included an analysis of lung cancer mortality in relation to "other cobalt exposures", which included exposure to cobalt alone or simultaneously with agents other than WC-Co. Because of the potential for confounding by WC-Co in other production areas and co-exposure to other lung carcinogens, which could not be fully adjusted for, the exposure metric

assessed in this study was considered uninformative for this evaluation. Similarly, although the study by Wild et al. (2000) used a detailed JEM to assess exposure–response in relation to exposure to WC-Co, the exposure group considered most relevant for the current evaluation was workers "ever employed" or "only employed" in the production of cobalt powder, who may have also been exposed separately to tungsten carbide powders. This exposure subgroup analysis was of limited informativeness because individuals in the "ever employed" category had potential exposure to other lung carcinogens during work in other production areas of the plant (there were only two lung cancer deaths in the more informative category of "only exposed").

Reviews and critiques of the exposure assessment methodology for studies of cobalt exposure and lung cancer in other industries (Moulin et al., 1993, 2000; Tüchsen et al., 1996; Grimsrud et al., 2005; Sauni et al., 2017) are described by the Working Group in Section 1.6.1. These studies varied in exposure assessment quality, and most had potential misclassification of cobalt exposure and confounding by co-exposure to other lung carcinogens. None of these studies found positive associations with cobalt exposure for which confounding by co-exposure to other lung carcinogens could be ruled out.

Among the studies reflecting exposures of the general population (as opposed to occupational exposures), the study by White et al. (2019) linked modelled census tract-level estimates of cobalt concentrations in outdoor air for a single year to each woman's address at enrolment. This failed to account for temporal trends in outdoor air concentrations of metals within census tracts.

One study within the prospective Sister Study cohort used cobalt concentrations in toenail cuttings as biological markers of exposure (Niehoff et al., 2021). In this study, toenail cuttings were obtained at baseline, thus enabling assessment of exposure before the onset of disease. Although one-time measurement of metal concentrations in toenail cuttings may not represent cumulative exposures to cobalt during biologically relevant time periods of exposure, they appear to be moderately correlated within individuals over a period of several years (see Section 1.6.1). A strength of the exposure assessments in the general-population studies was that much more extensive information was provided on individual-level covariates and quantitative estimates of exposure to other environmental contaminants. Although not a reflection of low-quality exposure assessment, the small range of exposures in the general-population studies may limit the informativeness of exposure–response analyses.

2.5.2 Lung cancer

In two high-quality studies of hard-metal manufacturing facilities, where there was some overlap in populations, elevated standardized mortality ratios or odds ratios for lung cancer were observed in workers "ever exposed" to cobalt with no exposure to tungsten carbide (Moulin et al.,1998; Wild et al., 2000). The study by Moulin et al. (1998) included an analysis of lung cancer mortality in relation to "other cobalt exposures", which included cobalt alone or simultaneous exposure to cobalt and agents other than WC-Co. Because exposures were defined as "ever" working in the departments where there was no tungsten carbide exposure, rather than "only" working in those departments, there is potential for confounding by exposure to WC-Co and co-exposure to other lung carcinogens. This study did not present exposure–response analyses for "other cobalt exposures", although it did control for categorical metrics of WC-Co in analyses of "other cobalt exposures". In addition, the study did not control for exposure to other carcinogens, and there was no information provided about the extent or intensity of exposure to them. Therefore, after careful consideration, the Working Group viewed this

study as uninformative for the present evaluation. Similarly, although the study by Wild et al. (2000) observed elevated lung cancer risks among workers ever employed in the production of cobalt and tungsten carbide powders, workers in this subgroup may have worked in other production areas that had potential for exposure to WC-Co and co-exposure to other lung carcinogens. There were only two deaths in the more informative category of "only exposed" in powder production. The Working Group considered this study to be uninformative for the evaluation because of the strong potential for exposure to WC-Co and other confounding exposures. In the occupational studies of other industries involving cobalt exposures, some of which had confounding or limited statistical precision, no consistent elevated risks of lung cancer were observed (Moulin et al., 1993, 2000; Tüchsen et al., 1996; Grimsrud et al., 2005; Sauni et al., 2017).

2.5.3 Breast cancer

One occupational study and three population-based studies examined breast cancer incidence after exposure to cobalt. The occupational study did not find an excess of breast cancer incidence among women employed in two Danish porcelain factories (Tüchsen et al., 1996). Limitations of this study included low statistical power and an inability to control for important breast cancer risk factors, including parity and age at first birth. In the study on the relation between residential exposure to cobalt and breast cancer within the Sister Study population, there was no clear gradient of increasing risk with increasing exposure (White et al., 2019). Limitations of this study included the low levels and small range of cobalt exposures and the use of broad proxies in the exposure assessment. The study on cobalt concentrations in toenail cuttings and breast cancer within the Sister Study population did not find associations with increasing levels of cobalt exposure (Niehoff et al., 2021). This study was also limited by low cobalt concentrations and a small range of cobalt exposures, as well as the considerations regarding the use of measurements of metal concentrations in toenail cuttings discussed in Section 2.5.1. One case–control study in an area of Mexico where metal exposures were thought to be high did not find a positive association between urinary cobalt concentrations and breast cancer (Mérida-Ortega et al., 2022).

2.5.4 Other cancers and all cancers combined

In total, five epidemiological studies reported on the association between cobalt exposure and the risk of cancer of the oral cavity, larynx, oesophagus, and oesophageal precursor lesions (O'Rorke et al., 2012; Moulin et al., 1993; Rogers et al., 1993; Sauni et al., 2017; Pan et al., 2021). Although suggestive evidence of positive associations for some cancer sites or subsites was reported in some studies, interpretation of evidence from these studies was limited by inconsistent findings and small numbers of cases.

Other cancer types (digestive organs, pancreas, kidney, urinary bladder, prostate, uterus, ovary, and brain) and melanoma, non-melanoma skin cancer, lymphoma, osteosarcoma, and leukaemia were assessed in three cohort studies (Moulin et al., 1993; Tüchsen et al., 1996; Sauni et al., 2017) and one nested case–control study (Rodrigues at al., 2020). Mostly, the risk estimates were imprecise and not substantially elevated. Tüchsen et al. (1996) reported an increased standardized incidence ratio for cancer of the uterine cervix among all exposed workers and for cancer of the uterine corpus among the unexposed workers. In the study by Moulin et al. (1993), the standardized mortality ratio for brain cancer was increased both among all workers and among a subpopulation of workers born in France. Overall, there were possible co-exposures in all three studies

that were not adjusted for in the analyses, therefore these studies are of moderate quality and informativeness.

Of the five studies assessing the risk of all cancers combined (Moulin et al., 1993; Tüchsen et al., 1996; Sauni et al., 2017; Duan et al., 2020; Li et al., 2021a), only one population-based study found a significant increase related to cobalt exposure in one of three adjusted models of analysis (Duan et al., 2020). A limitation of this study was the relatively short follow-up period, which yielded a small number of death outcomes. The exposure assessment was based on one-time urine analyses, which may not have reflected cobalt exposures over a biologically relevant time period.

Although several studies examined risks of other cancers, these studies did not provide an adequate basis for evaluation because of low statistical power and the small number of studies for each cancer type. Results of analyses of all cancers combined are generally less informative for carcinogenicity evaluations because this is a heterogeneous outcome.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Cobalt metal

3.1.1 Mouse

Inhalation

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were exposed by inhalation (whole-body) to cobalt metal-particle aerosol concentrations of 0, 1.25, 2.5, or 5 mg/m^3 (concentrations were based on the findings from a 13-week study) for untreated controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, 98.2% ± 0.6%; mass median aerodynamic diameter, MMAD, 1.5–2.0 μm; geometric standard deviation, GSD, 1.6–1.9 μm) for 6 hours plus T_{90} (12 minutes; the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation) per day, 5 days per week for 105 weeks (NTP, 2014). Surviving mice were killed at age 109–111 weeks. At study termination, survival of male mice was 39/50, 31/50, 29/50, and 25/50, and for female mice was 36/50, 36/50, 27/50, and 26/50, for the controls and groups at the lowest, intermediate, and highest concentration, respectively. These survival rates included one male in the group at the intermediate concentration, three males at the highest concentration, and two females at the lowest concentration that died during the last week of the study. Survival of males in groups at the intermediate or highest concentration was significantly less than that of controls. The mean body weights of the males and females at the highest concentration were significantly decreased, and at least 10% less than those of the control groups after weeks 85 and 21, respectively. Abnormal breathing and thinness were noted in exposed male and female mice. Tissue burden studies were conducted only in females; lung cobalt concentrations and burdens increased with increasing exposure concentrations and were greater than those of controls. The values of maximum cobalt lung burdens observed in the 2-year studies indicated that lung overload was not reached in these studies. All mice underwent complete necropsy with histopathological evaluation.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) [P = 0.011, Cochran–Armitage test]. The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 7/50 (14%), 11/49 (22%), 15/50 (30%), and 3/50 (6%), respectively, and was significantly

Table 3.1 Studies of carcinogenicity in experimental animals exposed to different forms of cobalt

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	Tumour incidence	Significance	Comments
Cobalt metal				
Full carcinogenicity Mouse, $B6C3F_1$/N (M) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 39, 31, 29, 25	*Lung* Bronchioloalveolar adenoma (includes multiple) 7/50 (14%), 1 1/49 (22%), 15/50 (30%)*, 3/50 (6%) Bronchioloalveolar carcinoma (includes multiple) 11/50 (22%), 38/49 (78%)*, 42/50 (84%)*, 46/50 (92%)* Bronchioloalveolar carcinoma, multiple 3/50, 18/49*, 24/50*, 36/50* Bronchioloalveolar adenoma or carcinoma (combined) 16/50 (32%), 41/49 (84%)*, 43/50 (86%)*, 47/50 (94%)*	[$P = 0.011$, Cochran–Armitage trend test] *$P = 0.016$, poly-3 test [$P = 0.0448$, Fisher exact test] $P < 0.001$, poly-3 trend test; [Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test] [$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.01$, poly-3 test [$P \leq 0.002$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, adequate number of mice, adequate duration of exposure and observation, and multiple doses based on a 3-month study were used. Other comments: MMAD, 1.5–2.0 µm; GSD, 1.6–1.9 µm. Survival rates in the groups exposed to 2.5 and 5 mg/m^3 significantly less than that of control. Historical controls: Bronchioloalveolar adenoma (includes multiple): inhalation studies 39/300 (13.0 ± 4.2%), range, 8–20%; all routes 145/950 (15.3 ± 6.2%), range, 2–26%. Bronchioloalveolar carcinoma (includes multiple): inhalation studies 59/300 (19.7 ± 3.4%), range, 16–24%; all routes 132/950 (13.9 ± 7.1%), range, 4–24%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 90/300 (30.0 ± 5.5%), range, 26–40%; all routes 263/950 (27.7 ± 5.7%), range, 16–40%.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, $B6C3F_1$/N (F) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 36, 36, 27, 26	*Lung* Bronchioloalveolar adenoma (includes multiple) 3/49 (6%), 9/50 (18%), 8/50 (16%), 10/50 (20%)* Bronchioloalveolar carcinoma (includes multiple) 5/49 (10%), 25/50 (50%)*, 38/50 (76%)*, 43/50 (86%)* Bronchioloalveolar carcinoma, multiple 1/49, 7/50*, 20/50**, 24/50** Bronchioloalveolar adenoma or carcinoma (combined) 8/49 (16%), 30/50 (60%)*, 41/50 (82%)*, 45/50 (90%)*	$P = 0.037$, poly-3 trend test *$P = 0.024$, poly-3 test [$P = 0.0387$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test] [$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0317$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.001$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, adequate number of mice, adequate duration of exposure and observation, and multiple doses based on a 3 mo study were used. Other comments: MMAD, 1.5–2.0 µm; GSD, 1.6–1.9 µm. Historical controls: Bronchioloalveolar adenoma (includes multiple): inhalation studies 16/299 (5.4 ± 3.7%), range, 2–12%; all routes 54/949 (5.7 ± 3.6%), range, 0–12%. Bronchioloalveolar carcinoma (includes multiple): inhalation studies 13/299 (4.4 ± 4.3%), range, 0–10%; all routes –38/949 (4.0 ± 3.6%), range, 0–14%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 28/299 (9.4 ± 4.8%), range, 2–16%; all routes – 90/949 (9.5 ± 4.8%), range, 2–22%.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 17, 20, 16, 16	*Lung* Bronchioloalveolar adenoma (includes multiple) 2/50 (4%), 10/50 (20%)*, 10/50 (20%)*, 14/50 (28%)**	$P = 0.011$, poly-3 trend test [$P = 0.015$, Cochran–Armitage trend test] *$P \leq 0.018$, poly-3 test [$P = 0.0139$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0009$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female rats used, adequate number of rats; adequate duration of exposure and observation, and multiple doses based on a 3-mo study were used. Other comments: MMAD, 1.4–2.0 μm; GSD, 1.6–1.9 μm. Body weights of rats treated with 2.5 and 5 mg/m^3 were ≥ 10% less than those of controls after weeks 99 and 12, respectively. Historical controls: Bronchioloalveolar adenoma (includes multiple): 5/100 (5.0 ± 1.4%), range, 4–6%. Bronchioloalveolar carcinoma (includes multiple): 0/100. Bronchioloalveolar adenoma or carcinoma (combined): 5/100 (5.0 ± 1.4%), range, 4–6%. Cystic keratinizing epithelioma: 0/100. Adrenal medulla, benign pheochromocytoma: 25/100 (25 ± 7.1%), range, 20–30%. Adrenal medulla, malignant pheochromocytoma: 2/100 (2.0% ± 2.8%), range, 0–4%. Adrenal medulla, benign or malignant pheochromocytoma: 27/100 (27 ± 9.9%), range, 20–34%. Pancreatic islets, adenoma: all routes, 0/100. Pancreatic islets, carcinoma: all routes, 2/100 (2 ± 2.8%), range, 0–4%. Pancreatic islets, adenoma or carcinoma (combined): 2/100 (2.0 ± 2.8%), range, 0–4%.
		Bronchioloalveolar carcinoma (includes multiple) 0/50, 16/50 (32%)*, 34/50 (68%)*, 36/50 (72%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	
		Bronchioloalveolar carcinoma, multiple 0/50, 6/50*, 14/50**, 30/50**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0133$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 2/50 (4%), 25/50 (50%)*, 39/50 (78%)*, 44/50 (88%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.0001$, 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014) (cont.)		Cystic keratinizing epithelioma 0/50, 1/50 (2%), 0/50, 1/50 (2%)	NS	
		Adrenal medulla Benign pheochromocytoma (includes bilateral) 15/50 (30%), 23/50 (46%), 37/50 (74%)*, 34/50 (68%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	
		Benign pheochromocytoma, bilateral 4/50 (8%), 13/50 (26%)*, 22/50 (44%)**, 21/50 (42%)**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P < 0.01$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Malignant pheochromocytoma (includes bilateral) 2/50 (4%), 2/50 (4%), 9/50 (18%)*, 16/50 (32%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P = 0.03$, poly-3 test) [$P = 0.0256$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0002$, Fisher exact test]	
		Malignant pheochromocytoma, bilateral 0/50, 0/50, 0/50, 7/50 (14%)*	*$P \leq 0.01$, poly-3 test [$P = 0.0062$, 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014) (cont.)		Benign or malignant pheochromocytoma (combined) 17/50 (34%), 23/50 (46%), 38/50 (76%)*, 41/50 (81%)*	*P* < 0.001, poly-3 trend test [*P* < 0.001, Cochran–Armitage trend test] **P* < 0.001, poly-3 test [*P* < 0.0001, Fisher exact test]	
		Pancreatic islets Adenoma 0/50, 1/50 (2%), 6/48 (13%)*, 3/49 (6%)	**P* = 0.015, poly-3 test [*P* = 0.0117, Fisher exact test]	
		Carcinoma 2/50 (4%), 1/50 (2%), 5/48 (10%), 6/49 (12%)	*P* = 0.021, poly-3 trend test NS, poly-3 test; [Fisher exact test]	
		Adenoma or carcinoma (combined) 2/50 (4%), 2/50 (4%), 10/48 (20%)*, 9/49 (18%)**	*P* = 0.002, poly-3 trend test [*P* = 0.007, Cochran–Armitage trend test] **P* = 0.013, poly-3 test [*P* = 0.0113, Fisher exact test] ***P* = 0.022, poly-3 test [*P* = 0.0235, Fisher exact test]	
		Kidney Renal tubule adenoma or carcinoma (standard and extended evaluation, combined) 3/50, 1/50, 1/50, 7/50	*P* = 0.023, poly-3 trend test [*P* = 0.039, Cochran–Armitage trend 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 35, 26, 24, 25	*Lung* Bronchioloalveolar adenoma (includes multiple) 2/50 (4%), 7/50 (14%), 9/50 (18%)*, 13/50 (26%)**	$P = 0.002$, poly-3 trend test [$P = 0.022$, Cochran–Armitage trend test] *$P = 0.016$, poly-3 test [$P = 0.0256$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0019$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan; male and female rats used, adequate number of rats, adequate duration of exposure and observation, and multiple doses based on a 3-mostudy were used. Other comments: MMAD, 1.4–2.0 µm; GSD, 1.6–1.9 µm. Survival rate in the group exposed to 2.5 mg/m^3 was significantly less than that of control, body weights of rats exposed to 2.5 or 5 mg/m^3 were ≥ 10% less than those of controls after weeks 57 and 21, respectively. Historical controls: Bronchioloalveolar adenoma: 2/100 (2.0 ± 2.8%), range, 0–4%. Bronchioloalveolar carcinoma (includes multiple): 0/100. Bronchioloalveolar adenoma or carcinoma (combined): 2/100 (2.0 ± 2.8%), range, 0–4%. Cystic keratinizing epithelioma: 0/100. Adrenal medulla, benign pheochromocytoma: 7/100 (7 ± 7.1%), range, 2–12%. Adrenal medulla, malignant pheochromocytoma: 1/100 (1 ± 1.4%), range, 0–2%. Adrenal medulla, benign or malignant pheochromocytoma (combined): 8/100 (8 ± 5.7%), range, 4–12%. All organs, mononuclear cell leukaemia: 35/100 (35 ± 4.2%), range, 32–38%. Pancreatic islets, adenoma or carcinoma (combined): 2/100 (2.0 ± 0.0%), range, 2%.
		Bronchioloalveolar carcinoma (includes multiple) 0/50 (0%), 9/50 (18%)*, 17/50 (34%)**, 30/50 (60%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0013$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 2/50 (4%), 15/50 (30%)*, 20/50 (40%)**, 38/50 (76%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0005$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Cystic keratinizing epithelioma 0/50, 4/50 (8%), 1/50 (2%), 2/50 (4%)	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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014) (cont.)		*Adrenal medulla* Benign pheochromocytoma (includes bilateral) 6/50 (12%), 12/50 (24%), 22/50 (44%)*, 36/50 (72%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0003$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Benign pheochromocytoma, bilateral 2/50 (4%), 4/50 (8%), 8/50 (16%)*, 19/50 (38%)**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0458$, Fisher exact test] **$P < 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Malignant pheochromocytoma (includes bilateral) 0/50, 2/50 (4%), 3/50 (6%), 11/50 (22%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0073$, Fisher exact test]	
		Malignant pheochromocytoma, bilateral 0/50, 1/50, 1/50, 4/50 (8%)*	*$P \leq 0.05$, poly-3 test	
		Benign or malignant pheochromocytoma (combined) 6/50 (12%), 13/50 (26%), 23/50 (46%)*, 40/50 (80%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0002$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014) (cont.)		*Pancreatic islets* Adenoma or carcinoma (combined) 1/50, 0/50, 0/50, 3/50 *All organs* Mononuclear cell leukaemia 16/50 (32%), 29/50 (58%)***, 28/50 (56%)**, 27/50 (54%)*	NS NS, poly-3 trend test [$P = 0.036$, Cochran–Armitage trend test] *$P = 0.019$, poly-3 trend test [$P = 0.0214$, Fisher exact test] **$P = 0.013$, poly-3 test [$P = 0.0131$, Fisher exact test] ***$P = 0.007$, poly-3 test [$P = 0.0077$, Fisher exact test]	
Full carcinogenicity Rat, Hooded (M) 2–3 mo 119 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0, 28 mg once 10, 10 NR, average survival: 71 wk	*Injection site* Rhabdomyofibrosarcoma or sarcoma (NOS) (combined) 0/10, 4/10* Rhabdomyofibrosarcoma 0/10, 3/10 Sarcoma (NOS) 0/10, 1/10	*[$P < 0.05$, Fisher exact test] [NS] [NS]	Principal strengths: the duration of observation was adequate, the rats were randomly allocated in groups. Principal limitations: small number of rats per group, use of single dose, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: Co metal powder in 0.4 mL fowl serum was injected once into the left thigh of the rat; control rats received fowl serum alone. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hooded (F) 2–3 mo 122 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0, 28 mg once 10, 10 NR, average survival: 71 wk	*Injection site* Fibrosarcoma, rhabdomyosarcoma, or rhabdomyofibrosarcoma (combined) 0/10, 5/10* Fibrosarcoma 0/10, 3/10 Rhabdomyosarcoma 0/10, 1/10 Rhabdomyofibrosarcoma 0/10, 1/10	 *[$P < 0.02$, Fisher exact test] [NS] [NS] [NS]	Principal strengths: rats randomly allocated in groups, the duration of observation was adequate. Principal limitations: small number of rats per group, use of single dose, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: Co metal powder in 0.4 mL fowl serum was injected once into the left thigh of the rat; control rats received fowl serum alone. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.
Full carcinogenicity Rat, Hooded (F) 2–3 mo 105 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0 (control, Zn powder), 0 (control, W powder), 28 mg once 5, 5, 10 NR, NR, 1 (average survival: 43 wk)	*Injection site* Rhabdomyofibrosarcoma, fibrosarcoma, or sarcoma (NOS) (combined) 0/5, 0/5, 8/10* Rhabdomyofibrosarcoma 0/5, 0/5, 5/10 Sarcoma (NOS) 0/5, 0/5, 2/10 Fibrosarcoma 0/5, 0/5, 1/10	 *[$P = 0.007$, Fisher exact test] [NS] [NS] [NS]	Principal strengths: the duration of observation was adequate. Principal limitations: small number of rats per group, only one sex, use of single dose, lack of untreated control group, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: two groups served as controls, one group of 5 rats received Zn powder and another group of 5 rats received W powder in the same manner as the exposed group. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 3 mo Wen et al. (2020)	Intramuscular implantation Co metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 3 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Neoplasms or inflammation (combined) 0/8, 4/8*	*[P = 0.0385, Fisher exact test]	Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the Co-exposed group to serve as surgery sham controls.
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 6 mo Wen et al. (2020)	Intramuscular implantation Co metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 6 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Neoplasms or inflammation (combined) 0/8, 6/8*	*[P = 0.0035, Fisher exact test]	Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the Co-exposed group to serve as surgery sham controls.
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 12 mo Wen et al. (2020)	Intramuscular implantation Cobalt metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 12 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Rhabdomyosarcoma or spindle cell tumours (NOS) (combined) 0/8, 7/8* Spindle cell tumours (NOS) 0/8, 5/8* Rhabdomyosarcoma 0/8, 2/8	*[P = 0.0007, Fisher exact test] *[P = 0.0128, Fisher exact test] [NS]	Principal strengths: the duration of exposure and observation was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the cobalt-exposed group to serve as surgery sham controls.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Age not specified 8 mo Hansen et al. (2006)	Subcutaneous implantation Co metal as bulk, NR None Diameter 6.5 mm, height 1 mm 1× for 6 or 8 mo 10 NR	*Implantation site* Sarcoma 0/10	NA	Principal strengths: the duration of exposure and observation was considered adequate. Principal limitations: statistics not provided, no controls, only one sex, small number of rats per group, each rat was exposed to the two test agents concurrently. Other comments: this group of 10 male rats was subcutaneously implanted with Co as bulk material on the right side of the vertebral column and implanted as nanoparticulate material on the left side in the paravertebral muscle (see study below).
Full carcinogenicity Rat, Sprague-Dawley (M) Age not specified 8 mo Hansen et al. (2006)	Intramuscular implantation Co metal as nanoparticulate material: average size 120 nm (range, 50–200 nm), NR (Sigma Chemicals, Deisenhofen, Germany) None 60–100 mg 1× for 6 or 8 mo 10 NR	*Implantation site* Sarcoma (NOS) (at 8 mo) 5/6	NA	Principal strengths: the duration of exposure and observation was considered adequate. Principal limitations: statistics not provided, no controls, only one sex, small number of rats per group, each rat was exposed to the two test agents concurrently. Other comments: this group of 10 male rats was implanted with Co as nanoparticulate material on the left side of the vertebral column in the paravertebral muscle and subcutaneously implanted with as bulk material on the right side (see study above).
Full carcinogenicity Rat, Sprague-Dawley (F) NR [bw, 120–140 g] 12 mo Jasmin & Riopelle (1976)	Intrarenal injection Co metal, NR (reagent grade) Glycerin (0.05 mL) 0, 5 mg Single injection into each pole of the right kidney 16, 18 NR	*Kidney* Tumours 0/16, 0/18	NA	Principal limitations: only one dose and sex used, number of rats at start unclear, short duration, step section method not used for histopathological examination, small number of rats per group.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Soluble cobalt(II) salts				
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1, 3 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 22, 31, 24, 20	*Lung* Bronchioloalveolar adenoma 9/50 (18%), 12/50 (24%), 13/50 (26%), 18/50 (36%)* Bronchioloalveolar carcinoma 4/50 (8%), 5/50 (10%), 7/50 (14%), 11/50 (22%)* Bronchioloalveolar adenoma or carcinoma (combined) 11/50 (22%), 14/50 (28%), 19/50 (38%), 28/50 (56%)*	 *P* = 0.005, life-table trend test *P* = 0.018, logistic regression trend test *P* = 0.029, Cochran–Armitage trend test **P* = 0.024, life-table test *P* = 0.027, logistic regression test *P* = 0.035, Fisher exact test *P* = 0.004, life-table trend test *P* = 0.006, logistic regression trend test *P* = 0.021, Cochran–Armitage trend test **P* = 0.031, life-table test *P* = 0.033, logistic regression test *P* = 0.045, Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.001, life-table test; logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.5–1.6 µm; GSD, 2.3 µm. Mean body weights of 3 mg/m^3 mice (M) were less than those of controls from week 96 until the end of the study. Historical controls: Bronchioloalveolar adenoma: 141/947 (14.9 ± 7.0%), range, 6–36%. Bronchioloalveolar carcinoma: 75/947 (7.9 ± 5.7%), range, 0–16%. Bronchioloalveolar adenoma or carcinoma (combined): 205/947 (21.7 ± 8.0%), range, 10–42%.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 34, 37, 32, 28	*Lung* Bronchioloalveolar adenoma 3/50 (6%), 6/50 (12%), 9/50 (18%), 10/50 (20%)* Bronchioloalveolar carcinoma 1/50 (2%), 1/50 (2%), 4/50 (8%), 9/50 (18%)* Bronchioloalveolar adenoma or carcinoma (combined) 4/50 (8%), 7/50 (14%), 13/50 (26%)*, 18/50 (36%)**	*P* = 0.014, life-table trend test *P* = 0.024, logistic regression trend test *P* = 0.045, Cochran–Armitage trend test **P* = 0.016, life-table test *P* = 0.024, logistic regression test *P* = 0.036, Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test) **P* < 0.01, life-table test; logistic regression test; Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* = 0.016, life-table test, logistic regression test, Fisher exact test ***P* < 0.001, logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, multiple-dose study, adequate number of mice, adequate duration of exposure and observation. Other comments: MMAD, 1.5–1.6 µm; GSD, 2.3 µm. For all exposed groups body weights were significantly greater than those of controls. Historical controls: Bronchioloalveolar adenoma: 61/939 (6.5 ± 3.2%), range, 0–14%. Bronchioloalveolar carcinoma: 38/939 (4.1 ± 3.2%), range, 0–12%. Bronchioloalveolar adenoma or carcinoma (combined): 97/939 (10.3 ± 3.7%), range, 0–16%.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h+ T90 (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 17, 15, 21, 15	*Lung* Bronchioloalveolar adenoma 1/50 (2%), 4/50 (8%), 1/48 (2%), 6/50 (12%)	$P = 0.042$, life-table trend test	Principal strengths: GLP study, study covered most of lifespan, male and female rats used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.4–1.6 µm; GSD, 2.1–2.2 µm. Historical controls: Bronchioloalveolar adenoma: 17/654 (2.6 ± 3.6%), range, 0–10%. Bronchioloalveolar carcinoma: 6/654 (0.9% ± 1.0%), range 0–2%. Bronchioloalveolar adenoma or carcinoma (combined): 23/654 (3.5 ± 3.7%), range, 0–10%. Adrenal medulla, benign pheochromocytoma: 163/623 (26.2 ± 13.2%), range 0–50%. Adrenal medulla, benign, complex, or malignant pheochromocytoma (combined): 176/623 (28.3 ± 12.0%), range, 8–50%.
		Bronchioloalveolar carcinoma 0/50, 0/50, 3/48 (6%), 1/50 (2%)	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 1/50 (2%), 4/50 (8%), 4/48 (8%), 7/50 (14%)*	$P = 0.027$, life-table trend test $P = 0.032$, logistic regression trend test $P = 0.038$, Cochran–Armitage trend test *$P = 0.030$, life-table test $P = 0.029$, logistic regression test $P = 0.030$, Fisher exact test	
		Adrenal medulla Benign pheochromocytoma 14/50 (28%), 19/50 (38%), 23/49 (47%)*, 20/50 (40%)	NS, life-table trend test; logistic regression trend test; Cochran–Armitage trend test *$P = 0.041$, Fisher exact test	
		Benign, complex, or malignant pheochromocytoma (combined) 15/50 (30%), 19/50 (38%), 25/49 (51%), 20/50 (40%)	NS, life-table trend test; logistic regression trend test; Cochran–Armitage trend test *$P < 0.05$, logistic regression test; 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 28, 25, 26, 30	*Lung* Bronchioloalveolar adenoma 0/50, 1/49 (2%), 10/50 (20%)**, 9/50 (18%)*	*P* = 0.003, life-table trend test *P* = 0.001, logistic regression trend test *P* = 0.002, Cochran–Armitage trend test) **P* < 0.003, life-table test; logistic regression test *P* = 0.001, Fisher exact test ***P* < 0.001, life-table test; logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan; male and female rats used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.4–1.6 µm; GSD, 2.1–2.2 µm. Historical controls: Bronchioloalveolar adenoma: 7/650 (1.1 ± 1.6%), range, 0–4%. Bronchioloalveolar carcinoma: 0/650. Bronchioloalveolar adenoma or carcinoma (combined): 7/650 (1.1 ± 1.6%), range, 0–4%. Adrenal medulla, benign pheochromocytoma: 35/608 (5.8 ± 4.9%), range, 0–14%. Adrenal medulla, benign, complex, or malignant pheochromocytoma (combined): 39/608 (6.4 ± 4.4%), range, 2–14%.
		Bronchioloalveolar carcinoma 0/50, 2/49 (4%), 6/50 (12%)*, 6/50 (12%)*	*P* < 0.05, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.05, life-table test; logistic regression test; Fisher exact test	
		Bronchioloalveolar adenoma or carcinoma (combined) 0/50, 3/49 (6%), 15/50 (30%)*, 15/50 (30%)*	*P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.001, life-table test; logistic regression test; Fisher exact test	
		Adrenal medulla Benign pheochromocytoma 2/48 (4%), 1/49 (2%), 3/50 (6%), 8/48 (17%)*	*P* < 0.01, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.05, logistic regression test; 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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1998) (cont.)		Benign, complex, or malignant pheochromocytoma (combined) 2/48 (4%), 1/49 (2%), 4/50 (8%), 10/48 (21%)*	$P < 0.001$, logistic regression trend test; Cochran–Armitage trend test $P = 0.001$, life-table trend test *$P < 0.02$, life-table test; logistic regression test; Fisher exact test	
Full carcinogenicity Rat, Wistar albino (M) About 4 wk 12 mo Shabaan et al. (1977)	Subcutaneous injection Cobalt(II) chloride ($CoCl_2$); purity, NR Physiological saline 0, 4 mg/100 g bw 1× in 2 courses of 5 days separated by a 9 day interval 20, 20 19, 11	*Subcutaneous tissue* Fibrosarcoma 0/20, 8/20*	*[$P = 0.0016$, Fisher exact test]	Principal strengths: adequate duration and schedule of exposure. Principal limitations: statistics not provided, only one sex, small number of rats per group, the control group was also used in the experiment below, use of single dose, necropsy was limited to macroscopic lesions only. Other comments: none of the 9 treated rats who died during the study (almost 50%) were examined. Most tumours induced have been described as developing at the injection site; however, this study also showed occurrence of tumours remote from the site of injection.
Full carcinogenicity Rat, Wistar albino (M) About 4 wk 8 mo Shabaan et al. (1977)	Subcutaneous injection Cobalt(II) chloride ($CoCl_2$); purity, NR Physiological saline 0, 4 mg/100 g bw 1× in 2 courses of 5 days separated by a 9 day interval 20, 20 20, 16	*Subcutaneous tissue* Fibrosarcoma 0/20, 6/20*	*[$P = 0.010$, Fisher exact test]	Principal strengths: adequate duration and schedule of exposure. Principal limitations: statistics not provided, only one sex, small number of rats per group, the control group was also used in the experiment above, use of single dose, necropsy was limited to macroscopic lesions only. Other comments: the 4 rats that died throughout the study were not examined. Most tumours induced have been described as developing at the injection site; however, this study also showed occurrence of tumours remote from the site of injection.

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	Tumour incidence	Significance	Comments
Co-carcinogenicity Rat, Wistar (M + F combined) 3 mo Lifetime Zeller (1975)	Subcutaneous injection Cobalt(II) chloride hexahydrate ($CoCl_2·6H_2O$), NR NR 5 (DEN), 5 (DEN) + 0.5 ($CoCl_2·6H_2O$) mg/kg bw 1×/wk for 43 wk 24, 24 NR	*All sites* Total tumours 19/24, 18/24	[NS]	Principal strengths: male and female rats used. Principal limitations: only one dose, no untreated control group was available, data combined for both sexes, small number of rats per group, limited reporting of the study, no statistics reported. Other comments: DEN (5 mg/kg bw) injected subcutaneously 1×/wk for 43 wk, no tumours were observed in another control group of 12 rats [sex distribution unspecified] treated with $CoCl_2·6H_2O$ only.
Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide				
Full carcinogenicity Mouse, Swiss (F) 2–3 mo 751 days Gilman & Ruckerbauer (1962)	Intramuscular injection Cobalt(II) oxide (CoO), NR 10% suspension in aqueous penicillin G procaine (60 000 IU) 0, 10 mg/site once 51, 50 NR, 12	*Injection site* Tumour 0/48, 0/46 *Lung* Adenoma 0/48, 2/46 (4%)	NA NS	Principal strengths: the duration of observation was adequate, adequate number of mice. Principal limitations: one sex and only one dose were used, neither microphotographs nor histopathology description provided. Other comments: particle size, < 5 µm.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0 (untreated), 0 (vehicle), 2, 10 mg/kg bw 1×/2 wk for 18 treatments and then 1×/4 wk from the 19th to the 39th treatment, over 2 yr 50, 50, 50, 50 NR	*Lung* Total tumours 0/50, 0/50, 1/50, 5/50* Bronchioloalveolar adenoma 0/50, 0/50, 0/50, 2/50 Bronchioloalveolar adenocarcinoma 0/50, 0/50, 0/50, 1/50 Adenocarcinoma 0/50, 0/50, 0/50, 2/50 Benign squamous epithelial tumour 0/50, 0/50, 1/50, 0/50	[P = 0.047, Cochran–Armitage trend test] *[P = 0.0281, Fisher exact test] [NS] [NS] [NS] [NS]	Principal strengths: multiple-dose study, male and female rats used, adequate number of rats used, randomly allocated in groups, adequate duration of exposure and observation. Principal limitations: no statistical analysis was performed, the particle size was potentially not appropriate for this route of exposure. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range. Total tumours were bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, adenocarcinoma, and benign squamous epithelial tumours.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0 (untreated), 0 (vehicle), 2, 10 mg/kg bw 1×/2 wk for 18 treatments and then 1×/4 wk from the 19th to the 39th treatment, over 2 yr 50, 50, 50, 50 NR	*Lung* Bronchioloalveolar adenoma 0/50, 0/50, 1/50, 0/50 Bronchioloalveolar carcinoma 0/50, 0/50, 0/50, 1/50	[NS] [NS]	Principal strengths: multiple-dose study, male and female rats used, adequate number of rats used, randomly allocated in groups, adequate duration of exposure and observation. Principal limitations: no statistical analysis was performed, the particle size was potentially not appropriate for this route of exposure. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Subcutaneous injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 2 mg/kg bw 5×/wk for 2 yr 10, 10 NR, NR	*Injection site* Malignant histiocytoma or sarcoma (NOS) (combined) 0/10, 5/10*	 *[P = 0.0163, Fisher exact test]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, survival and body weight data not reported, only one dose. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Subcutaneous injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 10 mg/kg bw 1×/wk for 2 yr 10, 10 NR, NR	*Injection site* Malignant histiocytoma or sarcoma (NOS) (combined) 0/10, 4/10	 [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, survival and body weight data not reported, only one dose. Other comments: CoO, 76.7% Co; ~80% of particles are in the 5–40 µm range.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo Up to 519 days Gilman & Ruckerbauer (1962)	Intramuscular injection Cobalt(II) oxide (CoO) powder, spectroscopically pure Aqueous penicillin G procaine (90 000 IU) 0, 30 mg/site (thigh) once 10, 10 NR, 260 days (average survival)	*Injection site* Rhabdomyosarcoma 0/10, 5/10*	*[P = 0.0163, Fisher exact test]	Principal strengths: rats randomly allocated in groups, the duration of observation was adequate. Principal limitations: statistics not provided, data combined for both sexes, small number of rats per group, use of single dose, limited reporting of study details. Other comments: particle size, ≤ 5 µm.
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo ≤ 342 days Gilman (1962)	Intramuscular injection Cobalt(II) oxide (CoO), spectroscopically pure Aqueous penicillin G procaine 20 mg/site (thigh) once 32 5	*Injection site* Sarcoma, mostly rhabdomyofibrosarcoma 12/24	NA	Principal strengths: adequate number of rats used, randomly allocated in groups, the duration of observation was adequate. Principal limitations: lack of control group, use of single dose, data combined for both sexes, histopathological confirmation was not consistently performed. Other comments: particle size, ≤ 5 µm. Among the effective number (24) of rats (those surviving ≥ 90 days), 5 were treated in both thighs and 19 in one thigh.
Full carcinogenicity Rat, Sprague-Dawley (M + F combined) 10 wk Lifetime Steinhoff & Mohr (1991)	Intraperitoneal injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 200 mg/kg bw 3× at intervals of 2 mo 20, 20 NR, NR	*Injection site* Total tumours 1/20, 14/20* Malignant histiocytoma 1/20, 10/20* Sarcoma (NOS) 0/20, 3/20 Malignant mesothelioma 0/20, 1/20	*[P < 0.0001, Fisher exact test] *[P = 0.0017, Fisher exact test] [NS] [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, data for both sexes combined, small number of rats per group, survival and body weight data not reported. Other comments: the control and treated groups of 20 rats included 10 male and 10 female. CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.

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	Tumour incidence	Significance	Comments
Co-carcinogenicity Rat, Sprague-Dawley (F) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 20 (benzo[*a*]pyrene), 20 (benzo[*a*]pyrene) + CoO 10 or 20 mg/kg bw CoO: 10 mg/kg bw, 1×/wk for a total of 7 treatments; then 20 mg/kg bw every 14 days for a total of 20 treatments (total dose, 470 mg/kg bw). Benzo[*a*]pyrene: 20 mg/kg bw, 1×/wk; starting from the 8th treatment dose given every 14 days for a total of 10 treatments (total dose, 200 mg/kg bw) 20, 20 NR	*Lung* Carcinomas (all) 1/20, 9/20* Squamous cell carcinoma 1/20, 8/20* Adenocarcinoma 0/20, 1/20	 *[$P = 0.0042$, Fisher exact test] *[$P = 0.0098$, Fisher exact test] [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: small number of rats, only one dose and sex, survival and body weight data not reported, no statistics reported, the particle size was potentially not appropriate for this route of exposure. Other comments: the alternating period between treatment with CoO and benzo[*a*]-pyrene was 4 days, the control group was treated with benzo[*a*]pyrene alone in physiological saline. CoO, 76.7% Co; ~80% of particles in the 5–40 µm range.
Full carcinogenicity Hamster, Syrian golden (M) 2 mo Lifetime Wehner et al. (1977)	Inhalation (whole-body exposure) Cobalt(II) oxide (CoO), reported as "purified" Clean air 0, 10 mg/m^3 7 h/day, 5 days/wk for life 51, 51 NR	*All sites combined* Total tumours (malignant) 1/51, 2/51 Reticulum cell sarcoma 0/51, 1/51 Carcinoma 0/51, 1/51	 [NS] [NS] [NS]	Principal limitations: only one sex, poor survival, limited reporting of the study, only one dose, no statistics reported. Other comments: median survival, 16.6 mo in cobalt-exposed hamsters vs 15.3 mo in controls.

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	Tumour incidence	Significance	Comments
Co-carcinogenicity Hamster, Syrian golden(M) 2 mo Lifetime Wehner et al. (1977)	Inhalation (whole-body exposure) Cobalt(II) oxide (CoO), reported as "purified" Clean air Cigarette smoke + 0 (CoO), cigarette smoke + 10 (CoO) mg/m^3 7 h/day, 5 days/wk for life 51, 51 NR	*All sites combined* Total tumours (benign) 5/51, 8/51 Polyps 0/51, 1/51 Squamous cell papilloma 0/51, 1/51 *Adrenal cortex*: adenoma 4/51, 5/51 *Vascular system*: haemangioma 0/51, 1/51	 [NS] [NS] [NS] [NS] [NS]	Principal limitations: only one sex, limited reporting of the study, only one dose, no statistics reported. Other comments: cigarette smoke exposure (3×/day for 10 min, nose-only) in modified Hamburg II smoking machine; twice before and once after the 7 h CoO exposure or sham dust exposure. Median survival, 21.6 mo in CoO + cigarette smoke-exposed hamsters vs 19.3 mo in cigarette smoke only-exposed controls.
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo ≤ 365 days Gilman (1962)	Intramuscular injection Cobalt(II) sulfide (CoS), spectroscopically pure Aqueous penicillin G procaine 20 mg/site (thigh) once 30 1	*Injection site* Sarcoma, mostly rhabdomyofibrosarcoma 28/29	 NA	Principal strengths: adequate number of rats used, randomly allocated in groups, the duration of observation was adequate. Principal limitations: lack of control group, data combined for sexes, use of single dose, histopathological confirmation was not consistently performed. Other comments: particle size, ≤ 5 µm. Among the effective number of rats (rats surviving ≥ 90 days), nearly all (27) were treated in both thighs and 2 in one thigh.
Full carcinogenicity Rat, Sprague-Dawley (F) NR [body weight, 120–140 g] 12 mo Jasmin & Riopelle (1976)	Intrarenal injection Cobalt(II) sulfide (CoS), NR (reagent grade) Glycerin 0, 5 mg Single injection into each pole of the right kidney 16, 20 NR	*Kidney* Tumours 0/16, 0/20	 NA	Principal limitations: only one dose and sex, number of rats at start unclear, short duration, step section method not used for histopathological examination, small number of rats per group. Other comments: glycerin, 0.5 mL.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Hamster, NR (M + F combined) 9 wk ≤ 110 wk Farrell & Davis (1974)	Intratracheal instillation Cobalt(II,III) oxide (Co_3O_4), NR Gelatin in saline 0, 4 mg 1×/wk [assumed] for 30 wk 50, 50 (25 M and 25 F/group) 43, 43	*Respiratory tract* Total tumours 5/44, 2/48 *Alveoli* Tumours 0/44, 2/48	 [NS] [NS]	Principal limitations: data combined for both sexes, histopathological examination was limited to the respiratory tract, only one dose, no statistical analysis reported. Other comments: particle size, 0.5–1.0 µm. The week after the last injection, the intratracheal instillations were started (1×/wk [assumed] for 30 wk); Co_3O_4 was suspended in 0.2 mL of 0.5% gelatin in saline; controls were instilled with 0.2 mL of 0.5% gelatin in saline only; the hamsters were observed for an additional 43–68 wk.
Initiation promotion (tested as promoter) Hamster, NR (M + F combined) 9 wk ≤ 110 wk Farrell & Davis (1974)	Intratracheal instillation Cobalt(II,III) oxide (Co_3O_4), NR Gelatin in saline DEN + vehicle, DEN + 4 mg Co_3O_4 1×/wk [assumed] for 30 wk 50, 50 (25 M and 25 F/group) 33, 39	*Respiratory tract* Total tumours 30/48, 37/48 *Nasal cavity* Tumours 1/48, 2/48 Carcinoma 0/48, 1/48 Papilloma 7/48, 12/48 *Trachea:* papilloma 27/48, 33/48 *Bronchi*: tumours 0/48, 1/48 *Alveoli*: tumours 7/48, 5/48	 [NS] [NS] [NS] [NS] [NS] [NS]	Principal limitations: data combined for both sexes, histopathological examination was limited to the respiratory tract, only one dose, no statistical analysis reported. Other comments: particle size, 0.5–1.0 µm. DEN treatment (0.5 mg/0.25 mL saline) by subcutaneous injection 1×/wk for 12 wk; the week after the last injection, the intratracheal instillations were started (1×/wk [assumed] for 30 wk); Co_3O_4 was suspended in 0.2 mL of 0.5% gelatin in saline. Controls were instilled with 0.2 mL of 0.5% gelatin in saline only. The hamsters were observed for an additional 43–68 wk.

bw, body weight(s); Co, cobalt; DEN, diethylnitrosamine; F, female; GLP, Good Laboratory Practice; GSD, geometric standard deviation; IU, International Unit; M, male; min, minute; MMAD, mass median aerodynamic diameter; mo, month; NA, not applicable; No., number; NOS, not otherwise specified; NR, not reported; NS, not significant; T_{90}, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation; Ta, tantalum; vs, versus; W, tungsten; wk, week; yr, year; Zn, zinc.

increased (P = 0.016, poly-3 test) [P = 0.0448, Fisher exact test] in the group at the intermediate concentration, exceeding the upper bound of the range observed in historical controls from this laboratory (inhalation studies, 39/300; 13.0 ± 4.2%; range, 8–20%; and all routes: 145/950; 15.3 ± 6.2%; range, 2–26%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test]. The incidence of bronchioloalveolar carcinoma (includes multiples) was significantly increased (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] in all exposed groups (control, 11/50; lowest concentration, 38/49; intermediate concentration, 42/50; highest concentration, 46/50). In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [P < 0.001, Cochran–Armitage test], with the incidence (control, 3/50; lowest concentration, 18/49; intermediate concentration, 24/50; highest concentration, 36/50) being significantly increased ($P \leq 0.01$, poly-3 test) [$P \leq 0.002$, Fisher exact test] in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the increase in incidence (control, 16/50; lowest concentration, 41/49; intermediate concentration, 43/50; highest concentration, 47/50) being significant (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] at all concentrations.

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.037, poly-3 test). The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 3/49 (6%), 9/50 (18%), 8/50 (16%), and 10/50 (20%), respectively, and significantly increased (P = 0.024, poly-3 test) [P = 0.0387, Fisher exact test] in the group at the highest concentration. The incidence of bronchioloalveolar adenoma (includes multiples) in all exposed groups exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 16/299; 5.4 ± 3.7%; range, 2–12%; all routes: 54/949; 5.7 ± 3.6%; range, 0–12%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test] with the increase in incidence (control, 5/49; lowest concentration, 25/50; intermediate concentration, 38/50; highest concentration, 43/50) being significant (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] at all concentrations. In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [P < 0.001, Cochran–Armitage test], with the incidence (control, 1/49; lowest concentration, 7/50; intermediate concentration, 20/50; highest concentration, 24/50) being significantly increased in all exposed groups (lowest concentration, $P \leq 0.05$, poly-3 test [P = 0.0317, Fisher exact test]; intermediate and highest concentration, $P \leq 0.01$, poly-3 test [P < 0.001, Fisher exact test]). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test] with the increase in the incidence (control, 8/49; lowest concentration, 30/50; intermediate concentration, 41/50; highest concentration, 45/50) being significant at all concentrations (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test].

Regarding non-neoplastic lesions in treated males and females, bronchioloalveolar epithelial hyperplasia and cytoplasmic vacuolization of the bronchioloalveolar epithelium occurred in the epithelium of the peri-acinar region of the lung, which encompassed the terminal bronchioles, associated alveolar ducts, and immediately adjacent alveoli (NTP, 2014). Moreover, instances of alveolar epithelial hyperplasia, alveolar proteinosis, alveolar histiocytic infiltration, bronchiolar epithelial erosion, and suppurative inflammation occurred. In general, the severity

of these non-neoplastic lesions increased with increasing exposure concentration. In the nasal cavity, larynx, and trachea, non-neoplastic changes also occurred in both treated males and females. [The Working Group noted that this was a well-conducted study that complied with GLP, both sexes were used, the duration of exposure and observation was adequate, there was a sufficient number of animals per group, and multiple concentrations based on a 3-month study were tested.]

3.1.2 Rat

(a) Inhalation

In a well-conducted study that complied with GLP, groups of 50 male and 50 female Fischer 344/NTac rats (age, 5–6 weeks) were exposed by inhalation (whole-body) to cobalt metal-particle aerosol concentrations of 0, 1.25, 2.5, or 5 mg/m^3 (concentrations were based on the findings from a 13-week study) for untreated controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, 98.2% ± 0.6%; MMAD, 1.4–2.0 μm; GSD, 1.6–1.9 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks (NTP, 2014). Surviving rats were killed at age 109–110 weeks. At study termination, survival of male rats was 17/50, 20/50, 16/50, and 16/50, and for female rats was 35/50, 26/50, 24/50, and 25/50 (including one rat that died during the last week of the study), for the controls and groups at the lowest, intermediate, and highest concentration, respectively. Survival of females at the intermediate concentration was significantly less than that of controls. The mean body weights of rats in the groups at the intermediate and highest concentration were at least 10% less than those of controls after weeks 99 and 12, respectively, for male rats, and weeks 57 and 21, respectively, for female rats. Abnormal breathing and thinness were noted in exposed male and female rats. Tissue burden studies were conducted only in females; lung cobalt concentrations and burdens increased with increasing exposure concentrations and were significantly greater than in controls. The values of maximum cobalt lung burdens observed in the 2-year studies indicated that lung overload was not reached in these studies. All rats underwent complete necropsy with histopathological evaluation.

In male rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.011, poly-3 test) [P = 0.015, Cochran–Armitage test]. The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 2/50, 10/50, 10/50, and 14/50, respectively, and significantly increased (lowest and intermediate concentration, $P \leq 0.018$, poly-3 test [P = 0.0139, Fisher exact test]; highest concentration, $P < 0.001$, poly-3 test [P = 0.0009, Fisher exact test]) in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 16/50; intermediate concentration, 34/50; highest concentration, 36/50) being significantly increased ($P < 0.001$, poly-3 test) [$P < 0.001$, Fisher exact test] in all exposed groups. In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [$P < 0.001$, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 6/50; intermediate concentration, 14/50; highest concentration, 30/50) being significantly increased (lowest concentration, $P \leq 0.05$, poly-3 test [P = 0.0133, Fisher exact test]; intermediate and highest concentration, $P \leq 0.01$, poly-3 test, [$P < 0.0001$, Fisher exact test]) in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the increase in incidence (control, 2/50; lowest concentration, 25/50; intermediate concentra-

tion, 39/50; highest concentration, 44/50) being significant ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test] at all concentrations. Cystic keratinizing epithelioma was observed only in the groups at the lowest (1/50, 2%) and highest (1/50, 2%) concentration. Incidence of cystic keratinizing epithelioma exceeded the incidence observed in historical controls from this laboratory (all routes including current study: 0/100). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 15/50 (30%), 23/50 (46%), 37/50 (74%), and 34/50 (68%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P < 0.001$, Fisher exact test] at the intermediate and highest concentration. The incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) in all treated groups exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 25/100; 25 ± 7.1%; range, 20–30%). In addition, there was a significant positive trend in the incidence of bilateral benign pheochromocytoma of the adrenal medulla [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 4/50 (8%), 13/50 (26%), 22/50 (44%), and 21/50 (42%), respectively, and significantly increased (lowest concentration, $P \leq 0.05$, poly-3 test [$P < 0.01$, Fisher exact test]; intermediate and highest concentration; $P \leq 0.01$, poly-3 test, [$P < 0.0001$, Fisher exact test]) in all exposed groups, and exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 25/100; 25.0% ± 7.1%; range, 20–30%) at the intermediate and highest concentration. There was a significant positive trend in the incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 2/50 (4%), 9/50 (18%), and 16/50 (32%), respectively, and significantly increased at the intermediate ($P < 0.001$, poly-3 test) [$P = 0.0256$, Fisher exact test] and highest concentration ($P < 0.001$, poly-3 test) [$P = 0.0002$, Fisher exact test]. [The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002).] The incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) in the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; 2.0% ± 2.8%; range, 0–4%). In addition, the incidence of bilateral malignant pheochromocytoma of the adrenal medulla at the highest concentration, which was 7/50 (14%), was significantly increased ($P \leq 0.01$, poly-3 test) [$P = 0.0062$, Fisher exact test], exceeding the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; 2.0% ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 17/50 (34%), 23/50 (46%), 38/50 (76%), and 41/50 (81%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test] at the intermediate and highest concentration. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in all treated groups exceeded the upper bound of the range observed in historical controls (all routes: 27/100; 27 ± 9.9%; range, 20–34%). There was a significant increase ($P = 0.015$, poly-3 test) [$P = 0.0117$, Fisher exact test] in the incidence of pancreatic islets adenoma (control, 0/50;

lowest concentration, 1/50; intermediate concentration, 6/48; highest concentration, 3/49) in the group at the intermediate concentration, with incidence at all concentrations exceeding the incidence observed in historical controls from this laboratory (all routes: 0/100). There was a significant positive trend in the incidence of pancreatic islets carcinoma (P = 0.021, poly-3 test). Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 1/50 (2%), 5/48 (10%), and 6/49 (12%), respectively, and the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls (all routes: 2/100; 2 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of pancreatic islets adenoma or carcinoma (combined) (P = 0.002, poly-3 test) [P = 0.007, Cochran–Armitage test]. The incidence of pancreatic islets adenoma or carcinoma (combined) in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 2/50 (4%), 10/48 (20%), and 9/49 (18%), respectively, and significantly increased at the intermediate (P = 0.013, poly-3 test) [P = 0.0113, Fisher exact test] and highest concentration (P = 0.022, poly-3 test) [P = 0.0235, Fisher exact test]. At the intermediate and highest concentration, incidence exceeded the upper bound of the range observed in historical controls (all routes: 2/100; 2.0 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of renal tubular adenoma or carcinoma (combined) (P = 0.023, poly-3 test) [P = 0.039, Cochran–Armitage test] for standard evaluations (single-sections) and extended evaluations (step sections) (combined), with the incidence being control, 3/50; lowest concentration, 1/50; intermediate concentration, 1/50; and highest concentration, 7/50. The incidence of renal tubular adenoma or carcinoma (combined) for standard evaluations (single-sections) of controls and groups at the lowest, intermediate, and highest concentration was 0/50, 1/50 (2%), 0/50, and 4/50 (8%), respectively. The incidence of renal tubular adenoma or carcinoma (combined) for standard evaluations observed in historical controls was only 1/100 (range, 0–2%).

In female rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.002, poly-3 test) [P = 0.022, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 2/50 (4%), 7/50 (14%), 9/50 (18%), and 13/50 (26%), respectively, and significantly increased at the intermediate (P = 0.016, poly-3 test) [P = 0.0256, Fisher exact test] and highest concentration (P < 0.001, poly-3 test) [P = 0.0019, Fisher exact test]. The incidence of bronchioloalveolar adenoma (includes multiples) at all concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; included current study, 2.0 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 9/50; intermediate concentration, 17/50; highest concentration, 30/50) being significantly increased in all exposed groups (lowest concentration, P < 0.001, poly-3 test [P = 0.0013, Fisher exact test]; intermediate and highest concentration, P < 0.001, poly-3 test [P < 0.0001, Fisher exact test]). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the increase in the incidence (control, 2/50; lowest concentration, 15/50; intermediate concentration, 20/50; highest concentration, 38/50) being significant (lowest concentration, P < 0.001, poly-3 test [P = 0.0005, Fisher exact test]; intermediate and highest concentration, P < 0.001, poly-3 test [P < 0.0001, Fisher exact test]) at all concentrations. Cystic keratinizing epithelioma was observed in all exposed groups, with incidence of

controls and groups at the lowest, intermediate, and highest concentration being 0/50, 4/50 (8%), 1/50 (2%), and 2/50 (4%), respectively. The incidence of cystic keratinizing epithelioma in all treated groups exceeded the incidence observed in historical controls from this laboratory (all routes, included current study: 0/100). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test] with the incidence of controls and groups at the lowest, intermediate, and highest concentration being 6/50 (12%), 12/50 (24%), 22/50 (44%), and 36/50 (72%), respectively, and significantly increased at the intermediate ($P < 0.001$ poly-3 test [$P = 0.0003$, Fisher exact test] and highest concentration ($P < 0.001$ poly-3 test) [$P < 0.0001$, Fisher exact test]. In addition, the incidence of bilateral benign pheochromocytoma of the adrenal medulla in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 4/50 (8%), 8/50 (16%), and 19/50 (38%), respectively, and significantly increased at the intermediate ($P \leq 0.05$, poly-3 test) [$P = 0.0458$, Fisher exact test] and highest concentration ($P \leq 0.01$ poly-3 test) [$P < 0.0001$, Fisher exact test]. The incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) in all exposed groups, and of bilateral benign pheochromocytoma of the adrenal medulla at the intermediate and highest concentration, exceeded the range observed in historical controls from this laboratory (all routes: 7/100; 7 ± 7.1%; range, 2–12%). There was a significant positive trend in the incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 2/50 (4%), 3/50 (6%), and 11/50 (22%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P = 0.0073$, Fisher exact test] at the highest concentration. [The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only.] The incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) in the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 1/100; 1 ± 1.4%; range, 0–2%). In addition, the incidence of bilateral malignant pheochromocytoma of the adrenal medulla in the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 1/50 (2%), 1/50 (2%), and 4/50 (8%), and significantly increased in the group at the highest concentration ($P \leq 0.05$, poly-3 test). There was a significant positive trend in the incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 6/50 (12%), 13/50 (26%), 23/50 (46%), and 40/50 (80%), respectively, and significantly increased at the intermediate ($P < 0.001$, poly-3 test) [$P = 0.0002$, Fisher exact test] and highest concentration ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test]. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in all exposed groups exceeded the range observed in historical controls from this laboratory (all routes: 8/100; 8 ± 5.7%; range, 4–12%). The incidence of pancreatic islets adenoma or carcinoma (combined) in the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 0/50, 0/50, and 3/50 (6%), respectively, and at the highest concentration exceeded the range observed in historical controls from this laboratory (all routes: 2/100; 2.0 ± 0.0%; range, 0–2%). There was a significant positive trend in the incidence of mononuclear cell leukaemia (all organs) [$P = 0.036$, Cochran–Armitage test]. The incidence of mononuclear cell leukaemia (all

organs) in the controls and groups at the lowest, intermediate, and highest concentration was 16/50 (32%), 29/50 (58%), 28/50 (56%), and 27/50 (54%), respectively, and significantly increased at the lowest ($P = 0.007$, poly-3 test) [$P = 0.0077$, Fisher exact test], intermediate ($P = 0.013$, poly-3 test) [$P = 0.0131$, Fisher exact test], and highest concentration ($P = 0.019$, poly-3 test) [$P = 0.0214$, Fisher exact test], exceeding the range observed in historical controls for all concentrations from this laboratory (all routes: 35/100; 35 ± 4.2%; range, 32–38%).

Regarding non-neoplastic lesions, the incidence of alveolar epithelial hyperplasia, alveolar proteinosis, chronic active inflammation in the lung, and bronchiolar epithelial hyperplasia in all exposed groups of male and female rats was significantly higher than that in the control groups. The incidence of medullary hyperplasia in the adrenal gland was significantly increased in female rats in the groups at the lowest and intermediate concentration; the incidence of this lesion was significantly decreased in male rats in groups at the intermediate and highest concentration. [The Working Group noted that this was a well-conducted study that complied with GLP, males and females were used, the duration of exposure and observation was adequate, there was a sufficient number of animals per group, and multiple concentrations based on a 3-month study were tested.]

(b) Intramuscular injection

Groups of 10 male and 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal microparticles (spectroscopically pure [no further details provided], 400 mesh; ranging from 3.5 × 3.5 μm to 17 × 12 μm, with several long narrow particles of 10 × 4 μm; clumps of 100 × 100 μm were present as well; suspended in 0.4 mL fowl serum) at a dose of 0 (controls, injected with 0.4 mL fowl serum alone at the same site) or 28 mg by single intramuscular injection into the left thigh muscle (Heath, 1956), and were then observed for up to 119 weeks for males and 122 weeks for females. Tumour-bearing rats were killed a few weeks after tumour appearance. [The Working Group determined the average survival time in treated males (71 weeks) and treated females (61 weeks); survival of the controls was not reported.] The first tumours were observed macroscopically at 5 months and the last ones by 12 months. All the tumours occurred at the injection site.

In male rats, there was a significant increase [$P < 0.05$, Fisher exact test] in the incidence of rhabdomyofibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) at the injection site in the treated group (control, 0/10; treated, 4/10).

In female rats, there was a significant increase [$P < 0.02$, Fisher exact test] in the incidence of fibrosarcoma, rhabdomyosarcoma, or rhabdomyofibrosarcoma (combined) at the injection site in the treated group (control, 0/10; treated, 5/10) (Heath, 1956). [The Working Group noted that the duration of observation was adequate, and the animals were randomly allocated in groups. However, the number of rats per group was small, a single dose was tested, the reporting of study details was limited, the vehicle used was serum that was not from the same species, and statistical analysis was not performed. The Working Group suggested that rhabdomyofibrosarcoma could be, in this case, a myofibroblastic reaction in association with rhabdomyosarcoma other than a dual differentiation of a sarcoma after the evaluation of the microphotographs.]

Groups of 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal micro-particles (spectroscopically pure [no further details provided], 400 mesh; ranging from 3.5 × 3.5 μm to 17 × 12 μm, with large numbers of long narrow particles of the order of 10 × 4 μm; suspended in 0.4 mL fowl serum) at a dose of 28 mg by single intramuscular injection into the right thigh muscle (Heath, 1956), and then observed for up to 105 weeks.

Tumour-bearing rats were killed a few weeks after tumour appearance. Five control rats were treated with zinc powder (spherical particles ranging from 1.5 to 44 μm diameter, with most of the particles having diameters between 4 and 20 μm, and no clumps were observed; suspended in 0.4 mL fowl serum) at a dose of 28 mg by intramuscular injection, and another 5 control rats were treated with tungsten powder (rectangular particles, with sizes ranging from 5 × 5 μm diameter to 50 × 50 μm diameter, with most of them having diameters between 8 × 12 μm and 10 × 30 μm, and some clumps up to 170 × 170 μm were observed; suspended in 0.4 mL fowl serum) at a dose of 28 mg by intramuscular injection. [The Working Group determined the average survival time for cobalt-treated rats (43 weeks); the survival of the controls was not reported.]

The first tumours were observed macroscopically at 5 months and the last ones by 12 months. All the tumours occurred at the injection site. There was a significant increase [P = 0.007, Fisher exact test] in the incidence of rhabdomyofibrosarcoma, fibrosarcoma, or sarcoma (NOS) (combined) at the injection site in the treated group (zinc powder controls, 0/5; tungsten powder controls, 0/5; treated, 8/10) (Heath, 1956). [The Working Group noted that the duration of observation was adequate. However, the number of animals per group was small, only one sex and dose was used, there was no untreated control group, the reporting of study details was limited, the vehicle used was serum that was not from the same species, and statistical analysis was not performed. The Working Group suggested that rhabdomyofibrosarcoma could be, in this case, a myofibroblastic reaction in association with rhabdomyosarcoma other than a dual differentiation of a sarcoma.]

A group of 30 male Hooded rats (age, 2–3 months) were treated with cobalt metal powder (spectroscopically pure [no further details provided]; suspended in 0.4 mL fowl serum) at a dose of 28 mg by single intramuscular injection into the right thigh muscle. A control group of 15 male rats received a single intramuscular injection of 0.4 mL fowl serum alone (Heath, 1960). A number of rats (not reported) were killed at daily intervals 1–28 days after injection, or at 2-week intervals up to 20 weeks after injection [no further details provided]. [The Working Group noted that the survival of controls and treated rats was not reported but that the number of treated animals was adequate. However, the duration of exposure and observation was short since some rats were killed on a daily basis during the first month of the study, only one sex and dose was used, the number of controls was small, no data on tumour incidence were given for controls, the vehicle used was serum that was not from the same species, the reporting of study details was limited, and statistical analysis was not conducted. Therefore, the Working Group considered the study inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.]

(c) Intramuscular or subcutaneous implantation

In a study using embedded metals (modelling "shrapnel wounds"), eight groups of 8 male Sprague-Dawley rats (age, ≥ 44 days) were treated with a single intramuscular implantation of four cobalt metal pellets (cylinders, 1 mm in diameter and 2 mm in length) (purity, > 99.99%) or tantalum (surgical-sham controls), with two pellets inserted into the gastrocnemius muscle of each limb, and were analysed for changes in the gastrocnemius muscle at 1, 3, 6, or 12 months. (Wen et al., 2020). No untreated controls were used.

No tumours or inflammation of the gastrocnemius muscle were observed in any of the tantalum-implanted rats or in rats in the cobalt-implanted group analysed after 1 month (incidence, 0/8). Tumours or inflammation (combined) of the gastrocnemius muscle were observed starting 3 months post-implantation in

the group implanted with cobalt (incidence, 4/8) [$P = 0.0385$, Fisher exact test], and the incidence of tumours or inflammation (combined) significantly increased over time: 6/8 in the group implanted with cobalt analysed at 6 months and 7/8 [all lesions were tumours] in the group analysed at 12 months [$P = 0.0035$ and $P = 0.0007$, Fisher exact test, respectively]. Tumours identified in the group implanted with cobalt analysed at 12 months were described as 5 spindle cell tumours [NOS] and 2 rhabdomyosarcomas; tumour types found at earlier time points were not identified (Wen et al., 2020). [The Working Group noted that the duration of exposure and observation was adequate. However, only one sex was used, there was a small number of animals per group and no untreated controls, and statistical analysis was not provided.]

One group of 10 male Sprague-Dawley rats [age at start, not specified] were treated with a single subcutaneous implantation of cobalt metal as bulk material (diameter, 6.5 mm; height, 1 mm) on the right side of the vertebral column, and a single intramuscular implantation of nanoparticulate cobalt metal (60–100 mg; average size, 120 nm; range, 50–200 nm; the particles covered an area of approximately 2 cm^2) on the left side in the paravertebral muscle of each rat, and followed for 6 or 8 months (Hansen et al., 2006). A 12-month observation period had been planned; however, one rat of this group died 8 months post-implantation, thus all remaining rats were killed at 8 months. There was no control group. Histological examination of the implantation sites showed a high incidence of malignant mesenchymal tumours (sarcomas, NOS) around nanoparticulate material implantation sites (tumour incidence, 5/6 at 8 months). Most tumours were localized intramuscularly (i.e. at the original implantation site). Malignant mesenchymal tumours (sarcomas) were not observed around the bulk material implantation sites (tumour incidence, 0/10). [The Working Group noted that the duration of exposure and observation was considered adequate. However, each rat was exposed to the two test agents concurrently, the number of animals was small, statistical analysis was not provided, only one sex was used, and the study did not include a control group.]

(d) Intrarenal injection

A group of 18 female Sprague-Dawley rats [number of rats at start unclear], weighing 120–140 g [age, not reported], were treated with a single intrarenal injection of 5 mg cobalt metal [powder] (reagent grade) [purity, not reported] suspended in 0.05 mL glycerin into each pole of the right kidney (Jasmin & Riopelle, 1976). In addition, a group of 16 female rats were treated with injections of 0.05 mL glycerin only into each pole of the kidney (controls). After 12 months, all rats were necropsied; no tumours were observed in the kidneys of cobalt-exposed or control rats. [The Working Group noted the use of only one dose and sex, the short duration of exposure and observation, and the small number of animals per group. Moreover, the Working Group noted that the authors did not mention the use of step sections in evaluating small kidney tumours.]

(e) Intrathoracic injection

Two groups of 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal microparticles (spectrographically pure [no further details provided]; particle size: 400 mesh; 3.5 × 3.5 µm to 17 µm × 12 µm, with many long narrow particles of the order of 10 × 4 µm; suspended in serum [species, not reported]) at a dose of 28 mg by single intrathoracic injection through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group), and then observed for up to 28 months (Heath & Daniel, 1962). Within 3 days post-injection, 6/10 rats injected through the diaphragm and 2/10 rats injected through the intercostal space had died. The remaining rats in the group injected through the diaphragm

survived for between 11 and 28 months, and the group injected through the intercostal space survived for between 7.5 and 17.5 months.

Of the 12 rats that survived the injection, four rats (two in each group) developed intrathoracic sarcomas (three of mixed origin, including rhabdomyosarcomatous elements, and one rhabdomyosarcoma arising in the intercostal muscles). Three of these tumours appeared to have originated in the cardiac muscle, which was thoroughly intermingled with tumour cells. These three tumours also had certain histological features in common: all had myosarcomatous histopathology with occasional neoplastic giant cell transformation and haemangiosarcomatous changes (Heath & Daniel, 1962). [The Working Group noted the appropriate duration of the experiment and the malignant features of the tumours. However, the Working Group noted the lack of controls, the small number of animals per group, the low number of rats surviving the treatment, and the use of only one dose and sex. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.]

3.2 Soluble cobalt(II) salts

3.2.1 Cobalt(II) sulfate heptahydrate

(a) Mouse

Inhalation

In a well-conducted study that complied with GLP undertaken by the National Toxicology Program (NTP, 1998) and also reported by Bucher et al. (1999), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were exposed by inhalation (whole-body) to aqueous aerosols at concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt(II) sulfate heptahydrate for controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, approximately 99%; MMAD, 1.5–1.6 μm; GSD, 2.3 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. No effects on survival were observed in exposed males or females compared with controls. At study termination, survival of male mice was 22/50, 31/50, 24/50, and 20/50, and for female mice was 34/50, 37/50, 32/50, and 28/50 for the controls and groups at the lowest, intermediate, and highest concentration, respectively. The mean body weights were significantly higher in all groups of females exposed to cobalt(II) sulfate heptahydrate from week 20 to 105 and lower in males at the highest concentration from week 96 to 105 compared with controls, but changes were within 10% of controls for both males and females. All mice underwent complete necropsy with histopathological evaluation.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.005, life-table test; P = 0.018, logistic regression test; P = 0.029, Cochran–Armitage test). The incidence of bronchioloalveolar adenoma for controls and groups at the lowest, intermediate, and highest concentration was 9/50 (18%), 12/50 (24%), 13/50 (26%), and 18/50 (36%), respectively, and significantly increased at the highest concentration (P = 0.024, life-table test; P = 0.027, logistic regression test; P = 0.035, Fisher exact test). The incidence of bronchioloalveolar adenoma in the group at the highest concentration was at the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 141/947; 14.9 ± 7.0%; range, 6–36%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (P = 0.004, life-table test; P = 0.006, logistic regression test; P = 0.021, Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 4/50 (8%), 5/50 (10%), 7/50 (14%), and 11/50 (22%), respectively, and significantly increased at the highest concentration (P = 0.031, life-table test; P = 0.033, logistic regression test; P = 0.045, Fisher exact test), exceeding the upper bound of the range

observed in historical controls from this laboratory (inhalation studies: 75/947; 7.9 ± 5.7%; range, 0–16%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$, life-table test, logistic regression test, and Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 11/50 (22%), 14/50 (28%), 19/50 (38%), and 28/50 (56%), respectively, and significantly increased at the highest concentration ($P < 0.001$, life-table test, logistic regression test, and Fisher exact test), exceeding the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 205/947; 21.7 ± 8.0%; range, 10–42%).

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma ($P = 0.014$, life-table test; $P = 0.024$, logistic regression test; $P = 0.045$, Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 3/50 (6%), 6/50 (12%), 9/50 (18%), and 10/50 (20%), respectively, and significantly increased ($P = 0.016$, life-table test; $P = 0.024$, logistic regression test; $P = 0.036$, Fisher exact test) at the highest concentration. The incidence in the groups at the intermediate and highest concentrations also exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 61/939; 6.5 ± 3.2%; range, 0–14%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma ($P < 0.001$, life-table test, logistic regression test, and Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 1/50 (2%), 1/50 (2%), 4/50 (8%), and 9/50 (18%), respectively, and significantly increased ($P = 0.007$, life-table test; $P = 0.009$, logistic regression test; $P = 0.008$, Fisher exact test) at the highest concentration, which exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 38/939; 4.1 ± 3.2%; range, 0–12%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$; life-table test, logistic regression test, and Cochran–Armitage test), with the increase in the incidence for controls and groups at the lowest, intermediate, and highest concentration being 4/50 (8%), 7/50 (14%), 13/50 (26%), and 18/50 (36%), respectively, and significantly increased at the highest ($P < 0.001$; life-table test, logistic regression test, and Fisher exact test) and intermediate concentrations ($P = 0.016$; life-table test, logistic regression test, and Fisher exact test). The incidence in the groups at the intermediate and highest concentrations also exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 97/939; 10.3 ± 3.7%; range, 0–16%).

Regarding non-neoplastic lesions, in all exposed groups of males and females, the incidence of cytoplasmic vacuolization of the bronchi was significantly higher than that in the control groups. The incidence of diffuse histiocytic cell infiltration in males and of focal histiocytic cell infiltration in females at the highest concentration was significantly greater than that in the controls. In addition, there was a significant increase in the incidence of non-neoplastic lesions of the nasal cavity and larynx in both treated males and females (NTP, 1998; Bucher et al., 1999). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, males and females were used, there was a sufficient number of animals per group, and multiple concentrations were tested.]

(b) Rat

Inhalation

In a well-conducted study that complied with GLP undertaken by the NTP (1998) and also reported by Bucher et al. (1999), groups of 50 male and 50 female Fischer 344/N rats (age,

6 weeks) were exposed by inhalation (whole-body) to aqueous aerosols of cobalt(II) sulfate heptahydrate at concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3 for controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, approximately 99%; MMAD, 1.4–1.6 μm; GSD, 2.1–2.2 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. No effects on survival or mean body weight were observed in exposed males or females compared with controls. At study termination, survival of male mice was 17/50, 15/50, 21/50, and 15/50, and for female mice was 28/50, 25/49, 26/50, and 30/50 for the controls and groups at the lowest, intermediate, and highest concentration, respectively. All rats underwent complete necropsy with histopathological evaluation.

In male rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.042, life-table test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 4/50 (8%), 1/48 (2%), and 6/50 (12%), respectively. Incidence in the group at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 17/654; 2.6 ± 3.6%; range, 0–10%). The incidence of bronchioloalveolar carcinoma for the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 0/50, 3/48 (6%), and 1/50 (2%), respectively. No statistically significant increase in the incidence of bronchioloalveolar carcinoma was observed; however, the incidence at the intermediate concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 6/654; 0.9 ± 1.0%; range, 0–2%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P = 0.027, life-table test; P = 0.032, logistic regression test; P = 0.038, Cochran–Armitage test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 4/50 (8%), 4/48 (8%), and 7/50 (14%), respectively, and significantly increased (P = 0.030, life-table test; P = 0.029, logistic regression test; P = 0.030, Fisher exact test) at the highest concentration. The incidence of bronchioloalveolar adenoma or carcinoma (combined) at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 23/654; 3.5 ± 3.7%; range, 0–10%). The incidence of benign pheochromocytoma of the adrenal medulla for the controls and groups at the lowest, intermediate, and highest concentration was 14/50 (28%), 19/50 (38%), 23/49 (47%), and 20/50 (40%), respectively, and significantly increased (P = 0.041, Fisher exact test) at the intermediate concentration. The incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla for the controls and groups at the lowest, intermediate, and highest concentration was 15/50 (30%), 19/50 (38%), 25/49 (51%), and 20/50 (40%), respectively, and significantly increased (P < 0.05, logistic regression test, Fisher exact test) at the intermediate concentration. The incidence for the group at the intermediate concentration was also slightly above the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 176/623; 28.3 ± 12.0%; range, 8–50%). [The Working Group noted that complex pheochromocytoma is an adrenal medullary tumour, characterized histologically by a pheochromocytoma component and a coexisting, variably differentiated neural component composed of ganglioneuroma and/or neuroblastoma, nerve fibres, and Schwann cells (Martinez & Mog, 2001). The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002). The Working Group also noted the absence of a concentration–response relationship, the significant increase only at the intermediate concentration, and the high background of these tumours,

and considered that this increase may or may not have been treatment-related.]

In female rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.003, life-table test; P = 0.001, logistic regression test; P = 0.002, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 1/49 (2%), 10/50 (20%), and 9/50 (18%), respectively, and significantly increased at the intermediate (P < 0.001, life-table test, logistic regression test, Fisher exact test) and highest concentration (P = 0.003, life-table test, logistic regression test; P = 0.001, Fisher exact test). The incidence of bronchioloalveolar adenoma at the intermediate and highest concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 7/650; 1.1 ± 1.6%; range, 0–4%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (P = 0.033, life-table test; P = 0.023, logistic regression test; P = 0.022, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 2/49 (4%), 6/50 (12%), and 6/50 (12%), respectively, and significantly increased in the groups at the intermediate and highest concentrations (P < 0.05, life-table test, logistic regression test, Fisher exact test). The incidence of bronchioloalveolar carcinoma at all concentrations exceeded the incidence observed in historical controls from this laboratory (inhalation studies: 0/650). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001; life-table test, logistic regression test, Cochran–Armitage test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 3/49 (6%), 15/50 (30%), and 15/50 (30%), respectively, with the increase in incidence being significant at the intermediate and highest concentrations (P < 0.001, life-table test, logistic regression test, Fisher exact test). The incidence of bronchioloalveolar adenoma or carcinoma (combined) at all concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 7/650; 1.1 ± 1.6%; range, 0–4%). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (P = 0.006, life-table test; P = 0.004, logistic regression test; P = 0.003, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 2/48 (4%), 1/49 (2%), 3/50 (6%), and 8/48 (17%), respectively, and significantly increased (P < 0.05; logistic regression test, Fisher exact test) at the highest concentration. The incidence of benign pheochromocytoma of the adrenal medulla at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 35/608; 5.8 ± 4.9%; range, 0–14%). There was a significant positive trend (P ≤ 0.001, life-table test, logistic regression test, Cochran–Armitage test) in the incidence of benign, complex, or malignant pheochromocytoma of the adrenal medulla (combined). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 2/48 (4%), 1/49 (2%), 4/50 (8%), and 10/48 (21%), respectively, with the incidence being significantly increased (P < 0.02; life-table test, logistic regression test, Fisher exact test) at the highest concentration. The incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 39/608; 6.4 ± 4.4%; range, 2–14%). [The Working Group noted that complex pheochromocytoma is an adrenal medullary tumour, characterized histologically by a pheochromocytoma component and a coexisting, variably differentiated neural component composed of ganglioneuroma and/or neuroblastoma, nerve fibres, and Schwann cells

(Martinez & Mog, 2001). The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002).]

Regarding non-neoplastic lesions, in all exposed groups of male and female rats, the incidence of alveolar proteinosis, alveolar epithelial metaplasia, granulomatous alveolar changes, and interstitial fibrosis in the lung was significantly higher than in the controls. The incidence of hyperplasia of the adrenal medulla was not significantly increased in exposed males or females (NTP, 1998; also reported by Bucher et al., 1999). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, males and females were used, there was an adequate number of animals per group, and multiple concentrations were tested.]

3.2.2 Cobalt(II) chloride

(a) Rat

(i) Subcutaneous injection

In one study, in a first experiment, two groups of 20 male Wistar albino rats (age, about 4 weeks) were treated with cobalt(II) chloride [purity not given] at a dose of 0 (control) or 4 mg/100 g bw suspended in physiological saline by daily subcutaneous injection into the central abdominal wall, in two courses of 5 days separated by a 9-day interval, and then observed for about 12 months (Shabaan et al., 1977). Survival rates at the end of the 12th month were 19/20 and 11/20 for controls and treated rats, respectively. Surviving rats were killed after 12 months and only tumours that were visible macroscopically were assessed microscopically; however, rats that died during the experiment were not examined.

There was a significant increase [$P = 0.0016$, Fisher exact test] in the incidence (control, 0/20; treated, 8/20) of fibrosarcomas of the subcutaneous tissue. Some fibrosarcomas were pleomorphic in appearance with numerous mitoses, and one had the appearance of a fibromyxosarcoma. Four rats developed fibrosarcomas at a distance from the sites of injection; these tumours were more pleomorphic than those developing in the vicinity of the injection sites. There was no evidence of metastases in any of the rats. [The Working Group noted that the duration and schedule of exposure were considered adequate. However, the number of animals per group was small, the control group was also used for another experiment (see experiment below), only one sex and dose was used, the necropsy was limited to macroscopic lesions only, and statistical analysis was not reported.]

In the same study by Shabaan et al. (1977), in a second and concurrent experiment, two groups of 20 male Wistar albino rats (age, about 4 weeks) were treated with cobalt(II) chloride [purity not given] at doses of 0 (control) [same control group as the first experiment] or 4 mg/100 g bw suspended in physiological saline by daily subcutaneous injection into the central abdominal wall, in 2 courses of 5 days separated by a 9-day interval, and then observed for about 8 months. Survival rates at the end of the 8-month experiment were 20/20 and 16/20 for controls and treated rats, respectively. Surviving treated rats were killed after 8 months and only tumours that were visible macroscopically were assessed microscopically; however, rats that died during the experiment were not examined. There was a significant increase [$P = 0.010$, Fisher exact test] in the incidence (control, 0/20; treated, 6/20) of tumours [reported to be of the same nature as those described in the first experiment, i.e. fibrosarcomas of the subcutaneous tissue]. [The Working Group noted that the duration and the schedule of exposure were considered adequate. However, the number of animals per group was small, the control group was also used for another experiment (see experiment above), only one sex and dose was used, the necropsy was limited to

macroscopic lesions only, and statistical analysis was not reported.]

(ii) Co-carcinogenicity

In a study by Zeller (1975), Wistar rats (age, 3 months) were divided into groups, each containing 12 males and 12 females. Two groups received diethylnitrosamine (DEN) at a dose of 5 mg/kg bw injected subcutaneously under the dorsal skin once per week for 43 weeks. One of these two groups also received cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) [no further details reported] at a dose of 0.5 mg/kg bw. The total dose of DEN was 215 mg/kg bw in all groups, and that of cobalt chloride was 21.5 mg/kg bw. A control group of 12 rats [sex distribution, not reported] was treated with cobalt chloride alone at the same dose by subcutaneous injection. All rats were observed until their natural death [but no surviIal information between groups was reported]. No tumours developed in the group treated with cobalt chloride alone (control). No local tumours were observed in either DEN-treated group. The incidence of tumours of the respiratory tract (nasal cavity) was approximately twice the incidence of tumours of the liver in both DEN-treated groups. There was no significant difference in the incidence of respiratory tract tumours or in the incidence of liver tumours between DEN-treated groups. [The Working Group noted that only one dose was tested, no statistical analysis was performed, data were combined for males and females, the small number of animals per group, no untreated control group was used, and limited reporting.]

Ivankovic et al. (1972) investigated whether the carcinogenicity of ethylnitrosourea (ENU) in rats could be significantly increased by metal ions, including Co^{2+} (from cobalt(II) chloride). In a first experiment, adult BD strain rats [age at start and sex not reported] were treated with ENU at a dose of 25–90 mg/kg bw with copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) or cobalt(II) chloride hexahydrate at doses of 5–10 mg/kg bw [purity, not reported; group size unclear; no further details provided] by single intraperitoneal injection. After a mean induction time of 190 days, local sarcomas were observed in the abdominal cavity of 6/20 rats. In the control groups (exposed to the same dose of metal salt only or of ENU only) [no further details on these controls provided], no such lesions were reported. In a second experiment, rats were treated with ENU at a dose of 10 mg/kg bw per week with cobalt chloride at 1 mg/kg bw or copper sulfate at 1 mg/kg bw [age at start and sex, not reported; purity, not reported; group size unclear] by single subcutaneous injection. [The Working Group noted the small number of animals, the very limited reporting of the study including the unspecified number of control animals, the absence of details on group size and dosing regimen, and the lack of survival and body-weight data. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt chloride in experimental animals.]

Three groups of 70–72 female CBA × C57BL mice were treated with 0.5% 20-methylcholanthrene [a carcinogen] in a volume of 0.02 mL of benzene by application to a shaved area of the skin (in the interscapular region) once per week until the end of the study [presumably for life] (Finogenova, 1973). Mice were treated with cobalt(II) chloride hexahydrate at a dose of 0, 10, or 100 µg (in 0.1 mL saline) for the controls and groups at the lower and higher dose, respectively, by intraperitoneal injection, twice per week for 8 weeks. The first injection was administered on the same day as the 20-methylcholanthrene application, subsequent injections were administered 3 days later. [The Working Group noted that an adequate number of animals per group and multiple doses were used. However, this study used benzene (classified by IARC as *carcinogenic to humans*, Group 1) as a vehicle, had a short duration, used only one sex, and did not report on tumour incidence or multiplicity, but only on tumour latency. The study

was considered inadequate for the evaluation of the carcinogenicity of cobalt chloride in experimental animals.]

3.2.3 Cobalt(II) nitrate

Rabbit

Subcutaneous injection

Two female rabbits [not further specified] (age, at least 9 months) were treated with cobalt(II) nitrate (equivalent to 1 mg of cobalt metal) in 10 mL of saline (9 g/L) or cobalt(II) nitrate (equivalent to 1 mg cobalt metal) in 10 mL of purified deproteinized amniotic fluid by subcutaneous injection [injection site, not reported] once per day for 5 days (Thomas & Thiery, 1953). From the start of the injections, there was a dramatic decrease in body weight. The rabbits were killed after 37 days (cobalt nitrate in saline) and 22 days (cobalt nitrate in amniotic fluid). [The Working Group noted the limited reporting, the very small number of animals, the use of a single dose, and the lack of controls. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt nitrate in experimental animals.]

3.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

3.3.1 Cobalt(II) oxide

(a) Mouse

Intramuscular injection

A group of 50 female Swiss mice (age, 2–3 months) was treated with cobalt(II) oxide [purity not reported] at a dose of 10 mg per site, as a 10% suspension in aqueous penicillin G procaine, by single intramuscular injection into each thigh muscle using a 22-gauge needle (Gilman & Ruckerbauer, 1962; Gilman, 1962). The particles of cobalt oxide used were milled to a size of 5 μm or less by means of a laboratory pebble mill followed by air elutriation. A vehicle control group of 51 female Swiss mice (age, 2–3 months) was treated with aqueous penicillin G procaine (60 000 IU) alone by single intramuscular injection into both thigh muscles. The experiment was terminated 751 days post-injection and after 772 days for vehicle controls. Of the treated mice, 12 survived (12/50) until day-751, and no tumours were observed at the sites of injection in any of the 46 effective mice (those surviving 90 days post-injection) in the cobalt oxide-exposed group, but two pulmonary adenomas were found. [Neither microphotographs nor histopathology descriptions were provided.] No tumours were observed at the injection sites in 48 effective vehicle controls [survival not reported], 772 days post-injection. [The Working Group noted the duration of observation and the number of animals used per group were adequate, but only one sex and dose was used.]

(b) Rat

(i) Intratracheal instillation

Four groups each of 50 male and 50 female Sprague-Dawley rats (age, 10 weeks) were treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) in physiological saline (sodium chloride) at doses of 0, 0, 2, and 10 mg/kg bw for untreated controls, vehicle controls (physiological saline only), and groups at the lower and higher dose, respectively, by intratracheal instillation once every 2 weeks for the first 18 treatments, and then every 4 weeks from the 19th to 39th treatments over 2 years (Steinhoff & Mohr, 1991). The rats were allowed to live until natural death or were killed when moribund. They underwent full autopsy and organs and tissues were histologically examined. Regarding body weight and survival, there were no appreciable differences between rats in the groups treated with cobalt oxide and the controls.

In male rats, there was a significant positive trend in the incidence (untreated, 0/50; vehicle, 0/50; lower dose, 1/50; higher dose, 5/50) of all tumours of the lung combined [P = 0.047, Cochran–Armitage test]. The incidence of all tumours of the lung combined at the higher dose (5/50: bronchioloalveolar adenoma, 2/50; bronchioloalveolar adenocarcinoma, 1/50; adenocarcinoma, 2/50) [P = 0.0281, Fisher exact test] was significantly higher than that in the controls (1/50, benign squamous epithelial tumour at the lower dose). In the treated groups, the incidence of bronchioloalveolar adenoma, of bronchioloalveolar adenocarcinoma, of adenocarcinoma of the lung, or of benign squamous epithelial tumours of the lung was not significantly higher than that in the control groups.

In female rats, the incidence of bronchioloalveolar adenoma and of bronchioloalveolar carcinoma was not significantly higher than that in the controls. [The Working Group noted that an adequate number of rats per group was used, rats were randomly allocated in groups, the duration of exposure and observation was adequate, the schedule of exposure was adequate, multiple doses were tested, and males and females were used. The Working Group also noted that the particle size was potentially not appropriate for this route of exposure, and that no statistical analysis was performed.]

(ii) Subcutaneous injection

In two concurrent studies using groups of 10 male Sprague-Dawley rats (age, 10 weeks), two groups treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) in physiological saline (sodium chloride, 1 mL/kg bw per treatment) at doses of 0 (control) or 2 mg/kg bw by subcutaneous injection, 5 days/week for 2 years; two other groups were given the same type of cobalt(II) oxide in physiological saline at doses of 0 (control) or 10 mg/kg bw by subcutaneous injection, once per week for 2 years (Steinhoff & Mohr, 1991). The rats were allowed to live until natural death or were killed when moribund.

In the first study, the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site was significantly higher [P = 0.0163, Fisher exact test] in the group of rats treated with 2 mg/kg bw compared with controls (5/10 and 0/10, respectively). In the second study, there was no significant difference between the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site between the group of rats treated with 10 mg/kg bw and controls (4/10 and 0/10, respectively) (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate, and the schedule of exposure was adequate. However, the number of animals per group was small, only one sex and dose was used, survival and body-weight data were not included, and no statistical analysis was performed.]

(iii) Intramuscular injection

A group of 10 male or female Wistar rats [sex distribution, not reported] (age, 2–3 months) was treated with cobalt(II) oxide powder (particle size up to 5 μm) [purity, not reported] at a dose of 30 mg as a 10% suspension in an aqueous suspension of penicillin G procaine (90 000 IU) by single intramuscular injection per thigh (Gilman & Ruckerbauer, 1962). A control group of 10 male or female rats [sex distribution, not reported] received single injections of aqueous penicillin G procaine alone. [Survival of both the control and treated rats was not specified.] Control and treated rats were observed for up to 519 and 489 days, respectively.

Cobalt(II) oxide caused a significant [P = 0.0163, Fisher exact test] increase in the incidence of rhabdomyosarcoma (control, 0/10; treated, 5/10) in male and female (combined) Wistar rats compared with controls. All five tumours occurring at the site of cobalt oxide injection appeared to be of muscle cell origin. Metastases involving the lymph nodes and lung

occurred in four of these five cases. No tumours were observed at the sites of injection in any of the controls (Gilman & Ruckerbauer, 1962). [The Working Group noted that the duration of exposure was adequate. However, the number of animals per group was small, data for males and females were combined, a single dose was tested, there was limited reporting of the study, and statistical analysis was not performed.]

A group of 32 male or female Wistar rats (age, 2–3 months) [sex distribution, not reported] were treated with cobalt(II) oxide powder [purity not reported] (particle size up to 5 μm) at a dose of 20 mg per thigh by intramuscular injection, and then observed for up to 342 days (Gilman, 1962). No information was given on control groups. The effective number of rats (surviving at least 90 days after the start of the treatment) was 24 in the treated group; 5 rats in the treated group were injected in both thighs, and 19 in one thigh only, because of toxicity [no further details provided]. Five treated rats survived to the end of the experiment.

Cobalt oxide-induced sarcomas (mostly rhabdomyofibrosarcomas) were observed at the injection site, with an incidence of 12/24 for treated males and females combined. Most tumours in the cobalt oxide-treated group were pleomorphic, highly cellular sarcomas of striated muscle origin, showing frequent metastasis to the lung and lymph nodes. Both regional and distant metastases occurred in 25% of the rats in the treated group. In all cases, the primary tumours, once established, tended to grow rather rapidly and often to a very large size around the site of injection (Gilman, 1962). [The Working Group noted that the duration of exposure was adequate, a sufficient number of animals was used and randomly allocated in groups, and males and females were used. However, not all animals were necropsied, histopathological confirmation was not consistently performed, data for males and females were combined, a single dose was tested, and there was no control group.]

(iv) Intraperitoneal injection

Two groups each of 10 male and 10 female Sprague-Dawley rats (age, 10 weeks) were treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) at doses of 0 (controls, received saline only) or 200 mg/kg bw suspended in physiological saline by intraperitoneal injection, three times at intervals of 2 months, and then followed by lifetime observation (Steinhoff & Mohr, 1991).

The incidence of malignant histiocytoma at the injection sites of treated males and females combined was significantly higher [$P = 0.0017$, Fisher exact test] than that in the controls (control, 1/20; treated, 10/20); however, the incidence of sarcoma was not significantly higher than that in the controls (control, 0/20; treated, 3/20), nor was the incidence of malignant mesothelioma (control, 0/20; treated, 1/20). Total tumour incidence at the injection site (control, 1/20; treated, 14/20) was significantly increased [$P < 0.0001$, Fisher exact test] (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate as was the exposure schedule. However, the number of animals per group was small, survival and body-weight data were not included, data for males and females were combined, and statistical analysis was not performed.]

(v) Co-carcinogenicity

A group of 20 female Sprague-Dawley rats (age, 10 weeks) were treated with alternating treatments of: (a) cobalt(II) oxide microparticles (76.7% cobalt; "chemically pure" [purity, not reported]; ~80% of particles in the 5–40 μm range) in physiological saline at an initial dose of 10 mg/kg bw once per week and starting from the 8th treatment at a dose of 20 mg/kg bw every 14 days (total of 27 treatments, total dose of 470 mg/kg bw); and (b) benzo[*a*]pyrene in physiological saline at a dose of 20 mg/kg bw once per week and starting from the 8th treatment

at the same dose every 14 days (total of 10 treatments, total dose of 200 mg/kg bw) by intratracheal instillation (Steinhoff & Mohr, 1991). The alternating period between treatments with the two compounds was 4 days. A control group of 20 female rats was given similar treatment with benzo[*a*]pyrene and physiological saline. The rats were allowed to live until natural death or were killed when moribund.

The incidence of pulmonary carcinoma in the group treated with cobalt oxide plus benzo[*a*]-pyrene was significantly higher [$P = 0.0042$, Fisher exact test] than the control group treated with benzo[*a*]pyrene only (control, 1/20; treated, 9/20). The incidence of squamous cell carcinoma of the lung (control, 1/20; treated, 8/20) was significantly higher [$P = 0.0098$, Fisher exact test] in the treated group than in the controls. One adenocarcinoma of the lung was observed in the treated group, but none were observed in controls (0/20, 1/20) (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate as was the exposure schedule. The Working Group also noted that only one sex and dose was used, a small number of animals per group, survival and body-weight data were not included, and that the particle size was potentially not appropriate for this route of exposure]

(c) Hamster

Inhalation and co-carcinogenicity

In a study investigating full carcinogenicity, groups of 51 male Syrian golden hamsters (ENG:ELA strain) (age, 2 months) were exposed by inhalation (whole-body) to cobalt(II) oxide respirable aerosol (with a MMAD of 0.45 μm) [purity, unspecified, reported as "purified"] concentrations of 0 (control) or 10 mg/m^3 for 7 hours per day, 5 days per week, for life (Wehner et al., 1977). Median survival of treated hamsters was 16.6 months compared with 15.3 months for controls. No significant difference in the incidence of any tumour type was observed between the treated hamsters and controls.

In a concomitant co-carcinogenicity experiment using a similar study design, two groups of 51 male Syrian golden hamsters (age, 2 months) were exposed by inhalation to cigarette smoke (3 times per day for 10 minutes, nose-only exposure) (controls) or to cigarette smoke plus cobalt oxide aerosol (10 mg/m^3) for life. There was no significant difference in the incidence of any tumours, including lung tumours, between the group of hamsters exposed to cigarette smoke plus cobalt oxide and the controls. [The Working Group noted the limited reporting of the study, the poor survival of the groups of cobalt- exposed hamsters and controls, that only one dose was tested, and the use of one sex only.]

3.3.2 Cobalt(II) sulfide

Rat

(i) Intramuscular injection

A group of 30 male and female Wistar rats [sex distribution, not reported] (age, 2–3 months) was treated with cobalt(II) sulfide [purity, not given] (particle size up to 5 μm) at a dose of 20 mg per thigh by single intramuscular injection, and then observed for 365 days (Gilman, 1962). No information was given on control groups. The effective number of rats (those surviving at least 90 days after the start of the treatment) was 29; nearly all rats ($n = 27$) were treated in both the left and right thigh, but two rats received one dose only. Only one treated rat survived until the end of the experiment.

Sarcomas (mostly rhabdomyofibrosarcomas) were induced at the injection site in treated rats; tumour incidence was 28/29 for treated males and females combined. Several of the tumours examined were highly anaplastic and difficult to categorize; however, the predominant histological type was a striated muscle tumour. Both regional and distant metastases occurred in

55% of rats in the treated group. In all cases, the primary tumours, once established, tended to grow rather rapidly and often to a very large size around the site of injection (Gilman, 1962). [The Working Group noted that the duration of exposure was adequate, a sufficient number of animals was used and randomly allocated in groups, and both males and females were used. However, not all rats were necropsied, histopathological confirmation was not consistently performed, data for males and females were combined, a single dose was tested, and there was no control group.]

(ii) Intrarenal injection

A group of 20 female Sprague-Dawley rats [number of rats at start unclear], weighing 120–140 g [age, not reported] treated with cobalt(II) sulfide (reagent grade) [purity and particle size, not reported] at a dose of 5 mg suspended in 0.05 mL glycerin by single intrarenal injection into each pole of the right kidney. A group of 16 female rats receiving injections of 0.05 mL of glycerin alone into each pole of the kidney served as controls. After 12 months, all rats were necropsied; no tumours were observed in the kidneys of treated or control rats (Jasmin & Riopelle, 1976). [The Working Group noted the use of only one dose and sex, and the small number of animals per group. Moreover, the Working Group noted that the authors did not mention the use of step sections in evaluating small kidney tumours.]

3.3.3 Cobalt(II,III) oxide

Hamster

Intratracheal instillation and initiation–promotion

In an experiment to study the effects of particulates on carcinogenesis in the respiratory tract induced by DEN, two groups of 50 (25 males and 25 females) hamsters [strain, not reported] (age, 7 weeks), were treated with DEN at a dose of 0 (controls) or 0.5 mg in 0.25 mL of saline by subcutaneous injection once per week for 12 weeks (total DEN dose, 6 mg) (Farrell & Davis, 1974). One week after the last injection of DEN, both groups were treated with cobalt(II,III) oxide powder (particle size, 0.5–1.0 μm [purity, not reported]) at a dose of 4 mg suspended in 0.2 mL of 0.5% gelatin in saline by intratracheal instillation, once per week [assumed] for 30 weeks. Two other groups of 50 (25 males and 25 females) hamsters, treated with either DEN or saline by subcutaneous injection, plus gelatin-saline by intratracheal instillation, served as controls. At the end of treatment (at week 42), there were 39, 33, 43, and 43 hamsters [male and female combined, sex distribution not reported] still alive in the groups exposed to DEN plus cobalt oxide, DEN plus gelatin-saline, saline plus cobalt oxide, or saline plus gelatin-saline, respectively. The hamsters were then observed for an additional 43–68 weeks (total study duration, up to 110 weeks) after the last intratracheal instillation.

In the initiation–promotion study, the incidence of respiratory tract tumours at various sites in hamsters treated with DEN plus cobalt oxide was not significantly different from that in hamsters receiving DEN plus gelatin-saline. In the group treated by subcutaneous injections of saline plus intratracheal instillations of cobalt oxide, 2/48 hamsters [sex distribution not reported] developed pulmonary alveolar tumours, although this increase was not statistically significant. No such tumours were observed in the 44 control hamsters in the group given subcutaneous injections of saline plus intratracheal instillations of gelatin-saline (Farrell & Davis, 1974). [The Working Group noted that data were combined for males and females, only one dose was tested, statistical analysis was not performed, and that histopathological examination was limited to the respiratory tract.]

3.4 Other cobalt(II) compounds

No data were available to the Working Group.

3.5 Evidence synthesis for cancer in experimental animals

3.5.1 Cobalt metal

In a well-conducted study on inhalation (whole-body exposure) of cobalt metal microparticles in male and female B6C3F$_1$/N mice that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined) in males and females (NTP, 2014).

In a well-conducted study on inhalation (whole-body exposure) of cobalt metal microparticles in male and female Fischer 344/NTac rats that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), benign pheochromocytoma of the adrenal medulla, malignant pheochromocytoma of the adrenal medulla, and benign or malignant pheochromocytoma (combined) of the adrenal medulla in males and females. In males, there was a significant increase in the incidence, with a significant positive trend, of pancreatic islet adenoma or carcinoma (combined), a significant increase in the incidence of pancreatic islet adenoma, and a significant positive trend in the incidence of pancreatic islet carcinoma and renal tubule adenoma or carcinoma (combined). In females, there was a significant increase in the incidence of mononuclear cell leukaemia (NTP, 2014).

In one study in male and female Hooded rats treated with cobalt metal microparticles by intramuscular injection, there was a significant increase in the incidence of rhabdomyofibrosarcoma or sarcoma (NOS) (combined) at the injection site in males, and of fibrosarcoma and rhabdomyosarcoma or rhabdomyofibrosarcoma (combined) at the injection site in females (Heath, 1956). In another study in female Hooded rats treated with cobalt metal microparticles by intramuscular injection, there was a significant increase in the incidence of rhabdomyofibrosarcoma and fibrosarcoma or sarcoma (NOS) (combined) at the injection site (Heath, 1956).

In one study in male Sprague-Dawley rats treated with cobalt metal microparticles by subcutaneous implantation, there was no occurrence of tumours at the implantation site (Hansen et al., 2006).

In a study in male Sprague-Dawley rats treated with cobalt metal nanoparticles by intramuscular implantation, there was a high incidence of sarcomas (NOS) at the implantation site [but there was a lack of concurrent controls] (Hansen et al., 2006). In a study in male Sprague-Dawley rats treated with cobalt metal pellets by intramuscular implantation, there was a significant increase in the incidence of rhabdomyosarcoma or spindle cell tumours (NOS) (combined) of the limb gastrocnemius muscle (Wen et al., 2020).

In one study in female Sprague-Dawley rats treated with cobalt metal by intrarenal injection (Jasmin & Riopelle, 1976), there was no significant increase in the incidence of tumours.

One study in Hooded rats treated with cobalt metal microparticles by intrathoracic injection (Heath & Daniel, 1962) and one study where cobalt metal microparticles were administered by intramuscular injection (Heath, 1960) were judged to be inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.

3.5.2 Soluble cobalt(II) salts

(a) Cobalt(II) sulfate heptahydrate

In a well-conducted study on inhalation (whole-body exposure) of cobalt(II) sulfate heptahydrate aerosols in male and female B6C3F$_1$ mice that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined) in males and females (NTP, 1998).

In a well-conducted study on inhalation (whole-body exposure) of cobalt(II) sulfate heptahydrate aerosols in male and female Fischer 344/NTac rats that complied with GLP, there was a significant positive trend in the incidence of bronchioloalveolar adenoma, a significant increase in the incidence – with a significant positive trend – of bronchioloalveolar adenoma or carcinoma (combined), and a significant increase in the incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla in males. In females, there was a significant increase in the incidence – with a significant positive trend – of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), benign pheochromocytoma of the adrenal medulla, and benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla (NTP, 1998).

(b) Cobalt(II) chloride

In a study that included two experiments involving male Wistar rats treated with cobalt(II) chloride by subcutaneous injection, there was a significant increase in the incidence of fibrosarcoma of the subcutaneous tissue in both experiments (Shabaan et al., 1977).

In a study of co-carcinogenicity of cobalt(II) chloride (administered by subcutaneous injection) in male and female Wistar rats (Zeller, 1975), there was no significant increase in the incidence of tumours in males and females combined.

Two studies of co-carcinogenicity, in which cobalt(II) chloride was administered to BD strain rats (Ivankovic et al., 1972) or to female CBA × C57BL mice (Finogenova, 1973) by intraperitoneal injection, were judged to be inadequate for the evaluation of the carcinogenicity of cobalt(II) chloride in experimental animals.

(c) Cobalt(II) nitrate

One study in rabbits treated with cobalt(II) nitrate by subcutaneous injection (Thomas & Thiery, 1953) was judged to be inadequate for the evaluation of the carcinogenicity of cobalt(II) nitrate in experimental animals.

3.5.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

(a) Cobalt(II) oxide

In a study in male and female Sprague-Dawley rats treated with cobalt(II) oxide microparticles by intratracheal instillation, there was a significant increase in the incidence of bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, or adenocarcinoma (combined) of the lung in males, with a significant positive trend (Steinhoff & Mohr, 1991).

In two concurrent studies in male Sprague-Dawley rats treated with cobalt(II) oxide microparticles by subcutaneous injection, there was a significant increase in the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site in one study (Steinhoff & Mohr, 1991).

In two studies in male and female Wistar rats treated with cobalt(II) oxide microparticles by intramuscular injection, in one study there was a significant increase in the incidence of rhabdomyosarcoma at the injection site in males and females combined (Gilman & Ruckerbauer, 1962). In the other study there was a high incidence of sarcoma (mostly rhabdomyofibrosarcoma) at the

injection site in males and females combined [but there was a lack of concurrent controls] (Gilman, 1962).

In a study in male and female Sprague-Dawley rats treated with cobalt(II) oxide microparticles by intraperitoneal injection, there was a significant increase in the incidence of malignant histiocytoma, sarcoma (NOS), or malignant mesothelioma (combined), and of malignant histiocytoma (both at the injection site) in males and females combined (Steinhoff & Mohr, 1991).

In a study in Swiss mice treated with cobalt(II) oxide microparticles by intramuscular injection (Gilman, 1962; Gilman & Ruckerbauer, 1962), there was no significant increase in the incidence of tumours.

In a study of co-carcinogenicity in female Sprague-Dawley rats treated with cobalt(II) oxide microparticles with benzo[*a*]pyrene by intratracheal instillation (Steinhoff & Mohr, 1991), there was a significant increase in the incidence of malignant lung tumours (total) and squamous cell carcinoma of the lung compared with benzo[*a*]pyrene treatment only. In a study of full carcinogenicity and another concomitant study of co-carcinogenicity involving cobalt(II) oxide inhalation (whole-body exposure) in male Syrian golden hamsters, there was no significant increase in the incidence of tumours (Wehner et al., 1977).

(b) Cobalt(II) sulfide

In a study in male and female Wistar rats (combined) treated with cobalt(II) sulfide microparticles by intramuscular injection, there was a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) at the injection site in males and females combined [but there was a lack of concurrent controls] (Gilman, 1962).

In a study in female Sprague-Dawley rats treated with cobalt(II) sulfide by intrarenal injection (Jasmin & Riopelle, 1976), there was no significant increase in the incidence of tumours.

(c) Cobalt(II,III) oxide

In studies on intratracheal instillation and initiation–promotion in male and female hamsters treated with cobalt(II,III) oxide, there was no significant increase in the incidence of tumours (Farrell & Davis, 1974).

3.5.4 Other cobalt(II) compounds

No data were available to the Working Group.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

[The Working Group noted that there is a paucity of information available on the pharmacokinetics of the individual forms of cobalt considered in the present monograph.]

(a) Absorption

In a study of four volunteers by Foster et al. (1989), the average fractional depositions of inhaled radiolabelled (^{57}Co) cobalt(II,III) oxide particles with geometric mean diameters of 0.8 and 1.7 μm in the lung were 52% and 78%, respectively. [The Working Group noted that these data were also reported in Bailey et al. (1989).]

Cobalt and tungsten concentrations measured in exhaled breath condensates from workers involved in the production of diamond tools or hard-metal inserts, collected post-shift, were higher than those in condensate samples collected before the beginning of the work shift (Goldoni et al., 2004). Cobalt concentration measured in samples of urine collected from workers is also a surrogate indicator of cobalt lung absorption. Workers exposed to cobalt metal or cobalt oxide have increased urine cobalt concentrations after

conclusion of their work shifts, suggesting that lung absorption and systemic delivery occurred (Apostoli et al., 1994; Fujio et al., 2009; Martin et al., 2010; Princivalle et al., 2017). [The Working Group noted that this finding could also result from non-inhalation occupational exposure.] Other studies suggest that absorption of cobalt from the lungs is dependent on the solubility of the cobalt compound. Soluble forms of cobalt are more readily absorbed than insoluble forms (Christensen & Poulsen, 1994; Lison et al., 1994).

Large interindividual variability for absorption rates in humans has been reported after ingestion. Studies using radiolabelled cobalt(II) chloride indicate that gastrointestinal absorption of cobalt in humans varies from 5% to 45% (Paley & Sussman, 1963; Valberg et al., 1969; Sorbie et al., 1971; Smith et al., 1972; Holstein et al., 2015; Tvermoes et al., 2015). The fraction of ingested cobalt that is absorbed from the gastrointestinal tract depends on an individual's nutritional status, and the dose and form of cobalt to which they are exposed. Overnight fasting and iron deficiency increase cobalt absorption (Valberg et al., 1969; Sorbie et al., 1971; Smith et al., 1972). Serum ferritin levels were inversely correlated with blood cobalt concentrations in some studies (Bárány et al., 2005; Meltzer et al., 2010). Gastrointestinal uptake of soluble cobalt(II) chloride was higher than that seen after ingestion of cobalt oxide, a less soluble form of cobalt (Christensen et al., 1993). Gastrointestinal uptake of cobalt is higher in women than men (Christensen et al., 1993; Tvermoes et al., 2014). Uptake of carrier-loaded inorganic cobalt (cobalt(II) chloride) is lower than for carrier-free cobalt (Holstein et al., 2015).

Dermal absorption of cobalt was assessed experimentally in four participants who exposed one hand to either freshly mixed powder (5–15% cobalt) or waste dry powder from a hard-metal production facility for 90 minutes. Increased urinary cobalt concentrations were seen post-exposure with both cobalt sources (Scansetti et al., 1994). A cohort of five volunteers placed their hands in a used coolant that contained cobalt at 1600 mg/L for 1 hour. Urinary excretion of cobalt averaged 18.1 nmol (range, 6.8–34.3 nmol) during the 24-hour period before exposure and increased to 38.5 nmol (range, 14.2–61.4 nmol) in the 24-hour period after exposure. One participant had no apparent increase in urinary cobalt concentration after exposure (Linnainmaa & Kiilunen, 1997). [The Working Group noted that both studies provided limited information concerning the participants and forms of cobalt used.]

Occupational studies examining dermal absorption have focused on workers in hard-metal production facilities. [The Working Group noted that these studies can be informative regarding the distribution of cobalt after absorption from the skin.] These studies show associations between exposure to cobalt on the skin and concentrations in blood or urine (Scansetti et al., 1994; Klasson et al., 2017; Kettelarij et al., 2018a; Wahlqvist et al., 2020). Exposure to cobalt on the skin was statistically associated with levels of cobalt in the blood but not in the urine (Wahlqvist et al., 2020). Linear regression analysis of blood concentrations in an occupational cohort demonstrated significant positive relationships with cobalt skin concentrations and with cobalt air concentrations (Klasson et al., 2017). Increased cobalt skin concentrations were associated with increased cobalt concentrations in the blood or urine, suggesting that systemic absorption of cobalt occurred (Klasson et al., 2017; Kettelarij et al., 2018a).

(b) Distribution

Cobalt is broadly distributed throughout the human body. Cobalt is distributed to the serum, whole blood, liver, lung, kidney, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, and pancreas (Gerhardsson et al., 1984; Collecchi et al., 1986; Ishihara et al., 1987; Hewitt, 1988; Muramatsu &

Parr, 1988; Takemoto et al., 1991). [The Working Group noted that autopsy specimens collected from workers or individuals with cancer or other disease states have been used to assess the distribution of cobalt in humans.] Experimental studies involving intravenous administration of radiolabelled cobalt(II) chloride to volunteers have shown that the liver accumulates approximately 10–50% of the body burden of this metal (Smith et al., 1972; Jansen et al., 1996).

(c) Metabolism

Metabolism of cobalt consists of the formation of complexes with a variety of protein and non-protein ligands (see Section 4.2.1). Cobalt is not subject to direct metabolism by enzymatic pathways but gets distributed or excreted as the parent compound.

(d) Excretion

Studies in humans given cobalt chloride by intravenous injection have shown that most excreted cobalt is found in urine and faeces (Kent & McCance, 1941; Smith et al., 1972; Curtis et al., 1976). Similar excretion patterns are found after ingestion. For example, Sorbie et al. (1971) reported that 9–23% of an orally administered dose of radiolabelled cobalt(II) chloride was excreted in the urine within 24 hours of administration. Increased urinary cobalt excretion (23%–42%) was seen in individuals with iron deficiency (Sorbie et al., 1971). Aerosol exposure of human volunteers to radiolabelled cobalt oxide revealed that approximately 40% of the initial lung burden of inhaled cobalt was retained for 6 months after exposure. Cumulative elimination of 33% of the initial lung burden was found in the urine and 28% was found in the faeces (Foster et al., 1989).

Half-lives of elimination have also been estimated in several other studies. Finley et al. (2013) studied cobalt pharmacokinetics in volunteers who ingested cobalt at approximately 1000 µg per day (10–19 µg/kg per day) as cobalt(II) chloride for 31 days. Steady-state whole-blood and erythrocyte cobalt concentrations were achieved within 14–24 days. Clearance of cobalt from blood and serum followed a two-phase exponential decay curve, with an initial rapid phase followed by a slower second phase. The fast-phase half-life was 3 days. For the slower phase, the half-life was 16 days for serum and 39 days for whole blood, with 23% of the cobalt found in serum and 39% found in whole blood (Finley et al., 2013). Tvermoes et al. (2014) also reported that cobalt elimination from whole blood and serum also followed a two-phase exponential decay curve in volunteers ingesting 1 mg per day for 90 days. Elimination from erythrocytes correlated with their lifespan of 120 days.

(e) In vitro studies

Several relevant in vitro studies using human tissues were also identified. Absorption of powdered cobalt, suspended in artificial sweat, by human abdominal skin has been assessed in vitro (Filon et al., 2004, 2009).

Several studies have examined the dissolution of cobalt in vitro. Percutaneous permeation through intact skin was approximately 0.01 µg/cm^2 per hour with an average lag time of 1.6 hours, and was increased in abraded skin (Filon et al., 2009). Studies suggest that lung clearance depends on dissolution of cobalt particles in alveolar macrophages, resulting in cobalt delivery from the lung to the systemic circulation, with smaller particles having faster dissolution rates than larger particles (Kreyling et al., 1990).

Several studies have examined dissolution of cobalt in simulated body fluids. [The Working Group noted that these studies used cell-free artificial fluids that mimic those found in humans.] Dissolution of ultrafine cobalt powder in artificial lung fluid appears to be six times as high as that of standard cobalt powder (Kyono et al., 1992). Dissolution rates are also influenced by the chemical form of cobalt. Water-soluble forms of cobalt (cobalt(II) chloride and cobalt(II) sulfate)

have higher dissolution rates in artificial lung fluids than less soluble forms of cobalt, including cobalt(II,II) oxide and cobalt hydroxide oxide. In contrast, increased surface area of particles was not associated with enhanced cobalt release (Verougstraete et al., 2022). Dissolution rates in simulated gastric and intestinal fluids also reflect water solubility, with higher rates occurring with cobalt(II) chloride and cobalt(II) sulfate than cobalt(II,III) oxide or cobalt oxyhydroxide. Cobalt metal powder was very soluble in gastric fluid and poorly soluble in intestinal fluid (Danzeisen et al., 2020).

4.1.2 Experimental systems

(a) Absorption

(i) Oral administration

Gastrointestinal absorption varies depending on animal species and age. [The Working Group focused on monogastric species, including rodents, dogs, and swine.] Naylor & Harrison (1995) reported that gastrointestinal absorption of cobalt(II) citrate in Harwell Mouth Tumour (HMT) rats was about 25% and was lower in Dunkin-Hartley guinea-pigs after single-dose oral administration (10–100 µL: 1–10 kBq ^{57}Co, depending on animal age, from age 1 to 200 days). Younger animals showed higher intestinal absorption and retention than adult animals. Cobalt absorption was 3%–15% greater in young rats and guinea-pigs than in adult animals (Naylor & Harrison, 1995). Oral administration of a low dose of cobalt(II) chloride (≤ 229 µg/kg per day) to juvenile swine for 14 or 21 days did not affect average blood cobalt concentration (Suh et al., 2019).

(ii) Inhalation

The pulmonary absorption of inhaled cobalt metal is rapid in animals, and the predominant fraction of body burden is in the lung. Kyono et al. (1992) reported that in rats exposed to ultrafine cobalt powder aerosols (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles), cobalt concentrations in lung and blood were 6.42 µg/g (wet) and 28.94 µg/L, respectively, 2 hours after exposure to 2.12 ± 0.55 mg/m^3 for 4 days at 5 hours per day, suggesting that cobalt accumulated in the lung after inhalation and was transferred rapidly to the blood (Kyono et al., 1992). Studies have reported that the rate of translocation of cobalt(II,III) oxide from lung to blood varies depending on the size, density, and surface area of materials, but there were no significant species-related differences (Bailey et al., 1989; Kreyling et al., 1991). The initial translocation of cobalt particles of diameter 0.8 µm ranged from 0.4% per day in humans and baboons to 1.6% per day in HMT rats. Translocation of 1.7 µm particles was lower in all species and ranged from 0.2% per day in baboons to 0.6% per day in HMT rats (Bailey et al., 1989).

(iii) Other routes of administration

Lacy et al. (1996) reported that dermal application of 100 µL of 2% cobalt(II) chloride to Syrian hamsters resulted in low concentrations of cobalt in the urine 24 or 48 hours after application, suggesting some systemic distribution [The Working Group noted that limited details were available to determine if oral exposure may have occurred.] In addition, cobalt was retained at the site of dermal application 48 hours after it was applied on the skin (Lacy et al., 1996). A study of cobalt metal embedded in the muscle of Sprague-Dawley rats showed that the implanted metals rapidly solubilized; a considerable concentration of cobalt was found in the urine, and cobalt was also detected in the brain (Hoffman et al., 2021a, b). Intranasal administration of radioactive $^{57}Co^{2+}$ (0.8 ng cobalt(II) chloride, 15 µCi) in Sprague-Dawley rats showed that cobalt was absorbed by the olfactory mucosa and was transported to the olfactory bulb of the brain and retained at high concentrations in this tissue (Persson et al., 2003).

(b) Distribution

(i) Oral administration

In general, oral exposure of cobalt to mice or rats caused increased cobalt concentrations in the liver and kidney; however, increased cobalt concentrations were also found in the spleen, pancreas, heart, blood, bone, brain, testis, and intestine (Nation et al., 1983; Domingo et al., 1984; Bourg et al., 1985; Edel et al., 1994; Kirchgessner et al., 1994; Zheng et al., 2019). For example, after oral administration of cobalt(II) chloride at 500 ppm in drinking-water given to male Sprague-Dawley rats for 3 months, a significant increase of cobalt was found in the liver, kidney, heart, and spleen (Domingo et al., 1984). In male Sprague-Dawley rats given feed supplemented with cobalt(II) sulfate (20 mg and 40 mg/kg bw per day for 8 weeks), increased concentrations of cobalt were found in the three tissues examined: myocardium, followed by the skeletal muscle and blood (Clyne et al., 1990a, b). In male Sprague-Dawley rats whose feed was supplemented with cobalt(II) chloride, leading to exposures of 5 mg/kg bw or 20 mg/kg bw per day for 69 days, atomic absorption spectrophotometric analyses after 69 days revealed a dose-dependent tissue accumulation of cobalt in blood, bone, brain, hair, small intestine, kidney, liver, and testis (Nation et al., 1983).

In another study, groups of three pregnant OFA-Sprague-Dawley rats were treated by gavage with cobalt(II) sulfate at a dose of 25, 50, or 100 mg/kg bw on days 1–20 of gestation, and cobalt concentrations were measured in maternal and fetal blood. Cobalt concentrations increased dose-dependently in the maternal blood, and the compound was found to cross the placenta and appear in the fetal blood. The concentration of cobalt was higher in fetal than in maternal blood. For example, cobalt concentrations were about 0.8 mg/L and 2 mg/L in maternal blood and fetal blood, respectively, 24 hours after the last administration of cobalt(II) sulfate at 100 mg/kg bw (Szakmáry et al., 2001).

(ii) Inhalation

Generally, after inhalation exposure, cobalt accumulates and is retained in the lung, translocates to the blood, and distributes to extrapulmonary tissues (Rhoads & Sanders, 1985; Bailey et al., 1989; Kyono et al., 1992; NTP, 2014). Lung retention varies depending on the species and also on the nature of the particle (size, density, and surface area); it is generally greater for larger particles than for smaller particles (Bailey et al., 1989; Kreyling et al., 1991; NTP, 2014).

In a study by Kyono et al. (1992), rats were exposed to ultrafine cobalt aerosol (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles) at 2.12 mg/m^3, 5 hours per day for 4 days. They found that the cobalt concentration in the lung reached 6.42 µg/g (wet) 2 hours after the last exposure; the control level was 0.006 µg/g (wet). The cobalt concentration in the lung had decreased to 0.09 µg/g (wet) by 28 days post-exposure.

In an inhalation study by Bailey et al. (1989), humans and various species of experimental animals – including baboon, dog, guinea-pig, rat, hamster, and mouse – were exposed to cobalt(II,III) oxide with different particle sizes (0.8 and 1.7 µm). Generally, lung retention at 90 days and at 180 days was less for the smaller (0.8 µm) particles than for the larger (1.7 µm) particles in most species. However, retention varied between species, with humans having higher values than experimental animals. For instance, 6 months after inhalation of 0.8 µm cobalt particles, lung retention ranged from 1% of the initial lung deposit in mice and rats to 45% in humans and ranged from 8% of the initial lung deposit in rats to 56% in humans for the 1.7 µm cobalt particles (Bailey et al., 1989). Kreyling et al. (1991) reported that particle density influenced lung retention. Administration of cobalt(II,III) oxide by inhalation to baboons, dogs, and rats

showed that there was increased lung retention when they were exposed to 0.9 µm solid cobalt particles compared with 0.8 µm porous cobalt particles (Kreyling et al., 1991).

Patrick et al. (1994) measured the retention of radioactive cobalt isotope (^{57}Co) deposited in the lung by intratracheal instillation of cobalt(II) chloride or cobalt(II) nitrate at various doses, ranging from 0.006 to 5.4 µg depending on the species used. Species included baboon, guinea-pig, rat (all three species exposed for 100 days), hamster (exposed for 121 days), and dog (exposed for > 1000 days); dogs were treated with cobalt nitrate. Cobalt was deposited directly in the lung, but no significant accumulation of the compound was observed in other organs. In the same study, it was observed that lung retention and whole-body retention differed significantly between species. After 100 days, the fraction of radioactive cobalt remaining in the lung ranged from 0.13% (hamster) and 0.58% (rat) to 1.2% (estimated for dog) of the amount administered, while the proportion of cobalt remaining in the body ranged from 0.35% (hamster) to 3.2% (dog) of the amount administered.

In the inhalation studies conducted by the NTP (2014) using cobalt metal particulate aerosols, cobalt concentrations and burdens increased with increasing cobalt concentration in the 2-week, 3-month, and 2-year studies. In the 2-week study, concentrations increased in the liver, lung, kidney, bone, heart, serum, blood, and testis. Cobalt was distributed to extrapulmonary tissues, and a large amount of cobalt was accumulated in the liver. Cobalt tissue burdens in the liver were similar to or greater than those in lung (NTP, 2014).

(iii) Intraperitoneal and intravenous injection

Numerous studies using injections of Co^{2+} in rats and mice have demonstrated that cobalt distribution to multiple different tissues depends on the dosage and route of administration. Liver, lung, kidney, heart, bone, pancreas, the gastrointestinal tract, and muscle have been reported to be the target tissues (Stenberg, 1983; Llobet et al., 1988; Roshchin et al., 1989; Szebeni et al., 1989; Edel et al., 1994; Bingham et al., 1997; Horiguchi et al., 2004).

In other studies, Wistar rats treated with cobalt(II) chloride at a dose of 0.75 mg/kg bw by daily intraperitoneal injection for 3 weeks showed an accumulation of cobalt in the liver, kidney, brain, gastrointestinal tract, spleen, heart, and lung, with a predominance in the liver (14.4 µg/g) (Houeto et al., 2018). Llobet et al. (1988) also reported that in Sprague-Dawley rats treated with cobalt(II) chloride at a dose of 0.06 mmol/kg bw per day, 3 days/week for 4 weeks by intraperitoneal injection, cobalt was detected in the kidney, liver, heart, spleen, brain, and blood (Llobet et al., 1988). Szebeni et al. (1989) administered 2 mL of a solution containing 0.09–0.11 µCi ^{57}Co (cobalt(II) chloride) [final cobalt concentration was not specified] intravenously to Sprague-Dawley rats and detected cobalt in the liver, bone, heart, lung, kidney, gastrointestinal tract, and blood 2 hours after exposure. In a study by Edel et al. (1994), 24 hours after male Sprague-Dawley rats were treated with cobalt(II) chloride (10 ng/rat) by single intravenous injection, high concentrations of cobalt were detected in the lung, kidney, liver, and spleen, and about 0.06% of the administered dose was detected in the testis. In another group of rats treated with cobalt(II) chloride (10 ng/rat) by single intraperitoneal injection, high concentrations of cobalt were detected in the pancreas, kidney, small intestine, and liver at day 3 after injection, and cobalt was retained at high concentrations in the kidney, liver, and spleen at day 7 after injection. A third group was assessed 100 days after exposure by single intraperitoneal injection (lowest dose, 5 µg/rat; highest dose, 1 mg/rat); tissue distribution was affected by the dose, with the lowest dose leading to high cobalt concentrations in the spleen, pancreas, and bone, and the higher dose leading

to highest concentrations in the bone samples, indicating that the compound accumulates in this tissue. Bingham et al. (1997) gave ^{57}Co [not further specified] to male HMT rats (0.2 mL of 50 kBq/mL ^{57}Co) by intravenous administration and found that the highest concentrations of the compound were found in the kidney and liver, with transient accumulation in the prostate, but no accumulation in the testis.

(c) Metabolism

Cobalt is not subject to direct metabolism by enzymatic pathways.

(d) Excretion

(i) Oral administration

After oral administration of cobalt, faecal excretion is the major route of elimination (Reuber et al., 1994; Ayala-Fierro et al., 1999). In particular, Ayala-Fierro et al. (1999) found that 36 hours after oral administration of cobalt(II) chloride at a dose of 33.3 mg/kg bw by gavage to male Fischer 344 rats, about 74.5% of the substance was eliminated in the faeces.

(ii) Inhalation

Cobalt compounds are cleared rapidly from the lung. As shown through a two-compartment model of the relevant data, clearance of cobalt from the lung is biphasic, with a first rapid phase and a second slow phase. Elimination of cobalt from blood is also indicated to have a rapid phase and a slow phase. A study by Kreyling et al. (1986) exposed beagle dogs to radioactive particles of cobalt oxides of different sizes (0.3–2.7 µm) by inhalation and found that smaller particles were cleared more rapidly from the lung (Kreyling et al., 1986). In a study by Sisler et al. (2016a), mice were exposed to cobalt(II) oxide nanoparticles (NPs) at 10 or 30 mg/m^3 (72 ± 16 nm) for 6 hours per day for 4 days by inhalation. The lung burden after 1 hour of exposure was found to be 7.7 µg/lung and 18.7 µg/lung for exposure at the lowest and highest dose, respectively; less than 1% of the cobalt(II) oxide NPs remained in the lungs at 56 days post-exposure. Kyono et al. (1992) reported that in rats exposed to an ultrafine cobalt aerosol (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles), the clearance of cobalt was biphasic: in the first phase about 75% of the cobalt was cleared from the lungs within 3 days with a half-life of 53 hours, while in the second phase (occurring within 3–28 days) a slower clearance rate was evident with a half-life of 156 hours (Kyono et al., 1992). When Syrian golden hamsters were exposed to cobalt(II) oxide by inhalation for 60 days, it was found that cobalt was eliminated 6 days after exposure, and only about 27% remained in the carcass, lung, liver, and kidney (of which 23.3% was in the carcass), 24 hours after inhalation of cobalt oxide (Wehner & Craig, 1972).

To provide data on pulmonary retention, clearance, and systemic distribution of cobalt metal, toxicokinetic studies were performed in Fischer 334/N rats and B6C3F$_1$/N mice by the NTP (2014). Animals were exposed to cobalt metal particulate aerosol in an inhalation chamber. The details of the 2-week, 3-month, and 2-year exposure studies are as follows.

In the 2-week exposure studies, rats or mice were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days/week for 16–17 days. Cobalt concentrations were found to increase with increasing exposure levels of cobalt in all tissues examined, including the liver, lung, kidney, bone, heart, serum, blood, and testis. Cobalt was distributed to extrapulmonary tissues, and a large amount of cobalt was accumulated in the liver. Liver burdens were similar to or greater than those in the lung. In general, normalized tissue burdens did not increase or decrease with increasing exposure concentration. At 3 weeks post-exposure, cobalt concentrations were markedly reduced in the blood, serum, and lung.

Urinary cobalt concentrations increased with increasing cobalt exposure concentration. The clearance half-lives were 9.2–11.1 days (blood), 2.8–3.4 days (serum), and 4.2–5.6 days (lung) in rats. In mice, the half-lives were 4.1–7.3 days (blood), 2.9–3.7 days (serum), and 5.5–6.6 days (lung).

In the 3-month exposure studies, female rats were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, and female mice were exposed by inhalation to cobalt metal particulate aerosol at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. It was observed that lung cobalt concentrations and burdens increased with increasing exposure concentration. The lung cobalt burden data from the exposure phases of these studies were analysed using a two-compartment model. Lung cobalt burdens increased rapidly within the first few days, and steady states were reached in all groups by day 26 (rat) or day 40 (mouse). During the 42-day recovery period, lung cobalt burdens decreased rapidly during the first week, after which lung clearance of cobalt slowed significantly. Liver cobalt concentrations increased with exposure concentration at both time points (day 26 and 40). Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase profile. In the rapid phase, half-lives were 1.8–2.6 and 1.4–3.2 days for rats and mice, respectively, and were 19–23 and 27–39 days for rats and mice, respectively, in the slower lung clearance phase that followed. The lung cobalt burden data were analysed using a one-compartment model, and the resulting half-lives were 4.7–9.0 and 2.4–17 days, respectively, for rats and mice.

In the 2-year studies, female rats and mice were exposed by inhalation to cobalt metal particulate aerosol at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days/week for 105 weeks. Cobalt concentrations and burdens in the lung increased with increasing exposure concentration. The lung burden data from the exposure phases were analysed using a two-compartment model. In rats, lung burdens increased rapidly by day 4; in mice, lung burdens increased rapidly within the first 5–26 days, and all exposed groups of rats and mice reached steady states by day 184. The majority of the deposited cobalt (more than 95% in rats and more than 82% in mice) was cleared quickly. The half-lives in the rapid clearance phase were between 1.5–2.9 days (rat) and 1.1–5.2 days (mouse). In the slow clearance phase, half-lives for rats and mice were 789 and 409, 167 and 172, and 83 and 118 days, respectively, for the exposures at 1.25, 2.5, and 5 mg/m^3. Cobalt deposition rates for rats and mice were 1.4 and 1.2, 2.1 and 1.1, and 5.6 and 5.2 µg/day during the rapid clearance phase, respectively, and 0.018 and 0.027, 0.078 and 0.075, and 0.29 and 0.25 µg/day during the slow clearance phase, for the exposures at 1.25, 2.5, and 5 mg/m^3.

(iii) Intravenous injection

Intravenous injection studies in mice and rats have indicated rapid elimination of cobalt. The excretion was predominantly via the urine, but a small proportion (2–15%) was also eliminated via bile and faeces (Cikrt & Tichý, 1981; Gregus & Klaassen, 1986; Llobet et al., 1986; Ayala-Fierro et al., 1999; Weber et al., 2012).

Weber et al. (2012) intravenously administered a single dose of radioactive cobalt(II) chloride (11.2 kBq of $^{60}CoCl_2$) to Fischer 344 rats and found that the liver, gastrointestinal tract, and muscle were the tissues with the greatest burdens of cobalt. These tissues cleared quickly, and less than 3% of the recovered dose remained in the body 28 days after administration (67.6% was removed with a half-life of 1.9 hours, and the remaining amount was eliminated with a half-life of 45 hours).

After rats were exposed to cobalt(II) chloride by a single intravenous injection, 70% and

7% of the dose given was excreted via the urine and faeces, respectively, during the first 3 days (Onkelinx, 1976). Another study showed that after exposure of rats to cobalt(II) chloride by intravenous injection, 73% and 15% were excreted via the urine and faeces, respectively, during the 4 days after dosing (Gregus & Klaassen, 1986). After various species of experimental animals were exposed to a radioactive cobalt nitrate (^{57}Co-labelled cobalt(II) nitrate) solution by injection, over 60% of the ^{57}Co was excreted during the first day, and over 90% was excreted by 3 weeks (Bailey et al., 1989). Thirty days after various species of experimental animals were exposed to ^{60}Co-labelled cobalt(II) chloride by intravenous administration, a significant amount (70–85%) was excreted in the urine during the first day; however, the long-term whole-body retention times were significant, being 495, 309, 183, and 180 days in mouse, rat, monkey, and dog, respectively (Thomas et al., 1976).

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 cobalt metal (without tungsten carbide), including cobalt metal nanoparticles, and soluble cobalt(II) salts are electrophilic or can be metabolically activated to an electrophile; are genotoxic; induce oxidative stress; induce chronic inflammation; modulate receptor-mediated effects; induce immortalization; and alter cell proliferation, cell death, or nutrient supply. Evidence is also reported as to whether soluble cobalt(II) salts alter DNA repair or cause genomic instability; induce epigenetic alterations; or are immunosuppressive. Evidence is also summarized as to whether cobalt(II) oxide and cobalt(II,III) oxide (including cobalt oxide nanoparticles) are genotoxic; induce oxidative stress; or modulate receptor-mediated effects. Few data are described for the other key characteristics for cobalt metal, soluble cobalt salts, and cobalt oxides. Sparse data for the key characteristics of carcinogens are reported for cobalt sulfide and other cobalt(II) compounds.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

See Table 4.1.

No studies on cobalt–DNA adducts were available to the Working Group; however, there was one study on cobalt–protein adduct formation in an exposed population.

In an occupational study, Princivalle et al. (2017) assessed cobalt–haemoglobin adducts in the blood of a subset of Italian hard-metal workers employed at several plants where they were exposed to both cobalt metal powder and cobalt oxide in various mixing and sintering operations. They reported positive correlations between geometric mean cobalt concentrations in blood and cobalt–haemoglobin adducts ($r = 0.9650$; $P < 0.001$) in 22 workers. This correlation between cobalt–haemoglobin adducts and exposure to cobalt was also seen in a larger population ($n = 66$) from the same group of plants ($r = 0.8440$; $P < 0.001$). [The Working Group noted that there was little potential for exposure misclassification. This study was focused on cobalt exposure and excretion over short time periods (days to weeks). Co-exposure to WC-Co was probable in this group of hard-metal workers; however, there was a substantial subset of workers whose work tasks primarily involved the handling of cobalt powder before the synthesis of hard-metal composites.]

Shirakawa & Morimoto (1997) examined a population exposed only to hard metal (WC-Co). [The Working Group evaluated the study and deemed it uninformative because the population

was exposed only to WC-Co), which was inseparable from exposure to cobalt alone. There is potential for non-differential misclassification in the exposure groups. A key limitation was the lack of information on the exposure groups and how they were constructed, particularly the timing of when these groups were defined in relation to the outcome measures. There are exposure metrics reported in the multiple logistic regression that are not described in the methods. Co-exposure to tungsten carbide is probable.]

[The Working Group also evaluated but excluded a study by Amirtharaj et al. (2008) as uninformative because there was no in vivo exposure to cobalt.]

(ii) Human cells in vitro

No data on direct cobalt–DNA binding in human cells in vitro were available to the Working Group.

Primary erythrocytes from healthy volunteers were treated with ^{57}Co as a tracer. Chromatographic analysis showed that ^{57}Co co-migrated with haemoglobin (Simonsen et al., 2011). [The Working Group noted that this might indicate cobalt binding to haemoglobin protein.]

When cells were depleted of zinc, the extracted wildtype human recombinant p53 proteins (produced via a baculovirus expression system) lost their DNA-binding capacity. In human MCF7 cells in vitro, addition of extracellular Zn^{2+} (5 μM) renatured and reactivated the p53. The addition of Co^{2+} (125 μM cobalt(II) chloride) had a similar effect to that of Zn^{2+} (i.e. renaturation, reactivation, and restoration of the DNA-binding capacity of p53), suggesting that Co^{2+} can substitute for Zn^{2+} binding in p53 protein (Méplan et al., 2000). [The Working Group noted that this "substitution" might be considered protein binding.]

(iii) Acellular systems

Cobalt binding to human albumin is relevant to the clinical use of the albumin-cobalt-binding (ACB) assay to detect the presence of myocardial ischaemia. The ACB assay was developed based on decreased cobalt binding to "ischaemia-modified albumin (IMA)" compared with normal human albumin and has great specific negative predictive value to reliably exclude myocardial ischaemia when the assay readout is low. However, increased assay readout is of low specificity because IMA is seen in many diseases or conditions other than ischaemia (Lu et al., 2012).

Human serum albumin has four known metal binding sites: A and B, an N-terminal binding site (NTS), and cysteine 34 (Coverdale et al., 2018). Cobalt binds to the first three sites, with a preference for sites A and B over the NTS; the affinity of Co^{2+} binding was strongest at site B, followed by site A, and weakest at the NTS (Sokołowska et al., 2009). Within the NTS, the 3-histidine and α-amino group are essential moieties that interact with Co^{2+} (Cho et al., 2022).

Using serum albumin from patients with fatty liver and healthy controls, Amirtharaj et al. (2008) showed that cobalt binding was lower in patients with fatty liver. In both purified albumin and normal serum, hydroxyl radicals [produced by hydrogen peroxide (H_2O_2) and copper (Cu)] (representative of oxidative stress) decreased cobalt binding to human albumin in vitro, and this decrease was reversed by catalase (CAT). These data were confirmed by the use of various combinations of xanthine/xanthine oxidase (to generate superoxide radicals) with CAT or superoxidase dismutase (SOD) (to scavenge superoxide), demonstrating that superoxide radicals, did not affect cobalt–albumin binding. The binding is affected by production of hydroxyl radicals as shown by the incubation with copper sulfate ($CuSO_4$) and H_2O_2, or $CuSO_4$ and H_2O_2 and thiourea and mannitol (Amirtharaj et al.,

Table 4.1 Cobalt-derived adducts in exposed humans

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Co–Hb adducts	Blood, urine	Italy, serial biomonitoring, longitudinal (pre/post-work shift Co measurements)	Hard-metal manufacturing workers in several plants, exposed to cobalt(II) metal and cobalt(III) oxide powders and sintering operations (n = 66)	Positive correlation between geometric mean Co concentrations in urine, in blood, and Co–Hb adducts in 22 of the workers; r = 0.965; P < 0.001); n = 22 workers Additional similar finding in total cohort: positive correlation log Co in blood (µg/L) and Hb (ng/g globin): log Co concentration in blood, −0.2894 + 0.6758 (log Co concentration in globin); n = 66; r = 0.8440, P < 0.001)	NR	There was little potential for misclassification. This study was focused on Co exposure and excretion over short time periods (days to weeks). Co-exposure to WC-Co was likely.	Principalle et al. (2017)

Co, cobalt; Hb, haemoglobin; NR, not reported; WC-Co, cobalt with tungsten carbide; wk, week.

2008). [The Working Group noted that albumin has at least seven fatty acid-binding sites, including a high-affinity site (FA2) near site A (Simard et al., 2005), and removing fatty acids from purified albumin also decreased cobalt–albumin binding, but fatty acid removal did not exacerbate the hydroxyl radical-induced reduction in cobalt–albumin binding (Amirtharaj et al., 2008).]

Cobalt(II,III) oxide NPs (diameter, 50 nm) with a defined crystalline phase caused minor changes in the tertiary structure of human serum albumin, as measured by fluorescence spectroscopy (Arsalan et al., 2020), but did not alter the secondary structure of albumin (amount of α-helix, β-sheet, and random coil structures), as measured by circular dichroism. When clusters of cobalt(II,III) oxide NPs were set to diameters of 0.5, 1, 1.5, and 2 nm, a computer docking program (HEX 6.3 software) calculated that the highest binding energy with human serum albumin was from the 1.5-nm diameter cluster.

(b) Experimental systems

(i) Non-human mammals in vivo

No data on cobalt–DNA or cobalt–protein binding were available to the Working Group.

(ii) Non-human mammalian cells in vitro

BALB/3T3 mouse fibroblasts, or isolated cytosolic and nuclear extracts, were acutely exposed to different forms of cobalt (at similar cobalt concentrations) (Sabbioni et al., 2014b). The amount of cobalt binding to DNA was consistently greater for cobalt microparticles (^{60}Co-labelled; mean diameter, 2.2 µm; form aggregates up to 15 µm in size [which is close to the diameter of fibroblast cells]; specific surface area, 0.75 m^2/g) than for cobalt NPs (^{60}Co-labelled; mean diameter, 3.4 nm; mean aggregate size, 200 nm; range, 100–400 nm, Zeta potential, 14 ± 4.2 mV; specific surface area, 14.4 m^2/g), which in turn was much greater than the DNA binding of Co^{2+} ions (from Co^{57}-labelled cobalt(II) chloride hexahydrate; metal purity, 99.998%) (Sabbioni et al., 2014b). [The Working Group noted that the greater amount of cobalt–DNA binding in cells treated with cobalt NPs, compared with Co^{2+}, was partially explained by the greater cellular uptake of cobalt NPs and the higher ratio of cobalt in the nuclei/whole cell compared with equivalent concentrations of Co^{2+}.]

When rat Novikoff ascites hepatoma cells were exposed to 1 mM of cobalt(II) chloride in vitro for 8 hours, the cell viability was greater than 85% and DNA–protein cross-links were seen in electrophoresis results (Wedrychowski et al., 1986). The majority of cross-linked proteins were between 94 and 200 kDa. [The Working Group noted that no digital image analysis or statistical analysis was performed.]

(iii) Acellular systems

In an acellular system, different forms of crystallized synthetic DNA were soaked in a cobalt(II) chloride solution, and Co^{2+} exhibited binding exclusively to the N^7 position of guanine (Gao et al., 1993). The binding affinity was influenced by the microenvironment of the guanine bases, as Co^{2+} in the presence of magnesium bound to different sites in the synthetic sequences CGCGCG and CGCGTG. The binding of Co^{2+} to the N^7 position of guanine probably changes the conformation of either B-DNA or A-DNA (Gao et al., 1993).

When synthetic DNA was crystallized in the presence of Co^{2+} from cobalt(II) chloride, it was observed that Co^{2+} bound to only the N^7 positions of terminal guanines (not non-terminal positions) in the resulting B-DNA crystals (Labiuk et al., 2003). Furthermore, it was shown that cobalt does not bind CGC nor B-DNA in a stable manner (Labiuk et al., 2003).

Lu et al. (2012) demonstrated that myristate, which has albumin-binding characteristics similar to those of the physiological fatty acids stearate and oleate, decreased the binding capacity of Co^{2+} (from cobalt(II) chloride

Table 4.2 Genetic and related effects of cobalt in exposed humans

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)[a]	Covariates controlled	Comments	Reference
DNA single-strand breaks (alkaline elution method)	PBMC	Germany, 10 facilities where high air concentrations of Cd were expected, cross-sectional	Workers with mixed metal exposures (Co, Cd, and Pb) ($n = 78$)	Positive correlation between Co air concentrations and DNA-SSB ($P < 0.001$, $r = 0.401$); similarly, Co in urine correlated with DNA-SSB ($P < 0.001$; $r = 0.381$)	Age, sex, alcohol, smoking status	The potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to Pb and Cd (both also quantitatively assessed) is a strength. The air samples (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure. Limitation of the study: reliance on a single biological measure of exposure.	Hengstler et al. (2003)
DNA single-strand breaks (comet assay, alkaline elution)	PBMC	European, multiple factories, cross-sectional study of genotoxic outcomes by exposure and DNA repair capacity	Co-exposed ($n = 21$) WC-Co-exposed ($n = 26$) Non-exposed controls ($n = 26$)	Comet assay: *XRCC3* × smoking status, $P = 0.037$ *XRCC1*, $P = 0.053$	Genotypes, age, exposure, type of plant, smoking, interaction terms	The potential for differential exposure misclassification is low. There was likely exposure to other metals that was not considered. Estimated Co exposure: 20 µg cobalt/m^3. This finding is for mixed (Co and WC-Co) exposure.	Mateuca et al. (2005)
Micronucleus formation (CBMN assay)	Peripheral blood lymphocytes			↑ MNMC for WC-Co-exposed workers, $P = 0.011$ ↑ MNCB for Co only-exposed workers, $P = 0.022$		Attributed to WC-Co exposure. Attributed to Co-only exposure.	Mateuca et al. (2005)

Table 4.2 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)[a]	Covariates controlled	Comments	Reference
DNA strand breaks (comet assay)	Peripheral blood lymphocytes	Belgium, Norway, Finland, Sweden, and England, workers from several refinery factories exposed to Co-containing dust, cross-sectional	Co-exposed (n = 35/24*) WC-Co-exposed (n = 29) Non-exposed controls (n = 35/27*)	Difference NSS between exposed and controls	Creatinine, urine Vitamin E (α-tocopherol), serum Selenium, serum Independent variables: Exposure, plant type, Co-urine, age, smoking, vitamin E-serum, interaction between smoking and hard-metal exposures	Key limitation of the study: reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes. Co exposure concentration approximates current TLV-TWA (20 μg/m^3). Multiple regression for influence of independent variables on outcomes including plant type (WC-Co or Co-alone exposure). Reduced number of participants for Co-exposed workers and non-exposed controls after one plant dropped from study because of age differences.	De Boeck et al. (2000)
Micronucleus formation (CBMN assay)	Peripheral blood lymphocytes			MNMC: interaction term (smoking status by plant type) P = 0.0145, but for WC-Co plant. ↑ MNCB: influenced by Co-only plant exposure and smoking as an interaction term (P = 0.0011).			
DNA damage (comet assay)	Sperm DNA	China, partners of fertility clinic patients, cross-sectional	n = 516	No associations between urinary metal levels and sperm DNA integrity parameters after adjustment for multiple testing (FDR-adjusted P for trend, > 0.10)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Urine samples collected at two points close in time on the same day as semen sample limits the findings. Only outcome for Co-only exposure in the interaction.	Wang et al. (2016a)

↑, increased; BMI, body mass index; CBMN, cytokinesis-block micronucleus; Cd, cadmium; Co, cobalt; DNA-SSB, DNA single-strand break; FDR, false discovery rate; MNCB, micronucleated binucleated cell; MNMC, micronucleated mononucleated cell; NSS, not statistically significant; Pb, lead; PBMC, peripheral blood mononuclear cell; TLV-TWA, threshold limit value/time-weighted average; WC-Co, cobalt with tungsten carbide.

[a] +, positive; –, negative.

hexahydrate) to mature bovine serum albumin (with metal- and fatty acid-binding properties similar to those of human serum albumin). Because ischaemia also increases plasma fatty acid levels, ACB assay results might not be attributable to albumin changes. Other conditions that increase fatty acid levels would also decrease measured cobalt binding in the ACB assay.

Cobalt has been shown to bind to the globin moieties of bovine haemoglobin (Hoffman & Petering 1970) and sperm whale myoglobin (Sato et al., 1990).

4.2.2 Is genotoxic

The genotoxicity of cobalt and cobalt compounds was previously evaluated in *IARC Monographs* Volumes 52 (IARC, 1991) and 86 (IARC, 2006). The following is a summary of the studies relevant to key characteristic 2 – "is genotoxic" – and reported in Tables 4.2 to 4.5 and Table S4.6 (see Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618), with a particular emphasis on the studies in exposed humans, human cells in vitro, and experimental (non-human mammalian) systems.

(a) Humans

(i) Exposed humans

See Table 4.2.

Evidence for genotoxic effects in human populations exposed to cobalt included a cross-sectional study of German workers exposed to cobalt, cadmium, and lead (Hengstler et al., 2003). A positive correlation was observed between cobalt air concentrations and DNA single-strand breaks in blood mononuclear cells (r = 0.401; P < 0.001). Similarly, cobalt concentrations in urine positively correlated with DNA single-strand breaks (r = 0.381; P < 0.001). [The Working Group noted that the authors indicated that cobalt exposure was comparatively low (range, 0–10 μg/m³ air) compared with the German TRK permissible exposure limit value of 100 μg/m³. The Working Group also noted that DNA damage in the exposed individuals might be a secondary event due to DNA repair inhibition. DNA repair inhibition might also explain interactions between heavy metals since a decreased repair capacity will increase susceptibility to direct genotoxic effects. The Working Group noted that the potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to lead and cadmium, both also quantitatively assessed, was a strength. However, the Working Group noted that non-standard analytical approaches were used to assess confounding (e.g. Greenland, 1989). The air (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure.]

In another cross-sectional study of European factory workers across several plants, some with exposure to cobalt alone and others to WC-Co, and including non-exposed controls, Mateuca et al. (2005) examined several end-points of genotoxic damage, including DNA single-strand breaks measured in peripheral blood mononuclear cells (PBMCs) using the comet assay, and mono- and binucleated cells in whole blood using the cytokinesis-block micronucleus assay. Only the amount of micronucleated binucleated cells in the micronucleus assay was found to be significantly increased in the workers exposed to cobalt alone (P = 0.022). [The Working Group noted that the potential for differential exposure misclassification was low. There was probable exposure to other metals that was not considered.]

In a similar cohort to that described above by Mateuca et al. (2005), DeBoeck et al. (2000) assessed genotoxic end-points in a group of European factory workers from several sites, with exposure to either metallic tungsten carbide particles or cobalt metal alone, and compared them with non-exposed controls from the same plants, adjusting for age and smoking status. No statistically significant differences

Table 4.3 Genetic and related effects of cobalt in human cells in vitro

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt metal					
DNA single-strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+[b]	0.3 µg/mL (d_{50}, 4 µm)	Difficult to infer LEC from the graphic representation.	De Boeck et al. (1998)
Oxidative DNA damage (comet assay with Fpg enzyme)	Mononuclear leukocytes	–[b]	6 µg/mL (d_{50}, 4 µm)		De Boeck et al. (1998)
DNA strand breaks (alkaline elution)	Lymphocytes	+[b]	3 µg/mL (d_{50}, 4 µm)		Anard et al. (1997)
DNA single-strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+[b]	4.5 µg/mL (d_{50}, 4 µm)		Anard et al. (1997)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	+[b]	0.6 µg/mL (d_{50}, 4 µm)		Van Goethem et al. (1997)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Leukocytes	–	6 µg/mL (d_{50}, 4 µm)		De Boeck et al. (2003)
DNA strand breaks (alkaline elution assay)	Human osteoblast-like cells, HOS Osteoblast-like cells (immortalized, non-tumourigenic), TE85 (clone F-5)	–	6 µg/mL	Purity, 99.5%; d_{50} = 1–4 µm, A heavy metal W alloy [pure mixture of W (92%), Ni (5%), and Co (3%)] was also tested with positive (synergistic) results.	Miller et al. (2001)
Micronucleus formation (CBMN assay)	Lymphocytes	+[b]	0.6 µg/mL (d_{50}, 4 µm)		VanGoethem et al. (1997)
Micronucleus formation (CBMN assay)	Lymphocytes	(+)	3 µg/mL (d_{50}, 4 µm)	Difficult to infer LEC from the data presented.	De Boeck et al. (2003)
Micronucleus formation (CBMN assay)	Human osteoblast-like cells, HOS osteoblast-like cells (immortalized, non-tumourigenic), TE85 (clone F-5)	(+)	0.75 µg/mL	Purity, 99.5%; d_{50}, 1–4 µm. Statistically significant increase without concentration–response relationship. A heavy metal W alloy [pure mixture of W (92%), Ni (5%), and Co (3%)] was also tested with positive results.	Miller et al. (2001)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt metal NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	+	50 µM [3 µg/mL], 2 h	d_{50} = 246 nm (100–500 nm) [given that the authors designated them as NPs, it is presumed that ≥ 50% of the particles have one dimension < 100 nm].	Colognato et al. (2008)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Blood T-cells	+	3 µM [0.53 µg/mL], 4 h	d_{50}, 50 nm (30–70 nm). Concurrent assessment of Co dissolution in culture medium, cytotoxicity.	Jiang et al. (2012)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) DNA double-strand breaks (γH2AX assay)	Primary CD34+ haematopoietic stem cells and haematopoietic progenitor cells (isolated from cord blood)	(+) (+)	200 µM [11.8 µg/mL]	Size, 50–200 nm. Single concentration of Co metal NPs tested; the aim was to test protective effect of selenomethionine against Co metal NPs. Concomitant assessment of cytotoxicity, apoptosis, oxidative stress and modulation of DNA repair response	Zhu et al. (2021b)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	+	5 µg/mL	Mean diameter, 20 nm Only 2 concentrations tested; no positive control described	Wan et al. (2012)
DNA double-strand breaks (γH2AX assay)		+	10–15 µg/mL	Concomitant assessment of NP uptake by cells, cytotoxicity, oxidative stress, and gene expression (genes involved in DNA damage response). DNA damage was significantly attenuated by pre-treatment with antioxidants (NAC or catalase); dose- and time-dependent increased expression of RAD51, phosphorylated p53, and ATM.	Wan et al. (2012)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549; human bronchial epithelial cells, HBEC3-kt (immortalized bronchial epithelial cells) Human lung alveolar cell line, A549	(+) + (Fpg)	40 µg/mL, 3 h and 24 h 20 µg/mL, 24 h	Co metal NP purity, 99.8%; different dimensions and shapes (from spheres to parallelepipeds); average size, 25 ± 8.8 nm. Only 2 concentrations tested; only positive at the highest concentration tested; no concentration–response. Concurrent assessment of cytotoxicity, ROS formation.	Cappellini et al. (2018)
Micronucleus formation (CBMN assay)	Lymphocytes	+	20 µM [1.18 µg/mL]	d_{50}, 246 nm.	Colognato et al. (2008)
Soluble cobalt(II) salts					
Cobalt(II) chloride ($CoCl_2$)					
DNA strand breaks (fluorescence analysis of DNA unwinding)	Mononuclear leukocytes	(+)	50 µM [6.5 µg/mL]	One concentration tested. No cytotoxicity assessment.	McLean et al. (1982)
DNA strand breaks (alkaline sucrose gradient)	Human diploid fibroblasts, HSBP	(+)	5 mM [650 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA strand breaks (nick translation)	Human diploid fibroblasts, HSBP	(+)	10 mM [1300 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA strand breaks (nucleoid sedimentation)	Human diploid fibroblasts, HSBP	(–)	10 mM [1300 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA single strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+		Not possible to infer LEC from the graphic representation.	De Boeck et al. (1998)
DNA cleavage (topoisomerase II-mediated DNA cleavage)	Human breast cancer cell line, MCF7	(+)	200 µM [26 µg/mL], 24 h	A single concentration tested.	Baldwin et al. (2004)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Primary human fibroblasts	(+)	0.84 µM [0.11 µg/mL]	Very limited data presented for cobalt(II) chloride. Artificial salt solution (synthesized fluids) containing cobalt(II) chloride alone at 0.84 µm induced DNA damage but at a lower level than that produced by a Co–Cr alloy solution.	Davies et al. (2005)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Hepatocellular carcinoma-derived cell line, HepG2	(+)	10 µg/mL [77 µM], 48 h	Two concentrations (10 and 15 µg/mL).	Alarifi et al. (2013)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Blood T-cells	–	30 µM [3.9 µg/mL]	Concurrent assessment of Co dissolution in culture medium, cytotoxicity.	Jiang et al. (2012)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human keratinocyte cell line, HaCaT	+	40 µM [5.2 µg/mL], 6 h and 24 h	Concomitant intracellular Co determination and cytotoxicity assessment.	Gault et al. (2010)
DNA double-strand breaks (neutral comet assay)	Human lymphoma cell line, Jurkat (CD4+ T-cells obtained from a T-helper lymphoma)	(+)	5 mM [650 µg/mL], 48 h	Only positive at the highest concentration tested; no concentration–response [the use of $CoCl_2$ or $CoCl_2{\cdot}7H_2O$ is not specified].	Caicedo et al. (2008)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	–	100 µM [13 µg/mL], 2 h		Colognato et al. (2008)
DNA breaks Single-cell array-based halo assay (alkaline conditions)	Fetal fibroblast cells	(+)	10 µM [1.3 µg/mL], 1 h	Method developed by the authors [source of Co^{2+} not mentioned, no positive control, number of independent experiments/ replicates not specified, primary or immortalized cells not specified].	Qiao & Ma (2013)
DNA double-strand breaks (γH2AX assay)	Human lung carcinoma cell line, H460	+	300 µM [39 µg/mL]	Concurrent cell viability, apoptosis, and ROS assessment.	Patel et al. (2012)
DNA double-strand breaks (γH2AX assay)	Human colorectal cancer cell lines, HCT116, SW620, and LOVO cells	(+)	100 µM [13 µg/mL], 24 h	A single concentration of $CoCl_2$ tested.	Zhong et al. (2020)
Micronucleus formation (CBMN)	Lymphocytes	+	40 µM [5.2 µg/mL]		Colognato et al. (2008)
Sister-chromatid exchange	Lymphocytes	+	10 µM [1.3 µg/mL]		Andersen (1983)
Aneuploidy	Lymphocytes	+	3.7 µg/mL [28.5 µM]		Resende de Souza Nazareth (1976)
Cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$)					
DNA strand breaks (nucleoid sedimentation)	Human cervical cancer cell line, HeLa	+	50 µM [11.9 µg/mL]		Hartwig et al. (1990)
DNA strand breaks (alkaline elution)	Lymphocytes	(–)	25 µg/mL [105 µM]	A single concentration tested.	Anard et al. (1997)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme or hOGG1 enzyme)	Human bronchial epithelial cell line, BEAS-2B	+ + (Fpg) + (hOGG1)	2.5 µg/mL [10.5 µM], 2 h 1.25 µg/mL [5.25 µM], 2 h, Fpg 5 µg/mL [21 µM], 24 h, hOGG1	[No data for ROS production or oxidative stress markers.]	Uboldi et al. (2016)
DNA double-strand breaks (γH2AX)		+	2.5 µg/mL [10.5 µM]		Uboldi et al. (2016)
Chromosomal aberration, including translocations and aneuploidy (M-FISH)	Primary fibroblasts	+ (structural + numerical chromosomal aberration) + (aneuploidy)	1.3 ppb (metal ion species) [0.005 µM] 25 ppb [0.105 µM]	Co tested at physiological doses (found in peripheral blood of exposed humans). Numerical chromosomal aberrations were predominantly present. Structural aberrations such as translocations and dicentrics were not observed.	Figgitt et al. (2010)
Chromosomal aberration	Primary normal human bronchial epithelial cells, NHBE	+	175 µM [41.7 µg/mL], 24 h	Concomitant cytotoxicity and intracellular Co ion concentration measured. Comparison with Co oxide NPs: at similar intracellular Co ion levels, $CoCl_2$ induces more chromosome damage than Co oxide NPs. The most common aberration observed was chromatid lesions.	Xie et al. (2016)
Chromosomal aberration	Human primary bronchial fibroblast-derived cell line, WTHBF-6 (hTERT immortalized clonal cell line)	+	50 µM [11.9 µg/mL], 24 h	Concomitant cytotoxicity and intracellular Co ion concentration measured. Based on intracellular Co levels, exposure to soluble or particulate Co induced relatively similar levels of genotoxicity. The most common aberrations observed were chromatid lesions.	Smith et al. (2014)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Chromosomal aberration	Human urothelial cells, hTU1–38 (hTERT immortalized subclone of human urothelial hTU1cell line)	+	175 µM [41.7 µg/mL], 24 h	Co concentrations were chosen based on physiologically relevant exposure levels in patients who received hip implants (mean cobalt ion urine concentration, 75.40 µg/L). The most common aberration observed was chromatid lesions. Concomitant cytotoxicity and intracellular Co concentration assessment. Comparison with Co oxide NPs (see below).	Speer et al. (2017)
Micronucleus formation (CBMN assay)	Human bronchial epithelial cell line, BEAS-2B	+	1.25 µg/mL [5.25 µM]	Cytotoxicity/apoptosis reported.	Uboldi et al. (2016)
Cobalt(II) acetate tetrahydrate ($Co(CH_3CO_2)_2 \cdot 4H_2O$)					
Chromosomal aberration	Leukocytes	–	0.6 µg/mL [2.4 µM]		Voroshilin et al. (1978)
Cobalt(II) nitrate ($Co(NO_3)_2$)					
Chromosomal aberration	Diploid fibroblasts, WI_{38} and MRC_5	–	0.08 µM [0.015 µg/mL]		Paton & Allison (1972)
Chromosomal aberration	Mononuclear leukocytes	–	0.8 µM [0.15 µg/mL]		Paton & Allison (1972)
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	(+)	800 µg/mL, 4 h (soluble and unfiltered fractions)	Cobalt content, 21%. Only one concentration tested. Marked induction of DNA strand breaks seemed to coincide with significant reduction in cell number and thus cytotoxic activity of the cobalt compound.	Kirkland et al. (2015)
Oxidative DNA damage (comet assay with hOGG1 enzyme)		(-)			
Cobalt(II) octoate					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	+	800 µg/mL (soluble fraction); 200 µg/mL (total fraction), 4 h	Cobalt content, 17%.	Kirkland et al. (2015)
Oxidative DNA damage (comet assay with hOGG1 enzyme)		+	800 µg/mL, 4 h		Kirkland et al. (2015)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Insoluble cobalt (II or II,III) compounds					
Cobalt(II) oxide (CoO)					
Chromosome aberrations	Human primary normal bronchial epithelial cells, NHBE	+	0.1 µg/cm², 24 h	Size, ≤ 10 µm. Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Xie et al. (2016)
Chromosome aberrations	Human bronchial fibroblasts, WTHBF-6 (hTERT immortalized clonal cell line derived from primary human bronchial fibroblasts)	+	0.5 µg/cm², 24 h	Size (average), 1 µm (0.27–3.56 µm). Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Smith et al. (2014)
Chromosome aberrations	Human urothelial cells, hTU1–38 (hTERT immortalized subclone of human urothelial hTU1cell line)	+	1 µg/cm², 24 h	Particle size, ≤ 10 µm. Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Speer et al. (2017)
Cobalt(II,III) oxide (Co_3O_4)					
DNA strand breaks, and alkali-labile sites (comet assay) Oxidative DNA damage (comet assay with Fpg and hOGG11enzymes)	Human bronchial epithelial cell line, BEAS-2B	+ + (with Fpg or hOGG1)	10 µg/mL, 2 h 2.5 µg/mL, 24 h 2.5 µg/mL, 2 h and 24 h, Fpg 1.25 µg/mL, 2 h, and 24 h, hOGG1	Purity, 98.4%. Co_3O_4 particles were mainly aggregated and exhibited a polyhedral structure with heterogeneous sizes: 100–400 nm. Concomitant assessment of secondary physicochemical properties in cell culture medium (DLS) and cytotoxicity/apoptosis. Comparison with $CoCl_2 \cdot 6H_20$. [Very comprehensive study.] Oxidative DNA damage was observed after Co particles (10–1000 nm) (10–20 µg/mL) and $CoCl_2$ (1.25–10 µg/mL) treatment. No data for ROS production or oxidative stress markers.	Uboldi et al. (2016)
DNA double-strand breaks (γH2AX assay)		+	2.5 µg/mL		Uboldi et al. (2016)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	(+)	20 µg/cm²	Size, < 10 µm. Only 2 concentrations tested, positive at the lower concentration only, Good physicochemical characterization of particles. Concomitant evaluation of ROS and formation of 8-oxo-dG adducts.	Kain et al. (2012)
Oxidative DNA damage (comet assay with Fpg enzyme)		–	40 µg/cm²		
DNA strand breaks and alkali labile sites (comet assay)	Human bronchial epithelial cell line, BEAS-2B	(+)	40 µg/cm²	Size, < 10 µm. Only 2 concentrations tested; positive at the higher concentration only. Good physicochemical characterization of particles; concomitant evaluation of ROS and formation of 8-oxo-dG adducts.	Kain et al. (2012)
Oxidative DNA damage (comet assay with Fpg enzyme)		+	20 µg/cm²		
Micronucleus formation (CBMN assay)	Human bronchial epithelial cell line, BEAS-2B	+	1.25 µg/mL	Purity, 98.4%. Same comment as for DNA strand breaks results above.	Uboldi et al. (2016)
Cobalt(II) oxide (CoO) NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human bronchial epithelial cells, HBEC3-kt (immortalized bronchial epithelial cells)	(+)	60 µg/mL, 3 h	Metal purity, 99.99%; mainly spherical and fused into large agglomerates with a pristine dimension of 43 ± 9 nm. Only 2 concentrations tested; only positive at the higher concentration tested; no concentration–response. Concurrent assessment of cytotoxicity, ROS formation, [Quite comprehensive study.]	Cappellini et al. (2018)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	(+) (+) (Fpg)	60 µg/mL, 24 h 60 µg/mL, 24 h	Only positive at the highest concentration tested; no concentration–response relationship.	Cappellini et al. (2018)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt(II,III) oxide (Co_3O_4) NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549; human bronchial epithelial cells	–	60 µg/mL, 3 h and 24 h	Size, 51 ± 11 nm. Concurrent assessment of cytotoxicity, ROS formation.	Cappellini et al. (2018)
Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	– (Fpg)	60 µg/mL, 3 h and 24 h		Cappellini et al. (2018)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human hepatocellular carcinoma-derived cell line, HepG2; human colon cancer cell line, Caco-2 cells; human neuroblastoma cell line, SH-SY5Y	–	100 µg/mL, 24 h	Size, 47.0 ± 20.3 nm. Concomitant assessment of secondary physicochemical properties in cell culture medium (TEM and DLS); uptake analysis; cytotoxicity/apoptosis and oxidative damage assessment.	Abudayyak et al. (2017)
	Human lung alveolar cell line, A549	+	0.1 µg/mL, 24 h	Concentration–response relationship but low level of induction of DNA breaks.	
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Lymphocytes	(+)	100 µg/mL, 24 h	Diameter, 96.4 ± 0.57 nm. Characterization of secondary physicochemical properties. Concurrent assessment of cytotoxicity and oxidative stress. A single concentration tested.	Rajiv et al. (2016)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human bronchial epithelial cell line, BEAS-2B	+ + (Fpg sites)	40 µg/mL, 2 h and 24 h 5 µg/mL, 2 h and 24 h	Purity, ≤ 99.5%; elongated hexagonal shape; d_{50}, 22.1 ± 7.2 nm. Direct DNA damage: dose-dependent trend, statistically significant only at the higher concentration tested. Concurrent evaluation of cytotoxicity and immunotoxicity.	Cavallo et al. (2015)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	+ + (Fpg sites)	40 µg/mL, 2 h 20 µg/mL, 24 h 20 µg/mL, 2 h and 24 h	Purity, ≤ 99.5%; elongated hexagonal shape; d_{50}, 22.1 ± 7.2 nm.	Cavallo et al. (2015)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Hepatocellular carcinoma-derived cell line, HepG2	+	10 µg/mL, 24 h 5 µg/mL, 48 h	Polygonal shape; size, ~21 nm.	Alarifi et al. (2013)
Chromosomal aberration	Lymphocytes	(+)	100 µg/mL, 1 h	Diameter: 96.4 ± 0.57 nm. Characterization of secondary physicochemical properties. Concurrent assessment of cytotoxicity and oxidative stress. A single concentration tested.	Rajiv et al. (2016)
Organic cobalt(II) compounds					
Cobalt(II) resinate					
Chromosomal aberration	Lymphocytes	(–) with S9 (+) with S9	300 µg/mL, (–S9) 37.5 or 75 µg/mL, (+S9), 3 h	Purity, 83.7%. Data from 3 (–S9) and 4 (+S9) experiments. Cytotoxicity evaluated by mitotic index. Results difficult to interpret due to inconsistencies; the impact of cytotoxicity is unclear because of the confounding factor of precipitation.	Kirkland et al. (2015)

8-oxodG, 8-oxo-2′-deoxyguanosine; CBMN, cytokinesis-block micronucleus; Co, cobalt; Cr, chromium; d_{50}, median diameter; DLS, dynamic light scattering; Fpg, formamidopyrimidine DNA glycosylase; γH2AX, gamma-H2A histone family member X; HIC, highest ineffective concentration; hOGG1, human 8-oxoguanine DNA *N*-glycosylase-1; hTERT, human telomerase reverse transcriptase; LEC, lowest effective concentration; M-FISH, multicolour fluorescence in situ hybridization; NAC, *N*-acetyl cysteine; Ni, nickel; NP, nanoparticle; ppb, part per billion; ROS, reactive oxygen species; TEM, transmission electron microscopy; W, tungsten.

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

[b] Refers to the same experiment in which Co and Co with tungsten carbide were compared.

for measurements of DNA single-strand breaks or micronuclei formation in peripheral blood lymphocytes were found between exposed groups and controls. The frequency of micronuclei in binucleated cells (MNCB) was influenced by cobalt-only plant exposure and smoking as an interaction term (P = 0.0011). [The Working Group noted that a key limitation of this study was the reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes.]

The association between urinary metal levels and sperm DNA damage, as measured by comet assay, was assessed in partners of Chinese fertility clinic patients in a cross-sectional study by Wang et al. (2016a). No significant (or suggestive) associations were found between urinary cobalt levels and sperm DNA integrity parameters after adjustment for age, BMI, abstinence time, smoking status, daily cigarette consumption, and concentrations of urinary creatinine and other metals, nor for multiple testing. [The Working Group noted that collection of urine samples at two points close in time on the same day as semen sample collection limits the findings.]

[The Working Group noted that three of four studies reported some evidence of cobalt-associated genotoxicity, including DNA strand breaks and micronucleus formation in binucleated cells.]

Three other studies examined the effects of cobalt exposure in humans: L'vova et al. (1990), Gennart et al. (1993), and Wultsch et al. (2017). [After evaluation, the Working Group excluded these studies. In L'vova et al. (1990), the study description was considered inadequate for the evaluation of the exposure assessment and the findings. The study by Gennart et al. (1993) was deemed uninformative because of the population's mixed exposure to other carcinogens, including chromium and nickel, without any regression analysis to isolate the effects of cobalt. Similarly, the study by Wultsch et al. (2017) was excluded because the selected population was co-exposed to chromium and probably to other metals, including Ni, and no regression analysis was performed to isolate correlations with cobalt. The potential for differential exposure misclassification is low. In addition, the authors relied on a single biological measure of exposure.]

(ii) Human cells in vitro

The results of the genotoxic effects of exposure to cobalt metal or cobalt(II) salts, including cobalt-based NPs, in human cells are summarized in Table 4.3.

Cobalt metal

Cobalt metal exposure was able to induce DNA strand breaks in human primary lymphocytes (Anard et al., 1997; De Boeck et al., 1998) and in mononuclear leukocytes (Van Goethem et al., 1997), although a negative result was also reported in leukocytes (De Boeck et al., 2003). Cobalt exposure did not induce formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites in mononuclear leukocytes (De Boeck et al., 1998). Cobalt metal did not induce DNA breaks in non-tumourigenic osteoblast-like cells (Miller et al., 2001). However, it induced a significant and concentration-dependent increase in micronucleus formation frequencies in lymphocytes (Van Goethem et al., 1997; De Boeck et al., 2003) and in human osteoblast-like cells (Miller et al., 2001).

Soluble cobalt(II) salts

Cobalt(II) chloride induced DNA strand breaks in human primary leukocytes and lymphocytes (McLean et al., 1982; De Boeck et al., 1998). In addition, an artificial salt solution containing Co^{2+} at a concentration of 0.84 µM caused a low but significantly increased level of DNA damage, as assessed by alkaline comet assay, in human primary fibroblasts (Davies et al., 2005). However, more recent studies using the same assay reported no induction of DNA lesions in primary blood leukocytes (Colognato et al., 2008) and primary T-cells exposed to cobalt(II) chloride (Jiang et al., 2012).

The induction of DNA strand breaks after exposure to cobalt(II) chloride was shown in HSBP cells (normal human foreskin fibroblasts, diploid) through an alkaline sucrose gradient or nick translation (but not by nucleoid sedimentation assay) (Hamilton-Koch et al., 1986), and in fetal fibroblast cells (Qiao & Ma, 2013), as assessed by the single cell array-based halo assay developed by the authors. In line with these reports, Gault et al. (2010) showed the induction of DNA damage in a human keratinocyte cell line, as measured by comet assay. Weakly positive results were detected in the human hepatoma-derived HepG2 (Alarifi et al., 2013) and Jurkat CD4+ T-cell (Caicedo et al., 2008) lines, the latter using the neutral version of the comet assay that enables measurement of DNA double-strand breaks.

Cobalt(II) chloride induced sister-chromatid exchange (Andersen, 1983) and aneuploidy (Resende de Souza-Nazareth, 1976) in human primary lymphocytes. Using the cytokinesis-block micronucleus assay, a significant and concentration-dependent increase in micronuclei frequency and a parallel decrease in the cytokinesis-block proliferation index (a measure of cytostasis) were reported in human primary lymphocytes exposed to cobalt(II) chloride, suggesting clastogenic/aneugenic potential (Colognato et al., 2008).

More recently, Patel et al. (2012) investigated the effects of cobalt(II) chloride alone, nickel chloride ($NiCl_2$), or a mixture of the two on a human lung carcinoma cell line (H460) using the phosphorylation of histone H2AX (γH2AX foci formation) as a marker of double-strand breaks. A significant induction of γH2AX foci formation was reported for cobalt(II) chloride, and a concomitant induction of reactive oxygen species (ROS) was found. The measured reduction of double-strand breaks after pre-treatment with an antioxidant agent (*N*-acetyl cysteine) suggested that ROS formation may underlie the genotoxicity of cobalt(II) chloride (Patel et al., 2012) (see also Section 4.2.5 and Table 4.13). The capacity of a single concentration of cobalt(II) chloride (100 μM) to induce the formation of γH2AX foci was additionally explored in several *KRAS*-mutant intestinal cancer cell lines (HCT 116, SW620, and LoVo cells), resulting in positive responses (Zhong et al., 2020). Furthermore, the same cobalt(II) chloride concentration (with treatment referred to as mimicking a hypoxic environment in cancer cells) markedly downregulated expression levels of *RAD51* and the percentage of *RAD51* focus-positive cells, indicating compromised capacity for homologous recombination repair (see Section 4.2.3). Cobalt(II) chloride tested at a single concentration (200 μM) was also observed to have the capacity to impair human topoisomerase IIα activity in the MCF7 cell line (Baldwin et al., 2004), as assessed by topoisomerase II-mediated DNA cleavage assay. [The Working Group noted that the results of parallel acellular assays further supported the idea that this compound may act as a topoisomerase II poison, suggesting that this mechanism contributes to its genotoxicity.]

Cobalt(II) chloride hexahydrate did not induce DNA strand breaks in human primary lymphocytes (Anard et al., 1997). In contrast, this cobalt(II) salt generated DNA strand breaks in the HeLa cell line, as assessed by nucleoid sedimentation assay (Hartwig et al., 1990), and in the human bronchial epithelial (BEAS-2B) cell line, as assessed by comet assay (Uboldi et al., 2016). Furthermore, using a modified version of the comet assay with the formamidopyrimidine glycosylase (Fpg) and human 8-oxoguanine DNA *N*-glycosylase-1 (hOGG1) enzymes to detect oxidized bases, positive results were also reported, indicating induction of oxidative DNA damage (Uboldi et al., 2016) (see also Section 4.2.5 and Table 4.13).

The ability of cobalt(II) chloride hexahydrate to induce numerical and/or structural chromosomal alterations has been assessed in human primary fibroblasts (Figgitt et al., 2010) and

human primary normal bronchial epithelial cells (Xie et al., 2016), and in other cell lines representative of the respiratory (WTHBF-6 cells) and urothelial (hTU1-38 cells) tracts (Smith et al., 2014; Speer et al., 2017). Of note, in two studies, the concentration ranges for cell treatment were selected on the basis of physiologically relevant exposure levels measured in samples of peripheral blood (Figgitt et al., 2010) or urine (Speer et al., 2017) collected from patients who had received cobalt-containing implants. All studies that analysed unstable chromosomal damage showed significant induction of chromosomal aberrations, with chromatid breaks being the aberration most frequently observed (Smith et al., 2014; Xie et al., 2016; Speer et al., 2017). By contrast, Figgitt et al. (2010), using full multicolour fluorescence in situ hydridization to analyse translocation and aneuploidy, found predominantly numerical chromosomal aberrations in primary fibroblasts, suggesting that this cobalt(II) salt displays aneugenic potential. The positive results described by Uboldi et al. (2016) with an in vitro cytokinesis-block micronucleus assay and a γH2AX assay in the BEAS-2B cell line support the clastogenic and/or aneugenic effects of this soluble cobalt(II) salt in immortalized bronchial cells.

Cobalt(II) acetate tetrahydrate did not induce chromosomal aberrations in human primary lymphocytes (Voroshilin et al., 1978).

Cobalt(II) nitrate did not induce chromosomal aberrations in human primary mononuclear leukocytes (Paton & Allison, 1972) or diploid fibroblasts (WI-38 or MRC-5 cells).

Cobalt(II) sulfate heptahydrate caused a marked induction of DNA breaks (as assessed by comet assay) after exposure of the A549 cell line (lung alveolar epithelial-like cells) to a single concentration of the compound (800 μg/mL, 4 hours), but no oxidative DNA damage was detected after treatment with the hOGG1 enzyme (Kirkland et al., 2015). [The Working Group noted that induction of DNA damage seemed to coincide with cytotoxic activity of the compound, and therefore the results should be interpreted with caution.]

Cobalt(II) octanoate, at a concentration of 800 (soluble fraction) or 200 (total fraction) μg/mL, induced a significant increase in DNA strand breaks and oxidative DNA damage in A549 cells, as assessed by comet assay without and with the hOGG1 enzyme (Kirkland et al., 2015).

Insoluble cobalt(II or II,III) compounds

Cobalt(II) oxide particles have consistently been observed to have the capacity to induce chromosomal aberrations in different types of human cells. Speer et al. (2017) showed that cobalt(II) oxide particles (size, up to 10 μm), tested at physiologically relevant exposure levels, are more genotoxic than soluble cobalt (cobalt(II) chloride hexahydrate) in human immortalized urothelial (hTU1-38) cells. However, at similar intracellular cobalt ion concentrations, particulate and soluble cobalt induced similar extents of chromosomal damage, the most common being chromatid lesions. The results were in line with those previously reported in human immortalized bronchial fibroblasts exposed to cobalt oxide particles (d_{50}, 1 μm) when compared with soluble cobalt (Smith et al., 2014). Of note, the induction of chromosomal damage by soluble and insoluble cobalt was also observed by Xie et al. (2016) using human primary bronchial epithelial cells; however, at similar intracellular cobalt levels, the frequency of chromosomal aberrations induced by cobalt(II) oxide particles was lower than that induced by soluble cobalt(II) salt.

Cobalt(II,III) oxide particles differing in their physicochemical properties (e.g. size) have been observed to have slightly different genotoxicity outcomes. Cobalt(II,III) oxide particles with a high degree of purity, that were poorly soluble and had a polyhedral structure and constituted sizes ranging from 100 to 400 nm, induced micronuclei and the formation of γH2AX foci (a marker of DNA double-strand breaks) in the BEAS-2B

cell line (Uboldi et al., 2016). In addition, the authors reported the induction of primary DNA lesions and oxidative DNA damage (see also Section 4.2.5 and Table 4.13), as assessed by modified comet assay with Fpg and hOGG1 enzymatic cleavage. Comparison with the results obtained in parallel for a soluble cobalt(II) salt showed that the observed genotoxicity of cobalt(II,III) oxide particles is induced by the particles themselves and cannot be solely attributed to the release of Co^{2+} ions (Uboldi et al., 2016). In previous work, larger cobalt(II,III) oxide particles (size, < 10 µm) induced DNA breaks in the BEAS-2B and A549 cell lines, but only at a single concentration (Kain et al., 2012). However, oxidative DNA damage was solely detected in the BEAS-2B cells and at a single concentration of particles, as assessed by Fpg-modified comet assay, while some interference of particles with the Fpg enzyme was noted (see also Section 4.2.5).

Cobalt-based nanoparticles

Cobalt metal NPs have been assessed for genotoxicity in vitro using mainly conventional assays, such as the comet assay, the γH2AX assay, and micronuclei formation in human primary cells and lung cell lines. Exposure of human primary blood mononuclear cells to cobalt metal NPs (d_{50}, 246 nm, or d_{50}, 50 nm) has resulted in consistently increased levels of DNA strand breaks and alkali-labile sites, as assessed by comet assay (Colognato et al., 2008; Jiang et al., 2012), and (cobalt metal NPs: d_{50}, 246 nm) have induced micronuclei formation in cytokinesis-blocked lymphocytes (Colognato et al., 2008). Zhu et al. (2021b) reported the induction of DNA single- and double-strand breaks by a single concentration of cobalt metal NPs (size, 50–200 nm) in human primary haematopoietic stem cells and haematopoietic progenitor cells (isolated from cord blood). Positive findings have been also reported in cells representative of the respiratory tract. Cappellini et al. (2018) reported induction of Fpg-sensitive sites (assessed by Fpg-modified comet assay) after exposure of the A549 cell line and induction of DNA strand breaks in immortalized human bronchial epithelial cells to the highest tested concentration (40 µg/mL, for 3 and 24 hours) of cobalt metal NPs with different dimensions and shapes (average size, 25 ± 8.8 nm). Cobalt metal NPs were taken up by both cell lines, and parallel analyses showed that they induced ROS formation, which may mediate the observed genotoxicity (see also Section 4.2.5). In agreement with these findings, another study also showed that cobalt metal NPs (mean diameter, 20 nm) caused an increase in DNA single- and double-strand breaks, as assessed by comet and γH2AX assays, respectively (Wan et al., 2012). The observed genotoxicity was significantly attenuated by pre-treatment of the cells with ROS scavengers, which also abolished ROS induction (see also Section 4.2.5).

Cobalt(II) oxide NPs (size, 43 ± 9 nm) (Cappellini et al., 2018) were tested for genotoxicity in immortalized human bronchial epithelial cells and in the alveolar A549 cell line with positive results, as assessed by standard (tested in both cells) and Fpg-modified (tested in A549 cells only) comet assays for the highest concentration tested (Cappellini et al., 2018). Other end-points were simultaneously analysed, such as cytotoxicity and ROS formation (see also Section 4.2.5).

Cobalt(II,III) oxide NPs were assessed for genotoxicity in various human cell lines, mainly by comet assay (and modified versions of it with DNA repair enzymes), generating mixed results (Alarifi et al., 2013; Cavallo et al., 2015; Rajiv et al., 2016; Abudayyak et al., 2017; Cappellini et al., 2018). A study using human primary lymphocytes reported the induction of DNA strand breaks and chromosomal aberrations by cobalt(II,III) oxide NPs (diameter, 96.4 ± 0.57 nm), at a concentration of 100 µg/mL (Rajiv et al., 2016). In a study by Abudayyak et al. (2017), human hepatoma (HepG2), intestine (Caco-2), and nervous system (SH SY5Y) cell lines were exposed to dose ranges (up to 100 µg/mL) of

Table 4.4 Genetic and related effects of cobalt in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Cobalt metal (Co)							
Gene mutation (K-*Ras*, *Egfr*, *Tp53*)	Mouse, $B6C3F_1$/N (M/F)	Alveolar/ bronchiolar carcinomas	+	All exposed groups combined (1.25, 2.5, and 5 mg/m^3)	Inhalation, 2 yr	Evaluation of mutation spectra in cobalt-caused cancers. The spectra were compatible with oxidative damage.	NTP (2014)
	Rat, F344/ NTac (M/F)		+	All exposed groups combined (1.25, 2.5, and 5 mg/m^3)		Evaluation of mutation spectra in cobalt-caused cancers. The spectra were compatible with oxidative damage.	
Micronucleus formation	Mouse, $B6C3F_1$ (M/F)	Peripheral blood NCEs	–	10 mg/m^3	Inhalation, 90 days, 3 dose levels	Guideline-based study, no decrease in PCE/NCE ratio. No bone marrow toxicity observed but general toxicity observed at the highest dose.	NTP (2014)
8-Oxo-dG adduct formation (immunohistochemistry)	Mouse, $B6C3F_1$/N (M/F)	Lung	+	10 mg/m^3	Inhalation, 90 days		Ton et al. (2021)
Cobalt metal (Co) NPs							
Gene mutation (*Gpt* locus)	Mouse, G*pt* delta transgenic (M/F)	Lung	+	50 µg/mouse	Intratracheal installation	Only one dose level.	Wan et al. (2017)
Oxidative DNA damage (8-OHdG, ELISA)	Mouse, *Gpt* delta transgenic (M/F)	Lung	+	50 µg/mouse	Intratracheal installation	Only one dose level.	Wan et al. (2017)
Soluble cobalt(II) salts							
Cobalt(II) chloride ($CoCl_2$)							
Aneuploidy, pseudodiploidy, and hyperploidy	Hamster (M)	Bone marrow and testes	(+)	400 mg/kg bw	Intraperitoneal injection, total dose given to each hamster over 9 days	Only one dose level, limited reporting, not sure if $CoCl_2$ anhydrous or hexahydrate. In testes, significant effect seen only in metaphase I, not in metaphase II.	Farah (1983)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Cobalt(II) acetate ($Co(CH_3CO_2)_2$)							
Oxidative DNA base damage (GC-MS)	Rat, F344/NCr (M/F)	Kidney, liver, and lung	+	50 µmol/kg bw [8.85 mg/kg]	Single intraperitoneal injection, evaluation 2 or 10 days after dosing, 2 dose levels	The bases determined were typical products of hydroxyl radical attack, mainly in liver and kidney.	Kasprzak et al. (1994)
Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)							
DNA damage (comet assay)	Rat, Sprague-Dawley (M)	Liver	(+)	10.37 ± 0.38 mg/day per rat	Oral via drinking-water, 4 wk exposure	Only one dose resulting in other liver effects (enzyme induction).	Khalil et al. (2020)
Micronucleus formation	Mouse, BALB/c AnNCj (M)	Bone marrow	+	50 mg/kg bw	Single intraperitoneal injection, 3 dose levels	Dose–response relationship observed, PCE/NCE ratio was decreased at the highest dose.	Suzuki et al. (1993)
Micronucleus formation	Mouse, Swiss albino (M)	Bone marrow	+	11.25 mg/kg bw	single intraperitoneal injection, 24 or 48 h after dosing, 3 dose levels	Dose-dependent increase, more significant effects 48 h after dosing, PCE/NCE ratio was unchanged or rather increased (highest dose, 48 h after the dosing).	Rasgele et al. (2013)
Chromosomal aberration	Mouse, Swiss albino (M)	Bone marrow	(+)	20 mg/kg bw	Oral, evaluation 6, 12, 18, and 24 h after dosing, 3 dose levels, 1×	Low number of cells analysed. Increases in chromosomal aberration frequencies and polyploid cells at all treatment times (including 6 h) raises questions.	Palit et al. (1991a)
Chromosomal aberration	Rat, Sprague-Dawley CD (M)	Testis (spermatogonia)	–	30 mg/kg bw	Gavage, 28 days, 3 dose levels	100 and 300 mg/kg resulted in overt toxicity. No concurrent positive control, but the laboratory had history of positive responses with mitomycin C.	Kirkland et al. (2015)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Rat, Wistar albino (M)	Bone marrow	(+)	300 mg/L in drinking-water [~36 mg/kg, estimated by the Working Group]	Oral via drinking-water, 7 days, 3 dose levels	The study had limited reporting (e.g. no information on the number of cells analysed or PCE/NCE ratios). No positive control, micronucleus induction several times that in other studies.	Awoyemi et al. (2017)
Micronucleus formation	Rat, Sprague-Dawley (M)	Bone marrow	–	600 mg/kg bw	Gavage, 3 dose levels, 1×	Non-published guideline-based study report, cited in ECHA (2016, 2017).	Study report, Gudi et al. (1998) in ECHA, (2016, 2017)
Chromosomal aberration	Rat, Sprague-Dawley (M)	Bone marrow	–	600 mg/kg bw	Gavage, 3 dose levels, 1×	Non-published guideline-based study report, cited in ECHA (2016, 2017).	Study report, Gudi et al. (1998) in ECHA, (2016, 2017)
Oxidative DNA damage (8-OHdG, ELISA)	Rat, Sprague-Dawley (M)	Kidney	(+)	300 mg/L in drinking-water	Oral via drinking-water, 4 wk	Only one dose, water consumption not reported, general toxicity seen as decreased weight gain, weights not reported.	Abdel-Daim et al., 2020
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	(–)	1000 mg/kg bw per day	Gavage, 2–5 days, 3 dose levels	Design complied with OECD guidelines, but deaths and early kills at two highest dose levels compromised the compliance. Two highest doses exceeded MTD, all groups were not evaluated and exposure duration of remaining rats was reduced.	Kirkland et al. (2015)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Insoluble cobalt (II or II,III) compounds							
Cobalt(II) oxide (CoO)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 5 days, 3 dose levels	OECD guideline-based study, no major limitations.	Kirkland et al. (2015)
Cobalt tetraoxide (Co_3O_4)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 5 days, 3 dose levels	OECD guideline-based study, no major limitations.	Kirkland et al. (2015)
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 2–4 days, 3 dose levels	Design complied with OECD guidelines, but deaths and early kills compromised the compliance. Two highest doses exceeded MTD, all study groups were not evaluated, and exposure duration of remaining rats was reduced.	Kirkland et al. (2015)
Organic cobalt(II) compounds							
Micronucleus formation	Mouse, Swiss Ico: OF1 (IOPS Caw) (M/F)	Bone marrow	–	500 mg/kg cobalt(II) acetyl acetonate	Gavage, dosing after 24 h, 3 dose levels	Highest dose resulted in deaths.	Kirkland et al. (2015)
Micronucleus formation	Mouse, Swiss Ico: OF1 (IOPS Caw) (M/F)	Bone marrow	–	1500 mg/kg cobalt(II) resinate	Gavage, dosing after 24 h, 3 dose levels	Highest dose resulted in decrease in PCE/NCE ratio.	Kirkland et al. (2015)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; bw, body weight; ELISA, enzyme-linked immunosorbent assay; F, female; GC-MS, gas chromatography-mass spectrometry; *Gpt*, guanine phosphoribosyltransferase; HID, highest ineffective dose; LED, lowest effective dose; M, male; MTD, maximum tolerated dose; NCE, normochromatic erythrocyte; OECD, Organisation for Economic Co-operation and Development; PCE, polychromatic erythrocyte; wk, week; yr, year.

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

well-characterized cobalt(II,III) oxide NPs (size, 47.0 ± 20.3 nm), with no induction of DNA strand breaks observed. In contrast, a concentration-dependent increase in DNA breaks was observed in the A549 cell line, although within the low dose range of 0.1–100 µg/mL. In agreement with this finding, a previous study using the A549 and BEAS-2B cell lines showed that exposure to elongated hexagonal cobalt(II,III) oxide NPs (d_{50}, 22.1 ± 7.2 nm) resulted in a dose-dependent positive trend with a significant increase in DNA damage solely observed at the highest concentration tested (Cavallo et al., 2015). Alarifi et al. (2013) showed that low concentrations of polygonal NPs (size, approximately 21 nm) induced DNA damage in the HepG2 cell line. Cappellini et al. (2018) reported that cobalt(II,III) oxide NPs (size, 51 ± 11 nm) failed to induce DNA strand breaks and oxidative DNA damage in the A549 cell line and immortalized human bronchial epithelial cells, suggesting that cobalt(II,III) oxide NPs have lower genotoxic potential than do cobalt metal NPs and cobalt(II) oxide NPs (Cappellini et al., 2018).

Organic cobalt(II) compounds

Cobalt(II) resinate did not induce chromosomal aberrations in human primary lymphocytes. However, the presence of the liver S9 metabolic activation system significantly increased the frequency of chromosomal aberrations compared with the negative control (Kirkland et al., 2015). [The Working Group noted that the results are difficult to interpret due to inconsistencies among experiments, the impact of cytotoxicity, and the occurrence of precipitation.]

(b) Experimental systems

The genotoxicities of cobalt metal and cobalt(II) salts have been extensively assessed in experimental systems both in vivo and in vitro. In vivo data have been summarized in Table 4.4 according to the form of cobalt studied, including cobalt metal, soluble cobalt(II) salts, insoluble cobalt(II or II,III) compounds, and cobalt metal NPs. Table 4.5, Table S4.6, and Table S4.7 include in vitro genotoxicity studies in non-human mammalian cells, non-mammalian cells, and acellular systems, respectively (for Tables S4.6 and S4.7, see Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Most of the data come from the cobalt(II) salts, most notably soluble cobalt(II) chloride. The data on cobalt metal are more limited when compared with the cobalt(II) salts. Although this evaluation is focused on cobalt metal and divalent cobalt salts, relevant data relating to some other cobalt compounds (like trivalent cobalt compounds) are briefly reviewed but not included in the tables.

(i) Non-human mammals in vivo

See Table 4.4.

Cobalt metal

There is only one in vivo animal study available on the genotoxicity of cobalt metal. NTP (2014) assessed micronucleus formation in peripheral blood normochromatic erythrocytes of male and female mice after 3 months of whole-body inhalation exposure to metallic cobalt at concentrations of 0.625–10 mg/m^3. No increases in the frequencies of micronucleus formation were seen. Although exposure to the highest dose resulted in decreased body weight and lung toxicity, no bone marrow toxicity (measured as a change in the percentage of polychromatic erythrocytes, PCEs) was observed. Immunohistochemical analysis of 8-oxo-2′-deoxyguanosine (8-oxo-dG) in the lungs of both male and female mice at the highest dose (10 mg/m^3) revealed oxidative DNA damage to be significantly increased compared with non-exposed controls (see also Section 4.2.5) (Ton et al., 2021).

Analysis of the mutational spectra of lung tumours induced by cobalt metal in the NTP (2014) studies in mice and rats showed mutations

(especially G→T transversions) in K-*Ras*, *Tp53*, and *Egfr* genes compatible with oxidative DNA damage (Hong et al., 2015). [The Working Group noted that cobalt was one of just three carcinogens that showed specific carcinogen-induced genomic signatures when lung tumours caused by 20 different chemicals were analysed (Riva et al., 2020).]

Soluble cobalt(II) salts

Few in vivo genotoxicity studies have been reported that have investigated soluble cobalt(II) salts. Four studies performed using intraperitoneal administration of cobalt(II) salts have resulted in consistently positive genotoxic responses. These include an early study by Farah (1983), which reported increased numbers of hyperploid and pseudodiploid cells in the bone marrow of Syrian golden hamsters after repeated intraperitoneal administration of cobalt(II) chloride. In testis, an increased number of bivalents during meiotic metaphase I (but not during metaphase II) was reported. Suzuki et al. (1993) reported increased frequency of micronuclei in PCEs of BALB/c mice after single intraperitoneal administration of cobalt(II) chloride. In this study, cobalt chloride was shown also to enhance the genotoxicity of the known genotoxic agents 1,1-dimethylhydrazine and benzo[*a*]-pyrene, which was partly considered to be due to an acceleration of erythropoiesis (Suzuki et al., 1993). An increase in bone marrow PCE micronuclei frequencies in Swiss albino mice after intraperitoneal administration of cobalt(II) chloride has also been reported by Rasgele et al. (2013). The most significant increases were seen 48 hours after treatment.

Dose-dependent increases in oxidative DNA damage were seen in kidney, liver, and lung tissues in rats treated by intraperitoneal administration of cobalt(II) acetate 2 and 10 days before tissue collection. The most significant increases were seen in kidney and liver, which is in accordance with the distribution of soluble cobalt(II) salts after intraperitoneal administration (Kasprzak et al., 1994) (see also Table 4.4 and Section 4.2.5).

The data available relating to genotoxicity after oral administration are less clear. One study has reported DNA damage, as assessed by comet assay, in liver after 4-week exposure to cobalt(II) chloride in drinking-water (reported cobalt dose, 10.37 ± 0.38 mg/day per rat) (Khalil et al., 2020). [The Working Group noted that the study included only one dose level, which appeared to cause liver toxicity indicated by significant elevations in liver enzyme and bilirubin levels.] Palit et al. (1991a) reported dose- and time-dependent increases in chromosomal aberration frequencies in bone marrow in mice 6–24 hours after oral administration by gavage of a single dose of cobalt(II) chloride (0, 20, 40 or 80 mg/kg bw). Similar dose responses were reported at all sampling times tested. [The Working Group noted that this finding, suggesting similar effects at all stages of the cell cycle, is a rather uncommon finding for genotoxicants.] Also, the frequency of several polyploid cells was increased at all time points. [The Working Group noted that the increases in the number of polyploid cells starting at the 6-hour time point is hard to explain, which raises questions about the reliability of this study.] The same group has also published related studies evaluating the modifying roles of calcium and chlorophyllin on the clastogenicity of cobalt(II) chloride (Ghosh et al., 1991; Palit et al., 1991b). Since these studies report the same cobalt(II) chloride results as Palit et al. (1991a), they have not been included in Table 4.4. Kirkland et al. (2015), on the other hand, reported negative results from a set of guideline-based chromosomal aberration studies in rats after oral administration, by gavage, of different (soluble and insoluble) cobalt(II) salts: cobalt(II) sulfate heptahydrate (100, 300, or 1000 mg/kg bw per day), tricobalt tetraoxide (Co_3O_4, synonym of cobalt tetraoxide or cobalt(II,III) oxide) (200, 600, or 2000 mg/kg bw per day), or cobalt monoxide (CoO, synonym

Table 4.5 Genetic and related effects of cobalt in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Cobalt metal						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	+	30 µg/mL	OECD guideline 476, high level of cobalt powder precipitation was observed.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	350 µg/mL	OECD guideline 476, extracts of cobalt metal powder were tested.	Kirkland et al. (2015)
Cobalt metal NPs						
DNA strand breaks (comet assay)	Mouse (BALB/3T3), fibroblasts	+	NT	1 µM	No dose–response, but high cytotoxicity at highest doses.	Ponti et al. (2009)
Micronucleus formation	Mouse (BALB/3T3), fibroblasts	+	NT	1 µM		Ponti et al. (2009)
DNA strand breaks, comet assay	Rat, kidney, NRK cells	(+)	NT	100 µM	Diameter, 20–50 nm. Single dose only.	Liu et al. (2017)
DNA strand breaks (comet assay)	Rat, liver, BRL-1A cells	(+)	NT	10 µM	Median size, 30–70 nm. The study does not provide quantitative results from the comet assay.	Liu et al. (2016)
DNA damage (comet assay without Fpg enzyme)	Mouse, embryo, fibroblasts (wildtype *Ogg*$^{+/+}$ and MEF *Ogg*$^{-/-}$ cells)	–	NT	1 µg/mL	Size, < 50 nm. No change in % tail DNA	Annangi et al. (2015)
Oxidative DNA damage (comet assay with Fpg enzyme)		+ (*Ogg*$^{-/-}$) – (*Ogg*$^{+/+}$)	NT NT	0.1 µg/mL 1 µg/mL	Dose-dependent effect observed only in Ogg1 knockout (mouse embryonic fibroblast *Ogg1*$^{-/-}$) cells but not in wildtype cells.	Annangi et al. (2015)
DNA strand breaks (comet assay)	Rat, CD34+ haematopoietic stem cells and haematopoietic progenitor cells	(+)	NT	200 µM [11.8 µg/mL]	Size, 50–200 nm. One dose only.	Zhu et al. (2021b)
Oxidative DNA damage (8-OHdG, ELISA)		(+)	NT	200 µM	One dose only	Zhu et al. (2021b)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Activation of γH2AX		(+)	NT	200 µM	One dose only.	Zhu et al. (2021b)
Rtkn-GFP and Srxn1-GFP reporter activity	Mouse embryonic stem cells (mES)	↑ Rtkn-GFP ↑ Srxn1-GFP	NR	Co (0–10 µg/mL), CoO and Co_3O_4 (0–100 µg/mL), 24 h	Assessment of (geno)toxicity including oxidative stress using ToxTracker assay.	Cappellini et al. (2018)
Soluble cobalt(II) salts						
Cobalt(II) chloride (hydrate not specified)						
DNA strand breaks (alkaline sucrose gradient)	Chinese hamster, ovary	+	NT	5 mM [650 µg/mL]		Hamilton-Koch et al. (1986)
DNA strand breaks (nucleoid sedimentation assay)	Chinese hamster, ovary	–	NT	10 mM [1300 µg/mL]		Hamilton-Koch et al. (1986)
DNA–protein cross links	Rat, Novikoff ascites hepatoma cells	+	NT	1 mM [130 µg/mL]		Wedrychowski et al. (1986)
DNA damage, integrity of nuclear genome (qPCR assay)	Rat, neuronal PC-12 cells	–	NT	100 µM [13 µg/mL]	Negative response in nuclear genome, but positive mitochondrial DNA (which might be related to hypoxia).	Wang et al. (2000)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	(+)	NT	200 µMM [26 µg/mL]	Weak response and only one dose with high cytotoxicity.	Miyaki et al. (1979)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cell	–	NT	100 µM [13 µg/mL]		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, V79 *Gpt*⁺ transgenic cell line G10	–	NT	100 µM [13 µg/mL]		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, *Gpt*⁺ transgenic cell line G12	+	NT	50 µM [6.5 µg/mL]		Kitahara et al. (1996)
Sister-chromatid exchange	Mouse, macrophage-like cells, $P388D_1$	+	NT	100 µM [13 µg/mL]		Andersen (1983)
Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_20$)						
DNA strand breaks (comet assay)	Mouse (BALB/3T3), fibroblasts, mouse	+	NT	1 µM [0.24 µg/mL]		Ponti et al. (2009)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA degradation (flow cytometry)	Mouse, heart, HL-1	(+)	NT	500 µM [120 µg/mL]	One dose only. Glucocorticoids protected from the cobalt-induced DNA damage.	Cruz-Topete et al. (2016)
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y cells	–	NT	57.11 µg/mL		Amacher & Paillet (1980)
Gene mutation (*Hprt* locus, *8AG*-resistant mutation)	Chinese hamster, V79 cells	–	NT	9 µM [2 µg/mL]	Low dose compared with in V79 cells Hartwig et al. (1990).	Yokoiyama et al. (1990)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	+	NT	100 µM [24 µg/mL]		Hartwig et al. (1990)
Gene mutation (*Hprt* locus)	Mouse, mammary carcinoma, FM3A cells	(+)	NT	200 µM [48 µg/mL]	Modified HPRT assay, only weak response.	Morita et al. (1991)
Sister-chromatid exchange	Chinese hamster, V79 cells	+	NT	10 µM [2.4 µg/mL]		Hartwig et al. (1991)
Micronucleus formation	Mouse (BALB/c), bone marrow cells, PCE	–	–	50 µg/mL		Suzuki et al. (1993)
Micronucleus formation	Mouse (BALB/3T3), fibroblasts	–	NT	10 µM [2.4 µg/mL]		Ponti et al. (2009)
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)						
Micronucleus formation	Syrian hamster, embryo	+	NT	1 µg/mL		Gibson et al. (1997)
Cobalt(II) sulfate ($CoSO_4$)						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	100 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	120 µg/mL	Cobalt(II) 2-ethylhexanoate ($C_8H_{15}CoO_2$+).	Kirkland et al. (2015)
Insoluble cobalt(II or II,III) compounds						
Cobalt(II) sulfide (CoS)						
DNA strand breaks (alkaline sucrose gradient)	Chinese hamster, ovary, CHO cells	+	NT	10 µg/mL		Robison et al. (1982)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	–	NT	1 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, transgenic cell line G10	–	NT	1 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, transgenic cell line G12	+	NT	0.5 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	922 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Cobalt(II) oxide (CoO)						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	60 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Cobalt tetraoxide						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	2000 µg/mL without S9 750 µg/mL with S9	OECD guideline test.	Kirkland et al. (2015)
Cobalt dihydroxide						
Gene mutation, *Hprt* locus	Mouse, lymphoma L5178Y cells	–	+/–	30 µg/mL	Positive responses in 2 out of 3 experiments at the highest dose showing high cytotoxicity.	Kirkland et al. (2015)
Cobalt(II,III) oxide NPs						
DNA strand breaks (comet assay)	Rat, cardiomyocytes	+	NT	5 µg/mL	Co_3O_4 NPs; non-spherical; mean diameter, 17 nm; forming agglomerates of tens of NPs.	Savi et al. (2021)
Organic cobalt(II) compounds						
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y *Tk*± cells	–	–	100 µg/mL	Cobalt resinate ($C_{40}H_{58}CoO_4$): top two doses resulted in precipitation.	Kirkland et al. (2015)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y *Tk*$^{\pm}$ cells	+/–	–	91.625 µg/mL	Cobalt acetyl acetonate ($C_{10}H_{14}CoO_4$): positive responses mainly at the highest dose in the presence of significant cytotoxicity.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	70 µg/mL	Cobalt oxalate (CoC_2O_4): highest dose resulted in precipitation, cytotoxicity noted at lower concentrations as well.	Kirkland et al. (2015)

8AG, 8-azaguanine; FPG, formamidopyrimidine DNA glycosylase; *Gpt*, guanine phophoribosyltransferase; γH2AX, phosphorylated form H2A histone family member X; HID, highest ineffective dose; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase; LED, lowest effective dose; NT, not tested; OECD, Organisation for Economic Co-operation and Development; Ogg, 8-oxoguanine DNA glycosylase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCE, polychromatic erythrocyte; qPCR, quantitative polymerase chain reaction; *Tk*, thymidine kinase.

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

of cobalt(II) oxide) (200, 600, or 2000 mg/kg bw per day) for 2–5 consecutive days that did not result in treatment-related increases in chromosomal aberration frequencies. However, exposure to cobalt(II) sulfate or cobalt oxide resulted in mortalities, and thus the exposure duration was reduced from the original 5 days for the remaining animals (Kirkland et al., 2015). [The Working Group noted that due to high mortality, no chromosomal aberration frequency could be determined for some groups and that in other groups the number of cells examined was reduced.] No increase in spermatogonial chromosomal aberrations was observed in rats treated with cobalt(II) chloride hexahydrate by gavage for 28 days at doses of 3, 10, and 30 mg/kg bw per day (Kirkland et al., 2015). One additional study reported a frequency of bone marrow micronuclei in rats treated with drinking-water containing cobalt(II) chloride for 7 days that was many times that of controls (Awoyemi et al., 2017). [The Working Group noted that the frequency of micronucleus formation in PCEs of exposed rats was several times higher when compared with observations in other in vivo studies. In addition, no positive control was included, and no information on, for example, the numbers of cells counted or PCE/normochromatic erythrocyte ratios was provided.]

Existing cobalt assessment reports (OECD, 2014; ECHA, 2016, 2017) include OECD guideline-compatible bone marrow chromosomal aberration and micronucleus tests, with cobalt(II) chloride showing no increased incidence of chromosomal aberrations after oral doses of up to 600 mg/kg bw (cited as "Study report, Gudi et al., 1998"). The highest doses were very high and probably above the maximum tolerated dose. Some reductions in the mitotic index and the percentage of PCEs were reported. [The Working Group noted that since the original study report is not publicly available, full evaluation of this study was not possible.] One study showed increased 8-OHdG levels, a marker of oxidative DNA damage, in rat kidney after administration of cobalt(II) chloride. However, only one dose was tested (see also Section 4.2.5) (Abdel-Daim et al., 2020).

Cobalt metal nanoparticless

In mice, intratracheal administration of cobalt metal NPs (mean diameter, 20 nm) (single dose, 50 μg/mouse) resulted in increased frequencies of guanine phosphoribosyltransferase gene (*Gpt*) mutations in lung genomic DNA and increased 8-OHdG levels (see also Section 4.2.5) (Wan et al., 2017). Analysis of the mutational spectra revealed that exposure to cobalt metal NPs caused mainly G→C to T→A transversions, which is in agreement with oxidative DNA damage.

Organic cobalt(II) compounds

Kirkland et al. (2015) reported results of studies of bone marrow micronucleus formation after mice were treated with the organic cobalt compounds cobalt(II) resinate and cobalt(II) acetyl acetonate, orally administered twice at three different dose levels. No increase in the frequency of micronucleus formation was observed when the compounds were tested up to toxic levels.

(ii) Non-human mammalian cells in vitro

See Table 4.5.

Cobalt metal

In a guideline-based study, exposure to cobalt metal induced increased incidence of hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) mutations when tested with metabolic activation in mouse L5178Y lymphoma cells (Kirkland et al., 2015). Instead results were largely negative without metabolic activation. However, the cobalt metal powder clearly precipitated in culture medium, at multiple concentrations. Extracts (non-precipitated fractions) of cobalt metal powder, prepared from culture media buffered solutions, were also tested up to cytotoxic levels. The weak responses seen with cobalt metal

powder were not reproduced with extracts of the powder.

Soluble cobalt(II) salts

Cobalt(II) chloride (either anhydrous or hexahydrate) has been tested in several experimental systems for its ability to cause DNA strand breaks, gene mutations, or chromosomal damage. It has been shown to induce DNA strand breaks via an alkaline sucrose gradient (but not nucleoid sedimentation assay) and a reduction of cloning efficiency in Chinese hamster ovary cells (Hamilton-Koch et al., 1986). Fibroblasts from BALB/3T3 mice, as assessed by comet assay, showed increased levels of DNA damage after 2-hour exposure to subtoxic concentrations of cobalt(II) chloride (Ponti et al., 2009). Wang et al. (2000) reported increased DNA damage in mitochondrial, but not nuclear, DNA of the rat neuronal PC-12 cell line. Cobalt(II) chloride has been also reported to cause protein–DNA cross-links in Novikoff ascites hepatoma cells (Wedrychowski et al., 1986) and DNA degradation in the mouse cardiomyocyte cell line HL-1 (Cruz-Topete et al., 2016).

Studies concerning gene mutations caused by cobalt(II) chloride have shown inconsistent results. Cobalt(II) chloride hexahydrate induced gene mutations in the *Hprt* locus in the Chinese hamster V79 cell line in one study (Hartwig et al., 1990), whereas another study using cobalt(II) chloride showed only weakly positive results (Miyaki et al., 1979) and a third study reported negative results (Kitahara et al., 1996). Kitahara et al. (1996) studied induction of gene mutations in the *Gpt* locus in Chinese hamster *Gpt*$^+$ transgenic G10 and G12 cell lines: in G12 cells, clear induction of mutations was observed, whereas no induction was seen in G10 cells (Kitahara et al., 1996). Weak induction of mutations was observed in the mouse mammary carcinoma FM3A cell line, as assessed by modified *Hprt* assay, in a study by Morita et al. (1991). No increased gene mutation frequencies after exposure to cobalt(II) chloride hexahydrate were observed at the *Tk* locus in the mouse lymphoma L5178Y cell line (Amacher & Paillet, 1980) or at the *Hprt* locus (8-azaguanine-resistant) in the Chinese hamster V79 cell line (Yokoiyama et al., 1990). [However, the Working Group noted that the doses in Yokoiyama et al. (1990) were low compared with other gene mutation studies using cobalt(II) chloride hexahydrate.]

Cobalt(II) chloride has been reported to induce sister-chromatid exchange in the mouse P388D1 (Andersen, 1983) and Chinese hamster V79 cell lines (Hartwig et al., 1991). However, tests of micronucleus formation in BALB/3T3 mouse fibroblasts and in BALB/c mouse bone marrow cell suspensions have been negative (Suzuki et al., 1993; Ponti et al., 2009).

In addition to cobalt chloride, in vitro genotoxicity studies have been performed with other cobalt(II) compounds. Kirkland et al. (2015) reported a series of *Hprt* locus gene mutation studies in the mouse lymphoma L5178Y cell line using different (soluble and insoluble) cobalt(II) compounds, including cobalt sulfate, cobalt 2-ethyl hexanoate, cobalt sulfide, cobalt tetraoxide, cobalt(II) oxide, and cobalt dihydroxide; (organic) cobalt(II) oxalate; and some other cobalt compounds not considered relevant for this assessment (lithium cobalt dioxide, cobalt oxide hydroxide, and cobalt borate neodecanoate). Although sporadic and statistically significant increases in mutation frequencies were seen with some of these compounds, there were no dose–response relationships. The resulting increases in mutation frequencies were below biological significance, which was considered to be an increase of threefold over the mean level for historical controls.

Cobalt(II) sulfate heptahydrate has been reported to induce a dose-dependent increase in micronucleus formation in Syrian hamster embryo (SHE) cells (Gibson et al., 1997).

Insoluble cobalt(II or II,III) compounds

In earlier studies with cobalt(II) sulfide, Kitahara et al. (1996) reported induction of gene mutations at the *Gpt* locus in Chinese hamster *Gpt*$^{+}$ transgenic G12 cells, but not in G10 cells. Negative results were obtained in the *Hprt* locus of Chinese hamster V79 cells (Kitahara et al., 1996). Co-culture with H_2O_2 did not result in increased mutation frequencies. Cobalt(II) sulfide particles have also been reported to induce DNA strand breaks in Chinese hamster ovary cells (Robison et al., 1982).

Cobalt-based nanoparticless

Cobalt metal NPs (size range, 20–500 nm; peak, 80 nm) induced DNA damage (as assessed by comet assay) and micronucleus formation in BALB/3T3 mouse fibroblasts, which were not dose-dependent. The reported lack of dose-dependency may have been due to the cytotoxicity at higher doses. [The Working Group noted that genotoxic effects were associated with significant uptake of cobalt metal NPs into the cells, possibly via a Trojan horse-type mechanism (Ponti et al., 2009).] Exposure of the rat kidney NRK cell line to cobalt NPs (diameter, 20–50 nm) at a concentration of 100 µM for 4–48 hours resulted in DNA damage, as assessed by comet assay, and cytotoxicity in a study by Liu et al. (2017). The same group also reported time- and dose-dependent genotoxicity, as assessed by comet assay, in normal rat liver cells cultured in vitro; however, no quantitative results were provided (Liu et al., 2016). No increase in DNA damage, as assessed by comet assay, was observed in wildtype mouse embryonic fibroblasts (MEF *Ogg1*$^{+/+}$) and derived 8-oxoguanine DNA glycosylase (*Ogg1*) knockout (MEF *Ogg1*$^{-/-}$) cells after exposure to cobalt NPs (Annangi et al., 2015). However, when the comet assay was performed with Fpg enzyme to detect 8-OHdG, a dose-dependent increase in oxidative DNA damage was observed in knockout (MEF *Ogg1*$^{-/-}$) cells but not in wildtype cells (Annangi et al., 2015) (see also Section 4.2.5). In a study by Zhu et al. (2021b), exposure of rat CD34+ haematopoietic stem cells/haematopoietic progenitor cells to cobalt NPs (size, 50–200 nm) resulted in DNA damage (as assessed by comet assay), activation of DNA damage-responsive γH2AX, and increased 8-OHdG levels (see also Section 4.2.5).

Cobalt(II,III) oxide NPs (non-spherical; mean diameter, 17 nm; forming small agglomerates of tens of NPs) have been reported to induce dose-dependent DNA strand breaks in rat cardiomyocytes cultured in vitro, as assessed by comet assay (Savi et al., 2021).

Organic cobalt(II) compounds

Two organic cobalt(II) compounds, cobalt resinate and cobalt acetyl acetonate, were tested for their ability to induce mutations in the *Tk* locus, as assessed by mouse lymphoma L5178Y Tk$^{+/-}$ assay (Kirkland et al., 2015). Cobalt resinate yielded negative results, whereas cobalt acetyl acetonate gave equivocal results with some positive responses at the highest doses tested mainly without metabolic activation. Relatively high toxicity was also observed at these doses.

(iii) Non-mammalian experimental systems

See Table S4.6 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal

Cobalt metal has been tested in *Salmonella typhimurium* strains TA98 and TA100 (NTP, 2014; Kirkland et al., 2015) and in *Escherichia coli* strain WP2 (NTP, 2014) in reverse mutation assays. A weakly positive response was observed in the first study in strain TA98 without metabolic activation (NTP, 2014). In strain TA100, the result was equivocal in the absence of metabolic activation and negative in its presence. In *E. coli*, results remained negative both with and without metabolic activation (NTP, 2014). Kirkland et al. (2015) reported Ames test results, using *S.*

typhimurium strain TA98, from three individual laboratories that complied with GLP. All these tests gave negative results both with and without metabolic activation.

Soluble cobalt(II) salts

Numerous studies have tested cobalt(II) chloride (either anhydrous or hexahydrate) using non-mammalian experimental systems including bacteria (*S. typhimurium*, *E. coli*, and *Bacillus subtilis*), yeast (*Saccharomyces cerevisiae*), zebrafish (*Danio rerio*), earthworms (*Eisenia hortensis*), and fruit flies (*Drosophila melanogaster*). Reinardy et al. (2013) measured the induction of DNA damage in male zebrafish sperm by exposure to cobalt(II) chloride, as assessed by comet assay. Increased incidence of DNA strand breaks was observed immediately after exposure, but induction of DNA damage did not differ from controls when measured after a 6-day recovery period. Although there were also statistically significant changes in the expression of DNA repair genes, these changes were not dose-dependent (Reinardy et al., 2013). Induction of micronucleus formation and DNA strand breaks in coelomocytes of earthworms has been also reported after exposure to cobalt(II) chloride at dose levels close to the LD_{50} (Ciğerci et al., 2016).

Cobalt(II) chloride has been shown to induce mutations in *D. melanogaster mwh/flr* strain in multiple studies (Ogawa et al., 1994; Kaya et al., 2002; Demir et al., 2009, Vales et al., 2013, Ertuğrul et al., 2020). In yeast, cobalt(II) chloride (and its hexahydrate) has been reported to induce respiratory deficiency "petite" mutations and *Trp* conversions, but mostly negative results have been obtained for the induction of *Ilv* reverse mutations (Prazmo et al., 1975, Putrament et al., 1977, Egilsson et al., 1979; Fukunaga et al., 1982; Singh, 1983; Kharab & Singh, 1985; Kharab & Singh, 1987) (see Table S4.6 in Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Cobalt(II) chloride (anhydrous or hexahydrate) has been reported to be negative in multiple reverse mutation assays in several strains of *S. typhimurium* (TA98, TA100, TA102, TA1535, TA1538, and TA2637) (Tso & Fung, 1981; Mochizuki & Kada,1982; Arlauskas et al., 1985; Ogawa et al., 1986) (see Table S4.6 in Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Only positive findings were reported for the studies by Wong (1988) using strains TA98 and 1537 (without S9 mix), and by Pagano & Zeiger (1992) using strain TA97 in a preincubation assay. One study reported cobalt(II) chloride-induced mutagenesis in the *supF* transfer RNA gene of *E. coli* (Ogawa et al., 1999), whereas other available *E. coli* studies reported negative results (Kada & Kanematsu, 1978; Rossman et al., 1984; Arlauskas et al., 1985). Positive results were reported for one out of three mutation assays employing *B. subtilis* (Kanematsu & Shibata, 1980; Kanematsu et al., 1980); negative results were reported for the two other assays (Nishioka, 1975; Inoue et al., 1981). Chromosomal aberration test and comet assay results reported for a study of onion bulbs (*Allium cepa* L.) showed positive responses in one study at concentrations that also caused cytotoxicity (decreased mitotic index) (Yıldız et al., 2009).

Fewer data were available for other cobalt(II) salts. Cobalt(II) nitrate hexahydrate induced gene mutation and chromosomal deletion, non-disjunction, or mitotic recombination in *D. melanogaster* (Yeşilada, 2001). Cobalt(II) nitrate hexahydrate induced micronucleus formation and chromosomal aberration in onion bulbs (*A. cepa* L.) (Macar et al., 2020; Kalefetoğlu Macar et al., 2021). The concentration used in these studies resulted also in a significant reduction in mitotic index. Erturk et al. (2013) reported induction of DNA damage in maize (*Zea mays* L.) after exposure to cobalt(II) nitrate hexahydrate. In an older study, nitrate salt of cobalt – either cobalt(II) or cobalt(III) nitrate – induced

mutations in the chlorophyll genes of *Pisum abyssinicum* (Von Rosen, 1964).

Cobalt(II) sulfate heptahydrate induced reverse mutations (without metabolic activation) in *S. typhimurium* strain TA100, but not in TA98 or TA1535 (Zeiger et al., 1992). However, this positive finding in TA100 was not repeated in subsequent studies performed independently by three different laboratories (Kirkland et al., 2015). A slightly positive response was observed by Rec assay in *B. subtilis* (strain H17 Rec+) exposed to cobalt(II) sulfate (Kanematsu & Shibata, 1980; Kanematsu et al., 1980). Cobalt(II) sulfate induced DNA double-strand breaks in *E. coli* (Kumar et al., 2017). These strand breaks were not associated with the induction of oxidative stress; however, cobalt(II) sulfate was observed to effectively inhibit the ROS-induced DNA repair pathways (Kumar et al., 2017). Cobalt(II) sulfate has been also reported to induce chromosomal aberrations and aneuploidy in plants (*A. cepa*) (Gori & Zucconi, 1957).

Cobalt(II) acetate tetrahydrate caused a positive response in a reverse mutation assay in *E. coli* strain WP2 without metabolic activation (Maeda et al., 2021).

Cobalt-based nanoparticles

Cobalt metal NPs (diameter, < 50 nm) have been reported to induce mutations in *D. melanogaster* mainly via somatic recombination mechanisms (Vales et al., 2013; Ertuğrul et al., 2020). Cobalt metal NPs also caused DNA damage in *D. melanogaster* haemocytes, as assessed by comet assay (Ertuğrul et al., 2020).

Cobalt(II,III) oxide NPs induced DNA strand breaks, as assessed by comet assay, in eggplant (*Solanum melongena* L. cv. Violetta lunga 2) (Faisal et al., 2016). The effects occurred concurrently with an increase in ROS levels. [The Working Group noted that significant induction of apoptosis was also observed at all concentrations tested.] Negative results were obtained in *S. typhimurium* strain TA98, as assessed by reverse mutation assay, using two different sizes of cobalt(II,III) oxide NPs (average size, 10–30 and 80–150 nm) (Kong et al., 2020).

Organic cobalt(II) compounds

Two organic cobalt(II) compounds, cobalt resinate and cobalt acetyl acetonate, did not increase mutation frequencies in any of the five different *S. typhimurium* strains tested (Kirkland et al., 2015).

(iv) Acellular systems

See Table S4.7 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal or cobalt metal nanoparticles

Cobalt metal induced DNA strand breaks in a study using purified DNA from mouse 3T3 cells (Anard et al., 1997). Ultrafine cobalt particles induced free-radical (oxidative) damage to supercoiled (bacteriophage) plasmid DNA (Zhang et al., 1998). [The Working Group noted that the mean diameter of the particles was 20 nm, being in the nanosized range.]

Soluble cobalt(II) salts

In early experiments conducted using calf thymus DNA, cobalt(II) chloride displaced acridine orange from DNA when assessed by fluorescence polarization, indicating an interaction of cobalt with DNA (Richardson et al., 1981). However, the effect was weaker than with, for example, iron or copper. The effect correlated with that reported by Sirover & Loeb (1976) on the fidelity of DNA synthesis. In their study, Sirover & Loeb (1976) reported that Co^{2+} substitutes Mg^{2+} in DNA polymerase (derived from *E. coli*, sea urchin, and avian myeloblastosis virus), resulting in an increase in the error frequency during DNA replication. Cobalt(II) chloride has induced DNA cleavage of the human c-Ha-*RAS*-1 proto-oncogene, but only in the presence of H_2O_2 (Kawanishi et al., 1989a, b; Yamamoto et al., 1989).

Table 4.8 Altered DNA repair and genomic instability in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
8-oxoG repair activity	Blood mononuclear cells	German, cross-sectional	78 workers with mixed metal exposures (cobalt, cadmium, and lead)	↓ 8-oxoG repair with increasing cobalt exposure; inversely correlated with the level of DNA single-strand breaks (R = –0.427, Pearson test; P = 0.001)	Age, sex, alcohol, smoking status	The potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to lead and cadmium, both also quantitatively assessed, is a strength. The air samples (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure. A limitation of this study is the reliance on a single biological measure of exposure. Detailed description of the 8-oxoG repair data not shown.	Hengstler et al. (2003)
DNA repair	Autopsy samples of brain and lung tissue	Mexico, urban air pollution exposure vs 2 less-exposed locations	47 exposed and 12 controls from less polluted cities (adults and children)	↓ OGG1 expression in olfactory bulb with ↑ frontal concentrations of cobalt (prob. t = 0.0161) ↓ LIG1 expression in olfactory bulb with ↑ frontal concentrations of cobalt (prob. t = 0.0306)	Age	Cobalt and other metals were quantitatively assessed using accepted methods. The potential for misclassification of cobalt exposure is low. A limitation of this study is the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest. Age, sex, place of residency, cause of death, and time between death and autopsy noted. Cause of death was considered for all subjects to rule out infection, inflammatory events, drug exposure, brain ischaemia, and hypoxia. Cobalt measured quantitatively as predictor of outcomes.	Calderón-Garcidueñas et al. (2013)

↑, increase; ↓, decrease; 8-oxoG, 8-oxoguanine; LIG1, DNA ligase I; OGG1, 8-oxoguanine DNA glycosylase; prob., probability; vs, versus.

Chemical changes were observed in chromatin isolated from the human K562 cell line and isolated calf thymus DNA after exposure to cobalt(II) sulfate, but only in the presence of H_2O_2 (Nackerdien et al., 1991). Kumar et al. (2017) showed that exposure to cobalt(II) sulfate changes the ellipticity of the plasmid DNA and inhibits DNA synthesis (Kumar et al., 2017). Exposure to cobalt(II) sulfate induced production of reactive hydroxyl species and resulted in deoxyribose degradation, which was only observed with co-treatment with H_2O_2 (Moorhouse et al., 1985).

4.2.3 Alters DNA repair or causes genomic instability

With the exception of direct reversal repair, all major DNA repair pathways progress in the same order: recognition of erroneous DNA, recruitment of repair components, removal of the erroneous part, reconstruction of the DNA, and reinstatement of the repaired part into the rest of the DNA structure (Kelley & Fishel, 2016). Specific players for each step vary by the types of DNA damage and the repair pathways deployed. Among major repair pathways – single-strand break repair includes base excision repair (BER), nucleotide excision repair (NER, including global genome NER and transcription-coupled NER), and mismatch repair; and double-strand break repair includes homologous recombination and non-homologous end joining (NHEJ). The effects of exposure to cobalt(II) salts in various steps involved in NER and in the reinstatement step of homologous recombination were studied in human cells in vitro and acellular systems, whereas the effects on BER were studied only assessed using a bacterial enzyme. Studies on other DNA repair pathways were not available to the Working Group. Cobalt(II) salts also decreased the mRNA (but not protein) expression of retrotransposons in two human cancer cell lines, which could result in genomic instability.

(a) Humans

(i) Exposed humans

See Table 4.8.

Evidence for perturbations in DNA repair in human populations exposed to cobalt included a cross-sectional study of German workers exposed to cobalt, cadmium, and lead (Hengstler et al., 2003). 8-Oxoguanine repair activity in blood mononuclear cells decreased with increasing cobalt exposure and was inversely correlated with the levels of DNA single-strand breaks ($r = -0.427$; $P = 0.001$). A similar dose–response relationship for cobalt (5–10 μg/m³) was observed for inhibition of 8-oxoguanine repair as for induction of DNA single-strand breaks; that is, repair activity for 8-oxoguanine decreased at concentrations of cobalt > 4 μg/m³. [The Working Group noted that the exposure to cobalt was low (range, 0–10 μg/m³ air) compared with the German TRK permissible exposure limit value of 100 μg/m³. In addition, while co-exposure to several metals was noted, regression analyses were performed to isolate the effects of cobalt alone.]

Another study examined brain and lung tissue samples, obtained via autopsies, to assess urban air pollution effects, including from exposure of people from urban versus less-exposed (control) locations in Mexico to metals (Calderón-Garcidueñas et al., 2013). While the cobalt concentrations in control versus urban-exposed frontal lobes were not significantly different (controls, 15 ± 2 μg/g dry weight tissue; exposed, 17 ± 2 μg/g dry weight tissue), regression analyses revealed that increased frontal lobe concentrations of cobalt correlated with decreases in 8-oxoguanine DNA glycosylase (*OGG1*) expression, which encodes the enzyme responsible for the excision of 8-oxoguanine in the olfactory bulb (probability, $t = 0.0161$). Cobalt also correlated inversely with the LIG1 gene (encodes for DNA ligase I), which functions in DNA replication and BER processes, in

Table 4.9 Altered DNA repair and genomic instability in human cells in vitro, non-human mammalian cells in vitro, and acellular systems exposed to cobalt

Pathway	Steps in the DNA repair pathway					Overall repair and notes
	Recognition→	Recruitment→	Removal→	Reconstruction→	Reinstatement	
Single-strand break repair	Co^{2+} did not affect polynucleotide kinase phosphatase function (Whiteside et al., 2010)[a]	NR	NR	NR	NR	NR
Nucleotide excision repair[b]	$CoCl_2$ ↑ XPA (Cycs4) protein level (Liu et al., 2012a)[c] $CoCl_2$ ↓ mouse XPA activity (Asmuss et al., 2000a, b)[e] $Co(NO_3)_2 \cdot 6H_2O$ substitute zinc in XPA zinc finger (Kopera et al., 2004)[a]		$CoCl_2$ ↓ incision (Kasten et al., 1997)[d]	$CoCl_2$ ↓ polymerization (Kasten et al., 1997)[d]	$CoCl_2$ did not affect ligation (Kasten et al., 1997)[d]	$CoCl_2$ ↓ overall repair (Hartwig et al., 1991)[c] $CoCl_2$ ↓ 8-oxo-dGTPase activity of human MTH1 protein (Porter et al., 1997)[a]
Base excision repair	$CoCl_2$ ↓ 8-oxo-dGTPase activity of bacterial MutT protein (Porter et al., 1997)[f]	NR	NR	NR	NR	NR
Homologous recombination repair	NR	NR	NR	NR	$CoCl_2$ ↓ *RAD51* mRNA level (Zhong et al., 2020)[c] $CoCl_2$ ↓ RAD51-positive foci (indicating repair) (Zhong et al., 2020)[c]	NR

↑, increased or upregulated; ↓, decreased or downregulated; 8-oxo-dGTPase, 8-oxo-2′-deoxyguanosine-5′-triphosphatase; mRNA, messenger RNA; MTH1, 8-oxo-dGTPase activity of recombinant human MutT homologue 1; NR, not reported (no study found); XPA, xeroderma pigmentosum complementation group A.

[a] Acellular system with human cell components.

[b] XPA is involved in both recognition and recruitment.

[c] Human cell line(s).

[d] Human primary cells.

[e] Used an acellular experimental system with mammalian cell components.

[f] Used an acellular experimental system with bacterial components.

the olfactory bulb (probability, $t = 0.0306$). [The Working Group noted that while co-exposure to several metals was noted, regression analyses were performed for individual metals and did not account for co-exposures. Cobalt and other metals were quantitatively assessed using accepted methods. The potential for misclassification of cobalt exposure is low. A limitation of this study was the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest.]

[The Working Group noted that there were two studies of cobalt-exposed populations with some evidence of alterations in DNA repair.]

(ii) Human cells in vitro

See Table 4.9.

Non-cytotoxic concentrations of cobalt(II) chloride inhibited the incision and polymerization steps of NER of ultraviolet (UV)-induced DNA damage in primary human skin fibroblast cells, which were derived from a normal donor (Kasten et al., 1997). The incision of UV-induced DNA damage was inhibited by 2-hour exposure to cobalt(II) chloride at 50 µM, while incision at cyclobutane pyrimidine dimer sites was inhibited only at concentrations of 150 or 200 µM, suggesting that cobalt might affect the removal of (6–4) photoproducts more than the removal of pyrimidine dimers (Kasten et al., 1997). Co-induced inhibition of polymerization was reversed by bivalent magnesium, suggesting metal substitution. The ligation step was not affected by cobalt (Kasten et al., 1997).

The removal of UV-induced thymine dimers in the HeLa cell line was decreased by cobalt(II) chloride hexahydrate at non-cytotoxic concentrations without increasing UV-induced thymine dimers or strand breaks (Hartwig et al., 1991).

When the human lung cancer cell lines Calu-6 and SK-LU-1 were exposed to cobalt(II) chloride [source and purity not reported] at a concentration of 500 µmol/L for 24 or 48 hours, protein levels of HIF-1α and xeroderma pigmentosum complementation group A (XPA) were increased (Liu et al., 2012a). Cobalt(II) chloride-induced increases in HIF-1α and XPA protein levels were not seen in the H358 cell line, which has "high levels of XPA protein concomitant with enrichment of the HIF-1α protein at the XPA promoter", suggesting that the increase of XPA was via HIF-1α binding to the promoter of XPA (Liu et al., 2012a).

Exposure to cobalt(II) chloride at a concentration of 100 µM for 24 or 72 hours decreased *RAD51* messenger RNA (mRNA) levels and RAD51 foci-positive cells, respectively, suggesting impaired homologous recombination repair, while γH2AX foci-positive cells increased, suggesting an increase in double-strand breaks (see Section 4.2.2) (Zhong et al., 2020). All these effects were in human colorectal cancer cell lines with *K-RAS* mutation – HCT 116, SW620, and LoVo – with the exception that *RAD51* mRNA was not measured in the LoVo cell line. [The Working Group noted that cobalt(II) chloride might decrease DNA repair, because double-strand breaks would increase *RAD51* mRNA, which plays an important role in homologous recombination, rather than the observed mRNA decrease.] [The Working Group noted that hypoxia might be how cobalt(II) chloride decreased *RAD51* mRNA, because bevacizumab (humanized anti-VEGF monoclonal antibody) also induced hypoxia and decreased *RAD51* gene expression (Zhong et al., 2020).]

The human genome rearranges itself via mobile elements, which include transposons and retrotransposons. Transposons operate in a "cut-and-paste" fashion, while retrotransposons operate in a "copy-and-paste" fashion and consequently increase their copy numbers in the genome faster than transposons (Kim et al., 2012). The only autonomous and active retrotransposons are the long interspersed elements (LINEs), including LINE-1, LINE-2, and LINE-3. LINE-1 can contribute to carcinogenesis via

insertion into oncogenes and tumour suppressor genes, creating DNA damage, increasing genomic instability, and affecting immune function (Zhang et al., 2020). While subtoxic concentrations of cobalt(II) chloride increased *LINE-1* mRNA levels in human neuroblastoma BE(2)-M17 and HeLa cell lines, but not in primary human fibroblasts derived from ATM patients (denoted "HF D") or healthy individuals ("HF WT"), it did not significantly increase LINE-1 promoter activity or protein levels in any of the four cell types tested (Habibi et al., 2014). Furthermore, retrotransposition of LINE-1 was not increased in HeLa cells (El-Sawy et al., 2005; Habibi et al., 2014) or neuroblastoma cells (not tested in HF D or HF WT cells) (Habibi et al., 2014).

(iii) Acellular systems

See Table 4.9.

The 8-oxo-2′-deoxyguanosine-5′-triphosphatase (8-oxo-dGTPase) activity of recombinant human MutT homologue 1 (MTH1) protein was inhibited by 2.5-minute exposure to 6 mM Co^{2+} (cobalt(II) chloride) in the presence of 8 mM Mg^{2+} (magnesium acetate) (Porter et al., 1997). In the absence of Mg^{2+}, the natural activator of 8-oxo-dGTPase, Co^{2+} could restore roughly one third of the activity of MTH1, while other bivalent metals were much less effective or had no effect (Porter et al., 1997).

After preincubation with bivalent Co^{2+} [cobalt compound form, source, and purity were not reported] at a concentration of 200 μM for 10 minutes, whole-cell extracts from the human lung fibroblast MRC-5 cell line showed the same levels of phosphatase activity, kinase activity, and nick ligation of recombinant polynucleotide kinase phosphatase in repairing single-strand breaks on synthetic substrates as untreated whole-cell extracts (Whiteside et al., 2010). The substrates were double-stranded oligonucleotides with a nick in one strand, a single-stranded oligonucleotide, and a double-stranded oligonucleotide with nearly one half of its length being only one-stranded. With the placement of two different fluorophores and the addition of phosphate, the substrates enabled the investigation of different functions of polynucleotide kinase phosphatase.

p53 family members (p53, p63, p73, and their isoforms) are facilitators of DNA repair. p53 causes cell cycle arrest to allow time for repair, and p53 family members also control the transcription of many genes involved in DNA repair. Cobalt(II) chloride impaired the DNA-binding capacity of p53 protein in a study using purified protein and synthetic DNA. Cobalt(II) chloride decreased purified human p53 protein binding to supercoiled plasmid DNA that did not contain the p53 consensus binding site sequence (p53CON) as well as to p53CON in a linear DNA fragment (Paleček et al., 1999). Compared with $ZnCl_2$, cobalt(II) chloride had a weaker inhibitory effect on DNA–protein interaction (Paleček et al., 1999). DNA binding of p63 and p73 proteins was also inhibited by cobalt(II) chloride (Adámik et al., 2015). More specifically, the binding of p53 DNA binding domain, p63 protein, and p73 protein to the 474-base pair-long DNA fragment of p53CON was inhibited in each case by preincubating the proteins with cobalt(II) chloride (Adámik et al., 2015).

XPA proteins recognize DNA damage in global genome NER and recruit other DNA repair proteins in both global genome NER and transcription-coupled NER. Using a synthetic peptide representing the human XPA zinc finger sequence (XPAzf), Kopera et al. (2004) showed that Co^{2+} (from cobalt(II) nitrate hexahydrate) can substitute Zn^{2+} (from zinc(II) nitrate hexahydrate, $Zn(NO_3)_2{\cdot}6H_2O$) in XPAzf while keeping the XPAzf structure equivalent to the native zinc finger, although the interaction between Co^{2+} and XPAzf was 100 times weaker than Zn^{2+}. [The Working Group noted that the effects of Co^{2+} substitution on XPAzf function were not investigated and remain unclear.] Low oxidative activity

Table 4.10 Epigenetic alterations in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Extracellular vesicle miRNA	Maternal plasma	Multiple samples analysis within a prospective cohort study of pregnant women, USA	Subset (n = 184) of larger cohort of pregnant women with early and late pregnancy samples available	Inverse association between cobalt and miR-150-5p after multiple testing corrections; stronger effect estimate when evaluating late pregnancy miRNA levels (β, −0.20; 95% CI, −0.31 to −0.09 compared with β, −0.16; 95% CI, −0.24 to −0.07 for early pregnancy)	None	The potential for differential exposure misclassification is low. Exposure to other metals was considered. A limitation of this study is the reliance on a single biological measure of exposure.	Howe et al. (2021)

CI, confidence interval; miRNA, microRNA.

Table 4.11 Epigenetic alterations in human cells in vitro exposed to cobalt

End-point	Cell line	Results	Concentration	Comments	Reference
Cobalt(II) salts					
Histone post-translational modifications	Human embryonic kidney cells (HEK-293)	H3-Ser10 dephosphorylation and ↓ H3 pan-acetylation	50 µg/mL and 1000 µM for 24 h	Used two doses that are very similar	Verma et al. (2011)
	Human neuroepithelioma cells (SK-N-MC)	↑ H3K4me3, H3K9me2, H3K9me3, H3K27me3, H3K36me3, uH2A, and uH2B; ↓ H4ac	50 µg/mL and 1000 µM for 24 h		
Histone post-translational modifications	Human lung alveolar carcinoma cells (A549)	↑ H3K4me3, H3K9me2, H3K9me3, H3K27me3 and H3K36me3 as well as uH2A and uH2B at >200 mM; ↑ at 500 mM histone ubiquitination of di-uH2A; ↓ acetylation at histone H4 (AcH4)	200 µM for 24 h		Li et al. (2009)
	Human bronchial epithelial cells (BEAS-2B)	↑ H3K4me3, H3K9me2 and H3K27me3, ↓ AcH4 at 25, 50, 100 and 200 mM, whereas ↑ H3K9me3 only at 200 mM, H3K36me3 (50–200 mM), uH2A (150 mM) and di-uH2A (200 mM)			
Histone post-translational modifications	Human neuroblastoma cells (SHSY5Y)	↓ H3ac and H4ac	100~400 µM for 24 h		Guo et al. (2021)
m^{6}A modification	Human neuroblastoma cells (H4)	↓ expression of m^{6}A modification enzymes and inactivate demethylase to alter the m^{6}A methylation levels of genes	400 µM for 24 h	Did not measure the cytotoxic effects of $CoCl_2$ on H4 cells	Tang et al. (2020)
Histone post-translational modifications	Human renal proximal tubular cells (HK-2)	↑ H3K27ac	300 µM for 7 h	300 µM caused cytotoxic effects	Ha et al. (2018)
miRNA expression	Human umbilical vein endothelial cells (HUVECs)	↑ miR-21 expression	150 µM for 24 h	Only one dose	Xu et al. (2017)
miRNA expression	Human colorectal adenocarcinoma cells (HT-29)	↑ miR-210 expression	300 µM for 24 h	Only one dose	Nersisyan et al. (2021)
	Human colon adenocarcinoma cells (Caco-2)	↑ miR-210 expression	300 µM for 24 h	Only one dose	
miRNA expression	Human colon adenocarcinoma cells (Caco-2)	↓ miR-148a expression	300 µM for 24 h	Only one dose	Nersisyan et al. (2021)

↑, increased or upregulated; ↓, decreased or downregulated; ac, acetylation; H, histone; K, lysine; LEC, lowest effective concentration; m^{6}A, methylation of the adenosine base at the N^6 position of mRNA; me2, demethylation; me3, trimethylation; miRNA, microRNA; uH, ubiquitinated histone.

in the Co^{2+}–XPAzf complex was observed. [The Working Group noted that the functional effects of this are unclear.]

(b) Experimental systems

(i) Non-human mammals in vivo and non-human mammalian cells in vitro

No studies were available to the Working Group.

(ii) Acellular systems

See Table 4.9.

After 15-minute preincubation with Co^{2+} [cobalt form, source, and purity were not reported] (lowest effect concentration 100 μM), the DNA–protein binding activity of recombinant mouse XPA protein to UVC-damaged oligonucleotide was inhibited, as assessed by gel mobility shift assay (Asmuss et al., 2000a, b). [The Working Group considered that because of the presence of excess Zn^{2+} (simultaneous incubation with Zn^{2+} at 5 or 10 times the Co^{2+} concentration) largely prevented cobalt-induced XPA inactivation, the Co^{2+} effects might be via displacement of zinc in the zinc finger structure of the protein (Asmuss et al., 2000a).] In contrast, 15-minute preincubation of Co^{2+} at concentrations up to 1000 μM did not affect bacterial Fpg activity on oxidatively damaged supercoiled DNA derived from bacteriophage PM2 (Asmuss et al., 2000a, b).

In BER, the 8-oxo-dGTPase activity of recombinant bacterial MutT protein was inhibited by 5-minute exposure to 12 mM Co^{2+} (cobalt(II) chloride) in the presence of 8 mM Mg^{2+} (magnesium acetate) (Porter et al., 1997). In the absence of Mg^{2+}, the natural activator of 8-oxo-dGTPase, Co^{2+} could restore roughly three quarters the activity of MutT, while other bivalent metals (concentration range, 2.4–0.1 mM) were much less effective or had no effect (Porter et al., 1997).

4.2.4 Induces epigenetic alterations

(a) Human

(i) Exposed humans

See Table 4.10.

In a multiple-sample analysis as part of a prospective cohort study in pregnant women in the USA, Howe et al. (2021) examined maternal plasma for external vesicle microRNA, which contributes to maternal–fetal communication and is dysregulated during pregnancy complications. Within a subset (n = 184) of the larger cohort, an inverse association between cobalt levels (early pregnancy) and miR-150-5p (in late pregnancy) was reported after multiple testing corrections. The findings showed a stronger effect estimate when evaluating late pregnancy microRNA levels (β, −0.20; 95% CI, −0.31 to −0.09 compared with β, −0.16; 95% CI, −0.24 to −0.07 for early pregnancy).

(ii) Human cells in vitro

See Table 4.11.

Epigenetic modifications are stable and heritable alterations that are mainly driven by three tightly regulated and interconnected processes: DNA methylation, modification of histones, and regulation of non-coding RNAs that alter DNA accessibility and chromatin structure, thereby modulating gene expression patterns (Strahl & Allis, 2000; Robertson, 2005; Portela & Esteller, 2010; Chervona & Costa, 2012). [The Working Group noted that exposure to cobalt metal or cobalt(II) salts appears to induce epigenetic alterations; however, most of the results were in cancer cell lines.]

Exposure of human embryonic kidney (HEK-293) and neuroepithelioma (SK-N-MC) cell lines to cobalt(II) chloride at 50 μg/mL and 1000 μM for 24 hours caused dramatic dephosphorylation of serine 10 on histone H3 and decreased pan-acetylation of histone H3 (Verma et al., 2011).

Exposure to cobalt(II) chloride (at concentrations ≥ 200 μM) for 24 hours caused alterations in histone post-translational modifications (increased trimethylation of lysine 4, lysine 9, lysine 27, and lysine 36, and increased dimethylation of lysine 9 on histone H3; increased ubiquitination of histones H2A and 2HB; and decreased acetylation of histone H4) in human lung carcinoma (A549) or human bronchial epithelial (BEAS-2B) cell lines (Li et al., 2009). Co^{2+} may compete with iron (Fe^{2+}) for binding to JMJD2A, thus directly inhibiting JMJD2A demethylase activity and resulting in increased histone methylation. In addition, Co^{2+} may prevent de-ubiquitination, leading to increased ubiquitination of histone H2 (Li et al., 2009). GeneChip microarray results showed that exposure of A549 cells to cobalt(II) chloride at 200 μM for 24 hours caused alterations in the expression of multiple genes, including increased expression of genes in different functional classes, such as transcriptional activation (i.e. JMJD1A), cell defence (i.e. HMOX1 and BN1P3L), and DNA repair and cell cycle checkpoint control (i.e. GADD45A), and decreased expression of genes involved in tumour suppression (i.e. NBL1 and MTUS1) (Li et al., 2009).

Exposure of the human neuroblastoma SH-SY5Y cell line to cobalt(II) chloride (100–400 μM) for 24 hours caused decreased acetylation of histone H3 and H4 in a time- and dose-dependent manner. In addition, cobalt(II) chloride selectively decreased HAT activity and protein expression but had no effect on HDAC in the SH-SY5Y cell line (Guo et al., 2021). Exposure of the human renal proximal tubular epithelial cell line HK-2 to cobalt(II) chloride (300 μM) for 7 hours increased the level of acetylated lysine 27 residues on histone H3 (Ha et al., 2018). In addition, exposure to cobalt(II) chloride (400 μM for 24 hours) caused significant differences in the pattern of methylation of the adenosine base at the N^6 position of mRNA (m^6A) in the human brain neuroblastoma cell line H4 (Tang et al., 2020, 2022). Cobalt(II) chloride-induced ROS affected the m^6A modification of apoptosis-related genes by decreasing the expression of FTO, resulting in the activation of apoptosis (Tang et al., 2022). In addition, cobalt(II) chloride exposure induced changes in gene expression related to the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signalling pathway, AMP-activated protein kinase, insulin signalling, MAPK, and the axon guidance signalling pathway (Tang et al., 2020).

Exposure to cobalt(II) chloride also caused microRNA dysregulation. Exposure of primary human umbilical vein endothelial cells (HUVECs) to cobalt(II) chloride (150 μM) for 24 hours caused upregulation of miR-21, downregulation of programmed cell death 4 (PDCD4) protein expression, and attenuation of apoptosis (Xu et al., 2017). [The Working Group noted that miR-21 is one of the earliest identified cancer-promoting "oncomiRs", targeting numerous tumour suppressor genes associated with proliferation, apoptosis, and invasion (Si et al., 2007).] Exposure of the human colorectal cancer cell lines HT-29 and Caco-2 to cobalt(II) chloride (300 μM) for 24 hours caused consistent upregulation of miR-210; however, cobalt(II) chloride exposure caused downregulation of miR-148a in Caco-2 cells (Nersisyan et al., 2021). [The Working Group noted that miR-210 not only targets numerous prosurvival proteins and angiostatic factors, but also directly silences proteins essential for mitochondrial respiration and DNA repair (Devlin et al., 2011). In addition, miR-210 has been extensively studied in cancer progression (Bavelloni et al., 2017). It is known that miR-210 generally exhibits oncogenic properties, because it is frequently elevated in several cancers including breast, lung, head and neck, and pancreatic cancer, and glioblastoma (Bavelloni et al., 2017; Devlin et al., 2011; Qin et al., 2014). miR-148a is aberrantly expressed in a variety of tumours, which has been linked to tumour size, stage of development, metastasis,

Table 4.12 Oxidative stress in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Antioxidant enzyme activity (GSH, GPX, RG, CAT, and SOD)	Blood, urine, nails	Pakistan, Lahore district, cross-sectional	48 people from 4 different industrially contaminated areas with environmental exposure to chromium, cadmium, nickel, cobalt, lead, and zinc; 48 controls (not well defined); metals measured in urine, blood, and nails	↓ GPX with ↑ blood [cobalt] ($R = -0.358$; $P = 0.016$)	Age	Correlation for cobalt and effect; questionnaire used but statistics did not include covariates, except age.	Bibi et al. (2016)
8-OHdG adducts	Urine	European, multiple factories, cross-sectional study of genotoxic outcomes by exposure and DNA repair capacity	21 cobalt-exposed; 26 WC-Co-exposed; 26 non-exposed controls	For the total population, including controls ($n = 72$): ↑ 8-OHdG adducts for interaction term: exposure (cobalt alone or WC-Co) × smoking ($R^2 = 0.155$; $P = 0.003$) For the exposed only group ($n = 47$): ↑ 8-OHdG adducts for interaction term: type of plant (cobalt alone or WC-Co) × smoking ($R^2 = 0.173$; $P = 0.004$)	Genotypes, age, exposure, type of plant, smoking, interaction terms	The potential for differential exposure misclassification is low. There was likely exposure to other metals that was not considered. Cobalt exposure: 20 μg/m^3. Raw data not shown for adduct measures. No effect of repair gene polymorphisms.	Mateuca et al. (2005)

Table 4.12 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
MDA, 8-I	Blood	China, two sites (one former e-waste site, one control site)	Individuals (n = 62) exposed to lead, nickel, cobalt, mercury, copper, zinc, tin, and cadmium Non-exposed controls (n = 47)	Positive correlation between cobalt and 8-I in multilinear regression; B = 2176.57 (244.31–4108.83); P = 0.03 No significant association between cobalt and MDA	[Unclear as text states "different variables" considered in regression] [No statistical differences in age, height, weight, and BMI between exposed and controls]	There is potential for non-differential misclassification as the biological samples may not be reflective of historically relevant exposure. This study did assess and explicitly investigate other metals as co-exposures. The key limitation of this study is the reliance on a single biological sample. It is mentioned that the exposed group did not have drug or alcohol exposure, but it is unclear for the controls. Question about exposure measurement being reflective of historical exposure.	Xue et al. (2021)
MDA, 8-I	Blood	China, two sites (one former e-waste site, one control site)	Exposed (n = 69); non-exposed controls (n = 53) Significant ↑ blood concentrations of lead, nickel, cobalt, and mercury in exposed vs controls	In regression analyses, cobalt was positively correlated with 8-I (R = 0.456; P = 0.00) and MDA (R = 0.171; P = 0.161).	None	The potential for differential exposure misclassification is low with the measurements collected. Though other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses. A key limitation of this study is the reliance on a single biological measurement for exposure assessment. Although other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses.	Li et al. (2021b)

Table 4.12 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
8-OHdG adducts	Urine	Refinery workers from several factories in Belgium, Norway, Finland, Sweden, and England	Cobalt-exposed (n = 35/24*) WC-Co-exposed (n = 29) Non-exposed controls (n = 34/27)	NSS between exposed and controls Significant interaction effect for the type of plant (cobalt alone or WC-Co) and age (P = 0.0294)	Creatinine, urine Vitamin E (α-tocopherol), serum Selenium, serum Independent variables: exposure, plant type, cobalt in urine, age, smoking, vitamin E in serum, interaction between smoking and hard-metal exposures	A key limitation of this study is the reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes. Target cobalt: current TLV-TWA (20 mg/g creatinine). Multiple regression for influence of independent variables on outcomes including plant type (WC-Co or cobalt-alone exposure). *Reduced number of cobalt-exposed workers and non-exposed controls after one plant was dropped from study due to worker age differences.	De Boeck et al. (2000)

↓, decreased; ↑, increased; +, positive(ly); 8-I, 8-isoprostane; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BMI, body mass index; CAT, catalase; e-waste, electronic and/or electrical waste; GPX, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; NSS, not statistically significant; RG, reduced glutathione; SOD, superoxide dismutase; TLV-TWA, threshold limit value/time-weighted average; vs, versus; WC-Co, cobalt with tungsten carbide.

and prognosis (Li et al., 2016). miR-148a has also been reported to inhibit the migration, invasion, and proliferation of the colorectal cancer cell lines LoVo and SW480 in vitro (Zhao et al., 2019).]

(b) Experimental systems

(i) Non-human mammals in vivo

In the study by Tang et al. (2020), male C57BL/6 mice were intraperitoneally injected with 0, 4, 8, or 16 mg/kg bw of cobalt(II) chloride, once per day for 30 days. The results showed that the m^6A proportion of total RNA in the cortex of the cobalt(II) chloride-exposed mice was significantly decreased. Cobalt(II) chloride exposure caused alterations in m^6A methylation by dysregulating m^6A methyltransferases (METTLL3, METTL14, and WTAP) and demethylases (FTO and ALKBH5).

(ii) Non-human mammalian cells in vitro

Exposure of the mouse myoblast cell line C2C12 to 50 µg/mL of cobalt(II) chloride for 24 hours or hippocampal primary neurons (derived from P2 mouse pups) for 1 week caused dramatic dephosphorylation of serine 10 on histone H3 and decreased histone H3 pan-acetylation (Verma et al., 2011). Exposure of the rat pheochromocytoma PC-12 or mouse neuroblastoma Neuro-2a (N2a) cell lines to a single dose (300 µM) of cobalt(II) chloride for 24 hours caused decreased acetylation of histones H3 and H4 (Guo et al., 2021). Furthermore, exposure of the monkey kidney COS-7 cell line to cobalt(II) chloride also decreased global histone H3 and H4 acetylation levels in a dose-dependent manner. In addition, exposure to cobalt(II) chloride decreased acetylated histone enrichment within the proximal promoter region of the enzyme extracellular superoxide dismutase 3 (SOD3), leading to its decreased expression (Hattori et al., 2016).

4.2.5 Induces oxidative stress

(a) Humans

(i) Exposed humans

See Table 4.12.

In a study of people living in areas of the Islamic Republic of Pakistan that were industrially contaminated by multiple metals, including cobalt, Bibi et al. (2016) assessed a group of oxidative markers in blood, including glutathione (GSH), glutathione peroxidase (GPX), reduced GSH, CAT, and superoxide dismutase (SOD). Only GPX was associated with cobalt blood levels in correlation analyses, revealing an inverse correlation with cobalt exposure ($r = -0.358$; $P = 0.016$). [The Working Group noted that although other co-exposures were assessed, they were only considered individually in the analyses reported.]

In a cross-sectional study of European factory workers across several plants, some with exposure to cobalt alone and others to tungsten carbide particles (WC-Co), and including non-exposed controls, Mateuca et al. (2005) examined urinary 8-OHdG concentrations as a measure of systemic oxidative DNA damage. At the plants with cobalt-only exposure, no correlation was observed between urinary cobalt and urinary 8-OHdG concentrations. In multiple regression analyses of the total population, including the controls, a positive correlation was observed for 8-OHdG and the interaction term: any cobalt exposure (cobalt alone or WC-Co) × smoking ($R^2 = 0.155$; $P = 0.003$). When the exposed only group (either Co alone or WC-Co) was considered, regression models showed a positive correlation for 8-OHdG and the interaction term: type of plant (Co alone or WC-Co) × smoking ($R^2 = 0.173$; $P = 0.004$). [The Working Group noted that both findings were reported by the authors as having been observed only in the plants with WC-Co exposure and were thus uninformative.]

DeBoeck et al. (2000) assessed a similar cohort to that reported by Mateuca et al. of European factory workers from several plants, who were exposed to either WC-Co or cobalt metal alone (at different facilities) and compared oxidative stress markers in urine with non-exposed controls from the same plants, matching groups for age and smoking. No significant differences were seen between 8-OHdG concentrations in cobalt-exposed workers compared with controls. 8-OhdG was reported to be elevated in smokers who were exposed to hard-metal dust but not in those exposed to cobalt alone. [The Working Group noted that a significant interaction effect for the type of plant (exposure to cobalt alone or WC-Co) and age (P = 0.0294) was reported but attributed the findings to WC-Co exposure.]

In a Chinese study of e-waste-exposed populations living in close proximity to electronics recycling facilities, Xue et al. (2021) measured concentrations of multiple metals including cobalt and various end-points of interest, including levels of 8-isoprostane (8-I) and malondialdehyde (MDA) as indicators of an oxidative stress effect, in blood samples. A positive association for cobalt and 8-I in multilinear regression was reported (B, 2176.57; 95% CI, 244.31–4108.83; P = 0.03), but no significant association between cobalt and MDA was found. [The Working Group noted there is potential for non-differential misclassification, as the biological samples may not be reflective of historically relevant exposure. This study used multilinear models to adjust for co-exposures to other metals. The key limitation of this study was the reliance on single biological samples.]

In another similar study of e-waste-exposed populations in China, Li et al. (2021b) also measured multiple metals and 8-I and MDA in blood to identify evidence of oxidative stress. In regression analyses, cobalt exposure was positively correlated with 8-I (r = 0.456; P = 0.000) and MDA (r = 0.171; P = 0.161). [The Working Group noted that the potential for differential exposure misclassification is low with the measurements collected. Though other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses. The key limitation of this study was the reliance on a single biological measurement for exposure assessment.]

[The Working Group noted that three out of five studies of cobalt-exposed populations showed some evidence of oxidative stress.]

Scharf et al. (2014) examined the effects of cobalt and chromium in patients who had received metal hip replacement. [The Working Group deemed the study as uninformative because it was related to the effects of metal-on-metal implants, which were outside the scope of this evaluation.]

Walters et al. (2012) examined workers exposed to metalworking fluids. [The Working Group reviewed and excluded the study because 90% of the exposed workers also had elevated urinary levels of chromium, and effects attributable to cobalt exposure alone were not isolated using any statistical analyses. A key limitation of this study was the reliance on a single spot urine sample. Another limitation was the potential for exposure to other metals, including chromium and tungsten.] In addition, Arslan et al. (2011) measured oxidative stress in patients with malignant glioma compared with controls. [The Working Group reviewed and excluded the study because, although levels of multiple metals were measured, these metal levels were not used as predictor variables for the outcomes, and no differences in cobalt levels were observed between exposed individuals and controls. A key limitation of this study was the reliance on a single spot urine sample. The use of a single sample may not reflect the relevant exposure window, particularly as the sample was collected after the outcome.]

Table 4.13 Oxidative stress in human cells in vitro exposed to cobalt

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
Primary cells						
Cobalt(II) chloride ($CoCl_2$)						
Expression of HMOX1	Human primary PBMCs	–	No changes	24 µg/mL	Also tested Co metal (6 µg/mL) with same outcome.	Lombaert et al. (2013)
Mitochondrial superoxide	Human primary PBMCs	+	↑	20 µM	Only one end-point for oxidative stress measurement.	Chamaon et al. (2019)
ROS and GSH levels	Human dermal microvascular endothelial cells isolated from juvenile foreskin	+	↑ ROS level after 4 h and ↓ GSH after 24 h	0.5 mM	No. of samples was not specified.	Peters et al. (2007)
ROS level	Primary human MDMs; primary human pulmonary alveolar cells; human monocytic cell line U937	+	↑	100 µM		Nyga et al. (2015)
Oxidative DNA damage; 8-OHdG detection	Human diploid fibroblasts	–	No changes	250 µM		Ivancsits et al. (2002)
ROS level	Primary human dermal fibrobalsts	+	↑	100–300 µM		Xu et al. (2018a)
Cobalt(II) sulfate ($CoSO_4$)						
Superoxide radical (H_2O_2) formation	Human polymorphonuclear leukocytes obtained from healthy subjects	–	No changes	1 µM/2.5 × 10^5 cells for 30 min	$CoSO_4$ No. of samples was not specified. Only one concentration was tested.	Zhong et al. (1990)
Cobalt metal NPs						
Activities of SOD, GPX, and CAT	Human primary T-cells isolated from peripheral blood collected from healthy donors	+	↓	6 µM for 4 h	NP size, 30–70 nm Donor number was not specified. ↓ SOD, ↓ GPX, and ↓ CAT.	Jiang et al. (2012)
ROS level; GSH	Human dermal microvascular endothelial cells isolated from juvenile foreskin	+	↑ ROS level after 4 h and ↓ GSH after 24 h	25 µg/mL	NP size, 28 nm. No. of samples was not specified.	Peters et al. (2007)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level, 8-OHdG, GSH, and GPX activity	Human CD34+ haematopoietic stem cells/ haematopoietic progenitor cells were isolated from human cord blood vessels	+	↑ ROS level; ↓ GSH; ↓ GPX; ↑ 8-OHdG	200 µM	NP size, 50–200 nm. Decreased cell viability Selenomethionine partially attenuated NP-induced increase in ROS production, and restored the total antioxidant capacity and GSH level. No. of samples was not specified. IC_{50} for cell viability was 200 µM; 200 µM was the only concentration tested for oxidative stress.	Zhu et al. (2021a)
ROS level; GSH level; lipid peroxidation; HO-1 expression	Endothelial cells derived from human aorta (HAECs) and human umbilical vein (HUVECs)	+	↑ ROS level; ↓ GSH; ↑ lipid peroxidation; ↑ HO expression	20 µg/mL	NP size, 17 nm.	Alinovi et al. (2015)
ROS level	Human vascular endothelial HUVEC and HMEC-1 cells	+	↑	800 µM for 24 h	NP size, < 50 nm.	Zhu et al. (2021b)
Cobalt(II) oxide (CoO) NPs						
ROS level	Human primary lymphocytes from health subjects	+	↑	5 µg/mL	NP size, 62 ± 4 nm. Concentration-dependent increase, NAC has significant effects on preventing NP-induced cytotoxicity.	Chattopadhyay et al. (2015a)
Cobalt(II,III) oxide (Co_3O_4) NPs						
ROS level; lipid peroxides; CAT, SOD, and GSH activity	Human peripheral lymphocytes from healthy volunteers	+	↑ ROS level; ↑ lipid peroxides; ↓ CAT; ↓ SOD; and ↓ GSH	50–100 µg/mL for 24 h	NP size, 35.8 + 0.8 nm. Increased cytotoxicity ($n = 3$).	Rajiv et al. (2016)
ROS level	Primary human MDMs; primary human pulmonary alveolar cells; human monocytic cell line U937	+	↑	5 µg/mL	NP size, 2–60 nm. A comparable concentration of Co^{2+} ions did not exhibit such effects.	Nyga et al. (2015)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
Immortalized cell lines						
Cobalt(II) chloride ($CoCl_2$)						
Protein oxidation and nitration; HO-1, GPX, and CAT expression	MG-63 osteoblast-like cells	+	↑ Protein oxidation; ↑ nitration; ↓ HO-1 expression; ↑ GPX; no change to CAT	2.5–10 ppm		Fleury et al. (2006)
ROS level; HO-1 expression	MG-63 osteoblast-like cells	+	↑ ROS levels; ↑ HO-1 expression	200 µM		Li et al. (2017)
Protein oxidation	Macrophage cell line U937	+	↑	10 ppm	Statistical analysis was not clear.	Petit et al. (2005)
Protein nitration	Macrophage cell line U937	+	↑	10 ppm	Statistical analysis was not clear.	Petit et al. (2006)
ROS level	Human prostate cancer cell line PC-3M	+	↑	200 µM		Lu et al. (2007)
Cell viability; clonogenic survival; NAC addition	Human lung cancer cell line H460	+/–	H460 cells treated with cobalt(II) chloride at 300 and 400 µM for 48 h significantly decreased cell viability, at 100–300 µM for 48 h clonogenic survival significantly decreased; an antioxidant scavenger, was able to preserve cell viability and clonogenic survival	LEC was 300 µM for cell viability. LEC was 100 µM for clonogenic survival end-point.	This study did not directly analyse the effect on ROS production or oxidative stress markers.	Ma et al. (2011)
ROS level	Prostate cancer cell line PC-3	+	↑	21.91 mg/L	Only one end-point measured for oxidative stress.	Mahey et al. (2016)
GSH level	Human neuroblastoma cell line SHSY5Y	+	↓	300 µM		Olivieri et al. (2001)
GSH level	Human neuroblastoma cell line SHSY5Y	+	↓	300 µM		Olivieri et al. (2002)
GSH level	H460 human lung epithelial cells	+	↓	100, 200, 300 µM	NAC protective effects of cytoxicity and pro-oxidant Co effects	Luczak and Zhitkovich (2013)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level (indirect)	Human embryonic kidney cell line HECK293T	+	↓	$CoCl_2$ (0, 50, 100, 150, and 200 mM) and/or ascorbic acid (0, 50, 100, 150, and 200 mM) for 3 h	Indirect measurements: whereas ascorbic acid repressed Co(II)-induced OCT4 expression	Yao et al. (2014)
ROS level	Human lung epithelial cells H460	+	↑	150 μM		Patel et al. (2012)
GSH level; MDA formation	Human vascular endothelial cell line EA.hy926	+	↓ GSH; ↑ MDA	100–250 μM		Qiao et al. (2009)
HO-1, Mn-SOD, Cu/Zn-SOD, CAT, and GPX	Human macrophage-like cells U937	+	↑ HO-1; no change to Mn-SOD, Cu/Zn-SOD, CAT, and GPX	7.5 ppm	HO-1 was the only oxidative stress-related marker changed.	Tkaczyk et al. (2010)
ROS level	Human keratinocyte cell line HaCaT	+	↑	400 μM		Yang et al. (2011a)
ROS level	Human transfected trophoblast HTR-8/SVneo cells	+	↑			Wang et al. (2021)
Cobalt(II) sulfate ($CoSO_4$)						
ROS level	Human lung alveolar cancer cell line A549, and immortalized human bronchial epithelial cell line BEAS-2B	+	↑	2.5 mM		Ton et al. (2021)
Cobalt(II,III) oxide (Co_3O_4)						
Oxidative DNA damage measured by alkaline comet assay modified with the enzymes Fpg and hOGG1	Human bronchial epithelial cell line BEAS-2B	+ (with Fpg or hOGG1)	↑	2.5 μg/mL, 2 h and 24 h, Fpg 1.25 μg/mL, 2 h and 24 h, hOGG1	Oxidative DNA damage was observed after cobalt particles (10–1000 nm) (10–20 μg/mL) and $CoCl_2$ (1.25–10 μg/mL) treatment. No data for ROS production or oxidative stress markers.	Uboldi et al. (2016)
Cobalt(II,III) oxide (Co_3O_4) NPs						
Level of MDA; 8-OHdG, GSH	HepG2, A549, and SHSY5Y	+	↑ MDA; ↑ 8-OHdG; ↓ GSH	10 μg/mL		Abudayyak et al. (2017)
Level of MDA; 8-OHdG, GSH	Caco-2 cells	–	↑ MDA; ↑ 8-OHdG; ↓ GSH	10 μg/mL		Abudayyak et al. (2017)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
GSH, lipid hydroperoxide, ROS level, SOD, and CAT activity	Hepatocellular carcinoma-derived cell line HepG2	+	↑ ROS levels; ↑ lipid hydroperoxide; ↑ SOD; ↑ CAT; ↓ GSH	5–15 µg/mL	NP size, 21 nm.	Alarifi et al. (2013)
Intracellular and mitochondrial ROS levels	Human fetal hepatic L02 cells	+	↑ ROS; ↑ mitochondrial ROS	2.5 µg/mL	NP size, 20 nm.	Feng et al. (2020)
ROS level; lipid peroxidation, protein oxidation; HO-1 expression	Human lung alveolar cancer cell line A549	+	↑	50 µg/mL	NP size, < 50 nm.	Alinovi et al. (2017)
ROS level	Human endothelial EVC-304 cells and hepatocellular carcinoma-derived cell line HepG2	+	↑	LEC was 14.7 µg/mL for Co_3O_4 NPs; HIC was 99.2 µg/mL for $CoCl_2$	NP size, 45 nm. Under non-cytotoxic concentrations, Co_3O_4 NPs (14.7–88.2 µg/mL) induced a concentration-dependent increase in ROS generation in both cell lines, whereas $CoCl_2$ (12.4–99.2 µg/mL, the same amount of cobalt in an ionic form) showed no effect on ROS generation.	Papis et al. (2009)
Oxidative DNA damage (ROS level)	Human normal bronchial epithelial cell line BEAS-2B	+	↑	20 µg/cm^2	NP size, 9–62 nm.	Kain et al. (2012)
ROS level	Human lung alveolar cancer cell line A549	+	↑	40 µg/cm^2	NP size, 9–62 nm.	Kain et al. (2012)
ROS level	Human lung alveolar cancer cell line A549	+	↑	30 ppm	NP size, 20–75 nm. Only one end-point for oxidative stress.	Limbach et al. (2007)
ROS level, 8-OHdG level	Human lung alveolar cancer cell line A549	+	↑	5 µg/mL	NP size, 20 nm. NPs composed of 85–90% cobalt metal and 10–15% Co_3O_4.	Wan et al. (2012)
Oxidative DNA damage	Human lung alveolar cancer cell line A549 and human normal bronchial epithelial cell line BEAS-2B	+	↑	20 µg/mL in A549 cells; 5 µg/mL in BEAS-2B cells	NP size, 22.1 + 7.2 nm. Only comet assay, no other end-points for oxidative stress measurement.	Cavallo et al. (2015)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level; GSH; lipid peroxidation	Human breast cancer cell line MCF-7	+	↑	50 µM	NP size, 30 ± 20 nm. NAC, a precursor of GSH, restored GSH depletion and (BSO, an inhibitor of GSH synthesis pathway) aggravated the depletion of GSH.	Akhtar et al. (2017)
ROS level	Human leukaemia cell line K562	+	↑	142 µg/mL	NP size, 50 nm. Only one concentration and one end-point for oxidative stress.	Arsalan et al. (2020)
ROS level	Human leukaemia cell line K562, Jurkat cells, KG1-A cells	+	↑	1 µg/mL	NP size, 74 ± 8 nm. Dose-dependent effect (1–50 µg/µL).	Chattopadhyay et al. (2015b)
ROS level	Human monocytic cell line U937	+	↑	100 µM	NPs were composed of 90% cobalt metal and 10% Co_3O_4. NP size, 2–60 nm.	Xu et al. (2018b)
Cobalt(II) oxide (CoO) NPs						
ROS level; HIF-1α protein expression; MT3, NOS2, PTGS2(Cox2), and SOD3 gene expression	Human small airway epithelial cells, HSAEc	+	↑	25 µg/mL	NP size, 53.55 nm.	Sisler et al. (2016b)
Hypoxia-related studies: primary cells						
Cobalt(II) chloride ($CoCl_2$)						
HO-1	Human trophoblasts isolated from normal-term placentas	+	↑	250 µM	Also increased in 500 µM.	Ma et al. (2011)
Mitochondria-derived ROS	Human periodontal ligament stem cells	+	↑	200 µM, 72 h	Induced apoptosis.	He et al. (2018)
Hypoxia-related studies: immortalized cell lines						
Cobalt(II) chloride ($CoCl_2$)						
ROS level; COX-2	Human skin keratinocyte cell line HaCaT	+	↑	400 µM, 24 h		Yang et al. (2011b)
Cellular ATP	Human prostate carcinoma cell line DU145	+	↓	25 µM		Lee et al. (2006)
ROS level	Human colorectal carcinoma epithelium cell line HCT 116	+	↑	100 µM, 30 min		Seo et al. (2016)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
PLD activity; COX-2	Human astroglioma cell line U-87 MG	+	↑	200 µM	Results occurred in a dose- and time-dependent manner.	Ahn et al. (2007)
ROS level	Human glioma cells with oxidative phosphorylation-dependent (U251-MG) and glycolytic-dependent (D54-MG) phenotypes	+	↑	100 µM		Griguer et al. (2006)
Intracellular OH radical levels	Hepatocellular carcinoma-derived cell line HepG2	+	↓	100 µM, 24 h		Porwol et al. (1998)
ROS level	Human colorectal adenocarcinoma cell line Caco-2	+	↑	100 µM		Liu et al. (2012b)
ROS level	Hep3B wildtype and Hep3B depleted of mitochondrial DNA	+	↑	100 µM		Chandel et al. (1998)
ROS level; COX-2	Pulmonary artery smooth muscle cells	–	No changes	25 µM, 24 h	Induced cell proliferation.	Li et al. (2014)
HO-1, ROS level	Human microvascular endothelial cell line HMEC-1	+	↑	250 µM		Loboda et al. (2005)
ROS level	Human retinal ganglion cells	+	↑	100 µM, 24 h		Tulsawani et al. (2010)
ROS level	Trophoblast cell line HRT-8/SVneo	+	↑	500 µM		Zhao et al. (2014)
ROS level	Human skin keratinocyte cell line HaCaT	+	↑	750 µM, 6 h	Resulted in cytotoxicity of HaCaT cells.	Yang et al. (2018)
ROS level	Endothelial cell line EA.hy926	+	↑	300 µM	Induces excessive apoptotic cell death.	Tan et al. (2009)
ROS level	Trophoblast cell line HTR-8	+	↑	500 µM	Proliferation of H8 cells gradually decreased as the $CoCl_2$ treatment concentration increased.	Zheng et al. (2016)
ROS level	Hepatocellular carcinoma-derived cell line HepG2	–	No changes	200 µM	Increased mRNA levels of pro-fibrotic cytokines TGF-β1, α-SMA.	Hernández et al. (2020)
ROS level; mitochondrial ROS; lipid 8-Is; 4-HNE protein adducts; NF-κB activation	Human retinal epithelium cell line hRPE	+	↑	200 µM	Decrease cell viability after 12 h.	Cervellati et al. (2014)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level	Human retinal pigment epithelial cells (ARPE-19 cell line)	+	↑	600 µM, 24 h	Decreased cell viability.	Li et al. (2013)
ROS level; COX-2; phosphorylation of NF-κB p65 subunit	Human skin keratinocyte cell line HaCaT	+	↑	500 µM	Reduced cell viability and oversecretion of IL-6 and IL-8.	Yang et al. (2011a)
ROS level	Human cervical cancer cell line HeLa	+	↑	150 µM	Decreased cell proliferation.	Triantafyllou et al. (2006)
SOD; MDA	Spiral arterial smooth muscle cells	+	↑	50 µM, 24 h	Decreased cell viability, increased apoptosis.	Xiao et al. (2020)
LDH; ROS level	Human colorectal adenocarcinoma cell line Caco-2	+	↑	100 mM		Basavaraju et al. (2021)

↑, increased or upregulated; ↓, decreased or downregulated; 4-HNE, 4-hydroxynonenal; 8-I, isoprostane; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ATP, adenosine triphosphate; BSO, buthionine-(S,R)-sulfoximine; CAT, catalase; COX-2, cyclooxygenase-2; Cu/Zn-SOD, copper/zinc superoxide dismutase; Fpg, formamidopyrimidine DNA glycosylase; GPx, glutathione peroxidase; GSH, glutathione; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; HIF-1α, hypoxia-inducible factor-1α; HMOX1, haem oxygenase 1; HO, haem oxygenase; HO-1, haem oxygenase isoenzyme 1; hOGG1, human 8-oxoguanine DNA *N*-glycosylase-1; IC_{50}, half maximal inhibitory concentration; IL-6/8, interleukin-6/8; LDH, lactate dehydrogenase; LEC, lowest effective concentration; MDA, malondialdehyde; MDM, monocyte-derived macrophages; min, minute; Mn-SOD, manganese superoxide dismutase; MT3, metallothionein 3; NAC, *N*-acetyl cysteine; NF-κB, nuclear factor-kappa B; NOS2, nitric oxide synthase 2; PBMC, peripheral blood mononuclear cell; PLD, phospholipase D; ppm, parts per million; PTGS2(Cox2), prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2); ROS, reactive oxygen species; SOD, superoxide dismutase.

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

(ii) Human cells in vitro

See Table 4.13.

Primary cells

Cobalt metal or soluble cobalt(II) salts

A study by Lombaert et al. (2013) reported that in human PBMCs isolated from healthy donors (n = 3), exposure to cobalt metal (6.0 μg/mL) or 6.0 μg/mL of cobalt-equivalent cobalt(II) chloride (24 μg/mL) did not result in significant induction of the antioxidant enzyme haem oxygenase 1, which is encoded by the HMOX1 gene (Lombaert et al., 2013). Cobalt(II) sulfate at 1 μM per 2.5×10^5 cells did not stimulate superoxide radical (O_2^-) formation in human polymorphonuclear leukocytes obtained from healthy volunteers (Zhong et al., 1990) [The Working Group noted that the number of volunteers was not specified]; however, Chamaon et al. (2019) reported that in PBMCs isolated from healthy donors (n = 2), exposure to cobalt(II) chloride at concentrations of 20, 200, and 300 μM for 24 hours significantly increased mitochondrial superoxide radical (O_2^-) formation. An increase in ROS formation, using 5-(and 6-)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) was observed in human dermal fibroblasts after a 6-hour exposure to cobalt(II) chloride at 100–300 μM (Xu et al., 2018a).

Cobalt-based nanoparticles

Chattopadhyay et al. (2015a) reported that intracellular production of ROS increased by 1.25–4.18-fold in human lymphocytes isolated from healthy donors (n = 6) treated with cobalt(II) oxide NPs (size, 62 ± 4 nm) at 1–50 μg/mL. Pre-treatment with the antioxidant *N*-acetylene cystine attenuated cobalt(II) oxide NP-induced cytotoxicity, indicating that ROS play a role in the cytotoxicity induced by cobalt(II) oxide NPs (Chattopadhyay et al., 2015a). Similarly, Rajiv et al. (2016) showed that cobalt(II,III) oxide NPs (size, 35.8 ± 0.8 nm; 50–100 μg/mL) caused concentration-dependent cytotoxicity and oxidative stress in human lymphocytes isolated from healthy volunteers (n = 3). The evidence for oxidative stress included increases in ROS and lipid peroxidation levels, and decreases in CAT, SOD, and GSH levels (Rajiv et al., 2016).

Nyga et al. (2015) performed a comparison study of cobalt(II,III) oxide NPs (2–60 nm) and cobalt(II) chloride in primary human monocyte-derived macrophages from healthy donors (n = 6), in primary human pulmonary alveolar macrophages from tissues obtained after resection for lung carcinoma (n = 6), and in the human monocytic U937 cell line. Cobalt(II,III) oxide NPs (5–20 μg/mL) induced cytotoxicity and increased ROS levels after exposure for 3 and 6 hours in all three types of monocytic cell models, whereas cytotoxic effects were not observed after treatment with an equivalent concentration of cobalt(II) chloride. Exposure to cobalt(II,III) oxide NPs increased the level of the HIF-1α transcription factor, leading to the upregulation of HIF target genes, which include BNIP3, COX2, GLUT1, and HO1. The antioxidants ascorbic acid and GSH both prevented ROS generation; however, only ascorbic acid reduced HIF-1α levels and prevented cell death (GSH had no effect in preventing cell death) (Nyga et al., 2015). [The Working Group noted that an ROS-independent pathway could be also involved in cytotoxicity induced by cobalt-based NPs.]

In a study conducted in human dermal microvascular endothelial cells isolated from juvenile foreskin [The Working Group noted that the number of samples was not specified], both cobalt metal NPs (size, 28 nm; 25 and 50 μg/mL) and cobalt(II) chloride (0.5 and 1 mM) increased ROS generation after 4-hour exposure (Peters et al., 2007). Another study reported that treatment of human CD34+ haematopoietic stem cells/haematopoietic progenitor cells that were isolated from human cord blood vessels in the placentas of full-term infants with cobalt metal

NPs (size, 50–200 nm) at a concentration of 200 μM (IC_{50}) increased ROS and 8-OHdG levels and decreased GPX activity. [The Working Group noted that the number of samples was not specified.] The addition of selenomethionine partially attenuated cobalt metal NP-induced ROS overproduction and restored total antioxidant capacity and the level of GSH (Zhu et al., 2021a).

Induction of oxidative stress by cobalt metal NPs was also detected in two primary endothelial cells derived from human aorta (HAECs) and human umbilical vein (HUVECs) (Alinovi et al., 2015; Zhu et al., 2021b) as well as from the HMEC-1 cell line (Zhu et al., 2021b), as demonstrated by increased levels of ROS production and lipid and protein peroxidation, and decreased GSH levels (Alinovi et al., 2015; Zhu et al., 2021b). Similarly, Jiang et al. (2012) observed reduction in the activities of superoxide dismutase, glutathione peroxidase and CAT enzymes after a 4-hour exposure to cobalt metal NPs at 6 μM in human primary T-cells isolated from peripheral blood collected from healthy donors (Jiang et al., 2012).

Immortalized cell lines

Soluble cobalt(II) salts

Increases in ROS levels were detected in the following human cell lines after exposure to soluble cobalt(II) salts: prostate cancer PC-3M cells (Lu et al., 2007; Mahey et al., 2016), lung epithelial H460 cells (Patel et al., 2012), osteoblast-like MG-63 cells (Li et al., 2017), keratinocyte 926 cells (Yang et al., 2011a), lung cancer A549 and immortalized bronchial epithelial BEAS-2B cells (Ton et al., 2021), vascular endothelial EA.hy926 cells (Qiao et al., 2009), and transfected trophoblasts (HTR-8/SVneo) (Wang et al., 2021).

In human MG-63 (osteoblast-like) and U937 monocytic cells, soluble cobalt(II) salts increased protein oxidation and nitration, which are two markers of oxidative stress (Petit et al., 2005, 2006; Fleury et al., 2006).

Soluble cobalt(II) salts elevated the expression of antioxidant enzymes such as haem oxygenase 1 (HO-1) and GPX in human MG-63 (osteoblast-like) cells (Fleury et al., 2006; Li et al., 2017) and in U937 (macrophage) cells (Tkaczyk et al., 2010). Studies also showed that the oxidative stress effect induced by soluble cobalt(II) salts is cell type-dependent (Ivancsits et al., 2002; Chamaon et al., 2019).

Studies also showed some evidence that soluble cobalt(II) salts induce oxidative stress because antioxidants (*N*-acetyl cysteine, ascorbic acid, and melatonin) showed protective effects against cobalt(II)-induced cytotoxicity (Olivieri et al., 2001, 2002; Luczak & Zhitkovich, 2013; Yao et al., 2014; Uboldi et al., 2016).

Cobalt-based nanoparticles

Several studies found that in human alveolar carcinoma (A549) and human normal bronchial epithelial (BEAS-2B) cells cultured in vitro, cobalt(II,III) oxide NPs caused increased levels of intracellular ROS, gene expression of haem oxygenase 1, lipid peroxidation, and oxidative stress-associated DNA damage (Limbach et al., 2007; Kain et al., 2012; Wan et al., 2012; Cavallo et al., 2015; Alinovi et al., 2017; Cappellini et al., 2018). (The NPs tested in the Wan et al. study consisted of 85–90% cobalt metal NPs and 10–15% cobalt(II,III) oxide NPs.) [The Working Group noted that some studies reported that the addition of antioxidants attenuated the adverse effects induced by the NPs.]

In two hepatic cell lines, human hepatocarcinoma (HepG2) and human fetal hepatic (L02) cells, cobalt(II,III) oxide NPs increased levels of ROS and lipid hydroperoxide, SOD, and CAT activity; and decreased the concentration of GSH. Soluble cobalt(II) salts also induced cytotoxicity and oxidative stress, but the effects were less significant compared with the NPs (Papis

et al., 2009; Alarifi et al., 2013; Abudayyak et al., 2017; Feng et al., 2020).

The induction of oxidative stress by cobalt(II,III) oxide NPs has also been reported in the following cell lines: human breast cancer (MCF7) cells (Akhtar et al., 2017), as demonstrated by increased ROS production and reduced GSH; human leukaemia (K562) cells (Arsalan et al., 2020; Chattopadhyay et al., 2015b); and by cobalt(II) oxide NPs in human small airway epithelial cells (HSAEs) (Sisler et al., 2016b), as demonstrated by increased ROS levels. Xu et al. also observed an increase of ROS level in human monocytic cells (U937 cell line) treated with 100 μM cobalt nanoparticles composed of 90% cobalt metal and 10% Co_3O_4 (Xu et al., 2018b).

Cobalt(II) chloride-induced hypoxia

Oxidative stress has been assessed in studies that used cobalt(II) chloride as a means of chemically inducing hypoxia, mostly after exposure to concentrations ≥ 100 μM (e.g. Porwol et al., 1998), with little evidence of hypoxia induction reported at lower concentrations (Lee et al., 2006; Xiao et al., 2020). The expression of HIF-1α, a key regulator of hypoxia, was frequently assessed in these studies. In two studies conducted with human primary cells, cobalt(II) chloride increased HO-1 expression, a sensor of cellular oxidative stress (Ma et al., 2011), and levels of ROS within the mitochondria (He et al., 2018) in trophoblasts isolated from normal-term placentas and human periodontal ligament stem cells, respectively. Numerous studies conducted in a variety of human immortalized cell lines (e.g. Yang et al., 2011a; Liu et al., 2012b; Seo et al., 2016) demonstrated an ability of cobalt(II) chloride to stimulate an intracellular hypoxia-like condition by regulating the stability of HIF-1α, thus rapidly increasing levels of intracellular ROS or other markers of oxidative stress (see Table 4.13).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table S4.14 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal or cobalt-based nanoparticles

After 12 months of follow-up, Sprague-Dawley rats with cobalt metal cylinders (diameter, 1 mm; length, 2 mm) surgically implanted into the gastrocnemius muscle showed no significant alterations in thiobarbituric acid-reacting substances (TBARS) and GSH in serum (Kalinich et al., 2022). Ultrafine cobalt metallic particles administrated by intratracheal instillation (1 mg/rat) increased the levels of lipid peroxides in bronchoalveolar lavage fluid (BALF) from male Wistar rats (Zhang et al., 1998). Elevated MDA and 8-OHdG levels were shown in the temporal lobe and hippocampus of rats after exposure to cobalt metal NPs (intraperitoneal administration at 2–8 mg/kg bw per day for 20 days) (Zheng et al., 2019), and in the lungs of mice exposed to cobalt-based NPs (85–90% cobalt metal NPs and 10–15% Co_3O_4 NPs) at 50 μg/mouse by intratracheal instillation for up to 28 days (Wan et al., 2017). In addition, Nrf2 and HO-1 levels showed dose-dependent increases in rat hippocampus (Zheng et al., 2019). In line with these findings, increased HO-1 levels were found in the lungs of mice exposed to a single dose of cobalt(II) oxide NPs (20 μg/mouse) by oropharyngeal aspiration (Zhang et al., 2012). Ton et al. (2021) also reported on the levels of 8-OHdG in lungs of mice exposed via inhalation to cobalt metal for 90 days in a study conducted by the NTP (2014). Ultrafine tricobalt tetraoxide [cobalt(II,III) oxide] enhanced hydroxyl radical generation in rat BALF (Dick et al., 2003). [The Working Group noted that this study tested only a single dose, showing a clear limitation of the study.]

Soluble cobalt(II) salts

Cobalt(II) chloride has been shown to induce alterations in multiple oxidative stress-related markers – including MDA, superoxide, H_2O_2, nitric oxide, and AOPP (advanced oxidation protein products) – in a wide range of tissues and organs (brain, blood, liver, heart, and kidney) of rats, rabbits, and pigs (Kuno et al., 1980; Morita et al., 1982; Johansson et al., 1986; Wang et al., 1993; Daido & Aniya, 1994; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Sumbayev, 2001; Gonzales et al., 2005; Kalpana et al., 2008; Garoui et al., 2011, 2013; Ajibade et al., 2017; Awoyemi et al., 2017; Akinrinde & Adebiyi 2019; Oyagbemi et al., 2019). In addition, oxidative stress-related enzymes were also reported to respond substantially to cobalt(II) chloride exposure. For example, HO-1 activity was found to be induced significantly in the liver of mice, rats, and pigs (Numazawa et al., 1989a; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005). In contrast, GPX activity displayed significant changes with varied patterns in a large number of studies (Hatori et al., 1993; Daido & Aniya, 1994; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Ajibade et al., 2017; Awoyemi et al., 2017; Akinrinde & Adebiyi, 2019; Oyagbemi et al., 2019). In agreement with the trend reported for GPX caused by cobalt(II) chloride exposure, the activities/levels of a group of antioxidant enzymes had distinct responses to exposure. While several studies found that glutathione *S*-transferase (GST) activity (Daido & Aniya, 1994), GSH levels (Nordström et al., 1990; Christova et al., 2001), CAT activity (Christova et al., 2002), glutathione reductase activity (Christova et al., 2001, 2002), and PSH (protein thiol) levels (Oyagbemi et al., 2019) were increased, many studies reported that cobalt(II) chloride exposure decreased GST activity (Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Ajibade et al., 2017; Abdel-Rahman Mohamed et al., 2019; Akinrinde & Adebiyi, 2019; Oyagbemi et al., 2019), SOD activity (Hatori et al., 1993; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Awoyemi et al., 2017; Abdel-Rahman Mohamed et al., 2019; Akinrinde & Adebiyi, 2019), CAT activity (Llesuy & Tomaro, 1994; Christova et al., 2001, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Awoyemi et al., 2017; Abdel-Rahman Mohamed et al., 2019; Oyagbemi et al., 2019), and PSH (protein thiol) and NPSH (non-protein thiol) levels (Garoui et al., 2013; Ajibade et al., 2017). Furthermore, gene and/or protein expression responses related to oxidative stress have also been investigated by several studies. Increased mRNA and/or protein expression of HO-1/2, copper/zinc-SOD, and NOX2 were found in multiple organs of rats exposed to cobalt(II) chloride (Bauer et al., 1998; Gonzales et al., 2005; Guan et al., 2015; Nordquist et al., 2015; Abdel-Rahman Mohamed et al., 2019). In contrast, decreased Nrf2 levels were reported in the brain of cobalt(II) chloride-treated rats (Guan et al., 2015). [The Working Group noted that, with regard to study quality, most of the studies tested only a single dose, which was a clear limitation. However, the large number of reports counterbalanced the limitations. In addition, several studies showed dose- and time-dependent effects or presented multiple end-points related to oxidative stress, supporting the accuracy and reliability of the studies.]

Studies on cobalt(II) chloride hexahydrate also demonstrated that multiple oxidative stress markers were significantly altered in a wide range of tissues and organs in rat models. Namely, one or more alterations in MDA, H_2O_2, nitric oxide, ROS, protein oxidation, 8-OHdG, total antioxidant capacity (TAC), and protein carbonyl levels have been reported in a variety of studies (Shrivastava et al., 2008; Saxena et al., 2010; Akinrinde et al., 2016; Zheng et al.,

2019; Abdel-Daim et al., 2020; Oyagbemi et al., 2020). Additionally, cobalt(II) chloride hexahydrate exposure induced alterations in oxidative stress-related enzymes. For example, augmented activities of haem oxygenase (Stelzer & Klaassen, 1985), GST (Akinrinde et al., 2016), CAT, and MPO (Akinrinde et al., 2016; Abdel-Daim et al., 2020; Oyagbemi et al., 2020), as well as increased GSH (glutathione) levels with declining GSSG (glutathione disulfide) levels accompanied by an increased ratio of GSH/GSSG (Stelzer & Klaassen, 1985; Shrivastava et al., 2008; Saxena et al., 2010), were found in animals exposed to cobalt(II) chloride hexahydrate. In addition, increased Nrf2 and HO-1 protein levels were observed in the hippocampus and/or gastrocnemius muscle of rats after administration of cobalt(II) chloride hexahydrate by injection (Saxena et al., 2010; Zheng et al., 2019). There are also several studies that used other cobalt forms, such as cobalt(II) sulfate and cobalt(II) acetate. One study demonstrated that cobalt(II) sulfate exposure caused suppression of manganese-SOD activity in the myocardium of rats (Clyne et al., 2001). [The Working Group noted as a clear limitation that both studies tested only a single dose.] Cobalt(II) acetate exposure in rats resulted in a dose-dependent increase in oxidative DNA damage and higher levels of products of hydroxyl radical attack in the kidney, liver, and lung (Kasprzak et al., 1994).

[The Working Group noted that, with regard to the exposure routes of the studies summarized above, most experiments investigating soluble cobalt(II) salt-induced alterations in end-points related to the key characteristic oxidative stress were carried out by acute or subacute intraperitoneal and subcutaneous injection using a dose range of up to 80 mg/kg bw. There were also several experiments performed by oral administration (100–650 mg/L) and, on occasion, gavage or inhalation exposure were used.]

(ii) Non-human mammalian cells in vitro

Cobalt metal nanoparticles

Cobalt metal NPs increased levels of multiple oxidative stress markers and various oxidative stress-related enzymes. Increased ROS levels, the most common oxidative stress markers in most studies, were found in the rat RAW 264.7 cell line and Wistar rat LV myocytes (Zhang et al., 2012; Savi et al., 2021).

Soluble cobalt(II) salts

Soluble cobalt(II) salts, mainly cobalt(II) chloride, increased multiple oxidative stress markers (e.g. ROS and MDA), oxidative stress-related enzymes (e.g. SOD, CAT, and GSH), and gene/protein expression (e.g. HO-1 and NOX) in various non-human mammalian cells in vitro. [The Working Group noted that an increase in ROS levels was the most common oxidative stress marker assessed in cells exposed to cobalt(II) chloride (Karovic et al., 2007; Chen et al., 2010b, 2018b, 2019; Kamiya et al., 2008, 2010; Tong et al., 2012; Lan et al., 2013; Wang et al., 2013c; Wenker et al., 2013; Guan et al., 2015; Shweta et al., 2015; Lee et al., 2016; Zimmerman et al., 2018; Manu et al., 2019; Yin et al., 2021; Luo et al., 2022).] In addition to increased ROS, cobalt(II) chloride increased MDA levels in various types of cells, including rat primary hepatocytes, cardiomyocyte H9c2 cells, and PC-12 cells (Wang et al., 2013c; Chen et al., 2019; Manu et al., 2019; Yin et al., 2021). In line with the above findings, the levels or activities of oxidative stress-related enzymes – including haem oxidase (Maines & Sinclair, 1977), GSH and GSSG (Maines & Sinclair, 1977; Chen et al., 2010b; Guan et al., 2015; Wang et al., 2016a), SOD (Yang et al., 2011c; Tong et al., 2012, Wang et al., 2013c, 2016a; Yin et al., 2021), CAT (Tong et al., 2012; Wang et al., 2016a; Yin et al., 2021), and NOX2 (NADPH oxidase 2) (Chen et al., 2019) – were also significantly altered by cobalt(II) chloride exposure. In addition, cobalt(II) chloride induced changes in

Table 4.15 Chronic inflammation in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
COX-2; IL-1β	Autopsy samples of brain and lung tissue	Mexico, urban air pollution exposure vs 2 less-exposed locations	47 exposed and 12 controls (adults and children)	↓ COX-2 with ↑ frontal lobe Co ($R = -1.96 \times 10^4$; probability $t = 0.0024$) Effect of Co on IL-1β in brain NSS	Age	Co and other metals were quantitatively assessed using accepted methods. The potential for misclassification of Co exposure is low. A limitation of this study is the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest. Age, sex, place of residency, cause of death, and time between death and autopsy noted. Cause of death was considered for all subjects to rule out infection, inflammatory events, drug exposure, brain ischaemia, and hypoxia impact on mRNA levels of the inflammatory markers measured in the study. Co measured quantitatively as predictor of outcomes. While specific metal effects were explored through regression analysis for some brain findings (Table 3), other outcome comparisons between exposed individuals and controls for lung and brain sites found significant correlations between any lung metal concentrations and COX-2 or IL-1β lung expression.	Calderón-Garcidueñas et al. (2013)

Table 4.15 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Inflammatory infiltrate with fibrosis	Postmortem lung tissue and blood	Belgium, worker case report	Diamond polisher using Co-containing discs for 35 yr	+ Lung histology for active inflammation with multinucleated giant cells and a mononuclear inflammatory infiltrate in the alveolar septa with prominent fibrosis. ↑ Blood sedimentation rate (28 mm/h) and ↑ leukocyte count of 10 500/mm^3 (with 71% neutrophils)		The case report provides detailed qualitative narrative exposure history and quantitative measurements of Co in lung tissue for 1 patient. The key limitation is the nature of a case report with a sample size of 1.	Nemery et al. (1990)
Inflammatory markers	Serum	Belgium, Co refinery workers	82 Co-exposed males 82 non-exposed controls (age-matched)	↑ Sedimentation rate in exposed vs controls but NSS difference ↑ leukocyte count ($P < 0.05$) in Co-exposed vs controls	Controls were age, education, and SES matched	There is limited potential for non-differential misclassification in the exposure groups. This study focuses on comparisons between exposed and unexposed groups working at different companies. The key limitation of this study is the reliance on the qualitative exposure assessment approach (exposed/unexposed) based on location of employment. These findings might not be clinically relevant nor indicative of chronic inflammation.	Swennen et al. (1993)

Table 4.15 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Inflammatory and fibrotic markers (TGF-β and α-SMA)	Blood	China, 2 sites (1 former e-waste site, 1 control site)	Individuals (n = 62) exposed to Pb, Ni, Co, Hg, Cu, Zn, Sn, and Cd Non-exposed controls (n = 47)	In multiple linear regression analyses, Co was positively correlated with α-SMA (β = 323.18; P = 0.03) and TGF-β (β = 4814.69; P < 0.01) in blood from the exposed group	[Unclear as text states "different variables" considered in regression.] [No statistical differences in age, height, weight, and BMI between exposed individuals and controls]	There is potential for non-differential misclassification as the biological samples may not be reflective of historically relevant exposure. This study did assess and explicitly investigate other metals as co-exposures. The key limitation of this study is the reliance on a single biological sample. Study mentions that the exposed group did not have drug or alcohol exposure, but unclear about the controls. End-points measured as indicators of inflammation and fibrosis. Question about exposure measurement being reflective of historical exposure.	Xue et al. (2021)
Serum proteins	Serum	Czechia production workers, cross-sectional	38 Ni-exposed 35 Co-exposed Non-exposed controls	↑ A_1AT, A_2M, CPL, and LYS in Co-exposed compared with controls (P < 0.001 to P < 0.005)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1983)
Serum proteins	Serum	Czechia production workers, cross-sectional	38 Ni-exposed 35 Co-exposed Non-exposed controls	↑ A_2M, TRF, A_1AT, CPL, LYS, and A_1GP in Co-exposed compared with controls (P < 0.05 for TRF; P < 0.001 for all others)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1986b)

+, positive(ly); ↓, decreased; ↑, increased; A_1AT, alpha$_1$-anti-trypsin; A_2M, alpha$_2$-macroglobulin; A_1GP, alpha$_1$-glycoprotein; BMI, body mass index; Cd, cadmium; Co, cobalt; COX-2, cyclooxygenase-2; CPL, ceruloplasmin; Cu, copper; e-waste, electronic and/or electrical waste; IL-1β, interleukin 1 beta; Hg, mercury; LYS, lysozyme; mRNA, messenger RNA; Ni, nickel; NSS, not statistically significant; Pb, lead; α-SMA, α-smooth muscle actin; Sn, tin; TGF-β, transforming growth factor-β; TRF, transferrin; vs, versus; yr, year; Zn, zinc.

protein levels and/or mRNA expression of many antioxidants and ROS-generating enzymes, including GST, SOD, CAT, HO-1, NOX1/2, and iNOS (inducible nitric oxide synthase). Cobalt(II) chloride hexahydrate also induced oxidative stress markers. For instance, ROS and protein carbonyl levels were increased in rat RAW 264.7 cells (Zhu et al., 2017; Salloum et al., 2018). [The Working Group noted that most studies were relevant, because they presented more than one end-point related to oxidative stress.]

[The Working Group noted that cobalt(II) chloride and cobalt(II) chloride hexahydrate were commonly used as hypoxia mimics in many studies in which oxidative stress-related markers were assessed. Most of the studies had limitations related to the concentration used and end-point selected; however, the major findings of the studies conducted in non-human mammalian cells in vitro supported the conclusion that soluble cobalt(II) salts are oxidative stressors.]

4.2.6 Induces chronic inflammation

(a) Humans

(i) Exposed humans

See Table 4.15.

A Mexican autopsy study in brain and lung tissue samples investigated air pollution effects, including from metal exposure in people from urban versus less-exposed (control) locations (Calderón-Garcidueñas et al., 2013). There was a difference (not statistically significant) in cobalt concentrations in frontal lobe or lung tissue samples from urban-exposed versus control individuals. However, regression analyses revealed that increased frontal lobe concentrations of cobalt correlated inversely with gene expression of the cyclooxygenase-2 (COX2) enzyme, a pro-inflammatory marker (probability, $t = 0.0024$). A cobalt-dependent effect on the pro-inflammatory cytokine interleukin-1β (IL-1β) in brain tissue was not observed. COX-2 expression in the lung was also measured and found to be significantly elevated in the urban-exposed group versus controls ($P = 0.01$), but no significant correlations were found for any metal-specific effects. [The Working Group considered this study of air pollution mixtures to be minimally informative for the evaluation of cobalt with respect to this end-point.]

Nemery et al. (1990) described the autopsy findings of a Belgian diamond polisher who had worked using cobalt-containing polishing discs for 35 years. They reported mostly normal blood haematology and chemistry results, except for an elevated sedimentation rate of 28 mm/hour and a leukocyte count of 10 500/mm^3 with 71% neutrophils. The diamond polisher's lung histology showed active inflammation with multinucleated giant cells and a mononuclear inflammatory infiltrate in the alveolar septa upon necropsy. Prominent fibrosis was also observed. [The Working Group noted that this case report provided detailed qualitative narrative exposure history and quantitative measurements of cobalt in lung tissue for one patient. The key limitation was the nature of a case report with a sample size of one.]

In a study conducted in Belgium, Swennen et al. (1993) assessed health effects in 82 cobalt refinery workers employed for an average duration of 8 years, with a geometric mean TWA exposure to cobalt dust of 125 μg/m^3. This included 25% of the worker population with exposure > 500 μg/m^3. All study participants were grouped into the exposed or unexposed group on the basis of their location of employment. Within the exposed group, exposure was further assessed through inhalation (air samples) and all routes (biological measurements). Results were compared with an age-matched group of non-cobalt dust-exposed controls. Leukocyte counts were elevated in the cobalt-exposed group compared with controls (8.03 versus 6.73 × 10^9/L; $P < 0.05$). The sedimentation rate was also higher, but not statistically significant, in the

cobalt-exposed group compared with controls (5.00 versus 3.00 mm/hour). [The Working Group noted that these findings might not be clinically relevant nor indicative of chronic inflammation. There is limited potential for non-differential misclassification in the exposure groups. This study focused on comparisons between exposed and unexposed groups working at different companies. A potential limitation of this study was the reliance on the qualitative exposure assessment approach, which was based on the location of employment, but air sampling and biological monitoring confirmed good exposure contrast between the groups.]

In a Chinese study of e-waste-exposed populations living near former electronics recycling plants, Xue et al. (2021) measured concentrations of metals and assessed markers of inflammation and fibrosis in blood, including transforming growth factor-β and α-smooth muscle actin. In multiple linear regression analyses, cobalt was positively correlated with α-SMA (α-smooth muscle actin) (β = 323.18; P = 0.03) and TGF-β (transforming growth factor-β) (β = 4814.69; $P < 0.01$) in blood from the exposed group. [The Working Group noted that this study did assess other metals as co-exposures and that these were accounted for in the results for cobalt. However, a key limitation of this study was the reliance on a single biological sample and whether this was reflective of historical exposure.]

Other evidence of an altered inflammatory response in cobalt-exposed human populations was reported in two cross-sectional studies of cobalt production workers in Czechia compared with non-exposed, age-matched controls (Bencko et al., 1983, 1986b). In the earlier study (1983), average values of acute-phase reactant proteins including α1-anti-trypsin, α_2-macroglobulin, ceruloplasmin, and lysozyme were elevated in the cobalt-exposed individuals ($P < 0.001$ to $P < 0.005$). In the later study (1986), acute-phase reactant proteins were also assessed, of which, α1-anti-trypsin, α_2-macroglobulin, transferrin, ceruloplasmin, lysozyme, and α1-glycoprotein were all significantly elevated in the cobalt-exposed individuals compared with controls ($P < 0.05$ for transferrin; $P < 0.001$ for all others). [The Working Group noted that the key limitation of this study was the limited information on the exposure definition, which provided no information on the industry or occupation of the exposed workers, or the potential for co-exposure.]

[The Working Group noted that four out of six studies of cobalt-exposed populations showed some evidence of being possibly associated with chronic inflammation.]

Katsarou et al. (1997) examined Greek cement workers with co-exposure to chromium. [The Working Group evaluated and excluded the study as uninformative because no statistical analyses were performed to isolate the effects of cobalt alone. Furthermore, the study design makes the evaluation of exposure assessment challenging. The exposure was a controlled aspect of the experimental design; there is no potential for exposure misclassification.]

The study by Krakowiak et al. (2005) is a case report of a diamond-polishing disc former with 9 years of exposure to hard metal (WC-Co). [The Working Group reviewed but excluded the study as uninformative because the effects of cobalt alone were not examined.]

Walters et al. (2012) investigated metal-working fluid-exposed workers. [The Working Group reviewed but excluded the study because 90% of workers also had elevated urinary levels of chromium and effects attributable to cobalt exposure alone were not isolated using any statistical analyses.]

The study by Rizzato et al. (1994) is a case report of four workers exposed to hard metal. [The Working Group deemed the study uninformative and excluded it.]

Shirakawa & Morimoto (1997) assessed a population exposed to hard metal. [The Working Group evaluated but excluded the study as uninformative because the population

was exposed only to hard metal (WC-Co), which was inseparable from exposure to cobalt alone. Furthermore, there is potential for non-differential misclassification in the exposure groups. A key limitation was the lack of information on the exposure groups and how they were constructed, particularly the timing of when these groups were defined in relation to the outcome measures. There were exposure metrics reported in the multiple logistic regression that were not described in the methods.]

(ii) Human cells in vitro

Numerous studies have shown that cobalt compounds – including cobalt metal, soluble cobalt(II) salts (mostly cobalt(II) chloride), and cobalt-based NPs – have pro-inflammatory effects on human cells, which include the induction of cytokines/chemokines and cell surface activation markers and adhesion molecules (see Table S4.16, Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). [The Working Group noted that the studies using human cells in vitro present acute (short-term) end-points but may be relevant to chronic pro-inflammatory effects induced by various forms of cobalt in the context of potential repeated exposure over time.]

(b) Experimental systems

(i) Non-human mammals in vivo

[The Working Group noted that only those studies that include end-points ≥ 7 days are reported.]

Cobalt metal or cobalt-based nanoparticles

In a subchronic study conducted by the NTP, male and female Fischer 344/NTac rats and $B6C3F_1$ mice were exposed to cobalt metal by inhalation for 14 weeks (6 hours per day, 5 days per week) at concentrations of 0.625, 1.25, 2.5, 5, and (mice only) 10 mg/m^3. The incidence of chronic active inflammation was significantly increased in the lungs of male and female rats at all exposure levels, as was the incidence of alveolar histiocytic cell infiltration in male and female mice. The incidence of chronic active inflammation in the nose in male and female mice was significantly increased at concentrations of 5 and 10 mg/m^3.

In a chronic study conducted by the NTP, male and female Fischer 344/NTac rats and $B6C3F_1$ mice were exposed to cobalt metal by inhalation for 2 years (6 hours per day, 5 days per week) at concentrations of 1.25, 2.5, or 5 mg/m^3. The incidence of chronic active inflammation was significantly increased in the lungs of male and female rats at all exposure concentrations. The incidence of histiocytic cell infiltration (in alveoli) was significantly increased in the lungs of male and female mice at all exposure concentrations, as was the incidence of suppurative inflammation in male (2.5 and 5 mg/m^3) and female (5 mg/m^3) mice. In the nose, the incidence of chronic active inflammation in male and female rats, and of suppurative inflammation in male and female rats and mice, was significantly increased at all exposure concentrations (NTP, 2014). Transcriptomic profiling of bronchioloalveolar carcinomas from the cobalt-exposed mice showed significant alterations in molecular pathways related to immune response signalling including "IL-8 signalling", "melatonin signalling", "B cell receptor signalling", and "regulation of IL-2 expression in activated and anergic T lymphocytes", with pro-inflammatory IL-8 signalling being one of the most significantly altered (Ton et al., 2021).

In a study by McNamara & Williams (1981), cobalt metal discs (diameter, 5 mm; thickness, 2 mm) were surgically implanted intramuscularly in male Lister rats, and histological effects were assessed for up to 52 weeks. Breakdown of muscle fibres was observed near the site of implantation. Many inflammatory cells had infiltrated between fibres and there were several "lymphoid clumps" that were mainly composed of plasma

cells. In some areas, bloodborne lymphocytes were attached to the blood vessels.

In another implantation study, cobalt metal pellets were surgically implanted into the skeletal muscle of male Sprague-Dawley rats, which induced spindle-cell tumours as early as 3 months. The Gene Ontology (GO) of differentially expressed genes (DEGs) with increased expression 3 months after implantation were "leukocyte cell-cell adhesion", "leukocyte proliferation", "lymphocyte proliferation", "mononuclear cell proliferation", and "adaptive immune response". The transcriptomic profile and GO of DEGs with increased expression in muscle tissue closer to the implant were "cell activation", "leukocyte activation", "lymphocyte activation", "T cell activation", and "positive regulation of immune system process" at 1 month, and "immune system process", "immune response", "regulation of immune system process", "cell activation", and "leukocyte activation" at 3 months (Wen et al., 2020).

In a study by Hansen et al. (2006), male Sprague-Dawley rats were implanted intramuscularly with cobalt metal NPs (average size, 120 nm) or implanted subcutaneously with bulk cobalt metal (4.73 mm^{-1}) and assessed at 6, 8, or 12 months. For the bulk cobalt metal, but not the cobalt metal NPs, inflammatory infiltrates (composed of mononuclear cells and lymphocytes) and granulomas were reported in muscle tissue after exposure for 6 months.

In a study by Kalinich et al. (2022), male Sprague-Dawley rats were implanted intramuscularly with cobalt metal pellets (cylinders of 1 mm in diameter, 2 mm in length). Interferon-gamma (IFN-γ), IL-4, IL-5, IL-6, IL-10, and IL-13 protein levels were significantly increased in serum samples 3–12 months after exposure. Keratinocyte chemoattractant/human growth-regulated oncogene chemokine (KC/GRO) and tumour necrosis factor alpha (TNFα) protein levels did not change. Administration of ultrafine cobalt metal [the Working Group noted that this was probably cobalt metal NPs] to female Sprague-Dawley rats by single intratracheal instillation at 0.06, 0.3, 0.6 mg/100 g bw significantly increased the number of total cells, macrophages, neutrophils, and lymphocytes in BALF on day 1. However, no significant effects were observed in BALF at day 28 or 1 month after 4 instillations (administered once per month) of 0.06 mg/100 g bw (Lasfargues et al., 1995). In a study by Zhang et al. (1998), male Wistar rats were treated with ultrafine cobalt (consisting of cobalt metal and cobalt(II,III) oxide) [The Working Group noted that these were cobalt-based NPs with a diameter of 20 nm] administered by intratracheal instillation (1 mg/mL). There were significant increases in the number of total cells and neutrophils (and a decrease in macrophages) in BALF on days 1, 3, 7, 15, and 30, together with a significant increase in lymphocytes on days 15 and 30.

In *gpt* delta transgenic mice treated with cobalt metal NPs at a dose of 50 µg (size, 20 nm) by intratracheal instillation, lung inflammation and injury were induced. Neutrophils were significantly increased in BALF on days 1, 3, 7, and 28 (although the numbers recorded on days 7 and 28 were lower than on days 1 and 3). The chemokine (C-X-C motif) ligand 1 (CXCL1)/keratinocyte chemoattractant (KC) was significantly increased in BALF on days 1, 3, and 7. Infiltration of macrophages and neutrophils into lung tissue (alveolar space/septa and interstitium) was identified on day 7 by histological assessment, and inflammatory cells were still present 4 months post-exposure (Wan et al., 2017).

In a study by Viegas et al. (2022), male and female Sprague-Dawley rats were exposed for 4 hours to aerosols of cobalt metal and various other (soluble and insoluble) cobalt(II) compounds (including cobalt dihydroxide, cobalt monoxide, cobalt(II) sulfate heptahydrate (in Fischer 344 rats), tricobalt tetraoxide [cobalt(II,III) oxide], cobalt(II) sulfide, and cobalt(II) sulfate). Histopathological assessment

showed increased lung perivascular (inflammatory) oedema at day 1 and up to days 3, 5, 14, or 16 post-exposure, except after exposure to cobalt dihydroxide and cobalt(II) sulfate.

In a study by Burzlaff et al. (2022), Wistar rats were exposed by inhalation to tricobalt tetraoxide [cobalt(II,III) oxide] particles at a concentration of 5, 20, or 80 mg/m^3 for 6 hours per day for 28 days. Neutrophil numbers were significantly increased in BALF at exposure concentrations of 20 and 80 mg mg/m^3 at day 1 and at day 91 after the end of the exposure period in both males and females.

In a study by Sisler et al. (2016a), male C57BL/6 mice were exposed to cobalt(II) oxide NPs (72 ± 16 nm) administered by inhalation (whole-body) for 4 days at concentrations of 10 or 30 mg/m^3. Neutrophils, eosinophils, and lymphocytes were significantly increased in BALF of mice 1 day (together with IL-1β and IL-6, but not TNFα and KC/GRO) and 7 (but not 56) days after the last exposure at a concentration of 30 mg/m^3, whereas alveolar macrophages were significantly increased in BALF at all three time points (including day 56).

Soluble cobalt(II) salts

Male Sprague-Dawley rats were exposed to cobalt(II) chloride in drinking-water (300 mg/L per day) for 4 weeks, which induced kidney injury. There were significant increases in pro-inflammatory response protein biomarkers – nitric oxide, TNFα (tumour necrosis factor-α), MPO (myeloperoxidase), and CRP (C-reactive protein) – and mRNAs (NF-κB and IL-6) in kidney tissue (Abdel-Daim et al., 2020). In a study by Oyagbemi et al. (2019), peritubular and periglomerular inflammation were increased in the kidney of male Wistar rats exposed to cobalt(II) chloride in drinking-water (150 or 300 ppm) for 7 days, along with focal areas of glomerulonecrosis at the highest exposure concentration. In male Wistar rats exposed by gavage to cobalt(II) chloride hexahydrate (150 mg/kg bw) for 8 days, infiltration of kidney by inflammatory cells and increased levels of NF-κB in the heart and kidney were reported (Oyagbemi et al., 2020).

In a study by Awoyemi et al. (2017), mild infiltration of hepatic interstitium by inflammatory cells was observed in male Wistar rats exposed to cobalt(II) chloride in drinking-water (600 mg/L) for 7 days.

In one study, male Sprague-Dawley rats were treated with feed containing cobalt(II) chloride hexahydrate (12.5 mg/kg bw) for 7 days. There were no significant treatment-related changes in the levels of inflammatory cytokines IFNα, IFN-γ, or MCP-1 in BALF (Shukla et al., 2009).

In a study using transgenic mice with a conditional deletion of lung epithelium-derived HIF-1α (Saini et al., 2010), non-transgenic control mice (on a mixed C57BL/6 and FVB/N background) were exposed to cobalt(II) chloride (30 or 60 μg) via oropharyngeal aspiration, 5 days per week for 2 weeks. The mice exhibited lung injury with histopathological evidence of mild-to-moderate chronic bronchopneumonia (marked inflammation), which consisted of infiltrates of mixed inflammatory cells (including monocytes/macrophages, neutrophils, eosinophils, lymphocytes, and occasionally plasma cells) 72 hours after the last exposure. Total cell counts and absolute numbers of macrophages, lymphocytes, neutrophils, and eosinophils, as well as protein levels of some pro-inflammatory cytokines/chemokines (for example, RANTES) were increased in BALF, but the changes were not statistically significant compared with saline-treated mice. In another study, exposure of the same non-transgenic control male mice to cobalt(II) chloride (60 μg) by oropharyngeal aspiration induced a significant increase in total cell counts and number of macrophages in BALF after 5 or 10 doses, and of neutrophils, eosinophils, and lymphocytes after 5 (but not 10) doses (Proper et al., 2014).

In a metal sensitization study by Bonefeld et al. (2015), mice were sensitized dermally with

10% cobalt(II) chloride and then challenged dermally twice with 10% cobalt(II) chloride 3 weeks later. CD4+ (BrdU+) T-cells, CD8+ (BrdU+) T-cells, and CD19+ (BrdU+) B-cells were significantly increased in the draining lymph nodes after challenge. In a study by Tsui et al. (2020), male BALBalb/c mice were sensitized dermally with 5% cobalt(II) chloride hexahydrate on days 1 and 8, and then challenged by inhalation of 0.05% cobalt(II) chloride hexahydrate on days 15, 17, 19, 22, and 24. Neutrophils and eosinophils, as well as the chemokines MCP-1, MIP-2, and KC, were significantly increased (and macrophages decreased) in BALF 24 hours after the last challenge.

In a metal sensitization study, guinea-pigs were sensitized dermally with 5% cobalt(II) chloride on days 0, 2, 7, and 9 and then challenged by cobalt(II) chloride inhalation at 2.4 mg/m^3 for 6 hours per day for 2 weeks, starting on day 42. Neutrophils and eosinophils were significantly increased in BALF after the last challenge (Camner et al., 1993).

In a study by Johansson et al. (1984), male rabbits were exposed by inhalation to cobalt(II) chloride at concentrations of 0.4–0.6 mg/m^3 for 4–6 weeks (6 hours per day, 5 days per week), but no inflammation was observed. However, in another study by Johansson et al. (1983), there was a significant increase in alveolar macrophages (with increased metabolic activity) in BALF after 1 month of exposure. Chronic exposure of male rabbits to cobalt(II) chloride at a concentration of 2 mg/m^3 for 14–16 weeks (6 hours per day, 5 days per week) by inhalation also resulted in a significant increase in alveolar macrophages (with increased metabolic activity) in BALF (Johansson et al., 1986), and an increase in interstitial and intra-alveolar leukocytic and/or lymphocytic inflammation in the lung (Johansson et al., 1987).

Male and female Fischer 344/N rats and B6C3F$_1$ mice were subchronically exposed to cobalt(II) sulfate heptahydrate at concentrations of 0.3, 1, 3, 10, or 30 mg/m^3 by inhalation for 13 weeks (6 hours per day, 5 days per week). In male and female rats, incidence of inflammation (≥ 1 mg/m^3) and inflammatory polyps (≥ 10 mg/m^3) in the larynx, and of inflammation (≥ 3 mg/m^3) and histiocytic infiltrates (≥ 1 mg/m^3) in the lung, were significantly increased. In male and female mice, incidence of inflammation in the nose (≥ 3 mg/m^3) and larynx (≥ 10 mg/m^3), and of chronic inflammation (≥ 10 mg/m^3) and histiocytic infiltrates (≥ 0.3 mg/m^3) in the lung, were significantly increased (Bucher et al., 1990).

In a chronic study by the NTP, male and female Fischer 344/N rats and B6C3F$_1$ mice were exposed by inhalation to cobalt(II) sulfate heptahydrate at concentrations of 0.3, 1, or 3 mg/m^3 for 2 years (6 hours per day, 5 days per week). Incidence of granulomatous alveolar inflammation was significantly increased in the lung of male and female rats at all exposure concentrations (NTP, 1998; Bucher et al., 1999). Histological re-evaluation of the lungs of male rats reported a significant increase in the incidence of chronic active inflammation and histiocytosis (Ozaki et al., 2002). The incidence of diffuse and focal histiocytic cell infiltration was significantly increased in the lungs of male and female mice, respectively, at the highest exposure concentration. The incidence of suppurative inflammation in the nose was also significantly increased in male (3 mg/m^3) and female (1 mg/m^3) mice (NTP, 1998; Bucher et al., 1999).

(ii) Non-human mammalian cells in vitro

[The Working Group noted that in vitro studies in experimental systems with acute end-points were not included in the evaluation since they were not considered to be very relevant to chronic pro-inflammatory effects in humans.]

Table 4.17 Immunosuppression in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Ig subclasses	Serum	Czechia production workers, cross-sectional	38 nickel-exposed 35 cobalt-exposed Non-exposed controls	↑ IgA in cobalt-exposed group compared with controls ($P < 0.05$)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1983)
Ig subclasses	Serum	Czechia production workers, cross-sectional	38 nickel-exposed; 35 cobalt-exposed Non-exposed controls	↑ IgA in cobalt-exposed group compared with controls ($P < 0.05$) ↓ IgE in cobalt-exposed compared with controls (NSS)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1986b)

↓, decreased; ↑, increased; Ig, immunoglobulin; NSS, not statistically significant.

4.2.7 Is immunosuppressive

(a) Humans

(i) Exposed humans

See Table 4.17.

Evidence for immunomodulation in human populations exposed to cobalt has been reported in two cross-sectional studies in Czechia that compared cobalt production workers with non-exposed, age-matched controls (Bencko et al., 1983, 1986b). In the earlier study (1983), significantly increased average values were obtained for immunoglobulin A (IgA) in serum samples collected from cobalt-exposed workers compared with controls ($P < 0.05$).

In the later study (1986), levels of IgG, IgA, IgM, and IgE were measured; levels of IgA in cobalt-exposed workers were significantly higher than those of controls ($P < 0.05$). However, levels of IgE in cobalt-exposed individuals were lower than those of controls, although the difference was not statistically significant.

[The Working Group evaluated the study by Tilakaratne & Sidhu (2015) but excluded it as uninformative because it was a case report of T-cell populations from two cement workers with mixed exposure to chromium and nickel, without any regression analyses to isolate the effects of cobalt alone. The Working Group noted that the case report provides a narrative work history. Both cases reported probably also had exposure to chromate. The key limitations of this study were the lack of quantitative exposure data and the nature of a case report with a sample size of two.]

(ii) Human cells in vitro

[The Working Group noted that decreased cell proliferation and increased apoptosis/cytotoxicity of human leukocyte cell populations (including lymphocytes) in vitro induced by cobalt could be relevant to immunosuppression. All but one of the studies of human cells in vitro (described below) used primary cells.]

Wang et al. (1996) investigated cytotoxicity, T- and B-cell proliferation, and cytokine release by human peripheral blood mononuclear cells (PBMC) from whole blood after exposure to cobalt(III) chloride ($CoCl_3$). PBMC were exposed to cobalt(III) chloride (0.01, 0.1, 1.0, 10, 100 ng/mL) which was not cytotoxic at any concentration tested after a 72-hour exposure. However, phytohaemagglutinin-induced T-cell proliferation and lipopolysaccharide-induced B-cell proliferation were significantly inhibited by 48-hour exposure to cobalt(III) chloride at all tested concentrations and at ≥ 0.1 ng/mL, respectively. Furthermore, the release of the cytokines IL-2, IL-6, and IFN-γ by phytohaemagglutinin-stimulated PBMCs was significantly decreased after a 48-hour exposure to cobalt(III) chloride at all tested concentrations for IL-6 and IFN-γ, and at ≥ 0.1 ng/mL for IL-2. [The Working Group noted that the cobalt(III) chloride form has a valence state of +3 and, as such, is unstable/elusive. It is more likely that the cobalt(II) chloride form is responsible for observed effects.]

Akbar et al. (2011) studied the effects of cobalt(II) chloride on primary human lymphocytes in terms of cell viability, apoptosis, proliferation, and cytokine release. Lymphocytes were exposed to Co^{2+} (as cobalt(II) chloride) at concentrations of 0.1, 1, 10, or 100 µM for 24 or 48 hours. Exposure of resting lymphocytes to Co^{2+} at 100 µM resulted in a significant decrease in cell viability. This cytotoxic response was enhanced in lymphocytes stimulated with anti-CD3 antibody and observed after exposure to Co^{2+} at 10 µM for 24 and 48 hours, suggesting that activated cells are more sensitive to Co^{2+}-induced reductions in viability than resting lymphocytes. There was also a slight (but significant) increase in apoptosis of resting and CD3-activated lymphocytes after exposure to Co^{2+} at 100 µM for 24 and 48 hours. After 48-hour exposure, lymphocyte proliferation and cytokine (IFN-γ and IL-2) release by CD3-activated lymphocytes, with and without anti-CD28 antibody stimulation,

were significantly decreased after exposure to Co^{2+} at concentrations of 100 (proliferation), 0.1 (IL-2), or 10 (IFN-γ) μM.

Hagmann et al. (2013) investigated the effect of cobalt(II) chloride on primary human CD4+ T-lymphocytes with the aim of assessing lymphocyte viability, apoptosis/necrosis, and cell surface lymphocyte activation markers (CD25, CD38, CD69, and CD95). Co^{2+} (as cobalt(II) chloride) at a concentration of 1000 μg/L induced significant decreases in cell viability (with increases in the rates of apoptosis and necrosis) and the expression of CD25, CD38, and CD95 after exposure for 120 hours.

Bruzzese et al. (2016) treated peripheral blood lymphocytes isolated from whole blood and a human lymphoma CD4+ T-(CEM) cell line expressing $A_{2A}R$ with cobalt(II) chloride at concentrations of 50–800 μM to induce hypoxia. Resting peripheral blood lymphocytes and CEM cells exposed to cobalt(II) chloride for 24 hours showed a significant dose-dependent decrease in cell viability. Cells activated with phorbol 12-myristate 13-acetate plus phytohaemagglutinin and then treated with cobalt(II) chloride showed a greater dose-dependent decrease in cell viability than did resting cells.

In the study by Paladini et al. (2011), primary PBMCs extracted from whole-blood samples from 18 healthy donors were exposed to cobalt(II) chloride hexahydrate at concentrations of 42–336 μM for up to 6 days. Treatment with cobalt(II) chloride caused a significant reduction in T-lymphocyte numbers (84 μM cobalt(II) chloride reduced T-lymphocyte viability and increased apoptosis), and the expression of p53 in CD3+ T-lymphocytes was significantly increased 16 hours after exposure. Monocytes exposed to the same concentrations of cobalt(II) chloride did not display decreased cell viability (increased apoptosis), but p53 expression was significantly increased. Monocytes exposed to cobalt(II) chloride showed an increase in cytoplasmic $p21^{Cip1/WAF1}$, an inhibitor of pro-caspase-3 (an anti-apoptotic effect), which supports the results reported regarding the survival of monocytes treated with cobalt(II) chloride. Other experiments using monocytes showed that cobalt(II) chloride exposure increased NF-κB activity, as well as the expression and secretion of pro-inflammatory cytokines (TNFα and IL-1β), suggesting a role for cobalt(II) chloride in the activation of monocytes. The expression of human leukocyte antigen (HLA) class II molecules and the ability of the cells to capture and present antigens decreased over time with exposure, suggesting that cobalt(II) chloride maintains monocytes in a partially activated (but not fully differentiated) pro-inflammatory state. [The Working Group noted that decreased antigen presentation would dampen immune activation.]

Posada et al. (2015) studied the effects of Co^{2+} ions on primary human lymphocytes in terms of cell viability, apoptosis, proliferation, and cytokine production. Cell viability was measured 24, 48, and 120 hours after exposure to Co^{2+} at 0.1 μM (source of Co^{2+} not specified). [The Working Group noted that this concentration was chosen to mimic the metallosis that can occur in the local environment surrounding an implant.] The results showed that there were no significant changes in the numbers of lymphocytes or metabolic activity after exposure to Co^{2+} at 0.1 μM for 24 or 120 hours, but lymphocyte proliferation was significantly decreased after 48-hour (but not 120-hour) exposure. IL-2 secretion by lymphocytes after 24- and 48-hour exposure to Co^{2+} at 0.1 μM was significantly decreased, but significant alterations in the secretion of IL-6, IFN-γ, and TNFα were not reported.

Primary human PBMCs isolated from whole blood were exposed to cobalt metal NPs (size, < 50 nm), particles, or cobalt(II) chloride at a concentration of 10^{-5}, 10^{-6}, and 10^{-7} M to assess the resulting effects on the production of several cytokines. Exposure to cobalt particles induced a significant decrease in the release of IFN-γ,

TNFα, IL-10, IL-4, and IL-2 at all concentrations tested, and of IL-6 at 10^{-7} M. Exposure to cobalt metal NPs significantly decreased the production of IL-2 and IL-10 at all concentrations tested, and stimulated the release of TNFα at 10^{-6} and 10^{-7} M, and IFN-γ at 10^{-7} M. Exposure to cobalt(II) chloride significantly decreased the production of IL-10, IL-2, and TNFα at 10^{-5} M (Petrarca et al., 2006).

Verstraelen et al. (2014) assessed expression profiles of genes involved in immune signalling in human alveolar (A549) and bronchial (BEAS-2B) epithelial cells after exposure to cobalt(II) oxide NPs (size, 7.04 ± 0.81 nm) at concentrations of 9.1×10^{13}/mL [60.91 μg/mL] and 9.1×10^{12}/mL [6.091 μg/mL], respectively, for 3, 6, 10, 24 hours. Exposure to cobalt(II) oxide NPs at a concentration of 60.91 μg/mL was cytotoxic to BEAS-2B, but not A549, cells after exposure for 24 hours. BEAS-2B cells were more sensitive to the effects of cobalt(II) oxide NPs and had higher numbers of differentially expressed transcripts than A549 cells, including differentially expressed transcripts of immune markers, especially after 24-hour exposure. Cobalt(II) oxide NPs predominantly induced downregulation of the expression of genes involved in immune signalling over time after exposure. The downregulated genes included TLR6, HLA-DRB3, TIRAP, and HLA-A. The mRNA transcript that had the largest decrease was HLA-DRB3, which plays an important role in presenting peptides derived from extracellular proteins. [The Working Group noted that the decreased antigen presentation would dampen immune activation.] The TLR6 gene was suppressed in both cell types. In contrast, the ITGB2 and PAG1 genes, which encode a protein involved in the adhesion and migration of leukocytes and a protein thought to be involved in the regulation of T-cell activation, respectively, were upregulated.

(b) Experimental systems

(i) Non-human mammals in vivo

In a study by Chetty et al. (1979), Sprague-Dawley rats were treated with feed containing cobalt(II) chloride (0–300 ppm) with sufficient or deficient levels of iron. Rats that received cobalt(II) chloride at concentrations of ≥ 100 ppm (iron-sufficient and -deficient groups) had reduced body and thymus weights; however, non-lymphoid tissues such as liver, kidney, and heart were not affected. In exposed rats, there was also a significant decrease in sheep haemagglutinins (200 ppm, iron-sufficient group; ≥ 50 ppm, iron-deficient group) and antibody-producing cells as assessed by plaque-forming cell assays using sheep erythrocytes (≥ 100 ppm, iron-sufficient group; ≥ 50 ppm, iron-deficient group).

Nagai et al. (1989) examined the effects of cobalt(II) chloride on the production of specific IgM and polyclonal IgG antibodies as assessed by direct plaque-forming cell and reverse plaque-forming assays, respectively. Mice given cobalt(II) chloride hexahydrate (9.0, 0.9, or 0.45 mg/kg bw) by intraperitoneal injection every other day (for a total of three injections) and then immunized with sheep erythrocytes on the last injection day showed an increase in the number of IgM plaque-forming cells in spleen (9.0 and 0.9 mg/kg bw). However, numbers of polyclonal IgG plaque-forming cells (9.0 or 0.9 mg/kg bw) in the spleen did not differ from those in the control group.

(ii) Non-human mammalian cells in vitro

In a study by Shweta et al. (2015) in peritoneal macrophages and splenocytes from BALB/c mice exposed to cobalt(II) chloride (0.05 mM) for 24 hours, there was a significant decrease in the expression of TLR4 by macrophages and the cell surface activation markers CD25, CD40, and CD69 (but not CD95) by splenocytes; however, cobalt(II) chloride induced a significant increase in the secretion of pro-inflammatory TNFα and

Table 4.18 Modulation of receptor-mediated effects in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Thyroid hormone levels	Serum	Belgium, highly exposed cobalt refinery workers	82 cobalt-exposed men 82 non-exposed controls (age-matched)	Negative association for T3 with cobalt Mean 150.00 ng/dL in controls vs 140.18 ng/dL in exposed ($P < 0.05$) Effect of cobalt on FT4 and TSH	Controls were age-, education-, and SES-matched	There is limited potential for non-differential misclassification in the exposure groups. This study focuses on comparisons between exposed and unexposed groups working at different companies. A key limitation of this study is the reliance on the qualitative exposure assessment approach (exposed/unexposed) based on location of employment.	Swennen et al. (1993)
Reproductive hormones	Serum	China, partners of fertility clinic patients, cross-sectional	$n = 511$	No associations between urinary cobalt levels and serum reproductive hormones after adjustment for multiple testing (FDR-adjusted P for trend for each hormone > 0.50)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Urine samples collected at two close points in time on the same day as semen sample limits the findings.	Wang et al. (2016a)
Thyroid hormone levels	Blood	China, Hangzhou Birth Cohort Study, cross-sectional	$n = 915$ pregnant women	While the middle tertile of urinary cobalt was associated with a decrease in FT3 ($P < 0.05$), no associations between urinary cobalt levels and serum reproductive hormones in single-metal models were observed after adjustment for multiple testing (FDR-adjusted for each hormone, P for trend ranged from > 0.26 to 0.88)	Maternal age, education, household income, working in pregnancy, second-hand smoke in pregnancy, drinking in pregnancy, gestational age at measurement of thyroid hormone levels, parity, history of hyperglycaemia in pregnancy, and pre-pregnancy BMI	Single blood samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Co-exposures to other metals (arsenic, cobalt, selenium, manganese, and nickel) were evaluated in the statistical analyses if they produced statistically significant results ($P < 0.05$) when evaluated one at a time. Logistic regression results presented for manganese, nickel, and antimony with FT4.	Guo et al. (2018)

BMI, body mass index; FDR, false discovery rate; FT3, free triiodothyronine; FT4, free thyroxine; SES, socioeconomic status; T3, triiodothyronine; TSH, thyroid stimulating hormone; vs, versus.

IL-6 (but not IFN-γ). The expression of NF-κB by macrophages was also increased.

4.2.8 Modulates receptor-mediated effects

(a) Humans

(i) Exposed humans

See Table 4.18.

Swennen et al. (1993) assessed health effects in 82 workers at a cobalt refinery in Belgium who had an average duration of exposure of 8 years and a geometric mean TWA exposure to cobalt dust of 125 μg/m³. This included 25% of the worker population with exposure > 500 μg/m³. Results were compared with an age-matched group of non-cobalt dust-exposed controls. Mean triiodothyronine (FT3) concentrations were lower in the cobalt dust-exposed population (140.18 ng/dL) than in non-exposed controls (150.00 ng/dL) ($P < 0.05$). However, other thyroid hormone measurements, including free thyroxine (FT4) and thyroid stimulating hormone (TSH), did not differ between the groups.

In the Chinese Hangzhou Birth Cohort Study, Guo et al. (2018) examined thyroid hormone levels in 915 pregnant women grouped into tertiles of six metals: arsenic, cobalt, manganese, nickel, antimony, and selenium. While the middle tertile of urinary cobalt was associated with a decrease in free triiodothyronine (FT3) ($P < 0.05$), no associations between urinary cobalt levels and levels of reproductive hormones in serum in single-metal models were observed after adjustment for multiple testing (FDR-adjusted model; P for trend ranged from 0.26 to 0.88) across the hormones assessed. Covariates considered included maternal age, education, household income, working during pregnancy, exposure to second-hand smoke during pregnancy, drinking during pregnancy, gestational age at measurement of thyroid hormones, parity, history of hyperglycaemia during pregnancy, and pre-pregnancy BMI. [The Working Group noted that single blood samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Co-exposures to other metals (arsenic, cobalt, selenium, manganese, and nickel) were assessed in the statistical analyses if they produced statistically significant results ($P < 0.05$) when considered one at a time. Logistic regression results were presented for manganese, nickel, and antimony with free thyroxine.]

The association of urinary metal levels with reproductive hormones was assessed in partners of fertility clinic patients in a cross-sectional study in China by Wang et al. (2016a). No significant (or suggestive) associations were found between urinary cobalt levels and any hormones assessed after adjustment for age, BMI, smoking status, daily cigarette consumption, abstinence time, and concentrations of urinary creatinine and other metals, nor for multiple testing.

[The Working Group noted that there was only one of three studies of cobalt-exposed populations with some evidence related to receptor-mediated effects. The Working Group also noted that the occupational exposure levels to metals in Swennen et al. (1993) were probably much higher than the environmental exposure levels occurring in the two cohort studies.]

Johnstone et al. (2014) investigated exposure to trace metals as a predictor of benign uterine fibroid tumour development. [The Working Group evaluated this study but excluded it as uninformative because exposure to trace metals is an uncertain indicator, and while the authors reported higher cobalt concentrations in patients with fibroids compared with non-fibroid controls, it was not a predictor of a fibroid diagnosis.]

The study by Tilakaratne & Sidhu (2015) was a case report of T-cell populations from samples collected from two cement workers with mixed exposure to chromium and nickel. [The Working Group evaluated the study but excluded it as uninformative because it referred

to co-exposure and was without any regression analyses to isolate the effects of cobalt alone.]

(ii) Human cells in vitro

Cobalt(II) salts

The effects of cobalt(II) chloride on steroid hormone receptor signalling pathways, specifically ERα and PR, were studied in the ERα-positive human breast cancer cell line MCF7 (Martin et al., 2003; Cho et al., 2005). Similar to estradiol at 1 nm (24-hour exposure), exposure to cobalt(II) chloride at 1 μM (24-hour exposure) decreased ER mRNA and protein levels, increased pS2 (an estrogen-responsive gene) mRNA, increased PR mRNA and protein levels via interacting with the ER, and increased MCF7 cell proliferation (Martin et al., 2003). [The Working Group noted that cobalt(II) chloride, presumably in ion form, binds to ERα non-competitively and activates ERα through the hormone-binding (not DNA-binding) domain (Martin et al., 2003).] Investigating the mechanisms of hypoxia-induced ERα downregulation, Cho et al. (2005) used cobalt(II) chloride to induce cellular hypoxia in MCF7 cells and found that inhibition of HIF-α protein synthesis could partially prevent the cobalt(II) chloride (hypoxia)-induced ERα protein decrease. Protein kinase inhibitors of MAPK, P13K, and p38 did not prevent cobalt(II) chloride-induced downregulation of ERα (Cho et al., 2005).

Using a recombinant ER DNA-binding domain polypeptide, Predki & Sarkar (1992) restored the nonspecific DNA-binding property of the apo-polypeptide, which was previously inhibited by treatment with metal chelators, by dialysis with buffer containing zinc, cobalt, or cadmium (but not copper or nickel). [The Working Group noted that the authors did not specify the species on which the recombinant polypeptide was based. It was likely to be human recombinant ER.]

Retinoic acid receptor-related orphan receptor α (RORα) and peroxisome proliferator-activated receptor α (PPARα), both of which can have potential pro- and anticancer effects, were assayed in various human cells exposed to cobalt(II) chloride. After the human hepatoma cell line HepG2 was treated with cobalt(II) chloride, the increase in the level of *RORA* gene expression (encoding RORα) and overall protein production was statistically significant, although the increase in RORα transcriptional activity was not statistically significant (Chauvet et al., 2002). While the study by Chauvet et al. did not investigate downstream effects of RORα, cobalt(II) chloride was reported by Daoud et al. (2005) to result in decreased PPARα mRNA levels in isolated human placental trophoblast cells, where decreased PPARβ and PPARγ mRNA levels were also observed. [The Working Group noted that PPARα has several potential anticancer effects, including the induction of decreased pro-inflammatory transcription factors via interaction with the ER.]

Cobalt(II) chloride treatment or transfection of HIF-1α decreased constitutive androstane receptor (CAR) protein expression as well as *CAR* gene promoter activity in the human cancer cell lines AGS (gastric), SW480 (colon), and PC-3 (prostate) (HIF-1α was tested in AGS cells only) (Küster et al., 2010). [The Working Group noted that CAR, a tumour suppressor, also has pro-cancer activities (e.g. increases cell proliferation and metastasis), so the effects of decreased CAR expression remain unclear.]

Aryl hydrocarbon receptor nuclear translocator (ARNT) regulates many genes involved in tumour growth and angiogenesis. Exposure to either cobalt(II) chloride at 50 μM (chemically induced hypoxia) or 1% oxygen (1% oxygen hypoxia) for 24 hours increased ARNT protein levels in the human cell lines MCF7 (breast ductal carcinoma), Hep3B (hepatocellular carcinoma), and REPC (primary human kidney), while only cobalt(II) chloride (not 1% oxygen hypoxia) increased ARNT protein levels in HepG2 (hepatocellular carcinoma). In HeLa

(cervical carcinoma) and Kelly (human neuroblastoma) cells, neither cobalt(II) chloride nor 1% oxygen hypoxia altered ARNT protein levels (mRNA levels were not measured). The mRNA levels of ARNT did not always change in the same direction as the protein. ARNT mRNA levels were decreased by cobalt(II) chloride (but not 1% oxygen hypoxia) in MCF7 cells, decreased by both cobalt(II) chloride and 1% oxygen hypoxia in Hep3B cells, and unchanged in both HeLa and REPC cells (Wolff et al., 2013).

Cobalt has been reported to affect the receptors of two peptides that influence vascular tone: adrenomedullin (ADM), a vasodilator, and endothelin (ET), a vasoconstrictor. Cobalt(II) chloride increased mRNA levels of ADM but decreased the mRNA levels and mRNA stability of an ADM receptor component, receptor activity modifying protein 2 (RAMP2), in two human neuroblastoma cell lines (IMR-32 and NB-69) (Kitamuro et al., 2001). [The Working Group noted that in studies not using cobalt, ADM was induced by HIF-1α and displayed several pro-cancer effects, including anti-apoptosis signalling and stimulation of cell growth, and indirectly decreased immune responsiveness (Zudaire et al., 2003).] Like estradiol, cobalt(II) chloride increased mRNA and protein levels of endothelin-A receptor (ETAR) in several human breast cancer cell lines (Wülfing et al., 2005). The functions of endothelin and its receptors, including ETAR, include mitogenesis, anti-apoptosis, angiogenesis, synergism with growth factors, and promoting tumour cell growth, in addition to vasocontraction (Grant et al., 2003; Jovanović, 2018). [The Working Group noted that the effects of cobalt(II) chloride on receptors of ADM and ET are likely to contribute to carcinogenesis.]

(b) Experimental systems

(i) Non-human mammals in vivo

Male Sprague-Dawley rats given drinking-water containing cobalt(II) chloride at 2 mM for 10 days showed decreased mRNA of PPARα- and PPARα-regulated genes, such as pyruvate dehydrogenase kinase 4 (PDK4), muscle carnitine palmitoyltransferase I (mCPT I), and malonyl-CoA decarboxylase (MCD) in heart cells (Razeghi et al., 2001). [The Working Group noted the high dose of cobalt(II) chloride used in this study and that no water intake information was provided.]

PPARα and PPARγ (but not PPARδ) mRNA levels were increased in adipose tissue of male Slc:ICR mice fed a high-fat diet for 14 days. When cobalt(II) chloride was administered at 1, 3, 5, or 7.5 mg/kg bw per day for 10 days via subcutaneous injection after the high-fat diet began, both PPARα and PPARγ mRNA levels were restored to the same levels as in the group given standard diet (Kawakami et al., 2012). [The Working Group noted that the effective dose of cobalt(II) chloride was not specified in the results.]

Investigating the link between CAR and HIF-1α, Shizu et al. (2013) used cobalt(II) chloride to activate HIF-1α. In male C3H/HeN mice, administration of cobalt(II) chloride (40 mg/kg bw) by a single intraperitoneal injection increased CAR protein expression, nuclear CAR accumulation, CAR-mediated phenobarbital-responsive enhancer module transactivation, and gene expression of CAR target genes in the liver (Shizu et al., 2013).

(ii) Non-human mammalian cells in vitro

Cobalt(II) chloride treatment of ovarian granuloma cells collected from pigs caused decreased estradiol synthesis but had no effects on progesterone production (Grasselli et al., 2005). In contrast, treatment with cobalt(II) sulfate heptahydrate had no effect on 17β estradiol release by rat ovarian fragments (Roychoudhury et al.,

Table 4.19 Immortalization in mammalian cells in vitro exposed to cobalt

End-point	Species, tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Soluble cobalt(II) salts					
Cobalt(II) chloride ($CoCl_2$)					
Morphological transformation assay (type III foci)	Mouse, embryonic fibroblasts, BALB/3T3	(–)	50 µM [~6 µg/mL]	$CoCl_2 \cdot 6H_20$; 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Data in Figure 1, subfigure titles, and legend do not match text. Negative and several positive controls included.	Sabbioni et al. (2014a)
Morphological transformation assay (type III foci)	Mouse, embryonic fibroblasts, BALB/3T3	–	70 µM [~9.1 µg/mL]	$CoCl_2$; 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Negative and positive controls included.	Ponti et al. (2009)
Morphological transformation assay (type II foci; type III foci were not observed)	Mouse, embryonic fibroblasts, C3H10T1/2	(+)	5 µg/mL [~40 µM]	$CoCl_2$; 48 h exposure, 42-day growth period. Effective dose range induced ≥ 95% cytotoxicity. Dose-dependent response, negative and positive controls included.	Doran et al. (1998)
Cobalt(II) sulfate ($CoSO_4$)					
Reduced pH morphological transformation assay	Syrian hamster, embryo, SHE	(+)	0.125 µg/mL [~0.75 µM]	$CoSO_4$ hydrate; 24 h exposure. Source and purity of cobalt not reported. Similar magnitude of effect at all doses, effective dose range induced ≤ 50% cytotoxicity.	Kerckaert et al. (1996)
Cobalt(II) acetate ($Co(CH_3CO_2)_2$					
Enhancement of simian adenovirus SA7-induced transformation	Syrian hamster, embryo, SHE	(+)	200 µM [~35 µg/mL]	$(Co(CH_3CO_2)_2$; 18 h exposure, 25–30-day growth period. Source and purity of cobalt not reported. Only reported effects at a single dose. Only reported ratio of enhancement.	Casto et al. (1979)
Cobalt metal (Co) or cobalt metal NPs					
Morphological transformation assay (type II and III foci), clonal growth in soft agar, Tumour growth in female athymic nude mice	Human, osteosarcoma, HOS TE85 [*p53* mutation at codon 156]	–	3 µg/mL cobalt powder	Cobalt metal particle size: d_{50}, 1–4 µm); 24 h exposure, 35-day growth period. Dose range based on minimal to 60% cytotoxicity. Dose-dependent response in cytotoxicity. Negative and positive control included.	Miller et al. (2001)
Anchorage-independent colony growth on soft agar	Human, cervical adenocarcinoma, HeLa	(+)	0.05 µg/mL	NP size: d_{50}, 30 nm; hd_{50}, 505 nm; 5–12 wk exposure, 21-day growth period on soft agar. Indirect exposure of HeLa cells fed media conditioned by *Ogg*$^{-/-}$ mouse embryonic fibroblasts exposed to cobalt nanoparticles. Cobalt source specified, but not purity. No discussion of cytotoxicity following 5–10 wk of exposure. Dose-dependent response, negative control included.	Annangi et al. (2015)
	Mouse, embryonic fibroblasts, MEF (WT)	+	0.05 µg/mL		
	Mouse embryonic fibroblasts, MEF (*Ogg*$^{-/-}$)	+	0.05 µg/mL		

Table 4.19 (continued)

End-point	Species, tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Morphological transformation assay (type II and III foci)	Mouse, embryonic fibroblasts, C3H10T1/2	(–)	500 µg/mL	Cobalt metal particle size: d_{50}, < 5 µm; 42-day exposure and growth period. Particle size was determined visually by light microscopy, exposures continued beyond 48 h throughout 42-day growth period. Dose range based on minimal to > 95% cytotoxicity, negative and positive controls included.	Doran et al. (1998)
Morphological transformation (type III foci), following exposure as initiator or promoter in two-stage carcinogenesis bioassay	Mouse embryonic fibroblasts, BALB/3T3	+ –	10 µM [2.0 µg/mL] (initiator) 2.5 µM [0.5 µg/mL] (promoter)	NP size: d_{50}, 28 nm; hd_{50}, 141 nm; 72 h exposure as initiator, 9-day exposure as promoter, 42-day growth period. Cobalt source specified, but not purity. Dose range based on 50–80% cytotoxicity, negative and positive controls included.	Sighinolfi et al. (2016)
Morphological transformation assay (type III foci)	Mouse embryonic fibroblasts, BALB/3T3	(+) (+)	1 µM [~0.13 µg/mL] cobalt metal 5 µM [~0.65 µg/mL] cobalt metal NPs	Cobalt metal particles (hd_{50}, 2.2 µm), cobalt metal NPs (hd_{50}, 200 nm); 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Dose-dependent response, negative and several positive controls included. Data in Figure 1, subfigure titles, and legend do not match text.	Sabbioni et al. (2014a)
Morphological transformation assay (type III foci)	Mouse embryonic fibroblasts, BALB/3T3	+	7 µM [~1.4 µg/mL]	Cobalt metal NPs (d_{50}, 80 nm); 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Dose-dependent response, negative and positive controls included.	Ponti et al. (2009)
Cobalt(II) sulfide or disulfide (CoS or CoS_2)					
Morphological transformation assay (disordered colony morphology)	Syrian hamster, embryo, SHE	+ +/–	1 µg/mL CoS_2 particles 5 µg/mL CoS particles	CoS_2 crystalline particles (d_{50}, 1.25 µm), CoS amorphous particles (d_{50}, 2.0 µm); 48 h exposure, 20-day growth period. Cobalt source specified, but not purity. Dose range based on minimal to > 50% cytotoxicity. Dose-dependent response, negative control included, morphology scored by 3 blinded observers.	Abbracchio et al. (1982); Costa et al. (1982)

d_{50}, mean particle diameter; hd_{50}, hydrated mean particle diameter; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; wk, week; WT, wildtype.
[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

2014), but did decrease progesterone release by both rat ovarian fragments (Roychoudhury et al., 2014) and porcine primary ovarian granulosa cells (Kolesarova et al., 2010). Progesterone production by primary cultures of canine corpus luteum cells was also decreased after exposure to cobalt(II) chloride (Sousa et al., 2016).

Steroid production by male reproductive Leydig cells was decreased by cobalt(II) chloride. In Leydig cells collected from adult rats, cobalt(II) chloride decreased the maximum rate of luteinizing hormone (LH)-stimulated androgen production (Moger, 1983). By using inhibitors, Moger (1983) determined that the inhibition occurred between cAMP formation and the 3β-hydrogenase step. When the mouse Leydig tumour-derived MA-10 cell line was exposed to cobalt(II) chloride, progesterone production was decreased via increased ROS production, increased HIF-1α activity, and decreased basal mRNA levels of the specific enzyme cytochrome P450 side-chain cleavage ($P450_{scc}$) (Kumar et al., 2014). [The Working Group noted that in MA-10 cells, the steroidogenesis product is progesterone due to the lack of 17α hydroxylase.]

Exposure of Cos7 cells (SV40-transformed African green monkey renal cells) to either cobalt(II) chloride (50 μM, 24 hours) or hypoxia (1% oxygen, 24 hours) increased ARNT protein levels (Wolff et al., 2013).

Cobalt(II) chloride (100 μM) decreased mRNA levels of macrophage scavenger receptor 1 (MSR1) (via increasing HIF-1α expression) in a mouse cell line (RAW 264) (24-hour exposure) and in primary macrophages (8-hour exposure) collected from mouse peritoneal exudate (Shirato et al., 2009). [The Working Group noted that while decreased levels of MSR1 were associated with worse recurrence-free survival of patients with prostate cancer (Cao et al., 2017), the role of macrophage scavenger receptor 1 in carcinogenesis remains to be determined.]

4.2.9 Causes immortalization

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cobalt metal or cobalt metal nanoparticles

See Table 4.19.

Direct evidence is limited to a single study that was previously reported in *IARC Monographs* Volume 86 (IARC, 2006). Exposure of the human osteosarcoma (HOS) cells (TE85, cone F-5) to cobalt metal at subcytotoxic concentrations for 24 hours (3 μg/mL; mean particle diameter, 1–4 μm) did not increase transformation after a 35-day period of clonal growth in soft agar or tumourigenesis when cells were implanted in athymic nude mice (Miller et al., 2001). [The Working Group noted that simulated W/Ni/Co (a mixture of metals including cobalt) increased transformation frequency, anchorage-independent growth of transformed clones in soft agar, and formation of injection-site tumours in athymic nude mice (Miller et al., 2001).]

Indirect evidence is also available from the observation of a concentration- and duration-dependent increase in the anchorage-independent growth of HeLa cells in soft agar, when fed with media conditioned by MEF *OGG1*$^{-/-}$ cells exposed to cobalt metal NPs at concentrations of 0.05 and 0.1 μg/mL for up to 10 weeks (Annangi et al., 2015). [The Working Group noted that *hOGG1* encodes a protein responsible for the BER of oxidative 8-OHdG lesions. The Working Group also noted that the effects of conditioned media cannot be attributed to the presence of cobalt metal NPs specifically, as the potential confounding effects of cell-derived mediators such as matrix metalloproteinases 2 and 9, which were also increased, cannot be ascertained.]

Cobalt compounds

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

See Table 4.19.

Cobalt metal or cobalt metal nanoparticles

Exposure of MEF BALB/3T3 cells to cobalt metal at (non-cytotoxic and cytotoxic) concentrations of ≥ 0.13 µg/mL for 72 hours significantly increased transformation frequency as determined by a concentration-dependent increase in type III foci, which was inhibited by co-treatment with ascorbic acid at 50 µM (Sabbioni et al., 2014a). [The Working Group noted concerns, as described in Table 4.19, namely, apparent inconsistencies in the reporting of results that decrease confidence in the interpretation of these findings.] However, cobalt metal particles < 5 µm in diameter did not increase the incidence of type II or III foci formation in C3H10T1/2 cells after exposure at concentrations ≤ 500 µg/mL, despite significantly increased cytotoxicity (Doran et al., 1998). [The Working Group noted that it was impossible to remove particulates from cell monolayers, so cells were exposed throughout the 42-day growth period, greatly exceeding the intended 48-hour exposure period.]

Cobalt metal NPs (mean diameter, 0.08 µm) induced morphological transformation as determined by increased incidence of type III foci in MEF BALB/3T3 cells after 72-hour exposures at concentrations ≥ 1.4 µg/mL (Ponti et al., 2009); larger cobalt metal particles (diameter, 0.2 µm) significantly increased transformation after 72-hour exposures to concentrations ≥ 0.65 µg/mL, including non-cytotoxic and cytotoxic exposures, which was inhibited by co-treatment with 50 µM ascorbic acid (Sabbioni et al., 2014a). [The Working Group noted concerns with the study by Sabbioni et al. (2014a) as described in Table 4.19 and discussed above.] When used as the initiating agent in a two-stage transformation bioassay, cobalt metal NPs (mean diameter, 0.028 µm; hydrated mean diameter, 0.14 µm) at a concentration of 2.0 µg/mL increased transformation of BALB/3T3 cells after a 3-day exposure, in the absence of any promoter. When cobalt metal NP exposure was followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion, the number of transformed foci increased ninefold. Cobalt metal NPs were not effective as a promoter after 9-day exposure at the lower concentration of 0.5 µg/mL (Sighinolfi et al., 2016). [The Working Group noted that activity in the initiation phase might indicate that cobalt metal NPs can induce transformation in non-neoplastic cells. The Working Group also noted that the number of foci observed with 3-methylchloanthrene initiation followed by cobalt metal NP promotion was reduced, suggesting that cobalt metal NP treatment suppressed 3-methylchloanthrene-initiated colony formation, possibly due to increased cytotoxicity over the extended exposure duration.]

After 12 weeks of exposure at concentrations that were non-cytotoxic in the short-term, exposure of MEF cells deficient in Ogg1 (*Ogg1*$^{-/-}$) to cobalt metal NPs at a concentration of 0.05 µg/mL (mean diameter, 0.03 µm; hydrated mean diameter, 0.5 µm) induced morphological changes including cell elongation, but not in isogenic wildtype MEFs. After 10 weeks of exposure, there was a concentration-dependent increase in anchorage-independent soft agar colony formation in both wildtype and *Ogg1*$^{-/-}$ MEFs exposed to 0.05 or 0.1 µg/mL cobalt metal NPs; colony formation was greater in the *Ogg1*$^{-/-}$ versus wildtype MEFs, and colony formation increased after exposure to 0.1 µg/mL of cobalt metal NPs for only 5 weeks in *Ogg1*$^{-/-}$ but not wildtype MEFs (Annangi et al., 2015).

Soluble cobalt(II) salts

Cobalt(II) chloride did not increase morphological transformation (by evaluation of type III foci) in the MEF BALB/3T3 cell line after 72-hour

Table 4.20 Alterations in cell proliferation, cell death, or nutrient supply in human primary cells exposed to cobalt

End-point	Tissue, cell line	Results[a]	Concentration	Comments	Reference
Angiogenesis					
VEGF expression	Primary human fetal striatal neuroblasts	+	400 µM for 48 h		Ambrosini et al. (2015)
VEGF expression	Bone marrow mesenchymal stem cells	+	100 µM for 24 h	4 donors.	Nguyen et al. (2020)
VEGF expression	Fibroblasts and HUVECs	+	100 µM for 18 h	Statistical analysis was not performed.	Minchenko et al. (1994)
VEGF expression	Dental pulp-derived cells	+	300 µM, for 24 h	3 donors. Non-cytotoxic concentration.	Müller et al. (2012)
VEGF expression Decreased SDF-1 expression	Human endometrial stromal cells	+ +	10–100 µM for 48 h	22 donors.	Tsuzuki et al. (2012)
VEGF mRNA and protein expression	Retinal pigment epithelial cells	(+)	600 µM for 12 and 24 h		Gu et al. (2021)
VEGF expression	Retinal pigment epithelial cells	+	50–200 µM for 24 h	1 donor. Non-cytotoxic concentration.	Rosen et al. (2015)
VEGF expression	Nasal polyp fibroblasts	+	500 µM for 8 and 12 h	8 donors. VEGF was not increased after 24 h.	Lin et al. (2008)
VEGF expression	Periosteum-derived mesenchymal stem cells	+	50–200 µM for 7 day	$CoCl_2 \cdot 6H_20$. Cells obtained from periosteal biopsies from the proximal tibia of adolescent and adult patients during total knee replacement procedure or distraction osteogenesis.	Chai et al. (2018)
VEGF expression	HUVECs	+	100 µM for 24 h	Statistical analysis was not performed.	Namiki et al. (1995)
VEGF expression	HUVECs	+	50 µM for 4 and 24 h		Qiu et al. (2012)
VEGF expression Capillary-like tube structure formation	HUVECs	(+) +	100 µM for 24 h	Figure legend describing VEGF expression seems inverted.	Liu et al. (2009)
VEGF expression PECAM-1 expression	HUVECs	+ +	0.5–50 µM for 48 h 25 µM for 48 h	Non-cytotoxic concentrations.	Bosch-Rué et al. (2021)
VEGF expression	HUVECs	+	100 µM for 24 h		Wei et al. (2019)
VEGF expression	CD133+ cells from umbilical cord blood samples	+	100–200 µM		Zan et al. (2012)

Table 4.20 (continued)

End-point	Tissue, cell line	Results[a]	Concentration	Comments	Reference
Glycolysis					
ATP production through glycolysis	Skin fibroblasts	+	70 µM for 24 h	Non-cytotoxic concentration.	Vincent et al. (2008)
Cell proliferation					
Cell growth	Cholesteatoma keratinocytes	+	50–200 µM for 24 h		Zhang et al. (2021b)
Cell viability	Primary human fetal striatal neuroblasts	(+)	300–400 µM for 48 h	Cell viability used as an indirect marker of cell proliferation.	Ambrosini et al. (2015)
Cell viability	CD133+ cells from umbilical cord blood samples	(+)	50–200 µM for 72 h	Cell viability used as an indirect marker of cell proliferation.	Zan et al. (2012)
Cell viability increased CDK4 and cyclin D1 expression	HUVECs	+	20–200 µM for 24 h	Cell viability used as an indirect marker of cell proliferation.	Wei et al. (2019)
		+	100 µM for 24 h		
Cell number	Bone marrow mesenchymal stem cells	–	100 µM for 24 h	4 donors.	Nguyen et al. (2020)
Cell death					
Decreased apoptosis	Primary human umbilical vein endothelial cells (HUVECs)	+	100 µM for 24 h		Wei et al. (2019)
Increased Bcl-2 expression		+			
Decreased Bax and caspase-3 expression		+			
Decreased apoptosis	Primary human umbilical vein endothelial cells (HUVECs)	+	150 µM for 24 h	This effect of $CoCl_2$ is dependent on increased miR-21 expression.	Xu et al. (2017)
Decreased PDCD4 expression		+			

ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma-2; CDK4, cyclin-dependent kinase 4; HUVEC, human umbilical vein endothelial cell; mRNA, messenger RNA; PDCD4, programmed cell death protein 4; PECAM-1, platelet endothelial cell adhesion molecule-1; SDF-1, stromal cell-derived factor-1; VEGF, vascular endothelial growth factor.
[a] +, positive; –, negative; (+), positive in a study of limited quality.

exposures at concentrations ≤ 9.1 μg/mL, despite concentration-dependent increases in cytotoxicity as assessed by decreased relative plating efficiency (Ponti et al., 2009; Sabbioni et al., 2014a). [The Working Group noted that these negative studies were inconsistent with the positive results reported in earlier studies and summarized in *IARC Monographs* Volume 86 (IARC, 2006), although several quality and reporting concerns exist, as noted in Table 4.19, including a lack of concentration dependence in responses or effects only observed at concentrations associated with more than 90% cytotoxicity.] Briefly, concentration-dependent increases in the number of type II foci were observed in the MEF C3H10T1/2 cell line after 48-hour exposure to 5–20 μg/mL cobalt(II) chloride, concentrations that induced reductions of more than 95% in relative plating efficiency (Doran et al., 1998). An increased rate of morphological transformation was also observed in SHE cells, cultured at pH 6.7, after 24-hour exposure to ≥ 0.125 μg/mL cobalt(II) sulfate hydrate, including both subcytotoxic and cytotoxic concentrations, although the magnitude of effect was not concentration-related (Kerckaert et al., 1996). Similarly, 18-hour treatment with cobalt(II) acetate at 35 μg/mL enhanced simian adenovirus SA7-induced transformation in SHE cells (Casto et al., 1979).

Cobalt(II) sulfide

As reported previously in *IARC Monographs* Volume 86 (IARC, 2006), particles of crystalline CoS_2 and amorphous CoS (mean diameters, 1.25 μm and 2.0 μm, respectively) induced morphological transformation in a concentration-dependent manner in SHE cells after 48 hours of exposure to relatively noncytotoxic levels of 1 and 5 μg/mL, respectively, with greater transformation reported with crystalline versus amorphous forms, and at cytotoxic concentrations of 10 μg/mL (Abbracchio et al., 1982; Costa et al., 1982).

4.2.10 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Primary cells

See Table 4.20.

Cobalt(II) chloride has been frequently used to model the effects of chemically induced hypoxia because both cobalt(II) chloride and cobalt(II) sulfate induce responses similar to physiological hypoxia in cells (i.e. exposure to 1% oxygen), with the reported IC_{50} for induction of hypoxia-response element (HRE)-regulated genes around 10–30 μM for cobalt(II) sulfate, and VEGF induction occurring in a HIF-1α-dependent manner at concentrations ≤ 100 μM (Vengellur et al., 2003; Xia et al., 2009). In fact, the main hypoxia biomarker and signal transducer activated by cobalt(II) chloride is HIF-1α. [The Working Group noted that the mechanisms for HIF-1α activation are generally thought to involve replacing Fe^{2+} ions in the prolyl hydroxylase active centre leading to stabilization of HIF-1α protein, and possibly other activities.] Cobalt(II) chloride has been shown to induce hypoxia in 19 studies conducted using human primary cells (Table 4.20). In these studies, hypoxia effects induced by cobalt(II) chloride were associated with end-points related to key characteristic 10: "alters cell proliferation, cell death, or nutrient supply".

Regarding angiogenesis, increased VEGF mRNA and/or protein expression was shown in cells – such as bone marrow mesenchymal stem cells (Nguyen et al., 2020), dental pulp-derived cells (Müller et al., 2012), endometrial stromal cells (Tsuzuki et al., 2012), nasal polyp fibroblasts (Lin et al., 2008), and periosteum-derived mesenchymal stem cells (Chai et al., 2018)

– treated with cobalt(II) chloride at various concentrations (50–500 μM). Cobalt(II) chloride treatment (600 μM) of human retinal pigment epithelial cells (ARPE-19 cell line) significantly increased HIF-1α and VEGF mRNA and protein levels at 12 and 24 hours, respectively (Gu et al., 2021). Concentration-dependent increases in VEGF expression were observed in human retinal pigment epithelial cells (Rosen et al., 2015), human endometrial stromal cells (Tsuzuki et al., 2012), and CD133+ cells derived from human umbilical cord blood (Zan et al., 2012). In addition, different concentrations of cobalt(II) chloride (20–200 μM) were shown to induce VEGF expression in human umbilical vein endothelial cells (HUVECs) (Namiki et al., 1995; Liu et al., 2009; Qiu et al., 2012). In the study by Liu et al. (2009), cobalt(II) chloride also increased the formation of capillary-like tube structures, a marker of angiogenesis. In terms of nutrient supply, cobalt(II) chloride (70 μM) increased ATP production via glycolysis in human skin fibroblasts (Vincent et al., 2008) (Table 4.20). [The Working Group noted that for the most informative studies, with statistical significance, showing VEGF expression induced by cobalt(II) salts in human primary cells, cytotoxicity was controlled and/or concentration-dependent effects were observed (Müller et al., 2012; Vincent et al., 2008 ; Tsuzuki et al., 2012 ; Qiu et al., 2012 ; Zan et al., 2012 ; Rosen et al., 2015).]

Fewer studies have assessed the effects of cobalt(II) chloride on cell proliferation in human primary cells. Treatment of human cholesteatoma keratinocytes with cobalt(II) chloride induced cell proliferation, which was dependent on p-PDK1 and p-Akt signalling (Zhang et al., 2021b). Concentration-dependent increases in cell proliferation were observed in primary human fetal striatal neuroblasts (Ambrosini et al., 2015) and umbilical cord blood-derived CD133+ cells (Zan et al., 2012). [The Working Group noted that cell viability was assessed in both studies; this represents an indirect marker of cell proliferation.] Increased cell proliferation induced by cobalt(II) chloride in HUVECs involved increased CDK4 and CCND1 expression (Wei et al., 2019). On the other hand, no effects on cell proliferation were observed in bone marrow mesenchymal stem cells treated with cobalt(II) chloride (Nguyen et al., 2020). Only two studies showed inhibition of apoptosis by cobalt(II) chloride in HUVECs (Xu et al., 2017; Wei et al., 2019). In these studies, inhibition of apoptosis was associated with increased BCL2 and decreased BAX and caspase-3 expression and was dependent on increased miR-21 expression. All references are shown in Table 4.20.

Immortalized cells

See Table S4.21 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt(II) chloride has been extensively shown in 49 studies to be a hypoxia inducer in human immortalized cell lines. In these studies, increased HIF-1α expression was associated with hypoxia induced by cobalt(II) chloride. The hypoxic effects induced by cobalt(II) chloride were associated with end-points related to key characteristic 10: "alters cell proliferation, cell death, or nutrient supply".

Regarding angiogenesis, increased VEGF mRNA and/or protein expression was shown in studies with cobalt(II) chloride (50–200 μM) in several cell lines, such as choroidal vascular endothelial cells (Balaiya et al., 2013), human microvascular endothelial cells (Loboda et al., 2005), human Müller cells (Sears & Hoppe, 2005), and osteoblast-like cell lines (Kim et al., 2002) obtained from healthy tissue. In addition, several studies conducted in human retinal pigment epithelial cells (ARPE-19 cell line), have shown increased VEGF expression induced by different concentrations (100–300 μM) of cobalt(II) chloride (Oh et al., 2013; Park et al., 2015; Zhao et al., 2015; Wang et al., 2016b; Chang et al., 2017).

In this same cell line, cobalt(II) chloride (100 µM) induced downregulation of SIRT1 and acetylation of the pro-angiogenic factor HMGB1 (Chang et al., 2017).

Furthermore, cobalt(II) chloride (50–200 µM) increased VEGF mRNA and/or protein expression in several immortalized cell lines, such as endometrial carcinoma (Molitoris et al., 2009), cervical carcinoma (Osera et al., 2015), mesothelioma (Sato et al., 2014), colon adenocarcinoma (Law et al., 2012), pancreatic carcinoma (Wen et al., 2016), prostate carcinoma (Lee et al., 2006), and oral squamous carcinoma cells (Stewart et al., 2003). Such effects were also reported after treatment with cobalt(II) sulfate (100 µM) (Xia et al., 2009). Studies demonstrated that the effects of cobalt(II) chloride on VEGF expression were dependent on several factors including: HuR (human antigen R) (Osera et al., 2015); Yes, a member of the Src family of kinases (Sato et al., 2014); STAT, which binds the VEGF promoter (Gray et al., 2005); AMP-activated protein kinase (Lee et al., 2006); or PI3K signalling (Stewart et al., 2003). In the study by Maurage et al. (2009), cobalt(II) chloride (100 µM) also increased secretion of endocan (also known as endothelial cell-specific molecule 1), a pro-angiogenic factor, by a glioblastoma cell line. In terms of nutrient supply, cobalt(II) chloride (150 µM) induced the expression of GLUT-1 and/or hexokinase II in human cervical cancer (Cheng et al., 2013) and mammary cancer (Chen et al., 2010a) cells (see Table S4.21, Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Fewer studies have assessed the effects of cobalt chloride on cell proliferation in normal human cell lines. Concentration-dependent increases in cell proliferation were observed in ARPE-19 cells (Wang et al., 2016b). [The Working Group noted that cell viability was assessed, which represents an indirect marker of cell proliferation.] Increased proliferation of pulmonary artery smooth muscle cells, induced by cobalt(II) chloride, was dependent on PGI_2 downregulation and low levels of hydrogen sulfide (Li et al., 2014). In addition, studies demonstrated induction of cell proliferation using cobalt(II) chloride at different concentrations, and different neoplastic cell lines, such as gastric adenocarcinoma (Bi et al., 2010), cervical cancer cells (Cheng et al., 2013), pancreatic cancer cells (Chen et al., 2018a), and renal cell carcinoma (Zhang et al., 2017). Induction of cell proliferation involved increased ERK-MAPK signalling (Bi et al., 2010) and inhibition of p53 (Lee et al., 2001). On the other hand, cobalt(II) chloride induced concentration-dependent decreases in cell proliferation (cell viability) in colon cancer (Yang et al., 2016) and breast cancer (Barrak et al., 2020) cells. In addition, no alterations in cell proliferation were observed in studies conducted with cobalt(II) chloride in fetal mesencephalic neural progenitor cells (Milosevic et al., 2009), Hodgkin lymphoma (Kewitz et al., 2016), and ovarian cancer cell lines and clear cell carcinoma cells (Nunes et al., 2018). No effects (Xu et al., 2014) or increases (Cheng et al., 2013) in apoptosis were observed after treatment of hepatoma and cervical cancer cells with cobalt(II) chloride, respectively. All references are shown in Table S4.21 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

[The Working Group noted that the most informative data refer to the increased expression of VEGF in human primary cells and human immortalized cells induced by cobalt(II) chloride. Few of these available studies controlled for cobalt(II) chloride cytotoxicity. However, cytotoxic effects are normally seen with concentrations > 300 µM, while increased expression of VEGF was observed at lower concentrations.]

(b) Experimental systems

(i) Non-human mammals in vivo

Cobalt metal or cobalt metal nanoparticles

Cobalt metal microparticles induced an array of epithelial cell damage and proliferative responses in the respiratory tracts of male and female Fischer 344/N or Fischer 344/Ntac rats exposed to aerosols (MMAD, 1.4–2.0 μm) at 1.25 mg/m^3 for 2, 14, or 105 weeks (NTP, 2014), like that reported after exposure to cobalt(II) salts. Dose- and duration-dependent increases in hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar and bronchiolar epithelium, along with olfactory epithelial degeneration and hyperplasia, nasal respiratory epithelium necrosis and squamous metaplasia, and turbinate atrophy. Lung was the tissue most sensitive to injury after cobalt metal microparticle exposure, as significant effects in the trachea were not reported, unlike those observed after exposure to cobalt(II) salts (NTP, 2014). While injury was not observed after 1 day of exposure to cobalt metal NPs in male JcL:SD rats, 4 days of exposure to 2.12 ± 0.55 mg/m^3 aerosols (d_{50}, 20 nm; MMAD, 760 nm) appeared to induce a variety of similar lung pathologies evident by transmission electron microscopy, including bronchiolar hypertrophy and proliferation, bronchiolization of alveolar ducts, type II pneumocyte proliferation, and mitosis of fibroblasts (Kyono et al., 1992). In male Sprague-Dawley rats, bulk cobalt metal and cobalt metal NPs (d_{50}, 120 nm) were implanted on contralateral sides of the vertebral column (subcutaneously and intramuscularly, respectively), and subdermal lesions were assessed after 6 months due to early deaths or 8 months due to unacceptable lesion morbidity. In 4/10 rats, capsules with fibroblastic proliferations were described, which were described as having preneoplastic characteristics, along with increased proliferating cell nuclear antigen (PCNA) expression (Hansen et al., 2006). [The Working Group noted that several conduct and reporting issues were identified in this study, including lack of reporting on comprehensive histopathological lesion incidence, severity, or magnitude, incidence reported only in terms of numbers of rats with fibroblastic proliferation, and no description of PCNA evaluation or comparison method.]

Similar to the effects observed in rats, cobalt metal aerosols induced an array of epithelial cell damage and proliferative responses in the respiratory tracts of male and female B6C3F$_1$/N mice exposed at 1.25 mg/m^3 (MMAD, 1.4–2.0 μm) for 2, 14, or 105 weeks, with damage also evident in the larynx and lung after exposures at 0.625 mg/m^3 for 14 weeks (NTP, 2014). Dose- and duration-dependent increases in necrotic, hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar and bronchiolar epithelium, along with olfactory epithelial necrosis, atrophy, and hyperplasia, nasal respiratory epithelium degeneration and squamous metaplasia, and turbinate atrophy. Unlike in similarly exposed rats, but comparable with the results reported in B6C3F$_1$/N mice after exposure to cobalt(II) salts, larynx was the tissue most sensitive to injury, because squamous metaplasia was induced at the lowest concentrations after subchronic or longer exposure durations (NTP, 2014). Intratracheal instillations of cobalt metal NPs (d_{50}, 20 nm; hd_{50}, 260 nm) induced proliferative responses in the lung epithelial cells of male and female transgenic C57Bl/6J mice (*gpt*, lambda phage EG10 DNA), elevating Ki-67 and PCNA expression 1 and 16 weeks after exposure to 50 μg/mouse [~2 mg/kg bw] (Wan et al., 2017).

[The Working Group noted that Viegas et al. (2022) exposed male and female Crl:CD(SD) rats to a variety of (soluble and insoluble) cobalt compounds via nose-only inhalation for 4 hours (cobalt metal powder, cobalt(II) hydroxide, cobalt(II) sulfate, cobalt carbonate, tricobalt tetraoxide [cobalt(II,III) oxide], lithium cobalt oxide, or cobalt(II) sulfide). However, the authors

Table 4.22 Alterations in cell proliferation, cell death, or nutrient supply in non-human mammals in vivo exposed to cobalt

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Soluble cobalt(II) salts					
Cell proliferation					
Cobalt(II) chloride ($CoCl_2$)					
Epithelial cell damage and proliferative response (nodular aggregation of alveolar type II epithelial cells, and concentrations of type II cells in other areas of the lung, with some AAH characteristics)	Rabbit, lung	+ (4–6 wk) + (15 wk) + (16 wk)	0.4–0.6 mg Co^{2+}/m^3 0.4 ± 0.2 mg Co^{2+}/m^3 0.6 ± 0.5 mg Co^{2+}/m^3	$CoCl_2$ aerosol: MMAD, 1 µm. Cobalt source and purity not reported, evaluations were blinded to exposure condition, dose-dependent response, $n = 8$, rabbits housed individually, males evaluated, negative control included.	Johansson et al. (1992, 1987, 1984)
Epithelial cell proliferation (PCNA expression and BrdU incorporation)	Rat (Wistar), kidney (in ablation/infarction model of chronic kidney disease)	+ (8 day)	10 mg/kg per day (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, $n = 8$, males evaluated, positive and negative controls included.	Deng et al. (2010)
Epithelial cell proliferation (PCNA staining)	Wistar rats, kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, $n = 4–8$, females evaluated, negative control included.	Tanaka et al. (2005a)
Epithelial cell proliferative capacity (ODC and HO-1 activity by bioassay)	Rat (Wistar), liver	+ (6–36 h)	125 µmol/kg [92.6 mg/kg] (subcutaneous injection; single or multiple)	Cobalt source and purity not reported, subcutaneous injection route $n = 3–4$, dose- and duration-dependent responses, males evaluated, negative controls included.	Numazawa et al. (1989b)
Epithelial cell proliferation (BrdU staining)	Rat (SD), bladder	+ (6 days)	200 µM every 2 days, for 30 min (intravesical infusion)	Cobalt purity not reported, single dose evaluated, intravesical exposure route, $n = 6$, males evaluated, vehicle control included.	Buttyan et al. (2003)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Cobalt(II) sulfate ($CoSO_4$)					
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, AAH, metaplasia, interstitial fibrosis; bronchiolar epithelium regeneration Nose: olfactory epithelial atrophy, degeneration, metaplasia; respiratory epithelium hyperplasia, squamous metaplasia Larynx: ulcer, necrosis, squamous metaplasia	Rat (F344/N), lung, nose, and larynx	+ (13 wk) + (13 wk) + (13 wk) + (104 wk)	0.3 mg/m³ (larynx); 1.0 mg/m³ (lung); 10 mg/m³ (nose); 0.3 mg/m³ (lung, nose, and larynx)	$CoSO_4 \cdot 7H_2O$ aerosol: MMAD, 1–3 µm. Gold-standard for design, methodology, and reporting. Dose-dependent responses, negative controls included, 13 wk (n = 10), 104 wk (n = 50), males and females evaluated, evaluations were blinded to exposure condition.	Bucher et al. (1990, 1999)
Epithelial cell damage and proliferative response Lung: cytoplasmic vacuolization of the bronchiolar epithelium, regeneration; alveolar epithelial hyperplasia Nose: olfactory epithelial atrophy, degeneration, hyperplasia; respiratory epithelium squamous metaplasia Larynx: necrosis, squamous metaplasia Trachea: squamous metaplasia	Mouse ($B6C3F_1$/N), lung, nose, larynx, and trachea Mouse ($B6C3F_1$/N), lung, nose, larynx	+ (13 wk) + (13 wk) +/– (13 wk) + (104 wk) + (104 wk)	0.3 mg/m³ (lung and larynx); 3.0 mg/m³ (nose); 30 mg/m³ (trachea); 0.3 mg/m³ (lung and larynx); 1.0 mg/m³ (nose)	$CoSO_4 \cdot 7H_2O$: MMAD, 1–3 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay. Dose-dependent responses, 13 wk (n = 10), 104 wk (n = 50), males and females evaluated, negative controls included, evaluations were blinded to exposure condition.	Bucher et al. (1990, 1999)
Angiogenesis					
Cobalt(II) chloride ($CoCl_2$)					
VEGF protein expression, EPO mRNA by qRT-PCR	Rats (Wistar), kidney (in ablation/infarction model of chronic kidney disease)	+ (8 days)	10 mg/kg per day (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, n = 8, males evaluated, positive and negative controls included.	Deng et al. (2010)
VEGF and EPO mRNA levels by qRT-PCR, and VEGF protein by IHC	Rat (Wistar), kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, n = 4–8, females evaluated, negative control included.	Tanaka et al. (2005b)
VEGF mRNA expression by northern blot	Rat (Wistar), liver, heart, kidney, muscle Rat (Wistar), brain	+ (6–8 h) – (6–8 h)	60 mg/kg (subcutaneous injection)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, n = 5, males and females evaluated, negative control included.	Minchenko et al. (1994)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
VEGF mRNA expression by northern blot	Rat (Wistar), heart Rat (Wistar), brain and kidney	(+/–) (4 h) (–) (4 h)	1 mL of 50 or 75 mM solution [32 or 48 mg/kg] (subcutaneous injection)	Cobalt source and purity not reported, treatment dosage unclear [1 mL of 50 or 75 mM], single dose evaluated, subcutaneous injection route, (*n* = unclear), negative control included.	Ladoux & Frelin (1994)
Factor XIII by IHC and microvessel enumeration, HIF-1α and VEGF protein expression by western blot	Rat (SD), bladder	+ (6 days)	200 µM every 2 days, for 30 min (intravesical infusion)	Cobalt purity not reported, single dose evaluated, intravesical exposure route, *n* = 6, males evaluated, vehicle control included.	Buttyan et al. (2003)
VEGF, EPO, and HO-1 mRNA levels by qRT-PCR, VEGF protein by western blot	Rat (SD), kidney (unephrectomized Thy1 nephritis model)	+ (3 wk)	2.7 mg/kg every 3 days (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, *n* = 2–9, males evaluated, negative control included.	Tanaka et al. (2005b)
HIF-1α and VEGF mRNA by qRT-PCR and protein by western blot	Rat (SD), tibial bone	+ (unclear duration)	15 mg/kg per day (intraperitoneal injection)	Cobalt source and purity not reported, uncertain treatment duration, single dose evaluated, *n* = 8, females evaluated, negative control included.	Huang et al. (2015)
HIF-1α, VEGF, and EPO mRNA expression by RT-PCR and protein expression by western blot, and HIF-1α DNA-binding	Rat (SD), heart	+ (7 days)	12.5 mg/kg per day (oral gavage)	Co source and purity not reported, single dose evaluated, *n* = 3–10, negative control included.	Singh et al. (2010)
VEGF and EPO mRNA by qRT-PCR	Rat (SD), kidney (ischaemic renal injury model)	+ (days –10 to 3 post-injury)	2 mM in drinking-water [440 mg/kg per day]	Cobalt source and purity not reported, single dose evaluated, *n* = 2–3, males evaluated, vehicle control included.	Matsumoto et al. (2003)
Microvessel density by morphometric evaluation of new, immature arterioles	Rat (SD), heart	+ (5 wk)	75 mg/kg, 3×/wk (intraperitoneal injection)	Cobalt source and purity not reported, single dose evaluated, polycythemia observed, *n* = 8–10, males evaluated, vehicle control included.	Rakusan et al. (2001)
VEGF and GLUT1 protein expression in cerebrum microvessels by western blot, point counting of capillary density	Rat (SD), brain	+ (12 days) – (12 days)	2 mM in drinking-water [260 mg/kg per day]	Cobalt purity not reported, single dose evaluated, *n* = 12, males evaluated, vehicle control included, evaluations were blinded to exposure condition.	Badr et al. (1999)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Flt-1 and EPO mRNA by RNase protection assay, VEGF, and flt-1 mRNA expression	Rat (SD), liver Rat (SD), lung	+ (6 h) – (6 h)	60 mg/kg (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, $n = 6$, males evaluated, negative control included.	Sandner et al. (1997)
EPO mRNA by RNse protection assay, serum EPO concentrations, VEGF mRNA expression	Rat (SD), liver and kidneys Rat (SD), lung, liver, kidneys, and heart	+/– (6 h) – (6 h)	60 mg/kg (subcutaneous injection)	Cobalt source and purity not reported, limited method reporting, $n = 6$, males evaluated, negative control included.	Sandner et al. (1996)
HIF-1α and HIF-2α protein levels by western blot and/or IHC, VEGF and EPO mRNA levels by qRT-PCR	Rat (SHR/NDmcr-cp), kidney (diabetic nephropathy model)	+ (26 wk)	200 µM in drinking-water (1 mg/rat per day) [2.1 mg/kg per day]	Cobalt source and purity not reported, single dose evaluated, $n = 10$, males evaluated, negative control included.	Ohtomo et al. (2008)
HIF-1α protein level by western blot, VEGF and EPO mRNA expression by qRT-PCR	Mouse (BALB/c), brain and hippocampus	(+)	60 mg/kg (unspecified injection)	Cobalt source and purity not reported, uncertain treatment route, single dose evaluated, duration-independent, $n = 3–6$, males evaluated, negative control included.	Zhang et al. (2014)
HIF-1α and VEGF protein levels by IHC and western blot, and mRNA expression by qRT-PCR	Mouse (BALB/c), nose, nasal mucosa (in ovalbumin-induced allergic rhinitis model)	+ (29 days)	9.8 mg/kg per day (intraperitoneal injection)	Cobalt purity not reported, single dose evaluated, $n = 4–6$, negative control included.	Zhou et al. (2012)
Enumerating blood vessels and vessel density; no effects with $CoCl_2$ in absence of MatLyLu cells	Chicken, chorioallantoic membrane in whole egg	+ (5 days)	100 µM (implanted ring containing rat prostate tumour cells [MatLyLu] cells)	Cobalt purity not reported, single dose evaluated, $n = 5$, vehicle and negative control included.	Van Lieshout et al. (2003)
Skin flap necrosis by visual evaluation of margins, resulting from improved blood flow	Rat (Wistar), skin flap	(+) (13 wk pre-conditioning)	10% gel (skin application)	$CoSO_4 \cdot 7H_2O$. Cobalt source and purity not reported, observations were not blinded, single dose evaluated, macroscopic necrosis margins estimated visually, $n = 8$, "no treatment" control included but no gel vehicle control, males and females evaluated.	Bobek et al. (2005)
Apoptosis					
Cobalt(II) chloride ($CoCl_2$)					
TUNEL staining	Rat (Wistar), kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ ↓ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, $n = 4–8$, females evaluated, negative control included.	Tanaka et al. (2005a)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Apoptosis: TUNEL staining in a partially VEGF-dependent manner	Rat (SD), kidney (unnephrectomized Thy1 nephritis model)	+ ↓ (3 wk)	2.7 mg/kg every 3 days (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, n = 2–9, males evaluated, negative control included.	Tanaka et al. (2005b)
Cobalt metal or cobalt metal NPs					
Cell proliferation					
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, cytoplasmic vacuolization, interstitial fibrosis; bronchial epithelial hyperplasia, cytoplasmic vacuolization, erosion, necrosis Nose: olfactory epithelial atrophy, necrosis, degeneration, hyperplasia; respiratory epithelium cytoplasmic vacuolization, degeneration, squamous metaplasia; turbinate atrophy Larynx and trachea: squamous metaplasia, cytoplasmic vacuolization	Mouse, ($B6C3F_1$/N), lung and nose Mouse ($B6C3F_1$/N), lung, nose, and larynx Mouse ($B6C3F_1$/N), lung, nose, larynx, and trachea	+ (2 wk) + (14 wk) + (14 wk) + (105 wk)	2.5 mg/m³ (lung and nose); 0.625 mg/m³ (lung and larynx); 1.25 mg/m³ (nose); 1.25 mg/m³ (lung, nose, larynx, and trachea)	Aerosol particle size: MMAD, 1.4–2.0 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay, dose-dependent responses, survival-adjusted analyses: 2 wk (n = 5), 14 wk (n = 10), and 104 wk (n = 50), males and females evaluated, negative control included, evaluations were blinded to exposure condition.	NTP (2014)
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, interstitial fibrosis; bronchial epithelial hyperplasia, necrosis Nose: olfactory epithelial atrophy, necrosis, degeneration, hyperplasia, metaplasia; respiratory epithelium necrosis, hyperplasia, metaplasia; turbinate atrophy	Rat (F344/N), lung and nose Rat (F344/N), lung and nose Rat (F344/NTac), lung and nose	+ (2 wk) + (14 wk) + (14 wk) + (105 wk)	2.5 mg/m³ (lung and nose); 1.25 mg/m³ (lung); 2.5 mg/m³ (nose); 1.25 mg/m³ (lung and nose)	Aerosol particle size: MMAD, 1.4–2.0 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay, dose-dependent responses, survival-adjusted analyses: 2 wk (n = 5), 14 wk (n = 10), 104 wk (n = 50), males and females evaluated, negative control included, evaluations were blinded to exposure condition.	NTP (2014)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Epithelial cell damage and proliferative response: bronchiolar hypertrophy and proliferation, damage to cilia and bronchioalveolar duct junctions, bronchiolization of alveolar ducts; type II pneumocyte proliferation, interstitial oedema, fibroblast mitosis	Rat (SD, Jcl:SD), lung	(–) (1 days) (+) (4 days)	2.72 ± 0.44 mg/m^3 2.12 ± 0.55 mg/m^3	Aerosol NP size: d_{50}, 20 nm; MMAD, 760 nm. Cobalt purity not reported, no negative controls, procedures for pathological evaluation not described, *n* = 2–5, males evaluated, duration-dependent responses.	Kyono et al. (1992)
Fibroblast proliferation: encapsulated fibroblastic proliferation with preneoplastic characteristics, PCNA expression by IHC	Rat (SD), dermis	(+) (26–35 wk)	"Bulk" cobalt metal pellets subcutaneously; 60–100 mg cobalt metal NPs intramuscularly; agglomerate of particles of 10 µM size.	"Bulk" cobalt metal pellets (4.73 mm^1) and cobalt metal NPs (d_{50}, 120 nm) implanted on contralateral sides of vertebral column. Cobalt purity not reported, detailed lesion incidence and PCNA expression not clearly reported, *n* = 10, males evaluated, positive and negative controls included.	Hansen et al. (2006)
Altered cell proliferation and/or cell death: percentage of CD34$^+$ HSC/HPC by fluorescence-activated cell sorting	Rat (SD), bone marrow	(+ ↓) (6 wk)	1 mg/kg (3×/wk injections into right hip)	NP size: diameter, 50–200 nm. Cobalt purity and rat source not specified, hip injection as route of exposure, experimental allocation unclear (may be *n* = 6), males evaluated, negative control included.	Zhu et al. (2021a)
Epithelial cell damage and proliferative response: focal alveolar epithelial cell hyperplasia, LDH in BALF, proliferation rate by Ki-67 and PCNA IHC 1 wk; interstitial fibrosis, bronchiolization of the alveola, proliferation rate by Ki-67 and PCNA IHC 16 wk	Mouse (C57Bl/6J, *Gpt* transgenic, lambda phage EG10 DNA), lung	+ (1 wk) + (16 wk)	50 µg/mouse [~2 mg/kg] (intratracheal instillation) 50 µg/mouse [~2 mg/kg] (intratracheal instillation)	NP size: d_{50}, 20 nm; hd_{50}, 260 nm. Cobalt purity not specified, intratracheal route of exposure, *n* = 4–8, males and females evaluated, negative control included.	Wan et al. (2017)

AAH, atypical adenomatous hyperplasia; BALF, bronchoalveolar lavage fluid; BrdU, bromodeoxyuridine; CD34, cluster of differentiation 34; d_{50}, mean particle diameter; EPO, erythropoietin; flt, Fms related receptor tyrosine kinase 1; Gpt, guanine phosphoribosyltransferase; hd_{50}, hydrated mean particle diameter; HIC, highest ineffective concentration; HO-1, haem oxygenase isoenzyme 1; HPC, haematopoietic progenitor cell; HSC, haematopoietic stem cell; IHC, immunohistochemistry; LDH, lactate dehydrogenase; LEC, lowest effective concentration; min, minute; MMAD, mass median aerodynamic diameter; mRNA, messenger RNA; PCNA, proliferating cell nuclear antigen; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RNase, ribonuclease; RT-PCR, reverse transcription polymerase chain reaction; SD, Sprague-Dawley; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; VEGF, vascular endothelial growth factor; wk, week; yr, year.

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

presented only a summary severity score based upon the aggregation of four histopathological end-points from the upper respiratory tract. Due to the lack of primary data reporting and other quality concerns, this study was not considered for further evaluation.]

Soluble cobalt(II) salts

Cobalt(II) chloride disrupted the organization of type II pneumocytes in the lungs of male rabbits exposed by inhalation to Co^{2+} aerosol at 0.4–0.6 mg/m^3 (MMAD, 1 μm) for 4–16 weeks. The effect manifested by the nodular aggregation of alveolar type II epithelial cells in some regions of the distal lung, coinciding with decreasing proportions of these cells in adjacent alveoli, consistent with epithelial cell proliferation in response to alveolar injury (see Table 4.22) (Johansson et al., 1984, 1987, 1992). The authors observed that treatment-related effects in type I pneumocytes were less frequent, less severe, and appeared later in time compared with the aggregation observed in type II cells. [The Working Group noted that some aspects of the nodular aggregation reported after longer durations appear consistent with atypical adenomatous hyperplasia.] A greater degree and variety of epithelial cell damage and proliferative responses were observed in the respiratory tracts of male and female Fischer 344/N rats exposed by inhalation to cobalt(II) sulfate aerosols (MMAD, 1–3 μm) at 0.3 mg/m^3 for 13 weeks and 2 years, in inhalation bioassays. While concentration- and duration-dependent increases in hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar epithelium, along with olfactory epithelial degeneration and squamous metaplasia of the nasal respiratory epithelium, necrosis and squamous metaplasia of the larynx appeared earliest and at the lowest exposure concentrations assessed, in response to significant tissue injury (Bucher et al., 1990, 1999). Similarly, although generally less severe, pathology was also observed in the lungs, nasal passages, and larynx of male and female B6C3F$_1$/N mice that were similarly exposed, with the addition of squamous metaplasia appearing in a few animals after subchronic exposure to the highest concentrations (Bucher et al., 1990, 1999).

Increased epithelial cell proliferation and/or decreased apoptosis have been reported in rat kidney after cobalt(II) chloride administration via subcutaneous or intraperitoneal injection (Tanaka et al., 2005a, b; Deng et al., 2010), as well as in the urinary bladder after intravesical infusion (Buttyan et al., 2003).

Increased HIF-1α mRNA and protein expression have been widely reported after exposure to cobalt(II) chloride (single doses to multiple weeks) in several strains of rats and BALB/c mice by injection, gavage, and drinking-water (e.g. Ohtomo et al., 2008; Deng et al., 2010; Zhang et al., 2014; Huang et al., 2015). Because of this chemically induced hypoxia, cobalt(II) chloride has been used to investigate the effects of hypoxia-mediated protection in various rodent models of human diseases and tissue injury, including allergic rhinitis (Zhou et al., 2012), chronic kidney disease (Tanaka et al., 2005a, b; Deng et al., 2010), ischaemia (Rakusan et al., 2001; Matsumoto et al., 2003; Singh et al., 2010; Zhang et al., 2014), and bone fracture (Huang et al., 2015). Increased mRNA and/or protein levels of various angiogenic mediators induced by HIF-1α activation have also been reported, including VEGF and EPO (e.g. Minchenko et al., 1994; Matsumoto et al., 2003; Tanaka et al., 2005a, b; Ohtomo et al., 2008; Deng et al., 2010; Singh et al., 2010; Zhou et al., 2012; Zhang et al., 2014; Huang et al., 2015). However, in several studies evaluating effects in rat tissues 4–6 hours after treatment with 30–60 mg/kg cobalt(II) chloride administered via subcutaneous injection, mRNA expression of VEGF and the VEGF receptor Flt-1 increased marginally in the heart but not in the brain, kidney, liver, or lung (Ladoux & Frelin, 1994; Sandner et al., 1996, 1997).

Few studies reporting direct measures of angiogenesis after cobalt(II) chloride exposures were identified; increased microvessel formation coinciding with increased VEGF protein expression was observed in the bladders of Sprague-Dawley rats (Buttyan et al., 2003), increased formation of new, immature arterioles in the heart coincided with decreases in some measures of existing capillary supply (Rakusan et al., 2001), and no change in microvessel density in the cerebrum was reported despite increased VEGF protein expression (Badr et al., 1999). Interestingly, while exposure to cobalt(II) chloride alone had no effect on angiogenesis in a chick chorioallantoic membrane (CAM) model, the inclusion of cobalt(II) chloride-exposed MatLyLu cells (Dunning rat prostate tumour cells) increased both the number and density of blood vessels (Van Lieshout et al., 2003). [The Working Group noted that while cobalt(II) chloride appeared to widely stimulate the activities of HIF-1α and EPO, increased expression of downstream mediators of angiogenesis such as VEGF, and the ultimate stimulation of new vessel formation, may occur in a tissue-specific manner.] While studies using cobalt(II) chloride to induce hypoxia typically only assessed a single dose, multiple subcutaneous cobalt(II) chloride injections every 6–12 hours increased activity of the downstream HIF-1α target ornithine decarboxylase (ODC) in a greater-than-additive manner in male Wistar rat liver, compared with a single injection of 125 µmol/kg [92.6 mg/kg bw] (Numazawa et al., 1989b).

[The Working Group noted that Burzlaff et al. (2022), in experiments described only in the supplementary materials associated with their manuscript, exposed male and female Wistar (Han) rats via nose-only inhalation to cobalt(II) sulfate aerosols (MMAD, 1.6 µm) at 2 mg/m^3 for 28 days, and reported increased focal squamous metaplasia at the base of the epiglottis in most exposed rats. Owing to quality concerns regarding study reporting, this study was not considered for further evaluation.]

(ii) Non-human mammalian cells in vitro

Soluble cobalt(II) salts

While increased cyclin B1 content suggestive of increased cellular proliferation was reported in porcine ovarian granulosa cells exposed to high concentrations of cobalt(II) sulfate (90 µg/mL) (Kolesarova et al., 2010), no increases in cellular proliferation, or decreases in apoptosis inferred by alterations in relative plating efficiency (RPE), were reported by several studies in mouse embryonic fibroblasts (MEF) or immortalized RAW 264.7 macrophages exposed to cobalt(II) chloride at concentrations ≥ 2 µM (for examples see: Kerckaert et al., 1996; Doran et al., 1998; Ponti et al., 2009; Sabbioni et al., 2014a; Salloum et al., 2018; Zhang et al., 2021b). In other cell types, cobalt(II) chloride concentrations ≥ 100 µM induced cellular proliferation (Wenker et al., 2013), had no appreciable effects (Yang et al., 2004; Lu et al., 2009; Wu et al., 2012a), or failed to attenuate caspase-3 activation and apoptotic cell death (Yang et al., 2011d; Mo et al., 2016).

Cobalt(II) chloride has been extensively used in numerous studies as a means of chemically inducing hypoxia in cell lines from various mammalian species and many tissue types after exposures to concentrations ≥ 100 µM (e.g. Xia et al., 2009), with little evidence of any consistent concentration–response relationship at lower concentrations (Kuo et al., 2019). While HIF-1α protein expression or DNA-binding activity is commonly increased, along with increased expression and activity of downstream HIF-1α targets such as VEGF associated with increased measures of angiogenesis (e.g. Zhang et al., 2021b), cobalt(II) chloride exposure does not consistently increase HIF-1α mRNA expression levels (e.g. Kumar et al., 2012; Wang et al., 2013b). Cobalt(II) chloride exposure increased VEGF expression in primary cells isolated from

bovine and porcine ovaries (Grasselli et al., 2005; Zhang et al., 2011), rabbit retinal pigment epithelial cells (Wang et al., 2003), rat kidney cortical cells (Deng et al., 2010), and rat heart myocyte cultures (Ladoux & Frelin, 1994). In primary pancreatic islets isolated from C57Bl/6 mice, cobalt(II) chloride increased VEGF mRNA and protein expression, which was associated with an expanded number and network of endothelial cells (Sankar et al., 2019), with similar results also reported in bone marrow populations isolated from 129S1/SvlmJ mice (García-Román et al., 2010). Exosomes derived from RAW 264.7 macrophages that were stimulated with lipopolysaccharide and exposed to 200 μM cobalt(II) chloride induced neoangiogenesis when administered to a human endothelial cell line (EA.hy926) installed in Matrigel plugs in male Sprague-Dawley rats (Zhang et al., 2021b).

Cobalt metal nanoparticles

At concentrations of ≥ 100 μM, cobalt metal NPs (diameter, 50–500 nm) did not increase cellular proliferation, viability, or attenuate cell death in Sprague-Dawley rat primary bone marrow CD34+ haematopoietic stem and progenitor cells (Zhu et al., 2021a). Similar results were reported in several studies in MEFs (e.g. Miller et al., 2001; Ponti et al., 2009; Sabbioni et al., 2014a; Annangi et al., 2015; Sighinolfi et al., 2016).

Other relevant information

Swennen et al. (1993) assessed health effects in 82 cobalt refinery workers in Belgium with an on average 8-year duration of exposure and a geometric mean TWA exposure to cobalt dust of 125 μg/m^3. This included 25% of the refinery workers who had been exposed to > 500 μg/m^3. Results were compared with an age-matched group of non-cobalt dust-exposed controls. Of interest, three erythrocyte measurements were all significantly lower in the exposed population than controls: the total erythrocyte count (4.85 versus 5.48 × 10^{12}/L), haemoglobin (15.05 versus 15.59 g/100 mL), and haematocrit (44.03% versus 45.54%). [The Working Group noted that these minor differences in erythrocyte parameters were unlikely to be clinically relevant, but that cobalt often causes the opposite effects in erythrocytes.]

4.2.11 Multiple characteristics identified by transcriptomics (or other experimental approaches)

(a) Humans

(i) Human cells in vitro

See Table S4.23 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Soluble cobalt(II) salts

Several studies have assessed the transcriptional responses of various human cell lines at the mRNA expression and/or proteomics level, typically after exposures to high levels (e.g. 100–300 μM) of cobalt(II) chloride for up to 24 hours. In a study that assessed the extent of similarity in gene expression between cells after cobalt or hypoxia treatment, cobalt(II) chloride treatment of RKO colon carcinoma cells induced differential gene expression in 54 out of 150 "hypoxia upregulated" and 12 out of 76 "hypoxia downregulated" genes from a repository of hypoxia-related gene sets that were published on the MSigDB database (Sheffer et al., 2011). Genes that were upregulated impacted the carbohydrate metabolism and glycolysis, oxidoreductase activity, drug transport, cell survival, and angiogenesis pathways. Co-treatment with zinc(II) chloride was found to at least partially reverse expression in roughly one half of the hypoxia-associated genes and appeared to globally reverse the differential expression induced by cobalt(II) chloride exposure, when assessed by principal component analysis (PCA) (Sheffer et al., 2011).

[The Working Group noted that zinc supplementation may downregulate HIF-1/2 activity, and that the reverse of gene expression changes induced by cobalt(II) chloride may reflect zinc-mediated antagonism of HIF-1α activity.] In studies examining the effects of hypoxia induced by 24 hours of exposure to cobalt(II) chloride at 300 µM in differentiated enterocyte-like Caco-2 cells, activation of hallmark gene sets (described in Liberzon et al., 2015) associated with hypoxia induction via HIF signalling was reported – for example, protein tyrosine phosphatase, receptor type, f polypeptide, interacting protein, α 4 (*PPFIA4*) and B-cell CLL/lymphoma 2 apoptosis regulator (*BCL2*) – in addition to the TNFα-mediated NF-κB inflammation pathway, mTORC1 proliferation, and p53 cell cycle regulation (Knyazev et al., 2021). In a similar study, HIF-1α mRNA levels were not increased in Caco-2 cells but did increase in another colorectal adenocarcinoma cell line (HT-29), consistent with previous observations that HIF-1 may be regulated primarily by post-translational stabilization in a cell type-specific manner (Nersisyan et al., 2021).

Compared with another chemical method of hypoxia induction (oxyquinoline derivative), which stabilizes HIF-1α via inhibition of HIF prolyl hydroxylases, cobalt(II) chloride uniquely and independently stimulated dozens of genes in Caco-2 or HT-29 cells including elements involved in the oxidative stress and pro-inflammatory response, upregulation of anaerobic glycolysis (Knyazev et al., 2021), and the major histocompatibility complex class I antigen presentation pathway (Nersisyan et al., 2021). While more than 230 proteins were also differentially regulated, few changes in protein levels coincided with differential mRNA expression levels (Knyazev et al., 2021). [The Working Group noted this lack of concordance between mRNA and protein expression levels is not uncommon in such studies that typically assess a single high concentration of exposure at a single time point, and could result from several factors including the extent of protein regulation by post-translational modification, which underscores the difficulty of attempting to apply data from hypothesis-generating studies to specific hypothesis-testing applications.] Of the 17 genes previously reported by Benita et al. (2009) to be widely associated with hypoxia across various tissues, only 5 were found to be associated with cobalt(II) chloride exposure at both the mRNA and protein levels: aldolase, fructose-bisphosphate C (*ALDOC*), pyruvate dehydrogenase kinase 1 (*PDK1*), N-myc downstream regulated 1 (*NDRG1*), BCL2 interacting protein 3 (*BNIP3*), and prolyl 4-hydroxylase subunit α 1 (*P4HA1*) (Knyazev et al., 2021).

Concentrations of cobalt(II) chloride inducing 50% cytotoxicity in placental trophoblast cells (HTR-8/SVneo) broadly decreased intracellular levels of metabolite profiles including saturated and unsaturated fatty acids, GSH, amino acids, and tricarboxylic acid cycle intermediates such as malic and citric acid; effects on secreted metabolites were limited to decreased methionine, citramalic acid, and the two unsaturated fatty acids gamma-linolenic acid and conjugated linoleic acid, which also decreased internally (Chen et al., 2020). When the effect of a non-cytotoxic concentration of cobalt(II) chloride (20 µg/mL or approximately 150 µM) on the proteins secreted by BEAS-2B lung bronchial epithelial cells was investigated, 23 of the predicted 66 proteins in the extracellular "secretome" were found to be suppressed, with the most dramatic decreases reported in fibrillins 1 and 2, carboxypeptidase A4, biglycan, complement factor B, and cysteine rich transmembrane BMP regulator 1 (Malard et al., 2012). Only endoplasmic reticulum aminopeptidase 1 was induced, providing evidence that cobalt exposure generally reduces protein secretion in lung epithelial cells (Malard et al., 2012). Earlier gene expression studies evaluating supraphysiological LD_{50} concentrations (2 mM) in A549 cells reported differential expression of

genes involved in cobalt transport, transcription factors, the stress response, and cellular metabolism, including the induction of several HIF-1α target genes: aldolase, fructose-bisphosphate A (*ALDOA*), solute carrier family 2 member 1 (*SLC2A1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*), lactate dehydrogenase A (*LDHA*), BCL2 interacting protein 3 like (*BNIP3L*), phosphoglycerate kinase 1 (*PGK1*), and transferrin receptor (*TFRC*) (Malard et al., 2007). While this study assessed nine candidate genes coding for secreted proteins as potential biomarkers of cobalt(II) chloride exposure and confirmed that TIMP2 was significantly decreased in A549 cells, none of these candidates were differentially regulated after subcytotoxic exposures in BEAS-2B cells (Malard et al., 2012).

In neoplastic U266 multiple myeloma cells, treatment with cobalt(II) chloride at 100 μM induced approximately 30% cytotoxicity and upregulated the expression of genes associated with cellular development and death, as well as the immune response and B-cell activation, while genes involved in the biological processes regulating cell cycle, transcription, and kinase activity experienced both up- and downregulation (Bae et al., 2012). One of the most highly induced was oxidative stress induced growth inhibitor 1 (*OKL38*), a tumour suppressor gene that can inhibit neoplastic proliferation by inducing apoptosis. In U937 acute promonocytic leukaemia cells, cobalt(II) chloride at 50 μM induced accumulation of HIF-1α protein, coinciding with decreased content of proteins associated with physiological hypoxia and regulating cellular metabolism, cell proliferation, and differentiation; increases were only observed in the *NDRG1* gene (Han et al., 2006). In human HaCaT keratinocytes, non-cytotoxic cobalt(II) chloride concentrations (3 μg/mL or approximately 23 μM) also induced genes associated with HIF-1 activation such as *BNIP3* and *ALDOC*, as well as gene sets implicated in glycolysis and carbohydrate metabolism, and focal adhesion (Busch et al., 2010). While similar changes were also observed in liver Hep3B carcinoma cells exposed to cobalt(II) chloride at 100 μM, the authors noted that differences in gene expression were evident when compared with physiological hypoxia and cautioned against considering them equivalent (Vengellur et al., 2005).

Insoluble cobalt(II or II,III) oxide compounds

The effects of exposure to cobalt(II) oxide NPs in BEAS-2B lung bronchial epithelial cells and A549 alveolar adenocarcinoma cells at non-cytotoxic levels (6.09 μg/mL and 60.9 μg/mL, respectively) were assessed at the transcriptomic level (Verstraelen et al., 2014). Despite the lower concentration of cobalt(II) oxide NPs, there were approximately 100-fold more differentially expressed, and primarily downregulated, transcripts in BEAS-2B compared with A549 cells. In A549 cells, cobalt(II) oxide NPs primarily induced expression of seven genes associated with cellular metabolism: aryl hydrocarbon receptor nuclear translocator like 2 (*ARNTL2*), excision repair 4, endonuclease catalytic subunit (*ERCC4*), folylpolyglutamate synthase (*FPGS*), G protein nucleolar 3 like (*GNL3L*), hook microtubule tethering protein 3 (*HOOK3*), protein kinase cAMP-dependent type II regulatory subunit α (*PRKAR2A*), and zinc finger protein 721 (*ZNF721*). A similar induction in cellular metabolism was also observed in BEAS-2B cells, but the different genes were upregulated in a cell-specific manner: angiopoietin-like 4 (*ANGPTL4*), basic helix-loop-helix family member e40 (*BHLHE40*), endothelin 2 (*EDN2*), hexokinase 2 (*HK2*), pyruvate dehydrogenase kinase 1 (*PDK1*), protein phosphatase 1 regulatory subunit 3C (*PPP1R3C*), ribosomal modification protein rimK like family member A (*RIMKLA*), very low-density lipoprotein receptor (*VLDLR*) (Verstraelen et al., 2014), and both solute carrier family 2 member 1 (*SLC2A1*) and transferrin receptor (*TFRC*) which were reported by Malard et al. (2007) to be elevated in A549 cells after exposure to cobalt(II)

chloride. In addition, downregulation of several transcripts associated with immune system signalling were observed only in BEAS-2B cells, including toll-like receptor 6 (*TLR6*) and MHC (major histocompatibility complex) class I, A (*HLA-A*), with the most significant decrease noted in MHC class II, DR β 3 (*HLA-DRB3*) (Verstraelen et al., 2014).

(b) Experimental systems

(i) Non-human mammals in vivo

In Sprague-Dawley rats given drinking-water containing cobalt(II) chloride at a dose of 12.5 mg/kg bw per day for 7 days, proteomic analysis of plasma proteins indicated changes in proteins involved in lipid and mineral metabolism, as well as increased levels of albumin, apolipoproteins ApoA1 and ApoA4, and complement C3 (Ahmad et al., 2016).

Transcriptomic analysis was performed on lung bronchioloalveolar carcinomas induced in male and female $B6C3F_1$/N mice exposed via inhalation to cobalt metal aerosols (MMAD, 1.4–2.0 μm) at 5 mg/m^3 for 105 weeks, as reported by the NTP (2014), compared with spontaneous bronchioloalveolar carcinomas from unexposed mice, as well as normal, unaffected lung tissue from control mice (Ton et al., 2021). One significant difference in cobalt metal-induced bronchioloalveolar carcinomas compared with spontaneous bronchioloalveolar carcinomas or normal lung tissue was the identification of Kras as a primary regulator of downstream pathway activation, consistent with *Kras* mutations being present in 67% of cobalt metal-induced bronchioloalveolar carcinomas in mice compared with 27% of spontaneous lung tumours (Hong et al., 2015; Ton et al., 2021). [The Working Group noted that Kras mutations stimulate activation of downstream effectors via numerous pathways, including the Raf-MEK-MAPK signalling cascade.] Canonical pathways (as assessed by Ingenuity Pathway Analysis) uniquely altered in cobalt metal-induced, but not spontaneous, lung tumours included those related to MAPK signalling: integrin-linked kinase (ILK), p21-activated kinase (PAK), phosphoinositide 3-kinase/Akt (PI3K/AKT), ERBB, melatonin, and IL-8. Interestingly, amphiregulin (AREG) and epiregulin (EREG), ERBB family receptor ligands capable of stimulating both MAPK and PI3K/AKT pathways, were included among the most highly induced genes in cobalt metal-induced bronchioloalveolar carcinomas. Other pathways were overrepresented in both tumour types, including Rho family GTPases, signal transducer and activator of transcription 3 (STAT3), B-cell receptor, retinoic acid receptor, and xenobiotic metabolism. When expression profiles of cobalt metal-induced mouse bronchioloalveolar carcinomas were compared with the published transcriptomic data sets from human stage I lung adenocarcinomas, the most concordant canonical signalling pathways included Nop56p-associated pre-rRNA complex, 60S ribosomal subunit, dilated cardiomyopathy, focal adhesion, and hypertrophic cardiomyopathy (Ton et al., 2021).

(ii) Non-human mammalian cells in vitro

Cobalt(II) salts induced a variety of changes in gene expression when differentiated rat PC-12 adrenal pheochromocytoma cells were exposed to cobalt(II) acetate concentrations associated with 20% cytotoxicity (at < 20 μM), primarily involving an oxidative stress response with a marked increase in the HIF-1α downstream target haem oxygenase 1 (HMOX1) (Adams et al., 2015). When exposed to cobalt(II) chloride at concentrations associated with similar low cytotoxicity, changes in RNA and protein expression in the rat liver-derived cell lines H4-II-E-C3 and MH1C1 were generally consistent with those observed in human cell lines and in rodent studies in vivo, with modulation of pathways associated with responses to NRF2-mediated oxidative stress, acute-phase stress, and hypoxia-like stress.

Differential expression of genes downstream of HIF-1α signalling was reported and associated with changes in carbohydrate metabolism and other energy metabolism-related pathways (Permenter et al., 2013). Modulation of extracellular proteins and/or transcripts was also assessed, and while there were some thematic similarities with the "secretome" of human bronchial epithelial cells reported by Malard et al. (2012), no identical matches were described in rat liver cells (Permenter et al., 2013). Cobalt(II) salts were demonstrated to induce a transcriptomic response highly similar to physiological hypoxia in a HIF-1α-dependent manner in MEFs (wildtype and HIF-1α$^{-/-}$) exposed to 100 μM cobalt(II) chloride (Vengellur et al., 2003), while in BALB/3T3 fibroblasts exposed to cobalt(II) chloride at 1 μM, cobalt metal, or cobalt metal NPs (mean aggregate diameter, 450 nm or 500 nm, respectively), cobalt metal NPs induced the largest magnitude of differential RNA expression, involving the cell stress response and repair pathways (Papis et al., 2007). In another murine fibroblast cell line (PW), HIF-1α was induced at non-cytotoxic concentrations of cobalt metal NPs as low as 19 μM (mean diameter, 20 nm; hydrated mean diameter, 260 nm), while growth arrest and DNA damage inducible α (GADD45α) mRNA and protein expression increased at ≥ 75 μM in a HIF-1α-dependent manner (Feng et al., 2015).

4.2.12 Evaluation of high-throughput in vitro toxicity screening data

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 131 was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Cobalt(II) sulfate heptahydrate was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2022a).

The ToxCast/Tox21 high-throughput screening results are presented based on the assays that have been mapped to the key characteristics (Reisfeld et al., 2022). The detailed results are available in supplementary information for this volume (Annex 4, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, available from: https://publications.iarc.fr/618). Here, for brevity, assays for which there is a positive "hit call" are referred to as "active" assays. A summary of these results is given below as the number of active assays (without any caution flags)/total number of key characteristic-related assays for the chemical.

Cobalt(II) sulfate heptahydrate was active in two assays mapped for key characteristic 5, "induces oxidative stress". These results include the significantly increased activity of HIF-1α in the cervical cell line ME-180 at AC_{50} of 31.4 μM, and increased activity of nuclear factor erythroid 2-related factor 2 (NRF2) in the liver cell line HepG2 at AC_{50} of 36.7 μM. In addition, cobalt(II) sulfate heptahydrate was active in one assay mapped for key characteristic 8, "modulates receptor-mediated effects". The result showed increased activity of nuclear receptor subfamily 3, group C, member 1 (*NR3C1*, glucocorticoid receptor) in the cervical adenocarcinoma cell line HeLa at AC_{50} of 23.28 μM. [The Working Group noted that the purity of the compound evaluated could not be determined because the quality control grade is listed as "unknown/inconclusive" (NCATS, 2022).]

5. Summary of Data Reported

5.1 Exposure characterization

The agents evaluated in the present monograph include: metallic cobalt (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, and the relatively insoluble compounds cobalt(II) or cobalt(II,III) oxide and cobalt(II) sulfide. Cobalt is ubiquitous in the environment, generally occurring at low levels in rocks, soil and sediments, groundwater and surface water, and air as oxides, sulfides, arsenides, and sulfoarsenides. Anthropogenic activities such as mining, smelting and other related industrial processes, coal combustion, and vehicular traffic have resulted in elevated levels of cobalt and cobalt compounds in the environment. Cobalt is produced mainly as a by-product of the mining and processing of ores of other metals. The global production of cobalt from mines and refineries has increased steadily over the past two decades. Cobalt is used in many industries, including in the manufacture of cutting and grinding tools, in pigments and paints, coloured glass, medical implants, batteries, and electroplating. Its use has increased at an annual rate of approximately 5% since 2013, particularly driven by the production of lithium-ion batteries. Occupational exposure to cobalt is expected to occur predominantly during the refining of cobalt, in the production of cobalt metals and cobalt compounds, during the use of diamond–cobalt tools, during the production of dental materials, in plate painting with cobalt pigments, and during the manufacture of nickel–hydrogen batteries. The main route of occupational exposure to cobalt is expected to be the respiratory tract; however, skin exposure and inadvertent ingestion may also occur. Workers may be exposed to various cobalt compounds and cobalt metal powders together with other agents. For the general population, food is usually the primary source of cobalt exposure; exposure may also occur via medical implants. Blood, serum, and urinary concentrations of cobalt are commonly used as indicators of exposure. Cobalt levels in other biological matrices such as nails or exhaled breath condensate have also been used to estimate human exposure. Occupational exposure to cobalt is regulated in many countries, particularly the inhalable dust fraction in workplace air; monitoring via analysis of cobalt in biological matrices such as blood and urine has also been recommended by several regulatory agencies. Some environmental guidelines exist for cobalt in natural water sources, foodstuffs, and dietary supplements.

5.2 Cancer in humans

Several studies on cobalt exposure in humans were available. Two high-quality occupational cohort studies in the hard-metal industry were considered potentially informative and found positive associations with lung cancer. However, in these studies it was difficult to separate a cobalt-specific effect from the effect of co-exposure to WC-Co or other lung carcinogens present at the work sites. Five studies in other industries did not show a consistent association between cobalt exposure and risk of lung cancer. Several studies in the general population considered cobalt exposure in relation to many other cancer sites, including breast, and none of the studies found strong or consistent evidence for a positive association. Most of the studies in the general population were limited by the use of one-time exposure assessments, which may or may not have reflected biologically important windows of exposure and temporal variability. The studies in the general population also had a relatively small range of exposures to evaluate exposure–response relations. Overall, the available studies did not permit a conclusion to be drawn about the presence of a causal association between cobalt exposure and lung cancer or other cancers in humans. No informative studies were found that permitted the separation of the

effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II) or cobalt(II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

5.3 Cancer in experimental animals

5.3.1 Cobalt metal

Treatment with cobalt metal caused an increase in the incidence of tumours in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice (GLP).

Cobalt metal microparticles administered by inhalation (whole-body exposure) in one well-conducted GLP study in male and female $B6C3F_1$/N mice caused an increase in the incidence of bronchioloalveolar carcinoma in males and females.

Cobalt metal microparticles were administered by inhalation (whole-body exposure) in one well-conducted GLP study in male and female F344/NTac rats. Cobalt metal microparticles caused an increase in the incidence of bronchioloalveolar carcinoma and malignant pheochromocytoma of the adrenal medulla in males and females; of pancreatic islet adenoma or carcinoma (combined), and a positive trend in the incidence of pancreatic islet carcinoma and renal tubule adenoma or carcinoma (combined) in males; and of mononuclear cell leukaemia in females.

Cobalt metal microparticles administered by intramuscular injection in one study in female Hooded rats caused an increase in the incidence of rhabdomyofibrosarcoma, fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined). Cobalt metal microparticles administered by intramuscular injection in one study in male and female Hooded rats caused an increase in the incidence of rhabdomyofibrosarcoma or sarcoma (NOS) (combined) in males, and of fibrosarcoma and rhabdomyosarcoma or rhabdomyofibrosarcoma (combined) in females.

Cobalt metal pellets administered by intramuscular implantation in one study in male Sprague-Dawley rats caused an increase in the incidence of rhabdomyosarcoma or spindle cell tumours (NOS) (combined) of the limb gastrocnemius muscle. Cobalt metal nanoparticles administered by intramuscular implantation in one study in male Sprague-Dawley rats caused a high incidence of sarcomas (NOS) at the implantation site (but there was a lack of concurrent controls).

5.3.2 Soluble cobalt(II) salts

(a) Cobalt(II)sulfate

Treatment with cobalt(II)sulfate caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of two species in well-conducted studies that complied with GLP.

Cobalt(II) sulfate administered by inhalation (whole-body exposure) in one well-conducted study that complied with GLP in male and female $B6C3F_1$ mice caused an increase in the incidence of bronchioloalveolar carcinoma in males and females.

Cobalt(II) sulfate was administered by inhalation (whole-body exposure) in one well-conducted study that complied with GLP in male and female Fischer 344/NTac rats. Cobalt(II) sulfate caused an increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) in males; and an increase in the incidence of bronchioloalveolar carcinoma, and benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla in females.

(b) Cobalt(II) chloride

Treatment with cobalt(II) chloride caused an increase in the incidence of malignant neoplasms in a single experiment in one species (the rat).

Cobalt(II) chloride administered by subcutaneous injection in one study in male Wistar rats (including two experiments) caused an increase in the incidence of fibrosarcoma of the subcutaneous tissue in both experiments.

5.3.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

(a) Cobalt(II) oxide

Treatment with cobalt(II) oxide caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in more than one study in one species (the rat), carried out at different times or in different laboratories and/or under different protocols.

Cobalt(II) oxide microparticles administered by intratracheal instillation in one study in male and female Sprague-Dawley rats caused an increase in the incidence of bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, or adenocarcinoma of the lung (combined) in males.

Cobalt(II) oxide microparticles administered by subcutaneous injection in one study in male Sprague-Dawley rats caused an increase in the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site.

Cobalt(II) oxide microparticles administered by intramuscular injection in one study in male and female Wistar rats caused an increase in the incidence of rhabdomyosarcoma at the injection site in males and females combined. Cobalt(II) oxide microparticles were administered by intramuscular injection in one study in male and female Wistar rats. Cobalt(II) oxide microparticles caused a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) in males and females combined (but there was a lack of concurrent controls).

Cobalt(II) oxide microparticles administered by intraperitoneal injection in one study in male and female Sprague-Dawley rats caused an increase in the incidence of malignant histiocytoma, sarcoma (NOS), or malignant mesothelioma (combined), and of malignant histiocytoma, both at the injection site, in males and females combined.

(b) Cobalt(II) sulfide

Treatment with cobalt(II) sulfide caused a high incidence of malignant neoplasms in a single experiment in one species (the rat).

Cobalt(II) sulfide microparticles were administered by intramuscular injection in one study in male and female Wistar rats. Cobalt(II) sulfide microparticles caused a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) in males and females combined (but there was a lack of concurrent controls).

(c) Cobalt(II,III) oxide

Treatment with cobalt(II,III) oxide did not cause a carcinogenic effect in a single study.

Cobalt(II,III) oxide microparticles administered by intratracheal instillation in male and female hamsters did not cause a significant increase in the incidence of tumours in males and females combined.

5.3.4 Other cobalt(II) compounds

No data on other cobalt(II) compounds were available to the Working Group.

5.4 Mechanistic evidence

The Working Group considered multiple forms of cobalt in evaluating the mechanistic evidence of carcinogenicity. Appropriate particles of all sizes were grouped together on the basis of similar chemical forms of cobalt, since observed differences regarding the effects of particles of micron size and smaller on mechanistic end-points were deemed minor on the basis of evidence available to the Working Group.

Data were available on the absorption and distribution of cobalt in humans and experimental systems. Inhaled cobalt can be deposited and absorbed in the respiratory tract or cleared by mucociliary clearance and swallowed. Deposition, absorption, and clearance of cobalt in the respiratory tract is influenced by particle size and surface area, and solubility of the compound. More soluble forms of cobalt have a higher rate of absorption from the lungs and gastrointestinal tract. Dermal absorption of cobalt from intact skin is low (less than 1%) but is higher through abraded skin. Cobalt is primarily distributed to the serum, whole blood, liver, kidneys, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, pancreas, and other organs. Cobalt crosses the placenta and appears in fetal blood. Cobalt is not subject to metabolism by enzymatic pathways and is excreted primarily in the urine and faeces, with elimination half-lives of several hours to 1 week in humans.

Several studies in humans with either occupational or environmental exposure to cobalt were available to the Working Group. These studies provided some evidence of cobalt-induced effects on key characteristics of carcinogens for genotoxicity, induction of oxidative stress, and modulation of receptor-mediated effects. However, these studies did not identify the form of cobalt to which exposure occurred, therefore they have not been included in the summary. One occupational study with possible mixed exposure to cobalt metal and cobalt oxides was retained, and appears in the summary of cobalt metal, cobalt(II) oxide, and cobalt(II,III) oxide.

5.4.1 Cobalt metal

There is consistent and coherent evidence that cobalt metal exhibits key characteristics of carcinogens.

Cobalt metal is genotoxic. In exposed humans, evidence for genotoxicity is suggestive, as positive correlations were observed between urinary cobalt concentrations and DNA single-strand breaks and the frequency of micronuclei in blood binucleated cells in one occupational study. Consistent and coherent evidence for genotoxicity comes from multiple studies in human primary cells showing that cobalt metal induces DNA strand breaks and increases the frequency of micronucleus formation. Consistent and coherent evidence for genotoxicity also comes from experimental systems, including DNA and chromosomal damage in multiple studies in human cell lines, and in non-human mammalian experimental systems in vivo and in vitro.

Cobalt metal induces oxidative stress. No data were available in humans identified as exposed specifically to cobalt metal. There is consistent and coherent evidence of increased ROS levels or other oxidative stress biomarkers in multiple studies in human primary cells. One study showed oxidative DNA damage. Additional consistent and coherent evidence comes from in vivo rodent studies, in vitro studies showing oxidative stress and oxidative DNA damage in human cell lines, and several in vitro studies in non-human mammalian cell lines.

Cobalt metal induces chronic inflammation. No data were available in humans identified as exposed specifically to cobalt metal. There is consistent and coherent evidence of chronic inflammation from chronic rodent inhalation studies that demonstrated increased inflammation in the nose and larynx, and chronic active inflammation (and increased histiocytic infiltrates) in the lung. Shorter-term inhalation and implant studies in rodents provide further evidence of chronic inflammation after cobalt metal exposure.

Cobalt metal alters cell proliferation, cell death, or nutrient supply. No data were available in exposed humans. Cobalt metal induced progressive, proliferative lesions, including hyperplasia and metaplasia, in a dose-responsive manner in the upper and lower respiratory tract

of rats and mice after acute to chronic inhalation exposures. There was no evidence of increased cell proliferation or cell viability, or attenuated apoptosis, in several studies in non-human mammalian cell lines.

There is suggestive evidence that cobalt metal modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

There is suggestive evidence that cobalt metal causes immortalization. Mixed evidence from two studies showed an increase in anchorage-independent growth in neoplastic human cell lines. Additional evidence comes from several in vitro studies in murine embryonic fibroblast cell lines showing cobalt metal-induced morphological transformation. Cobalt metal also acted as an initiator in a single two-stage (initiator–promoter) in vitro assay.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.2 Soluble cobalt(II) salts

There is consistent and coherent evidence that soluble cobalt(II) salts exhibit key characteristics of carcinogens.

Soluble cobalt(II) salts are genotoxic. No data were available in exposed humans. There was consistent and coherent evidence based on multiple studies using human primary cells showing that cobalt(II) salts induce chromosomal aberrations, DNA strand breaks, and, in one study, sister-chromatid exchange. Consistent and coherent evidence for genotoxicity is also available from experimental systems, including studies in human cell lines. Several rodent studies using intraperitoneal administration of cobalt(II) salts consistently reported genotoxic effects. Oral exposure of rodents to cobalt salts, and studies using in vitro non-human mammalian and non-mammalian systems, provide mixed results.

Soluble cobalt(II) salts induce oxidative stress. No data were available in exposed humans. There is consistent and coherent evidence from numerous studies using immortalized human cell lines in vitro, rats and mice in vivo, and non-human mammalian cell lines in vitro of increased levels of oxidative stress biomarkers, including oxidative DNA damage. There is suggestive evidence in a few studies in human primary cells that yielded mixed results.

Soluble cobalt(II) salts induce chronic inflammation. No data were available in exposed humans. Consistent and coherent evidence of increased nasal and laryngeal inflammation, as well as chronic active inflammation (and histiocytic infiltrates) in the lung, is provided by subchronic and chronic inhalation studies in rodents. Increased levels of macrophages in bronchoalveolar lavage and markers of interstitial or intra-alveolar lung inflammation were also reported after subchronic inhalation exposures in rabbits, and acute exposures in mice and rats. In dermal sensitization studies, leukocyte accumulation was induced in both mice and guinea-pigs.

Soluble cobalt(II) salts are immunosuppressive. No data were available in exposed humans. There is consistent and coherent evidence of immunosuppression based on studies in experimental systems and suggestive evidence in human primary cells. Decreased thymus weight and antibody-producing cells in response to sheep erythrocyte stimulation were reported after oral exposure to cobalt salts in a single study in rats, while increased production of IgM but not IgG antibodies was reported in a single study in mice. Decreased toll-like receptor 4 expression was reported in a single study in primary mouse macrophages. In addition, cobalt(II) salts induced decreased lymphocyte viability, activation, proliferation, and/or cytokine expression in human primary cells in several studies, and in a single study in primary mouse splenocytes. Decreased expression of human leukocyte

antigen HLA class II molecules was reported in one study in human primary cells.

Soluble cobalt(II) salts alter cell proliferation, cell death, or nutrient supply. No data were available in exposed humans. Consistent and coherent evidence of increased vascular endothelial growth factor expression was reported in numerous studies in human primary cells, immortalized human cell lines, and rodent studies in vivo. Increased formation of capillary-like tube structures was reported in one study in primary human umbilical vein endothelial cells and two studies in mouse endothelial cell cultures. Stimulated cell proliferation, or increased cell viability, was seen in several studies in human primary cells or human cell lines, but the evidence for attenuated cell death was mixed. Cobalt(II) salts also induced progressive, proliferative lesions, including hyperplasia and metaplasia, in a dose-responsive manner in the upper and lower respiratory tract of rabbits, rats, and mice after acute to chronic inhalation exposures. Apoptosis was decreased in two in vivo rat studies.

There is suggestive evidence that soluble cobalt(II) salts at non-cytotoxic concentrations alter DNA repair, based on one study in human primary cells and on studies in experimental systems using human cell lines and acellular systems.

There is suggestive evidence that soluble cobalt(II) salts induce epigenetic alterations, based on a single study in human umbilical vein endothelial cells. Suggestive evidence is also available from several studies in human cell lines showing changes in histone acetylation, mRNA methylation, and altered expression of miRNAs after cobalt(II) salt treatment. Altered methylation of RNA was reported after repeated intraperitoneal administration in one mouse study. Several studies in non-human mammalian cells in vitro also revealed consistent evidence of decreased histone acetylation after exposure to cobalt(II) salts.

There is suggestive evidence that soluble cobalt(II) salts modulate receptor-mediated effects based on measurements of protein levels of (largely) individual receptors in human cell lines. Consistent with the observations in human cell lines, decreased peroxisome proliferator-activated receptor PPAR mRNA levels were reported in two in vivo rat studies.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.3 Cobalt(II) oxide

For cobalt(II) oxide, the mechanistic evidence is suggestive. While consistent and coherent evidence was reported for oxidative stress in experimental systems (described below), this was not supported by such evidence for other key characteristics of carcinogens.

There is suggestive evidence that cobalt(II) oxide is genotoxic based on one study in human primary cells and in multiple studies using human cell lines. In these studies, cobalt(II) oxide induced chromosome aberrations and DNA strand breaks. One in vivo study in rats showed negative results for chromosomal aberrations. Findings in non-human mammalian cells in vitro and in non-mammalian systems typically yielded mixed results in a small number of studies.

There is consistent and coherent evidence from two experimental systems that cobalt(II) oxide induces oxidative stress, including one in human cell lines and one in vivo study in rodents. There is suggestive evidence that cobalt(II) oxide induces oxidative stress in a single study in human primary cells.

There is suggestive evidence that cobalt(II) oxide modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.4 Cobalt(II,III) oxide

For cobalt(II,III) oxide, the mechanistic evidence is suggestive. While consistent and coherent evidence was reported for oxidative stress in experimental systems (described below), this was not supported by such evidence for other key characteristics of carcinogens.

There is suggestive evidence that cobalt(II,III) oxide is genotoxic, based upon induction of chromosome aberrations and DNA strand breaks in one study in human primary cells. There is suggestive evidence in human cell lines for DNA strand breaks and micronuclei formation. One oral study in rodents and one study in non-human mammalian cells reported negative findings.

There is consistent and coherent evidence that cobalt(II,III) oxide induces oxidative stress based on increased levels of reactive oxygen species in two studies in human primary cells, and increased measurements of oxidative stress, including oxidative DNA damage, in multiple studies in human cell lines. Similar effects were also reported in one in vivo rodent study.

There is suggestive evidence that cobalt(II,III) oxide modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

There are either no data or sparse data for cobalt(II,III) oxide for all other key characteristics.

5.4.5 Cobalt sulfide

There are either no data or sparse data for all key characteristics.

5.4.6 Other cobalt(II) compounds

There are either no data or sparse data for all key characteristics.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of cobalt metal (without tungsten carbide or other metal alloys) and of soluble and insoluble cobalt(II) and cobalt(II,III) compounds.

6.2 Cancer in experimental animals

6.2.1 Cobalt metal

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

6.2.2 Soluble cobalt(II) salts

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt(II) sulfate.

There is *limited evidence* in experimental animals for the carcinogenicity of cobalt(II) chloride.

6.2.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt(II) oxide.

There is *inadequate evidence* in experimental animals regarding the carcinogenicity of cobalt(II,III) oxide.

There is *limited evidence* in experimental animals for the carcinogenicity of cobalt(II) sulfide.

6.2.4 Other cobalt(II) compounds

There is *inadequate evidence* in experimental animals regarding the carcinogenicity of other cobalt(II) compounds.

6.3 Mechanistic evidence

There is *strong evidence* in human primary cells and in experimental systems that cobalt metal (including particles of all sizes) exhibits key characteristics of carcinogens.

There is *strong evidence* in human primary cells and in experimental systems that soluble cobalt(II) salts exhibit key characteristics of carcinogens.

For cobalt(II) and cobalt(II,III) oxides (including particles of all sizes), there is *limited* mechanistic evidence.

For cobalt(II) sulfide, there is *inadequate* mechanistic evidence.

For other cobalt(II) compounds, there is *inadequate* mechanistic evidence.

6.4 Overall evaluation

Cobalt metal (without tungsten carbide or other metal alloys) is *probably carcinogenic to humans (Group 2A)*.

Soluble cobalt(II) salts are *probably carcinogenic to humans (Group 2A)*.

Cobalt(II) oxide is *possibly carcinogenic to humans (Group 2B)*.

Cobalt(II,III) oxide is *not classifiable as to its carcinogenicity to humans* (*Group 3*).

Cobalt(II) sulfide is *not classifiable as to its carcinogenicity to humans* (*Group 3*).

Other cobalt(II) compounds are *not classifiable as to their carcinogenicity to humans* (*Group 3*).

6.5 Rationale

The Group 2A evaluation for cobalt metal (without tungsten carbide or other metal alloys) is based on *sufficient evidence* for cancer in experimental animals and *strong* mechanistic evidence in human primary cells. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice. There is *strong evidence* in human primary cells and in experimental systems that cobalt metal (including particles of all sizes) is genotoxic and induces oxidative stress. There is *strong evidence* in experimental systems that cobalt metal (including particles of all sizes) induces chronic inflammation and alters cell proliferation, cell death, or nutrient supply. There is *inadequate evidence* regarding cancer in humans.

The Group 2A evaluation for soluble cobalt(II) salts is based on *sufficient evidence* for cancer in experimental animals and *strong* mechanistic evidence in human primary cells. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms caused by soluble cobalt(II) sulfate in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice, and on an increase in the incidence of malignant neoplasms caused by soluble cobalt(II) chloride in a single experiment in one species (the rat). There is *strong evidence* in human primary cells and in experimental systems that soluble cobalt(II) salts are genotoxic, and alter cell proliferation, cell death, or nutrient supply. There is *strong evidence* in experimental systems that soluble cobalt(II) salts induce oxidative stress and chronic inflammation, and that they are immunosuppressive. There is *inadequate evidence* regarding cancer in humans.

The Group 2B evaluation for cobalt(II) oxide is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in more than one study in one species (the rat), carried out at

different times or in different laboratories and/or under different protocols. There is *limited* mechanistic evidence in exposed humans, in human primary cells, and in experimental systems for insoluble cobalt(II) oxide. While there is consistent and coherent evidence that insoluble cobalt(II) oxide (including particles of all sizes) induces oxidative stress in experimental systems, there is not for other key characteristics of carcinogens. There is *inadequate evidence* regarding cancer in humans.

The Group 3 evaluation for cobalt(II,III) oxide is based on *inadequate evidence* regarding cancer in humans and in experimental animals and on *limited* mechanistic evidence. There is *limited* mechanistic evidence in exposed humans, in human primary cells, and in experimental systems for cobalt(II,III) oxide. While there is consistent and coherent evidence that cobalt(II,III) oxide (including particles of all sizes) induces oxidative stress, there is not for other key characteristics of carcinogens.

The Group 3 evaluation for cobalt(II) sulfide is based on *inadequate evidence* regarding cancer in humans, on *limited evidence* for cancer in experimental animals, and on *inadequate* mechanistic evidence. The *limited evidence* for cancer in experimental animals is based on a high incidence of malignant neoplasms observed in a single experiment in one species (the rat).

The Group 3 evaluation for other cobalt(II) compounds is based on *inadequate evidence* regarding cancer in humans and in experimental animals, and on *inadequate* mechanistic evidence. No studies were available in experimental animals, and the few mechanistic studies were largely negative.

All classifications above should be presumed to apply to all size classes for the listed agents.

The evidence in humans is *inadequate* regarding the carcinogenicity of cobalt metal (without tungsten carbide or other metal alloys) and non-metallic forms of cobalt. Among the available human cancer studies, the studies of exposures to cobalt in the hard-metal industry did not permit separation of cobalt's effects from those of the cobalt and tungsten carbide composite, or other known or suspected lung carcinogens, in examining lung cancer risk, and other occupational studies were either confounded by other known lung carcinogens or did not show associations of cobalt with lung cancer. None of the four available studies in workers or the general population found convincing associations of cobalt with breast cancer risk. Five studies examining other cancer sites either sporadically found positive associations or were considered of low quality or uninformative. No informative studies were found that permitted the separation of the effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II or II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

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TRIVALENT AND PENTAVALENT ANTIMONY

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature, synonyms, trade names, molecular formulae, and relative molecular mass

Multiple agents are considered in this monograph as follows: the trivalent antimony compounds antimony(III) oxide (Sb_2O_3) [antimony trioxide], antimony(III) chloride ($SbCl_3$), antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$ or the anhydrous form $K_2Sb_2(C_4H_2O_6)_2$), antimony(III) sulfide (Sb_2S_3), trivalent antimony(III) ions (Sb^{3+}), and antimony(III) hydride (also known as stibine, SbH_3), and the pentavalent antimony compounds antimony(V) oxide (Sb_2O_5), meglumine antimoniate(V) ($C_7H_{17}NO_5{\cdot}H{\cdot}O_3Sb$), and antimony(V) in sodium stibogluconate ($C_{12}H_{38}Na_3O_{26}Sb_2$). Current and discarded or replaced Chemical Abstracts Service (CAS) registration numbers together with common synonyms and trade names of these agents are presented in Table 1.1. [It is noted by the Working Group that this is a non-exhaustive list of trivalent and pentavalent antimony compounds.]

Antimony(III) oxide can exist in various polymorphs, notably valentinite and senarmontite, with different chemical and physical characteristics. The aqueous speciation of antimony may be complex. The inorganic antimony(III) and antimony(V) species, which tend to predominate under reducing and oxidizing conditions, respectively, may also be variably protonated as a function of pH, complex with a variety of inorganic and organic ligands (notably sulfur-bearing ligands) and they may exist as polynuclear or polymeric moieties even at comparatively low total antimony concentrations (Guo et al., 2020). Many of the agents that commonly occur in the solid phase can exist in forms with various degrees of hydration, polymerization, or protonization. The names of some related agents for antimony(III) potassium tartrate, which also exhibits non-conformational isomerism, are therefore also recorded in Table 1.1. "Meglumine antimoniate(V)" and "antimony(V) in sodium gluconate" are terms that are widely used to refer to mixtures that may have ranges of compositions and molecular constituents of antimony and other compounds. [The Working Group notes that although chemical names may be reported as "synonyms" of a particular agent, such reporting may not always be accurate and relevant papers may be silent on characteristics, such as polymorphism, particle size, or trace element impurities, that may be critical to inform understanding of the reactivity or toxicity of the product overall (Gaultieri, 2017).]

Table 1.1 Registry numbers, synonyms and trade names, molecular formulae, and relative molecular masses for trivalent and pentavalent antimony, antimony metal, and antimonite ore

Chemical name (reference)	CAS No.[a]	Synonyms and trade names	Formula	Relative molecular mass
Antimony(III) oxide (IARC, 1989; ECHA, 2022a; NLM, 2022)	1309-64-4 1327-33-9 1317-98-2 (specific to valentinite) 12412-52-1 (specific to senarmontite)	IUPAC names: diantimony trioxide; (stibanyloxy) stibanediol Antimony trioxide; diantimony trioxide; antimony oxide; antimonous oxide; flowers of antimony Valentinite Trade names: CI 77052; CI Pigment White 11; antimony white; AP 50 Senarmontite	Sb_2O_3	291.50
Antimony(III) chloride (NCBI, 2022d)	10025-91-9 [8007-28-1; 12515-76-3; 39357-85-2; 59922-49-5]	IUPAC names: antimony trichloride (preferred); trichlorostibane (systematic) Antimony chloride; antimony(III) chloride; trichlorostibine; stibine, trichloro-; antimonous chloride; antimony butter; butter of antimony; caustic antimony	$SbCl_3$	228.11
Antimony(III) potassium tartrate (NCBI, 2022a; ECHA, 2022b)	28300-74-5 [1332-41-8; 1332-43-0; 16039-64-8; 26282-95-1]	IUPAC name: potassium antimony(3+) 2,3-dihydroxybutanedioate Tartaric acid, antimony potassium salt, trihydrate; antimony potassium tartrate; tartrated antimony; emetic, tartar; potassium antimonyl tartrate Trade and other names: Tartox	$C_8H_{10}K_2O_{15}Sb_2$; anhydrous form: $K_2Sb_2(C_4H_2O_6)_2$	667.87
Trivalent antimony(III) ions (NCBI, 2021a)	23713-48-6	Antimony(3+); antimony cation (3+); antimony(III) ION; antimony black	Sb^{+3}	121.760
Antimony(III) hydride (Grund et al., 2006; NCBI, 2022b)	7803-52-3	Stibine; stibine; antimony hydride; antimony trihydride; hydrogen antimonide	SbH_3	124.77
Antimony(III) sulfide (NCBI, 2023)	1345-04-6	Diantimony trisulfide, stibnite	Sb_2S_3	339.7
Antimony(V) oxide (NCBI, 2022c)	1314-60-9	IUPAC name: (dioxo-λ^5-stibanyl)oxy-dioxo- λ^5-stibane Antimony(V) oxide; antimony oxide (Sb_2O_5); antimony pentaoxide; antimony pentoxide; antimonic oxide; diantimony pentaoxide; stibic anhydride; Apox S; Nyacol AGO 40	Sb_2O_5	323.52
Meglumine antimoniate(V) (Chemical Abstracts Service, 2021; ChemSrc, 2022)	133-51-7 [161842-96-2]	D-Glucitol, 1-deoxy-1-(methylamino)-,trioxoantimonate(1-); glucitol, 1-deoxy-1-(methylamino)-, compd. with antimonic acid ($HSbO_3$)(1:1), D-; D-glucitol, 1-deoxy-1-(methylamino)-, compd. with antimonic acid ($HSbO_3$)(1:1); glucitol, 1-deoxy-1-(methylamino)-, antimonite(V); antimonic acid ($HSbO_3$), compd. with D-1-deoxy-1-(methylamino)glucitol (1:1) Trade and other names: Glucantime; Glucantim; 2168-rp; Protostib; RP 2168; MFCD01725422; EINECS 205-108-3	$C_7H_{17}NO_5 \cdot H \cdot O_3Sb$[b]	383.995[b]

Table 1.1 (continued)

Chemical name (reference)	CAS No.[a]	Synonyms and trade names	Formula	Relative molecular mass
Antimony(V) in sodium stibogluconate (Chemical Abstracts Service, 2021; NCBI, 2021b; SelleckChem, 2021)	16037-91-5 [35-03-0; 12001-86-4]	D-gluconic acid, 2,4:2′,4′-O-(oxydistibylidyne)bis-,sodium salt, hydrate(1:3:9); D-gluconic acid, 2,4:2′,4′-O-(oxydistibylidyne) bis-, Sb,Sb′-dioxide, trisodium salt, nonahydrate; D-gluconic acid, 2,4:2′,4′-O-[oxybis(oxidostibylidyne)]bis-, trisodium salt, nonahydrate; 1,3,2-dioxantimonane, D-gluconic acid deriv.; 1,3,2-dioxastibinane, D-gluconic acid deriv.; D-gluconic acid, cyclic ester with antimonic acid ($H_8Sb_2O_9$) (2:1), trisodium salt, nonahydrate; antimony gluconate sodium; antimony gluconic acid; antimony sodium gluconate; stibogluconate sodium; SSG Trade and other names: Myostibin; Pentostam; Solustin; Solusurmin; Stibanate; Stibatin; Solyusurmin; Stibinol; Stibanose; Lenocta	$C_{12}H_{38}Na_3O_{26}Sb_2$[c]	909.90[c]

CAS No., Chemical Abstracts Service registry number; CI, Colour Index; IUPAC, International Union of Pure and Applied Chemistry.

[a] Deleted CAS Nos are shown in square brackets.

[b] Meglumine antimoniate(V) is reported by Roberts et al. (1998) to be a complex mixture of compounds rather than a single compound.

[c] Sodium stibogluconate is reported by NCBI (2021b) to be a "D-gluconate adduct of indefinite composition containing between 30% and 34% of antimony(V)"; relative molecular mass is dependent upon degree of protonation and number of waters of hydration.

Molecular formulae and relative molecular masses of the agents under consideration are given in Table 1.1.

1.1.2 Chemical and physical properties of the pure substances

Chemical and physical properties of the agents are presented in Table 1.2. It is noted that many of these properties are dependent upon the polymorphic form of the agent, the particle size, and the level and nature of impurities in specific trade products.

1.1.3 Technical grade and impurities

Purities of commercially available trivalent and pentavalent antimony compounds have been noted to vary from 99% to 99.999% for antimony(III) oxide (ChemicalBook, 2021a; Sigma-Aldrich, 2022a), 98% to > 99% for antimony(III) chloride (NCBI, 2022d), 95% to 99.95% for antimony(III) potassium tartrate (ChemicalBook, 2021b; Sigma-Aldrich, 2022b), 98% to 99% for meglumine antimoniate(V) (ChemSrc, 2022; Albalawi et al., 2021), and > 90% to 99.9% for antimony(V) in sodium stibogluconate (ChemicalBook, 2021c).

1.2 Production and use

1.2.1 Production

Global primary mining production of antimony in 2020 amounted to around 111 000 tonnes, of which most originated in China (61 000 tonnes), the Russian Federation (25 000 tonnes), and Tajikistan (13 000 tonnes), with substantial production greater than 2000 tonnes noted also in Myanmar, the Plurinational State of Bolivia, and Australia (USGS, 2021). Antimony in mined ores is predominantly in the antimony(III) oxidation state. Antimony is a chalcophile element and its ores are dominated by the sulfide minerals stibnite (also known as antimonite; Sb_2S_3) (Filella et al., 2002) and, to a lesser extent, jamesonite ($Pb_4FeSb_6S_{14}$), as well as fahlore, a sulfosalt that is highly compositionally variable (including in sulfur, arsenic, copper, zinc, iron, silver, and mercury) (George et al., 2017), of which tetrahedrite ($Cu_{12}Sb_4S_{13}$) is an antimony-dominated end-member. The antimony(III) oxide polymorphs senarmontite and valentinite are also widely mined (Butterman & Carlin, 2004). Minerals with mixed oxidation-state antimony include cervantite ($Sb(III)Sb(V)O_4$) and stibiconite ($Sb(III)Sb(V)_2O_6(OH)$), such as are mined at the San José antimony mines, northern Mexico (White & Jenaro Gonzáles, 1946). Antimony often, but not exclusively, occurs in complex ores from which it may be removed or extracted as a by-product, made more profitable because antimony is widely considered to be a penalty element (i.e. a contaminant that reduces the value of a metal/metalloid product) in base metal (e.g. copper) concentrates (Lane et al., 2016; Haga et al., 2018). In the USA, recycling contributes to around 15% of national consumption (USGS, 2021) but is largely restricted to the production of antimonial lead for use in alloy production (Butterman & Carlin, 2004). Global recycling rates are estimated to be between 1% and 10% (European Commission, 2020a), although these rates are higher (28%) in the European Union (EU) (Filella et al., 2020). Among several other antimony compounds, the antimony(III) salts of acetic acid, antimony, and antimony-rich lead dross have been classified in the past as High Production Volume chemicals by the Organisation for Economic Co-operation and Development (OECD, 2009), whereas the only antimony compounds listed as such in 2022 were antimony metal and antimony(III) sulfide (OECD, 2022).

Production methods for antimony products are highly variable, depending upon the product and the nature and grade of the source. The overwhelming majority of the world's

Table 1.2 Chemical and physical properties of the pure agents containing antimony

Chemical name	Typical physical description	Melting point (°C)	Boiling point (°C)	Density (g/cm³)	Solubility
Antimony(III) oxide (IARC, 1989; Lide, 1992; National Research Council, 2000; European Commission, 2008; ILO, WHO, 2013)	White, odourless, crystalline powder	656	1550 (sublimes)	5.7–5.9 (valentinite); 5.2 (senarmontite)	Very slightly soluble in cold water; slightly soluble in hot water; soluble in potassium hydroxide, hydrochloric acid, and acetic acid; insoluble in organic solvents; pH-dependent solubility in water is 19.7, 25.6, and 28.7 mg/L at 20 °C and pH 5, 7, and 9, respectively
Antimony(III) chloride (Grund et al., 2006; NCBI, 2022d)	Colourless, orthorhombic, deliquescent	73.4	223	3.14	Very soluble in water: 987 g/100 g water at 25 °C; slightly soluble in ethanol, hydrochloric acid, and tartaric acid
Antimony(III) potassium tartrate (ATSDR, 2019; Lide, 1992)	Colourless crystals	100 (decomposes)	NA	2.6	Slightly soluble in water: 8.3 g/100 mL at 20 °C; soluble in glycerine; insoluble in alcohol
Trivalent antimony(III) ions	Aqueous species, not a phase	NA	NA	NA	See above, depends on matrix composition and nature of mobility controlling phases
Antimony(III) hydride (NCBI, 2022b; ATSDR, 2019)	Colourless gas under ambient conditions	−88	−18	2.204[a]	Slightly soluble in water: 4.1 g/L at 0 °C; soluble in carbon disulfide and ethanol
Antimony(III) sulfide (NCBI, 2022e)	Red or black crystals	550	NA	4.56	Practically insoluble in water
Antimony(V) oxide (Grund et al., 2006; NCBI, 2022c; ATSDR, 2019; ChemicalBook, 2022)	White to yellowish powder	380	NA	3.78	Very slightly soluble in water; soluble in alkalis; practically insoluble in nitric acid
Meglumine antimoniate(V) (ChemSrc, 2022; VWR, 2021)	White to yellow powder[b]	168–170	490.4	1.375	Greater than 0.38 g/100 mL[c]; predicted: 24 g/100 mL in water[d]
Antimony(V) in sodium stibogluconate (ChemicalBook, 2021c; ChemSrc, 2021c; SelleckChem, 2021)	Sodium stibogluconate is a pale yellow solid	673.6	NA	NA	Slightly soluble to soluble in water: 2 g/100 mL at 25 °C, 0.1 g/100 mL at 75 °C; insoluble in ethanol

NA, not available or not applicable.

[a] At −17 °C; density is strongly dependent on pressure and temperature.

[b] VWR page code 22 676 858; product ACRO461141000 (VWR, 2021).

[c] Inferred from Martins et al. (2009).

[d] Predicted solubility based on ALOGPS software (www.vcclab.org/lab/alogps/) (DrugBank, 2021).

production has been located at plants in China, the USA, Kyrgyzstan, France, the Plurinational State of Bolivia, and Belgium, notably including some countries with very limited, if any, mine production (Anderson, 2012). Primary product compounds are typically antimony(0) metal, antimony(III) oxide, or the antimony(V) compound sodium antimonate ($NaSbO_3$), with other antimony products produced from those forms. The dominant production methods include blast-furnace smelting, volatilization roasting, liquation, reduction, leaching/electrowinning, and precipitation (Butterman & Carlin, 2004), all of which typically require prior crushing, grinding, and/or sizing of the ore, and, in some countries, hand-sorting is or has been widespread. Blast-furnace smelting at temperatures of 1300–1400 °C, a dominant production process for 25–40% antimony concentrates, generates antimony(0) metal and relatively large volumes of solid waste, known as "slag" (Anderson, 2012). Volatilization roasting of typically antimony(III) stibnite ores with charcoal or coke at temperatures over 1000 °C, a dominant process for 5–25% antimony ore/concentrates (Anderson, 2012), results in direct recovery of antimony oxides from the produced vapour phase. The production of antimony(III) oxide requires careful control of the process, otherwise less marketable oxides, such as the mixed antimony(III)/antimony(V) oxide, antimony tetroxide (Sb_2O_4), are produced. The process is energy-intensive and releases sulfur dioxide, carbon dioxide, and other volatiles, including those bearing arsenic and mercury. Liquation is typically used for the production of antimony(III) sulfide, whereas reduction, precipitation, and leaching/electrowinning are more indicated for high-grade (45–60% antimony) ores/concentrates and for the production of high-quality antimony(0) metal (Butterman & Carlin, 2004; Anderson, 2012). Revolatilization of antimony(III) oxide or antimony(0) metal is often required to remove lead, arsenic, sulfur, iron, and copper impurities to attain antimony(III) oxide at the purities required for commercial purposes (Butterman & Carlin, 2004).

1.2.2 Uses

Antimony is included on the EU list of critical raw materials (European Commission, 2020b) and within the EU has been used in flame retardants (43%), lead–acid batteries (32%), lead alloys (14%), plastics (catalysts and stabilizers) (6%), and glass and ceramics (5%) (the values refer to usage over the period 2012–2016) (European Commission, 2020a). In the USA, antimony is principally used in flame retardants (42%), metal products (36%), and non-metal products, including ceramics, glass, and rubber products (22%) (USGS, 2021). [Information on major uses outside Europe and North America was unavailable to the Working Group.]

The major use of antimony(III) oxide is as a flame retardant in combination with halogenated compounds, forming pyrolysis-inhibiting halogenated antimonials in a wide variety of products, including "plastics, cable coatings, upholstered furniture, car seats, fabrics and household appliances" (European Commission, 2020a). Antimony(III) oxide is also used as a catalyst in the production of several types of plastic, notably polyethylene terephthalate (PET), which is widely used in plastic bottles used for drinking-water and soft drinks (Martin et al., 2010; Snedeker, 2014). Antimony(III) oxide can be used as a colourant in materials used in the production of food packaging; in plastics, rubber, paints, and enamels (European Commission, 2020a); and as a stabilizer in polyvinyl chloride (European Commission, 2020a). Antimony(III) sulfide is used as a lubricant in brake pads and clutch discs, and as an ammunition primer in explosives (European Commission, 2020a). Antimony(V), in the form of sodium hexahydroxoantimonate ($NaSb(OH)_6$), is used as a degassing agent in the production of glass, as well as in controlling glass colour. Antimony(V) oxide is used as a flame

retardant, including in textiles (Garcia, 1982; Leblanc, 1980; NCBI, 2022c).

Antimony(III) potassium tartrate has been principally used in the treatment of trypanosomiasis, schistosomiasis (Dziwornu et al., 2020), and leishmaniasis (Haldar et al., 2011), although other medicines are now generally preferred. Both meglumine antimoniate(V) and the antimony(V) product sodium stibogluconate are still widely used (McGwire & Satoskar, 2014) as the treatment of first choice (Haldar et al., 2011) for the treatment of leishmaniasis, a disease that is particularly prevalent in Andean Latin America, north Africa, the Middle East, western sub-Saharan Africa, and south Asia (Karimkhani et al., 2016). There is increasing use, or exploration of use, of the delivery of these agents within microspheres (Navaei et al., 2014) or as part of nanoparticulate biocomposites (Gélvez et al., 2018). These developments, together with exploration of alternative treatments, are being driven by concerns about adverse effects of and pathogen resistance to antimony-based medicines (Gélvez et al., 2021).

1.3 Detection and quantification

This section focuses on methods, and the key attributes of those methods, for the detection and quantification of antimony and antimony species in media relevant to assessing exposure and health risks. Antimony may be present in environmental media (e.g. water, air, soil, food, packaging, paint, water pipework, flame retardants, and consumer products) and biological media (e.g. blood, urine, organs, and other tissues) in target organisms.

Although several notable repositories of analytical methods for antimony and other analytes in environmental and biological media exist (see Table 1.3), selection of methods or protocols for the analysis of antimony or antimony speciation is typically made on the basis of a comparison of method characteristics against project-dependent data and data quality objectives (US EPA, 2006). Broader process-dependent controls on data quality include: sampling strategy and representativeness (Gy, 1995), sampling and preservation protocols, selection of analytical instrumentation, analytical and data reduction protocols, and total quality management protocols (Polya & Watts, 2017; APHA/AWWA/WEF, 1999; Ferreira et al., 2014). Yang et al. (2023) highlight the importance of ensuring that contamination from antimony-bearing plastic tubes into aqueous samples presented for instrumental analysis is minimized. Middleton et al. (2016) emphasize the need for adequate sample preparation, notably in the removal of surface contaminants from biological media such as toenail cuttings and hair before digestion.

1.3.1 Elemental analysis of antimony

Elemental analysis of antimony in environmental and biological media has been widely carried out by atomic absorption spectrophotometry (Bureau of Indian Standards, 2003), neutron activation analysis, inductively coupled plasma (ICP) mass spectrometry (ICP-MS), and anodic stripping voltammetry, as well as various colorimetric and titrimetric techniques (IARC, 1989). Electrothermal atomic absorption spectrophotometry (APHA/AWWA/WEF, 1999; Section 3113B) and ICP-MS (APHA/AWWA/WEF, 1999; Section 3125B) are particularly recommended as the analytical methods of choice for water, wastewater, and digests of solid media by the American Public Health Association, American Water Works Foundation, and Water Environment Foundation. The National Institute for Occupational Safety and Health (NIOSH) lists several methods (NIOSH, 2003a, b, c) for the analysis of total antimony in digested air particulates in NIOSH (2022). However, detection limits of as low as around 0.001 µg/g are now achievable after sample (e.g. human hair) digestion followed by instrumental analytical techniques such as ICP

Table 1.3 Analytical methods for the measurement of antimony or antimony chemical species in environmental or biological media[a]

Sample matrix (analyte)	Sample preparation (method)	Analytical technique (method)	LOD[b] (working range)	Comments	Reference
Air (total antimony)	Collection on filter (0.8 µm [pore size] cellulose ester membrane (MCE), or 5.0 µm [pore size] PVC membrane) followed by digestion in aqua regia	ICP-AES (NIOSH Method 7301)	0.192 µg/sample, 7.7 ng/mL ([5–2000 µg/sample])	Validated multi-element method. NIOSH (2017) notes "antimony [does] not form stable solutions in nitric acid when chloride from the PVC filters is present".	NIOSH (2003b)
Air (total antimony)	Collection on filter (0.8 µm [pore size] MCE membrane) followed by hot-block digestion in hydrochloric acid:nitric acid	ICP-AES (NIOSH Method 7303)	0.018 µg/mL (5000–50 000 µg/sample)	Partially validated multi-element method.	NIOSH (2003c)
Air (total antimony)	Collection on filter (MCE membrane, 37 mm diameter, 0.8 µm pore size) followed by microwave digestion in nitric acid	ICP-AES (NIOSH Method 7302)	0.4 µg/sample (2.5–750 µg/sample)	Validated multi-element method.	NIOSH (2014)
Air (total antimony)	Collection on internal capsule, cellulose-acetate dome with inlet opening, attached to 0.8 µm pore size MCE membrane filter and housed within a 2-piece, closed-face cassette filter holder, 37 mm diameter followed by hotplate digestion, microwave digestion, or hot-block extraction with nitric acid, nitric acid:perchloric acid, aqua regia, or nitric acid:hydrochloric acid	ICP-AES (NIOSH Method 7306)	0.11 µg/sample ([0.04–10 000 µg/m^3])	Validated multi-element method.	NIOSH (2015)
Air (total antimony)	Collection on 0.5 µm PVC filter, then treated with a mixture of 5 mL of 37% hydrochloric acid and 1 mL of 70% nitric acid, followed by ultrasonic shock for 30 min, and filtered using a Millipore filter membrane with a 0.22 µm pore size	ICP-MS	4.4 µg/sample	Spike recovery only 84.5%. NIOSH (2017) notes "antimony [does] not form stable solutions in nitric acid when chloride from the PVC filters is present".	Wu & Chen (2017)
Airborne particles (total antimony)	MCE filters (0.8 µm pore size); filters digested with nitric acid, sulfuric acid, and hydrogen peroxide; sampling via wipes and bulk methods also possible	ICP-AES (OSHA Method ID-125G)	4.2 µg/sample (0.28–100 µg/mL)	Validated multi-element method.	OSHA (2002)
Airborne particles (total antimony)	MCE filters (0.8 µm pore size); filters digested to give a final acid concentration of 4% nitric acid and 32% hydrochloric acid; sampling via wipes and bulk methods also possible	ICP-AES (OSHA Method ID-206)	3.5 µg/sample (0.47–100 µg/mL)	Validated multi-element method.	OSHA (1991)

Table 1.3 (continued)

Sample matrix (analyte)	Sample preparation (method)	Analytical technique (method)	LOD[b] (working range)	Comments	Reference
Biological media (inorganic and organic antimony species)	A variety of extraction methods noted including various combinations of: methanol/water, citric acid, sodium hydroxide/acetic acid, EDTA, TBAOH, acetonitrile, chloroform, cellulase, protease, aqua regia, and sulfuric acid	Separation by HPLC, GC, or HG-GC; detection by HPLC-ICP-AES, ES-MS, UV spectroscopy, NMR, HG-GC-ICP-MS, HG-CT-GC-ICP-MS, or HPLC-ES-MS; HPLC-ICP-MS; HPLC-ES-MS/MS		A critical review of published methods. Highlights challenges of achieving quantitative extraction, and of species instability and preservation, during sample processing and analysis.	Hansen and Pergantis (2008)
Biological media (antimony speciation)	Freeze-dried plant material was ground to powder, pressed into 5 mm diameter, 2–3 mm thick pellets under a nitrogen atmosphere	XANES (LODs not reported); quantitative speciation information obtained from plant samples with antimony species concentrations between ~1 and 1600 µg/g	NR	Method provided quantitative information about concentrations of trivalent and pentavalent antimony, and TMSb, in plant roots and shoots.	Ji et al. (2017)
Biological media (total antimony)	Low-temperature (85 °C) digestion with nitric acid followed by careful addition of 30% hydrogen peroxide solution; evaporated to dryness and reconstituted with 5% nitric acid	ICP-MS (USGS NWQL Method B-9001-95)	0.3 µg/L in digest (> 0.01 µg/g)	Multi-element method. Applicable to aquatic biological tissue and aquatic plant material only.	US EPA (1996)
Blood (total antimony)	Preserve 10 mL of sample with heparin; digest in 3:1:1 (v/v/v) nitric acid: perchloric acid: sulfuric acid	ICP-AES (NIOSH Method 8005)	1 µg/100 g blood (10–10 000 µg/100 g blood)	Validated multi-element method.	NIOSH (1994)
Blood (total antimony)	Sample (0.5 mL) microwave digested with 3 mL of 70% nitric acid	ICP-MS	0.06 µg/L	Recovery, 90.0%.	Wu & Chen (2017)
Hair (total antimony)	Sample (0.4 g) washed with 1:200 v/v Triton X-100, acetone and then deionized water. Oven-dried at 75 °C for 24 h and then microwave digested with 70% nitric acid and then diluted with 1% hydrochloric acid and an indium internal standard solution	ICP-MS	0.0004 µg/g	Recovery rate, 81.0%.	Wu & Chen (2017)

Table 1.3 (continued)

Sample matrix (analyte)	Sample preparation (method)	Analytical technique (method)	LOD[b] (working range)	Comments	Reference
Solids (including airborne particles) (antimony speciation)	Extraction with hydroxylammonium chlorhydrate aqueous solution assisted by ultrasound	HPLC-HG-AFS	In extracts: trivalent antimony, 0.9 µg/L; pentavalent antimony, 1.2 µg/L	Extraction efficiencies greater than 90% reported. Relative standard deviations better than 10% for a 6 µg/g certified reference material.	Bellido-Martín et al. (2009)
Solids (antimony speciation)	PET bottle section of size 0.5 cm × 0.5 cm × 0.2 mm used without further preparation	XANES (speciation information obtained from a PET sample with 0.3 µg/g antimony)		Method provided qualitative information about the nature, particularly oxidation state of antimony species in PET.	Martin et al. (2010)
Solids (antimony speciation)	Samples mounted in epoxy resin and polished	XANES with LCF, EXAFS		XANES/LCF used to quantitatively determine antimony speciation in glasses. EXAFS used to identify pentavalent antimony in the aluminium tetrahedral site of anorthite.	Miller et al. (2019)
Tissue (total antimony)	Sample 1 g of tissue; digestion in 3:1:1 (v/v/v) nitric acid:perchloric acid:sulfuric acid	ICP-AES (NIOSH Method 8005; working range; LOD)	0.2 µg/g tissue (2–2000 µg/g tissue)	Validated multi-element method.	NIOSH (1994)
Toenails (total antimony)	Samples washed, air-dried, and then digested in 9:1 nitric acid:hydrochloric acid and diluted with deionized water	ICP-QQQ		Multi-element method.	White et al. (2020)
Urine (total antimony)	Urine samples to be refrigerated for short-term (several days) storage or frozen at ≤ –20 °C (if to be stored for longer)	ICP-DRC-MS (CDC Method 3004.1)	0.04 µg/L (lower)	Multi-element method. Based on Mulligan et al. (1990).	CDC (2006, 2013)
Urine (total antimony)	Sample (10 mL) centrifuged and upper solution mixed with indium internal standard and 1% v/v hydrochloric acid and nitric acid; creatinine measured separately and antimony reported as µg/g creatinine	ICP-MS	0.03 µg/L	Recovery rate, 90.2%.	Wu & Chen (2017)
Water (inorganic antimony species)	Water samples diluted with water or hydrochloric acid	HPLC-HG-AFS	Trivalent antimony, 0.3 µg/L; pentavalent antimony, 0.4 µg/L ([500–5000 µg/L])	Addition of hydrochloric acid noted to result in oxidation of trivalent antimony to pentavalent antimony during storage.	González de las Torres et al. (2020)

Table 1.3 (continued)

Sample matrix (analyte)	Sample preparation (method)	Analytical technique (method)	LOD[b] (working range)	Comments	Reference
Water (inorganic antimony species)	Water samples centrifuged and diluted with 2% nitric acid	HPLC-ICP-OES	Trivalent antimony, 24.9–32.3 µg/L; pentavalent antimony, 36.2–46.0 µg/L (12.5–5000 µg/L for each species)	Recoveries of 90–105%.	Moreno-Andrade et al. (2020)
Water (inorganic antimony species)	Water samples filtered through 0.2 mm pore-size cellulose-acetate membrane, flash frozen, and separated by anion exchange chromatography	AEC-ICP-MS	NR (but species concentrations as low as 0.04 µg/L reported)	Reported quantification of all of trivalent and pentavalent antimony, trithioantimonate, and tetrathioantimonate.	Guo et al. (2020)
Water (inorganic antimony species)	FBA method	FBA-HG-AFS	6 ng/L (100–2000 ng/L)	Recovery rates, 90–114%. Throughput of 54 samples/h.	Lima et al. (2020)
Water (inorganic antimony)	Pre-treatment/concentration with SPE cartridges	HPLC-ICP-MS and FT-ICR MS		Used to identify a new antimony species, trimethylmonothioantimony.	Yin et al. (2022)
Water (total antimony)	Water (or digest of a solid material) diluted with hydrochloric acid and made up to 2% v/v nitric acid	AA (direct aspiration; EPA Standard Method 204.1)	200 µg/L (optimal, 1000–40 000 µg/L)		US EPA (1978a)
Water (total antimony)	Water (or digest of a solid material) diluted with hydrochloric acid and made up to 2% v/v nitric acid	AA (graphite furnace; EPA Standard Method 204.2)	3 µg/L (optimal, 20–300 µg/L)		US EPA (1978b)

AA, atomic absorption; AEC, anion exchange chromatography; AES, atomic emission spectrometry; AFS, atomic fluorescence spectroscopy; CDC, Centers for Disease Control and Prevention; CT, cryotrapping; DRC, dynamic reaction cell; EDTA, ethylenediaminetetraacetic acid; EPA, United States Environmental Protection Agency; EXAFS, extended X-ray absorption fine structure; FBA, flow-batch analysis; FT-ICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; HG, hydride generation; HPLC, high-performance liquid chromatography; HPLC-ES-MS, high-performance liquid chromatography with electrospray mass spectrometry; ICP-AES, inductively coupled argon plasma atomic emission spectroscopy; ICP, inductively coupled plasma; LCF, linear combination fitting; LOD, limit of detection; MCE, mixed cellulose ester; min, minute; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; NMR, nuclear magnetic resonance; NR, not reported; NWQL, National Water Quality Laboratory; OES, optical emission spectrophotometer; OSHA, Occupational Safety and Health Administration; PET, polyethlyene terephthalate; PVC, polyvinyl chloride; QQQ, (triple) quadrupole mass spectrometry; SPE, solid-phase extraction; TBAOH, tetrabutylammonium hydroxide; TMSb, trimethylantimony; USGS, United States Geological Survey; UV, ultraviolet; v, volume; XANES, X-ray absorption near edge structure.

[a] This is not a comprehensive nor a systematically selected list, but rather an indicative list of: (i) methods validated by NIOSH, the United States EPA, OSHA, or other relevant organizations; and (ii) methods illustrating the use and characteristics of different sampling, preparation, and instrumental analytical techniques.

[b] LODs are as reported and would be expected to vary depending upon instrument model, operation conditions, and calibration standard selection.

triple quadrupole mass spectrometry (Nouioui et al., 2018), and field-portable-X-ray fluorescence spectrometry has been demonstrated to be a very effective and rapid screening tool for solid plastic consumer products with antimony concentrations in the range of 100 to 60 000 µg/g (Turner & Filella, 2017). Time-of-flight secondary ion mass spectrometry has been shown to be of value in determining antimony concentrations for particle surface and bulk (Kappen et al., 2017), as well as having the potential to be applied to the analysis of single particles.

1.3.2 Speciation analysis of antimony in air, water, urine, soil, sediment, consumer products, and human tissues

Solid-phase antimony speciation may be determined in bulk samples by X-ray diffraction (Gržeta et al., 2002), when phases are sufficiently crystalline. Although more expensive, neutron powder diffraction, synchrotron-based X-ray diffraction, and X-ray absorption spectroscopy, including X-ray absorption near edge structure and extended X-ray absorption fine structure spectroscopy, collectively provide the means of accurately characterizing bond distances and coordination of antimony in solid phases at a molecular level, and of determining local- and long-range structures (Miller et al., 2019; Mayer et al., 2020). Micro-synchrotron-based X-ray fluorescence together with X-ray absorption near edge structure spectroscopy provides the means of mapping antimony speciation at the micrometre scale in, for example, PET bottles (Martin et al., 2010) and household dusts (Walden, 2010). The isotope ^{121}Sb is suitable for Mössbauer spectroscopy (Gržeta et al., 2002). A variety of extraction methods (e.g. the Bioaccessibility Research Group of Europe Unified Bioaccessibility Method) are used to measure operationally defined solid-phase antimony speciation, for example, to assess bioaccessibility in the gastrointestinal tract (Denys et al., 2012). Smichowski (2008) reviewed methods to determine antimony speciation in extracts of airborne particles. Reviews of methods to determine inorganic and organic speciation of antimony in biological media are available (e.g. see Hansen & Pergantis, 2008).

Aqueous-phase antimony speciation is routinely accomplished by hyphenated techniques involving liquid chromatographic separation of the chemical species followed by detection by atomic fluorescence spectrometry (González de las Torres et al., 2020), ICP-optical emission spectrometry (Moreno-Andrade et al., 2020), or ICP-MS (Guo et al., 2020). ICP-MS and ICP-optical emission spectrometry have typical limits of detection (LODs) of 0.5 and 50 µg/L, respectively, which may be comparable to or higher than concentrations observed in many waters. Sufficient sensitivity, together with challenges regarding comprehensively separating a wide range of antimony species and ensuring their stability during storage before analysis, are key limitations to these techniques (as summarized in Moreno-Andrade et al., 2020). Fang et al. (2020) reported high-resolution imaging of antimony(III) distribution in soil pore waters. Aqueous antimony speciation may be complex, so many methodologies focus on types of species – for example, antimony(III) or antimony(V) – or operationally defined classes of species (e.g. Chen et al., 2020). Independent verification of species otherwise determined by retention time is important (Guo et al., 2020).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence, including dietary and consumer products

Antimony is a naturally occurring element. Its abundance in the Earth's continental crust is estimated to be around 0.2 µg/g . Most antimony deposits are formed by hydrothermal activity. They are found in geological formations of a wide range of ages, although they tend to

be concentrated in the Palaeozoic and Cenozoic, and – particularly in China – predominantly in the Devonian (Grund et al., 2006; Dill et al., 2008; Seal et al., 2017).

Although antimony can exist in various oxidation states – including antimony(–III), antimony(0), antimony(III), antimony(IV), and antimony(V) – in environmental, biological, and geochemical samples, it exists mainly as antimony(III) and antimony(V). Antimony ores are dominated by antimony(III) minerals, the antimony(III) sulfide stibnite, and the antimony(III) oxide valentinite. These compounds of antimony are commonly found in ores of copper, silver, and lead. Oxidation of antimony ores may result in the formation of antimony(V)-bearing minerals such as natrojarosite ($NaFe_3(SO_4)_2(OH)_6$) or iron–antimony (oxy)hydroxides (Álvarez-Ayuso et al., 2022), or mixed antimony(III)–antimony(V) oxide minerals. Antimony – as either antimony(III) or, to a lesser extent, antimony(V) – is also a common minor or trace component of coal and petroleum (Filella et al., 2002). Antimony(V) readily substitutes for aluminium(III) in anorthite feldspar ($CaAl_2Si_2O_8$) (Miller et al., 2019), a very common mineral in the continental crust, meaning that even small concentrations of antimony(V) in anorthite could account for a substantial proportion of all antimony in the continental crust (Miller et al., 2019). Antimony in coal may be in either the antimony(III) or antimony(V) oxidation states, while (coal-derived) fly-ash leachates have similarly been found to contain antimony predominantly as either antimony(III) (Narukawa et al., 2005) or antimony(V) (Miravet et al., 2006). Miravet et al. (2007) measured predominantly antimony(III) in antimony leached from volcanic ash from the Copahue volcano, Argentina.

Releases of antimony, including both antimony(III) and antimony(V) compounds, into the environment occur as a result of natural processes, such as the weathering of rocks, soil runoff, wind-blown dust, volcanic eruptions, sea spray, forest fires, and from biogenic sources, as well as from anthropogenic sources, including mining and the processing of ores, industry (e.g. glass, dyestuff, ceramics, and fire retardants), coal combustion, metal production (e.g. copper), and refuse incineration (Pacyna, 1984; Mok & Wai, 1990; ATSDR, 1992; Slooff et al., 1992). Biological accumulation of antimony is low in algae and no antimony accumulation is produced during deep-water oceanic circulation. The few data available point to antimony accumulation near the soil surface, concentrations decreasing with increasing depth. There is little evidence of biomagnification of antimony in food-chains represented by the soil–vegetation–invertebrate–insectivore pathway of grasslands and little indication of significant accumulation by herbivorous mammals, despite marked contamination of their diets (Filella et al., 2002).

Nriagu (1989) estimated that natural sources of antimony emissions into the air and their median percentage contributions were as follows: wind-borne soil particles [32.5%], volcanoes [29.6%], sea spray [23.3%], forest fires [9.2%], and biogenic sources [12.1%]. However, anthropogenic activities have significantly affected the atmospheric flux of antimony over the past 2000 years (Shotyk et al., 1996; Barbante et al., 2004; Hong et al., 2009). Nriagu (1989) estimated that in 1983, anthropogenic sources contributed 59% of antimony emissions into the air, although this figure is questioned by Shotyk et al. (2005), who estimated that the ratio of anthropogenic to natural antimony emissions may be around 10 or more. Several studies have also indicated a positive correlation between locally increased concentrations of antimony in air, soil, and water and road traffic (European Commission, 2008), while the general population is exposed to low levels of antimony mainly in ambient air and food. Individuals can also be exposed to antimony(III) in PET water bottles or from consumer products containing antimony flame retardants, including textiles, toys, and aircraft

and automobile seat covers (ATSDR, 2019). Table 1.4 summarizes occurrences of antimony in environmental matrices, foodstuffs, drinking-water, and consumer products.

(a) Air

Releases of antimony to the atmosphere occur from natural and anthropogenic sources, which include coal combustion, smelting, and refining as major sources (Belzile et al., 2011). Global atmospheric antimony emissions from anthropogenic activities were estimated to be around 2232 tonnes in 2005 and then decreased gradually to about 1904 tonnes in 2010, with fuel combustion as the major source category. Asia and Europe accounted for about 57% and 24%, respectively, of the global total emissions, with China, the USA, and Japan ranked as the top three emitting countries (Tian et al., 2014). The United Kingdom (UK) Heavy Metals Monitoring Network reported that the mean air concentration of antimony in winter 2017–2018 was 1.84 ng/m^3 (Goddard et al., 2019). Belzile et al. (2011) reviewed published data on ambient air in Europe, North and South America, Asia, Oceania, Atlantic and Pacific Ocean islands, and the Arctic area. They found that air concentrations of antimony varied from a few picograms per cubic metre in remote areas to a few nanograms per cubic metre in urban areas and that values were much higher at contaminated sites (e.g. at a site close to a lead smelter, the average was 146 ng/m^3, with a range of 5.2–1210 ng/m^3).

Atmospheric particulate matter (PM) has also been found to be enriched with antimony. Pinto et al. (2015) reported that antimony is predominantly associated with PM with diameters less than approximately 2.5 μm ($PM_{2.5}$), indicating that antimony originates mainly from anthropogenic sources, such as industry and road traffic. Higher antimony levels in high-density traffic areas probably result from emissions from automobile engines and the abrasion of tyres, brake linings, and other automotive components that use antimony alloys (Belzile et al., 2011). Ramírez et al. (2020) have reported that the main sources contributing to antimony in PM smaller than approximately 10 μm (PM_{10}) are unidentified industry (62%), road dust (14%), fossil-fuel combustion and forest fires (12%), the iron and steel industry (7%), and traffic emissions (4%). A recent study reported that the annual average air PM_{10} mass concentration of antimony was 1.31 ng/m^3 at urban sites in Grenoble, France (Borlaza et al., 2021).

(b) Soil

Antimony occurs naturally as a trace element in soil, with its main source being the weathering of soil parent materials. Contamination of the soil leads to increased concentrations of antimony. Most of the antimony released into the environment is released to land (ATSDR, 2019). The soil concentration of antimony varies greatly because of variations in background concentrations, which reflect mineralization and parent material differences, and varying degrees of anthropogenic influence. Observed concentrations can be scale-dependent, and spatial distributions can be highly heterogeneous. Antimony contamination in soils occurs frequently on and around mining and smelting sites, often co-occurring with arsenic (Wilson et al., 2010).

On the basis of a national geochemical survey of soil in the USA, Smith et al. (2013) reported that the mean concentration of antimony was 0.84 mg/kg, while the range was from below the LOD (< 0.05 mg/kg) to 630 mg/kg. The average antimony concentration in the surface soils of the Bosten Lake Basin in central Asia was 1.05 mg/kg (Ma et al., 2019). However, in a region with geogenic antimony contamination (i.e. naturally occurring antimony) in Kutahya, Türkiye, the mean soil concentration of antimony was found to be 13.5 mg/kg (Guney et al., 2019).

Filella et al. (2002) reviewed published data on soils and found that antimony concentrations

Table 1.4 Occurrence of antimony in environmental matrices, foodstuffs, drinking-water, and consumer products

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Environmental samples in air, soil, water, and sediment							
Air, PM_{10} samples	United Kingdom, 25 sites, winter 2017/2018	123	1.84 (0.13–8.02) ng/m³	1.21 ng/m³	ICP-MS (0.002 ng/m³)	Monitoring sites around the United Kingdom Heavy Metals Monitoring Network; strong correlations were observed between Sb, Ba, and Cu concentrations	Goddard et al. (2019)
Air, PM_{10}, urban sites	Grenoble, France, 2017–2018	~390	Annual mean: 1.31 (25th, 0.33; 75th, 0.93) ng/m³		ICP-MS (NR)	Annual means: As, 0.33 ng/m³; Pb, 4.42 ng/m³; Cr, 1.65 ng/m³; Cd, 0.07 ng/m³; Cu, 8.5 ng/m³; Ni, 0.91 ng/m³	Borlaza et al. (2021)
Soil	USA, 2007–2010	4841	0.84 (< 0.05–630; SD, 9.1) mg/kg	0.57 (25th, 0.37; 75th, 0.80) mg/kg	ICP-MS (0.05 mg/kg)	National geochemical survey; means: Pb, 25.8 mg/kg; Cr, 36 mg/kg; Cd, 0.3 mg/kg; Cu, 17.9 mg/kg; Ni, 17.7 mg/kg; Sr, 159 mg/kg	Smith et al. (2013)
Soil	Bosten Lake Basin, central Asia, NR	48	1.05 (0.68–1.47; SD, 0.20) mg/kg		ICP-MS (0.05 mg/kg)	Means: As, 9.86 mg/kg; Pb, 17.1 mg/kg; Cr, 49.6 mg/kg; Cd, 0.15 mg/kg; Cu, 17.9 mg/kg; Ni, 23.1 mg/kg; Hg, 20.6 ng/g	Ma et al. (2019)
Soil	Kutahya, Türkiye, NR	53	13.5 (0.44–76.0) mg/kg		ICP-MS (NR)	Means: As, 182 mg/kg; Hg, 108 µg/kg	Guney et al. (2019)
Soil, around coal mines	Anhui, China, NR	33	4.0 (2.9–7.7) mg/kg		ICP-OES (0.0084 mg/kg)		Qi et al. (2011)
Soil, abandoned arsenic-containing mine	Hunan, China, 2019	190	6.5 (1.1–76.9; SD, 9.6) mg/kg	3.5 mg/kg	ICP-OES (NR)	Means: As, 394 mg/kg; Pb, 44.3 mg/kg; Sr, 232 mg/kg	Ran et al. (2021)
Soil, mining area	Zijiang River basin, Hunan, China, 2017	135	18.1 (3.8–251.0; SD, 32.7) mg/kg	9.7 mg/kg	ICP-OES (NR)	Means: As, 8.9 mg/kg; Cr, 90.3 mg/kg; Pb, 29.5 mg/kg	Zhang et al. (2019)
Soil, near mining areas	Central China, 2013	54	36.7 (9.6–111) mg/kg		AAS (NR)	Means: Cd, 0.472 mg/kg; Pb, 193 mg/kg; As, 89.0 mg/kg	Fan et al. (2017)
Soil, former mining sites	Sudetes, Poland, NR	6	(12.8–195) mg/kg		ICP-MS (NR)	As, 56–50 000 mg/kg	Lewińska et al. (2018)

Table 1.4 (continued)

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Soil, mining and smelting area	Hunan, China, NR	9	(100.6–5045) mg/kg		ICP-AES (0.63 µg/L)		He (2007)
Soil, mining area	Hunan, China, 2012	29	3061 (74.2–16 389; SD, 3715) mg/kg		ICP-MS (NR)	Means: As, 216 mg/kg; Cd, 1.93 mg/kg; Pb, 48.3 mg/kg; Cu, 23.6 mg/kg	Li et al. (2014)
Soil, shooting range	Alaska, USA, 2010–2015	12	(< LOD to 281.3) mg/kg		ICP-MS (5.9 mg/kg)	As, 12.9–21.0 mg/kg; Cr, 114–150 mg/kg; Pb, 19.5–16 410 mg/kg	Barker et al. (2019)
Soil, shooting range	Østre Toten, Norway, 2010	9	(40–123) mg/kg		ICP-MS (0.06 µg/L)	Pb, 356–1112 mg/kg; Cu, 41–88 mg/kg	Okkenhaug et al. (2016)
Soil, shooting ranges	Norway, 6 locations, NR	18	(< 0.001–830) mg/kg		ICP-MS (0.05 µg/L)	Pb, 20–13 000 mg/kg; Cu, 10–5200 mg/kg	Mariussen et al. (2017)
Soil, shooting ranges	Switzerland, 7 locations, NR	7	(500–13 800) mg/kg		ICP-MS (0.04 µg/L)		Johnson et al. (2005)
River water	Bytomka River, Poland, 2014–2015	60		0.91 (range, 0.36–1.78) µg/L	ICP-MS (0.05 µg/L)	Middle part of the river current below the water surface	Jabłońska-Czapla & Zerzucha (2019)
River-bottom sediments	Bytomka River, Poland, 2014–2015	60		33.6 (range, 5.9–98.1) mg/kg		Top 0–5 cm of river-bottom layer	
Surface water	Lowland dam reservoir, Poland, 2018	20		1.01 (range, 0.67–1.58) µg/L	ICP-MS (0.01 µg/L)	As: median, 1.96 µg/L	Jabłońska-Czapla & Grygoyć (2020)
Bottom sediments	Lowland dam reservoir, Poland, 2018	25		1.86 (range, 0.32–3.30) mg/kg		As: median, 33.4 mg/kg	
Surface water, mineralized areas	Alaska, USA, 2005–2007	2	(2.7–4.2) µg/L		ICP-MS (~5 µg/L)	Background (upstream) concentration	Ritchie et al. (2013)
Streambed sediments	Alaska, USA, 2005–2007	2	(91–968) mg/kg			Background (upstream) concentration	
Groundwater	Bangladesh, nationwide 1998–1999	112	(0.0015–1.8) µg/L		ICP-MS (0.0015 µg/L)	Water from tubewells; As, 0.0007–0.64 mg/L	Frisbie et al. (2002)

Table 1.4 (continued)

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Groundwater	England and Wales, nationwide (23 areas), NR	989	0.18 (< 0.05–3.43) µg/L	< 0.05 µg/L	ICP-MS (0.05 µg/L)	As: mean, 3.2 µg/L; median, < 1 µg/L	Shand et al. (2007)
Groundwater	Ibadan metropolis, Nigeria, 2016	210	13.5 ± 15.0 to 33.2 ± 36.8 µg/L		ICP-OES (NR)	Suggesting influence of geogenic factors; As, 2.17 ± 3.49 to 33.8 ± 37.2 µg/L	Etim (2017)
River water, mining areas	Nandan, China, NR	27	727 ± 820 µg/L		ICP-MS (NR)	River water of mine; As, 185 µg/L; Pb, 34 µg/L; Sr, 752 µg/L	Li et al. (2018)
River water further downstream from mining areas	Nandan, China, NR	6	192 ± 100 µg/L			River water (further downstream); As, 15 µg/L; Pb, 1 µg/L; Sr, 518 µg/L	
Sediments, mining areas	Nandan, China, NR	27	5942 ± 7337 mg/kg			Sediment in mining area; As, 11 577 mg/kg; Pb, 3406 mg/kg; Sr, 323 mg/kg	
Sediments, further downstream from mining areas	Nandan, China, NR	6	1696 ± 879 mg/kg			Sediment (further downstream); As, 5045 mg/kg; Pb, 2168 mg/kg; Sr, 229 mg/kg	
Discharge water, shooting ranges	Norway, 6 locations, NR	7	(0.34–65) µg/L		ICP-MS (0.05 µg/L)	Pb, 1.9–176 µg/L; Cu, 11–415 µg/L	Mariussen et al. (2017)
Subsurface soil water, shooting range		19	0.4 ± 0.33 to 150 ± 93 µg/L			Pb, 3.0–2500 µg/L; Cu, 1.3–900 µg/L	
Food and drinking-water							
Potable water	Greece, 19 locations, 2012	74	(< 0.01–2.12) µg/L		ICP-MS (0.01 µg/L)	Significant correlation: Sb/As (r = 0.61)	Andra et al. (2014)

Table 1.4 (continued)

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Water bottled in PET	Brands of bottled water from 28 countries, NR	132		0.33 (range, 0.001–2.57) µg/L	ICP-MS (NR)		Krachler & Shotyk (2009)
Water bottled in PET	Japan, NR	170	0.43 ± 0.52 µg/L		ICP-MS (0.2 µg/L)		Suzuki et al. (2000)
Water bottled in PET	Ontario, Canada, 2005	12	0.16 ± 0.09 µg/L		ICP-SF-MS (0.03 ng/L)	Natural water	Shotyk et al. (2006)
		3	0.16 ± 0.03 µg/L			Deionized water	
	Germany, 2005	6	0.36 ± 0.05 µg/L		ICP-SF-MS (0.03 ng/L)	Brand A	
		3	0.63 ± 0.02 µg/L				
		6	0.26 ± 0.02 µg/L			Brand B	
		6	0.30 ± 0.04 µg/L			Brand C	
	Brands of bottled water from 11 other European countries, 2005	35		0.343 µg/L	ICP-SF-MS (0.03 ng/L)		
Water bottled in PET	Türkiye, 2007	70	0.47 (0.29–1.23) µg/L	0.38 µg/L	ICP-MS (0.004 µg/L)	As: 1.71 (0.12–30.6) µg/L; median, 0.44 µg/L	Güler & Alpaslan (2009)
Water bottled in PET	Hungary, NR	37	0.26 ± 0.16 µg/L		ICP-SF-MS (0.7 ng/L)	Still mineral water	Keresztes et al. (2009)
		29	0.40 ± 0.22 µg/L			Sparkling mineral water	
Water bottled in PET	Monterrey, Mexico, NR	12	1.05 (0.28–2.30) µg/L		HG-AFS (0.113 µg/L)		Chapa-Martínez et al. (2016)
Water bottled in PET	Poland, NR	30	0.37 (0.08–1.15) µg/L	0.28 µg/L	ICP-MS (0.061 µg/L)	Flavoured bottled drinking-waters	Lorenc et al. (2020)
		12	0.39 (0.20–0.61) µg/L	0.43 µg/L		Functional bottled drinking-waters	
Water bottled in metals	Germany, NR	1	24.4 µg/L		ICP-MS (NR)	Pewter hip flask; consists of Cu–Sb alloy	Krachler & Shotyk (2009)
		1	5.0 µg/L			Stainless-steel flask	
Canned meat	Lublin, Poland, 2017	14	0.0268 (0.0028–0.0724) µg/g		ICP-MS (0.023 ng/g)		Kowalska et al. (2020)

Table 1.4 (continued)

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Canned fish		16	0.0377 (0.0014–0.0830) μg/g				
Various foods Milk and dairy products	Secondary school students, Hong Kong Special Administrative Region, China, 2000	100		< 0.001 (range, < 0.001–0.009) μg/g 0.001 (range, 0.0004–0.004) μg/g	ICP-MS (0.001 μg/g)	Cooked cereal and cereal products, vegetables, fruits, meat, poultry, eggs and their products, and seafood	Cheung Chung et al. (2008)
Rice grain	Zijiang River basin, including a mining area, China, 2017	135	0.027 ± 0.055 μg/g	0.007 (range, 0.002–0.408) μg/g	ICP-MS (0.001 μg/g)	Mean: Cu, 2.013 μg/g); Ni, 1.332 μg/g; Cr, 0.571 μg/g; Cd, 0.283 μg/g; As, 0.241 μg/g; Pb, 0.145 μg/g	Zhang et al. (2020)
Brown rice	Central China, near mining areas, 2013	54	5.175 (1.987–10.320) μg/g		AAS (NR)	Cd, 0.103 μg/g; Pb, 0.131 μg/g; As, 0.524 μg/g	Fan et al. (2017)
Wild fish	Gaotang Lake in the coal-mining area, China, NR	28	0.017 μg/g (range, 0.015–0.018 μg/g) in crucian carp to 0.040 μg/g (range, 0.018–0.083 μg/g) in common carp		AFS (0.5 ng/g)	Wet-weight basis; 6 fish species, including crucian carp, bighead carp, silver carp, tilapia, common carp, and grass carp; Cu, 2.47 μg/g; Cr, 1.60 μg/g; Pb, 0.122 μg/g; Co, 0.060 μg/g; As, 0.048 μg/g; Cd, 0.029 μg/g; Hg, 0.018 μg/g in common carp	Cheng et al. (2019)
Total diet study	Adults, Hong Kong Special Administrative Region, China, 2010–2011	600	≤ 0.004 μg/g		ICP-MS (0.001 μg/g)	Total diet study; 150 most commonly consumed food items were selected	Chen et al. (2014)
Total diet study	France, NR	998	≤ 0.0024 μg/g		ICP-MS (0.001 μg/g)	First total diet study; 300 individual food items	Leblanc et al. (2005)
Total diet study	France, 2007–2009	1319	≤ 0.0089 μg/g		ICP-MS (0.001 μg/g)	Second total diet study; 212 individual food items	Arnich et al. (2012)

Table 1.4 (continued)

Sample type	Location and collection date	No. of samples	Concentration of antimony		Analytical method (LOD)	Co-exposures and other relevant information	Reference
			Mean (range or percentile) or ± SD	Median (range or percentile)			
Consumer products							
Children's toys	Lebanon, NR	30	9.0 (< LOD to 159; SD, 31.2) µg/g		NR	Plastic toys	Korfali et al. (2013)
		23	2.1 (< LOD to 10 µg/g; SD, 6.2) µg/g			Modelling clays	
Cosmetics	Araraquara, Brazil, NR	3	12.7 ± 0.2 µg/g		AAS (0.3 µg/g)	Orange blush	Barros et al. (2016)
		3	14.5 ± 1.2 µg/g			Pink blush	
		3	9.1 ± 0.9 µg/g			Purple eye shadow	
Cosmetics	Spain and Germany, NR	12	(< 0.0013–75.6) µg/g		ICP-OES (0.0013 µg/g)	Kohl eyeliners; As, < 0.0008–12.6 µg/g; Pb, 1.73–410 807 µg/g; Cd, 0.0006–20.75 µg/g	Navarro-Tapia et al. (2021)
Cigarettes	France, NR	6	(1.2–1.5) ng/mL		ICP-MS (LOQ 0.1 ng/mL)	E-cigarette liquids	Beauval et al. (2017)
		96	(< 0.11–0.47) pg/mL puff		ICP-MS (LOQ 0.11 pg/mL puff)	Aerosols from 1 model of e-cigarette	

AAS, atomic absorption spectroscopy; AES, atomic emission spectroscopy; AFS, atomic fluorescence spectrometry; As, arsenic; Ba, barium; Cd, cadmium; Cr, chromium; Cu, copper; e-cigarette, electronic cigarette; Hg, mercury; HG, hydride generation; ICP, inductively coupled plasma; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; Ni, nickel; NR, not reported; OES, optical emission spectrometry; Pb, lead; PET, polyethylene terephthalate; PM_{10}, particulate matter, inhalable particles with diameters 10 µm and smaller; Sb, antimony; SD, standard deviation; SF-MS, sector field mass spectrometry; Sr, strontium.

ranged from 1.1 to 360 µg/g dry weight in areas around a smelter. Studies in and around mining areas have also reported mean soil concentrations of antimony ranging from 4.0 to 36.7 mg/kg (µg/g) (Qi et al., 2011; Fan et al., 2017; Zhang et al., 2019; Ran et al., 2021). Soil concentrations of antimony ranged from 12.8 to 195 mg/kg at former mining sites in the Sudetes in south-western Poland (Lewińska et al., 2018), and from 100.6 to 5045 mg/kg in a mining and smelting area in Hunan, China (He, 2007). Concentrations of antimony also averaged 3061 mg/kg in highly polluted soils of the mining area in Hunan, China (Li et al., 2014).

Soil contamination by antimony released from corroding ammunition or bullets also occurs, because antimony is used as a hardening agent in lead bullets (Ackermann et al., 2009; Hockmann et al., 2014; Melo et al., 2014). Several studies have reported that average soil concentrations of antimony in shooting ranges can be up to 13 800 mg/kg (Johnson et al., 2005; Okkenhaug et al., 2016; Mariussen et al., 2017; Barker et al., 2019).

(c) Water and sediments

The presence of antimony in ground- and surface water results primarily from rock weathering, soil runoff, and anthropogenic sources (Filella et al., 2002; Seal et al., 2017), including from mining and smelting, shooting ranges, and road-bound traffic dust from brake pads and tyres (ATSDR, 2019).

Filella et al. (2002) reviewed published data for fresh water, marine waters, and estuaries and found that typical concentrations of total dissolved antimony were usually < 1.0 µg/L in non-polluted waters, while the data for sediments revealed a range from < 0.05 µg/g dry weight in uncontaminated areas to 12 500 µg/g dry weight in areas around a smelter. Antimony is not considered to be a highly reactive element in oceans, with concentrations of the order of 200 ng/L (Filella et al., 2002). Studies on water and sediments at the bottom of bodies of water have also reported that median concentrations of antimony were 0.91 µg/L in water and 33.6 mg/kg in sediment of the Bytomka River, Poland (Jabłońska-Czapla & Zerzucha, 2019), and 1.01 µg/L in water and 1.86 mg/kg in sediment of the Kozłowa Góra dam reservoir, Poland (Jabłońska-Czapla & Grygoyć, 2020). Ritchie et al. (2013) reported that in Alaska, USA, the background (upstream) concentrations of antimony in Stampede Creek and Slate Creek surface waters and sediments from mineralized areas ranged between 2.7 and 4.2 µg/L and 91 and 968 mg/kg, respectively.

WHO reported that antimony concentrations in groundwater, surface water, and drinking-water were typically < 0.001, < 0.2, and < 5 µg/L, respectively (WHO, 2017). Frisbie et al. (2002) similarly found that the concentrations of antimony in groundwaters ranged from 0.0015 to 1.8 µg/L in Bangladesh. Shand et al. (2007) also reported antimony concentrations in the ranging from 0.1 to 1.0 µg/L in the majority of groundwaters in the UK.

However, antimony concentrations of > 100 µg/L in surface waters have been found near mining operations in various countries (Bolan et al., 2022). Etim (2017) reported mean concentrations of antimony in groundwater ranging between 13.5 and 33.2 µg/L in the shallow groundwater system of Ibadan metropolis, south-western Nigeria, suggesting an influence of geogenic factors. In China, the mean concentration of dissolved antimony in mining areas was 727 µg/L in river waters and 5942 mg/kg in sediments. The highest dissolved antimony concentration (5475 µg/L) in river water was found at the outfall of antimony ore flotation drainage (Li et al., 2018). The concentration ranges of antimony in discharge water and subsurface soil water were 0.34–65 and 0.4–150 µg/L, respectively, at six shooting ranges located in Norway (Mariussen

et al., 2017). Conventional water treatment processes do not remove antimony (WHO, 2017).

(d) Food and drinking-water

Belzile et al. (2011) reviewed published data on drinking-water and found that antimony concentrations in drinking-water were < 1.0 µg/L, except for some bottled waters that contained antimony at higher concentrations under extended storage conditions. Of 19 geographical locations in Greece, antimony concentrations in potable water systems were < 1.0 µg/L at 17 locations, 1.27 µg/L in Oreokastro, and 2.12 µg/L in Filippoi, Kavala (Andra et al., 2014).

Antimony(III) oxide is widely used as a building-block catalyst in the production of PET, with the resin containing residual antimony ranging from 200 to 300 mg/kg (Carneado et al., 2015). Previous studies have reported a wide range of antimony concentrations in water packaged in plastic PET bottles (0.001–2.6 µg/L; Suzuki et al., 2000; Shotyk et al., 2006; Güler & Alpaslan, 2009; Keresztes et al., 2009; Krachler & Shotyk, 2009; Chapa-Martínez et al., 2016; Lorenc et al., 2020), and in stainless steel and pewter flasks (5.0 and 24.4 µg/L, respectively) (Krachler & Shotyk, 2009). Some studies have also identified leaching of antimony from plastic PET bottles under certain environmental conditions (high temperatures, sunlight, and increased storage time outdoors), with concentrations in bottled water reaching as high as 2.6–14.4 µg/L, depending on conditions (Westerhoff et al., 2008; Cheng et al., 2010; Hureiki & Mouneimne, 2012; Fan et al., 2014; Al-Otoum et al., 2017).

In terms of food, Belzile et al. (2011) reviewed published data and found that concentrations in food are generally well below 1.0 µg/g on a dry-weight basis, suggesting that there should be little concern in terms of antimony uptake from food. Studies have also reported that the mean concentrations of antimony in canned meat and fish sold at a hypermarket in Lublin, Poland, were 0.027 and 0.038 µg/g, respectively (Kowalska et al., 2020). Median concentrations of antimony in food samples analysed did not exceed the LODs (0.001 µg/g for solid food and 0.1 µg/L for liquid food) in a diet study of secondary-school students conducted in Hong Kong Special Administrative Region, China (Cheung Chung et al., 2008). Mean concentrations of antimony in food samples analysed did not exceed 0.004 µg/g in the first Hong Kong Total Diet Study (TDS) (Chen et al., 2014), 0.0024 µg/g in the first French TDS (Leblanc et al., 2005), and 0.0089 µg/g in the second French TDS (Arnich et al., 2012). In rice grain gathered at the Zijiang River basin (including a mining and smelting area), China, mean concentrations of antimony were found to be 0.027 µg/g (range, 0.002–0.408 µg/g; Zhang et al., 2020). One study reported a high concentration of 5.175 µg/g (range, 1.987–10.320 µg/g) in brown rice gathered near mining areas in central China (Fan et al., 2017). In wild fish caught in Gaotang Lake, China, which is in a coal-mining area, mean concentrations of antimony ranged between 0.017 µg/g (range, 0.012–0.024 µg/g) and 0.040 µg/g (range, 0.018–0.083 µg/g) on a wet-weight basis (Cheng et al., 2019).

(e) Consumer products

Antimony(III) oxide is used as a plastic catalyst and in flame retardants, commonly in combination with brominated compounds, such as decabromodiphenyl oxide. As a flame retardant, it is used in plastics, paints, textiles, adhesives, and rubbers (Jayjock et al., 2015). As a fastener, opacifier, or a fining agent, antimony is also found in paints and enamels for ceramics and glassware, and in paint pigments in plastic materials (Turner & Filella, 2020). Antimony(III) oxide is found in several consumer products in which it is used for non-flame-retardant purposes, including children's toys, children's jewellery, and several PET product types, such as food trays and water bottles (Jayjock et al., 2015). Antimony is found also in cosmetics (Barros et al., 2016; Navarro-Tapia et al., 2021).

Jayjock et al. (2015) reviewed published data on consumer products, showing that concentrations of antimony varied from a few micrograms per gram in carpets or bedding to 30% (300 000 µg/g) in rubber and other elastomers. It was also reported by Butterman & Carlin (2004) that rubber and other elastomers are flame-retarded with halogen compounds and between 5% to as much as 30% antimony(III) oxide. However, the concentration of antimony in polyvinyl chloride straps, which infants might swallow, was found to be between 0.389 and 0.722 µg/g. Other studies have reported that the mean concentration of antimony was 9.0 µg/g in plastic toys and 2.1 µg/g in modelling clays for children (Korfali et al., 2013). In cosmetics, the concentration of antimony was found to range from < 0.0013 to 75.4 µg/g (Barros et al., 2016; Navarro-Tapia et al., 2021).

Antimony was found in liquids used in electronic cigarettes (range, 1.2–1.5 ng/mL) and in the aerosols that they produce (range, < 0.11–0.47 pg/mL puff); however, it was not found in smoke emitted by conventional cigarettes (Beauval et al., 2017).

1.4.2 Occupational exposure

Inhalation of airborne dust is considered to be the most significant route of occupational exposure to antimony. However, oral exposure through hand-to-mouth contact and direct dermal contact with antimony dust (e.g. direct handling, either by contamination of skin surfaces or by dermal deposition of airborne dust) are also concerns (ATSDR, 1992, 2019; European Commission, 2008; Saerens et al., 2019). Occupational exposure to antimony appears to be highest for workers involved in the production, formation, and processing of antimony and antimony(III) oxide. Workers in battery-formation areas in lead storage battery plants may also be exposed to high levels of antimony dust and stibine (see also Table 1.5, Table 1.6, Table 1.7, and Table 1.8).

Exposures may include a combination of several antimony compounds. In the atmosphere, the predominant forms of antimony are considered to be antimony(III) oxide and other oxides (to a lesser extent). In contrast, when dissolved in aqueous media, antimony is largely in the pentavalent-positive (+V) oxidation state. In addition, interconversion is possible in the environment as well as in vivo. Thus, studies on antimony exposure in workers have focused on antimony in general and not on specific valence states or species of antimony (Saerens et al., 2019).

The EU has reported on occupational exposure concentrations estimated on the basis of typical and worst-case inhalation, via personal air sampling and dermal-exposure scenarios in common occupational settings with antimony(III) oxide exposure, using data from measurements and modelling by RISKOFDERM (risk assessment of occupational dermal exposure to chemicals) (European Commission, 2008; Table 1.5). Only 1 of the 12 dermal-exposure scenarios (exposure in processing as a flame retardant in textiles) was modelled using RISKOFDERM. For dermal exposure in production of antimony(III) oxide, there was a sufficient number of measurements. For the other dermal-exposure scenarios, analogous/surrogate data were used. The highest inhalation exposure was estimated to occur during the production of antimony(III) oxide, particularly during the conversion process, followed by the final handling process in cases in which workers did not use respiratory protective equipment. In cases in which the workers used respiratory protective equipment, the highest exposure level was found to occur during raw material handling of flame retardants, in the production of plastics, and in the formulation stage for flame retardants in textiles. The highest dermal exposure was estimated to occur during the processing of flame retardants in textiles, followed by final

Table 1.5 Occupational exposure to antimony(III) oxide via inhalation and dermal exposure based on measured and modelled data in occupational settings in several European countries

Occupational setting	Process	Inhalation exposure (mg/m³)				Dermal exposure (µg/cm² per day)	
		Median (typical)		90th percentile (RWC)		Median	90th percentile
		With RPE[a]	Without RPE	With RPE[a]	Without RPE		
Production of antimony(III) oxide	Conversion[b]	0.027	0.54	0.15	2.9	5.2	16
	Refuming[c]	0.012	0.23	0.047	0.94	12	22
	Final handling[d]	0.040	0.79	0.110	2.1	18	31
Use as catalyst in production of PET	Powder handling	0.002	NA	0.026	NA	3.4[e]	5.8[e]
Use as flame retardant in production of plastics	Raw-material handling	0.13	NA	0.57	NA	6.8[e]	12[e]
Use as flame retardant in textiles	Formulation	0.13[f]	NA	0.57[f]	NA	4.5[g]	7.8[g]
	Processing	< 0.001	NA	0.001	NA	11[h]	72[h]
	Further handling	Negligible	NA	Negligible	NA	0.08	0.35
Use in pigments, paints, coatings, and ceramics	Loading and mixing[i]	0.036[f]	NA	0.16[f]	NA	2.3[g]	3.9[g]
Use as flame retardant in production of rubber	Formulation	0.051[f]	NA	0.22[f]	NA	2.3[g]	3.9[g]
	Processing	0.064[j]	NA	0.14[j]	NA	1.8[a]	3.1[a]
Use in production of crystal glass	Cutting	0.003[k]	NA	0.015[k]	NA	3[l]	11[l]

NA, not applicable; PET, polyethylene terephthalate; RPE, respiratory protective equipment; RWC, realistic worst case.

[a] With the use of RPE, the assigned protection factor was 10 for a P2 mask and 20 for a P3 mask. Use of RPE was mandatory in all surveyed plants. The median exposure level was called "typical" and the 90th percentile exposure level "worst case" exposure.

[b] Refers to conversion of antimony metal to antimony oxides and covers work tasks like loading of furnace with antimony ingots, supervision of operating conditions, and routine inspections and adjustments.

[c] Refuming (if crude antimony metal feedstock is used) is done to adjust the chemical and physical properties of the product. This is achieved by feeding the material through a furnace which has a free flow of air into the transfer ducting.

[d] Final handling covers all kind of work tasks at places where the final product, antimony(III) oxide, is handled, like weighing, packaging etc.

[e] Surrogate data extrapolated from data measured during bag filling at a plant producing antimony(III) oxide.

[f] Contains analogous/surrogate data measured during raw-material handling in the plastics sector.

[g] Recalculated from dermal exposure values of antimony(III) oxide production.

[h] Exposure levels were estimated from the model RISKOFDERM.

[i] Loading and mixing covers emptying of big bags, mixing, etc.

[j] Extrapolated from exposure to antimony(III) oxide measured in rubber fumes and rubber-process dust in the British rubber industry.

[k] Analogous data from the lead Risk Assessment Report (LDAI, 2006) were used.

[l] Analogous data on lead exposure (Wheeler & Sams, 1999) for estimation of dermal exposure to antimony(III) oxide were used.

From European Commission (2008).

Table 1.6 Antimony concentrations in samples of air, blood, urine, and hair of metal-exposed workers and administrative staff, by type of industry, in Taiwan, China

Type of industry	No. of samples	Mean concentration of antimony ± standard deviation[a]			
		Air (mg/m³)	Blood (µg/L)	Urine (µg/g creatinine)	Hair (µg/g)
Glass-manufacturing plant					
Workers	55	0.14 ± 0.01	0.78 ± 0.21	5.60 ± 1.24	0.10 ± 0.01
Administrative staff	20	0.007 ± 0.001	0.60 ± 0.11	2.55 ± 0.71	0.06 ± 0.01
Antimony(III) oxide-manufacturing plant					
Workers	14	2.51 ± 0.57	3.88 ± 1.10	27.15 ± 6.00	5.66 ± 3.66
Administrative staff	9	0.04 ± 0.01	1.07 ± 0.87	2.09 ± 0.55	0.04 ± 0.004
Engineering plastic-manufacturing plant					
Workers	22	0.21 ± 0.06	2.17 ± 0.48	7.48 ± 1.30	0.32 ± 0.05
Administrative staff	13	0.004 ± 0.001	0.49 ± 0.05	1.86 ± 0.55	0.04 ± 0.004
Total					
Workers	91	0.52 ± 0.88	1.61 ± 1.25	9.28 ± 6.31	1.00 ± 2.35
Administrative staff	42	0.012 ± 0.015	0.602 ± 0.0140	2.26 ± 0.68	0.048 ± 0.041

[a] Measurement results were significantly different between workers and administrative staff ($P < 0.001$) for all plants and all sample types.
From Wu & Chen (2017).

Table 1.7 Measurement of antimony in human biological specimens from workers in various types of industries at different production stages

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or ± SD	Median (range)		
Smelting						
Non-ferrous smelter producing antimony(V) oxide and sodium antimoniate, Belgium, NR	Urinary Sb	26	12.3 ± 5.0 µg/g creatinine 11.3[a] ± 1.56 µg/g creatinine		Wet process; end-of-shift sampling.	Bailly et al. (1991)
	Urinary Sb	14	110 ± 76 µg/g creatinine 91[a] ± 1.90 µg/g creatinine		Dry process; end-of-shift sampling.	
Secondary smelting of Pb for birdshot production, Italy, NR	Urinary Sb	18	5.9 (0.1–19.8) µg/L	2.8 µg/L	14 production workers, 2 technical employees, and 2 warehouse workers. End-of-shift sampling.	Lovreglio et al. (2018)
Production of antimony compounds						
Production of Sb_2O_3, Newcastle, United Kingdom, NR	Faeces	6	[2215] (1290–3510) ppm [µg/g] in dry faeces		Packaging department.	Oliver (1933)
Production of Sb_2O_3, Republic of Korea, 1995	Urinary Sb	11	182.7 ± 40.2 µg/g creatinine		Oxidation process; end-of-shift sampling.	Kim et al. (1997)
	Urinary Sb	10	137.1 ± 54.6 µg/g creatinine		Packing process; end-of-shift sampling.	
Production of Sb_2O_3, Republic of Korea, NR	Urinary Sb	12	410.8[a] ± 4.1 µg/g creatinine		Workers directly exposed to Sb_2O_3 through manufacturing processes.	Kim et al. (1999)
	Urinary Sb	22	112.5[a] ± 2.2 µg/g creatinine [b]		Workers in the same factory producing Sb_2O_3 as a major product, but not near the sources of Sb.	
Glass manufacturing						
Glass manufacturing containing Sb_2O_3, Germany, NR	Blood Sb	109		1.0 (0.4–3.1) µg/L	End-of-shift sampling; Pb, 250 (70–680) µg/L.	Lüdersdorf et al. (1987)
	Urinary Sb	109		1.9 (0.2–15.7) µg/L	End-of-shift sampling; Pb, 38 (7–110) µg/L.	
Textile industry						
Industrial plant producing fireproof textiles containing Sb_2O_3, Italy, NR	Urinary Sb	24	0.46 (0.16–1.77) µg/L		Finishing and intermediate inspection operators; end-of-shift sampling.	Iavicoli et al. (2002)
	Urinary Sb	15	0.18 (0.10–0.29) µg/L		Jet operators; end-of-shift sampling.	

Table 1.7 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or ± SD	Median (range)		
Polymerization process of polyester fibre containing Sb_2O_3, Egypt, NR	Urinary Sb	22	13 (10–19) µg/L			El Shanawany et al. (2017)
Battery industry						
Production of lead batteries containing Sb_2O_3 and SbH_3, Germany, NR	Blood Sb	7		2.6 (0.5–3.4) µg/L	Grid-casting area, exposure to Sb_2O_3.	Kentner et al. (1995)
	Urinary Sb	7		3.9 (2.8–5.6) µg/g creatinine	Grid-casting area; end-of-shift sampling.	
	Blood Sb	14		10.1 (0.5–17.9) µg/L	Formation area, exposure to Sb_2O_3 and SbH_3.	
	Urinary Sb	14		15.2 (3.5–23.4) µg/g creatinine	Formation area; end-of-shift sampling.	
LBRWs and ERWs, Ghana, NR	Urinary Sb	64	0.75[a] (0.05–17) µg/g creatinine		First morning void sampling; LBRW; As, 75 µg/L[a]; Ni, 3.1 µg/L[a]; Pb, 1.8 µg/L[a]; Se, 24 µg/L[a]; Cr, 0.25 µg/g creatinine.[a]	Dartey et al. (2017)
	Urinary Sb	64	0.16[a] (0.03–7.6) µg/g creatinine		First morning void sampling; ERW; As, 101 µg/L[a]; Ni, 2.9 µg/L[a]; Pb, 1.1 µg/L[a]; Se, 32 µg/L[a]; Cr, 0.23 µg/g creatinine.[a]	
E-waste recycling industry						
Recycling workers, Sweden, 2007–2009	Blood Sb	50		2.2 (1.8–3.8) µg/L	Ni, 0.99 µg/L; Mn, 11 µg/L.	Julander et al. (2014)
	Urinary Sb	53		0.18 (0.054–0.9) µg/L	First morning void sampling; Ni, 1.8 µg/L; Mn, 11 µg/L.	
Recycling workers, Germany, 2017–2018	Urinary Sb	49	0.26[a] (0.15–2.4) µg/L	0.15 µg/L	End-of-shift sampling[a]; As, 1.96 µg/L; Cd, 0.16 µg/L; Cr, 0.10 µg/L; Co, 0.32 µg/L; Ni, 0.74 µg/L; Hg, 0.38 µg/L.	Gerding et al. (2021)
Firefighting						
Firefighters, Florida, USA, 2009	Urinary Sb	20	0.063 µg/g creatinine[a]	0.059 (0.027–0.285) µg/g creatinine	Department A, firefighters did not wear trousers made from Sb-containing fabric.	CDC (2009); de Perio et al. (2010)
	Urinary Sb	41	0.054 µg/g creatinine[a]	0.048 (0.017–0.366) µg/g creatinine	Department B, firefighters did wear trousers made from Sb-containing fabric.	

Table 1.7 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or ± SD	Median (range)		
Firefighters, New York, USA, 2001	Urinary Sb	318	0.203 µg/L[a]		All firefighters.	Edelman et al. (2003)
	Urinary Sb	95	0.381 µg/L[a]		Special operation command firefighters (i.e. rescue, squad, and marine units).	

As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; ERW, electronic repair worker; Hg, mercury; LBRW, lead battery repair worker; Mn, manganese; Ni, nickel; NR, not reported; Pb, lead; ppm, parts per million; Sb, antimony; SD, standard deviation; Se, selenium.

[a] Geometric mean (geometric SD).

[b] Mean urine Sb concentration was significantly different ($P < 0.01$) from the mean value for workers who were directly exposed to Sb_2O_3.

Table 1.8 Occupational exposure to antimony measured in the air in various types of industries at different production stages

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or mean ± SD μg/m³	Median (range) μg/m³		
Smelting						
Non-ferrous smelter producing Sb_2O_5 and sodium antimoniate, Belgium, NR	Personal air, Sb dusts	26	86 ± 78 68 ± 1.94[a]		Wet process (handling a solution or a wet paste containing Sb); one whole work shift sampling.	Bailly et al. (1991)
	Personal air, Sb dusts	14	927 ± 985 594 ± 2.62[a]		Dry process (grinding, sieving, and packaging dry Sb products); one whole work shift sampling.	
Production of antimony compounds						
Production of Sb_2O_3, USA, 1960	Stationary air, Sb dusts	28	138 000		Bagging area.	Cooper et al. (1968)
Production of Sb_2O_3, Republic of Korea, 1995	Personal air, total Sb dusts	11	(8.3–40.0)		Oxidation process; whole 8 h work shift sampling.	Kim et al. (1997)
	Personal air, respirable Sb dusts	11	(2.0–3.8)		Oxidation process; whole 8 h work shift sampling.	
	Stationary air, total Sb dusts	11	22.3 ± 4.4		Oxidation process; whole 8 h work shift sampling.	
	Stationary air, respirable Sb dusts	11	2.8 ± 0.4		Oxidation process; whole 8 h work shift sampling.	
	Personal air, total Sb dusts	10	(22.3–402.5)		Packing process; whole 8 h work shift sampling.	
	Personal air, respirable Sb dusts	10	(1.7–5.0)		Packing process; whole 8 h work shift sampling.	
	Stationary air, total Sb dusts	10	280.6 ± 64.2		Packing process; whole 8 h work shift sampling.	
	Stationary air, respirable Sb dusts	10	3.4 ± 0.6		Packing process; whole 8 h work shift sampling.	
Production of Sb_2O_3, Republic of Korea, NR	Personal air, Sb dusts	12	766 ± 1850[a]		Workers directly exposed to Sb_2O_3 through manufacturing processes; more than 4 h sampling.	Kim et al. (1999)
	Personal air, Sb dusts	22	NT		Workers in the same factory producing Sb_2O_3 as a major product, but not near the sources of Sb.	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or mean ± SD µg/m³	Median (range) µg/m³		
Glass manufacturing						
Glass manufacturing containing Sb_2O_3, Germany, NR	Personal air, Sb dusts	4	(< 50–840)		Batch bunker; 2 h sampling.	Lüdersdorf et al. (1987)
	Personal air, Sb dusts	4	< 50		Melting area; 2 h sampling.	
	Stationary air, Sb dusts	4	(40–290)		Batch bunker; 2 h sampling.	
	Stationary air, Sb dusts	4	(< 5–5)		Melting area; 2 h sampling.	
Textile industry						
Industrial plant producing fireproof textiles containing Sb_2O_3, Italy, NR	Personal air, Sb dusts	26	0.12 ± 0.11		Finishing and intermediate inspection operators; whole 8 h work shift sampling.	Cavallo et al. (2002)
	Personal air, Sb dusts	15	0.052 ± 0.038		Jet operators; whole 8 h work shift sampling.	
Industrial plant producing fireproof textiles containing Sb_2O_3, Italy, NR	Personal air, Sb dusts	24	0.11 ± 0.07		Finishing and intermediate inspection operators; whole 8 h work shift sampling during 5-day period.	Iavicoli et al. (2002)
	Personal air, Sb dusts	15	0.05 ± 0.04		Jet operators; whole 8 h work shift sampling during 5-day period.	
Battery industry						
Production of lead batteries containing Sb_2O_3 and SbH_3, Germany, NR	Personal air, Sb dusts	7		4.5 (1.2–6.6)	Grid-casting area, exposure to Sb_2O_3; over 3 h sampling.	Kentner et al. (1995)
	Personal air, Sb dusts and volatile SbH_3	14		12.4 (0.6–41.5)	Formation area, exposure to Sb_2O_3 and SbH_3; over 3 h sampling.	
E-waste recycling industry						
Recycling workers, Sweden, 2007–2009	Personal air, Sb dusts	77	0.21 ± 2.3[a] (0.0041–1.1)		Inhalable fraction according to EN 481; 10 h work shift, sampling during 5 h. Geometric means: Pb, 7.0 µg/m³; Ni, 0.49 µg/m³; Cr, 0.45 µg/m³; Cd, 0.18 µg/m³; Co, 0.07 µg/m³; As, 0.04 µg/m³; Hg, 0.01 µg/m³.	Julander et al. (2014)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Antimony concentration		Comments	Reference
			Mean (range) or mean ± SD μg/m³	Median (range) μg/m³		
Recycling workers, Germany, 2017–2018	Personal air, Sb dusts	40	0.091[b]	0.075 (0.051–0.34)	Inhalable fraction; during disassembly work. Geometric means: As, 0.033 μg/m³; Cd, 0.017 μg/m³; Cr, 0.20 μg/m³; Co, 0.041 μg/m³; Ni, 0.27 μg/m³; Hg, 0.47 μg/m³.	Gerding et al. (2021)
	Stationary air, Sb dusts	21	0.067[b]	0.062 (0.047–0.17)	Inhalable fraction; during disassembly work. Geometric means: As, 0.031 μg/m³; Cd, 0.009 μg/m³; Cr, 0.12 μg/m³; Co, 0.035 μg/m³; Ni, 0.11 μg/m³; Hg, 0.46 μg/m³.	
	Personal air, Sb dusts	4	0.076[b]	0.077 (0.064–0.088)	Respirable fraction; during disassembly work. Geometric means: As, 0.025 μg/m³; Cd, 0.008 μg/m³; Cr, 0.077 μg/m³; Co, 0.021 μg/m³; Ni, 0.076 μg/m³.	
	Stationary air, Sb dusts	12	0.064[b]	0.062 (0.047–0.14)	Respirable fraction; during disassembly work. Geometric means: As, 0.033 μg/m³; Cd, 0.007 μg/m³; Cr, 0.110 μg/m³; Co, 0.034 μg/m³; Ni, 0.08 μg/m³.	

As, arsenic; Cd, cadmium; Cr, chromium; Co, cobalt; e-waste, electronic and/or electrical waste; Hg, mercury; Ni, nickel; NR, not reported; NT, not tested; Pb, lead; Sb, antimony; SD, standard deviation.

[a] Geometric mean ± geometric SD.

[b] Geometric mean.

handling in the production of antimony(III) oxide (see Table 1.5) (European Commission, 2008).

The French National Institute for Research and Occupational Safety (INRS) provided data for air measurements of antimony in industrial sectors and job titles (INRS, 2022). By industrial sector, the manufacture of chemicals and chemical products showed the highest arithmetic mean (AM) value in the breathing zones of workers from personal air sampling (AM, 5.66 mg/m^3), followed by the manufacture of textiles (AM, 0.222 mg/m^3), the wholesale and retail trade (repair of motor vehicles and motorcycles) (AM, 0.145 mg/m^3), and the manufacture of rubber and plastic products (AM, 0.095 mg/m^3) (Table 1.9). By job title, plant and machine operators and assemblers had the highest arithmetic mean value (AM, 0.566 mg/m^3), followed by cleaners and helpers (AM, 0.235 mg/m^3) (Table 1.10).

In a cross-sectional study, Wu & Chen (2017) reported antimony exposure among employees in industries in Taiwan, China. In total, 91 workers and 42 office administrators (all men) from two glass-manufacturing plants, one antimony(III) oxide-manufacturing plant, and two engineering plastic-manufacturing plants were recruited. Table 1.6 shows antimony levels in samples of stationary air, blood, urine, and hair, by industry type for workers and administrative staff. The mean (± standard deviation, SD) antimony concentration in the air samples measured for the antimony(III) oxide-manufacturing plant was the highest (2.51 ± 0.57 mg/m^3), being approximately 18 times that for the glass-manufacturing plants (0.14 ± 0.01 mg/m^3) and 12 times that for the engineering plastic-manufacturing plants (0.21 ± 0.06 mg/m^3). The antimony concentrations in the blood, urine, and hair of workers at the antimony(III) oxide-manufacturing plant were also the highest. The antimony concentrations in the blood, urine, and hair of employees were significantly correlated with the concentrations in air samples, with coefficients of 0.713, 0.870, and 0.865 ($P < 0.01$), respectively. The measured antimony concentrations in air and in blood, urine, and hair samples were significantly lower among administrative staff than among workers ($P < 0.001$).

Several scenarios or situations regarding exposure to antimony and antimony compounds in occupational settings are summarized below. Detailed data relating to human tissues, air, and/or biological monitoring are shown in Table 1.7, Table 1.8, and Table 1.11. Occupational exposure may arise in various industrial sectors such as in smelting, the production of antimony compounds and of other metals, glass manufacture, textile production, battery manufacture, and electronic and/or electrical waste (e-waste) processing. Workers may be exposed to various antimony compounds in the workplace and may also have co-exposure to other agents, including arsenic, chromium, lead, cadmium, selenium, nickel, cobalt, and mercury (see Table 1.7, Table 1.8, and Table 1.11). Specifically, in the processing of mined materials, co-exposures may be expected to commonly include arsenic, lead, and silica, among other agents (see Table 1.4). The potential for co-exposure to known or suspected human carcinogens (IARC, 2022) has been reported in various occupational studies (see Table 1.12) [Exposure assessments involving detailed characterization of antimony speciation and co-occurrence of other agents, notably in airborne dusts, were limited.]

(a) Smelting

Smelter workers in northern Sweden were compared with a group of individuals from a nearby area who had no occupational exposure. The individuals studied consisted of a group of 76 deceased men who worked as copper-smelter workers at the Rönnskär smeltery and who died after April 1975. As controls, 25 age-matched men were selected from rural (Burträsk and Jörn) and urban (Stockholm) areas. In the lung tissue of exposed workers, median antimony

Table 1.9 Distribution of air concentrations of antimony dust in industrial sectors, 2000–2020, French National Institute for Research and Occupational Safety

Industrial sector	Antimony concentration in personal air (mg/m^3)				Antimony concentration in stationary air (mg/m^3)			
	N	AM	Median	IQR	N	AM	Median	IQR
Manufacture of textiles	10	0.222	0.012	< LOQ–0.252	8	0.008	NA	NA
Manufacture of chemicals and chemical products	26	5.66	1.37	0.356–6.22	10	0.814	0.677	0.568–1.09
Manufacture of rubber and plastics products	27	0.095	< LOQ	< LOQ–0.016	14	0.003	< LOQ	< LOQ to < LOQ
Manufacture of other non-metallic mineral products	14	0.009	< LOQ	< LOQ to < LOQ	4	NA	NA	NA
Manufacture of basic metals	23	0.007	< LOQ	< LOQ–0.007	60	0.003	< LOQ	< LOQ–0.004
Manufacture of fabricated metal products, except machinery and equipment	76	0.01	0.004	< LOQ–0.011	11	0.003	< LOQ	< LOQ–0.005
Manufacture of computer, electronic, and optical products	11	< LOQ	< LOQ	< LOQ to < LOQ	11	< LOQ	< LOQ	< LOQ to < LOQ
Manufacture of electrical equipment	10	0.013	0.008	< LOQ–0.017	26	0.012	< LOQ	< LOQ–0.014
Manufacture of machinery and equipment	20	0.005	0.001	< LOQ–0.008	18	0.002	< LOQ	< LOQ–0.001
Manufacture of other transport equipment	19	0.005	< LOQ	< LOQ–0.003	10	< LOQ	< LOQ	< LOQ to < LOQ
Water supply; sewerage, waste management, and remediation activities	79	0.008	< LOQ	< LOQ–0.005	62	0.004	< LOQ	< LOQ to < LOQ
Construction	10	0.005	< LOQ	< LOQ to < LOQ	NA	NA	NA	NA
Wholesale and retail trade: repair of motor vehicles and motorcycles	16	0.145	0.01	0.002–0.038	10	0.009	< LOQ	< LOQ–0.006
Financial and insurance activities	24	< LOQ	< LOQ	< LOQ to < LOQ	17	0.006	< LOQ	< LOQ to < LOQ
Administrative and support service activities	22	0.001	< LOQ	< LOQ to < LOQ	NA	NA	NA	NA
Human health and social work activities	35	0.004	< LOQ	< LOQ to < LOQ	NA	NA	NA	NA
Arts, entertainment, and recreation	9	0.029	NA	NA	1	NA	NA	NA

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements; NA, not applicable.
From INRS (2022).

Table 1.10 Distribution of air concentrations of antimony dust according to job title, 2000–2020, French National Institute for Research and Occupational Safety

Job title	Antimony concentration in personal air (mg/m³)				Antimony concentration in stationary air (mg/m³)			
	N	AM	Median	IQR	N	AM	Median	IQR
Environmental protection professionals	5	< LOQ	NA	NA	6	< LOQ	NA	NA
Technicians and associate professionals	29	0.004	< LOQ	< LOQ–0.006	9	0.007	NA	NA
Clerical support workers	8	0.01	NA	NA	NR	NR	NR	NR
Craft and related trades workers	166	0.012	<LOQ	<LOQ–0.005	59	0.013	< LOQ	< LOQ to < LOQ
Building and related trades workers, excluding electricians	13	0.005	< LOQ	< LOQ to < LOQ	NR	NR	NR	NR
Metal, machinery, and related trades workers	90	0.019	0.003	< LOQ–0.009	47	0.014	< LOQ	< LOQ–0.002
Handicraft and printing workers	16	0.011	< LOQ	< LOQ–0.018	12	< LOQ	< LOQ	< LOQ to < LOQ
Food-processing, wood-working, and garment and other craft and related trades workers	47	0.002	< LOQ	< LOQ to < LOQ	NR	NR	NR	NR
Plant and machine operators, and assemblers	85	0.566	0.007	< LOQ–0.047	56	0.067	< LOQ	< LOQ–0.01
Elementary occupations	64	0.028	< LOQ	< LOQ–0.004	30	0.003	< LOQ	< LOQ–0.001
Cleaners and helpers	7	0.235	NA	NA	NR	NR	NR	NR
Labourers in mining, construction, manufacturing, and transport	8	0.009	NA	NA	1	NA	NA	NA
Garbage collectors and other unskilled workers	49	0.002	< LOQ	< LOQ–0.001	29	0.002	< LOQ	< LOQ–0.001

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements; NA, not applicable; NR, not reported.
From INRS (2022).

Table 1.11 Measurement of antimony in human tissue samples from workers in the smelting and mining industries

Occupational group/job type, location, and time period	Sample type	No. of samples	Antimony concentration		Co-exposures (ng/g wet weight) and other relevant information	Reference
			Mean (range) (ng/g dry weight)	Median (ng/g wet weight)		
Smelting						
Copper smelter, Sweden, 1930–1982	Lung	76	NR	280	Death after April 1975: Cr, 410; Cd, 162; Pb, 140; As, 38; Se, 151; Co, 16	Gerhardsson et al. (1985)
Copper or lead smelter, Sweden, 1930–1980s	Lung	85	NR	260	Death after April 1975: Cr, 450; Cd, 166; Pb, 140; As, 35; Se, 152; Co, 17	Gerhardsson et al. (1988)
Production of antimony compounds and other metals						
Uranium miners, Germany, 1957–1992	Lung	6	NR (218–1180)	NR	Underground workers: As, 136–956; Cr, 2.7–41.2; Co, 157–525; Se, 410–1180	Wiethege et al. (1999)
	Lung	7	NR (40–340)	NR	Other workers: As, 17–95; Cr, 1.1–3.1; Co, 46–86; Se, 420–880	

As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; NR, not reported; Se, selenium.

Table 1.12 Occupational co-exposures with antimony in various processes

Process	Agent to which co-exposure was reported (IARC classification[a])							Study design	
	Arsenic (Group 1)	Cadmium (Group 1)	Chromium[b] (Group 1)	Nickel (Group 1)	Lead[c] (Group 2A)	Polonium-210[d] (Group 1)	Asbestos (Group 1)	Case–control studies	Cohort studies
Antimony smelting	✓				✓				Jones (1994); Schnorr et al. (1995)
Tin smelting	✓	✓			✓	✓			Binks et al. (2005); Jones et al. (2007)
Glass production	✓	✓	✓	✓	✓		✓	Wingren & Axelson (1987, 1993)	

[a] IARC (2022)
[b] Chromium(VI).
[c] Lead compounds, inorganic.
[d] Internally deposited α-particle-emitting radionuclides.

concentrations were approximately 9 and 15 times as high as those in the control group (280 ng/g wet weight compared with 32 ng/g wet weight in Burträsk and Jörn, and 19 ng/g wet weight in Stockholm), respectively (Gerhardsson et al., 1985). An additional study was conducted on 85 deceased smelter workers who worked at the same smeltery, which continued collection of tissues after the study of Gerhardsson et al. (1985), and a similar or the same mean concentration (260 or 280 ng/g wet weight) was found in lung tissue (Gerhardsson et al., 1988; Gerhardsson & Nordberg, 1993).

Another study of non-ferrous-smelter workers involved in the production of antimony(V) oxide and sodium antimonate reported that the mean (± SD) antimony concentration in the personal air of dry-process workers (927 ± 985 μg/m^3) (involved in grinding, sieving, and packaging dry antimony products) was approximately 11 times that of wet-process workers (86 ± 78 μg/m^3) (handling a solution or a wet paste containing antimony). The mean concentration of antimony in urine samples from dry-process workers was approximately 9 times that of wet-process workers. In addition, a significant correlation ($r = 0.86$, $P < 0.0001$) was found between personal air concentrations and increased antimony concentrations in urine (Bailly et al., 1991).

(b) Production of antimony compounds and other metals

In eastern Germany, antimony was found in the lung tissue of uranium miners exposed to ^{222}Rn and dust, even when they had stopped working more than 20 years before death. The antimony concentrations in individuals working underground (range, 218–1180 ng/g dry weight) were also higher than the reference values (range, 190–320 ng/g dry weight) (Wiethege et al., 1999).

Oliver (1933) investigated workers at an antimony-processing plant in Newcastle-upon-Tyne, UK. Six men were engaged in oxide manufacture, four of whom had done the job for 13 years but had been antimony smelters for many years before oxide production began. The two other men had worked with antimony for 2 and 3 years. Examination of faeces indicated that antimony intake had occurred, with concentrations of antimony ranging from 1290 to 3510 ppm [mg/kg] in dry faeces. The mean antimony concentration in four men employed for 13 years [2377.5 mg/kg] was higher than that of two men employed for 3 years or less [1890 mg/kg]. [The Working Group noted that this study was of limited informativeness because of its small sample size and when the assessment was made.]

At an antimony(III) oxide-manufacturing plant in Texas, USA, workers were exposed to crude ore and, primarily, antimony(III) oxide. The mean air concentration was 138 mg/m^3 in the area concerned with bagging operations and 0.08–75 mg/m^3 in other locations (Cooper et al., 1968).

At an antimony(III) oxide-manufacturing plant in Kyeonggi-do, Chungnam, Cheonnam, Republic of Korea, there were two workplaces where the processing of antimony(III) oxide occurred according to the work type (oxidation and packing). The workers in the oxidation area were exposed to antimony fumes during the process of antimony oxidation and exposed to antimony(III) oxide-product dusts while packing the powders into bags in the packing area. The packing area had local ventilation, but the oxidation area did not. The mean concentrations of antimony in personal air in the packing area (range, 22.3–402.5 μg/m^3) were higher than those in the oxidation area (range, 8.3–40.0 μg/m^3). The mean (± SD) stationary air concentration of total antimony dust in the packing area (280.6 ± 64.2 μg/m^3) was approximately 13 times as high as that in the oxidation area (22.3 ± 4.4 μg/m^3). However, the mean air concentrations of antimony in respirable dust and urine samples collected from workers between the packing and oxidation areas did not differ significantly (Kim et al., 1997).

Workers directly exposed to antimony(III) oxide at a manufacturing facility were compared with those at the same facility who did not work near sources of antimony. A second control group of volunteers without occupational exposure to antimony was also examined. The mean concentration of antimony in personal air for the exposed workers was 766 μg/m^3, and personal exposure concentrations for the control workers and volunteer controls were not measured. The geometric mean antimony concentrations in urine were 410.8, 112.5, and 27.8 μg/g creatinine for the exposed workers, control workers, and volunteer controls, respectively (Kim et al., 1999).

In workers at a factory where birdshot was produced by a secondary smelting of lead, the mean urinary antimony concentration was 5.9 μg/L in exposed workers. All measurements were higher than the LOD for exposed workers, whereas only 22% of measurements for the control group were higher than the LOD (Lovreglio et al., 2018).

(c) Glass manufacture

The production of glass involves the use of antimony. Exposure to antimony oxides occurs primarily in sectors of the glass industry where traditional, non-mechanized techniques are used, such as in the production of crystal and other art glasses (IARC, 1993). The stationary air concentration of antimony dust was 2 μg/m^3 or less in the glass-manufacturing department of a plant that produced hypodermic syringes from glass containing small amounts of antimony (Burroughs & Horan, 1981).

Lüdersdorf et al. (1987) reported that the mean stationary air concentrations of antimony at two glass-producing factories were between < 5 and 290 μg/m^3. The personal air concentrations of antimony that the workers were exposed to were between < 50 and 840 μg/m^3. The median antimony concentrations in blood and urine samples collected from exposed workers were 1.0 and 1.9 μg/L, respectively. Median antimony concentrations in blood and urine samples collected from unexposed controls were 0.6 and 0.4 μg/L, respectively. In analyses of antimony concentrations in blood samples collected from workers in four subgroups (i.e. melters, batch mixers, craftsmen, and glass washers), statistically significant differences were observed for the batch mixers (median, 1.1 μg/L) and glass washers (median, 1.1 μg/L) when compared with unexposed controls ($P < 0.05$), but not when compared with the melters (median, 0.8 μg/L) and craftsmen (median, 0.7 μg/L). In urine, higher values were found almost exclusively in samples from individuals working as batch mixers (median, 5.0 μg/L), and statistically significant differences ($P < 0.05$) were observed compared with the melters (median, 0.9 μg/L), craftsmen (median, 0.9 μg/L), and glass washers (median, 1.2 μg/L).

(d) Textile industry

Exposure to antimony has been reported in the textile industry, including among workers in an industrial plant producing fireproof textiles for car upholstery in Italy. Finishing operators unloaded fabric from the transportation trolleys and prepared it for finishing. Once per week, they prepared the antimony(III) oxide-based flame/fire-retardant suspension by mixing fireproofing and binding products. This mixture was then pumped automatically into a tank and used to impregnate the fabric. On the day of sampling, they were assisted by an intermediate inspection operator who checked the product after finishing with the flame retardant and sometimes sampled the textile. At the same time, jet operators were dyeing the raw textiles approximately 20 m away from the finishing plant. The concentrations of antimony in personal air and the urine samples collected from workers were significantly higher among the finishing and intermediate inspection operators than among the jet operators ($P < 0.05$) (Cavallo et al., 2002; Iavicoli et al., 2002), see Table 1.7 and Table 1.8. [Data from the study by

Iavicoli et al, (2002) pertinent to the absorption, distribution, metabolism, and excretion of antimony are detailed in Section 4.1.3(a)]. In Kafr El Dawwar, Beheira, Egypt, workers exposed to antimony(III) oxide while working on the polymerization of polyester in a polyester fibre plant were surveyed (antimony(III) oxide is used as a catalyst in polymerization). The mean concentrations of antimony in urine samples were 13 μg/L in the workers and less than the LOD (< 10 μg/L) in controls (El Shanawany et al., 2017).

(e) Battery industry

At a lead battery-production facility in Germany, workers in the grid-casting and formation areas were surveyed regarding the significance of exposure to antimony and antimony(III) oxide. Antimony(III) oxide dust arose during the casting of the plates during the skimmings of the melt. Stibine was generated while charging the battery plates at the formation area, particularly at the end of the charging process or upon overcharging. The workers in the formation area were exposed to both stibine and antimony(III) oxide, but the casters were exposed only to antimony(III) oxide. The median antimony exposure concentrations of workers in the formation area were approximately 3 to 4 times as high (personal air, 12.4 μg/m^3; blood, 10.1 μg/L; urine, 15.2 μg/g creatinine) as those of workers in the casting area (personal air, 4.5 μg/m^3; blood, 2.6 μg/L; urine, 3.9 μg/g). Median antimony concentrations in blood and urine samples from workers in both the formation and casting areas were considerably greater than the values for occupationally non-exposed individuals (blood, < 1 μg/L; urine, < 0.5 μg/L) (Kentner et al., 1995).

Antimony concentrations in urine samples from lead battery- and electronic-repair workers in Kumasi, Ghana, were measured. The job tasks of the lead battery-repair workers included the charging of lead batteries, the breaking of batteries to replace damaged batteries and the repair of lead plates, and replacement of the lead terminals of batteries by welding. The main job tasks of the electronic-repair workers were dismantling, soldering, and welding, and the reassembly of electronic equipment, including televisions, radios, video players, and computers. The geometric mean urinary antimony concentrations were approximately 5 times as high for the lead battery-repair workers (0.75 μg/g creatinine) as for the electronic-repair workers (0.16 μg/g creatinine) (Dartey et al., 2017).

(f) E-waste recycling industry

Julander et al. (2014) reported antimony exposure levels of recycling workers at three companies involved in e-waste recycling in Sweden. The workers recycled goods such as television sets and computers, electronic tools, toys, and small and large household appliances, and their main tasks were the dismantling, handling, inspection, and transportation of goods. The geometric means of personal air inhalable fraction antimony concentrations were 0.21 μg/m^3 for recycling workers and 0.0085 μg/m^3 for unexposed office workers. The median antimony concentrations in blood and urine samples collected from recycling workers were 2.2 μg/L and 0.18 μg/L, respectively, and 2.2 μg/L and 0.12 μg/L for unexposed office workers. The concentration in the personal air inhalable fraction correlated with concentrations of antimony in both blood ($r = 0.49$; $P = 0.019$) and urine ($r = 0.49$; $P = 0.017$) samples. No significant differences were detected between blood and urine samples from recycling workers and the unexposed office workers.

At the e-waste recycling workshops – where recycling workers mainly performed disassembly of cathode ray tubes and liquid crystal display screens, and sorting of small electronic devices such as consumer electronics – the geometric mean antimony concentrations in urine samples from recycling workers and unexposed controls were 0.26 and 0.36 μg/L, respectively. The difference between the two groups was not significant

(Gerding et al., 2021). [The Working Group noted the lack of occupational exposure data on antimony in e-waste recycling activities in low- and middle-income countries.]

(g) Firefighters

Antimony(III) oxides and antimony(V) oxides have been used as flame retardants in textiles, and uniforms made from fabric containing antimony are commonly worn by firefighters in the USA. NIOSH surveyed the exposure levels of firefighters to antimony and reported that the geometric mean urinary antimony concentrations for firefighters who did not wear trousers made from antimony-containing fabric (department A), firefighters who wore trousers made from antimony-containing fabric (department B), and the general population were 0.063, 0.054, and 0.126 µg/g creatinine, respectively. Means of the log-transformed antimony concentrations in urine samples from participants in departments A and B did not differ significantly; nevertheless, they were significantly lower than that of the general population ($P < 0.001$) (CDC, 2009; de Perio et al., 2010).

Elevated antimony concentrations were reported in urine samples collected from firefighters after they had attended the collapse of the World Trade Center in New York, USA, on 11 September 2001. The adjusted geometric mean (adjusted for covariates of age, race, creatinine, and log cotinine using analysis of covariance) was 0.203 µg/L for all 318 firefighters and 0.165 µg/L for 47 controls. A significant difference was observed between firefighters and controls ($P < 0.01$). By unit assignment, urinary antimony concentrations were 0.381 µg/L for 95 special operations command firefighters (i.e. rescue, squad, and marine units) and 0.169 µg/L for 195 other exposed firefighters (e.g. ladder and engine); the difference was statistically significant ($P < 0.01$) (Edelman et al., 2003).

1.4.3 Exposure of the general population, including biomonitoring levels

Exposure of the general population to a variety of forms of antimony may occur through multiple routes, given the natural occurrence of antimony in the environment, its emission from industrial plants, and its use in the manufacture of certain consumer products and medicines (ATSDR, 2019) (see Section 1.4.1 of the present monograph). Exposure to antimony may result from the ingestion of food and drinking-water (Belzile et al., 2011), the inhalation of PM containing antimony in ambient air (e.g. among those residing in close proximity to urban and industrial sources of antimony pollution) (Cao et al., 2016), contact with consumer products for which manufacture involves the use of antimony (e.g. polymers such as those used in food and drink packaging) (Belzile et al., 2011), and through the treatment of leishmaniasis (Miekeley et al., 2002).

Concerning the antimony species to which general populations are exposed, as discussed in Section 1.1.2 and Section 1.4.1 of the present monograph, antimony naturally occurs in multiple valence states, a variety of forms are used in industrial applications, and antimony is used for the treatment of leishmaniasis, which is particularly relevant to low- and middle-income countries with a high prevalence of the disease. [The Working Group noted that most of the general-population exposure data reviewed consisted of measurements of total antimony concentrations (e.g. in urine), which reflect exposure to any of the individual agents within the scope of this evaluation. For population-based studies, without information on the exposure source, it is difficult to attribute total antimony concentrations to a particular agent.]

Measurement of the total antimony concentration in urine is routinely used to assess recent exposures to different forms of antimony. Among the participants surveyed by the United States

National Health and Nutrition Examination Survey (NHANES) (CDC, 2021) in three later cycles – 2011–2012, 2013–2014, and 2015–2016 (> 2500 people/cycle) – median urinary antimony concentrations ranged from 0.041 (in 2013–2014) to 0.047 µg/L (in 2011–2012), a decrease compared with concentrations observed in the six earlier cycles conducted between 1999–2000 and 2009–2010, in which median concentrations ranged from 0.13 (in 1999–2000) to 0.05 µg/L (in 2009–2010). The 95th percentiles of urinary antimony concentrations for both sexes between 2011 and 2016 ranged from 0.188 to 0.201 µg/L. In the 2015–2016 NHANES cycle, median total urinary antimony concentrations were 0.053 and 0.042 µg/L for male and female participants, respectively, and 0.063, 0.060, and 0.044 µg/L in the age groups 6–11, 12–19, and ≥ 20 years, respectively (ATSDR, 2019). The 95th percentile of total antimony concentrations in spot urine samples collected from 5576 members of the Canadian general population (age, 3–79 years), surveyed from 2009 to 2011 as part of the Canadian Health Measures Survey, was 0.17 µg/L (Saravanabhavan et al., 2017). While there was a paucity of antimony biomonitoring data from general populations in low- and middle-income countries, the 95th percentile of total antimony concentrations in spot urine samples collected from 357 adults from western Kenya, recruited between 2016 and 2019, was 0.46 µg/L (Watts et al., 2021). The urinary antimony concentrations in all the population-based surveys described in this section were considerably lower than those reported in exposed workers (e.g. Table 1.6 and Table 1.7 in Section 1.4.2 of this monograph).

Other studies have quantified antimony concentrations in whole blood, plasma, and other tissues in the general population. The median total antimony concentration in plasma from 419 participants from a Spanish population was 2.23 µg/L, and a positive correlation was observed with age (Henríquez-Hernández et al., 2020). Hoet et al. (2021) reported an upper reference limit for total antimony in blood of < 0.08 µg/L, which was the method LOD for their study of a population in Belgium. Stojsavljević et al. (2021) did not detect (limit of quantification, 0.057 ng/g) antimony in a study of placental tissues taken after placental delivery in a survey of 105 healthy pregnant White women in Serbia.

Several population-based dietary surveys have provided estimates of total antimony intakes using the TDS method. These include Yaoundé, Cameroon (mean dietary exposure estimate, MDE, 0.014–0.034 µg/kg body weight (bw) per day, lower bound–upper bound) (Gimou et al., 2014), France (MDE, 0.03–0.04 µg/kg bw per day) (Arnich et al., 2012), Hong Kong Special Administrative Region, China (MDE, 0.016–0.039 µg/kg bw per day) (Chen et al., 2014), northern Italy (MDE, 0.050 µg/kg bw per day) (Filippini et al., 2020), and the UK (adults, MDE, 0.032–0.033 µg/kg bw per day; toddlers, age 1.5–4.5 years: 0.075–0.077 µg/kg bw per day; young people, age 4–18 years, 0.049–0.050 µg/kg bw per day; elderly people, 0.027 µg/kg bw per day) (Rose et al., 2010). Therefore, all the aforementioned population-based studies have reported mean dietary intakes well below the tolerable daily intake (TDI) of 6 µg/kg bw per day for drinking-water derived by WHO (WHO, 2003).

Elevated concentrations of antimony in biological specimens and estimated daily intakes have been reported in specific populations. These include patients being treated for leishmaniasis with antimony(V) (Miekeley et al., 2002), people living near mining areas (Wu et al., 2011; Ye et al., 2018; Guo et al., 2021a), cigarette smokers and e-cigarette users (Badea et al., 2018; Olmedo et al., 2021), and those exposed to second-hand smoke (Richter et al., 2009). For example, adults living in close proximity to an antimony mine in Xikuangshan, Hunan, China – the world's largest antimony mine – were estimated to have antimony intakes of 554 µg per day assuming a body weight of 60 kg [9.23 µg/kg bw per day],

which exceeds the WHO TDI of 6 µg/kg bw per day (Wu et al., 2011). Co-exposure to arsenic is also often present in such populations (Wu et al., 2011; Fan et al., 2017; Guo et al., 2021a). Although antimony is known to migrate from PET drinking bottles (Makris et al., 2013), the amount released does not appear likely to result in daily intakes that exceed the WHO TDI of 6 µg/kg bw per day. Estimates accounting for recommended daily water consumption were shown to be dependent on time (duration of storage) and temperature, ranging from 19 ng/kg bw per day [0.019 µg/kg bw per day] for a 72 kg adult after 1 month of storage at 6 °C to 259 ng/kg bw per day [0.26 µg/kg bw per day] for a 10 kg child after 12 months of storage at 40 °C (Zmit & Belhaneche-Bensemra, 2019).

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

The occupational exposure limit (Table 1.13) in the USA for antimony and its compounds (CAS No. 7440-36-0, measured as antimony) in air is 0.5 mg/m^3 as a time-weighted average (TWA) over an 8-hour (Occupational Safety and Health Administration, OSHA; American Association of Governmental Industrial Hygienists) or up to 10-hour (NIOSH) work shift (OSHA, 2021), broadly as recommended by NIOSH (1978) on the basis of a working lifetime of exposure with a 40-hour work week. Similar limits are prescribed for Canadian Jurisdictions (e.g. Ontario) (Ontario Ministry of Labour, Training and Skills Development, 2020), although "ALARA" (as low as reasonably achievable) is indicated for the production of antimony(III) oxide by the Ontario Ministry of Labour, Training and Skills Development (2020). Lower limits exist in Sweden (0.25 mg/m^3), Latvia, and Romania (0.20 mg/m^3), and Japan (0.1 mg/m^3) (International Antimony Association, 2021; IFA, 2021a), while a lower limit of 0.02 mg/m^3 is under consideration by NIOSH and OSHA in the USA (International Antimony Association, 2021). The German Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA 2021b, c) reports 8-hour occupational exposure limits separately for antimony(III) oxide (CAS No. 1309-64-4) and antimony(III) hydride (CAS No. 7803-52-3) for more than 20 jurisdictions. German Technical Rules for Hazardous Substances (TRGS 900) have indicated an occupational exposure limit of 0.006 mg/m^3 for respirable antimony in the form of antimony(III) oxide or antimony(III) sulfide (BAuA, 2018; International Antimony Association, 2021). OSHA (2021) also reports a value for antimony of 50 mg/m^3 for "immediately dangerous to life or health", a parameter "established (1) to ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment and (2) to indicate a maximum level above which only a highly reliable breathing apparatus, providing maximum worker protection, is permitted" (NIOSH, 2019).

(b) Air, water, soil, consumer products, and food

For non-occupational settings, selected regulatory and/or screening values for air, water, soil, food, pharmaceuticals, and toys are summarized in Table 1.14.

Ontario, Canada, set a 24-hour ambient air quality concentration target for antimony of 25 µg/m^3 (Government of Ontario, 1990).

WHO (2003) has published a guideline value of 20 µg/L for total antimony in drinking-water, and this remains the WHO guideline value in 2022. The United States Environmental Protection Agency (US EPA) has recommended a maximum contaminant level for antimony in drinking-water in the USA of 0.006 mg/L (i.e. 6 µg/L) (Office of the Federal Register, 2021; US EPA, 2021). Canada has adopted the same maximum admissible concentration (Health Canada, 2019).

Table 1.13 Occupational exposure limits for antimony and antimony compounds in various countries

Country	Antimony and its compounds (except stibine) CAS No. 7440-36-0 (as Sb)		Antimony(III) oxide CAS No. 1309-64-4 (as Sb)		Antimony(III) hydride (stibine) CAS No. 7803-52-3	
	Limit value		Limit value		Limit value	
	8 h (mg/m³)	Short-term (mg/m³)	8 h (mg/m³)	Short-term (mg/m³)	8 h (mg/m³)	Short-term (mg/m³)
Australia	0.5		0.5		0.51	
Austria	0.5[a]	1.5[a]	0.1[b]	0.4[b]	0.5	2.5
Belgium	0.5				0.52	
Canada (Ontario)	0.5		ALARA[c]	ALARA[c]		
Canada (Quebec)	0.5		0.5		0.51	
China	0.5					
Denmark	0.5	1.0			0.25	0.5
Finland	0.5		0.5			0.26[d]
France	0.5				0.5	
Germany (AGS)			0.006[e]	0.048[d]		
Hungary	0.5	2	0.1	0.4	0.5	2
Ireland	0.5					
Japan (JSOH)	0.1					
Japan (MHLW)			0.1			
Latvia	0.2	0.5[d]	1			
New Zealand	0.5		0.1		0.51	
Norway	0.5				0.25	
Poland	0.5				0.5	1.5[d]
Romania	0.2	0.5[d]			0.2	0.5[d]
Singapore	0.5		0.5		0.51	
Republic of Korea	0.5		0.5		0.5	
Spain	0.5				0.5	
Sweden	0.25[a]		0.25[f]		0.3	
Switzerland	0.5[a]		0.1[b]		0.5	0.5
The Netherlands	0.5				0.5	
USA (NIOSH)	0.5				0.5	
USA (OSHA)	0.5				0.5	
United Kingdom	0.5		0.5		(0.52)[g]	(1.6)[g]

AGS, Ausschuss für Gefahrstoffe [Committee on Hazardous Substances]; ALARA, As Low as Reasonably Achievable; CAS No., Chemical Abstracts Service Registry Number; JSOH, Japanese Society of Occupational Health; MHLW, Ministry of Health, Labour and Welfare; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration.

[a] Inhalable fraction.

[b] Inhalable aerosol.

[c] Exposure by all routes should be carefully controlled to levels as low as possible.

[d] 15-minute average value.

[e] Respirable fraction.

[f] Inhalable dust.

[g] The United Kingdom Advisory Committee on Toxic Substances has expressed concern that, for the OELs shown in parentheses, health may not be adequately protected because of doubts that the limit was soundly based.

From IFA (2021a, b, c).

Table 1.14 Environmental regulations and guidelines for antimony and antimony compounds

Regulation/guideline	Country or location	Value and units	Reference
Air			
24 h Local Air Quality Regulation Standard 24 h Ambient Air Quality Criteria target Annual Ambient Air Quality Criteria target	Canada, Ontario	25 µg/m^3 0.6 µg/m^3 0.12 µg/m^3	Government of Ontario (1990) CAREX Canada (2022)
Water			
WHO guidelines for drinking-water quality	World	20 µg/L	WHO (2003)
Guidelines for Canadian drinking-water quality	Canada, Ontario	0.006 mg/L	Health Canada (2019)
European Council directive on the quality of water intended for human consumption	European Union	5 µg/L	European Council (1998)
Standards for drinking-water quality, national standard of the People's Republic of China	China	5 µg/L	Standardization Administration of the People's Republic of China (2006)
Australian drinking-water guidelines 6, version 3.4, updated October 2017	Australia	3 µg/L	NHMRC, NRMMC (2017)
National standards for drinking-water quality	Pakistan	≤ 5 µg/L	Government of Pakistan (2008)
National primary drinking-water regulations	USA	6 µg/L	US EPA (2021)
Soil			
Soil screening values	United Kingdom	37 mg/kg dry weight (as Sb)	Martin et al. (2020)
Canadian soil quality guidelines	Canada	20 mg/kg (agricultural, parkland) 40 mg/kg (commercial, industrial)	Canadian Council of Ministers of the Environment (2007)
Food			
European Food Safety Authority Scientific Panel opinion	European Union	40 µg/kg of food (as Sb)	EFSA (2004)
Consumer products			
Permitted daily exposure as impurity in pharmaceuticals	USA	120 µg/g; 1200 µg/day (oral) 9 µg/g; 90 µg/day (parenteral) 2 µg/g; 20 µg/day (inhalation)	USP (2017)
Permitted concentration in toy materials	Sweden	45 mg/kg (dry, brittle, powdery, or flexible toy material) 11.3 mg/kg (liquid or sticky toy material)	KIFS (2017)

Sb, antimony.

In the EU (European Council, 1998) and China (Standardization Administration of the People's Republic of China, 2006), regulatory maximum concentrations of antimony in drinking-water (in the EU, water intended for human consumption is defined as "cover[ing] all water either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes, regardless of its origin and whether it is supplied from a distribution network, from a tanker, or in bottles or containers; and all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption") are 5 µg/L (European Commission, 2018), although it is recognized that this longstanding regulatory value is lower than the WHO (2003) guideline value. Australia (NHMRC, NRMMC, 2017) has an even lower guideline value of 3 µg/L. In contrast, many low- and middle-income countries have either adopted a default WHO (2003) or earlier guideline value in their regulations (e.g. Pakistan, ≤ 5 µg/L) (Government of Pakistan, 2008), or do not seem to have any explicit regulatory value for antimony in drinking-water (e.g. India and Bangladesh) (Bureau of Indian Standards, 2012; PHED, 2015). WHO (2018) noted that 76 out of 104 countries and territories considered have set a regulatory/guideline value, with 62 of them setting a value lower than the WHO guideline value, the median value (set by 50 countries) being 5 µg/L.

The UK Environment Agency reported a soil screening value for total antimony of 37 mg/kg (dry weight) based on soil ecotoxicity (Martin et al., 2020). The Canadian Council of Ministers of the Environment (2007) reported use-dependent soil quality guidelines for Canada of 20 mg/kg for agricultural and residential or parkland uses, and 40 mg/kg for commercial and industrial uses. Bolan et al. (2022) cited highly variable values for the bioavailability of antimony in soils depending on the nature and distribution of antimony phases, indicating the future utility of chemical species-dependent soil quality guidelines for antimony.

The European Food Safety Authority recommended a restriction of 0.04 mg/kg of antimony(III) oxide as antimony in food (EFSA, 2004), on the basis of a 10% allowance of a WHO (2003)-recommended TDI for antimony of 0.006 mg/kg bw per day. According to Norwegian Ministry of Health and Care Services (2021) regulations, food-contact plastic materials and articles must not release antimony in quantities giving rise to antimony concentrations in excess of 0.04 mg/kg food.

The United States Pharmacopeia (USP, 2017) indicated limits of 120, 9, and 2 µg/g for total antimony as a trace impurity in pharmaceuticals to be administered via oral, parenteral, and inhalation routes, respectively, on the basis of recommended permitted daily exposures of 1200, 90, and 20 µg per day for each of those routes, respectively (USP, 2017).

To limit migration of antimony from toys, the Swedish Chemicals Agency has set regulations on the upper permissible concentrations of antimony in toys of 45 mg/kg for dry, brittle, powdery, or flexible toy material, and 11.3 mg/kg for liquid or sticky toy material (KIFS, 2017).

Notwithstanding concerns over toxicity, antimony(III) oxide continues to be used as a flame retardant because of the poorer performance of potential substitutes, notably aluminium trihydrate and magnesium hydroxide (European Commission, 2020a).

1.5.2 Reference values for biological monitoring

A summary of selected reference values for human exposure biomonitoring is given in Table 1.15. Saravanabhavan et al. (2017) reported a reference value (RV95) for total antimony in urine of 0.17 µg/L (95% confidence interval, CI, 0.15–0.19 µg/L) (children and adults) on the

basis of the 95th percentile of observations made of a Canadian population. Equivalent reference values have also been reported for populations in Germany (children, 0.3 µg/L; Schulz et al., 2009; adults, 0.2 µg/L; Göen et al., 2020, Heitland & Köster, 2006), Italy (0.095 µg/L; Aprea et al., 2018), the UK (0.26 µg/L; Morton et al., 2014), Belgium (0.236 µg/L; Hoet et al., 2013), and France (0.32 µg/L; Fréry et al., 2011; 0.41 µg/L, Nisse et al., 2017). The International Labour Organization and WHO (ILO, WHO, 2006) indicated that pregnant women should not be exposed to antimony (i.e. the urinary antimony concentration should not exceed the non-exposed reference limit of 0.8 nmol/L [0.1 µg/L]).

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 Epidemiological studies of cancer in humans

The Working Group identified 16 key studies of cancer in humans for which a critical appraisal of exposure assessment methods was undertaken; these included 8 cohort studies and 8 case–control studies. Of these studies, two case–control studies investigated exposure to antimony(III) oxide, one prospective and one retrospective cohort study investigated antimony-containing ores, two retrospective cohort studies investigated antimony-containing feedstock from smelting, and the remaining studies either did not, or did not intend to, specify the specific agent (this last group being population-based – rather than occupationally based – investigations). The critiques undertaken for each study in relation to different aspects of exposure assessment, in addition to the identification of the specific agent under investigation, are tabulated in Table S1.16 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

(a) Exposure assessment methods

(i) Cohort studies

Several investigations were conducted on workers employed at a tin smelter in the UK (Binks et al., 2005; Jones et al., 2007), an antimony smelter in the UK (Jones, 1994), and an antimony smelter in south Texas, USA (Schnorr et al., 1995). The tin smelter in the UK, in operation from 1937 through 1991, processed tin ore concentrates and raw ore as feedstock to produce high-purity tin, lead, copper, cadmium, antimony, and silver; after 1967, the smelter underwent major upgrades, including the introduction of ventilation systems (Binks et al., 2005; Jones et al., 2007). At the antimony smelter in the UK, work practices and raw materials changed over time, including the sourcing of the ore, although most of the sulfide ore came from South Africa and contained about 60% antimony and up to 0.5% arsenic (Jones, 1994). At the antimony smelter in the USA, there was little change in process operations from 1930 until it closed in 1979; the primary source of ore was composed mainly of antimony oxide or antimony sulfide (Schnorr et al., 1995). Schnorr et al. (1995) presented summaries by operation of 8-hour area (n = 12) and personal sampling (n = 50) measurements for antimony and arsenic collected in 1975 and 1976, respectively. Overall, the surveys showed that personal exposures to antimony for workers involved in various operations at the smelter exceeded the OSHA permissible exposure limit (PEL) of 500 µg/m^3 for antimony; all measured exposures were below the OSHA standard for arsenic at the time (OSHA PEL, 500 µg/m^3). Across operations, personal exposures (n = 6) were highest for antimony (range, 90–3100 µg/m^3; geometric mean, 1498 µg/m^3) and arsenic (range, 8–37 µg/m^3; geometric mean,

Table 1.15 Biomonitoring guidance and reference values for antimony and antimony compounds

Regulatory/guideline or reference value	Country/ location	Value and units	Reference
Urine			
Occupational biomonitoring reference value (biological reference value, end-of-shift after several previous shifts)	Germany	0.2 µg/L	Göen et al. (2020)
Federal Environment Agency biomonitoring reference value for children	Germany	0.3 µg/L	Schulz et al. (2009)
Biomonitoring reference value RV95 for adults	Belgium	0.236 µg/L	Hoet et al. (2013)
	Italy	0.095 µg/L	Aprea et al. (2018)
	France	0.32 µg/L or 0.25 µg/g creatinine	Fréry et al. (2011)
	United Kingdom	0.26 µg/L	Morton et al. (2014)
Biomonitoring reference value RV95 for children and adults	Canada	0.17 µg/L	Saravanabhavan et al. (2017)
	USA	0.18 µg/g creatinine (survey 2015–2016)	CDC (2021)
Biomonitoring reference value for men, 75th percentile	Wuhan, China	0.19 µg/L (spot)	Wang et al. (2019a)
Blood			
Reference values for adults (non-fasting sample)	Belgium	< 0.08 µg/L[a]	Hoet et al. (2021)
Serum			
Biomonitoring reference value RV95 for urban and rural men and women	Andalusia, Spain	2.29–2.4 µg/L	Henríquez-Hernández et al. (2020)

RV95, 95th percentile of the substance of interest at a specific time point rounded off within its 95% CI.

[a] Reference limit is below the limit of detection.

19 μg/m³) among workers operating the oxide furnace. [The OSHA PEL for arsenic is currently 10 μg/m³.]

These prospective and retrospective cohort studies applied standard methods in comparing all-cause or cause-specific mortality rates of male worker populations with national and regional population rates (Jones, 1994; Schnorr et al., 1995; Binks et al., 2005). Cohorts were restricted to include workers employed for at least 3 (Jones, 1994; Schnorr et al., 1995) or 12 months (Binks et al., 2005). Jones (1994) also categorized the cohort into four occupational groups (antimony workers, maintenance workers, zircon workers, and others) and computed standardized mortality ratios (SMRs) for each group, stratified by initial employment before and after 1 January 1961. In addition, for lung cancer mortality, Binks et al. (2005) and Jones (1994) performed subcohort analyses using quantitative (i.e. time-related) variables (e.g. years of employment, years since entering employment, and date of first employment). In a follow-up study of lung cancer mortality among workers assessed previously by Binks et al. (2005), an exposure assessment was conducted that relied on over 20 000 occupational hygiene measurements from area and personal sampling conducted between 1972 and 1991 (with lead most often measured) to estimate annual average levels of antimony and other contaminants in seven process areas of the smelter (Jones et al., 2007). For earlier periods dating back to 1937, three extrapolation methods were applied by: applying the mean of the three earliest years for which data were available, applying a linear increasing trend from baseline values to values higher by two-fold in the early 1940s, and applying a linear increasing trend to values higher by two-fold in 1960 and subsequently declining to one half of the baseline in 1937. Under each scenario, this exposure matrix (with three dimensions: time, jobs, and exposure level) was coupled to work histories obtained from personnel records to generate estimates of cumulative exposures to antimony (mg year/m³). Cumulative exposures to other contaminants (arsenic, cadmium, lead, and polonium-210) were also computed.

Two population-based cohort studies relied on measurements of metals in spot urine samples collected from adults (age, ≥ 20 years) participating in the United States Centers for Disease Control and Prevention NHANES 1999–2014 survey to assess associations between metal exposures and cancer mortality. The earlier study assessed the impact of antimony on mortality with no assessment of exposures to other metals (Guo et al., 2016), whereas the later study assessed the impact of 10 metals including cobalt, antimony, and tungsten (with creatinine concentration added as a continuous covariate to the regression models that were applied) (Duan et al., 2020). In addition to models that assessed each metal separately, Duan et al. (2020) assessed the metal mixture using weighted quantile sum (WQS) analyses.

In the Sister Study (a cohort of 50 884 women in the USA, recruited between 2003 and 2009, who had a sister who had received a diagnosis of breast cancer), modelled estimates of metal (antimony, cobalt, arsenic, cadmium, chromium, lead, manganese, mercury, nickel, and selenium) concentrations in air (μg/m³) at the census tract-level from the 2005 US EPA National Air Toxics Assessment (NATA) were linked to participants' geocoded residences at baseline (White et al., 2019). In addition, the metal mixture was assessed using WQS regression. In another investigation of the Sister Study cohort that applied a race/ethnicity-stratified case–cohort study design, concentrations of 15 metals, including antimony and cobalt, were measured in toenail cuttings collected at baseline (Niehoff et al., 2021). Two exposure metrics were applied: a continuous variable with results reported for an interquartile range increase in metal concentration and tertiles. A quantile-based g-computation

approach was used to assess metal mixtures in a model, with each metal categorized into tertiles.

(ii) Case–control studies

Two case–control studies (Wingren & Axelson, 1987, 1993) investigated the risk of death from stomach, colon, and lung cancer in relation to occupational exposure to antimony in the glass-working industry in Sweden in the context of antimony(III) oxide exposure (the agent present in this industry). Both studies used the registered title of occupation recorded on individuals' (men aged ≥ 45 years) death certificates collected from 11 parishes and supplemented them with information collected from questionnaires completed by seven glassworks in the same parishes. These questionnaires included questions on the consumption of metals, both at the time the study was conducted and 25 years earlier. In the earlier of these two analyses (Wingren & Axelson, 1987), three exposure definitions were investigated: any glasswork employment, six categories of glassworkers by task, and exposure categories according to metal consumption (with antimony grouped with arsenic and lead). In the later study (Wingren & Axelson, 1993), the same data were used, but analyses were presented for individual metals, with exposure categorized as employment in a glassworks with no, low, or high consumption.

Three case–control studies used multi-element biomonitoring to assess antimony exposure: one in Poland investigated plasma concentrations of antimony in relation to *BRCA1*-related breast cancer incidence among women who were *BRCA1* mutation carriers (Kotsopoulos et al., 2012), and two studies investigated urinary antimony concentrations in relation to thyroid cancer incidence in Shenzhen, China (Liu et al., 2021) or breast cancer incidence in northern Mexico (Mérida-Ortega et al., 2022). None of these studies investigated a specific scenario of exposure to antimony, instead measuring total antimony (with more than 10 other elements) at a single time point in biological specimens. In the study by Kotsopoulos et al. (2012), blood draws were undertaken for 79% of participants with breast cancer after diagnosis but before treatment, and in the studies by Liu et al. (2021) and Mérida-Ortega et al. (2022), urine samples were collected from all participants around the time of interview, which was post-diagnosis for cases.

Two case–control analyses conducted as part of the same study in Spain investigated residential proximity to point sources of antimony and other pollutants in relation to incidence of colorectal cancer (García-Pérez et al., 2020) and stomach cancer (García-Pérez et al., 2021). The current residential addresses of study participants were geocoded, and distances to facilities on the European Pollutant Release and Transfer Register with information about antimony releases to water, air, and soil were calculated and categorized into different buffers (within 1, 1.5, 2, 2.5, or > 3 km). To account for a minimum latency period of 10 years for these cancers, only facilities that were in operation at least 10 years before recruitment were included. In a case series [case–case comparison study] (Kresovich et al., 2019) of 696 women with a breast cancer diagnosis (2005–2008) enrolled in the Breast Cancer Care in Chicago study, exposures were assessed using modelled estimates of ambient air levels of antimony at the census tract-level from the 2002 US EPA NATA that were linked to the geocoded residences of participants in the same year.

(b) Critical review of exposure assessment

(i) Cohort studies

In the prospective and retrospective occupational cohort studies conducted on workers exposed to antimony (Jones, 1994; Schnorr et al., 1995; Binks et al., 2005), the time-dependent exposure variables (e.g. years of employment) were probably computed with little error. However, limitations in the use of these metrics are that they are not specific to a particular contaminant

and do not account for differences in the magnitude of exposure. Schnorr et al. (1995) presented industrial hygiene data that had been collected in 1975 and 1976 as part of a NIOSH evaluation, although they were not used in the analysis. Personal sampling data indicated that employee exposures to antimony in most operations at the smelter exceeded the PEL on the days that were sampled. There were also exceedances (based on the OSHA PEL at the time) for arsenic among workers operating the oxide furnace. The study of lung cancer mortality among workers at a tin smelter in the UK (Jones et al., 2007), the same cohort as assessed in Binks et al. (2005), relied on historical occupational hygiene measurements from area and personal sampling to generate a job-exposure matrix (JEM), which was coupled to worker histories for estimating cumulative exposures to antimony. To assess the uncertainty in the exposure assessment, three different approaches were applied to model exposures during periods for which no industrial hygiene data were available. Notwithstanding these advantages, the JEM was limited by variability in the quality and quantity of the exposure data over the 20-year period, the use of measurements that may have been collected under "worst-case" sampling strategies (probably resulting in exposure estimates that were biased high), and the use of area samples, which probably underestimated personal exposures.

Both population-based cohort studies of adults (age, ≥ 20 years) participating in NHANES used measurements of metals in spot urine samples (with creatinine correction) to assess associations between antimony (Guo et al., 2016) and cobalt, antimony, and tungsten (Duan et al., 2020), and cancer prevalence and mortality. Because cobalt, antimony, and tungsten have relatively short half-lives in urine, one limitation of the exposure assessments in these two studies was that a single urine sample was used; hence, the measured concentrations may have reflected recent rather than long-term exposure relative to the total exposure period of interest. Further, the exposure estimates were probably imprecise due to intra-individual variation in urinary antimony concentrations over time (Wang et al., 2019a). A key difference between these NHANES studies relates to their assessment of co-exposures. Whereas the earlier study focused on antimony alone (Guo et al., 2016), the later study assessed a metal mixture (cobalt, antimony, tungsten, and seven other metals) using WQS analyses (Duan et al., 2020).

Both of the studies that were conducted among the Sister Study cohort in the USA assessed metals both individually and as mixtures (White et al., 2019; Niehoff et al., 2021). The exposure metrics in the study by White et al. (2019) linked modelled census tract-level estimates of outdoor air levels of antimony and cobalt from the US EPA NATA for a single year to residential addresses at enrolment. Hence, the exposure assessment failed to capture temporal trends in outdoor air levels of metals and did not account for variation in levels within census tracts. Further, there was no consideration of residential mobility, although a sensitivity analysis, restricted to women who did not move during the follow-up period, was conducted. Another investigation of the Sister Study cohort used toenail cuttings to assess exposures to cobalt and antimony (together with 13 other metals) (Niehoff et al., 2021). Toenails as an exposure biomarker offer advantages as they are non-invasive and typically represent exposures to metals 3–12 months before sampling (Gutiérrez-González et al., 2019) as compared with the shorter exposure window reflected in urine samples. However, in the study by Niehoff et al. (2021), antimony measurements from cuttings collected at baseline may not have represented exposures during the at-risk period for all women, because follow-up ranged from 0.1 to 13.5 years (average follow-up, 7.5 years) and a relatively weak intrapersonal temporal correlation of 0.18 in toenail cutting measurements taken an average of 7.6 years apart has

been observed in this study population (O'Brien et al., 2019). Other general considerations with the use of toenails as biomarkers of metal exposures are that they are highly susceptible to exogenous contamination and require adherence to rigorous preparatory and cleaning protocols before analysis (Middleton et al., 2016).

(ii) Case–control studies

Both of the glassworks studies conducted in Sweden (Wingren & Axelson, 1987, 1993) were limited by the same exposure assessment methodology. Individuals were linked by occupation to glassworks in the same geographical area from which only qualitative estimates on antimony exposure were available. No direct, individual-level exposure assessments were undertaken. While the later of the two analyses was separated by exposure to individual metals, correlations between antimony and other metals – particularly lead – were too strong to allow the interpretation of antimony-specific results.

The case–control studies conducted in Poland (Kotsopoulos et al., 2012) and China (Liu et al., 2021), in which the exposure assessments consisted of total antimony measurements for plasma and urine samples, respectively, were both subject to the same major limitations. Primarily, total antimony measurements were assessed on a single occasion – after diagnosis for all participants recruited by Liu et al. (2021) and for 79% of the participants recruited by Kotsopoulos et al. (2012) – meaning that reverse causation could not be ruled out.

The case–control study conducted in Spain in relation to colorectal cancer (García-Pérez et al., 2020) and stomach cancer (García-Pérez et al., 2021) used the same proxy exposure assessment approach for both cancers, using geospatial data to calculate residential proximities to point sources of antimony pollution. The industries included on the register were required to report pollutant releases to water, air, and soil, implying several possible exposure routes that could be considered. While the timing of exposures implied that they occurred at least 10 years before participants received a diagnosis, the proxy nature of this exposure assessment probably resulted in non-differential exposure misclassification. The study by Kresovich et al. (2019) was also probably subject to non-differential misclassification, because the exposure assessment relied on area-level modelled estimates of outdoor air levels of antimony linked to participants' residential addresses.

1.6.2 Mechanistic studies in humans

The Working Group identified 23 key mechanistic studies for which a critical appraisal of exposure assessment methods was undertaken, of which three investigated exposures to meglumine antimoniate(V), seven investigated antimony(III) oxide (one included antimony(V) oxide), two investigated antimonite ore (one included an aforementioned antimony(III) oxide study), and the remaining studies either did not, or did not intend to, specify the specific agent. The critiques undertaken for each study in relation to different aspects of exposure assessment and, where possible, the specific agents investigated are tabulated in Table S1.17 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

(a) Exposure assessment methods

Three studies (Hantson et al., 1996; Torrús et al., 1996; Costa et al., 2018) investigated exposure of patients with leishmaniasis to treatment with meglumine antimoniate(V) and subsequently assessed genotoxic (Hantson et al., 1996) and pro-inflammatory (Torrús et al., 1996; Costa et al., 2018) end-points. In all three studies, known doses of meglumine antimoniate(V) were administered for a known duration at known intervals relative to outcome measurements.

In a study by Guo et al. (2018), whole-blood samples were collected via venepuncture during routine health examinations, to assess associations between cobalt and antimony concentrations in plasma and thyroid hormones among a relatively large sample of pregnant women in China. Linear regression analyses were applied using natural log-transformed thyroid hormone levels as the dependent variable and tertiles of blood metal concentrations as the independent variable. Co-exposures were investigated if models assessing metals individually produced statistically significant results ($P < 0.05$); logistic regression results for simultaneous assessment of multiple metals were presented for the association between metals (manganese, nickel, and antimony) and free thyroxine (FT4). In another study (Kirmizi et al., 2021), exposures to antimony and seven other metals were investigated in fasting blood samples collected from women recruited from gynaecology outpatient clinics, who had or did not have polycystic ovary syndrome (PCOS). Differences in mean metal levels between women with and without PCOS were assessed using Student *t*-tests, and Spearman's correlation analysis was used to assess relationships between metals and glucose metabolism parameters and oxidative, antioxidative, and pro-inflammatory markers.

Five studies employed biomonitoring of total antimony concentrations with simultaneous determination of one or more other elements in urine samples from the general population (Tellez-Plaza et al., 2014; Scinicariello & Buser, 2016; Domingo-Relloso et al., 2019), men attending a fertility clinic (Wang et al., 2016), and women early in pregnancy (Margetaki et al., 2021), and investigated associations with a myriad of mechanistic end-points. In the majority of these studies, cross-sectional associations were explored between antimony concentrations in single spot urine samples (or the average of two closely timed samples) (Wang et al., 2016) and end-points measured in biological specimens collected on the same occasion. In one study (Tellez-Plaza et al., 2014), prospective associations were also investigated between total antimony in spot urine samples and oxidative stress markers in blood samples collected ~10 years later. Except for the study by Margetaki et al. (2021), in which urinary concentrations were adjusted for specific gravity, creatinine correction was applied to account for urinary dilution. Another unique aspect of this study was that Margetaki et al. (2021) assessed the potential for co-exposures to cadmium and lead using Bayesian kernel machine regression (BKMR).

Several studies conducted among workers with occupational exposure to antimony and other metals to assess associations with mechanistic end-points have used a variety of different exposure assessment methods (i.e. area sampling, personal sampling in the breathing zones of workers, and/or biomonitoring). A cross-sectional study evaluated associations between antimony(III) oxide exposures, assessed using personal air sampling, and genotoxic parameters (micronucleus formation and sister-chromatid exchange) in peripheral lymphocytes among two groups of men ("high" exposure group, $n = 17$; "low" exposure group, $n = 6$) working at an industrial plant producing fireproof textiles (Cavallo et al., 2002). In this study, multiple personal exposure measurements were collected over a work week (Monday to Friday) with the antimony(III) oxide-based flame retardant applied on Thursday; an earlier study by Iavicoli et al. (2002) indicated that sampling was conducted over the entire work shift "in most cases" or was divided into two 4-hour subsamplings. Differences in markers of oxidative DNA damage were compared between exposure groups, as well as with an unexposed group ($n = 23$). Another study employed area sampling along with biomonitoring to assess associations between antimony and immunological markers – leukocytes, lymphocytes, monocytes, and serum immunoglobulin (Ig)G, IgA, and IgE levels – among 91 workers and 42 office

administrators (all men) at glass-, antimony(III) oxide-, and engineering plastic-manufacturing factories (Wu & Chen, 2017). In this study, air samplers were deployed at work sites and administrative offices during work shifts (5–7 hours in duration), and hair, blood, and first-void urine samples were collected. By factory, differences in average antimony concentrations in samples of air, blood, urine, and hair were observed between workers and administrative staff.

An early occupational study of workers at an antimony plant processing crude ore (stibnite) into antimony(III) oxide reported on assessments for pneumonoconiosis, along with results of area air sampling and biological monitoring of antimony in urine samples (concentrations reported without adjustment for creatinine concentrations) (Cooper et al., 1968). Five studies (Kim et al., 1999; Goi et al., 2003; El Shanawany et al., 2017; Riffo-Campos et al., 2018; Bai et al., 2021) used spot urinary concentrations to assess associations between antimony exposure and mechanistic end-points among workers with suspected occupational exposure to antimony. Kim et al. (1999) investigated differences in immunological end-points between workers (n = 12) exposed to antimony dusts and fumes during the production of antimony(III) oxide and both unexposed workers (n = 22) from the same factory and unexposed controls (n = 33) not employed in the factory. Higher antimony exposure among workers was supported by significantly ($P < 0.01$) elevated geometric mean total antimony concentrations in spot urine test samples relative to the other two groups, previous physician diagnoses of dermal manifestations that resolved after withdrawal from antimony-exposed tasks, and antimony concentrations detected in workplace air (although this was not assessed for either of the two "unexposed" groups). In a similar design, El Shanawany et al. (2017) compared genotoxicity and oxidative stress end-points between 25 workers exposed to antimony(III) oxide at a polyester-production facility and 25 non-exposed controls. Exposures between these groups were further assessed by comparing mean total antimony concentrations in spot urine test samples. El Shanawany et al. (2017) also used duration of employment as an exposure metric.

In a cross-sectional study that collected spot urine samples, investigators assessed associations between antimony exposures, along with 22 other metals and polycyclic aromatic hydrocarbon (PAH) metabolites (with creatinine correction), and mosaic loss of chromosome Y (mLOY) among 888 coke-oven plant workers (all men) (Bai et al., 2021). Three different regression methods were applied including generalized linear models, least absolute shrinkage and selection operator (LASSO) regression, and BKMR to account for mixtures. Deng et al. (2019) also used LASSO analysis in a study that examined associations between antimony and 22 other metals in urine samples (collected either pre- or post-shift) and microRNA (miRNA) expression levels among 122 workers at a coke-oven plant – working at the top, side, or bottom of the coke oven – at a steel factory in China and 238 workers from other locations (e.g. offices) presumed to be unexposed. Another cross-sectional study examined associations between antimony in urine (from a single sample and with no correction for dilution) and lysosomal enzymes in plasma among 26 art-glass workers and 50 unexposed controls (all men) (Goi et al., 2003). While urinary levels of arsenic were also measured, there was no assessment for co-exposures in the statistical analyses. Riffo-Campos et al. (2018) investigated associations between urinary levels of antimony and three other metals (arsenic, cadmium, and tungsten) (with creatinine correction) and subclinical atherosclerosis among 73 workers (all men) at a car assembly plant, assessed during follow-up visits. Correlation analysis was performed to assess relationships between metal exposures and the atherosclerosis markers. In addition, DNA Infinium Methylation 450 K data were available

for a smaller (n = 23) subset of participants who provided blood samples after fasting for assessment of DNA methylation markers [timing of blood draw, i.e. at baseline or during a follow-up visit, is unclear]. Relationships between differentially methylated regions with respect to subclinical atherosclerosis in coronary, carotid, and femoral territories, metal concentrations, sociodemographic variables, and different cell types were assessed using a big-data approach (i.e. bump hunter methodology). A cross-sectional study quantified pro-inflammatory markers and immune responses of antimony miners (Lobanova et al., 1996) and compared them with a group of "unexposed" gold miners. Qualitative occupational status was the primary exposure metric. The quantification of antimony in dust was reported to have been undertaken previously, but limited information was presented. A cross-sectional study (Potkonjak & Pavlovich, 1983) examined lung inflammation in a group of smelters exposed to dust consisting of up to 88% antimony(III) oxide and antimony(V) oxide, in addition to lower concentrations of silica, ferric oxide, and arsenic oxide. Qualitative occupational status was the primary exposure metric, with no exposure contrast or control group available. However, end-points were compared between those with more than and less than 9 years of employment.

Finally, a cross-sectional study (Alrashed et al., 2021) conducted among participants in a case–control study on recurrent pregnancy loss investigated the association between genotoxicity and oxidative stress markers and spot concentrations of total antimony in blood. Continuous metrics were used to investigate associations. The sources of antimony exposure and the specific agents that participants were exposed to were not specified.

(b) Critical review of exposure assessment

The three studies investigating mechanistic end-points in relation to exposure to treatment with meglumine antimoniate(V) in patients with leishmaniasis (Hantson et al., 1996; Torrús et al., 1996; Costa et al., 2018) all had no major limitations as the exposure assessment consisted of known antimony(V) exposure doses, durations, and timing relative to outcome measurements.

The five studies investigating associations between spot urinary concentrations of total antimony due to environmental sources and mechanistic end-points (Tellez-Plaza et al., 2014; Scinicariello & Buser, 2016; Wang et al., 2016; Domingo-Relloso et al., 2019; Margetaki et al., 2021), in addition to the cross-sectional study that measured antimony concentrations in spot blood samples (Alrashed et al., 2021), all shared common limitations. Except for one study (Tellez-Plaza et al., 2014), all associations investigated were cross-sectional, with the timing of exposure assessment the same as that of outcome measurements. None of the studies assessed co-exposures, except that of Margetaki et al. (2021), which assessed cadmium and lead in addition to antimony using BKMR, and that of Alrashed et al. (2021), which quantified (but did not adjust for) arsenic. Because all studies measured urinary antimony concentrations in populations that were not knowingly exposed to elevated levels of antimony, e.g. through occupation or medication use, no information was available on the sources or durations of the exposures to antimony. While urinary total antimony is an appropriate biomarker for recent exposure, the exposure assessments in these studies that relied on measurements made at single time points provided little information about long-term exposure or temporal variability. In one of two cross-sectional studies that collected blood samples (Kirmizi et al., 2021), co-exposures to arsenic, chromium, cadmium, mercury, lead, copper, and zinc were also measured. In the

other study (Guo et al., 2018), co-exposures to arsenic, selenium, manganese, and nickel were considered when the models for metals, assessed one at a time, produced statistically significant ($P < 0.05$) results. However, like the aforementioned studies, these investigations were cross-sectional, and antimony concentrations in blood (like urinary antimony concentrations) reflect recent exposure (ATSDR, 2019).

The investigation by Cooper et al. (1968) of workers exposed to antimony at an antimony plant that processed crude ore reported air levels of antimony in bagging operation areas and other locations, but provided few details except that the exposure assessment was conducted in 1966 and covered many locations and different environmental conditions. Measurements of antimony in urine samples collected from workers were also reported but were not corrected for creatinine concentrations. A key strength of the study by Cavallo et al. (2002) of workers exposed to antimony(III) oxide was the collection over the work week of personal exposure measurements in the breathing zone using accepted sampling and analysis methods. While different numbers of samples were collected for the two exposure groups compared because of differences in shift schedules (with more samples collected for workers in the lower exposure group), additional details would be needed to determine how this may have influenced the results. No other exposures were assessed in this study.

Ten other occupational studies (Potkonjak & Pavlovich, 1983; Lobanova et al., 1996; Kim et al., 1999; Goi et al., 2003; El Shanawany et al., 2017; Wu & Chen, 2017; Guo et al., 2018; Riffo-Campos et al., 2018; Deng et al., 2019; Bai et al., 2021) benefited from knowledge of potential exposures to antimony in specific workplaces. Goi et al. (2003) classified art-glass workers on the basis of their use of arsenic trioxide (As_2O_3) or antimony(III) oxide. The contrasts between total antimony concentrations in spot urine samples from exposed workers and unexposed comparison groups in the studies by Kim et al. (1999) and El Shanawany et al. (2017) provided evidence of ongoing or recent exposure among the worker groups. The study by Kim et al. (1999) further benefited from information on previous diagnoses of dermal manifestations attributed to antimony exposure among exposed workers and antimony concentrations measured in their workplace air, although no comparison air measurements were available for the "unexposed" groups. The study by Wu & Chen (2017) was also multifaceted in its exposure assessment, conducting area monitoring and biological monitoring by collecting blood, urine, and hair samples. However, there was little information about the area monitoring; no information was provided regarding over what time period monitoring was conducted (e.g. on a single day or several days in a single week) or how many air samplers were deployed at each location. Notwithstanding the strengths described, these studies had limitations. Except for one study by Riffo-Campos et al. (2018), study designs were cross-sectional, with the exposures and end-points of interest assessed at the same time, which limited the inferences that could be drawn. Furthermore, reliance on single measurements of antimony in urine or blood provides little information about long-term exposure and, due to within-worker variability in exposure, probably resulted in imprecise exposure estimates. Thus, these assessments may not reflect average exposures and probably introduced non-differential exposure misclassification. In addition, only three (Guo et al., 2018; Deng et al., 2019; Bai et al., 2021) of the eight studies assessed the potential for confounding effects attributable to co-exposures to other metals by applying methods for assessing mixtures in the statistical analyses that were performed. A strength of the study by Riffo-Campos et al. (2018) was the application of a big-data approach (bump hunter methodology) to assess relationships between differentially methylated DNA regions with respect to subclinical atherosclerosis in

coronary, carotid, and femoral territories, metal concentrations, sociodemographic variables, and different cell types among a subgroup of workers (Riffo-Campos et al., 2018). Both Lobanova et al. (1996) and Potkonjak & Pavlovich (1983) provided compelling evidence of probable high antimony exposures among the workers investigated and, in the case of Lobanova et al. (1996), an "unexposed" group of miners was used for comparison. However, both studies were limited by a lack of quantitative exposure contrasts and the high likelihood of co-exposures in both mining and smelting operations.

2. Cancer in Humans

This section comprises a review of the evidence from the studies of cancer in humans exposed to antimony. In the previous evaluation by the *IARC Monographs* programme in 1989, the available data were inconclusive for the evaluation of the carcinogenicity of antimony(III) oxide and antimony(III) sulfide in humans (IARC, 1989). No studies of cancer in humans were included in that evaluation; therefore, all the available studies of cancer in humans are evaluated for the first time in the present volume. The epidemiological evidence for evaluation of the carcinogenicity of trivalent antimony consists of a relatively small number of population-based studies and studies of occupational exposure. The studies of occupational exposure included one of glassworkers (Wingren & Axelson, 1993), two of antimony-smelter workers (Jones, 1994; Schnorr et al., 1995), and one of tin-smelter workers (Jones et al., 2007). There were two retrospective case–control studies using residential distance from industrial sources emitting antimony as the exposure metric, one for colorectal cancer (García-Pérez et al., 2020) and one for stomach cancer (García-Pérez et al., 2021). Studies within a large prospective cohort of women with a sister who had received a diagnosis of breast cancer (the Sister Study) examined breast cancer risk in relation to baseline residential antimony air pollution levels (White et al., 2019) and breast cancer risk in relation to antimony concentrations in toenail cuttings (Niehoff et al., 2021). A small study compared plasma antimony concentrations between *BRCA1* mutation carriers with breast cancer and controls (Kotsopoulos et al., 2012). Another study compared estimated antimony levels in residential air for women with estrogen receptor-negative and progesterone receptor-negative (ER/PR-negative) breast cancer and for women with estrogen receptor-positive and/or progesterone receptor-positive (ER/PR-positive) breast cancer (Kresovich et al., 2019). There were four studies of urinary concentrations of antimony measured in spot urine samples. Two of these were case–control studies on thyroid and breast cancer (Liu et al., 2021; Mérida-Ortega et al., 2022), and two were general population-based studies on the association with total cancer mortality (Guo et al., 2016; Duan et al., 2020).

Workers in smelting processes are exposed to antimony(III) oxide and antimony(III) sulfide. After detailed review of the occupational cancer studies, it was unclear whether there was any exposure to pentavalent antimony among workers in smelting operations. While the types and routes of antimony exposure measured from biological samples cannot be easily determined, these measures probably represent all routes of exposures, including ingestion through diet or hand-to-mouth contact, as well as the inhalation of agents in workplace air. No epidemiological studies have yet considered exposures to pentavalent antimony, i.e. that would occur through injection of drugs for the treatment of leishmaniasis.

The outcomes examined in occupational studies were exclusively related to cancer mortality rather than incidence. For lung cancer, for which survival time tends to be shorter than

for other cancers, this is a reasonable proxy for lung cancer incidence. However, this is not the case for many other types of cancer and is particularly problematic when evaluating all types of cancers combined, which comprise a heterogeneous group of outcomes. The case definitions for the incidence studies tended to be more precise and based on histological confirmation.

2.1 Cancer of the lung and other parts of the respiratory tract

See Table 2.1.

Four occupational studies investigated lung cancer mortality among workers with potential exposure to antimony. Two cohort studies were conducted among antimony-smelter workers – one in north-eastern England, UK, (Jones, 1994) and another in Texas, USA (Schnorr et al., 1995). A further study of cohort of tin-smelter workers in Humberside, UK, included analyses by duration of service (Binks et al., 2005) but did not contribute to the present evaluation, because it was not specific to antimony exposure. However, a later study of the same cohort estimated antimony exposure on the basis of air sampling and personal monitoring data (Jones et al., 2007). A case–control study of glass blowers in Sweden, in which exposure assessment was based solely on job title (Wingren & Axelson, 1987), lacked risk estimation specific to antimony exposure and was considered uninformative.

Jones (1994) examined lung cancer mortality among workers at an antimony smelter in north-eastern England that produced antimony metal and antimony alloys up to 1970. Production of antimony(III) oxide increased, beginning in the 1950s, and became exclusive after 1973. The smelter also produced arsenical antimony, and the ore [raw material] that was processed varied over time. Ores contained up to 0.5% of arsenic, and arsenic metal and arsenic oxide were additionally produced by the plant (for an unspecified period). Although the smelter began operations in the early part of the 20th century, records of individuals who left employment before 1961 were not available. A cohort of 1452 men employed at the smelter for at least 3 months was identified, including all active employees as of January 1961 and workers who joined after that date until the end of follow-up in 1992. Thirty-two employees could not be traced and were excluded from the analysis, yielding an analytical cohort of 1420. [The Working Group noted that some analyses were presented separately for the active employees in 1961 and those hired later; however, the number of workers in each group was not specified.] Smelter workers were exposed to a variety of agents, including lead, antimony and its oxides, arsenic and arsenic oxides, sulfur dioxide, and PAHs. Regional mortality rates were used to calculate expected deaths. The population was divided into four occupational groups: antimony workers, maintenance workers, zircon workers, and others (this category included office workers and management staff). Because permanent job transfers occurred, an antimony worker was defined as any person who worked in the antimony plant for at least 3 months regardless of present or last occupation.

Using regional referent rates (Tyne and Wear, UK), the standardized mortality ratio for lung cancer was elevated for antimony workers [1.5; 95% CI, 1.1–2.1] and for maintenance workers [1.9; 95% CI, 1.1–3.0]. No statistically significant excesses were found for zircon workers or the category of "other" workers. Among antimony workers, this excess was largely among those hired before 1961 ([SMR, 2.2; 95% CI, 1.5–3.0]) and those who died 21–30 years after first employment ([SMR, 2.4; 95% CI, 1.5–3.6]). Higher observed versus expected numbers of lung cancer deaths were additionally identified for maintenance workers hired before 1961 ([SMR, 2.3; 95% CI, 1.2–3.8]), but not after. [The Working Group noted that the workers actively employed in 1961 represent a survivor population

Table 2.1 Epidemiological studies on exposure to antimony and cancer of the lung and other parts of the respiratory tract

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones (1994) North-eastern England 1961–1992 Cohort	1420 men working in an antimony-manufacturing plant, employed on 1 January 1961 or after, for ≥ 3 mo Comparisons were made between the worker population relative to the national (England and Wales) and local (Tyneside conurbation 1961–1973, Tyne and Wear 1974–1983) populations Exposure assessment method: based on company employment records; subcohort grouping by job type, calendar period of first employment, years of exposure, and years since first exposure	Lung, mortality	Occupational group (SMR):			Age, calendar year	*Exposure assessment critique*: Key strengths include: exposures assessed before the development of the outcome; duration of exposure, calendar period of first employment, and years since first exposure probably assessed with minimal error; and stratification in occupational groups with presumably similar exposures. Key limitations include: the potential for exposures to other IARC Group 1 carcinogens (e.g. arsenic); exposure metrics not specific to a particular contaminant; and time-dependent exposure metrics account for duration, but not magnitude, of exposure. *Other strengths*: comparisons with other types of workers in the plant. *Other limitations*: no individual antimony exposure estimates.
			Antimony workers	37	[1.5 (1.1–2.1)]		
			Maintenance workers	15	[1.9 (1.1–3.0)]		
			Zircon workers	5	[0.6 (0.2–1.3)]		
			Others (including office and management)	6	[1.0 (0.4–2.0)]		
		Lung, mortality	Antimony workers, hire year (SMR):				
			Hired before 1961	32	[2.2 (1.5–3.0)]		
			Hired after 1961	5	[0.5 (0.2–1.2)]		
		Lung, mortality	Antimony workers, years since first exposure (SMR):				
			< 1 yr	0	[0 (0–18.3)]		
			1–5 yr	0	[0 (0–3.7)]		
			6–10 yr	3	[1.4 (0.4–3.9)]		
			10–20 yr	7	[0.9 (0.4–1.8)]		
			21–30 yr	20	[2.4 (1.5–3.6)]		
			31–40 yr	6	[1.9 (0.8–4.0)]		
			41–50 yr	1	[1.1 (0.1–5.5)]		
			> 50 yr	0	[0 (0–18.3)]		
		Lung, mortality	Maintenance workers, hire year (SMR):				
			Hired before 1961	12	[2.3 (1.2–3.8)]		
			Hired after 1961	3	[1.1 (0.3–2.9)]		

Table 2.1 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnorr et al. (1995) Texas, USA Enrolment, 1937–1971/follow-up, 1989 Cohort	1014 men employed for ≥ 3 mo at an antimony smelter (91 White and 923 Spanish-surnamed) Exposure assessment method: comparisons of worker mortality rates with the general population (assumed to be unexposed): both national and Texas ethnicity-specific cancer rates; workers grouped into job categories based on company records; additional metric was duration of employment	Respiratory tract, mortality	Smelter employees (SMR, USA rates for White men):			Age, calendar time	*Exposure assessment critique*: A key strength was that exposures were assessed before the development of the outcome. Exposure was corroborated with area and personal air sampling. Key limitations include the potential for exposures to other IARC Group 1 carcinogens (e.g. arsenic and cadmium) and exposure metrics are not specific to a particular contaminant. *Other strengths*: area and personal air monitors established antimony concentrations exceeding OSHA standards; analysed duration of employment; standardization to ethnicity-specific state-wide rates. *Other limitations*: no individual antimony exposure estimates; potential confounding by other metals/metalloids, and other factors such as smoking. *Other comments*: 91.5% of study cohort was Spanish-surnamed; Spanish surnames identified using data from 1980 census.
			All	34	0.81 (0.56–1.13)[a]		
		Lung, mortality	Smelter employees (SMR, USA rates for White men):				
			All	30	[0.75 (0.52–1.06)][a]		
		Lung, mortality	Smelter employees (SMR, Texas, rates for White and Spanish-surnamed men):			Age, calendar time, race/ethnicity	
			All employees	28	[1.39 (0.94–1.96)][a]		
			Spanish-surnamed employees	25	[1.40 (0.93–2.04)][a]		
			White employees	3	[1.27 (0.32–3.46)][a]		
		Lung, mortality	Duration of employment (SMR, Texas rates for White and Spanish-surnamed men):				
			< 5 yr	11	[0.83 (0.43–1.44)][a]		
			5–10 yr	8	[2.24 (1.04–4.26)][a]		
			> 10 yr	9	[2.73 (1.33–5.01)][a]		
			Trend-test *P*-value, < 0.005				

Table 2.1 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2007) Humberside, UK Enrolment, 1967–1995/follow-up, 2001 Cohort	1462 men employed for ≥ 12 mo at Capper Pass tin smelter Exposure assessment method: > 20 000 measurements were used to generate JEM with annual averages by process and non-process area for 1972–1991; three methods were applied to estimate exposures for earlier years by: (A) applying the mean of the 3 earliest years in which data were available; (B) applying a linear increasing trend from baseline values to values higher by 2-fold in the early 1940s; or (C) applying a linear increasing trend as in (B) to values higher by 2-fold in 1960 and subsequently declining to one half of the baseline in 1937); annual averages were linked to work histories to generate estimates of cumulative exposure under each scenario	Lung, mortality	Cumulative exposure (mg year m^{-3}), back-extrapolated using method A (ERR):			Age, calendar year	*Exposure assessment critique*: Key strengths include: exposures assessed before the development of the outcome; the creation of a JEM based on area and personal air sampling measurements for antimony and four other metals coupled to worker histories; and multiple methods for extrapolating exposures to earlier periods. Key limitations include: use of data from worst-case scenario sampling, which may have overestimated exposure; area air sampling measurements likely underestimate personal exposures; varying quality and quantity of the data over the 20 yr period; and no assessment for co-exposures in the statistical analyses. *Other strengths*: individual antimony exposure estimates. *Other limitations*: potential for confounding by other metals/metalloids, and other factors such as smoking.
			Unweighted	62	0.21 (−0.24 to 0.65)		
			Weighted	62	1.66 (0.56–3.77)		
			Trend-test *P*-value, 0.48 (unweighted), 0.004 (weighted)				
		Lung, mortality	Cumulative exposure (mg year m^{-3}), back-extrapolated using method B (ERR):				
			Unweighted	62	0.12 (−0.22 to 0.47)		
			Weighted	62	1.18 (0.28–3.08)		
			Trend-test *P*-value, 0.59 (unweighted), 0.013 (weighted)				
		Lung, mortality	Cumulative exposure (mg year m^{-3}), back-extrapolated using method C (ERR):				
			Unweighted	62	0.11 (−0.22 to 0.44)		
			Weighted	62	1.20 (0.35–2.09)		
			Trend-test *P*-value, 0.59 (unweighted), 0.016 (weighted)				

CI, confidence interval; ERR, excess relative risk; JEM, job-exposure matrix; mo, month; OSHA, Occupational Safety and Health Administration; SMR, standardized mortality ratio; US, United States; yr, year.

[a] The CIs for this study are 90%.

in which long-term employees may be over-represented and those who left employment before 1961 because of disability or death would not be included. The study relied on job classification as a measure of antimony exposure and thus does not include quantitative exposure estimates for antimony metal and antimony(III) oxide, or assess important co-exposures such as arsenic, an agent that is classified in IARC Group 1, *carcinogenic to humans*, with *sufficient* evidence for cancer of the lung. While no adjustment was made for cigarette smoking in the analysis, the elevated standardized mortality ratios were specific to antimony workers and maintenance workers but not to zircon workers or the category of "other" workers, which suggests that confounding by cigarette smoking was unlikely to explain the elevated standardized mortality ratios observed among antimony workers.] Reports from McCallum (1989) and Doll (1985) refer to data from antimony-process workers in the north of England that fitted the description of Jones (1994) but with scant information. [The Working Group did not consider the reports from Doll (1985) and McCallum (1989) in its evaluation because of the sparse description of the studies, probable overlap with Jones (1994), and, for McCallum (1989), the lack of quantitative risk estimates.]

An occupational cohort study was conducted at an antimony smelter in Texas, USA, that produced both antimony metal and antimony oxide from ore that probably contained primarily antimony(III) oxide or antimony(III) sulfide (see Sections 1.1 and 1.2; Schnorr et al., 1995). The study cohort included 91 White and 923 Spanish-surnamed men (n = 1014 workers) who worked a minimum of 3 months between 1 January 1937 and 1 July 1971. Spanish-surnamed members of the cohort were identified by matching the surnames of study participants to Spanish surnames on a computer tape from the 1980 census. Work histories, including start and end dates of employment but not job title or department, were obtained through 1975 from employment and payroll records. Vital status as of 31 December 1989 was obtained from the Social Security Administration, Internal Revenue Service, Veterans Administration, Health Care Finance Administration, National Death Index, and other state and local sources. Only 3.3% of Spanish-surnamed and 2.2% of White workers had unknown vital status as of 31 December 1989. Employment records were reviewed through 1975. Additionally, NIOSH conducted area sampling and personal air monitoring in 1975 and 1976, respectively. Using White men in the USA as the standard, the standardized mortality ratio for lung cancer was 0.75 ([95% CI, 0.52–1.06]). Using rates for Spanish-surnamed and White Texas populations as the standard, the standardized mortality ratios for lung cancer were 1.39 ([95% CI, 0.94–1.96]) overall, and 0.83 ([95% CI, 0.43–1.44]), 2.24 ([95% CI, 1.04–4.26]), and 2.73 ([95% CI, 1.33–5.01]) for < 5, 5–10, and > 10 years of employment, respectively (excluding 2 deaths occurring in the 1950s before the availability of state rates). Standardized mortality ratios did not differ markedly for Spanish-surnamed compared with White men, although the lung cancer mortality rates were lower for Spanish-surnamed men in the population than for White men. [The Working Group considered the ethnicity-specific mortality rates for men in Texas to be more representative of the study population than rates in the USA.]

The primary source of ore was from Mexico, and the ore was composed largely of antimony oxide and sulfide. The composition of antimony ore from Mexico measured in bulk samples collected in 1975 was 31% antimony, 0.054% arsenic, 0.46% sulfur, and 0.136% lead. On the basis of results of the NIOSH surveys reported by Schnorr et al. (1995), the geometric mean values of area air samples for antimony taken in 1975 and personal air samples in 1976 averaged 551 and 747 μg/m^3, respectively; on average, personal air samples exceeded the OSHA PEL of

500 μg/m³. The concurrently measured arsenic levels were 2 and 5 μg/m³, respectively, which were well below the OSHA standard at the time of 500 μg/m³. Schnorr et al. (1995) used an OSHA risk assessment model to estimate that an excess of 0.6 deaths from lung cancer would be expected for the 1014 workers exposed to arsenic for 6.8 years. [The Working Group noted that, although the observed trend towards increasing risk by duration of employment is compelling, it does not consider specific job tasks or estimated levels of exposure. The study lacked the ability to adjust for co-exposures including arsenic, an IARC Group 1 lung carcinogen. Air concentrations for arsenic exposure overall were orders of magnitude lower than those for antimony (with some exceedances based on the current OSHA PEL of 10 μg/m³), and plant processes were considered to be fairly consistent over time. Modelling studies have estimated exposure–response relations between relatively low levels of occupational exposure to arsenic in air and lung cancer (e.g. Hertz-Picciotto & Smith, 1993; Lubin et al., 2008); however, the lowest ranges of arsenic exposure for which study data were available in those studies exceeded the arsenic air levels reported in this study. On the basis of these data, the Working Group concluded that the low levels of arsenic present in the facility would be unlikely to fully explain the strong positive associations with duration of employment, although some role for arsenic cannot be ruled out. Tobacco smoking was a potential confounder. In earlier reports, smoking prevalence rates were lower in men with Spanish surnames than in other men, thus the inclusion of a Spanish-surnamed standard population reduced the likelihood of confounding, which would not be expected given the magnitudes of the exposures and unlikely association with tobacco smoking.]

Jones et al. (2007) added quantitative estimates of cumulative antimony exposure to the analysis of the cohort previously reported by Binks et al. (2005) of workers involved in the production of high-purity tin and antimony/lead alloy. The cohort included men who were employed for at least 12 months at a tin smelter in Humberside, UK, between 1 November 1967 and 28 July 1995, and followed until 31 December 2001 ($n = 1462$). Expected numbers of deaths were computed from national lung cancer mortality rates among men (England and Wales). Individual exposure estimates were derived from a JEM using data from area air and personal air sampling carried out between 1972 and 1991. Exposures were back-extrapolated to earlier years using three exposure models (scenarios A, B, and C). Scenario A used constant back-extrapolation and the mean levels for the earliest 3 years of sampling. Scenario B used a linearly increasing trend for back-extrapolation from baseline levels (in scenario A) to values in the 1940s that were higher by two-fold. Scenario C used levels of antimony that were greater by 2-fold than those at baseline in 1960, then back-extrapolated declining levels to one half of baseline by 1937. Estimated cumulative antimony exposure was unrelated to lung cancer mortality in the unweighted analysis of all three exposure models. Poisson regression models assessed exposures weighted for the assumption that exposures decreased with time since exposure and attained age; associations with lung cancer were detected in models that weighted cumulative exposure with excess relative risks of 1.66 (90% CI, 0.56–3.77; $P = 0.004$), 1.18 (90% CI, 0.28–3.08; $P = 0.013$), and 1.20 (90% CI, 0.35–2.09; $P = 0.016$) per mg year/m³ of cumulative exposure for scenarios A, B, and C, respectively, on the basis of smoothed weights. [The Working Group noted that the authors reported that "stepwise" weights produced similar results, but the data were not shown. The Working Group noted that while there were advantages to the JEM-based individual exposure estimation, the quantity and quality of exposure data varied over time. In particular, exposure to specific antimony compounds may have varied over time because

of differences in the composition of the feedstock processed at the smelter. Co-exposures to other metals or metalloids such as arsenic were not adjusted for in the analysis. Risk estimates for arsenic exposure appeared lower than those for antimony; however, it was not possible to fully distinguish the independent effects of arsenic. In addition, the analysis did not include adjustment for other potentially confounding factors such as tobacco smoking; but this was not considered an important confounder in this study by the Working Group, given the magnitude of the antimony exposure concentrations and that they were unlikely to be associated with tobacco smoking.]

2.2 Cancer of the stomach, colon, rectum, and other digestive organs

See Table 2.2.

A death certificate-based case–control study on stomach and colon cancer was conducted in an area of Sweden where numerous glassworks were located (Wingren & Axelson, 1993). Two occupational cohort studies on mortality of antimony-smelter workers in the UK and USA were conducted (Jones, 1994; Schnorr et al., 1995). Case–control studies on the association between residence near antimony-emitting industrial facilities and cancer of the stomach and colorectal cancer were carried out in the context of a population-based multicase–control study of common tumours in Spain (MCC-Spain) (García-Pérez et al., 2020, 2021).

Wingren & Axelson (1993) conducted a case–referent [case–control] study on deaths from stomach and colon cancer at age 45 years or older among male residents of seven parishes where many glassworks facilities were located. A total of 3523 deceased men were included in the analysis. Deaths from stomach and colon cancers (number not specified) were selected as cases, and referents [controls] included men who had died from causes other than cancer or cardiovascular disease. Levels of exposure to antimony among glassworkers were categorized as none, low, and high, on the basis of information about the volume of metal used (kg/year) and number of employees provided by each of the glassworks facilities, with the referent group defined as decedents who had not worked in glassworks. There was no apparent association between level of use of antimony and stomach cancer, although positive associations were found for use of other metals, including copper, nickel, and manganese. For colon cancer, the odds ratio (OR) for glassworkers in the "no antimony exposure" category was 1.4 (95% CI, 0.6–3.3), the low-exposure category was 1.8 (95% CI, 0.8–13.8), and the high-exposure category was 5.0 (95% CI, 2.6–9.6), compared with decedents who had not been glassworkers. A positive association with colon cancer was also found for lead exposure levels, which were positively correlated ($r = 0.76$) with antimony exposure levels. [The Working Group noted that the exposure assessment was unspecific on the basis of reported antimony use at each facility, and the analyses did not account for co-exposures to other metals, including inorganic lead, which is considered by IARC to have *limited* evidence for stomach cancer in humans. An earlier death certificate-based study of similar design (Wingren & Axelson, 1987) was considered uninformative, because the results were presented for glassworkers overall and without antimony-specific exposure assessment.]

In the study of antimony-smelter workers in north-eastern England, UK (Jones, 1994), described in Section 2.1, there were only 5 observed and 9.4 expected deaths from stomach cancer in the total population (SMR, [0.5]; 95% CI, [0.2–1.2]), and only 2 observed and 4.8 expected deaths from stomach cancer among workers assigned to the antimony department (SMR, [0.4]; 95% CI, [0.1–1.4]). Results for cancers of the colon, rectum, and other digestive organs

Table 2.2 Epidemiological studies on exposure to antimony and cancer of the stomach, colon, rectum, and other organs of the digestive tract

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wingren & Axelson (1993) South-east Sweden 1950–1982 Case–control	Cases: number of cases not reported; deaths of men from stomach and colon cancer aged ≥ 45 yr, residents of 7 parishes where glassworks provided information about metal use Controls: number of controls not reported; deaths of men from causes other than cancer or cardiovascular disease aged ≥ 45 yr, residents of 7 parishes where glassworks provided information about metal use Exposure assessment method: based on job type within glass industry (in the context of antimony(III) oxide), supplemented by information on metal consumption across job types; exposure to individual metals by type of production assessed via questionnaire received from 7 glassworks	Stomach, mortality	Exposure to antimony (OR):			Age at death (45–64, 65–74, or ≥ 75 yr)	*Exposure assessment critique*: This study used an improved metal-specific exposure assessment metric compared to its earlier counterpart. However, the exposure assessment undertaken was still qualitative in design, which did not permit proper quantification of numerous co-exposures to other metals.
			No glasswork employment	NR	1		
			None	NR	2.0 (1.3–3.1)		
			Low	NR	1.6 (0.9–2.6)		
			High	NR	0.8 (0.3–2.0)		
		Colon, mortality	Exposure to antimony (OR):				
			No glasswork employment	NR	1		
			None	NR	1.4 (0.6–3.3)		
			Low	NR	1.8 (0.8–13.8)		
			High	NR	5.0 (2.6–9.6)		

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones (1994) North-east England 1961–1992 Cohort	1420 men employed at an antimony smelter for ≥ 3 mo and traced for mortality through 1992 Exposure assessment method: comparisons were made in the mortality experience between the worker population relative to the national (England and Wales) and local (Tyneside conurbation 1961–1973, Tyne and Wear 1974–1983) populations (presumed to be unexposed); subcohort grouping by job type, based on company records	Stomach, mortality	Occupational group (SMR): Antimony workers Maintenance workers Zircon workers Others (including office and management) Total	 2 1 2 0 5	 [0.4 (0.1–1.4)] [0.6 (0.0–2.9)] [1.1 (0.2–3.7)] [0 (0–3.1)] [0.5 (0.2–1.2)]	Age, calendar year	*Exposure assessment critique*: Key strengths include: exposures assessed before the development of the outcome; duration of exposure, calendar period of first employment, and years since first exposure were likely assessed with minimal error; and stratification in occupational groups with presumably similar exposures. Key limitations include: the potential for exposures to other IARC Group 1 carcinogens (e.g. arsenic); exposure metrics are not specific to a particular contaminant; and time-dependent exposure metrics account for duration, but not magnitude, of exposure. *Other strengths*: low loss to follow-up; major products were antimony metal, antimony alloys, and antimony(III) oxide.

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones (1994) North-east England 1961–1992 Cohort (cont.)							*Other limitations*: very small sample size; potential exposure to arsenic, sulfur dioxide, lead, and polycyclic aromatic hydrocarbons; no quantitative exposure data available.
Schnorr et al. (1995) Texas, USA Enrolment, 1937–1971/follow-up, 1989 Cohort	1014 men employed ≥ 3 mo at an antimony smelter (91 White and 923 Spanish-surnamed) Exposure assessment method: records; comparisons in worker mortality rates between the national population (assumed to be unexposed) and Texas-specific ethnic cancer rates	Stomach, mortality	Smelter employees (SMR, USA rates for White men): All	10	1.49 (0.71–2.74)	Age, calendar time	*Exposure assessment critique*: A key strength was that exposures were assessed before the development of the outcome. Exposure was corroborated with area and personal air sampling. Key limitations include: (1) The potential for co-exposures to IARC Group 1 carcinogens (e.g. arsenic and cadmium); and (2) exposure metrics are not specific to a particular contaminant. *Other strengths*: two industrial hygiene studies conducted at the smelter estimated relative levels of exposure to antimony and arsenic.
		Stomach, mortality	Smelter employees (SMR, Texas rates for White and Spanish-surnamed men): All	7	1.24 (0.50–2.55)	Age, calendar time, race/ ethnicity	
		Liver, biliary tract, and gall bladder, mortality	Smelter employees (SMR, USA rates for White men): All	7	3.17 (1.27–6.52)	Age, calendar time	
		Liver, biliary tract, and gall bladder, mortality	Smelter employees (SMR, Texas rates for White and Spanish-surnamed men): All	6	1.58 (0.57–3.44)	Age, calendar time, race/ ethnicity	
		Colon and rectum, mortality	Smelter employees (SMR, USA rates for White men): All	2	0.12 (0.01–0.45)	Age, calendar time	

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnorr et al. (1995) Texas, USA Enrolment, 1937–1971/follow-up, 1989 Cohort (cont.)							*Other limitations*: no individual exposure assessment; Spanish-surnamed rates were best available but may not have been fully representative of referent rates for the cohort. *Other comments*: rates for Spanish-surnamed men were only available for 1970–1974 and 1980–1984 and were interpolated for the other years (deaths from the 1950s were excluded)

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
García-Pérez et al. (2020) Spain 2008–2013 Case–control	Cases: 557 histologically confirmed cases of colorectal cancer diagnosed among residents of 11 provinces, aged 20–85 yr Controls: 2948 population-based controls selected from administrative records of primary health-care centres frequency-matched on sex, region, and age Exposure assessment method: a geospatial analysis using shortest distance from current residence to registered point sources of antimony pollution using 4 separate proxies of exposure	Colon and rectum, incidence	Residential proximity to industries releasing antimony (OR): Reference area (> 3.0 km) ≤ 1 km ≤ 1.5 km ≤ 2.0 km ≤ 2.5 km ≤ 3.0 km	 NR 11 46 76 97 105	 1 5.05 (2.10–12.19) 4.37 (2.69-7.10) 4.15 (2.61–6.60) 6.36 (4.05–9.98) 5.30 (3.45–8.15)	Province of residence (random effect), sex, age, BMI, family history of colorectal cancer, tobacco smoking, educational level, physical activity in leisure time, total energy intake, alcohol consumption, vegetable intake, red and processed meat intake	*Exposure assessment critique*: Strengths included: coupling residential proximity to industrial sources to an emissions inventory database; an assessment of calendar year of operation of facilities and sensitivity analysis accounted for potential errors in exposure assessment due to residential mobility. Limitations included: the indirect/proxy nature of this exposure assessment; exposure metrics are not specific to contaminants (except for strategy 4 classification); no assessment of potential for co-exposures; and use of pollutant emissions data for a single year. *Other strengths*: large case–control study; extensive information on covariates. *Other limitations*: method for adjustment for multiple comparisons referenced but not described.

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
García-Pérez et al. (2020) Spain 2008–2013 Case–control (cont.)		Colon and rectum, incidence	Residential proximity to industries releasing antimony, sensitivity analysis with long-term residents (OR): Reference area (> 3.0 km) ≤ 1 km ≤ 1.5 km ≤ 2.0 km ≤ 2.5 km ≤ 3.0 km	 NR 10 38 68 85 91	 1 5.99 (2.36–15.20) 3.97 (2.33–6.76) 4.46 (2.68–7.45) 6.57 (4.01–10.78) 5.17 (3.23-8.27)	Province of residence (random effect), sex, age, BMI, family history of colorectal cancer, tobacco smoking, educational level, physical activity in leisure time, total energy intake, alcohol consumption, vegetable intake, red and processed meat intake	
García-Pérez et al. (2021) Spain 2008–2013 Case–control	Cases: 137 histologically confirmed cases of stomach cancer diagnosed among residents of 9 provinces, aged 20–85 yr Controls: 2664 population-based controls selected from administrative records of primary health-care centres frequency-matched on sex, region, and age	Stomach, incidence	Residential proximity to industries releasing antimony (OR): Reference area (> 3.0 km) ≤ 1.5 km ≤ 2.0 km ≤ 2.5 km ≤ 3.0 km	 NR 8 9 12 14	 1 6.18 (2.29–16.63) 3.88 (1.43–10.57) 5.01 (1.97–12.71) 4.82 (1.94–12.01)	Province of residence (random effect), sex, age, family history of stomach cancer, tobacco smoking, educational level	*Exposure assessment critique*: Strengths included: coupling residential proximity to industrial sources to an emissions inventory database; an assessment of calendar year of operation of facilities and sensitivity analysis accounted for potential errors in exposure assessment due to residential mobility.

Table 2.2 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
García-Pérez et al. (2021) Spain 2008–2013 Case–control (cont.)	Exposure assessment method: a geospatial analysis using shortest distance from current residence to registered point sources of antimony pollution using 4 separate proxies of exposure	Stomach, incidence	Residential proximity to industries releasing antimony, sensitivity analysis with long-term residents (OR):			Province of residence (random effect), sex, age, family history of stomach cancer, tobacco smoking, educational level	Limitations include: the indirect/proxy nature of this exposure assessment; exposure metrics are not specific to contaminants (except for strategy 4 classification); no assessment of potential for co-exposures; and use of pollutant emissions data for a single year. *Other strengths*: large case–control study; extensive information on covariates. *Other limitations*: 43 pollutants analysed; method for adjustment for multiple comparisons referenced but not described; although ORs are significant, based on relatively small numbers of cases.
			Reference area (> 3.0 km)	NR	1		
			≤ 1.5 km	7	6.53 (2.24–19.07)		
			≤ 2.0 km	8	4.36 (1.52–12.54)		
			≤ 2.5 km	10	4.66 (1.71–12.68)		
			≤ 3.0 km	11	3.81 (1.43–10.18)		
		Stomach (non-cardia tumours), incidence	Residential proximity to industries releasing antimony (OR):				
			Reference area (> 3.0 km)	NR	1		
			≤ 1.5 km	5	6.10 (1.81–20.50)		
			≤ 2.0 km	6	4.70 (1.48–14.89)		
			≤ 2.5 km	9	7.44 (2.60–21.26)		
			≤ 3.0 km	10	6.16 (2.18–17.43)		
			Residential proximity to industries releasing antimony, sensitivity analysis with long-term residents (OR):				
		Stomach (non-cardia tumours), incidence	Reference area (> 3.0 km)	NR	1		
			≤ 1.5 km	5	8.18 (2.35–28.51)		
			≤ 2.0 km	6	6.49 (2.04–20.60)		
			≤ 2.5 km	8	7.85 (2.59–23.84)		
			≤ 3.0 km	8	5.10 (1.69–15.41)		

BMI, body mass index; CI, confidence interval; IARC, International Agency for Research on Cancer; mo, month; NR, not reported; OR, odds ratio; SMR, standardized mortality ratio; yr, year.

were not reported. [The Working Group noted that the workers actively employed in 1961 represent a survivor population in which long-term employees may be over-represented, and that those who left employment before 1961 because of disability or death would not be included. The observed and expected numbers of stomach cancer deaths were very small, and observed and expected numbers of deaths were not reported for other digestive organs. The exposure assessment was based on department assignment only and was not specific to a particular agent. It also did not account for co-exposures, including inorganic lead, which is considered by IARC to have *limited* evidence for stomach cancer in humans.]

In the study of antimony-smelter workers in Texas, USA, by Schnorr et al. (1995), standardized mortality ratios for stomach, liver, and gall bladder cancer, and cancers of the colon and rectum, were analysed using US White male referent rates and referent rates obtained from the Texas State Health Department for liver and biliary tract stomach cancer for Spanish-surnamed men and White men in Texas (see Section 2.1). The study found a significant excess in mortality from cancers of the liver, biliary tract, and gall bladder (SMR, 3.17; 95% CI, 1.27–6.52; 7 deaths) and a non-significant excess for stomach cancer (SMR, 1.49; 95% CI, 0.71–2.74) in analyses using US rates. When using the Texas ethnicity-specific rates as the comparison, the standardized mortality ratio attributable to cancers of the liver, biliary tract, and gall bladder was 1.58 (n = 6 deaths; 95% CI, 0.57–3.44) and for stomach cancer was 1.24 (n = 7 deaths; 95% CI, 0.50–2.55). There were only 2 deaths from cancers of the colon and rectum, with 16.19 expected based on US referent rates (expected numbers were not calculated based on Texas ethnicity-specific rates). [The Working Group noted that ethnicity-specific rates for Texas provide the more appropriate referent population because mortality patterns are known to be very different for Hispanic people and non-Hispanic people in the USA. Major limitations of the study included lack of job history or individual exposure assessment and relatively small population size, which limited statistical power for identifying potential excess risks associated with antimony exposure for these sites. Arsenic exposure is also a potential confounding exposure for liver cancer.]

An additional occupational cohort study was identified as potentially relevant for evaluating the carcinogenicity of antimony in relation to the digestive tract. Binks et al. (2005) studied workers employed at a tin smelter in north Humberside, UK, that processed a variety of tin ore concentrates and residues to produce high-purity tin as its main product, and lead, copper, cadmium, antimony, and silver as secondary products. Workers were potentially exposed to a variety of substances, including tin, lead, antimony, arsenic, cadmium, sulfur dioxide, natural-series radionuclides, and combustion products. [This study was judged to be uninformative, because no analyses were presented that could distinguish cancer risks associated with antimony from risks associated with other exposures. The cohort was updated with additional exposure information by Jones et al. (2007), but the results were reported only for lung cancer].

Case–control studies of cancer of the stomach and colorectal cancer were conducted in the context of a population-based multicase–control study of common tumours in Spain (MCC-Spain) to assess the possible associations with residential proximity to industrial installations, according to categories of industrial groups and specific pollutants released by the plants (García-Pérez et al., 2020, 2021). The MCC-Spain study included five types of tumour (breast, colorectum, leukaemia, prostate, and stomach) diagnosed among men and women aged 20–85 years recruited in 2008–2013 in 11 provinces of Spain. Population-based controls (n = 3440) for the entire MCC-Spain study were randomly selected from the administrative records of the primary health-care centres located within the hospital catchment areas and

were frequency-matched on the overall distribution of cases of each type of cancer by age (in 5-year age groups), province of residence, and sex. Exposure assessment for individual toxic substances was conducted by identifying industrial facilities that came into operation at least 10 years before the midpoint of the recruitment period in the study provinces. Toxic emissions to air and water by these facilities was determined from the European Pollutant Release and Transfer Register. To assess the relationship between residential proximity to industries releasing specific industrial pollutants and the cancer of interest, an exposure variable was calculated for each participant and each toxicant released by facilities within varying buffer sizes of their residence. Analyses were conducted to assess the change in risk of cancers of the colorectum and stomach with increasing proximity to industrial facilities releasing specific toxicants, and there was extensive control for person-level confounding exposures on the basis of interview data. Numerous toxicants were investigated, and only those with statistically significant odds ratios and numbers of cases and controls ≥ 10 were reported. [The Working Group noted that the proxy nature of this exposure assessment, which used geospatial data to calculate residential proximity to point sources of antimony pollution, probably resulted in non-differential exposure misclassification.]

A total of 557 cases and 2948 controls from 11 provinces were included in the case–control study of residential exposure to industrial emissions and colorectal cancer (García-Pérez et al., 2020). Among the toxicants studied, the highest odds ratios for stomach cancer were observed for individuals residing near industries releasing antimony – at ≤ 1 km (OR, 5.05; 95% CI, 2.10–12.19), ≤ 1.5 km (OR, 4.37; 95% CI, 2.69–7.10), ≤ 2 km (OR, 4.15; 95% CI, 2.61–6.60), ≤ 2.5 km (OR, 6.36; 95% CI, 4.05–9.98), and ≤ 3 km (OR, 5.30; 95% CI, 3.45–8.15) – compared with those residing > 3 km from industries releasing antimony. Similar results were obtained in the sensitivity analysis considering only long-term residents. [The Working Group noted that although there was extensive control for person-level confounding exposures, there was no control in the antimony analyses for confounding exposures to other toxicants or industrial types, some of which were highly associated with colorectal cancer. For example, positive associations were reported for residential proximity to the organic chemical industry at ≤ 1 km and for the inorganic chemical industry at ≤ 2 km; antimony was one of numerous toxicants associated with both industries. In addition, 43 pollutants were analysed, and the method for adjustment for multiple comparisons was referenced but not described. Confidence intervals for the risk estimates were wide, reflecting statistical imprecision.]

A total of 137 cases of stomach cancer and 2664 MCC-Spain study controls from 9 provinces were included in the case–control study on residential exposure to industrial emissions and stomach cancer (García-Pérez et al., 2021). Elevated odds ratios were observed for individuals residing near industries releasing antimony – at ≤ 1.5 km (OR, 6.18; 95% CI, 2.29–16.63), ≤ 2 km (OR, 3.88; 95% CI, 1.43–10.57), ≤ 2.5 km (OR, 5.01; 95% CI, 1.97–12.71), and ≤ 3 km (OR, 4.82; 95% CI, 1.94–12.01) – compared with those residing > 3 km from industries releasing antimony; similar results were obtained in the sensitivity analysis for stomach cancer considering only long-term residents and for non-cardia stomach cancer. There was no apparent trend towards higher risks associated with residing at shorter distances from the antimony source. [The Working Group noted that, as for the earlier study by García-Pérez et al. (2020), it is difficult to determine whether the observed increased risks of stomach cancer in this study are associated specifically with antimony exposure or result from uncontrolled confounding with other exposures to other pollutants or industries.

Confidence intervals for the risk estimates were wide, reflecting statistical imprecision.]

2.3 Cancer of the breast

See Table 2.3.

Studies on the potential association between antimony and breast cancer have used diverse exposure metrics and designs; some studies included subgroups of high-risk women, and others examined variation among breast cancer subtypes. Exposure metrics included antimony concentrations in plasma (Kotsopoulos et al., 2012), estimated antimony concentrations in ambient air (Kresovich et al., 2019; White et al., 2019), and antimony concentrations in toenails (Niehoff et al., 2021) and urine (Mérida-Ortega et al., 2022). One small case–control study was conducted in a cohort of women positive for *BRCA1* mutations (Kotsopoulos et al., 2012), and two studies (White et al., 2019; Niehoff et al., 2021) were conducted within a large prospective cohort of women whose sister had received a diagnosis of breast cancer (the Sister Study). One study compared antimony concentrations in women with ER/PR-negative versus ER/PR-positive breast cancers identified from a population-based case series [case–case comparison study] of incident cases of breast cancer (Kresovich et al., 2019). One population-based case–control study was conducted in an area of Mexico where metal exposures in the environment were considered to be high (Mérida-Ortega et al., 2022).

A case–control study within a registry cohort of *BRCA1*-mutation carriers, conducted in Poland, examined the relationship between antimony concentrations in plasma samples from 48 women who developed breast cancer and from 96 controls who did not (Kotsopoulos et al., 2012). The primary goal of the study was to determine whether dietary and environmental components (as assessed by measurement of 14 micronutrients and trace elements) influenced breast cancer risk in women with hereditary high risk. Blood samples were drawn from breast cancer cases shortly before or after diagnosis, but before chemotherapy, radiotherapy, or surgery. Two controls were selected for each case, matched on year of birth and oophorectomy status. Mean concentrations of antimony did not differ between cases (6.84 µg/L) and controls (6.75 µg/L). However, there appeared to be a positive association between plasma antimony concentrations (analysed by tertile) and breast cancer risk, with an estimated odds ratio of 1.71 (95% CI, 0.68–4.31) in the middle tertile and 2.43 (95% CI, 1.00–5.91) in the highest tertile (*P* for trend, 0.05). [The Working Group noted that the potential association between breast cancer and exogenous agents among women positive for *BRCA1* mutations is of interest, because the mechanisms of cancer may differ between women with high risk and women with average risk. Limitations of the study included very small sample size, the exposure assessment being conducted at only one point in time, and the potential for antimony concentrations to have been influenced by disease status.]

A case-series [case–case comparison] study compared estimated residential antimony levels in ambient air in 147 women with ER/PR-negative breast cancer and 549 women with ER/PR-positive (either or both receptors present) breast cancer (Kresovich et al., 2019). Metal exposure was based on the US EPA NATA level for census tract of residence in 2002, which was 3–6 years before diagnosis. In analyses by quintile of exposure, odds ratios were elevated in higher quintiles of antimony exposure for ER/PR-negative compared with ER/PR-positive women. Comparing the highest with the lowest quintiles, the odds ratio was 1.8 (95% CI, 0.9–3.7; *P* for trend, 0.05). [The Working Group noted that although the authors describe this as a case-series study, it might more accurately be described as a case–case comparison study. This design was of limited usefulness, because it does not directly

Table 2.3 Epidemiological studies on exposure to antimony and cancer of the breast

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kotsopoulos et al. (2012) Poland 2009–2011 Case–control	Cases: 48 cases selected from a prospective study of *BRCA1*-mutation carriers; blood drawn before or shortly after diagnosis Controls: 96 controls selected from a prospective study of *BRCA1*-mutation carriers, matched on year of birth (± 2 yr) and oophorectomy (yes/no) Exposure assessment method: quantitative measurements; plasma biomonitoring of total antimony and other trace elements and micronutrients was employed on a single occasion, which differed in timing, in cases and controls	Breast, incidence	Antimony levels (OR): ≤ 6.07 µg/L 6.08 to ≤ 7.11 µg/L > 7.11 µg/L Trend-test *P*-value, 0.05	 9 16 23	 1 1.71 (0.68–4.31) 2.43 (1.00–5.91)	Year of birth, oophorectomy status	*Exposure assessment critique*: Plasma micronutrients were examined within a population of *BRCA1*-mutation carriers to determine whether dietary and environmental exposures influence risk in high-risk women. *BRCA1* helps maintain genomic integrity through repair of DNA double-strand breaks. Limitations: the large proportion of cases included for whom exposure assessment was undertaken at a single time point post-diagnosis severely limits the quality of this exposure assessment. Furthermore, information on the source of antimony exposure was lacking. *Other limitations*: blood samples were drawn shortly before or after diagnosis, but before chemotherapy, radiotherapy, or surgery, thus, plasma levels may have been influenced by disease status.

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kresovich et al. (2019) Chicago 2005–2008 Case series [case–case comparison study]	Cases: 696; incident breast cancers among women (aged 30–79 yr) with a diagnosis of a first primary in situ or invasive breast cancer, self-identified as non-Latina White, non-Latina Black, or Latina living in the metropolitan Chicago area at time of diagnosis, enrolled in the Breast Cancer Care in Chicago study and evaluated for ER/PR status: ER/PR-negative if negative for both ER and PR (n = 147); ER/PR-positive otherwise (n = 549) Exposure assessment method: records-based; total ambient inhalation exposure to antimony was quantitatively estimated at the census tract-level using US EPA NATA data that account for mobile and stationary sources of exposure, but do not include indoor sources or other occupational exposures	Breast, incidence	Quintiles of residential airborne antimony in ER/PR-negative vs ER/PR-positive cases (OR): Quintile 1 (< 0.02 ng/m^3) Quintile 2 (0.02–0.03 ng/m^3) Quintile 3 (0.03–0.04 ng/m^3) Quintile 4 (0.04–0.06 ng/m^3) Quintile 5 (> 0.06 ng/m^3) Trend-test P-value, 0.05	 NR NR NR NR	 1 1.3 (0.7–2.5) 1.7 (0.8–3.3) 2.1 (1.1–4.1) 1.8 (0.9–3.7)	Age, race/ethnicity, education, BMI, income, census tract affluence and disadvantage, reproductive factors	*Exposure assessment critique*: Non-differential exposure misclassification likely. Timing of exposure measurement may be outside the relevant time window of exposure for cancer outcome under study. Census tract-level concentrations are broad proxies for personal exposures. The reliance on residential address at a single point in time may have introduced non-differential exposure misclassification. Strengths include consideration of co-exposure to other metals in ambient air analyses. *Other strengths*: high proportion of ER/PR-negative cases (21%) giving enough power to detect etiological heterogeneity. *Other limitations*: 11% of participants were excluded due to missing residential history and 20% lacked information on tumour ER/PR status.

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, July 2015 Cohort	2587 breast cancer cases; prospective cohort study of 50 884 women (aged 35–74 yr) who had a sister with a diagnosis of breast cancer but no prior breast cancer at enrolment (Sister Study), followed through July 2015 Exposure assessment method: assessment of antimony exposure was made for a single year in time quantitatively based on address at enrolment before the development of the outcome; annual census tract estimates of metal concentrations in air (μg/m³) for antimony, along with arsenic, cadmium, cobalt, chromium, lead, manganese, mercury, nickel, and selenium from the US EPA 2005 NATA were linked to participants' geocoded residences at baseline and categorized into quintiles for analysis	Breast, incidence	Quintiles of residential airborne antimony concentration (HR):			Age, race (non-Hispanic White, other), education, annual household income, marital status, parity (continuous), census tract median income, geographical region	*Exposure assessment critique*: A key strength was that weighted quantile sum regression was used to assess metal mixtures. Key limitations include: non-differential exposure misclassification likely, as neither temporal trends in outdoor metal levels nor residential mobility was accounted for in the exposure assessment, and potential for within-census tract variability in outdoor air levels of metals also likely introduced error in the exposure assessment. *Other strengths*: large prospective cohort study; address ascertained at baseline; extensive covariate information. An independent validation study in California found good agreement between monitored data and certain air toxics in the 2005 NATA data release. *Other limitations*: for exposure analysis, only the exposure levels at the enrolment residence were considered.
			Quintile 1	489	1		
			Quintile 2	509	1.0 (0.91–1.2)		
			Quintile 3	551	1.1 (0.99–1.3)		
			Quintile 4	557	1.1 (1.0–1.3)		
			Quintile 5	462	0.95 (0.83–1.1)		
			Trend-test *P*-value, 0.9				
		Breast, incidence	Quintiles of residential airborne antimony concentration, premenopausal women (HR):				
			Quintile 1	101	1		
			Quintile 2	95	0.81 (0.61–1.1)		
			Quintile 3	113	0.86 (0.65–1.1)		
			Quintile 4	130	0.95 (0.72–1.2)		
			Quintile 5	95	0.69 (0.51–0.94)		
			Trend-test *P*-value, 0.1				
		Breast, incidence	Quintiles of residential airborne antimony concentration, postmenopausal women (HR):				
			Quintile 1	388	1		
			Quintile 2	414	1.1 (0.95–1.3)		
			Quintile 3	436	1.2 (1.0–1.4)		
			Quintile 4	425	1.2 (1.0–1.4)		
			Quintile 5	367	1.0 (0.88–1.2)		
			Trend-test *P*-value, 0.5				

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, July 2015 Cohort (cont.)		Breast, ER-positive vs non-cases, incidence	Quintiles of residential airborne antimony concentration (HR):			Age, race (non-Hispanic White, other), education, annual household income, marital status, parity (continuous), census tract median income, geographical region	
			Quintile 1	291	1		
			Quintile 2	301	1.06 (0.90–1.25)		
			Quintile 3	320	1.15 (0.97–1.35)		
			Quintile 4	306	1.09 (0.93–1.29)		
			Quintile 5	269	0.97 (0.82–1.16)		
		Breast, ER-negative vs non-cases, incidence	Quintiles of residential airborne antimony concentration (HR):				
			Quintile 1	65	1		
			Quintile 2	36	0.53 (0.35–0.81)		
			Quintile 3	55	0.79 (0.54–1.16)		
			Quintile 4	69	1.08 (0.75–1.55)		
			Quintile 5	36	0.53 (0.34–0.83)		
		Breast, ER-positive vs ER-negative cases, incidence	Quintiles of residential airborne antimony concentration (HR):				
			Quintile 1	NR	1		
			Quintile 2	NR	1.99 (1.27–3.11)		
			Quintile 3	NR	1.45 (0.95–2.20)		
			Quintile 4	NR	1.01 (0.68–1.50)		
			Quintile 5	NR	1.83 (1.14–2.92)		

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico 2003–2009 Cohort	50 884 women (aged 35–74 yr) who had a sister with a diagnosis of breast cancer but no prior breast cancer at enrolment (Sister Study); case–cohort study design evaluated 1495 incident breast cancers (all non-Hispanic Black women and a random sample of non-Hispanic White women) and 1605 women randomly sampled from the cohort, stratified by race/ethnicity Exposure assessment method: concentrations of 15 metals, including antimony, were measured in toenail cuttings collected at baseline and categorized into tertiles for analysis	Breast, incidence	Antimony levels (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	*Exposure assessment critique*: Key strengths include: (1) exposures were assessed before the development of the outcome; and (2) analyses considered the metal mixture. Limitations were that non-differential exposure misclassification was likely, and that metals in toenails cuttings typically represent exposures 3–12 mo before sampling (Gutiérrez-González et al., 2019), and hence a single toenail specimen may not have represented average exposure during the follow-up period. *Other strengths*: cases and controls were drawn from a large, national prospective study population; the case–control study had a large sample size with extensive covariate information.
			Tertile 1 (< 9.8 ng/g)	504	1		
			Tertile 2 (9.8–19.0 ng/g)	519	1.04 (0.86–1.25)		
			Tertile 3 (> 19.0 ng/g)	472	0.93 (0.77–1.13)		
		Breast (ER-positive), incidence	Antimony levels (HR):				
			Tertile 1 (< 9.8 ng/g)	373	1		
			Tertile 2 (9.8–19.0 ng/g)	381	1.03 (0.84–1.27)		
			Tertile 3 (> 19.0 ng/g)	339	0.92 (0.74–1.13)		
		Breast (ER-negative), incidence	Antimony levels (HR):				
			Tertile 1 (< 9.8 ng/g)	64	1		
			Tertile 2 (9.8–19.0 ng/g)	66	1.05 (0.71–1.54)		
			Tertile 3 (> 19.0 ng/g)	56	0.79 (0.52–1.18)		
		Breast, incidence	Antimony levels, non-Hispanic White (HR):				
			Tertile 1 (< 9.8 ng/g)	433	1		
			Tertile 2 (9.8–19.0 ng/g)	424	1.05 (0.86–1.29)		
			Tertile 3 (> 19.0 ng/g)	395	0.96 (0.78–1.18)		

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico 2003–2009 Cohort (cont.)		Breast, incidence	Antimony levels, non-Hispanic Black (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	
			Tertile 1 (< 9.8 ng/g)	71	1		
			Tertile 2 (9.8–19.0 ng/g)	95	0.86 (0.57–1.29)		
			Tertile 3 (> 19.0 ng/g)	77	0.56 (0.37–0.84)		
Mérida-Ortega et al. (2022) Northern Mexico 2007–2011 Case–control	Cases: 452 histopathologically confirmed breast cancer cases from main public and academic hospitals, aged ≥ 18 yr, no personal history of other type of cancer, ≥ 1 yr residence in study area, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations , matched by age to cases (± 5 yr)	Breast, incidence	Antimony quartile (µg/g creatinine):			Age, schooling, estrogenic index, alcohol consumption, BMI	*Exposure assessment critique*: A key limitation of this study is the reliance on a single spot urine sample. The use of a single sample may not reflect the relevant exposure window, particularly as the sample was collected after the outcome. A strength of this study was the consideration of co-exposure to other metals and trace elements. *Other strengths*: population-based case–control study design; area studied has natural contamination by metals in water and the largest non-ferrous metal-processing site worldwide; exposure to other metals accounted for in the statistical analysis.
			Quartile 1	NR	1		
			Quartile 2	NR	0.70 (0.47–1.05)		
			Quartile 3	NR	0.37 (0.24–0.57)		
			Quartile 4	NR	0.92 (0.63–1.35)		
			Trend-test *P*-value, 0.269				
		Breast, incidence	Mixture 1 (high loadings of chromium, nickel, antimony, aluminium, lead, and selenium), natural log-transformed (ln-µg/g creatinine) (OR):				
			Per unit increase, all women	444	1.15 (1.06–1.25)		
			Per unit increase, premenopausal women	178	1.13 (0.98–1.29)		
			Per unit increase, postmenopausal women	266	1.20 (1.08–1.33)		

Table 2.3 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mérida-Ortega et al. (2022) Northern Mexico 2007–2011 Case–control (cont.)	Controls: 439 women with ≥ 1 yr residence in study area with no personal history of cancer, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations, matched by age to cases (± 5 yr) Exposure assessment method: this study assessed antimony exposure (all routes) in urine samples collected at a single point in time; in addition, exposure to other metals and trace elements was assessed						*Other limitations*: urine samples collected after diagnosis, leading to potential for reverse causation; spot urine sample may not reflect exposures during biologically relevant time period.

BMI, body mass index; *BRCA1*, BRCA1 DNA repair associated; CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; mo, month; NATA, National Air Toxics Assessment; NR, not reported; OR, odds ratio; PR, progesterone receptor; US EPA, United States Environmental Protection Agency; vs, versus; yr, year.

estimate the risk of breast cancer associated with antimony exposure. It is included here because the results for antimony exposure in women with ER/PR-negative and ER/PR-positive breast cancer can be compared with the findings of White et al. (2019), described below. An additional limitation of this study was that the exposure assessment was conducted a relatively short time before diagnosis.]

One cohort study investigated the potential association between residential exposure to metallic air pollutants (including antimony) in ambient air and breast cancer risk (White et al., 2019). This study was conducted within a nationwide prospective cohort study of 50 884 breast cancer-free women who had a sister with breast cancer recruited in 2003–2009 (the Sister Study). The US EPA NATA, a database that provides nationwide modelled airborne-concentration information on hazardous air toxics at the census-tract level (US EPA, 2005), was linked to the geocoded baseline residence of each study participant at recruitment and categorized in quintiles. A total of 2587 cases of breast cancer (including both invasive breast cancer and ductal carcinoma in situ), diagnosed before 31 July 2015, were included in the study. In analyses by quintiles of antimony exposure in the overall sample, slightly elevated risks of breast cancer were observed for antimony in quintile 3 (hazard ratio, HR, 1.1; 95% CI, 0.99–1.3) and quintile 4 (HR, 1.1; 95% CI, 1.0–1.3) compared with quintile 1; however, no excess risk was observed in quintile 5. No significant trends were observed for all breast cancers, or premenopausal or postmenopausal breast cancer. However, in the analysis of premenopausal breast cancer, hazard ratios in quartiles 2–5 were all below 1 and the hazard ratio for quintile 5 was 0.69 (95% CI, 0.51–0.94). Results were similar in subgroup analyses by ER status, with some evidence for an inverse, non-monotonic association with antimony concentrations among women with ER-negative breast cancer. The upper bounds of the 95% confidence intervals were below 1 in both quintile 2 (HR, 0.53; 95% CI, 0.35–0.81) and quintile 5 (HR, 0.53; 95% CI, 0.34–0.83). An analysis that compared ER-positive versus ER-negative cases found significantly elevated hazard ratios for quintiles 2 and 5, largely driven by inverse associations with increasing antimony exposures in ER-negative cases. [The Working Group noted that the strengths of this study included the large population and prospective study design. However, the exposure assessment quality was limited, because antimony air levels were estimated at one point in time on the basis of residence at diagnosis and because of the potential for measurement error in the exposure assessment. The results of the analysis by ER subtype contrasted with the findings of the study by Kresovich et al. (2019), which reported positive associations with increasing quintiles of antimony exposure for women with ER/PR-negative versus ER/PR-positive breast cancer.]

A case–cohort study within the Sister Study cohort investigated the relationship between antimony levels in toenail cuttings and incident breast cancer (Niehoff et al., 2021). Toenail cuttings were collected from Sister Study participants at the time of enrolment, and concentrations of 15 metals in toenail samples were measured for 1495 cases diagnosed through September 2017 and a race-stratified random subcohort of 1605 women (including 107 women who received a diagnosis of breast cancer after enrolment and 1498 women who remained breast cancer-free through follow-up). There was no evidence of a positive association between antimony levels and breast cancer among all cases and controls combined (HR in tertile 2, 1.04; 95% CI, 0.86–1.25; and HR in tertile 3, 0.93; 95% CI, 0.77–1.13). Similar exposure–response patterns were observed in analyses stratified by ER status and race (tests for trend were not reported for these analyses). [The Working Group noted the strengths of the study with respect to design and sample size. The exposure assessment

from a single point in time was a limitation, and there was only a twofold difference in the cut point values used to define the lowest (< 9.8 ng/g) and highest (> 19.0 ng/g) exposure categories. Sample sizes were small for some subgroup analyses, resulting in wide confidence intervals, for ER-negative breast cancers and cancers among non-Hispanic Black women.]

A population-based case–control study in Mexico examined associations between certain metals or metalloids and incident breast cancer in a region that has naturally high levels of metals in water, and houses the world's fourth largest non-ferrous metal-processing facility (Mérida-Ortega et al., 2022). Women with histopathologically confirmed breast cancer (n = 499) were identified from public hospitals in several states in northern Mexico and were matched on age (± 5 years) to controls. [The Working Group noted that the authors did not mention how the case group was selected from the larger case pool of 1045 histopathologically confirmed cases.] Controls were identified from a national population-based survey. Interviews were conducted to obtain covariate information, and height and weight were measured. Metals were measured in first morning-void urine samples near the time of interview and before any treatment had begun for the women with breast cancer (on average, 2 months after diagnosis). After excluding cases and controls with exceptionally low or high creatinine concentrations, 452 cases and 439 controls remained in the analysis. Odds ratios were calculated for creatinine-adjusted metal concentrations, both individually in models and grouped together using principal component analysis to assess mixture patterns. [The Working Group noted the somewhat imprecise age-matching as a limitation, and the high response rate (> 90%) of both cases and controls, and good control for confounding factors – e.g. body mass index (BMI), endogenous estrogen exposure, and alcohol consumption – as strengths.]

Antimony concentrations in urine were higher (although not significantly) among controls than among cases. In exposure–response analyses, antimony alone was not associated with breast cancer: the odds ratio for the highest quartile was 0.92 (95% CI, 0.63–1.35). Antimony was correlated most strongly with chromium (r = 0.72), lead (r = 0.56), and nickel (r = 0.52), and these metals – together with aluminium and tin – loaded highly on the first principal component. A positive association was observed for the mixture defined by this principal component (OR, 1.15; 95% CI, 1.06–1.25), which was slightly more pronounced among postmenopausal women (OR, 1.20; 95% CI, 1.08–1.33). [The Working Group noted that, while none of the studied metals has been found by IARC to have *limited* or *sufficient* evidence for breast cancer in humans, the relatively high correlations between antimony and other carcinogenic metals make it difficult to ascribe the positive findings to any particular metal. It is notable that in the analyses for individual metals, only tin was positively associated with breast cancer.]

2.4 Cancer of the thyroid and other sites, including all cancers combined

See Table 2.4.

A total of two cohort studies were identified that examined mortality from all cancers combined using NHANES. An additional hospital-based case–control study of thyroid cancer was conducted in China. An occupational cohort study of tin-smelter workers (Binks et al., 2005), which examined mortality from all cancers as well as specific cancers, was considered uninformative because it lacked information specific to antimony.

Guo et al. (2016) published results from a mortality follow-up study of NHANES, a survey selected by a stratified, multistage probability

Table 2.4 Epidemiological studies on exposure to antimony and cancer of the thyroid and other sites, including all cancers combined

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Guo et al. (2016) USA Enrolment, 1999–2010/ follow-up, 2011 Cohort	7781; aged ≥ 20 yr, full covariate and metal data available from the NHANES survey followed for mortality through 2011 (average follow-up, 6.04 yr); mean baseline age, 45.2 yr among those alive at the end of follow-up and 67.25 yr among those who died Exposure assessment method: quantitative measurements; exposure assessment relied on spot urine samples; measurements were made using standard methods of analysis	All cancers combined, mortality	Creatine-adjusted urine concentrations (μg/g creatinine) (HR): Quartile 1: ≤ 0.048 (reference) Quartile 2: > 0.048–0.075 Quartile 3: > 0.075–0.121 Quartile 4: > 0.121 Trend-test *P*-value, 0.487	 23 31 43 48	 1 0.90 (0.49–1.62) 0.85 (0.46–1.59) 1.08 (0.63–1.86)	Age, sex, race/ ethnicity, smoking, drinking, marital status, educational level, PIR, BMI, hypertension, diabetes, eGFR, urinary creatine, lead, cadmium	*Exposure assessment critique*: Key limitations include: use of urine samples because the half-life of antimony in urine is of the order of several days to a couple of weeks (Wang et al., 2019a; CDC, 2017); use of single spot antimony levels in urine samples subject to substantial intra-individual variability; no assessment of co-exposures in the analyses. General comments: RR estimates are also provided for self-reported history of cancer (exposure measures after the outcome). *Other strengths*: biomarker of antimony exposure; large sample size. *Other limitations*: potential for exposure misclassification as urine is a short-term biomarker; all cancers combined is a heterogeneous outcome; mortality does not reflect incidence of cancers with low mortality rates.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Duan et al. (2020) USA Enrolment, 1999–2014/ follow-up, 2015 Cohort	26 056 participants aged ≥ 20 yr, not pregnant; with full covariate, mortality, and metal data drawn from the NHANES 1999–2014 survey sample of 82 091 participants; followed for mortality through 2015 (mean follow-up, 7.4 yr); mean age at baseline, 45.9 yr Exposure assessment method: exposure to antimony through all routes was assessed quantitatively from a single urine sample; WQS estimates were made of the metal mixture (including urinary levels of barium, cadmium, caesium, molybdenum, lead, titanium, cobalt, tungsten, and uranium, and blood levels of mercury, lead, and cadmium)	All cancers combined, mortality	Urine concentrations (μg/L) (RR): Median, 0.07 (IQR, 0.04–0.12) Trend-test *P*-value, 0.044	560	1.31 (1.01–1.70)	Sex, age, age^2, ethnicity, urinary creatinine, education, PIR, cotinine category, BMI, physical activity, CVD, diabetes	*Exposure assessment critique*: Key strengths include: single urine samples were collected before the development of the outcomes. Key limitations include: urinary levels of antimony have relatively short half-lives and hence, reflect recent rather than long-term exposure; single spot antimony levels in urine samples are subject to substantial intra-individual variability (Wang et al., 2019a; CDC, 2017). Non-differential exposure misclassification likely. Co-presence and relative weights of other metals were examined (however, other possible carcinogenic exposures were not assessed). *Other strengths*: metals considered as single elements and as a mixture taking into account collinearity; participants. drawn from the general population of the USA, relatively large sample size.
		All cancers combined, mortality	Urine concentrations (μg/L) (RR): Per unit increase	560	1.20 (0.87–1.65)	Sex, age, age^2, ethnicity, urinary creatinine, education, PIR, cotinine category, BMI, physical activity, CVD, diabetes, 9 other urinary metals (barium, cadmium, cobalt, caesium, molybdenum, lead, titanium, tungsten, and uranium)	
		All cancers combined, mortality	Urine WQS mixture (μg/L) (RR): Per unit increase	560	1.60 (1.02–2.52)	Sex, age, age^2, ethnicity, urinary creatinine, education, PIR, cotinine category, BMI, physical activity, CVD, diabetes	

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Duan et al. (2020) USA Enrolment, 1999–2014/ follow-up, 2015 Cohort (cont.)							*Other limitations*: the relatively short follow-up period yielded a small number of death outcomes; potential for exposure misclassification: concentrations in the urine may not reflect the actual exposure; most metals have a short half-life, which reflects recent exposure; "all cancers combined" is a heterogeneous outcome; mortality does not reflect incidence of cancers with low mortality rates.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Liu et al. (2021) Shenzen, China 2017–2019 Case–control	Cases: 111 with diagnosis of thyroid tumour Controls: 111 healthy controls matched on age (± 2 yr) and sex without thyroid abnormalities Exposure assessment method: quantitative assessment based on multi-element urinary biomonitoring, including total antimony, was employed on a single occasion after study recruitment	Thyroid (papillary thyroid microcarcinoma/ carcinoma), incidence	Urinary concentrations (μg/L) (OR): Quartile 1 Quartile 2 Quartile 3 Quartile 4 Trend-test *P*-value, 0.939	 NR NR NR NR	 1 0.71 (0.44–1.15) 0.53 (0.26–1.10) 0.08 (0.01–0.56)	Age, sex, BMI, duration of residence in Shenzen, smoking, drinking, education, household income, household renovation status	*Exposure assessment critique*: Limitations: urine sampling/analysis undertaken at a single time point post-diagnosis severely limits the quality of this exposure assessment: potential for reverse causation. Furthermore, information on the source of antimony exposure was lacking, making the findings difficult to interpret. *Other strengths*: histopathological confirmation of thyroid cancer; biomarker of exposure. *Other limitations*: hospital-based, case–control design with limited description of control group and small sample size; potential for exposure misclassification as urine is a short-term biomarker and taken after the diagnosis.

BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IQR, interquartile range; NHANES, National Health and Nutrition Examination Survey; NR, not reported; OR, odds ratio; PIR, poverty-to-income ratio; RR, relative risk; WQS, weighted quantile sum; yr, year.

algorithm of the non-institutionalized population every 2 years from 1999 to 2010. The study by Guo et al. (2016) included 7781 participants aged 20 years or older, with a mean age of 45.2 years at baseline among those who remained alive and 67.25 years among those who died during the follow-up period. Participants were among the one third of individuals (aged over 6 years) randomly selected for measurement of antimony and other elements by ICP-MS in spot urine samples. Participants with missing data regarding sociodemographic and lifestyle factors, health conditions (BMI or estimated glomerular filtration rate), or urinary creatinine, or with abnormal urinary antimony concentrations, were excluded. The follow-up period ended on 31 December 2011, with an average follow-up of 6.04 years. Using the National Death Index, Centers for Medicare and Medicaid, Social Security Administration, and death certificates, 145 cancer deaths were identified. In Cox proportional hazard models weighted for the sampling design, quartiles of urinary antimony concentrations were unrelated to mortality from all malignant neoplasms, including in models adjusted for a large and varied number of potential confounders. [The Working Group noted that there is intraperson variability in a single urine measurement and that it represents short-term exposure; mortality from all malignant neoplasms is a heterogeneous outcome and does not reflect incidence of cancers with low fatality rates.]

Duan et al. (2020) subsequently examined 26 056 participants from NHANES 1999–2014, with an average age of 45.9 years, for whom mortality data were obtained until 31 December 2015 from the National Center for Health Statistics. Those pregnant, aged less than 20 years at enrolment, or with missing data for metals, covariates, or mortality were excluded. A multi-element analysis was performed of urinary metals including antimony, barium, cadmium, cobalt, caesium, molybdenum, lead, titanium, tungsten, and uranium, and also whole-blood lead, mercury, and cadmium. Concentrations of these elements were examined in relation to mortality from all cancers combined together with outcomes (cardiovascular disease and all causes). With an average follow-up of 7.4 years, there were 560 deaths from all malignant neoplasms. In the single-element covariate-adjusted Poisson model, antimony was associated with an elevated relative risk of all-cancer mortality (relative risk, RR, 1.31; 95% CI, 1.01–1.70), although this was not statistically significant after adjustment for multiple comparisons (false discovery rate-adjusted *P*-value, 0.168) or after adjustment for the other urinary metals listed above (RR, 1.20; 95% CI, 0.87–1.65). Using a mixtures approach (WQS regression) of all the urinary metals analysed, the relative risk for the mixture was 1.60 (95% CI, 1.02–2.52) with a weight of 13% for antimony. [The Working Group noted that there is intraperson variability in a single urine measurement and that it represents short-term exposure; all-cancer mortality is a heterogeneous outcome and not representative of the incidence of non-fatal malignancies.]

Liu et al. (2021) published results from a case–control study of 111 patients with thyroid cancer from Peking University Shenzhen Hospital and Shenzhen People's Hospital in Shenzhen, China, enrolled from September 2017 to September 2019. Cases included patients with histologically diagnosed papillary thyroid microcarcinoma or carcinoma. Controls were matched 1:1 (on age ± 2 years and sex) and enrolled from community health centres in the area. Concentrations of 12 elements – including antimony, chromium, manganese, cobalt, nickel, arsenic, selenium, molybdenum, cadmium, mercury, thallium, and lead – were measured in urine samples after 8 hours of fasting. In conditional logistic regression models adjusted for age, sex, BMI, duration of residence in Shenzhen, smoking, drinking, education level, household income, and house renovation status, no association was observed

across quartiles of urinary antimony concentrations (quartile 2: OR, 0.71; 95% CI, 0.44–1.15; quartile 3: OR, 0.53; 95% CI, 0.26–1.10; quartile 4: OR, 0.08; 95% CI, 0.01–0.56; as compared with quartile 1, *P* for trend, 0.939) in the single-element model. [This study also included a case group of nodular goitre confirmed via B-ultrasound and tested for associations with thyroid function tests. Multi-element models were performed only for the combined group of thyroid cancer and nodular goitre. The Working Group was unable to assess whether hospitalized cases were representative of the population of the area and from which controls were drawn, and no response rates were provided. Measurement of urinary antimony was carried out after diagnosis; it is a relatively short-term measure and there is intraperson variability in a single measurement.]

2.5 Evidence synthesis for cancer in humans

The epidemiological evidence base for the evaluation of the carcinogenicity of trivalent antimony consists of both occupational and population-based studies. The occupational studies include one death certificate-based study of workers exposed to antimony in glassworks, two occupational cohort studies in antimony-smelter workers, and one in tin-smelter workers. There were two retrospective case–control studies using residential distance from an industrial emission source as the exposure metric, one for stomach cancer and one for colorectal cancer, and a prospective cohort study on breast cancer examining risk in relation to baseline residential air pollution levels. Three studies on breast cancer in women used single time-point toenail-cutting, plasma, or urine samples to estimate individual exposure to antimony. Two general-population studies examining total cancer mortality in relation to a one-time urine antimony concentration, one case–case study of breast cancer, and one case–control study on thyroid cancer, were reviewed but not considered informative for this evaluation. No studies were identified that specifically evaluated cancer risk associated with pentavalent antimony in humans. It was unclear whether this form of antimony was present in any of the occupational or other studies considered in this evaluation.

2.5.1 Quality of exposure assessment for antimony and co-exposures

Quality of the 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 Section 1.6.1.

The quality of exposure assessment methods varied among the occupational cohort studies, but each was found to have limitations with respect to defining antimony exposure and addressing co-exposure to arsenic and other lung carcinogens. Among the lung carcinogens of concern in these studies (see Table 1.12 and Section 1.6.1), arsenic was the most ubiquitous and of primary concern for interpreting the results. Wingren & Axelson (1993) classified decedents as glassworkers or not on the basis of occupations listed on death certificates, and estimated intensity of exposure to antimony and other metals, including arsenic, on the basis of qualitative use estimates from individual glassworks facilities. The study of antimony-smelter workers by Jones (1994) had no quantitative data on antimony, arsenic, or other co-exposures; co-exposure to arsenic is of particular concern in this facility where arsenical antimony is also manufactured. This study did report job history data, which were used to stratify exposures by four department assignments reflecting probable exposure to antimony. The study of antimony-smelter workers by Schnorr

et al. (1995) used duration of total employment at the smelter as the metric in exposure–response analyses but had no information on job titles. A strength of this study was the availability of antimony and arsenic air concentrations from industrial hygiene surveys conducted in 1975–1976. Although there were no quantitative data from earlier decades when members of the study cohort were employed, processes and materials were considered to be fairly consistent over time. Among the occupational cohort studies, the study of tin-smelter workers by Jones et al. (2007) had the most detailed and highest-quality exposure assessment. Individual-level exposure estimates were derived from a JEM using air sampling and personal monitoring antimony measurements. None of the occupational cohort studies had information on smoking history, a potential confounder in analyses of lung cancer risk. Although all the occupational studies had limitations in the quality of exposure assessment, they were considered particularly important in the evaluation because occupational exposures are generally orders of magnitude higher than population exposures, and even the crudest exposure metrics, such as duration of employment, reflect biologically relevant time periods. The wide range of potential exposures across jobs and individuals may also allow for meaningful exposure–response analyses.

Two case–control studies used geospatial data to calculate residential proximity to point sources of antimony pollution (García-Pérez et al., 2020, 2021). The proxy nature of this exposure assessment probably resulted in non-differential exposure misclassification. Exposure metrics were similarly developed for residence near specific types of industrial facility and residence near facilities emitting certain categories of industrial pollutants, including any agents categorized in IARC Group 1 (*carcinogenic to humans*), 2A (*probably carcinogenic to humans*), or 2B (*possibly carcinogenic to humans*). Analyses of associations with antimony did not control for these other exposure metrics. In a prospective study, White et al. (2019) linked modelled census tract-level estimates of outdoor air levels of antimony for a single year to each woman's address at enrolment, which may have resulted in misclassification by not accounting for temporal trends and variations in outdoor air levels of metals within census tracts. One study, the prospective Sister Study cohort (Niehoff et al., 2021), used antimony concentrations in toenail cuttings as a biological marker of exposure. In this study, toenail cuttings were obtained at baseline, thus enabling assessment of exposure before the onset of disease. Toenail cuttings may reflect more long-term concentrations than urine or blood for substances like antimony that are excreted in a few days to weeks and have relatively short half-lives in the blood. However, one-time measurement of toenail antimony may not represent cumulative exposures to antimony during biologically relevant time periods of exposure, although levels appear to be moderately correlated within individuals over a period of several years (see Section 1.6.1). A strength of the exposure assessment in the general-population studies described here is the much more extensive information on individual-level covariates and quantitative estimates of exposure to other environmental contaminants. Although not a reflection of low-quality assessment, the small range of exposures in the general-population studies included may limit the informativeness of exposure–response analyses.

2.5.2 Lung cancer

Of the cancer sites for which data in humans were available, the most evidence was available for lung cancer. All three occupational cohort studies found that lung cancer mortality was elevated compared with the general population, and one also found positive associations with antimony exposure (Jones, 1994; Schnorr et al., 1995; Binks et al., 2005; Jones et al., 2007). Two

studies were of antimony-smelter workers. One defined antimony exposure by job classification and found significantly elevated standardized mortality ratios in analyses of subgroups with early periods of employment and latency of 21–30 years (Jones, 1994). The second observed elevated standardized mortality ratios for lung cancer using ethnicity-specific referent rates, with an increasing trend by duration of exposure (Schnorr et al., 1995). A study of tin-smelter workers that used a JEM to estimate individual exposure reported trends of increasing risk with increasing cumulative antimony exposures weighted by time since exposure and attained age. The Working Group viewed the consistent results among these studies as important in evaluating carcinogenicity. A major concern was lack of ability to adjust for co-exposure to recognized lung carcinogens (e.g. those classified by IARC in Group 1), especially arsenic, which was present in all three studies. Tin-smelter workers in the study by Jones et al. (2007) had a wide range of exposures to other metals. The study by Schnorr et al. (1995) was viewed by the Working Group as having the strongest evidence for an association between lung cancer and antimony exposure, because antimony levels measured in 1975 and 1976 were orders of magnitude greater than arsenic levels, and arsenic levels were generally well below the lowest ranges of exposures at which elevated antimony has been associated with lung cancer (Hertz-Picciotto & Smith, 1993; Lubin et al., 2008). There is some question as to whether the levels of arsenic measured in 1975 and 1976 can be extrapolated to reflect earlier exposures. It may be reasonable to make inferences about historical relative and absolute levels of antimony and arsenic exposure in this facility from more recent data because the sources of the ore and processes were consistent over time. Jones (1994) lacked individual exposure estimates for antimony workers, and practices and materials changed over time. It was possible to compare risk with other workers in the plant, thus minimizing concerns about confounding by other factors such as tobacco smoking, which is not typically a concern in occupational studies of high exposures and where smoking is unlikely to be strongly associated with the exposures. However, the concern about arsenic remained because the ore contained higher levels of arsenic and because of the production of arsenical antimony at the smelter. Jones et al. (2007) estimated risks associated with other co-exposures such as arsenic but did not adjust for these in their analysis. Risk estimates for arsenic were lower than for antimony and imprecise for other elements, which strengthened the evidence for antimony. Thus, although positive associations were observed between antimony exposure and lung cancer mortality in all three occupational studies of smelter workers, the possibility that findings were attributable to confounding by co-exposure to arsenic or to other known lung carcinogens could not be reasonably excluded.

2.5.3 Other cancer sites

Few studies were available for other cancer types. Elevated standardized mortality ratios for some digestive tract cancers were reported in the study by Schnorr et al. (1995), but risk estimates were imprecise. In population-based case–control studies of colorectal cancer (García-Pérez et al., 2020) and stomach cancer (García-Pérez et al., 2021), high odds ratios were observed in all exposure quartiles on the basis of distance of residence from antimony emission sites. However, lack of exposure gradients with decreasing distance from an emission source, misclassification of individual exposure based on a very crude exposure metric, and lack of control for confounding by broader categories of exposure, such as industry type, precluded a causal interpretation.

Three studies on breast cancer used biomarkers as the exposure metric in populations that were not selected for occupational

exposure (Kotsopoulos et al., 2012; Niehoff et al., 2021; Mérida-Ortega et al., 2022), and two studies used area-based estimates of antimony levels in air (Kresovich et al., 2019; White et al., 2019). Collectively, these studies found little evidence for increased risk of breast cancer associated with antimony exposure, and all were found to have limitations in exposure assessment. Two of these studies were considered particularly relevant by the Working Group, because they were conducted in a very large and well-characterized prospective study (the Sister Study) (White et al., 2019; Niehoff et al., 2021). However, in both studies, the ability to detect a positive association was limited by use of a one-time exposure measure and the relatively small range of antimony exposure in the population.

Two general-population studies examined total cancer mortality, a heterogeneous outcome, in relation to a one-time urine antimony concentration (Guo et al., 2016; Duan et al., 2020). One study found no association (Guo et al., 2016), and another found elevated risk associated with urinary antimony in a mixture model, which was not statistically significant after adjusting for multiple comparisons. One case–control study on thyroid cancer measured urine levels after diagnosis (Liu et al., 2021). Thus, studies on cancer of the thyroid and other sites were minimally informative, too few in number, and did not provide consistent evidence for this evaluation.

3. Cancer in Experimental Animals

No data on pentavalent antimony were available to the Working Group. The available data concerned trivalent antimony only.

See Table 3.1.

3.1 Mouse

Antimony(III) oxide

Inhalation

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$/N mice (age, 6 weeks) were exposed by inhalation (whole-body) to aerosols of antimony(III) oxide (purity, > 99.9%; mass median aerodynamic diameter, 0.9–1.5 µm; geometric standard deviation, 1.7–2.2) at a concentration of 0, 3, 10, or 30 mg/m^3 for the control group and at the lowest, intermediate, and highest concentration, respectively, for 6 hours plus T_{90} (12 minutes; the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation) per day, 5 days per week, for up to 105 weeks (NTP, 2017). Survival of males and females at the intermediate and highest concentration was less than that of controls, primarily because of bronchioloalveolar carcinomas and lung inflammation in males, and malignant lymphoma and lung inflammation in females. At study termination, survival was 38/50, 30/50, 27/50, and 17/50 in males, and 36/50, 31/50, 26/50, and 15/50 in females, for the control group and at the lowest, intermediate, and highest concentration, respectively. At the highest concentration, antimony(III) oxide reduced body weights by between 10% and 25% in males (starting from week 73) and by 10% in females (after week 85) compared with controls. Lung burden was assessed at multiple time points in female B6C3F$_1$/N mice. On the basis of measured lung burden and estimation of clearance half-lives, lung overload occurred at 10 and 30 mg/m^3. Lung overload did not occur at 3 mg/m^3. All mice underwent complete necropsy with histopathological evaluation, except one male at the lowest concentration and one female in the control group.

Table 3.1 Studies of carcinogenicity in mice and rats exposed to antimony(III) oxide

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$/N (M) 6 wk 105 wk NTP (2017)	Inhalation (whole-body exposure) Antimony(III) oxide, > 99.9% Air 0, 3, 10, 30 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 38, 30, 27, 17	*Lung* Bronchioloalveolar carcinoma (includes multiple) 4/50 (8%), 18/50 (36%)*, 20/50 (40%)*, 27/50 (54%)* Bronchioloalveolar adenoma or carcinoma (combined) 13/50 (26%), 29/50 (58%)*, 28/50 (56%)*, 34/50 (68%)* Bronchioloalveolar adenoma (includes multiple) 10/50 (20%), 14/50 (28%), 9/50 (18%), 14/50 (28%) *Skin* Fibrous histiocytoma 0/50, 1/50 (2%), 1/50 (2%), 4/50 (8%)* Fibrosarcoma 0/50 (0%), 0/50 (0%), 2/50 (4%), 0/50 (0%) Fibrous histiocytoma or fibrosarcoma (combined) 0/50, 1/50 (2%), 3/50 (6%), 4/50 (8%)*	$P < 0.001$, poly-3 trend test [Cochran–Armitage trend test] *$P < 0.001$, poly-3 test $P < 0.001$, poly-3 trend test [Cochran–Armitage trend test] *$P < 0.001$, poly-3 test NS $P = 0.012$, poly-3 trend test *$P = 0.039$, poly-3 test NS $P = 0.023$, poly-3 trend test *$P = 0.039$, poly-3 test	Principal strengths: GLP study; males and females used; adequate duration of exposure and observation; multiple doses used; and adequate number of animals per group. Other comments: MMAD, 0.9–1.5 µm; GSD, 1.7–2.2. Historical controls: Bronchioloalveolar adenoma: inhalation studies 35/250 (14.0% ± 3.7%), range, 10–20%; all routes 83/550 (15.1% ± 5.9%), range, 8–26%. Bronchioloalveolar carcinoma: inhalation studies 42/250 (16.8% ± 5.4%), range, 8–22%; all routes 75/550 (13.6% ± 6.4%), range, 4–22%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 69/250 (27.6% ± 2.6%), range, 26–32%; all routes 147/550 (26.7% ± 6.5%), range, 16–38%. Skin, fibrous histiocytoma and fibrosarcoma: inhalation studies 1/250 (0.4% ± 0.9%), range, 0–2%; all routes 2/550 (0.4% ± 0.8%), range, 0–2%. Skin, fibrous histiocytoma or fibrosarcoma (combined): inhalation studies 2/250 (0.8% ± 1.1%), range, 0–2%; all routes 5/550 (0.9% ± 1.0%), range, 0–2% (includes one sarcoma).

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$/N (F) 6 wk 105 wk NTP (2017)	Inhalation (whole-body exposure) Antimony(III) oxide, > 99.9% Air 0, 3, 10, 30 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 36, 31, 26, 15	*Lung* Bronchioloalveolar adenoma (includes multiple) 1/50 (2%), 10/50 (20%)*, 19/50 (38%)**, 8/50 (16%)*	[$P < 0.001$, Cochran–Armitage trend test] *$P < 0.01$, poly-3 test **$P \leq 0.001$, poly-3 test	Principal strengths: GLP study; males and females used; adequate duration of exposure and observation; multiple doses used; and adequate number of animals per group. Other comments: MMAD, 0.9–1.5 µm; GSD, 1.7–2.2. Historical controls: Bronchioloalveolar adenoma: inhalation studies 12/249 (4.8% ± 2.7%), range, 2–8%; all routes, 27/549 (4.9% ± 3.5%), range, 0–10%. Bronchioloalveolar carcinoma: inhalation studies 17/249 (6.8% ± 3.7%), range, 2–10%; all routes 24/549 (4.4% ± 3.5%), range, 0–10%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 28/249 (11.3% ± 5.5%), range, 6–18%; all routes 50/549 (9.1% ± 5.2%), range, 2–18%. All organs, malignant lymphoma (all organs): inhalation studies 63/250 (25.2% ± 8.4%), range, 14–36%; all routes 109/550 (19.8% ± 7.9%), range, 12–36%. Skin, squamous cell carcinoma of skin: inhalation studies 0/250; all routes, 0/550.
		Bronchioloalveolar carcinoma (includes multiple) 2/50 (4%), 14/50 (28%)**, 11/50 (22%)*, 11/50 (22%)*	[$P = 0.016$, Cochran–Armitage trend test] **$P < 0.001$, poly-3 test *$P \leq 0.003$, poly-3 test	
		Bronchioloalveolar adenoma or carcinoma (combined) 3/50 (6%), 22/50 (44%)*, 27/50 (54%)*, 18/50 (36%)*	$P = 0.019$, poly-3 trend test *$P < 0.001$, poly-3 test	
		All organs Malignant lymphoma 7/50 (14%), 17/50 (34%)*, 20/50 (40%)**, 27/50 (54%)**	$P < 0.001$, poly-3 trend test *$P = 0.013$, poly-3 test **$P < 0.001$, poly-3 test	
		Skin Squamous cell carcinoma 0/50, 0/50, 0/50, 2/50	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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar Han[Crl:WI(Han)] (M) 6 wk 105 wk NTP (2017)	Inhalation (whole-body exposure) Antimony(III) oxide, > 99.9% Air 0, 3, 10, 30 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 30, 30, 28, 18	*Lung* Bronchioloalveolar adenoma (includes multiple) 3/50 (6%), 4/50 (8%), 6/50 (12%), 8/50 (16%) Bronchioloalveolar carcinoma 0/50, 0/50, 2/50, 0/50 Bronchioloalveolar adenoma or carcinoma (combined) 3/50 (6%), 4/50 (8%), 8/50 (16%), 8/50 (16%) *Adrenal medulla* Benign pheochromocytoma 1/49 (2%), 0/50 (0%), 2/49 (4%), 7/50 (14%)*	NS NS NS $P < 0.001$, poly-3 trend test *$P = 0.030$, poly-3 test	Principal strengths: GLP study; males and females used; adequate duration of exposure and observation; multiple doses used; and adequate number of animals per group. Other comments: MMAD, 0.9–1.5 µm; GSD, 1.7–2.2. Historical controls: Bronchioloalveolar adenoma and bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 4/150 (2.7% ± 3.1%), range, 0–6%; all routes 4/299 (1.3% ± 2.4%), range, 0–6%. Bronchioloalveolar carcinoma: inhalation studies 0/150; all routes 0/299. Adrenal medulla, benign pheochromocytoma: inhalation studies 5/149 (3.4% ± 4.2%), range, 0–8%; all routes 6/297 (2.0% ± 3.1%), range 0–8%.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar Han[Crl:WI(Han)] (F) 6 wk 105 wk NTP (2017)	Inhalation (whole-body exposure) Antimony(III) oxide, > 99.9% Air 0, 3, 10, 30 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 39, 38, 28, 20	*Lung* Bronchioloalveolar adenoma (includes multiple) 0/50 (0%), 2/50 (4%), 6/50 (12%)*, 5/50 (10%)* Cystic keratinizing epithelioma or squamous cell carcinoma (combined) 0/50, 0/50, 0/50, 3/50 (6%) Cystic keratinizing epithelioma 0/50, 0/50, 0/50, 2/50 Squamous cell carcinoma 0/50, 0/50, 0/50, 1/50 *Adrenal medulla* Benign pheochromocytoma 0/49, 2/49 (4%), 2/49 (4%), 6/50 (12%)* Benign or malignant pheochromocytoma (combined) 0/49, 2/49 (4%), 2/49 (4%), 7/50 (14%)*	$P = 0.029$, poly-3 trend test *$P \leq 0.021$, poly-3 test NS NS $P = 0.004$, poly-3 trend test *$P = 0.009$, poly-3 test $P < 0.001$, poly-3 trend test *$P = 0.004$, poly-3 test	Principal strengths: GLP study; males and females used; adequate duration of exposure and observation; multiple doses used; and adequate number of animals per group. Other comments: MMAD, 0.9–1.5 µm; GSD, 1.7–2.2. Historical controls: Bronchioloalveolar adenoma, cystic keratinizing epithelioma, or squamous cell carcinoma (combined) of the lung: inhalation studies 0/150; all routes 0/300. Adrenal medulla, benign pheochromocytoma: inhalation studies 1/148 (0.7% ± 1.2%), range, 0–2%; all routes 5/297 (1.7% ± 1.5%), range, 0–4%. Adrenal medulla, benign or malignant (combined) pheochromocytoma: inhalation studies 2/148 (1.4% ± 2.4%), range, 0–4%; all routes 7/297 (2.4% ± 2.0%), range, 0–4%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar-derived albino (M) 8 mo 71–73 wk Groth et al. (1986)	Inhalation (whole-body exposure) Antimony(III) oxide, 80% antimony (contaminants including: titanium, < 3%; tin, 0.2%; lead, 0.2%; cerium, 0.014%; arsenic, 0.004%) Air 0, 45.0–46.0 mg/m^3 (TWA) 7 h/day, 5 days/wk, for 52 wk 75, 75 22, 22	*Thyroid gland* Total tumours 12/84, 15/83 *Pituitary gland* Total tumours 14/59, 15/62	NS NS	Principal strengths: high number of animals per group; males and females used; adequate duration of exposure and observation. Principal limitations: statistical test NR, only one dose tested; number of deaths and number killed not clearly separated; tumour status not always accurately stated. Other comments: survival data were given for animals (75 male and 75 females per group) designated for the core cohort scheduled for removal 20 wk after the final exposure; tumour data were presented for all study animals, which includes the core group and 15 interim removals (5 males and 5 females each at 6, 9, and 12 mo).

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar-derived albino (F) 8 mo 71–73 wk Groth et al. (1986)	Inhalation (whole-body exposure) Antimony(III) oxide, 80% antimony (contaminants including: titanium, < 3%; tin, 0.2%; lead, 0.2%; arsenic, 0.004%) Air 0, 45.0–46.0 mg/m^3 (TWA) 7 h/day, 5 days/wk for 52 wk 75, 75 39, 31	*Lung* Total tumours 0/89, 19/89* Squamous cell carcinoma 0/89, 9/89* Scirrhous adenocarcinoma 0/89, 5/89* Bronchioloalveolar adenoma or carcinoma (combined) 0/89, 11/89* Total tumours (animals examined in weeks 41–72) 0/69, 19/70* *Thyroid gland* Total tumours 5/86, 6/88 *Pituitary gland* Total tumours 46/81, 50/77	 *$P \leq 0.001$, test NR [$P < 0.0001$, Fisher exact test] *[$P = 0.0016$, Fisher exact test] *[$P = 0.0295$, Fisher exact test] *[$P = 0.0004$, Fisher exact test] *$P \leq 0.001$, test NR [$P < 0.0001$, Fisher exact test] NS NS	Principal strengths: high number of animals per group; males and females used; adequate duration of exposure and observation. Principal limitations: statistical test NR; only one dose used; number of deaths and number killed not clearly separated; tumour status not always accurately stated. Other comments: survival data were given for animals (75 males and 75 females per group) designated for the core cohort scheduled for removal 20 wk after the final exposure; tumour data were presented for all study animals, which includes the core group and 15 interim removals (5 males and 5 females each at 6, 9, and 12 mo); the lung tumour types included squamous cell carcinoma, bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and scirrhous adenocarcinoma.
Full carcinogenicity Rat, F344 (CDF F344 Crl BR) (M) 8 wk 24 mo Newton et al. (1994)	Inhalation (whole-body exposure) Antimony(III) oxide, 99.68 ± 0.10% Air 0, 0.06, 0.51, 4.50 mg/m^3 6 h/day, 5 days/wk for 52 wk 52, 52, 53, 52 [56–58%], [56–58%], [58%], [56%]	*Lung* Pulmonary carcinoma 1/52, 0/52, 0/53, 1/52	 [NS]	Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; adequate number of animals per group. Other comments: sections of the heart, nasal turbinates, larynx, trachea, lung, and peribronchial lymph nodes, from all animals, were examined histologically; exact number of surviving animals NR.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344 (CDF F344 Crl BR) (F) 8 wk 24 mo Newton et al. (1994)	Inhalation (whole-body exposure) Antimony(III) oxide, 99.68 ± 0.10% Air 0, 0.06, 0.51, 4.50 mg/m^3 6 h/day, 5 days/wk for 52 wk 49, 52, 54, 50 [48%], [40%], [66%], [60%]	*Lung* Pulmonary carcinoma 0/49, 0/52, 1/54, 0/50	[NS]	Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; adequate number of animals per group. Other comments: sections of the heart, nasal turbinates, larynx, trachea, lung, and peribronchial lymph nodes, from all animals, were examined histologically; exact number of surviving animals, NR.
Full carcinogenicity Rat, F344 (CDF) (F) 19 wk 25–28 mo (12–15 mo observation period post-exposure) Watt (1983)	Inhalation (whole-body exposure) Antimony(III) oxide, 99.4% (major impurities, 0.02% arsenic, 0.2% lead) Air 0, 1.6, 4.2 mg/m^3 6 h/day, 5 days/wk for 13 mo 42, 44, 45 NR, NR, NR	*Lung* All tumours 1/13, 1/17, 14/18* Scirrhous adenocarcinoma 0/13, 0/17, 9/18* Squamous cell carcinoma 0/13, 0/17, 2/18 Bronchioloalveolar adenoma 0/13, 1/17, 3/18	[$P < 0.001$, Cochran–Armitage trend test] *$P < 0.01$, test NR [$P < 0.0001$, Fisher exact test] [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.01$, test NR [$P < 0.003$, Fisher exact test] NS NS	Principal limitations: the use of one sex only; two rats per inhalation cage; rats treated in the same room as miniature swine; statistical test NR; and number of rats intentionally killed or number of early deaths in the groups NR. Other comments: number at start is the effective number of animals; data reported for the animals killed 12–15 mo after the end of exposure.

F, female; GLP, Good Laboratory Practice; GSD, geometric standard deviation; M, male; min, minute; MMAD, mass median aerodynamic diameter; mo, month; NR, not reported; NS, not significant; T_{90}, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation; TWA, time-weighted average; wk, week.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar carcinoma and of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$, poly-3 test [$P < 0.001$, Cochran–Armitage test]). The incidence of bronchioloalveolar carcinoma (control, 4/50; lowest concentration, 18/50; intermediate concentration, 20/50; and highest concentration, 27/50), and of bronchioloalveolar adenoma or carcinoma (combined) (control, 13/50; lowest concentration, 29/50; intermediate concentration, 28/50; and highest concentration, 34/50), was significantly increased in all treated groups ($P < 0.001$, poly-3 test). The incidence of bronchioloalveolar adenoma at the lowest and the highest concentration – both 14/50 (28%) – exceeded the upper bound of the range observed in historical controls from this laboratory: inhalation, 35/250 (14.0% ± 3.7%); range, 10–20%; and all routes, 83/550 (15.1% ± 5.9%); range 8–26%. There was a significant positive trend in the incidence of fibrous histiocytoma of the skin ($P = 0.012$, poly-3 test). The incidence of fibrous histiocytoma of the skin – control, 0/50; lowest concentration, 1/50 (2%); intermediate concentration, 1/50 (2%); and highest concentration, 4/50 (8%) – was significantly increased ($P = 0.039$, poly-3 test) at the highest concentration, and exceeded the upper bound of the range observed in historical controls from this laboratory: inhalation, 1/250 (0.4% ± 0.9%); range, 0–2%; and all routes, 2/550 (0.4% ± 0.8%); range 0–2%. There was a significant positive trend in the incidence of fibrous histiocytoma or fibrosarcoma (combined) of the skin ($P = 0.023$, poly-3 test). The incidence of fibrous histiocytoma or fibrosarcoma (combined) of the skin was significantly increased ($P = 0.039$, poly-3 test) at the highest concentration compared with controls – control, 0/50; lowest concentration, 1/50 (2%); intermediate concentration, 3/50 (6%); and highest concentration, 4/50 (8%) – and exceeded the upper bound of the range (at the intermediate and highest concentration) observed in historical controls from this laboratory: inhalation, 2/250 (0.8% ± 1.1%); range, 0–2%; and all routes, 5/550 (included one sarcoma) (0.9% ± 1.0%); range, 0–2%. The incidence of fibrosarcoma of the skin at the intermediate concentration – 2/50 (4%) – exceeded the upper bound of the range observed in historical controls from this laboratory: inhalation, 1/250 (0.4% ± 0.9%); range, 0–2%; and all routes, 2/550 (0.4% ± 0.8%); range, 0–2%.

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiple) [$P < 0.001$, Cochran–Armitage test], of bronchioloalveolar carcinoma (includes multiple) [$P = 0.016$, Cochran–Armitage test], and of bronchioloalveolar adenoma or carcinoma (combined) ($P = 0.019$, poly-3 test). The incidence of bronchioloalveolar adenoma (control, 1/50; lowest concentration, 10/50; intermediate concentration, 19/50; and highest concentration, 8/50), of bronchioloalveolar carcinoma (control, 2/50; lowest concentration, 14/50; intermediate concentration, 11/50; and highest concentration, 11/50), and of bronchioloalveolar adenoma or carcinoma (combined) (control, 3/50; lowest concentration, 22/50; intermediate concentration, 27/50; and highest concentration, 18/50), was significantly increased in all exposed groups of females ($P < 0.01$, poly-3 test). There was a significant positive trend in the incidence of malignant lymphoma ($P < 0.001$, poly-3 test). The incidence of malignant lymphoma was significantly increased ($P \leq 0.013$, poly-3 test) in all treated groups – control, 7/50 (14%); lowest concentration, 17/50 (34%); intermediate concentration, 20/50 (40%); highest concentration, 27/50 (54%) – exceeding the upper bound of the range (at the intermediate and high concentration) in historical controls from this laboratory: inhalation, 63/250 (25.2% ± 8.4%); range, 14–36%; and all routes, 109/550 (19.8% ± 7.9%); range, 12–36%. The incidence of squamous cell carcinoma of the skin at the highest concentration – 2/50 (4%) – exceeded the incidence in historical controls

from this laboratory (inhalation, 0/250; and all routes, 0/550).

Regarding non-neoplastic lesions of the lung, there was a significant increase in the incidence of hyperplasia of the alveolar epithelium and of bronchiolar epithelium in all exposed groups of males and females (NTP, 2017). [The Working Group noted that this was a well-conducted GLP study using an adequate number of mice per group, both sexes, multiple concentration groups, and an adequate duration of exposure and observation.]

3.2 Rat

3.2.1 Antimony(III) oxide

Inhalation

In a well-conducted study that complied with GLP, groups of 50 male and 50 female Wistar Han [Crl:WI(Han)] rats (age, 6 weeks) were exposed by inhalation (whole-body) to aerosols of antimony(III) oxide (purity > 99.9%; mass median aerodynamic diameter, 0.9–1.5 µm; geometric standard deviation, 1.7–2.2) at a concentration of 0, 3, 10, or 30 mg/m^3 for the control group and at the lowest, intermediate, and highest concentration, respectively, for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks or less (NTP, 2017). Survival of females at the intermediate and highest concentration was less than that of controls, primarily because of lung proteinosis. In males, the negative trend in survival was primarily attributed to lung inflammation and proteinosis. At study termination, survival was 30/50, 30/50, 28/50, and 18/50 in males, and 39/50, 38/50, 28/50, and 20/50 in females, for the control group and at the lowest, intermediate, and highest concentration, respectively. Body weight of males at the highest concentration was less (10% or more) than that of controls after week 69 until the end of the study. Body weight of females at the lowest, intermediate, and highest concentration was 10% or less than that of controls by weeks 99, 81, and 65, respectively; and females at the intermediate and highest concentration continued to lose body weight (20% and 28% less than controls, respectively) until the end of the study. Lung burden was assessed at multiple time points for female rats. On the basis of measured lung burden and estimation of clearance half-lives, lung overload occurred at 10 and 30 mg/m^3. Lung overload did not occur at 3 mg/m^3. All rats underwent complete necropsy with histopathological evaluation (NTP, 2017).

In male rats, there was no significant increase in the incidence of lung tumours in treated rats compared with controls. The incidence of bronchioloalveolar adenoma (includes multiple) – 3/50 (6%), 4/50 (8%), 6/50 (12%), and 8/50 (16%) – and bronchioloalveolar adenoma or carcinoma (combined) – 3/50 (6%), 4/50 (8%), 8/50 (16%), and 8/50 (16%) – for the control group and at the lowest, intermediate, and highest concentration, respectively, exceeded the upper bound of the range observed in historical controls for bronchioloalveolar adenoma and brochioloalveolar adenoma or carcinoma (combined) from this laboratory: inhalation, 4/150 (2.7% ± 3.1%); range, 0–6%; and all routes, 4/299 (1.3% ± 2.4%); range, 0–6%. The incidence of bronchioloalveolar carcinoma at the intermediate concentration – 2/50 (4%) – exceeded the incidence observed in historical controls from this laboratory: inhalation, 0/150; all routes, 0/299. There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla in males ($P < 0.001$, poly-3 test), with the incidence – control, 1/49 (2%); lowest concentration, 0/50; intermediate concentration, 2/49 (4%); and highest concentration, 7/50 (14%) – being significantly increased ($P = 0.030$, poly-3 test) at the highest concentration, and exceeding the upper bound of the range observed in historical controls from this laboratory – inhalation, 5/149 (3.4% ± 4.2%); range, 0–8%; and all routes, 6/297 (2.0% ± 3.1%); range, 0–8%.

In female rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiple) (P = 0.029, poly-3 test). The incidence of bronchioloalveolar adenoma – control, 0/50; lowest concentration, 2/50 (4%); intermediate concentration, 6/50 (12%); and highest concentration, 5/50 (10%) – was significantly increased in groups at the intermediate and highest concentration ($P \leq 0.021$, poly-3 test), exceeding the incidence in historical controls from this laboratory: inhalation, 0/150; all routes, 0/300. In females at the highest concentration there was also one squamous cell carcinoma (incidence, 1/50) and two cystic keratinizing epitheliomas of the lung (incidence, 2/50). There was a significant positive trend in the incidence of cystic keratinizing epithelioma or squamous cell carcinoma (combined) ($P = 0.006$, poly-3 trend test), and incidence exceeded that in historical controls from this laboratory: inhalation, 0/150; all routes, 0/300. There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla and of benign or malignant pheochromocytoma (combined) of the adrenal medulla in female rats ($P = 0.004$ and $P < 0.001$, respectively; poly-3 test). [The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess based only on histomorphology (see Patterson et al., 1995; Thompson, 2002).] The incidence of benign pheochromocytoma of the adrenal medulla – 0/49, 2/48 (4%), 2/49 (4%), and 6/50 (12%) – and of benign or malignant pheochromocytoma (combined) – 0/49, 2/49 (4%), 2/49 (4%), and 7/50 (14%) – for the control group and at the lowest, intermediate, and highest concentration, respectively, was significantly increased at the highest concentration (P = 0.009 and P = 0.004, respectively; poly-3 test). This exceeded the upper bound of the range observed in historical controls from this laboratory: for benign pheochromocytoma of the adrenal medulla, inhalation, 1/148 (0.7% ± 1.2%); range, 0–2%; and all routes, 5/297 (1.7% ± 1.5%); and for benign or malignant pheochromocytoma (combined), inhalation, 2/148 (1.4% ± 2.4%); range, 0–4%; and all routes, 7/297 (2.4% ± 2.0%); range, 0–4% (NTP, 2017).

Regarding non-neoplastic lesions, there was a significant increase in the incidence of alveolar epithelial hyperplasia of the lung, bronchiolar epithelial hyperplasia of the lung, respiratory epithelium hyperplasia of the nasal cavity, and medullary hyperplasia of the adrenal gland in exposed males and females (NTP, 2017). [The Working Group noted that this was a well-conducted GLP study using an adequate number of rats per group, both sexes, multiple concentration groups, and an adequate duration of exposure and observation.]

In another study, groups of 90 male and 90 female Wistar rats (age, 8 months) were exposed by inhalation (whole-body) to antimony(III) oxide (80% antimony; with contaminants including: titanium, < 3%; tin, 0.2%; lead, 0.2%; cerium, 0.014%; and arsenic, 0.004%) at a concentration of 0 (control) or 45.0–46.0 mg/m^3 (TWA) for 7 hours per day, 5 days per week, for 52 weeks (exposures did not occur on a few days, because of holidays or mechanical breakdowns) (Groth et al., 1986). Groups of 5 males and 5 females were killed at 6, 9, and 12 months after initiation of the exposure. The remaining rats were killed 18–20 weeks after exposure ended. There was no difference in survival between groups of treated male or female rats and their respective controls. Mean body weights were consistently decreased in treated males after 26 weeks of exposure. A total of 22 control males, 22 treated males, 39 control females, and 31 treated females were killed at the end of the study (at 71–73 weeks). The total numbers of rats killed between weeks 0 and 73 were 84 control males, 83 treated males, 86 control females, and 88 treated females.

In female rats, the first lung tumours were seen in 2 out of 5 treated rats (one adenoma and one squamous cell carcinoma) killed at 53 weeks.

A total of 19/70 (27%; $P \leq 0.001$ [statistical test not reported] [$P < 0.0001$, Fisher exact test]) treated females examined between weeks 41 and 72 developed lung tumours, compared with 0/69 controls. The incidence of lung tumours in treated females was: squamous cell carcinoma, 9/89 [$P = 0.0016$, Fisher exact test]; scirrhous carcinoma [scirrhous adenocarcinoma], 5/89 [$P = 0.0295$, Fisher exact test]; and bronchioloalveolar adenoma or carcinoma (combined) [incidence of adenoma and incidence of carcinoma was unspecified], 11/89 [$P = 0.0004$, Fisher exact test], compared with 0/89 controls.

No lung tumours were observed in treated males or in any male or female controls. The incidence of tumours in tissues or organs other than the lung was not significantly different in treated and control rats of either sex (Groth et al., 1986). [The Working Group noted that statistical tests were used but analyses were undefined, only one concentration was tested, the number of deaths and number killed were not clearly separated, and tumour status was not always accurately stated. The control groups of males and females were the same as in the study on antimony ore concentrate by the same authors (see below).]

In another study, groups of approximately 50 male and 50 female Fischer 344 (CDF F344 Crl BR) rats (age, 8 weeks) were exposed by inhalation (whole-body) to antimony(III) oxide dust (purity, 99.68% ± 0.10%; mass median aerodynamic diameter, mean ± SD, 3.76 ± 0.84 μm; geometric standard deviation, 1.79 ± 0.32) at a concentration of 0, 0.06, 0.51, or 4.50 mg/m^3 for the control group and at the lowest, intermediate, and highest concentration, respectively, for 6 hours per day, 5 days per week, for 52 weeks, followed by a 12-month observation period (Newton et al., 1994). There were about 50 rats per group (males: 52, 52, 53, and 52; and females: 49, 52, 54, and 50; for the control group and at the lowest, intermediate, and highest concentration, respectively). Numerical values for survival were not reported, but survival [read from figures] was 56–58%, 56–58%, 58%, and 56% for males, and 48%, 40%, 66%, and 60% for females in the control group and at the lowest, intermediate, and highest concentration, respectively. Body weights of females and males were unaffected by exposure. All survivors were killed at 24 months (12 months post-exposure) and complete gross examinations were performed.

Three lung carcinomas were reported, two in males (one in the controls and one in the group at the highest concentration) and one in females (at the intermediate concentration). These tumours were assessed as comparable in incidence in all groups and were within the range reported for historical controls. No other primary neoplasms were seen in the lungs of treated rats. Microscopic changes in the lungs after treatment were limited to interstitial inflammation, increased alveolar macrophages, perivascular lymphoid aggregates, fibrosis, and foreign material (Newton et al., 1994). [The Working Group noted that this study used an adequate number of rats per group, both sexes, multiple concentrations, and an adequate duration of exposure and observation, but the numbers of rats at study termination was not reported.]

In another study reported in a doctoral dissertation, groups of female Fischer 344 (CDF) rats (age, 19 weeks) were exposed by inhalation (whole-body) to antimony(III) oxide dust (purity, 99.4%; arsenic, 0.02%; lead, 0.2%) at a concentration of 0, 1.6, or 4.2 mg/m^3 (as antimony), for the control group and at the lower and higher concentration, respectively, for 6 hours per day, 5 days per week, for 13 months or less, followed by a 12–15-month observation period without exposure (Watt, 1983). Group sizes were 42, 44, and 45 for the control group and at the lower and higher concentration, respectively. Mean body weight in treated groups at start of the study was slightly higher than that of controls but, after exposure and towards the end of the study, it was similar to that of controls. Small groups of rats [number of rats per group not reported]

were either killed or died [not clearly specified], and histopathology was assessed after 3, 6, 9, or 12 months of exposure. Groups of rats [number of rats per group not reported] were also killed 2 months after the end of exposure and at the end of the 12-month post-exposure period. However, the largest groups were killed at 12–15 months after the end of treatment.

In the rats killed 12–15 months after the end of exposure, there was a significant positive trend in the incidence of lung tumours [$P < 0.001$, Cochran–Armitage test]. The incidence of lung tumours (control group, 1/13; lower concentration, 1/17; and higher concentration, 14/18) was significantly increased at the higher concentration ($P < 0.01$ [statistical test not reported; $P < 0.0001$, Fisher exact test]). There was a significant positive trend in the incidence of scirrhous carcinoma [scirrhous adenocarcinoma] of the lung [$P < 0.001$, Cochran–Armitage test]. The incidence of scirrhous carcinoma (control group, 0/13; lower concentration, 0/17; and higher concentration, 9/18) was significantly increased in rats at the higher concentration ($P < 0.01$ [statistical test not reported; $P < 0.003$, Fisher exact test]). The incidence of scirrhous carcinoma was also increased in rats at the higher concentration that died or were killed between 2 months after the end of exposure and at the end of the 12-month post-exposure period (control, 0/6; higher concentration, 5/7) ($P < 0.05$ [test not reported; $P < 0.02$, Fisher exact test]) (Watt, 1983). [The Working Group noted that only female rats were used, there were 2 rats per inhalation cage, rats were treated in the same room as miniature swine, the statistical analyses used were not specified, and the numbers of rats intentionally killed and numbers of early deaths were not reported.]

3.2.2 Antimony ore concentrate

Inhalation

Groups of 90 male and 90 female Wistar rats (age, 8 months) were exposed by inhalation (whole-body) to antimony ore concentrate (antimony, 46%; titanium, < 4%; aluminium, 0.5%; tin, 0.2%; lead, 0.3%; iron, 0.3%; arsenic, 0.08%) [the Working Group noted that the antimony ore was principally antimony(III) sulfide; however, the amount of antimony(III) sulfide contained in the test agent was not reported] at a concentration of 0 (control) or 36.0–40.1 mg/m^3 (TWA) for 7 hours per day, 5 days per week, for 52 weeks (Groth et al., 1986). Groups of 5 males and 5 females were killed at 6, 9, and 12 months after initiation of the exposure. The remaining rats were killed between 18 and 20 weeks after the end of exposure. There was no difference in survival between the groups treated with antimony ore concentrate and the control groups for either sex. Body weights were reduced from week 26 onwards in females treated with the ore concentrate. [The Working Group noted that only one concentration was tested, statistical tests were used but analyses undefined, the numbers of deaths and numbers killed were not clearly separated, and tumour status was not always accurately stated. The control groups of males and females were the same as those used in the study on antimony(III) oxide by the same authors (see above). In addition, the Working Group noted that the test article characterization did not specify the amount of antimony forms (e.g. antimony(III) sulfide) within the ore concentrate; therefore, the Working Group considered that the study was inadequate for the evaluation of the carcinogenicity of trivalent antimony in experimental animals.]

3.3 Evidence synthesis for cancer in experimental animals

3.3.1 Pentavalent antimony

No data on pentavalent antimony were available to the Working Group.

3.3.2 Trivalent antimony

(a) Antimony(III) oxide

The carcinogenicity of antimony(III) oxide has been assessed in one well-conducted study that complied with GLP in male and female mice exposed by inhalation (whole-body), in one well-conducted study that complied with GLP in male and female rats exposed by inhalation (whole-body) (NTP, 2017), and in three additional studies: two in male and female rats (Groth et al., 1986; Newton et al., 1994) and one in female rats (Watt, 1983) exposed by inhalation (whole-body).

In a well-conducted study that complied with GLP in male and female B6C3F$_1$/N mice exposed to antimony(III) oxide by inhalation (whole-body), there was a significant increase, with a significant positive trend, in the incidence of bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined), in males and females; of fibrous histiocytoma of the skin, and fibrous histiocytoma or fibrosarcoma (combined) of the skin, in males; and of bronchioloalveolar adenoma, and malignant lymphoma (all organs), in females (NTP, 2017).

In a well-conducted study that complied with GLP in male and female Wistar rats exposed to antimony(III) oxide by inhalation (whole-body), there was a significant increase, with a significant positive trend, in the incidence of bronchioloalveolar adenoma, benign pheochromocytoma of the adrenal medulla, and benign or malignant pheochromocytoma (combined) of the adrenal medulla in females, and of benign pheochromocytoma of the adrenal medulla in males. There was also a significant positive trend in the incidence of cystic keratinizing epithelioma or squamous cell carcinoma (combined) of the lung in females (NTP, 2017).

In a study in male and female Wistar rats exposed to antimony(III) oxide by inhalation (whole-body), there was a significant increase in the incidence of squamous cell carcinoma, scirrhous adenocarcinoma, and bronchioloalveolar adenoma or carcinoma (combined) of the lung in exposed females (Groth et al., 1986).

In a study in female Fischer 344 (CDF) rats exposed to antimony(III) oxide by inhalation (whole-body), there was a significant increase in the incidence of lung tumours and of scirrhous adenocarcinoma of the lung (Watt, 1983).

In a study in male and female Fischer 344 (CDF) rats exposed to antimony(III) oxide by inhalation (whole-body), there was no significant increase in the incidence of tumours (Newton et al., 1994).

(b) Antimony ore concentrate

A study in male and female Wistar rats exposed to antimony ore concentrate by inhalation (whole-body exposure) was considered inadequate for the evaluation of the carcinogenicity of trivalent antimony in experimental animals (Groth et al., 1986).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption and distribution

(a) Humans

(i) Inhalation

Trivalent antimony

A study by Wu & Chen (2017) reported on the absorption and distribution of antimony in 91 male workers from various manufacturing plants who were exposed to antimony(III) oxide or sodium antimonite in Taiwan, China, with 42 male administrators as a control group. Antimony distributed to blood, hair, and urine. The levels were higher in workers than in the control group and were correlated with antimony concentrations in the air of work sites (see also Sections 1.4.2 and 1.6.2). (Wu & Chen, 2017). Kentner et al. (1995) examined the levels of antimony in blood and urine samples from workers involved in lead battery production. Among 7 workers from the casting area exposed to antimony(III) oxide and 14 workers from the formation area exposed to both stibine and antimony(III) oxide (all men), the median levels of antimony in personal air samples were 4.5 and 12.4 μg/m^3, respectively. Both antimony compounds exhibited similar pulmonary absorption and renal elimination. The median blood and urinary antimony concentrations were 2.6 μg/L and 3.9 μg/g creatinine, and 10.1 μg/L and 15.2 μg/g creatinine, for the formation workers and casters, respectively (Kentner et al., 1995). Measurements of antimony in blood were 5–10 times as high in a group of port workers exposed to trivalent antimony from heavyweight vehicular traffic as in workers in two control groups. [The Working Group noted that exposure levels were not provided.] More than 60% antimony was found in the erythrocyte fractions of blood samples in all three groups (Quiroz et al., 2009).

In a group of seven workers accidentally exposed to radioactive antimony (^{125}Sb) oxide aerosols, antimony was found to be mainly accumulated in the lungs (Garg et al., 2003).

Metallic antimony

Gerhardsson et al. (1982) reported tissue concentrations of antimony in the lung, liver, and kidney from a group of 40 deceased smelter workers in Sweden compared with a control group of 11 deceased people without occupational exposure. The median value of antimony in lung tissue from smelter workers was 315 μg/kg, 12 times that in controls. For lung tissue, there was no trend towards decreased antimony concentrations with time after cessation of exposure, indicating long-term retention. Antimony concentrations in the liver and kidney of workers did not differ significantly from those of controls (Gerhardsson et al., 1982). The distributions and concentrations of 27 trace elements, including antimony, in human lung and lymph nodes were investigated in eight deceased individuals who were assumed to have been exposed to airborne dust. [The Working Group noted that exposure information was not provided.] While some elements were homogeneously distributed over a lung pair and showed few differences in interindividual concentrations, antimony and a few other elements were not homogeneously distributed. The content of antimony in hilar lymph nodes (60–479 ng/g wet tissue) was higher than that in lung (3.5–48.2 ng/g wet tissue). The non-homogeneity and the concentration increased with age, suggesting the accumulation of atmospheric contaminants (Vanoeteren et al., 1986a, b, c).

(ii) Other exposure routes

Trivalent antimony

Accidental ingestion of antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$; 0.85–2.5 g) was reported for four patients; in three patients, the blood antimony levels increased during the first 12 hours; in one patient, the peak level was reached on admission. Antimony content was measured in one deceased individual, and high concentrations were found in the liver, gall bladder, and gastrointestinal tract. The estimated total body pool of antimony was 15–20 mg, which was a very small portion of the ingested dose (Lauwers et al., 1990). In one woman who intentionally ingested an unknown quantity of antimony(III) sulfide, concentrations of antimony in blood and urine remained elevated (blood, > 0.1 µg/100 mL; urine, > 1 µg/g creatinine) 1 week after ingestion, whereas antimony was undetectable in the bile and gastric fluid 100 hours after ingestion (Bailly et al., 1991).

Pentavalent antimony

In five healthy adult volunteers, after a single intramuscular injection of antimony(V) (as the drug Ulamina) at a dose of 5 mg/kg bw, the mean plasma concentration of total antimony [antimony(III) plus antimony(V)] after 15 minutes was 0.59 µg/mL, the C_{max} was 1.1 µg/mL, and the T_{max} was 1.3 hours (Vásquez et al., 2006).

Metallic antimony

A study aiming to establish a reference level for antimony in Irish infants showed the presence of low concentrations of antimony in the serum (0.09–0.25 µg/L) and urine (median, 0.42 ng/mg creatinine; upper 95% centile, 2.6 ng/mg creatinine) of 100 healthy infants (Cullen et al., 1998). Another study obtained a reference profile of 60 elements in 68 healthy Chinese men with death recorded as due to accidents but without obvious antimony exposure history. The lung showed the highest antimony concentration, followed by the liver, thyroid, and stomach (Zhu et al., 2010).

(b) Experimental systems

(i) Inhalation

Trivalent antimony

Both Newton et al. (1994) and the National Toxicology Program (NTP) (NTP, 2017) conducted studies of subchronic and chronic toxicity after inhalation of trivalent antimony.

In the study by Newton et al. (1994), groups of 50 male and 50 female Fischer 344 rats were exposed to antimony(III) oxide by inhalation (whole-body) at a concentration of 0 (control), 0.25, 1.08, 4.92, or 23.46 mg/m^3 for 6 hours per day, 5 days per week for 13 weeks, followed by a 27-week recovery period. In the lung, there was an initial rapid accumulation phase, followed by a second slower accumulation phase after 2–4 weeks. No steady-state level of antimony was reached. In the study of chronic toxicity, groups of 65 male and 65 female rats were exposed to antimony(III) oxide at a concentration of 0 (control), 0.06, 0.51, or 4.50 mg/m^3 for 12 months, followed by a 12-month recovery period. Elevated antimony concentrations were detected in erythrocytes, but not in the plasma. Antimony was present in the faeces. Lung burdens increased with increasing exposure concentration, and steady-state levels were reached 6 months after exposure. Pulmonary clearance of antimony was dependent on lung burden. For example, after the 12-month treatment, antimony lung burdens in male rats were 11.5, 132.0, and 1420 µg/g in the 0.06, 0.51, and 4.50 mg/m^3 exposure groups, respectively, and decreased to 0.4, 8.1, and 554 µg/g, respectively, after the 12-month recovery period. Similar trends were observed in female rats. The half-lives ranged from 2 to 10 months for the groups at 0.06, 0.51, and 4.50 mg/m^3 (Newton et al., 1994).

In a study conducted by the NTP (2017), groups of 5 female Wistar Han rats and groups of

5 female B6C3F$_1$/N mice were exposed by inhalation (whole-body) to antimony(III) oxide at a concentration of 0, 3.75, 7.5, 15, 30, or 60 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 2 weeks, followed by a 4-week recovery period. Total antimony lung burdens and blood antimony concentrations increased with increasing exposure concentration in rats and mice. Kinetic parameters were determined using lung burden data obtained at the end of the 2-week exposure and 4-week post-exposure periods. Clearance half-lives in the lung ranged from 73 to 122 days in rats and 47 to 62 days in mice. The shortest half-life was for the lowest exposure concentration, but no clear concentration–response trend was observed. Deposition rates were approximately proportional or slightly less than proportional to exposure concentrations. Deposition rates increased 15-fold in rats and 13-fold in mice when exposure concentration increased 16-fold. Steady-state lung burdens in rats and mice were not reached during the post-exposure period. Steady-state lung burdens were expected to be reached after about five clearance half-lives. The expected half-lives were estimated to be 365–610 days in rats and 235–310 days in mice. Kinetic analysis of blood antimony concentration in mice indicated that the elimination half-life was between 22 and 32 days, and the concentration–response trend proportional to exposure was similar to that observed in the lung. In rats, blood antimony concentrations increased during the recovery period, indicating that antimony entered the bloodstream. In rats, blood concentration was 0.8% of lung concentration at the end of the 30 mg/m^3 exposure, and 2% of lung concentration at 4 weeks post-exposure. In mice, blood concentration was 0.005% of lung concentration at the end of the 30 mg/m^3 exposure, and 0.005% of lung concentration at 4 weeks post-exposure.

In the long-term study of chronic toxicity performed by the NTP (2017), groups of 5 female Wistar Han [Crl:WI(Han)] rats and groups of 5 female B6C3F$_1$/N mice were exposed by inhalation (whole-body) to antimony(III) oxide at a concentration of 0 (control), 3, 10, or 30 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 79 weeks. Total antimony(III) oxide lung burdens and blood antimony concentrations increased with increasing exposure concentration in both rats and mice. Antimony(III) oxide particles were found in the lungs, nose, larynx, trachea, and bronchial, mediastinal, and mandibular lymph nodes of exposed mice. Lung burdens in rats at 3 or 10 mg/m^3 reached steady state; however, lung burdens in rats at 30 mg/m^3 and in all groups of treated mice increased steadily over time and did not reach steady state. In rats, lung deposition rates were proportional to exposure concentration and were indicative of deposition efficiencies that increased from approximately 3% to 5% of the inhaled antimony(III) oxide. The lung clearance half-lives in rats were 136, 203, and 262 days for the groups at 3, 10, and 30 mg/m^3, respectively. The reduced pulmonary clearance at higher concentrations (10 and 30 mg/m^3) was probably associated with lung overload. In rats, two thirds of the antimony dose to the lung was cleared over the course of the study in the group exposed to 3 mg/m^3. Only half of the antimony(III) oxide dose to the lung was cleared over the course of the study in the groups exposed to 10 and 30 mg/m^3. In rats and mice, blood antimony concentrations increased with exposure concentration. While blood concentrations increased with exposure duration in rats, they did not consistently increase over time in mice. The rate of clearance from the blood increased as exposure concentrations (and lung burdens) increased (NTP, 2017).

Pentavalent antimony

In an inhalation study in Syrian hamsters, both trivalent and pentavalent antimony accumulated in erythrocytes (Felicetti et al., 1974). However, an in vitro study showed that trivalent and pentavalent antimony had a notable affinity

for erythrocytes, but did not bind to haemoglobin (Hb) (Wu et al., 2018).

(ii) Oral administration and intraperitoneal injection

Trivalent antimony

Field voles (bred in the laboratory) were given feed containing antimony(III) oxide at a concentration of 500 or 6700 mg/kg diet. Antimony was detected in all three tissues examined, i.e. liver, lung, and kidney. The highest antimony concentration was in the liver (Ainsworth et al., 1991). Groups of 15 male and 15 female rats were given drinking-water containing antimony(III) potassium tartrate at a concentration of 0 (control), 0.5, 5, 50, or 500 ppm for 13 weeks. Ten additional rats were included in the control groups and groups at 500 ppm for a further 4-week recovery period after the 13-week exposure period. Tissue antimony concentrations were dose-related and followed the order: erythrocytes > spleen/liver > kidney > brain/fat > serum. After the 4-week recovery period, antimony concentrations in all tissues (except in the spleen) decreased in the group at 500 ppm (Poon et al., 1998). In a study by Hiraoka (1986), antimony was detected in the liver, spleen, lung, kidney, hair, bone, and blood of rats given feed containing 1.0% (w/w) antimony(III) oxide for 12 weeks. After exposure, the highest concentration was detected in the blood followed by the spleen. After a 12-week recovery period, 52% of antimony detected after the exposure period remained in the blood and 76% remained in the spleen, indicating slow excretion from the body (Hiraoka, 1986).

Fischer 344/N rats or B6C3F$_1$ mice were treated with antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$) in the drinking-water for 14 days, or as 12 intraperitoneal injections over 16 days, or as intraperitoneal injections every other day for 90 days (Dieter et al., 1991; NTP, 1992). In the 14-day drinking-water study, rats were exposed to antimony(III) potassium tartrate at a dose of 0 (control), 16, 28, 59, 94, or 168 mg/kg bw once per day. In rats, antimony was detected in the blood, kidney, heart, spleen, and liver, but without a clear dose–response relationship. Concentrations of antimony in the blood were about three times as high as those in other tissues examined. In the 16-day intraperitoneal injection study, rats were treated with antimony(III) potassium tartrate at a dose of 0 (control), 1.5, 3, 6, 11, or 22 mg/kg bw, and mice at doses of 0, 6, 13, 25, 50, or 100 mg/kg bw. Antimony was detected at dose-dependent concentrations in the blood, kidney, heart, spleen, and liver of rats. In mice, antimony was detected in the liver and spleen, but not in the blood, heart, or kidney. In the 90-day intraperitoneal injection study, rats and mice were exposed to antimony(III) potassium tartrate at a dose of 0, 1.5, 3, 6, 12, or 24 mg/kg bw, 3 times per week for 90 days. Dose-dependent accumulation of antimony was found in the blood, liver, kidney, spleen, and heart of rats, with the highest tissue concentration being detected in the spleen. Antimony was also detected in the liver and spleen of mice. Blood antimony concentrations in exposed rats in the 14-day drinking-water study (15–20 μg/g) were about twice those in exposed rats of the 16-day intraperitoneal injection study (4–12 μg/g). Since the rats in the 14-day study were given drinking-water that contained antimony at doses that were approximately 10-fold those administered to rats in the 16-day intraperitoneal injection study, absorption of the compound appeared to be lower when administered in drinking-water than when administered intraperitoneally, suggesting that antimony(III) potassium tartrate has poor absorption when given orally or low bioavailability via the gastrointestinal tract (Dieter et al., 1991; NTP, 1992).

Pentavalent antimony

Borborema et al. (2013) reported that in BALB/c mice treated with a single dose of radioactive pentavalent antimony by intraperitoneal injection, the uptake rate of radioactive antimony

was 0.02–1.6% after 72 hours. The blood profile indicated that antimony was rapidly absorbed and distributed, and slowly eliminated. Higher uptake of antimony was detected in the liver, and elimination occurred primarily through biliary excretion after liver processing, with a small proportion being excreted by the kidneys. Antimony did not accumulate in the brain, lungs, heart, or uterus.

Fernandes et al. (2013) reported that in Swiss and BALB/c mice treated with a pentavalent antimonial (a drug to treat leishmaniasis) at a dose of 200 mg/kg bw by gavage, there was rapid absorption, with a serum C_{max} of 2.9 mg/L and T_{max} of 1.3 hours, and liver accumulation. Ribeiro et al. (2010) reported that when three beagle dogs were treated with a single dose of the anti-leishmanial meglumine antimoniate(V) at a dose of 100 mg/kg bw (as antimony) by nasogastric intubation, the C_{max} of serum antimony was 7.5 mg/L, T_{max} was 0.9 hours, and mean residence time was 2.6 hours. Antimony was not detected in erythrocytes and was present exclusively in the serum.

(iii) Other exposure routes

Trivalent antimony

Syrian golden hamsters were treated with antimony(III) oxide particles (suspended in 0.9% saline) at a dose of 1.52 mg/kg bw by intratracheal instillation. After the exposure, antimony was detected at significant concentrations in the lung and liver, with smaller amounts in the kidney, stomach, and trachea. Pulmonary elimination was found to be biphasic. In an initial phase, about 20% of antimony(III) was eliminated during the first 20 hours. The calculated half-lives were about 40 hours for the initial phase and 20–40 days for the second phase (Leffler et al., 1984).

Pentavalent antimony

Rhesus monkeys were treated with meglumine antimoniate(V) at a dose of 5 or 20 mg/kg bw per day by intramuscular injection for 21 days. Antimony speciation in plasma on post-treatment days 1–9 indicated that although total antimony concentrations declined, the proportion of antimony(V) remained at 11–20%, whereas that of antimony(III) increased from 5% on day 1 to 50% on day 9. On post-treatment days 55 and 95, antimony was detected in various tissues, including the thyroid, liver, gall bladder, and spleen (Friedrich et al., 2012).

In Wistar rats treated with a single dose of meglumine antimoniate(V) at a concentration of 75 mg/kg bw by intramuscular injection, a sharp fall in antimony blood concentration (half-life, 0.6 hours) was observed, and antimony was cleared from the body within 6–12 hours. Exposure of rats to meglumine antimoniate(V) at an antimony(V) concentration of 300 mg/kg bw by subcutaneous injection once per day for 21 days resulted in a steady increase of antimony in the blood. Three months after dosing, antimony blood concentrations fell from 51 µg/g on day 22 to 36 µg/g on day 126. Antimony was found to accumulate in the spleen, bone, thyroid, kidney, and liver, with the highest amount being detected in the spleen. Antimony was mainly detected in whole blood but not plasma (Coelho et al., 2014).

In Wistar rats treated with a single intravenous injection of antimony(III) potassium tartrate at a dose of 1.72 mg/kg bw as antimony or given feed containing antimony(III) potassium tartrate, potassium pyroantimonate(V), or antimony(III) oxide at a dose of about 30 mg/kg bw per day as antimony for up to 17 weeks, antimony was distributed in the blood, principally accumulating in erythrocytes, and was integrated into Hb, regardless of oxidative state (III) or (V) (Wu et al., 2018).

A group of beagle dogs was treated with meglumine antimoniate(V) (Glucantime) at a dose of 100 mg/kg bw, equivalent to an antimony dose of 27.2 mg/kg bw, by intravenous injection initially, by intramuscular injection after 30 days, and by subcutaneous injection

after a further 30 days. After intravenous injection, the plasma concentration of total antimony decreased rapidly: about 78% of the antimony administered was excreted in the urine in the first 3 hours. After intramuscular and subcutaneous injections, plasma concentrations were similar, showing a rapid absorption phase, with a half-life of 40 minutes and T_{max} of 80 minutes (Valladares et al., 1996).

4.1.2 Metabolism

(a) Humans

The data on the metabolism of trivalent or pentavalent antimony in humans were limited.

Conversion of the valence state of antimony has been observed in clinical studies. One study showed that both trivalent and pentavalent antimony were detected in the urine of patients with leishmaniasis who had been treated with meglumine antimoniate(V) (Glucantime) (Miekeley et al., 2002). [The Working Group noted that the effects of *Leishmania* infection on the interconversion/metabolism of trivalent and pentavalent antimony are unclear.] About 23.3% systemic conversion to trivalent antimony was observed after pentavalent antimony was administered at a dose of 5 mg/kg bw by single intramuscular injection in 5 healthy adult volunteers (Vásquez et al., 2006). However, Patterson et al. (2003) reported that in cultured human keratinocytes, conversion of antimony from the pentavalent oxidation state to the trivalent oxidation state was not detected.

Treatment of human blood (collected from healthy donors) with pentavalent antimony showed that in the presence of glutathione (GSH), pentavalent antimony was reduced to trivalent antimony in the plasma and in the cytoplasm of erythrocytes. Trivalent antimony was found to be unstable and could be re-oxidized to pentavalent antimony (López et al., 2015).

(b) Experimental systems

Conversion of pentavalent antimony to trivalent antimony was observed in one study in rhesus monkeys (see Section 4.1.1(iii)). After administration of meglumine antimoniate(V) by intramuscular injection at a dose of 5–20 mg/kg bw once per day for 21 days, both trivalent and pentavalent antimony were detected in the plasma. The proportion of pentavalent antimony remained at 11–20% on post-treatment days 1–9, but that of trivalent antimony increased from 5% on post-treatment day 1 to 50% on day 9 (Friedrich et al., 2012).

In one study, two in vitro experiments showed interconversion of the valence states of antimony. Pentavalent antimony from a pentavalent antimony-based drug (sodium stibogluconate, Pentostam) was reduced to trivalent antimony in the human macrophage cell line Mono Mac 6 (Hansen et al., 2011).

Other in vitro experiments showed that thiols such as GSH prompted the reduction of pentavalent antimony to trivalent antimony, and that the oxidation–reduction reaction is favoured by acidic pH and elevated temperature (Frézard et al., 2001; Ferreira et al., 2003; Quiroz et al., 2013; Barrera et al., 2016).

4.1.3 Excretion

(a) Humans

Trivalent and pentavalent antimony are eliminated in the urine, regardless of the route of exposure (inhalation, injection, or ingestion). Increases in urinary concentrations of antimony have been reported in workers occupationally exposed to trivalent antimony by inhalation (Lüdersdorf et al., 1987; Kentner et al., 1995; Kim et al., 1999; Iavicoli et al., 2002; Dartey et al., 2017; El Shanawany et al., 2017) and in workers exposed to pentavalent antimony, including antimony(V) oxide and sodium antimonate(V) (Bailly et al., 1991). In general, urinary excretion

in workers exposed to antimony was related to the level of exposure.

(i) Inhalation

Trivalent antimony

Kim et al. (1999) reported that concentrations of antimony in urine were higher in workers directly exposed to antimony(III) oxide. Urinary antimony concentrations of 411, 113, and 28 μg/g creatinine were reported in workers exposed to antimony (mean air level, 766 μg/m^3), employees who worked in the same factory but not near the source of antimony, and healthy volunteers not exposed to antimony, respectively.

Analysis of urine samples collected from workers after exposure to antimony(III) by inhalation (7 workers from the casting area exposed to antimony(III) oxide and 14 workers from the lead plates stibine-formation production area exposed to both stibine and antimony(III) oxide) indicated that both trivalent antimony compounds showed similar levels of pulmonary absorption and renal elimination (see also Section 1.4.2). The median concentrations of antimony in urine of workers from the two groups were 15.2 and 3.9 μg/g creatinine, respectively. The half-life of renal elimination was approximately 4 days (Kentner et al., 1995). Lüdersdorf et al. (1987) measured the concentrations of antimony in blood and urine samples from 109 workers employed in four different fields of a glass-producing industry using trivalent antimony. Median concentrations were 0.4–3.1 μg/L (blood) and 0.2–15.7 μg/L (urine), depending on the fields in which the individuals worked (see also Section 1.4.2(c)). Iavicoli et al. (2002) assessed the personal exposure values for airborne antimony(III) oxide and urinary concentrations of antimony of workers exposed to antimony(III) oxide (see also Section 1.4.2(d)). Mean urinary concentrations were 0.31–0.35 μg/L; however, the personal exposure values (antimony < 0.01–0.55 μg/m^3) were much lower than international occupational standards (see Section 1.5) (Iavicoli et al., 2002). In a group of 7 workers accidentally exposed to radioactive antimony (^{125}Sb) oxide aerosols, pulmonary biphasic clearance was observed. There was a first rapid clearance phase of 7 days followed by a slower second phase. Half-lives were 600–1100 days for non-smokers and 1700–3700 days for smokers (Garg et al., 2003).

Pentavalent antimony

Bailly et al. (1991) showed that urinary excretion of antimony was related to the intensity of exposure by the analysis of urine samples of 22 workers in a smelter producing antimony(V) oxide and sodium antimonate(V) (see also Section 1.4.2(a)). A significant correlation was observed between the airborne concentrations of antimony and the concentrations in urine samples collected from workers post-shift (r = 0.83, P < 0.0001). It was estimated that after 8 hours exposure at 500 μg/m^3 the increase in urinary antimony concentration at the end of the shift amounted on average to 35 μg/g creatinine (Bailly et al., 1991).

(ii) Other exposure routes

Trivalent antimony

The urinary excretion of antimony was investigated in four patients who had accidentally ingested antimony(III) potassium tartrate (850–2500 mg/patient). Urinary excretion occurred in 8 hours, and the total amount of antimony excreted by the four patients during the first 3 days was 2 mg (Lauwers et al., 1990).

Pentavalent antimony

In patients treated with meglumine antimoniate(V) at a dose of 5 mg/kg bw per day by intramuscular injection for 30 or 60 days, the urinary concentration of antimony reached 60 mg/g creatinine 24 hours after administration. Rapid blood clearing occurred during the first 3 days after administration, and more than 50% of antimony was excreted by urine. The half-life for this rapid excretion phase was 24–72 hours,

and the half-life was greater than 50 days for the slow phase that followed (Miekeley et al., 2002). After administration of pentavalent antimony at a dose of 5 mg/kg bw by single intramuscular injection to five adult volunteers, it was reported that antimony elimination was biphasic; the first rapid elimination phase was about 4 hours, and the second slower phase extended for more than 24 hours. About 60% of the drug remained to be eliminated 24 hours after administration (Vásquez et al., 2006).

(b) Experimental systems

In Sprague-Dawley rats treated with antimony(III) trichoride by single intravenous or intraperitoneal injection, about 45–55% of the administered antimony was excreted within 4 days, with most being eliminated within the first day via both faeces and urine. Depletion of GSH before antimony exposure decreased faecal excretion and increased urinary excretion (Bailly et al., 1991). In Wistar rats, 2 hours after being treated with antimony(III) potassium tartrate at a dose of 50 µmol/kg bw by single intravenous injection, 55% of the administered antimony was excreted into the bile, and GSH promoted the process (Gyurasics et al., 1992).

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 trivalent and pentavalent antimony are electrophilic or can be metabolically activated to an electrophile; are genotoxic; induce oxidative stress; induce chronic inflammation; and alter cell proliferation, cell death, or nutrient supply. Evidence is also reported as to whether trivalent and pentavalent antimony alter DNA repair or cause genomic instability; induce epigenetic alterations; are immunosuppressive; modulate receptor-mediated effects; or cause immortalization.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

Trivalent or pentavalent antimony compounds

In studies of acute toxicity, Wistar rats were treated with antimony(III) potassium tartrate at a dose of 1.72 mg/kg bw by intravenous injection and killed 10 minutes or 1, 4, 24, or 48 hours after exposure. At 10 minutes after exposure, most of the antimony was distributed in erythrocytes, with antimony levels being much higher in erythrocytes than in plasma or Hb, indicating that the in vivo distribution of trivalent antimony was initially determined by the membrane proteins on erythrocytes or the free-state fraction in the cytoplasm of erythrocytes, but not by Hb and plasma proteins (Wu et al., 2018). However, the profile curves of antimony in erythrocytes and Hb virtually overlapped 4–48 hours after exposure and were significantly higher than that of antimony in plasma. At 48 hours after exposure, 99% of antimony in the blood was sequestered by erythrocytes and over 93% was integrated into Hb. The metabolized form of antimony, rather than the parental form, might be responsible for the increased levels of antimony in Hb (Wu et al., 2018).

In studies of subchronic toxicity, rats were given feed containing antimony(III) potassium tartrate, potassium pyroantimonate(V), or antimony(III) oxide at a dose of 500 mg/kg bw for

13 weeks, followed by 1–4 weeks of recovery. Both trivalent and pentavalent antimony were predominantly sequestered by erythrocytes and structurally integrated into Hb after biotransformation (Wu et al., 2018).

(ii) Non-human mammalian cells in vitro

Trivalent or pentavalent antimony compounds

Erythrocytes have been shown to sequester antimony. Concentrations of trivalent and pentavalent antimony significantly increased in erythrocytes after exposure to elevated levels of trivalent or pentavalent antimony in an erythrocyte-incubation system (Wu et al., 2018). Exposure of rat cardiac myocytes to antimony(III) potassium tartrate at a concentration of 50 or 100 μM for 4 hours induced alterations in thiol homeostasis (including decreased protein thiol levels) and adenine nucleotide status (Tirmenstein et al., 1997).

(iii) Acellular systems

Trivalent or pentavalent antimony compounds

The interaction of trivalent and pentavalent antimony with herring fish DNA was measured by Li et al. (2011). The findings showed that trivalent antimony had a strong binding affinity to DNA, but no interaction of pentavalent antimony with DNA was observed. Wu et al. (2018) showed that binding of the parent forms of trivalent and pentavalent antimony, at a concentration of 100, 200, 300, or 400 μM, to Hb was negligible in a pure-Hb (acellular) system. This indicated that Hb, the most abundant protein in erythrocytes, did not contribute to the sequestration of the parent forms of antimony compounds by erythrocytes. The interaction (binding) of trivalent and pentavalent antimony with bovine serum albumin (BSA) was reported by Verdugo et al. (2017) and Gu et al. (2021). Verdugo et al. (2017) showed that BSA protein aggregates and conformational changes were increased in the presence of trivalent antimony, when compared with pentavalent antimony, and that GSH was protective against the effects of trivalent antimony on BSA protein conformational changes by modifying the interaction. However, Gu et al. (2021) showed that both trivalent and pentavalent antimony forms were able to competitively bind to BSA, and that the binding of either to BSA caused changes in the secondary structure of BSA.

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

See Table 4.1.

The association between urinary antimony metal concentrations and sperm DNA damage, as measured by comet assay, of partners of patients at a fertility clinic in China was assessed in a cross-sectional study by Wang et al. (2016). No significant (or suggestive) associations were found between urinary antimony concentrations and sperm DNA integrity parameters after adjustment for age, BMI, abstinence time, smoking status, daily cigarette consumption, urinary creatinine, and other metals, nor for multiple testing. [The Working Group noted that although the sample size in this study was large (n = 1052), the range of antimony exposure levels in urine was small (first urine sample: median, 0.17 μg/L; interquartile range, 0.12–0.23; and second urine sample: median, 0.17 μg/L; interquartile range, 0.11–0.23), reducing the ability of the study to detect an exposure–response effect.]

In a study of 25 antimony-exposed rayon workers involved in polyester polymerization in Egypt, DNA damage in whole blood, expressed as an increase in apurinic/apyrimidinic sites, was compared with controls after adjusting for age and smoking status. Antimony exposure was found to significantly predict apurinic/apyrimidinic sites of DNA damage ($P < 0.001$) (El Shanawany et al., 2017).

Table 4.1 Genetic and related effects of trivalent or pentavalent antimony in exposed humans

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
DNA damage (comet assay)	Sperm DNA	China, partners of patients at fertility clinic, cross-sectional		No associations between urinary Sb levels and sperm DNA integrity parameters after adjustment for multiple testing (FDR-adjusted P for trend > 0.10)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Exposure assessment critique: Urine samples collected at two close points in time on the same day as semen sample limits the findings.	Wang et al. (2016)
DNA damage (increased apurinic/ apyrimidinic sites)	Whole blood	Egypt, rayon workers, cross-sectional	25 rayon workers; 25 non-exposed controls	Significant positive correlation between urinary Sb levels and the quantity of DNA damage (in the form of increased apurinic/ apyrimidinic sites) among workers ($r = 0.873$, $P < 0.001$)	Age, smoking	Exposure assessment critique: The evidence of Sb exposure contrast presented in this study is compelling and supported by occupational information and urinary biomonitoring. However, the lack of dilution correction of urinary Sb measurements and little information on co-exposures in the occupation under investigation does slightly weaken the informativeness of the findings. Those with a history of use of medicinal products containing Sb and exposure to other known genotoxic agents were excluded and smoking was quantified. However, information on other co-exposures in this occupation was lacking. Misclassification not suspected.	El Shanawany et al. (2017)

Table 4.1 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
DNA damage (comet assay, TM measured) Oxidative DNA damage, Fpg enzyme-modified comet assay, TM measured) Micronucleus formation Sister-chromatid exchange	PBLs	Rome, Italy, workers producing fire-retardant textiles, cross-sectional	23 antimony(III) oxide-exposed workers; Group A (high), $n = 17$; Group B (low), $n = 6$; 23 non-exposed controls	NS between exposed and controls Fpg enzyme-modified TM compared with non-enzyme-modified TM; increased in Group A ($P = 0.002$) NS between exposed and controls NS between exposed and controls	Age, smoking	Exposure assessment critique: A key strength was the collection of multiple personal exposure measurements in the breathing zones of textile workers over the work week. However, unclear if the differences in the number of repeated measurements between the two exposure groups may have influenced the results. Exposures were assessed at the same time as the end-points of interest. There is potential for co-exposures to other metals or carcinogens, although none were mentioned or evaluated. Misclassification not suspected. [The Working Group noted very low exposure; 0.12 μg/m^3 in "high" exposure group.] PEL, 500 μg/m^3.	Cavallo et al. (2002)
Micronucleus formation Chromosomal aberration, sister-chromatid exchange	PBLs	Belgium, patient with leishmaniasis, case report	Treatment with meglumine antimoniate(V)	8- to 9-fold increase post- vs pre-treatment for binucleated cells No change pre- vs post-treatment		Exposure assessment critique: Few/no limitations noted as an exact, consistent, and known dose of the agent was administered to the patient. However, information on potential impurities in the treatment was not reported. The addition of Sb biomonitoring would provide information on internal dose of the agent at the various study time points. No statistics shown.	Hantson et al. (1996)

Table 4.1 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Micronucleus formation	Peripheral blood	China, coke-oven workers, cross-sectional	$n = 888$	No effect of Sb on frequency of micronucleus formation	Age, alcohol drinking status (ever vs never), and smoking pack-years	Exposure assessment critique: Single spot urine samples were used to assess exposures to Sb and other metals, which likely introduced non-differential exposure misclassification. Mixture effects of metals and urinary PAH metabolites and PAH adducts were assessed using LASSO regression and BKMR analyses.	Bai et al. (2021)
mLOY (genomic instability)				Significant associations of increasing urinary levels of Sb with elevated mLOY (marked by decreased mLRR). Remained significant in the multiple-exposure model [β (95% CI) = −0.0058 (−0.0096 to 0.0019)], $P = 0.004$			

BKMR, Bayesian kernel machine regression; BMI, body mass index; CI, confidence interval; FDR, false discovery rate; Fpg, formamidopyrimidine DNA glycosylase; LASSO, least absolute shrinkage and selection operator; mLOY, mosaic loss of chromosome Y; mLRR, median log R ratio; NS, not significant; PAH, polycyclic aromatic hydrocarbon; PBL, peripheral blood lymphocyte; PEL, permissible exposure level; Sb, antimony; TM, tail moment; vs, versus.

In a cross-sectional study of Italian workers producing fireproof textiles who were exposed to antimony(III) oxide at two exposure levels (high, Group A; low, Group B) and compared with non-exposed controls, no differences were found in the frequency of sister-chromatid exchange and micronucleus formation between exposed workers and controls in peripheral blood lymphocytes (PBLs) after controlling for age and smoking. There was no difference in tail moment, as measured by comet assay, between antimony-exposed and non-exposed control groups (Cavallo et al., 2002). [The Working Group noted the low antimony exposure concentration, relatively small sample size (exposed and controls, n = 23), and lack of information provided about occupational co-exposures, which reduced the informativeness of this study.]

In a case report from Belgium of a patient with leishmaniasis undergoing antimony therapy (pentavalent antimony), Hantson et al. (1996) noted a post-treatment increase of 9-fold in the frequency of micronucleus formation in PBLs (upon bone marrow biopsy) but no change in either the induction of chromosomal aberration or the frequency of sister-chromatid exchange. [The Working Group noted that this provocative antimony finding may have been confounded by potential immunosuppression caused by visceral *Leishmania* infection. Thus, the genotoxicity could have been caused by the lack of immune surveillance or impaired repair capacity.]

In a study of coke-oven workers with mixed exposure to PAHs and multiple metals, including antimony, conducted in China, Bai et al. (2021) assessed associations between antimony exposure and micronucleus formation in PBLs and found no antimony-associated effects. [The Working Group noted that a strength of the study was in the use of LASSO regression and BKMR analyses to assess mixture effects, and the large sample size (n = 888).]

A study by Alrashed et al. (2021) examined heavy metal-induced genotoxicity and oxidative stress in women with recurrent pregnancy loss. [The Working Group deemed the study to be uninformative because the authors failed to control for confounding by abnormally high levels of gonadotropins and other hormones in the pregnancy-loss group.] [The Working Group noted that two of five studies in exposed humans showed some evidence of antimony-induced genotoxicity.]

(ii) Human cells in vitro

See Table 4.2.

Trivalent antimony compounds

In studies using primary cells, exposure of human PBLs to non-cytotoxic concentrations of antimony(III) oxide (0.5 μM) or antimony(III) chloride (1 μM) for 24 hours caused a significant increase in the frequency of sister-chromatid exchange (Gebel et al., 1997). Exposure of human PBLs to antimony(III) chloride (5 μM) caused a significant increase in DNA damage, as measured by comet assay, and in the frequency of micronucleus formation, but was negative for oxidative DNA damage (see Section 4.2.5), and no cytotoxicity was observed at concentrations of ≤ 50 μM (Schaumlöffel & Gebel, 1998). Treatment of human PBLs with antimony(III) oxide at a concentration of 100 μg/mL for 68 hours induced a significant increase in chromosomal damage (as measured by cytogenetic assay) with metabolic activation in cells from two donors, and without metabolic activation in cells from one donor (Elliott et al., 1998). Treatment of human leukocytes with a non-cytotoxic concentration of antimony(III) sodium tartrate (2.3×10^{-9} M) for 48 hours caused a significant increase in the incidence of chromosome breakage (Paton & Allison, 1972). In addition, 4-hour treatment with antimony(III) chloride at concentrations of > 50 μM caused a significant increase in the frequency of micronucleus

Table 4.2 Genetic and related effects of trivalent or pentavalent antimony in human cells in vitro

End-point	Cell type or line	Results[a]	Concentration (LEC or HIC), exposure duration	Comments	Reference
Trivalent antimony compounds					
Antimony(III) oxide (Sb_2O_3)					
Sister-chromatid exchange	Human PBLs	+	0.5 µM, 24 h	No positive control. 5 µM is cytotoxic.	Gebel et al. (1997)
Chromosomal aberration	Human PBLs	+	100 µg/mL, 68 h	Positive control. Tested cells from two donors. Positive with metabolic activation (+liver S9) in cells from both donors and without metabolic activation (–liver S9) in cells from one donor. Reported no decrease in mitotic activity.	Elliott et al. (1998)
Antimony(III) chloride ($SbCl_3$)					
Sister-chromatid exchange	Human PBLs	+	1 µM, 24 h	No positive control. 10 µM is cytotoxic.	Gebel et al. (1997)
Micronucleus formation	Human bronchial epithelial (BES-6) cells	+	> 50 µM, 4 h	LC_{50} of 80 µM (BES-6) and 40 µM (fibroblasts).	Huang et al. (1998)
	Human fibroblasts	+		Apoptosis and DNA fragmentation not detected.	
Micronucleus formation	Human PBLs	+	5 µM, 24 h	No positive control. No cytotoxicity (≤ 50 µM).	Schaumlöffel & Gebel (1998)
DNA damage (comet assay)		+		Co-exposure with antioxidants (SOD and CAT) with no effect on micronucleus formation [no role of oxidative stress in DNA-damage induction or chromosomal aberration].	
Induction of γH2AX	Human hepatoblastoma (HepG2) cells	+	100 µM, 24 h	97% cell viability (HepG2).	Kopp et al. (2018)
	Human colorectal epithelial adenocarcinoma (LS-174T) cells	+	250 µM, 24 h	72% cell viability (LS-174T).	
Decreased repair of irradiation-induced DNA double-strand breaks	Human cervical cancer (HeLa S3) cells	+	50 µM, 8 h		Koch et al. (2017)
Antimony(III) sodium tartrate ($C_8H_4Na_2O_{12}Sb_2$)					
Chromosomal aberration	Human leukocytes	+	2.3 nM, 48 h	Single dose tested. No positive control. 1×10^8 M is cytotoxic.	Paton & Allison (1972)

Table 4.2 (continued)

End-point	Cell type or line	Results[a]	Concentration (LEC or HIC), exposure duration	Comments	Reference
Pentavalent antimony compounds					
Potassium antimonate(V) ($KSbO_3$)					
Micronucleus formation	Human PBLs	+	360 µM, 24 h	PHA-stimulated. Tested cells from two donors.	Migliore et al. (1999)
Meglumine antimoniate(V) ($C_7H_{17}NO_5 \cdot H \cdot O_3Sb$)					
DNA damage (comet assay)	Human PBLs	–	15 mg/mL, 3 and 24 h		Lima et al. (2010)

CAT, catalase; γH2AX, phosphorylation of histone H2AX; HIC, highest ineffective concentration; LC_{50}, median lethal concentration; LEC, lowest effective concentration (units as reported); PBL, peripheral blood lymphocyte; PHA, phytohaemagglutinin; SOD, superoxide dismutase.

[a] +, positive; –, negative.

formation in primary human fibroblasts. The LC_{50} (median lethal concentration) was 40 μM for human fibroblasts, but no apoptosis or DNA fragmentation was detected (Huang et al., 1998).

In studies using cell lines, 4-hour treatment with antimony(III) chloride at concentrations of > 50 μM caused a significant increase in the frequency of micronucleus formation in the human bronchial epithelial cell line BES-6. The LC_{50} was 80 μM, and no apoptosis was observed after the 4-hour exposure period (Huang et al., 1998). In a high-throughput screening assay, exposure of the HepG2 cell line to antimony(III) chloride at a concentration of 100 μM (which resulted in 97% cell viability) or of the LS 174T cell line at a concentration of 250 μM (72% cell viability) for 24 hours significantly increased γH2AX induction, indicating that antimony(III) chloride caused DNA damage in the cells (Kopp et al., 2018).

Pentavalent antimony compounds

Exposure of phytohaemagglutinin-stimulated human PBLs from two donors to potassium antimonate ($KSbO_3$) at a concentration of 360 μM for 24 hours caused a significant increase in the induction of micronucleus formation, which was greater by 7- to 10-fold than in the control cells (Migliore et al., 1999).

Exposure of human PBLs to meglumine antimoniate(V) (Glucantime) at a concentration of 3.25, 7.5, or 15 mg/mL (corresponding to a pentavalent antimony concentration of 1.06, 2.12, or 4.25 mg/mL, respectively) for 3 or 24 hours did not cause DNA damage, as measured by comet assay (Lima et al., 2010).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

Trivalent antimony compounds

In an assay for unscheduled DNA synthesis using hepatocytes from male rats exposed by gavage to a single dose of antimony(III) oxide (at 3200 or 5000 mg/kg bw), sampled either 2 or 16 hours after treatment, no increase in net nuclear grains or the percentage of cells exhibiting evidence of DNA repair was observed (Elliott et al., 1998).

Elliott et al. (1998) reported that no statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes (PCEs) were observed in the bone marrow of CD-1 mice 24 or 48 hours after a single exposure to antimony(III) oxide at a dose of 5000 mg/kg bw by gavage or after repeated exposure to doses of 400, 667, or 1000 mg/kg bw by gavage once per day for 7, 14, or 21 days (Elliott et al., 1998). In a series of experiments performed in male and female Swiss Albino mice, Gurnani et al. (1992a, b, 1993) assessed the clastogenic effects of antimony(III) oxide and antimony(III) chloride. No clastogenic effects (i.e. chromosomal aberration) were observed in the bone marrow of male and female mice at 6, 12, 18, or 24 hours after a single exposure by gavage to antimony(III) oxide at doses of 400, 667.7, or 1000 mg/kg bw (Gurnani et al., 1992a). However, repeated exposures of male mice to the same doses of antimony(III) oxide for 7, 14, or 21 days significantly increased the frequency of chromosomal aberration in the bone marrow (except for the 21-day exposure to 1000 mg/kg bw, which was lethal) (Gurnani et al., 1992a, 1993), but did not cause sperm-head abnormalities in germ cells (Gurnani et al., 1993). Instead, acute exposure to a single dose of antimony(III) chloride at 70, 140, or 233.33 mg/kg bw caused a significant increase in the frequency of chromosomal aberration in the bone marrow of female mice at 6, 12, 18, and 24 hours after gavage (Gurnani et al., 1992b).

Table 4.3 Genetic and related effects of trivalent or pentavalent antimony in non-human mammals in vivo

End-point	Tissue, cell type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Trivalent antimony compounds					
Antimony(III) oxide (Sb_2O_3)					
Chromosomal aberration	Bone marrow of Swiss albino mice	–	1000 mg/kg bw	Gavage, 6, 12, 18, or 24 h, single dose	Gurnani et al. (1992a, 1993)
	Bone marrow of Swiss albino male mice	+	400 mg/kg bw per day	Gavage, 7, 14, or 21 days, repeated doses	
Sperm head abnormalities	Germ cells of Swiss albino male mice	–	1000 mg/kg bw per day		
Micronucleus formation in PCEs	Bone marrow of CD-1 mice	–	5000 mg/kg bw	Gavage, 24 or 48 h, single dose	Elliott et al. (1998)
		–	1000 mg/kg bw per day	Gavage, 7, 14, or 21 days, repeated doses	
DNA repair (unscheduled DNA synthesis assay)	DNA from hepatocytes of male APfSD rats	–	5000 mg/kg bw	Gavage, 2 or 16 h, single dose	
Chromosomal aberration or micronucleus formation	Bone marrow of SD rats	–	1000 mg/kg bw per day	Gavage, 21 days, repeated doses	Kirkland et al. (2007)
Micronucleus formation in normochromatic erythrocytes	Mature erythrocytes from peripheral blood of $B6C3F_1$/N mice	+	30 mg/m^3	Inhalation, 6 h/day, 5 days/wk for 12 mo	NTP (2017)
DNA damage (comet assay)	Lung tissue of $B6C3F_1$/N mice	+			
	PBLs of $B6C3F_1$/N mice	–			
Micronucleus formation in PCEs	Reticulocytes from peripheral blood of Wistar Han rats	–	30 mg/m^3		
DNA damage (comet assay)	Lung tissue of Wistar Han rats	–			
	PBLs of Wistar Han rats	–			
Antimony(III) chloride ($SbCl_3$)					
Chromosomal aberration	Bone marrow of Swiss female albino mice	+	70 mg/kg bw	Gavage, 6, 12, 18, or 24 h, single dose	Gurnani et al. (1992b)
Antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$ or $K_2Sb_2(C_4H_2O_6)_2$)					
Chromosomal aberration (chromatid gaps, chromatid breaks, and centric fusions)	Bone marrow of male rats	+	2 mg/kg bw	Intraperitoneal injection, 6, 24, or 48 h (single dose) or for 5 consecutive days (repeated doses), 20 days	El Nahas et al. (1982)

Table 4.3 (continued)

End-point	Tissue, cell type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Pentavalent antimony compounds					
Meglumine antimoniate(V) ($C_7H_{17}NO_5 \cdot H \cdot O_3Sb$)					
DNA-damage scores and frequency of nucleoids (comet assay)	PBLs and peritoneal macrophages of Swiss mice	+	212.5 mg/kg bw	Intraperitoneal injection, 3 h (macrophages) or 24 h (PBLs and bone marrow), 20 days	Lima et al. (2010)
Micronucleus formation in PCEs	Bone marrow of Swiss mice	+			
DNA-damage scores and frequency of nucleoids (comet assay)	PBLs of Swiss mice	+	425 mg/kg bw	Intraperitoneal injection, 24 h, 20 days	Cantanhêde et al. (2015)
Micronucleus formation in PCEs	Bone marrow of Swiss mice	+			
DNA-damage scores and frequency of nucleoids (comet assay with Fpg enzyme; oxidative DNA damage)	PBLs of BALB/c mice	+	20 mg/kg bw per day	Intraperitoneal injection, 20 days	Moreira et al. (2017)
Micronucleated PCEs	Bone marrow of BALB/c mice	+			
DNA-damage scores and frequency of the highest nucleoid classes (3 and 4) (comet assay with Fpg enzyme; oxidative DNA-damage)	PBLs of Swiss mice	+	810 mg/kg bw	Single intraperitoneal injection, 24 h	de Jesus et al. (2018)
Micronucleated PCEs	Bone marrow of Swiss mice	+			

bw, body weight; Fpg, formamidopyrimidine DNA glycosylase; HID, highest ineffective dose; LED, lowest effective dose; mo, month; PBL, peripheral blood leukocyte; PCE, polychromatic erythrocyte; SD, Sprague-Dawley; wk, week.

[a] +, positive; –, negative.

Kirkland et al. (2007) also did not find any chromosomal aberration or an increased frequency of micronucleus formation in the bone marrow of Sprague-Dawley rats after repeated exposure to antimony(III) oxide at doses of 250, 500, or 1000 mg/kg bw per day by gavage for 21 days. [The Working Group noted that exposure of the bone marrow was demonstrated by toxicokinetic data.]

Inhalation exposure of male and female B6C3F$_1$/N mice to antimony(III) oxide at a concentration of 3, 10, or 30 mg/m^3 for 12 months caused a significant increase in the frequency of micronucleus formation in mature erythrocytes (normochromatic erythrocytes), on the basis of significant statistical trend tests and significantly elevated frequencies of micronucleated normochromatic erythrocytes at the highest exposure concentration (NTP, 2017). There was also a significant increase in the percentage of reticulocytes (polychromatic erythrocytes, PCEs). In addition, significantly increased DNA damage (as measured by comet assay) was observed in the lung tissue of male and female mice, but not in PBLs (NTP, 2017). No genotoxic effects were observed in reticulocytes of Wistar Han rats (both sexes) after exposure to antimony(III) oxide at a concentration of 3, 10, or 30 mg/m^3 for 12 months (NTP, 2017). [The Working Group noted that concentrations of antimony in the blood of rats were much higher than those in mice; however, exposure to antimony(III) oxide did not increase the frequency of micronucleus formation in reticulocytes of rats.]

Exposure of male rats to tartar emetic (antimony(III) potassium tartrate; 36.5% trivalent antimony) at a dose of 2, 8.4, or 14.8 mg/kg bw by single intraperitoneal injection caused significant increases in the frequency of chromosomal aberrations such as chromatid gaps, chromatid breaks, and centric fusions in bone marrow at 6, 24, or 48 hours after treatment (except for the 48-hour exposure to 14.8 mg/kg bw), as did repeated intraperitoneal injections for 5 consecutive days (El Nahas et al., 1982).

Pentavalent antimony compounds

In Swiss mice exposed to meglumine antimoniate(V) (Glucantime) at a concentration of 425 or 810 mg/kg bw by single intraperitoneal injection, significant increases in DNA damage scores and the frequency of nucleoids, as measured by comet assay, were observed (including oxidative DNA damage measured by formamidopyrimidine DNA glycosylate, Fpg, enzymatic digestion) in PBLs (de Jesus et al., 2018), and significantly increased frequency of micronucleated PCEs, as assessed by micronucleus assay, in bone marrow after 24-hour exposure (Cantanhêde et al., 2015; de Jesus et al., 2018). Acute intraperitoneal injection of Swiss mice with Glucantime at a dose of 212.5, 425, or 850 mg/kg bw caused significantly increased DNA damage scores and frequencies of nucleoids in PBLs (at 24 hours) and resident peritoneal exudate macrophages (at 3 hours), as measured by comet assay, and significantly increased frequencies of micronucleated PCEs in bone marrow (at 24 hours) (Lima et al., 2010). In addition, exposure of BALB/c mice to Glucantime by intraperitoneal injection at a dose of 20 mg/kg bw per day for 20 days caused a significant increase in DNA damage scores and frequencies of nucleoids in PBLs, as measured by comet assay (with Fpg enzymatic digestion indicative of oxidative DNA damage), and of micronucleated PCEs in bone marrow (Moreira et al., 2017). [The Working Group noted that pentavalent antimony can be metabolized or converted to its more toxic trivalent form.]

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Trivalent antimony compounds

Exposure of mouse embryonic stem cell lines, transfected with a series of green fluorescent protein reporters (ToxTracker assay), to six trivalent antimony compounds – antimony(III)

Table 4.4 Genetic and related effects of trivalent or pentavalent antimony in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]	Concentration (LEC or HIC), exposure duration	Comments	Reference
Trivalent antimony compounds					
Antimony(III) chloride ($SbCl_3$)					
DNA damage (comet assay)	Chinese hamster, lung, V79	+	1 µM, 24 h	Cell viability > 90% at 20 µM.	Gebel et al. (1998)
Micronucleus formation	Chinese hamster, lung, V79	+	25 µM, 24 h		
DNA damage (alkaline elution)	Chinese hamster, lung, V79	+	10 µM, 24 h	No results for 20 µM exposure.	
Micronucleus formation	Chinese hamster, ovary, CHO-K1	+	> 50 µM, 4 h	LC_{50} of 180 µM (DNA fragmentation and apoptosis observed after 4 h exposure).	Huang et al. (1998)
Decreased repair of irradiation-induced DNA double-strand breaks	Chinese hamster, ovary, CHO-K1	+	0.2 mM, 2 h	Mean cytotoxic concentration, 0.21 mM.	Takahashi et al. (2002)
Sister-chromatid exchange	Chinese hamster, lung, V79	+	5 µg/mL, 28 h	Cytotoxic concentration, 20 µg/mL.	Kuroda et al. (1991)
Antimony(III) oxide (Sb_2O_3)					
Sister-chromatid exchange	Chinese hamster, lung, V79	+	0.17 µg/mL, 28 h		Kuroda et al. (1991)
Antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$ or $K_2Sb_2(C_4H_2O_6)_2$)					
Decreased repair of irradiation-induced DNA double-strand breaks	Chinese hamster, ovary, CHO-K1	+	0.4 mM, 2 h	Mean cytotoxic concentration, 0.12 mM. Most of the cells treated with 0.4 mM antimony(III) potassium tartrate lost proliferative capacity.	Takahashi et al. (2002)
Pentavalent antimony compounds					
Antimony(V) chloride ($SbCl_5$)					
Sister-chromatid exchange	Chinese hamster, lung, V79	–	35 µg/mL, 28 h		Kuroda et al. (1991)
Antimony(V) oxide (Sb_2O_5)					
Sister-chromatid exchange	Chinese hamster, lung, V79	–	40 µg/mL, 28 h		Kuroda et al. (1991)
Trivalent or pentavalent antimony compounds					
Sb_2O_3, Sb_2S_3, $SbCl_3$, $Sb_2(C_2H_4O_2)_3$,$SbC_6H_9O_6$, and $Sb_2K_2C_8H_4O_{12}$ (trivalent), and $SbCl_5$, $KSb(OH)_6$, $NaSbO_3$, $NaSb(OH)_6$, and Sb_2O_5 (pentavalent)					
DNA damage (Bscl2 and Rtkn genes in ToxTracker assay)	Mouse embryonic stem cells	–	1–4.24 µg/mL (trivalent) and 3 to ~45 µg/mL (pentavalent), 24 h	Wild-type cells from C57/BL6 B4418 mice.	Boreiko et al. (2021)

Bscl2, BSCL2 lipid droplet biogenesis associated, serpin; HIC, highest ineffective concentration; LC_{50}, median lethal concentration; LEC, lowest effective concentration; Rtkn, rhotekin.

[a] +, positive; –, negative.

oxide, antimony(III) sulfide, antimony(III) chloride, antimony tris (ethylene) glycolate ($Sb_2(C_2H_4O_2)_3$), antimony triacetate ($SbC_6H_9O_6$), and antimony potassium tartrate ($Sb_2K_2C_8H_4O_{12}$) – did not induce activation of the Bscl2 and Rtkn reporter genes, which would have been indicative of DNA damage (genotoxicity) or impaired DNA replication (Boreiko et al., 2021).

Treatment of the Chinese hamster lung cell line V79 with antimony(III) chloride at a concentration of 1 or 10 µM for 24 hours resulted in significantly increased DNA damage, as measured by comet assay and alkaline elution, respectively, and treatment with antimony(III) chloride at 25 µM significantly increased the frequency of micronucleus formation (Gebel et al., 1998). Treatment of V79 cells with antimony(III) chloride or antimony(III) oxide at concentrations up to 20 or 0.34 µg/mL, respectively, induced a significant increase in the frequency of sister-chromatid exchange after exposure for 28 hours (Kuroda et al., 1991). Moreover, 4-hour treatment of the Chinese hamster ovary cell line CHO-K1 with antimony(III) chloride at concentrations of > 50 µM significantly increased the frequency of micronucleus formation. The LC_{50} was 180 µM, but DNA fragmentation and apoptosis were detected after the 4-hour exposure (Huang et al., 1998).

Pentavalent antimony compounds

Similarly to trivalent antimony compounds, in the study by Boreiko et al. (2021), five pentavalent antimony compounds – antimony(V) chloride ($SbCl_5$), potassium hexahydroxoantimonate ($KSb(OH)_6$), sodium antimonate, sodium hexahydroxoantimonate, and antimony(V) oxide – did not induce a DNA damage response, as assessed by ToxTracker assay, in mouse embryonic stem cells exposed for 24 hours.

Treatment of the V79 cell line with antimony(V) chloride or antimony(V) oxide at concentrations up to 35 or 40 µg/mL, respectively, did not increase the frequency of sister-chromatid exchange after a 28-hour exposure (Kuroda et al., 1991).

(iii) Non-mammalian experimental systems

See Table 4.5.

Trivalent antimony compounds

Acute exposure of *Chironomus sancticaroli* to antimony(III) oxide at 50 or 800 µg/L for 48 hours, or subchronic exposure at 50 µg/L for 8 days, caused significantly increased DNA damage scores, as measured by comet assay (Morais et al., 2019).

Exposure of budding yeast (*Saccharomyces cerevisiae*) to trivalent antimony at 0.08–1 mM caused various forms of DNA damage including chemical modification of DNA bases, replication-associated DNA lesions, DNA double-strand breaks, and telomere damage (Litwin et al., 2021). Trivalent antimony also induced both replication-dependent and -independent DNA lesions in yeast, and trivalent antimony-induced DNA damage triggered the formation of RAD52 foci (Litwin et al., 2021).

Exposure to antimony(III) chloride or antimony(III) oxide caused DNA damage, as assessed by rec assay in *Bacillus subtilis*, which was reflected by the greater inhibition zone length of the Rec^- strain of *B. subtilis* than that of the wildtype (Rec^+) strain (Kanematsu et al., 1980; Kuroda et al., 1991). However, these trivalent antimony compounds did not exhibit mutagenicity, as assessed by spot tests, in *Escherichia coli* and *Salmonella typhimurium* strains with or without metabolic activation (liver S9) (Kanematsu et al., 1980; Kuroda et al., 1991; Elliott et al., 1998). In contrast, Nishioka (1975) reported that exposure to antimony(III) chloride did not cause DNA damage, as assessed by rec assay in *B. subtilis*. Antimony(III) chloride was not genotoxic, as assessed by SOS chromotest with *E. coli* strain PQ37 (Lantzsch & Gebel, 1997). Exposure to antimony(III) chloride did not cause mutagenicity, as assessed by *umu* genotoxicity assay

Table 4.5 Genetic and related effects of trivalent or pentavalent antimony in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]	Concentration (LEC, HIC, or range)	Comments	Reference
Trivalent antimony compounds					
Antimony(III) oxide (Sb_2O_3)					
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	0.05 M		Kanematsu et al. (1980)
Escherichia coli B/r WP2 *try*$^-$ and WP2 *hcr*$^-$ *try*	DNA reverse mutation	–	0.05 M		
Salmonella typhimurium his$^-$ strains TA98, TA100, TA1535, TA1537, and TA1538	DNA reverse mutation	–	0.05 M		
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	0.3–1.1 µg/disc		Kuroda et al. (1991)
Salmonella typhimurium TA98 and TA100	DNA reverse mutation	–	0.43–1.71 µg/plate	With and without metabolic activation (liver S9).	
Escherichia coli WP2P and WP2P*uvrA*		–	10 000 µg/plate	With and without metabolic activation (liver S9).	Elliott et al. (1998)
Salmonella typhimurium TA98, TA100, TA1535, and TA1537	DNA reverse mutation	–	10 000 µg/plate	With and without metabolic activation (liver S9).	
Antimony(III) chloride ($SbCl_3$)					
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	–	0.05 M		Nishioka (1975)
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	0.01 M		Kanematsu et al. (1980)
Escherichia coli B/r WP2 *try*$^-$ and WP2 *hcr*$^-$ *try*	DNA reverse mutation	–	0.01 M		
Salmonella typhimurium his strains TA98, TA100, TA1535, TA1537, and TA1538	DNA reverse mutation	–	0.01 M		
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	6.3–23 µg/disc		Kuroda et al. (1991)
Salmonella typhimurium TA98 and TA100	DNA reverse mutation	–	625–5000 µg/plate	With and without metabolic activation (liver S9).	
Escherichia coli PQ37	SOS chromotest	–	11–707 µM		Lantzsch & Gebel (1997)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]	Concentration (LEC, HIC, or range)	Comments	Reference
Salmonella typhimurium TA1535/pSK1002 (*umu* test)	DNA mutation	–	1.6×10^6 to 8.2×10^4 M	With and without metabolic activation (liver S9).	Yamamoto et al. (2002)
Salmonella typhimurium TA98 and TA100	DNA reverse mutation	–	1 mM	With and without metabolic activation (liver S9).	Kubo et al. (2002)
Antimony(III) potassium tartrate ($C_8H_{10}K_2O_{15}Sb_2$ or $K_2Sb_2(C_4H_2O_6)_2$)					
Salmonella typhimurium TA97, TA98, TA100, and TA1535	DNA reverse mutation	–	10–10 000 mg/plate	With and without metabolic activation (liver S9).	NTP (1992)
Pentavalent antimony compounds					
Antimony(V) chloride ($SbCl_5$)					
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	–	0.05 M		Nishioka (1975)
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	NR (0.03 mL)		Kanematsu et al. (1980)
Escherichia coli B/r WP2 *try*$^-$ and WP2 *hcr*$^-$ *try*	DNA reverse mutation	–	NR (0.03 mL)		
Salmonella typhimurium his strains TA98, TA100, TA1535, TA1537, and TA1538	DNA reverse mutation	–	NR (0.03 mL)		
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	+	65–260 µg/disc	Killing zone was not observed with negative results. With and without metabolic activation (liver S9).	Kuroda et al. (1991)
Salmonella typhimurium TA98 and TA100	DNA reverse mutation	–	54–864 µg/plate		
Antimony(V) oxide (SbO_5)					
Bacillus subtilis H17 (Rec$^+$, *arg*$^-$ *try*$^-$); M45 (Rec$^-$, *arg*$^-$ *try*$^-$)	DNA damage, rec assay	–	60 µg/disc	Killing zone was not observed with negative results. With and without metabolic activation (liver S9).	Kuroda et al. (1991)
Salmonella typhimurium TA98 and TA100	DNA reverse mutation	–	50–200 µg/plate		

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

[a] +, positive; –, negative.

with or without metabolic activation (liver S9) (Yamamoto et al., 2002). Kubo et al. (2002) also reported that exposure to antimony(III) chloride did not cause mutagenicity, as assessed by Ames test with *S. typhimurium* strains TA98 and TA100 with or without metabolic activation (liver S9). Antimony(III) potassium tartrate was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with or without metabolic activation (liver S9) (NTP, 1992).

Pentavalent antimony compounds

Exposure of invertebrates (*Chironomus tentans* larvae) to sediment spiked with antimony(V) oxide nanoparticles (NPs) at a concentration of 5000 µg/kg for 10 days caused DNA strand breakage (Oberholster et al., 2011).

Exposure to antimony(V) chloride caused DNA damage, as assessed by rec assay in *B. subtilis* (Kanematsu et al., 1980; Kuroda et al., 1991). However, antimony(V) chloride and antimony(V) oxide compounds were not observed to be mutagenic, as assessed by spot tests in *E. coli* and *S. typhimurium* strains with or without metabolic activation (liver S9) (Kanematsu et al., 1980; Kuroda et al., 1991). In contrast, Nishioka (1975) reported that exposure to antimony(V) chloride did not cause DNA damage, as assessed by rec assay in *B. subtilis*.

4.2.3 Alters DNA repair or causes genomic instability

(a) Humans

(i) Exposed humans

See Table 4.1.

In a study of coke-oven workers with mixed exposure to PAHs and multiple metals including antimony in China, Bai et al. (2021) measured mLOY in blood as an indicator of genomic instability. The calculation of mLOY was based on genome-wide single-nucleotide polymorphism genotyping and expressed as median log R ratio-Y. The results of this study consistently suggested significant positive dose–response relationships between urinary antimony levels and mLOY. [The Working Group noted that single spot urine samples were used to assess exposures to antimony, tungsten, and cobalt. Exposure and outcomes were assessed at the same time. A strength of the study was that mixture effects of metals and urinary PAH metabolites and adducts were assessed using LASSO regression and BKMR analyses.]

(ii) Human cells in vitro

Trivalent antimony compounds

Exposure of the human lung carcinoma A549 cell line to antimony(III) chloride at 250, 350, or 500 µM for 24 hours caused significant impairment of nucleotide excision repair (NER), as demonstrated by inhibition of the removal of ultraviolet C irradiation-induced cyclobutane pyrimidine dimers (Grosskopf et al., 2010). However, antimony(III) chloride did not affect the repair of benzo[*a*]pyrene diol epoxide- and 6–4 photoproduct-induced DNA adducts after exposure for 2 hours, or the removal of benzo[*a*]pyrene diol epoxide-induced DNA adducts (Grosskopf et al., 2010). Moreover, antimony(III) chloride exposure caused significantly decreased gene and protein expression of the NER pathway component XPE, and trivalent antimony interacted with the zinc finger domain of another NER protein, XPA. In a cellular system using A549 cells, the association/dissociation of XPA to/from damaged DNA was diminished in the presence of antimony(III) chloride, suggesting that trivalent antimony interferes with proteins involved in the NER pathway (Grosskopf et al., 2010).

Exposure of the HeLa S3 cell line to antimony(III) chloride at a concentration of 50 µM for 8 hours decreased repair of γ-irradiation-induced DNA double-strand breaks. BRCA1 and RAD51 were identified as molecular targets of antimony(III) chloride, suggesting that, in

addition to non-homologous end-joining, homologous recombination may also be impaired by exposure to antimony(III) chloride (Koch et al., 2017).

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

Trivalent antimony compounds

Pre-treatment of Chinese hamster ovary cells with trivalent antimony (antimony(III) chloride or antimony(III) potassium tartrate) at a concentration of 0.2, 0.4, 0.6, or 0.8 mM for 2 hours inhibited the repair (rejoining) of DNA double-strand breaks induced by γ-irradiation (Takahashi et al., 2002) [The Working Group noted that significant inhibition of repair was observed after exposure to antimony(III) chloride at 0.2 mM and antimony(III) potassium tartrate at 0.4 mM, but that the mean cytotoxic doses were 0.21 and 0.12 mM, respectively, and that most of the cells treated with antimony(III) potassium tartrate at 0.4 mM lost their proliferative capacity.]

(iii) Non-mammalian experimental systems

Trivalent antimony compounds

Trivalent antimony inhibited DNA double-strand break repair not only by non-homologous end-joining, but also by homologous recombination, two pathways that are important for the repair of DNA double-strand breaks (Litwin et al., 2021). Trivalent antimony exposure also strongly affected the morphology of microtubule (actin) filaments in yeast (Litwin et al., 2021). Alterations in the microtubule cytoskeleton (genomic instability) may cause nuclear disorganization and inhibit DNA repair in budding yeast, and regulation of astral microtubules may be coordinated with pathways that maintain genome integrity (Estrem & Moore, 2019).

4.2.4 Induces epigenetic alterations

(a) Humans

(i) Exposed humans

See Table 4.6.

In a study conducted in China of 360 healthy men employed as coke-oven workers who were exposed to PAHs and multiple metals including antimony, Deng et al. (2019) measured the expression of miRNAs in plasma to explore their associations with urinary metals and PAHs. The selection of miRNAs (let-7b-5p, miR-126-3p, miR-142-5p, miR-150-5p, miR-16-5p, miR-24-3p, miR-27a-3p, miR-28-5p, miR-320b, and miR-451a) was based on previous observations that the expression of these miRNAs is inversely associated with the PAH response, genetic damage, and oxidative stress. After adjusting for urinary PAHs and other metal exposures, antimony was significantly associated with a 13% decrease in all the miRNAs in both single- and multiple-metal models. [The Working Group noted that the strength of this study was that antimony was significantly associated with miRNA expression, not only in single-metal models adjusted for PAH exposure, but also in multiple-metal models simultaneously adjusted for PAHs and other metals. However, the Working Group noted that the correlation between biomarkers of genetic damage or oxidative stress (DNA strand-break levels in lymphocytes, cytokinesis-blocked micronuclei, and indicators of oxidative stress) and miRNAs was adjusted for the sum of metals and PAHs, thus it was not possible to discriminate the effect of antimony alone. In addition, the Working Group noted that the urinary levels of antimony in the exposed group were not different ($P = 0.447$) from those in the control group.]

In a subset of the Aragon Workers Health study conducted in Spain, Riffo-Campos et al. (2018) used DNA Infinium Methylation 450 K data obtained from whole-blood samples from 23 of 73 middle-aged men without clinically evident cardiovascular disease to identify differentially

Table 4.6 Epigenetic alterations in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
miRNAs	Plasma	China, coke-oven workers, cross-sectional	$n = 360$	Inverse association of urinary Sb concentration with 10 miRNA expression measurements ($P < 0.05$)	PAHs, other metals		Deng et al. (2019)
DMRs	Whole blood	Spain, Aragon Workers Health Study, cross-sectional	DNA Infinium Methylation 450 K data were obtained from 23 out of 73 middle-aged men without clinically evident CVD	Of 303 genes annotated to metal DMRs, 42 were uniquely associated with Sb exposure; among the nearest genes to the identified DMRs, 46% of the metal-DMR genes overlapped with atherosclerosis-DMR genes ($P < 0.001$).	Adjusted by age, smoking status, BMI, hypertension, dyslipidaemia, and diabetes	Exposure assessment critique: Single urine samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Urinary biomarkers of Sb represent recent exposure. Reliance on a single urine sample likely resulted in imprecise estimates of (recent) exposure. Sb levels are in normal range. Relationships between DMRs with respect to subclinical atherosclerosis in coronary, carotid, and femoral territories, metal concentrations, sociodemographic characteristics, and different cell types were evaluated using a big-data approach (i.e. bump hunter methodology). Sb effect is not characterized as positive or negative, rather as an overlapping of genes. Qualitative description of data only.	Riffo-Campos et al. (2018)

Table 4.6 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
DNA methylation and hydroxymethylation	Leukocytes from frozen specimens	USA, Strong Heart Cohort Study	48 Strong Heart Study participants for which selected metals had been measured in urine at baseline and DNA from leukocytes was available from 1989–1991 (visit 1) and 1998–1999 (visit 3)	Positive cross-sectional associations for Sb with global DNA methylation and hydroxymethylation were found, although the association with global DNA hydroxymethylation was weaker. *P*-values NR, but all CIs go through 1. Positive prospective associations for Sb with global DNA methylation in two different models Using urinary Sb above and below 0.27 µg/g creatinine: OR, 1.93 (95% CI, 1.07–3.47); OR, 2.15 (95% CI, 1.15–4.01) *P*-values, NR	Age, adiposity, smoking, and metal exposure	Exposure assessment critique: Biomonitoring being undertaken at a single time point limits the quality of this exposure assessment. Information on the source of Sb exposure was not available.	Tellez-Plaza et al. (2014)

BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DMR, differentially methylated region; miRNA, microRNA; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; Sb, antimony.

methylated regions. Of 303 genes annotated to differentially methylated regions associated with exposure to metals, 42 were uniquely associated with antimony exposure.

Tellez-Plaza et al. (2014) assessed the association between antimony exposure and global DNA methylation and hydroxymethylation in leukocytes from frozen blood samples collected from participants in a US cohort study of cardiovascular disease. Also collected were baseline urine samples in which metal levels, including antimony, were measured. After controlling for age, adiposity, smoking, and metal exposure in cross-sectional analyses, odds ratios were elevated for associations between antimony and global DNA methylation and hydroxymethylation, although the association with hydroxymethylation was weaker (or lower). Although *P*-values were not reported, all confidence intervals overlapped. However, in a prospective analysis examining associations between urinary antimony levels and global DNA methylation, a positive association for antimony exposure was observed in two different models when using a urine antimony cut-point of 0.27 µg/g creatinine, giving odds ratios of 1.93 (95% CI, 1.07–3.47) and 2.15 (95% CI,1.15–4.01) (*P*-values not reported). [The Working Group noted that all the three studies above showed some evidence of antimony-induced epigenetic effects in exposed humans.]

(ii) Human cells in vitro

Trivalent antimony compounds

Exposure of the human bronchial epithelial cell line BEAS-2B to antimony(III) chloride at a concentration of 10–25 µM for 24 hours caused apoptosis through significant trivalent antimony-induced sirtuin 1 (SIRT1) gene downregulation and protein degradation, which was also linked to trivalent antimony-induced oxidative stress (increased reactive oxygen species, ROS) (see Section 4.2.5) and ERK (also known as MAPK1) activation (Zhao et al., 2018). SIRT1 can deacetylate histones and several non-histone substrates (Lin & Fang, 2013); furthermore, it acts as either a tumour suppressor or promoter depending on its targets in specific signalling pathways or cancers (Lin & Fang, 2013).

Exposure of human keratinocytes to trivalent antimony at a concentration of 3 µM for 1 week caused significant downregulation of miR-203, miR-143, and miR-146a. miR-203 is an inhibitor of cell proliferation, whereas miR-143 and miR-146a are inducers (Phillips et al., 2016).

Chronic exposure of the LNCaP cell line to low (non-cytotoxic) concentrations of antimony(III) potassium tartrate at a concentration of 1 or 2 µM for 20 weeks induced upregulation of the long non-coding RNA PCA3, which targets the miR-132-3P/SREBP1 (also known as SREBF1) signalling pathway, resulting in a significant increase in cell growth/proliferation and colony formation (Guo et al., 2021b).

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group

(ii) Non-human mammalian cells in vitro

Trivalent antimony compounds

Exposure of CGR8 mouse embryonic stem cells to antimony(III) chloride at a concentration of 5 µM for 24 hours caused significantly decreased levels of 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine in both DNA and RNA (Xiong et al., 2017). The decrease in 5-hydroxymethylcytosine has been reported to be associated with early stages of carcinogenesis in rat liver where critical epigenetic modifications may occur (Lian et al., 2015).

4.2.5 Induces oxidative stress

(a) Humans

(i) Exposed humans

See Table 4.7.

In the cross-sectional study by Cavallo et al. (2002) (see Section 4.2.2 and Table 4.1) involving exposure to antimony(III) oxide, oxidative DNA damage was assessed by tail moment measurements of PBLs, as measured by Fpg enzyme-modified comet assay, compared with non-enzyme-modified tail moment measurements. The Fpg-modified tail moment measurements were significantly increased in the "high dose" antimony-exposed workers (group A) after adjusting for age and smoking ($P = 0.002$), suggesting oxidative DNA damage. [The Working Group noted the low antimony exposure concentration, relatively small sample size (exposed and controls, $n = 23$), and lack of information provided about occupational co-exposures, which reduced the informativeness of this study.]

In the study by El Shanawany et al. (2017), antimony exposure (exposed and controls, $n = 25$) was reported to significantly predict apurinic/apyrimidinic sites of DNA damage (see Section 4.2.2) but not total oxidant capacity in blood (Table 4.7), suggesting that antimony may have direct genotoxic effects independent from causing oxidative damage.

In a cross-sectional general-population study in Spain, several urinary markers of oxidative stress (oxidized to reduced glutathione ratio GSSG/GSH, malondialdehyde (MDA), and 8-oxo-deoxyguanine) were examined in 1440 participants and compared with urinary levels of metals, including antimony. No statistically significant differences in geometric mean ratios for any of the three oxidative stress end-points were observed when comparing the first and fifth quintiles of urinary antimony exposure (Domingo-Relloso et al., 2019).

In a study of 50 patients with cutaneous leishmaniasis in Saudi Arabia, markers of oxidative stress before and after administration of sodium stibogluconate and meglumine antimoniate(V) by intramuscular injection were assessed and compared with responses in 30 healthy controls (Seif & Al-Mohammed, 2021). For superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), all values were decreased in patients with cutaneous leishmaniasis compared with controls ($P < 0.001$). All values returned to close to normal ranges after antimony treatment ($P < 0.001$). Other measures of oxidative enzyme activity including nitric oxide, L-arginase, myeloperoxidase, adenosine deaminase, and MDA were all elevated before treatment compared with controls ($P < 0.001$–0.0001) except for GSH, which was markedly lower than control values ($P < 0.0001$). After antimony treatment, all values (except GSH) declined markedly towards normal values ($P < 0.001$–0.0001). [The Working Group noted that a strength of this study was its relatively large size. The study was unusual in comparing the effect of antimony on a cohort of people with systemic parasitic disease with healthy controls. However, the interpretation was complex, given the perturbations related to oxidative stress that are associated with leishmaniasis itself.]

Two studies reviewed by the Working Group examined the potential association between heavy metal-induced oxidative stress in women with either recurrent pregnancy loss (Alrashed et al., 2021) (see also Section 4.2.2) or PCOS (Kirmizi et al., 2021). [Both studies were deemed uninformative by the Working Group. Alrashed et al. (2021) failed to control for confounding by abnormally high levels of gonadotropins and other hormones in the pregnancy-loss group. Kirmizi et al. (2021) assessed a population with PCOS, which is reported to be associated with chronic low-dose inflammation and oxidative stress, thus it is difficult to assess the antimony-induced effect.]

(ii) Human cells in vitro

Trivalent antimony compounds

Primary erythrocytes from a healthy donor were treated with antimony(III) potassium tartrate (2 mM) for 1 hour resulting in the rapid

Table 4.7 Oxidative stress in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Total oxidant capacity	Whole blood	Egyptian rayon workers, cross-sectional	25 rayon workers, 25 non-exposed controls	No Sb effect The mean TOCs for workers and controls were 0.28 ± 0.11 and 0.27 ± 0.07 mmol/L, respectively (t = 0.167, P = 0.868)	Age, smoking	Exposure assessment critique: The evidence of Sb exposure contrast presented in this study is compelling and supported by occupational information and urinary biomonitoring. However, the lack of dilution correction of urinary Sb measurements and little information on co-exposures in the occupation under investigation does slightly weaken the informativeness of the findings. Those with a history of use of medicinal products containing Sb and exposure to other known genotoxic agents were excluded, and smoking was quantified. However, information on other co-exposures in this occupation was lacking. Misclassification not suspected.	El Shanawany et al. (2017)
Oxidative stress markers (GSSG/GSH, MDA, 8-oxo-dG)	Urine	Spain, general population, cross-sectional	n = 1440	No Sb effect NS differences in GMRs in any of the 3 end-points when comparing 1st and 5th quintile urinary Sb exposure	Diabetes, smoking	Exposure assessment critique: Population-based cross-sectional nature of the study and biomonitoring being undertaken at a single time point limits the quality of this exposure assessment. Information on the source of Sb exposure was not available. No details given for statistical methods; report regressions performed but no details.	Domingo-Relloso et al. (2019)

Table 4.7 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Oxidative stress markers (SOD, CAT, GPX) Oxidative enzyme activity (L-arg, MPO, ADA, GSH, NO, MDA)	Blood	Saudi Arabia, patients with CL Evaluation of pre/post markers after intramuscular injections of sodium stibogluconate and meglumine antimoniate(V)	50 patients with CL; 30 healthy controls	All values ↓ in patients with CL compared with controls; $P < 0.001$ All values return to normal range post-Sb treatment; $P < 0.001$ Untreated patients with CL: all values markedly ↑ compared with controls ($P < 0.001$–0.0001); except for GSH, ↓ compared with controls, $P < 0.0001$ All values mildly elevated in Sb-treated compared with controls; $P < 0.01$–0.0001 Except GSH; lower than controls ($P < 0.001$)		Strength of this study was its relatively large size.	Seif & Al-Mohammed (2021)

↓, decreased; ↑, increased; ADA, adenosine deaminase; CAT, catalase; CL, cutaneous leishmaniasis; GMR, geometric mean ratio; GPX, glutathione peroxidase; GSH, glutathione; GSSG/GSH, oxidized to reduced glutathione ratio; L-arg, L-arginase; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; NS, not significant; 8-oxo-dG, 8-oxo-deoxyguanine; Sb, antimony; SOD, superoxide dismutase; TOC, total oxidant capacity.

movement of trivalent antimony across the cell membrane and the formation of intracellular trivalent antimony–GSH complexes, which may protect the cells against trivalent antimony-induced oxidative damage (Sun et al., 2000).

Poon & Chu (2000) showed that exposure of human erythrocytes to trivalent antimony (0.2–1.2 mM) for 5 minutes inhibited the activity of glutathione-*S*-transferase with a 50% inhibition concentration (IC_{50}) of 0.05 mM, which was not reversed by the antioxidant *N*-acetylcysteine (NAC) and may promote oxidative stress (Poon & Chu, 2000).

Antimony(III) chloride (10–25 µM) significantly decreased SIRT1 messenger RNA (mRNA) and protein expression in human bronchial epithelial (BEAS-2B) cells and increased apoptosis after 24 hours of exposure. Treatment with the antioxidant NAC restored SIRT1, which decreased apoptosis, suggesting a role for ROS in the suppression of SIRT1 (Zhao et al., 2018).

Viana et al. (2021) showed that exposure of the human acute promyelocytic leukaemia cell line NB4 to antimony(III) oxide (300, 400, and 500 µg/mL) for 72 hours caused increased cytotoxicity and ROS levels.

In the human bladder cancer EJ cell line, exposure to antimony(III) potassium tartrate (0.8 µM) for 48 hours caused significant induction of ROS and mitochondrial damage – with significantly decreased matrix metalloproteinase, mitochondrial respiratory enzyme complex (I/II/III/IV) activity, and ATP levels, and an increased ADP/ATP ratio – and the induction was inhibited by NAC exposure (Lou et al., 2021).

In human A549 lung cells, antimony(III) chloride (200 µM) significantly increased autophagy (and cell death) after 24 hours; this was inhibited by NAC, suggesting that the autophagy was ROS-dependent (Zhao et al., 2017).

In the HEK-293 cell line, antimony(III) oxide (8 and 16 µM) significantly induced ROS (with increased apoptosis) in a dose-dependent manner, with the peak effect being at 6 hours after exposure. These effects were inhibited by NAC. Induction and nuclear translocation of NRF2 and GADD45B expression (with downstream activation/phosphorylation of MAPKs) were also increased, and GADD45B was shown to play a protective role in the induction of oxidative stress and apoptosis by antimony(III) oxide (Jiang et al., 2016).

In human acute promyelocytic leukaemia cells, antimony(III) oxide (1–3 µM) significantly induced ROS (with increased apoptosis) after exposure for 24 hours (Mann et al., 2006).

In the human macrophage cell line THP-1, antimony(III) potassium tartrate (105 µg/mL) significantly induced ROS (and early apoptosis), decreased intracellular GSH levels, and inhibited glutathione reductase (resulting in an accumulation of glutathione disulfide) after 4-hour exposure. Pentavalent antimony had no effect, suggesting that macrophages cannot reduce pentavalent antimony to trivalent antimony (Wyllie & Fairlamb, 2006).

Antimony(III) potassium tartrate inhibited cell proliferation after 72 hours of exposure in four human acute myeloid leukaemia cell lines – HL-60 (LC_{50}, 3.57 ± 1.25 µM), K562 (LC_{50}, 16.71 ± 6.07 µM), KG-1a (LC_{50}, 20.47 ± 4.85 µM), and U937 (LC_{50}, 14.92 ± 5.28 µM). Treatment of the HL-60 cells with antimony(III) potassium tartrate at 10 µM significantly decreased matrix metalloproteinase (at 8 hours) and increased apoptosis (at 24–48 hours) and ROS generation (at 24 hours), which was inhibited by NAC (Lecureur et al., 2002).

In HEK-293 cells, antimony(III) oxide significantly decreased cell viability (measured by mitochondrial activity; LC_{50} after 24-hour exposure, 9.15 µM), and more so than the pentavalent antimony compound potassium hexahydroxoantimonate. Although exposure to antimony(III) oxide at 1 and 5 µM or potassium hexahydroxoantimonate at 1, 5, and 10 µM for 24 hours significantly reduced ROS levels, a

trend of increasing ROS generation was observed with increasing concentrations of pentavalent or trivalent antimony (Verdugo et al., 2016).

Lösler et al. (2009) showed that antimony(III) oxide (5 μM) increased apoptosis in the human myeloid and lymphatic cell lines Loucy, CCRF-CEM, and HL-60, but not K562, after exposure for 16 days or less. Apoptosis was enhanced after exposure to antimony(III) oxide at 1 or 5 μM for 7 or 14 days in the presence of DL-buthionine-(*S,R*)-sulfoximine, which is a modulator of the cellular GSH redox system and an inhibitor of the GSH-synthesis enzyme γ-glutamylcysteine synthetase (an enzyme involved in oxidative stress detoxification). The incubation with DL-buthionine-(*S,R*)-sulfoximine also enhanced the loss of mitochondrial membrane potential in HL-60 cells after 48 hours of exposure to antimony(III) oxide at 10 or 20 μM (Lösler et al., 2009).

As mentioned in Section 4.2.2, exposure of primary human PBLs to antimony(III) chloride at 5 μM for 24 hours significantly increased DNA damage and the frequency of micronucleus formation. However, these genotoxic effects were not inhibited by co-exposure to SOD or CAT, suggesting that oxidative stress may not play a role in the induction of DNA and chromosomal damage by antimony(III) chloride (Schaumlöffel & Gebel, 1998). [The Working Group noted that this study showed that ~8% of the dose of trivalent antimony was oxidized to pentavalent antimony after 24 hours.]

Chronic exposure of LNCaP cells to antimony(III) potassium tartrate at low (non-cytotoxic) concentrations of 1 or 2 μM for 20 weeks had no effect on intracellular ROS production (Guo et al., 2021b).

Pentavalent antimony compounds

In human whole blood and isolated polymorphonuclear leukocytes, exposure to sodium stibogluconate at 1, 10, or 100 μg/mL for 1 hour significantly enhanced the generation of ROS induced by the protein kinase C activator phorbol myristate acetate (PMA) or zymosan. It was also observed that in isolated polymorphonuclear leucocytes, sodium stibogluconate caused a concentration-dependent increase in superoxide production induced by PMA. The effect of sodium stibogluconate (at 10 μg/mL only) was also observed on superoxide production induced by zymosan, and to a lesser extent by platelet-activating factor, or *N*-formylmethionine-leucyl-phenylalanine (Rais et al., 2000).

Redox reactivity involving the partial reduction of pentavalent antimony to trivalent antimony (in the presence of GSH) was observed in human blood plasma after a 45-minute exposure of whole blood to potassium hexahydroxoantimonate (200 ng/mL). This pentavalent antimony compound also significantly reduced the GSH:glutathione disulfide ratio and GPX activity, and increased SOD activity (López et al., 2015).

Poon & Chu (2000) showed that exposure of human erythrocytes to sodium stibogluconate for 5 minutes did not inhibit the activity of glutathione-*S*-transferase (Poon & Chu, 2000).

(b) Experimental systems

(i) Non-human mammals in vivo

Trivalent antimony compounds

In a study of subchronic toxicity, groups of male and female Sprague-Dawley rats were given drinking-water containing antimony(III) potassium tartrate at a concentration of 0.5, 5, 50, or 500 ppm for 13 weeks. Hepatic glutathione-*S*-transferase and ethoxyresorufin-*O*-deethylase activity were significantly increased in the liver of the groups at 500 ppm (Poon et al., 1998).

Antimony(III) chloride at a dose of 250 μmol/kg bw administered by subcutaneous injection potently induced haem oxygenase levels in the liver and kidney of male Sprague-Dawley rats (peak, 16 hours) (Drummond & Kappas, 1981).

Male Kunming mice were treated with antimony(III) oxide at a dose of 15 mg/kg bw by gavage for 60 days. The results showed that total antioxidant capacity was significantly decreased and levels of MsrA, MsrB1, and MDA increased in the testis after exposure. Levels of SOD were also decreased, but the change was not statistically significant compared with the control group (Wu et al., 2021).

Kunming mice treated with antimony(III) chloride by intraperitoneal injection (40 mg/kg bw) for 28 days exhibited significantly decreased levels of SOD and GPX, and increased levels of MDA, in liver mitochondria (Wang et al., 1998).

Zhang et al. (2021) reported that the expression of inducible nitric oxide synthase (iNOS) was significantly increased in the brain tissue of male ICR mice treated with antimony(III) potassium tartrate trihydrate at doses of 10 or 20 mg/kg bw by intraperitoneal injection for 8 weeks.

Pentavalent antimony compounds

Phorbol myristate acetate- or zymosan-induced ROS were increased in blood samples collected on day 21 from BALB/c mice treated with sodium stibogluconate at doses of 100, 200, or 400 mg/kg bw by subcutaneous injection on days 14, 16, and 18 (Rais et al., 2000).

Induction of oxidative DNA damage in PBLs of Swiss mice was observed 24 hours after administration of meglumine antimoniate(V) (Glucantime) at a dose of 810 mg/kg bw by intraperitoneal injection (see Section 4.2.2). The induction was inhibited by the antioxidants genistein (administered by gavage 3 days prior to meglumine antimoniate(V)) and ascorbic acid (administered by intraperitoneal injection either as co-exposure with meglumine antimoniate(V) or 24 hours after meglumine antimoniate(V)) (de Jesus et al., 2018).

Exposure of BALB/c mice to meglumine antimoniate(V) administered by intraperitoneal injection at a dose of 20 mg/kg bw per day for 20 days caused a significant increase in oxidative DNA damage in PBLs (see Section 4.2.2). SOD and CAT activities were significantly increased and GPX activity was decreased in serum, which are indicative of oxidative stress (Moreira et al., 2017).

Oxidative stress was induced in the heart, liver, spleen, and brain (no significant effects in kidney) of CF-1 mice treated with meglumine antimoniate(V) at a dose of 20, 60, or 120 mg of antimony(V)/kg per day by subcutaneous injection for 3 consecutive days. The oxidative stress biomarkers measured – protein carbonylation (in heart, spleen, and brain at the highest exposure level) and lipid peroxidation, as assessed by MDA levels (in liver and brain at all exposure levels) – were significantly increased. Imbalances in SOD activity (decreased in heart and brain and increased in spleen at doses of 20 and 60 mg of antimony(V)/kg) and decreases in CAT activity (decreased in liver and brain at all exposure levels) were also observed (Bento et al., 2013).

Antimony(V) chloride, at a dose of up to 250 µmol/kg bw administered by intravenous injection, did not induce haem oxygenase in the liver and kidney of male Sprague-Dawley rats (Drummond & Kappas, 1981).

(ii) Non-human mammalian cells in vitro

Trivalent antimony compounds

Treatment of rat cardiac myocytes with antimony(III) potassium tartrate at a concentration of 100 µM for 4 and 18 hours significantly increased lipid peroxidation (as measured via the release of thiobarbituric acid-reactive substances), which was inhibited by pre-treatment with antioxidants, including vitamin E. Antimony(III) potassium tartrate (50 and 100 µM) also significantly decreased GPX activity as early as 1 hour, increased oxidized GSSH levels at 2 hours (at 100 µM), and decreased GSH levels at 4 hours. The addition of GSH partially protected the cells from lipid peroxidation induced by antimony(III) potassium

tartrate (100 μM) after 18 hours of exposure or less (Tirmenstein et al., 1995).

Exposure of mouse embryonic stem cell lines to six trivalent antimony compounds for 24 hours significantly induced an oxidative stress response (indicated by activation of the Srxn1 and Blvrb gene reporters relevant to a Nrf2 response and ROS production), as measured by ToxTracker reporter assay (see also Section 4.2.2) at non-cytotoxic concentrations: antimony(III) oxide (peak response, 0.32–0.96 μg/mL), antimony(III) sulfide (peak response, 0.56–1.4 μg/mL), antimony(III) chloride (peak response, 0.2–0.8 μg/mL), antimony triacetate (peak response, 2.12 μg/mL), antimony(III) potassium tartrate (peak response, 0.4–0.6 μg/mL), and antimony tris (ethylene) glycolate (peak response, 0.35–0.7 μg/mL) (Boreiko et al., 2021).

Exposure of the rat PC12 cell line to antimony(III) potassium tartrate trihydrate at concentrations of 25–100 μM for 24 hours significantly increased ROS and MDA levels, resulting in increased apoptosis and autophagy (via inhibition of Akt/mTOR signalling), which was reversed by treatment with the antioxidant NAC (Wang et al., 2019b).

Similarly, exposure of PC12 cells to antimony(III) potassium tartrate trihydrate at 50 or 75 μM for 24 hours significantly induced apoptosis (at 75 μM only) and the activation (phosphorylation) of cyclic adenosine monophosphate response element-binding (CREB) protein (via JNK activation), which is protective against oxidative stress/apoptosis by regulating target genes such as uncoupling protein-2 and Nrf2 (Zhi et al., 2020).

Treatment of neonatal rat cardiac myocytes with antimony(III) potassium tartrate at non-cytotoxic concentrations (5 and 10 μM) for 18 hours significantly increased GSH levels and haem oxygenase 1 (HMOX1) activity, indicative of increased oxidative stress (Snawder et al., 1999).

Exposure of the rat C6 glioma cell line (astrocytes) to antimony(III) potassium tartrate trihydrate at concentrations of > 0.625 μmol/L for 24 hours significantly increased iNOS protein and mRNA levels (Zhang et al., 2021).

Pentavalent antimony compounds

In the study by Boreiko et al. (2021), exposure of mouse embryonic stem cells to two pentavalent antimony compounds – antimony(V) chloride (peak response, 1.6–4 μg/mL) and potassium hexahydroantimonate (38 μg/mL) – significantly induced an oxidative stress response (indicated by activation of the Srxn1 and Blvrb gene reporters) at non-cytotoxic doses, as assessed by ToxTracker assay, after exposure for 24 hours. Sodium antimonate induced a weak response (activation of Srxn1 only) at 3 μg/mL. Sodium hexahydroxoantimonate and antimony(V) oxide gave negative results for oxidative stress.

Treatment of mouse peritoneal macrophages with the non-cytotoxic pentavalent antimonial anti-leishmaniasis drug sodium antimony(V) gluconate at a concentration of 60 μg/mL for 24 hours induced significantly increased production of nitric oxide (only in the presence of interferon gamma) and intracellular ROS (Ghosh et al., 2013). In addition, significantly enhanced generation of ROS (after 3 and 6 hours) and nitric oxide (after 48 hours) was observed after exposure to sodium antimony(V) gluconate at 10 μg/mL (Mookerjee Basu et al., 2006).

4.2.6 Induces chronic inflammation

(a) Humans

(i) Exposed humans

See Table 4.8.

In a study of antimony-smelter workers from Serbia and Montenegro, Potkonjak & Pavlovich (1983) reported findings relating to 51 workers exposed to airborne dust containing high concentrations of antimony(III) oxide over a period of 9–31 years. Abnormalities observed by

Table 4.8 Chronic inflammation in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Pneumoconiosis	CXR	Serbia and Montenegro, Sb-smelting plant, cross-sectional	51 workers exposed to high concentrations of antimony(III) oxide (≤ 88% of Sb species present); 9–31 yr duration	Frequency of clinical outcomes On CXR: pulmonary punctate opacities in > 67% of patients On physical examination: 35% with upper airway irritation, 27% with conjunctivitis	None	[The Working Group noted that these represent clinical findings of chronic inflammation and are unique for Sb vs silica exposure.]	Potkonjak & Pavlovich (1983)
Pneumoconiosis	CXR	USA, Sb smelters, cross-sectional	28 workers exposed to Sb ore and antimony(III) oxide, CXR post-smelting	8/28 had suspect or clear CXR abnormalities with predominantly small pulmonary opacities No consistent PFT abnormalities	None		Cooper et al. (1968)

CXR, chest X-ray; PFT, pulmonary function test; Sb, antimony; vs, versus; yr, year.

chest X-ray were described as small pulmonary punctate opacities (primarily in the mid-lung fields) in > 67% of patients, consistent with pneumoconiosis, which arises from a chronic inflammatory response to respirable insoluble mineral dusts in the deep lung. These antimony-specific findings on chest X-ray were different from findings for antimony miners, who have a mixed exposure to silica and thus may develop the classic findings of silicosis. On physical examination, 35% of patients also had evidence of upper-airway irritation, and 27% had evidence of conjunctivitis, which is consistent with chronic exposure to irritant dust. [The Working Group noted that the chest X-ray findings reported may or may not indicate chronic lung inflammation in the absence of a clinical history and may have a broader differential diagnosis. But, given that this is a population finding (and not an individual observation) with exposure histories included, it is reasonable to ascribe the findings to a pro-inflammatory response.]

In another study of antimony smelters exposed to antimony(III) oxide at a plant in the USA, Cooper et al. (1968) described antimony pneumoconiosis, exhibiting predominantly small pulmonary opacities, in 8 of 28 workers. However, there were no consistent abnormalities in pulmonary function.

The effect of antimony exposure was examined in a population of women with PCOS (Kirmizi et al., 2021). [As also reported in Section 4.2.5, the Working Group deemed the study to be uninformative regarding either potential antimony-induced oxidative stress or chronic inflammation.]

In two case reports of patients with leishmaniasis, Costa et al. (2018) and Torrús et al. (1996) compared the effects of antimony-containing topical medication before and after treatment, without normal controls. [The Working Group considered the perturbed inflammatory state before treatment to be poorly suited to examination of the antimony effect. In addition, the two patients in the study by Torrús et al. (1996) were reported to have additional immunocompromised states, with one patient coinfected with HIV and hepatitis C, and one patient being treated with immune modifiers (post-kidney transplant), including cyclosporin (which is *carcinogenic to humans*, IARC Group 1).]

Lobanova et al. (1996) assessed the pathogenic effects of antimony exposure in miners, reporting various markers of inflammation. [The Working Group considered the study to be minimally informative, because standard pro-inflammatory markers of interest are not included, and no statistical methods or controls for other co-exposures are described.]

(ii) Human cells in vitro

Trivalent antimony compounds

Guildford et al. (2009) investigated the potential role of different types of metal NPs, including antimony(III) oxide NPs (size, 41–91 nm), in activating elements of the clotting system (with incubation for 30 minutes in platelet-rich human plasma) and human primary blood-derived monocytes/macrophages. It was observed that exposure of monocytes/macrophages to antimony(III) oxide NPs at 0.05 mg/mL (preconditioned with plasma) for 20 hours activated the monocytes/macrophages via the promotion of fibrin polymerization and the aggregation and fragmentation of platelets; however, cellular secretion of TNFα and PDGF-BB were not significantly increased. [The Working Group noted that nano-scale antimony compounds have the potential to induce a pro-inflammatory response via activation of monocytes/macrophages. The Working Group also noted that this is not a chronic effect.]

Pentavalent antimony compounds

HIV-1 transcription, replication, and viral production were significantly increased in human primary CD4+ T-cells and ex vivo primary thymic histocultures exposed to

sodium stibogluconate at concentrations of 50–500 µg/mL for 3–6 and 14 days, respectively. The effect of sodium stibogluconate was dose-dependent and occurred by cellular activation of NF-κB (also known as NFKB1), AP-1 (also known as JUND), and the Syk, Jun, and MAPK/ERK signalling pathway (Barat et al., 2007). [The Working Group noted that sodium stibogluconate induced T-cell activation, which enhanced HIV-1 infection, but the relevance to chronic inflammation is unclear.]

(b) Experimental systems

(i) Non-human mammalian in vivo

Trivalent antimony compounds

Exposure of male and female B6C3F$_1$ mice to drinking-water containing antimony(III) potassium tartrate at a dose of 407 mg/kg bw for 14 days induced focal areas of ulceration in the forestomach, with necrosis and inflammation of the squamous mucosa, which were most apparent in females. In contrast, exposure to antimony(III) potassium tartrate at a dose of 50 mg/kg bw administered by intraperitoneal injection over the course of 16 days (12 injections) induced increased hepatocellular necrosis and liver capsule inflammation and fibrosis, which were most apparent in male mice. No such effects were induced by antimony(III) potassium tartrate in F344/N rats (Dieter et al., 1991; NTP, 1992). [The Working Group noted that no statistics were reported.] In 90-day studies of subchronic toxicity, exposure of B6C3F$_1$ mice and F344/N rats to antimony(III) potassium tartrate by oral administration in drinking-water induced inflammatory cell infiltrates in the pancreas, intestine, and mesenteric lymph nodes in mice, but not in rats (dose and sex not specified). Exposure of male and female F344/N rats to antimony(III) potassium tartrate at doses of ≥ 6 mg/kg bw administered by intraperitoneal injection (every other day for 90 days) induced hepatocellular degeneration and necrosis (with a minimal inflammatory cell infiltrate), as well as liver capsule inflammation and fibrosis at doses of ≥ 3 mg/kg bw (Dieter et al., 1991; NTP,1992). [The Working Group noted that no statistics were reported.]

Male and female Fischer 344 rats were exposed by inhalation (whole-body) to antimony(III) oxide at a concentration of 0, 0.25, 1.08, 4.92, or 23.46 mg/m^3 for 6 hours per day, 5 days per week for 13 weeks (with or without an observation period of 27 weeks) in a study of subchronic toxicity, and to 0, 0.06, 0.51, or 4.5 mg/m^3 for 52 weeks (with or without an observation period of 1 year) in a study of chronic toxicity. Subchronic and chronic histopathological changes in the lung of males and females included increased numbers of alveolar/intra-alveolar macrophages and subacute–chronic interstitial and granulomatous inflammation, which were all mostly apparent at the highest exposure concentrations (Newton et al., 1994).

In subacute studies, Wistar Han rats and B6C3F$_1$/N mice were exposed by inhalation (whole-body) to antimony(III) oxide aerosol at a concentration of 0, 3.75, 7.5, 15, 30, or 60 mg/m^3 for 6 hours per day (5 days per week) for 12–13 exposure days during a 16–17-day period. Significantly increased incidence of chronic active inflammation in the lung was observed in male and female rats exposed to antimony(III) oxide at 30 and 60 mg/m^3, but not in mice (NTP, 2017).

In 2-year studies of chronic toxicity, Wistar Han rats and B6C3F$_1$/N mice were exposed by inhalation (whole-body) to antimony(III) oxide aerosols at a concentration of 0, 3, 10, or 30 mg/m^3 for 6 hours per day (5 days per week) for 105 weeks or less. The incidence of chronic active inflammation in the lung was significantly increased in male and female rats and mice at all exposure concentrations. The incidence of cellular infiltration of lymphocytes in the lung was significantly increased in male and female mice, in female rats at all exposure concentrations, and in male

rats at 3 and 10 mg/m^3. The incidence of suppurative alveolar and pleural inflammation was also significantly increased in rats and mice (in both males and females), respectively, at all exposure concentrations. There was a significantly increased incidence of chronic active inflammation in the (respiratory epithelium of the) nose of male mice (3 and 10 mg/m^3) and larynx of female rats (3 mg/m^3). The incidence of chronic active inflammation in the arteries of multiple organs/tissues combined (including pancreas, lung, kidney, mediastinum, and mesentery) was significantly increased in male and female rats at the highest exposure concentration. In addition, the incidence of chronic active inflammation in the epicardium of the heart was significantly increased in male and female mice at the two highest exposure concentrations, as was the incidence of chronic active inflammation of the forestomach in males at the highest exposure concentration (NTP, 2017).

Potkonjak & Pavlovich (1983) reported that exposure of female Wistar albino rats to antimony(III) oxide dust (50 mg) administered intraperitoneally or intratracheally induced non-collagenous (non-fibrotic) pneumoconiosis 2 months after the exposure.

Exposure of female Fischer 344 (CDF) rats to antimony(III) oxide by inhalation (whole-body) at a concentration of 1.6 ± 1.5 or 4.2 ± 3.2 mg/m^3 (6 hours per day and 5 days per week) for 1 year or less caused a significant increase in the incidence of multinucleated giant cells, cholesterol clefts, pigmented macrophages, and focal fibrosis in the lung (after 3–6 months of exposure), and chronic interstitial nephritis in the kidney (Watt, 1983).

In a study of subchronic toxicity, male and female Sprague-Dawley rats were given drinking-water containing antimony(III) potassium tartrate at a concentration of 0.5, 5, 50, or 500 ppm for 13 weeks. Monocytes were significantly increased in the blood of the female rats at 500 ppm (Poon et al., 1998).

Lung inflammation was not reported in rats and rabbits exposed to antimony(III) oxide (100–125 mg) by inhalation for ~50 and ~14 days, respectively, although the alveolar spaces in rabbits contained some macrophages (Gross et al., 1955b).

Pentavalent antimony compounds

Potkonjak & Pavlovich (1983) also reported that antimony(V) oxide dust (50 mg) administered intraperitoneally or intratracheally to female Wistar albino rats induced non-collagenous (non-fibrotic) pneumoconiosis 2 months after the exposure.

In BALB/c mice exposed to sodium antimony gluconate administered by a single intravenous injection (16 mg/kg bw), peritoneal macrophages isolated 2 days after injection exhibited significantly increased T-cell-stimulating activity (with increased interleukin-2 production), which is relevant to enhanced antigen presentation, and production of interleukin-12 and tumour necrosis factor alpha (TNFα) (Ghosh et al., 2013).

(ii) Non-human mammalian cells in vitro

Trivalent antimony compounds

Exposure of the rat C6 glioma cell line (astrocytes) to antimony(III) potassium tartrate trihydrate at a concentration of 0.1–5.0 μmol/L for 24 hours significantly increased cell activation/proliferation as well as phosphorylation/nuclear translocation of p65 (also known as RELA; NF-κB signalling) and expression of the pro-inflammatory cytokines interleukin IL-1β (also known as IL1B), IL-6, and TNFα at exposure concentrations of ≥ 1.25 μmol/L. Antimony also induced increased phosphorylation of TGFβ-activated kinase 1 (TAK1, also known as MAP3K7) thus increasing its activity, whereas TAK1 inhibition alleviated antimony(III) potassium tartrate trihydrate-induced p65 phosphorylation and subsequent C6 cell activation (Zhang et al., 2021). [The Working Group noted that the link between astrocyte proliferation and the activation of

NF-κB or TAK1 is missing, since no cell proliferation markers were investigated after the cells were treated with NF-κB or TAK1 inhibitors.]

Treatment of the murine haematopoietic Baf3 cell line with antimony(III) potassium tartrate (10 µg/mL) did not enhance IL-3-induced Jak2/Stat5 tyrosine phosphorylation or IL-3-induced proliferation (Pathak & Yi, 2001).

Pentavalent antimony compounds

Treatment of peritoneal macrophages derived from BALB/c mice with a non-cytotoxic dose of sodium antimony gluconate (60 µg/mL) induced significantly increased T-cell-stimulating activity (with increased IL-2 production) (after 24- and 72-hour exposures) and production of interleukin-12 and TNFα (after 24-hour exposure) (Ghosh et al., 2013). [The Working Group noted that acute immune activation may or may not be linked to chronic inflammation.]

Treatment of murine haematopoietic Baf3 cells with sodium stibogluconate (10 µg/mL) increased IL-3-induced Jak2/Stat5 tyrosine phosphorylation, and IL-3-induced Baf3 proliferation (at day 3) was significantly increased at concentrations of sodium stibogluconate between 0.3 and 200 µg/mL, with a maximal effect concentration of ~40 µg/mL (Pathak & Yi, 2001).

4.2.7 Is immunosuppressive

(a) Humans

(i) Exposed humans

See Table 4.9.

Kim et al. (1999) assessed antimony-induced alterations of the immune system – including the concentrations of the IgG subclasses, IgE, IL-2, interferon gamma (IFNG), and IL-4 – in samples of sera collected from workers (n = 34) exposed to antimony(III) oxide during manufacture. Serum IgG1 levels were significantly lower ($P < 0.05$) in workers in the group exposed to the highest concentration (n = 12) than those moderately (n = 22) and not exposed (control, n = 33). Serum IgE concentrations in the group exposed to the highest concentration were also significantly lower ($P < 0.05$) than the control (3.6-fold) group and the group exposed to the lowest concentration (5.2-fold). Workers in the group exposed to the highest concentration also had significantly lower serum interferon gamma ($P < 0.05$) and IL-2 levels than the group exposed to the lowest concentration and controls (although, for IL-2, the difference did not reach statistical significance).

In an NHANES study, which is a nationally representative survey of the civilian population in the USA, Scinicariello & Buser (2016) reported leukocyte telomere length in blood samples collected from study participants as a function of antimony exposure. [The Working Group noted that critically shortened telomeres may signal replicative senescence and apoptosis in cells.] Exploring several effect models – and considering covariates and adjustments including age, ethnicity, education, weight status, and lead exposure – higher antimony concentrations in urine were reported to be significantly associated with shorter leukocyte telomere length. Individuals in the third and fourth quartiles of the urinary antimony distribution had significantly shorter leukocyte telomere lengths (quartile 3, −4.78%; 95% CI, −8.42 to −0.90; and quartile 4, −6.11%; 95% CI, −11.04 to −1.00) than the lowest reference antimony quartile with evidence of a dose–response (P for trend, 0.03). [The Working Group noted that increased antimony-induced apoptosis of circulating leukocytes could be linked to immunosuppressive effects.]

Wu & Chen (2017) studied 99 industrial workers employed at five plants – involved in industries including the manufacture of glass and engineered plastics – exposed to antimony(III) oxide and 42 administrative staff (non-exposed controls) from the same plants. Mean urinary antimony concentrations in all industrial workers (9.28 µg/g creatinine) were significantly higher ($P < 0.001$) than non-exposed controls (2.26 µg/g

Table 4.9 Immunosuppression in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Ig subclasses, cytokines	Serum	Republic of Korea, Sb workers, cross-sectional	Group A, $n = 12$ high antimony(III) oxide exposure Group B, $n = 22$ moderate antimony(III)oxide exposure Group C, $n = 33$ non-exposed controls	↓ Serum IgG1 ($P < 0.05$) in Group A compared with Groups B or C ↓ Serum IgE ($P < 0.05$) in Group A compared to Groups B (5.2-fold) or C (3.6-fold) ↓ Serum IFNG ($P < 0.05$) and IL-2 (NS) in Group A compared with Groups B or C	None	Exposure assessment critique: The evidence of Sb exposure contrast presented in this study is very compelling and supported by multiple methods of exposure assessment. The differences in Sb exposure between comparison groups is indisputable.	Kim et al. (1999)
Leukocyte telomere length	Blood, leukocytes	USA, NHANES, cross-sectional		↓ LTL with increased Sb exposure; individuals in the 3rd and 4th quartiles of urinary Sb distribution had significantly shorter LTL (−4.78%, 95% CI, −8.42 to −0.90; and −6.11%, 95% CI, −11.04 to −1.00, respectively) compared with the lowest reference quartile with evidence of a dose–response (P for trend = 0.03)	3 models, multiple covariates and adjustments including age, ethnicity, education, weight status, and Pb	Exposure assessment critique: Population-based cross-sectional nature of the study and biomonitoring being undertaken at a single time point limits the quality of this exposure assessment. Information on the source of Sb exposure was not available.	Scinicariello & Buser (2016)

Table 4.9 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Ig subclasses	Serum	Taiwan, China, industrial workers, cross-sectional	$n = 99$ industrial workers from 5 plants $n = 14$ with higher Sb exposure compared with the others $n = 42$ administrative staff (non-exposed controls) from the same plants Mean urinary Sb in workers = 9.28 μg/g creatinine vs in controls = 2.26 μg/g creatinine ($P < 0.001$)	↓ Mean serum IgG, IgA, and IgE levels among all industrial workers compared with controls ($P \leq 0.001$) ↓ Monocyte counts for total participants ($n = 133$) with ↑ Sb levels in blood and urine ($P < 0.001$ and $P < 0.05$, respectively)	Authors note that the prevalence rates of smoking, drinking, betel nut chewing, and allergy were alike for the Sb-exposed workers and controls	Exposure assessment critique: Area air sampling was conducted during work shifts using standard methods. There were insufficient details about the strategy for collecting air (area) samples, particularly in terms of how many air samples were collected each day and whether sampling occurred on multiple days. Levels of Sb in air samples and in urine, blood, and hair were measured at the same time as the end-points of interest. Co-exposures to other metals and chemicals were not evaluated.	Wu & Chen (2017)

↓, decreased; ↑, increased; CI, confidence interval; Ig, immunoglobulin; IFNG, interferon gamma; IL-2, interleukin 2; LTL, leukocyte telomere length; NHANES, National Health and Nutrition Examination Survey; NS, not significant; Pb, lead; Sb, antimony.

creatinine), and mean serum IgG, IgA, and IgE levels in all industrial workers were significantly lower ($P \leq 0.001$). Monocyte counts for all study participants ($n = 133$) were negatively correlated with antimony in blood and urine ($P < 0.001$ and $P < 0.05$, respectively). [The Working Group noted that there were possible co-exposures to other metals and carcinogens in these plants that were not addressed in the analyses reported, but that the three studies showed some evidence of antimony-induced immunosuppression in exposed humans.]

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

In a study conducted by the NTP (2017) (see Section 4.2.6(b)(i)), chronic exposure to antimony(III) oxide by inhalation caused a significant increase in the incidence of cellular depletion in the thymus of male and female B6C3F$_1$/N mice at the two highest exposure concentrations (10 and 30 mg/m^3).

White Leghorn chick embryo eggs were injected with antimony(III) chloride or antimony(III) potassium tartrate on days 7 and 14 as follows: (a) 0.0 ppm (in 0.5 or 1.0 mL of vehicle), (b) 1.0 ppm (in 0.5 or 1.0 mL), and (c) 5.0 ppm (in 0.5 mL of vehicle). Blood was collected 20 days after injection. The results showed that injection of 1 ppm/mL antimony decreased the number of leukocytes by 47%, but that injection of 2 or 10 ppm/mL did not (Newkirk et al., 2014). [The Working Group noted that there were high standard deviations in this study.]

4.2.8 Modulates receptor-mediated effects

(a) Humans

(i) Exposed humans

See Table 4.10.

In the Hangzhou Birth Cohort Study conducted in China, thyroid hormone levels in 915 pregnant women were examined by tertiles of level of exposure to six metals: arsenic, cobalt, manganese, nickel, antimony, and selenium. For antimony, in a single-metal model, an inverse association was found with FT4 in blood across tertiles of antimony exposure after controlling for multiple testing (false discovery rate-adjusted P for trend, < 0.05). In a multiple-metal model, significantly decreased changes in percentage levels of thyroid hormones were demonstrated between FT4 and the third tertile of antimony exposure (–1.99%; 95% CI, –3.44% to 0.52%; P for trend, 0.006). Results of spline regression models indicated that FT4 decreased throughout the range of antimony concentrations with a significant linear trend (P for overall association, 0.001; P for nonlinearity, 0.716). Covariates considered included maternal age, education, household income, working during pregnancy, exposure to second-hand smoke in pregnancy, drinking during pregnancy, gestational age at measurement of thyroid hormones, parity, history of hyperglycaemia during pregnancy, and pre-pregnancy BMI (Guo et al., 2018).

In a study of 824 pregnant women in the Rhea Birth Cohort, conducted in Greece, Margetaki et al. (2021) assessed women with high (third tertile) urinary antimony concentrations and found that they had a 12.5% lower (95% CI, 1.8–22.0%) level of thyroid-stimulating hormone in blood than women in the first and second tertiles, which was statistically significant ($P < 0.05$). Covariates considered included trimester at blood sampling, maternal age, parity, smoking early in pregnancy, maternal education, maternal pre-pregnancy BMI, iodine status, and family history of thyroid disease. In a combined exposure model including other metals (cadmium and lead exposure), the thyroid-stimulating hormone effect reported was a change of –9.8% (95% CI, –19.7 to 1.3; $P < 0.1$).

The association of urinary metal concentrations with reproductive hormone levels was

Table 4.10 Modulation of receptor-mediated effects in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Thyroid hormone levels	Blood	China, Hangzhou Birth Cohort Study, cross-sectional	n = 915 pregnant women	Negative associations of tertiles of Sb exposure with FT4 ($P < 0.05$; FDR = 0.018) Negative associations of 3rd tertile of Sb exposure in multiple-metal model for FT4 (–1.99%, 95% CI, –3.44 to –0.52%; P for trend = 0.006) Results of spline regression models indicated that thyroid hormones decreased throughout the range of Sb concentrations with a significant linear trend (P for overall association = 0.001, P for nonlinearity = 0.716)	Maternal age, education, household income, working during pregnancy, second-hand smoke during pregnancy, drinking during pregnancy, gestational age at measurement of thyroid hormone levels, parity, history of hyperglycaemia in pregnancy, and pre-pregnancy BMI	Exposure assessment critique: Single blood samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Co-exposures to other metals (As, Co, Se, Mn, and Ni) were evaluated in the statistical analyses if they produced statistically significant results ($P < 0.05$) when evaluated one at a time. Logistic regression results presented for Mn, Ni, and Sb with FT4.	Guo et al. (2018)
Thyroid hormone levels	Serum	Greece, Rhea Birth Cohort	n = 824 pregnant women	↓ TSH with ↑ urinary Sb (3rd tertile); 12.5% ↓ TSH (95% CI, 1.8–22.0%) compared with women with lower (2nd and 1st tertile) urinary Sb ($P < 0.05$) in a single exposure model and -9.8% change (95% CI, –19.7 to 1.3) for a combined exposure model ($P < 0.1$)	Trimester at blood sampling, maternal age, parity, smoking early in pregnancy, maternal education and maternal pre-pregnancy BMI, iodine status, and family history of thyroid disease	Exposure assessment critique: Single urine samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Urinary biomarkers of Sb represent recent exposure. Co-exposures to Cd and Pb were evaluated using BKMR. Reliance on a single urine sample likely resulted in imprecise estimates of (recent) exposure.	Margetaki et al. (2021)
Reproductive hormone levels	Serum	China, partners of patients at a fertility clinic, cross-sectional	n = 511	No associations between urinary Sb levels and serum reproductive hormones after adjustment for multiple testing (FDR-adjusted P for trend) for each hormone ranging from > 0.50 to 0.99)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Exposure assessment critique: Urine samples collected at two close points in time on the same day as semen sample limits the findings.	Wang et al. (2016)

↓, decreased; ↑, increased; As, arsenic; BKMR, Bayesian kernel machine regression; BMI, body mass index; Cd, cadmium; CI, confidence interval; Co, cobalt; FDR, false discovery rate; FT4, free T4 (thyroxine); Mn, manganese; Ni, nickel; Pb, lead; Sb, antimony; Se, selenium; TSH, thyroid-stimulating hormone.

also assessed in the partners of patients at a fertility clinic in China in a cross-sectional study conducted by Wang et al. (2016). No associations between urinary antimony concentrations and serum reproductive hormones after adjustment for multiple testing were observed (with false discovery rate-adjusted *P* for trend for each hormone ranging from > 0.50 to 0.99). [The Working Group noted that cadmium and lead were also measured in urine samples, and co-exposures were assessed using BKMR analysis.] [The Working Group noted that two of the two studies that assessed the effects of antimony on different thyroid hormone measurements reported inverse associations.]

(ii) Human cells in vitro

Trivalent antimony compounds

Zhang et al. (2018a, b) demonstrated that exposure of androgen-dependent human prostate cancer cell lines to low (non-cytotoxic) doses of antimony(III) potassium tartrate for 48–72 hours (LNCaP cells, 0.5 μM; PC3 cells, 8 μM) significantly promoted cell growth/proliferation, invasion (into Matrigel), and migration, as assessed by wound-healing assay with PC3 cells only. In LNCaP cells, treatment with antimony(III) potassium tartrate triggered the phosphorylation of the androgen receptor, which transcriptionally regulated the expression of many androgen-associated genes, including upregulation of PSA and NKX3.1, thus mimicking androgen activity (Zhang et al., 2018a). In the study with PC3 cells, antimony(III) potassium tartrate regulated the expression of Ctbp2, which resulted in the transcriptional regulation of RhoC (a Rho GTPase family member) expression, increased ROCK1 kinase activity, and increased stability of the c-Myc oncogenic protein (Zhang et al., 2018b).

Chronic exposure of LNCaP cells to antimony(III) potassium tartrate at low (non-cytotoxic) concentrations of 1 and 2 μM for 20 weeks significantly increased cell growth/proliferation, colony formation, and triglyceride and cholesterol levels, indicative of lipid metabolic disequilibrium induced by upregulation of the long non-coding RNA PCA3, which targets the miR-132-3P/SREBP1 receptor signalling pathway, in response to antimony(III) potassium tartrate exposure (Guo et al., 2021b).

Patterson & Rice (2007) found that exposure of spontaneously immortalized keratinocyte (SIK) cells to antimony(III) potassium tartrate (5 μM) for 3 or 9 days resulted in the stabilization of the epidermal growth factor (EGF) receptor, which preserved its ability to signal and thus the proliferative capacity of the cells. In the presence of AG1478, an EGF receptor inhibitor, exposure to antimony(III) potassium tartrate for 3 days did not increase the colony-forming efficiencies of SIK cells and primary normal human epidermal keratinocytes, which indicates that antimony has an EGF receptor-dependent effect on cell proliferation. Treatment of the SIK cells with antimony(III) potassium tartrate also stabilized nuclear β-catenin protein and significantly altered (in the presence of EGF) β-catenin-dependent gene expression (Patterson & Rice, 2007).

Choe et al. (2003) used human MCF7 cells, which were stably transfected with the plasmid pTK-ERE containing a luciferase reporter gene under the control of an estrogen-responsive element (MCF7-ERE), and an E-Screen assay (also with MCF7 cells) to assess the estrogenicity of various heavy metals and their species. They found that treatment with antimony(III) chloride (1 μM) exhibited high estrogenicity in MCF7-ERE cells and the E-Screen assay after exposure for 36 hours and 6 days, respectively (Choe et al., 2003).

Comparison of the gene expression profiles of SIK cells exposed to trivalent arsenic (3 μM) or trivalent antimony (6 μM) for 1 week using next-generation sequencing revealed that both trivalent arsenic and antimony produced highly similar transcriptional responses in human keratinocytes (Phillips et al., 2016). mRNAs

downregulated by both antimony(III) and arsenic(III) included those encoding the leucine-rich repeats protein 1 (LRIG1), a negative regulator of EGFR signalling, and the ryanodine receptor 1 (RYR1), a positive regulator of calcium signalling that promotes cell differentiation (Phillips et al., 2016). Moreover, exposure of SIK cells to either trivalent antimony or arsenic also significantly attenuated bone morphogenetic protein-6 (BMP6)-induced DUSP2 and DUSP14 (Phillips et al., 2016), consistent with the maintenance of EGF receptor signalling.

Pentavalent antimony compounds

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

No data were available to the Working Group.

4.2.9 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Exposed humans

See Table 4.11.

Goi et al. (2003) assessed the activity of lysosomal glycohydrolases in plasma samples from a group of 26 Italian art-glass workers, of whom 16 were exposed to arsenic(III) oxide and 10 were exposed to antimony(III) oxide. No significant mean differences were found between the antimony-exposed workers and non-exposed controls ($n = 50$) in levels of the six enzymes measured (*N*-acetyl-β-D-glucosaminidase, β-D-glucuronidase, α-D-galactosidase, α-D-glucosidase, β-D-galactosidase, and α-D-mannosidase). There was also no evidence of metabolic, endocrinological, or haemolytic diseases, haemoglobinopathies, or diseases involving major organs. Furthermore, none of the workers had a family history of diabetes and all were HIV-negative.

(ii) Human cells in vitro

Trivalent antimony compounds

Goi et al. (2003) found that 24-hour exposure of human PBLs to trivalent, but not pentavalent, antimony (200–300 μg/L) caused significantly increased secretion of the lysosomal glycohydrolases *N*-acetyl-β-D-glucosaminidase and β-D-glucuronidase (Goi et al., 2003).

In vitro exposure of the human prostate cancer cell lines LNCaP and PC3 to antimony(III) potassium tartrate significantly promoted cell growth/proliferation and invasion, as assessed by Matrigel assay, and wound healing (Zhang et al., 2018a, b; Guo et al., 2021b), as well as lipid metabolic disorder (Guo et al., 2021b) (see Section 4.2.8).

Exposure of the human bladder cancer EJ cell line to low-dose antimony(III) potassium tartrate (0.8 μM) for 24–48 hours significantly increased cell proliferation, invasion (into Matrigel), and migration (in a wound-healing assay) (Lou et al., 2021). In addition, antimony(III) potassium tartrate significantly inhibited mitophagy in EJ cells by downregulating the expression of PINK1, Parkin, and p(ser65)-Parkin, which resulted in increased cell proliferation. Activation of the PINK–Parkin pathway by carbonyl cyanide 3-chlorophenylhydrazone significantly inhibited antimony(III) potassium tartrate-induced cell proliferation (Lou et al., 2021).

Treatment of human SIK cells with antimony(III) potassium tartrate (5 μM) or EGF alone for 3 days significantly increased their colony-forming efficiency by two- to three-fold compared with untreated (control) cultures. In SIK cells co-treated with both agents, the colony-forming efficiency increased by four-fold. (Patterson & Rice, 2007).

Pentavalent antimony compounds

No data were available to the Working Group.

Table 4.11 Alterations in cell proliferation, cell death, or nutrient supply in humans exposed to trivalent or pentavalent antimony

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Lysosomal glycohydrolase levels	Plasma	Italy, art glass workers, cross-sectional	*n* = 10 antimony(III) oxide-exposed workers *n* = 16 arsenic(III) oxide-exposed-workers *n* = 50 non-exposed controls	NS between exposed workers and controls for *N*-acetyl-β-D-glucosaminidase, β-D-glucuronidase, α-D-galactosidase, α-D-glucosidase, β-D-galactosidase, and α-D-mannosidase	There was no evidence of metabolic, endocrinological, or haemolytic diseases, haemoglobinopathies, or diseases involving major organs; none of the workers had a family history of diabetes, and all were HIV-negative	Exposure assessment critique: Single urine samples were used to assess exposures to metals concurrently with the assessment of plasma levels of lysosomal enzymes. No correction of Sb (and As) concentrations for urinary dilution. No assessment for co-exposure to As in the statistical analyses. [The Working Group noted that it seemed that the workers were parsed by either As or Sb exposure, although other potential co-exposures were not mentioned.]	Goi, et al. (2003)

As, arsenic; HIV, human immunodeficiency virus; NS, not significant; Sb, antimony.

(b) Experimental systems

(i) Non-human mammals in vivo

Trivalent antimony compounds

In studies of subacute toxicity, Wistar Han rats and B6C3F$_1$/N mice were exposed by inhalation (whole-body) to antimony(III) oxide aerosol at a concentration of 0, 3.75, 7.5, 15, 30, or 60 mg/m^3 for 6 hours per day (5 days per week) for 12–13 days during a 16–17-day period. A significantly increased incidence of squamous metaplasia of the epiglottis was observed in the larynx of male and female mice, but not rats, after exposure to the two highest concentrations (30 and 60 mg/m^3) (NTP, 2017).

In 2-year studies of chronic toxicity, Wistar Han rats and B6C3F$_1$/N mice were exposed by whole-body inhalation to antimony(III) oxide aerosols at a concentration of 0, 3, 10, or 30 mg/m^3 for 6 hours per day (5 days per week) for 105 weeks or less. The incidence of alveolar and bronchiolar epithelial hyperplasia, as well as lymphoid hyperplasia of the bronchial lymph nodes, was significantly increased in male and female rats and mice at all concentrations. Lymphoid hyperplasia of the mediastinal lymph nodes was significantly increased at all exposure concentrations in male and female rats, and at the two highest concentrations in male and female mice. Bone marrow hyperplasia (predominantly because of increased erythroid precursors) was significantly increased in male and female rats at the highest concentration, and bone marrow hyperplasia (myeloid) was significantly increased in male and female mice at the two highest concentrations. The incidence of squamous metaplasia of the alveolar epithelium (at the lowest concentration) and of the nasal respiratory epithelium (at the highest concentration) was significantly increased in female rats and mice, respectively. Hyperplasia and squamous metaplasia of the respiratory epithelium of the nose and hyperplasia of the adrenal medulla were also significantly increased in male and female rats at the highest concentration. In addition, the incidence of hyperplasia and squamous metaplasia of the respiratory epithelium of the epiglottis in the larynx and of hyperplasia of the squamous epithelium was significantly increased in male and female mice at the highest concentration. There was also a significant increase in the incidence of haematopoietic cell proliferation in the spleen of female mice at the highest concentration (NTP, 2017).

In a study of chronic toxicity by Groth et al. (1986), male and female Wistar rats were exposed by inhalation (whole-body) to antimony(III) oxide (TWA, 45 mg/m^3) or antimony ore (TWA, 36–40 mg/m^3) for 7 hours per day, 5 days per week for 52 weeks or less. After exposure to antimony(III) oxide for 6 months, alveolar wall hyperplasia was increased in female rats, but not in males. After 12 months of exposure, the incidence of cuboidal and columnar (alveolar wall) cell metaplasia was increased in female and male rats (although less so in males). Similar effects in terms of metaplasia were reported after exposure to antimony ore.

Newton et al. (1994) reported an increase in the incidence of bronchioloalveolar hyperplasia in female, but not male, Fischer 344 rats exposed to antimony(III) oxide for 1 year by inhalation (whole-body) at the highest exposure level (23.5 mg/m^3) after a 1-year observation period. Minimal to no bronchioloalveolar hyperplasia was observed in male and female rats after exposure for 13 weeks (with or without a 27-week observation period) or 1 year.

Increased incidence and severity of bone marrow myeloid hyperplasia was reported by Poon et al. (1998) in male and female Sprague-Dawley rats given drinking-water containing antimony(III) potassium tartrate at a concentration of 0.5, 5, 50, or 500 ppm for females, and 50 or 500 ppm for males for 13 weeks. The incidence of bone marrow erythroid hyperplasia was also increased, but only in males exposed at the

highest concentration (500 ppm), and to a lesser degree than the myeloid type.

In a 13-week study, antimony(III) potassium tartrate administered by intraperitoneal injection caused an increase in the incidence of bile duct hyperplasia in the liver of Fischer 344 (primarily male) rats at the highest dose (24 mg/kg bw) (Dieter et al., 1991, NTP, 1992).

Watt (1983) reported a significant increase in the incidence of bile duct proliferation and pneumocyte hyperplasia in female CDF rats exposed by inhalation (whole-body) to antimony(III) oxide (1.6 ± 1.5 and 4.2 ± 3.2 mg/m^3) for up to 1 year.

In tumour implantation studies, male BALB/c immunodeficient (nude) mice were given drinking-water containing antimony(III) potassium tartrate (0.1 or 1.0 μg/mL) or potassium tartrate hemihydrate ($C_4H_6K_2O_7$, control compound) for 14 days, and then inoculated subcutaneously and bilaterally with prostate cancer (LNCaP or PC3) cells (in Matrigel), after which exposure to antimony(III) potassium tartrate or potassium tartrate hemihydrate in drinking-water was continued. Tumour sizes and volumes were monitored until days 21 (PC3) and 23 (LNCaP) and were found to be greater in mice treated with antimony(III) potassium tartrate (Zhang et al., 2018 a, b). Similar findings were reported by Lou et al. (2021) for exposure to antimony(III) potassium tartrate (0.8 μmol/mL) in a tumour implantation study using the bladder cancer cell line EJ, with tumour size/volume monitored for > 24 days. In the study by Guo et al. (2021), male BALB/c (nude) mice were inoculated with LNCaP cells by subcutaneous injection and then given drinking-water containing antimony(III) potassium tartrate (1.0 μg/mL) or potassium tartrate ($K_2C_4H_40_6$, control compound). Tumour sizes and volumes were monitored to day 40 and were found to be greater in mice treated with antimony(III) potassium tartrate. Serum triglyceride and total cholesterol levels were also significantly increased in the group treated with antimony(III) potassium tartrate, consistent with lipid metabolic disequilibrium.

Zhang et al. (2021) reported that the expression of glial fibrillary acidic protein (GFAP) and iNOS, two critical protein markers of reactive astrogliosis, were significantly increased in the brain tissue of male ICR mice treated with antimony(III) potassium tartrate trihydrate at a dose of 10 or 20 mg/kg bw by intraperitoneal injection for 8 weeks, indicating that antimony induced astrocyte activation in vivo. Although antimony(III) potassium tartrate induced astrocyte activation and proliferation in vitro (in the rat C6 glioma cell line) (Zhang et al., 2021) (see below), no cell proliferation markers were investigated in the brain tissue of the antimony-exposed mice.

Pentavalent antimony compounds

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

Trivalent antimony compounds

Exposure of the rat C6 glioma cell line (astrocytes) to antimony(III) potassium tartrate trihydrate (0.1–5.0 μmol/L) for 24 hours significantly increased cell activation and proliferation, with increased expression of PCNA and cyclin D1 proteins (> 0.625 μmol/L). GFAP and iNOS protein and mRNA levels were also significantly increased (> 0.625 μmol/L) (Zhang et al., 2021).

Exposure of rat erythrocyte-derived phosphofructokinase (a rate-limiting enzyme of glycolysis in erythrocytes) to antimony(III) potassium tartrate hydrate (5 mM) for 3 minutes inhibited its activity by 95%. GSH and Hb partially protected phosphofructokinase against the inhibitory effect of antimony (most effectively at low antimony concentrations) (Poon & Chu, 1998).

Treatment of the Baf3 cell line with antimony(III) potassium tartrate (1–1000 μg/mL) did not result in detectable inhibition of protein tyrosine phosphatases, nor was enhanced

(IL-3-induced) proliferation observed (Pathak & Yi, 2001).

Pentavalent antimony compounds

Treatment of the Baf3 cell line with sodium stibogluconate (10–100 µg/mL) induced tyrosine phosphorylation of cellular proteins – including inhibition of the protein tyrosine phosphatases SHP-1, SHP-2, and PTP1B and increased (IL-3-induced) proliferation at concentrations between 0.3 and 200 µg/mL (maximal effect concentration, ~40 µg/mL) (Pathak & Yi, 2001). This suggests that only the pentavalent form of antimony acts as an inducer of Baf3 cell proliferation, which is inactivated when it is transformed into the trivalent form (Pathak & Yi, 2001).

Pathak et al. (2002) also showed that treatment of the NB4 cell line with sodium stibogluconate (25–100 µg/mL) inactivated SHP-1 protein tyrosine phosphatases.

4.2.10 Evidence on other key characteristics of carcinogens

(a) Causes immortalization

Exposure to antimony(III) acetate (1.6, 3.2, or 7.5 µM) significantly enhanced the frequency of transformation of Syrian hamster embryo cells by simian adenovirus SA7 (Casto et al., 1979).

(b) Multiple characteristics

(i) Human cells in vitro

In view of the chemical similarity of inorganic trivalent arsenic (arsenite) and antimony (antimonite), the responses of human epidermal keratinocytes to either trivalent antimony or arsenic were compared at the transcriptional and translational levels (Phillips et al., 2016, 2020). Human epidermal SIK cells were treated with sodium arsenate (Na_3AsO_4, 3 µM) or antimony(III) potassium tartrate (6 µM) for 7 days. Results from next-generation sequencing showed that exposure to trivalent arsenic and antimony induced highly similar transcriptional responses in SIK cells (Phillips et al., 2016). Furthermore, the gene expression changes were almost entirely in the same direction for the two treatments, although the degrees of change were sometimes different (Phillips et al., 2016). Both sodium arsenate and antimony(III) potassium tartrate induced suppression of RYR1 and LRIG1, as well as bone morphogenic protein-6 activity, which are linked to decreased differentiation and maintenance of EGF-dependent proliferative capacity. Both compounds also inhibited many miRNAs, including miR-203 (a suppressor of proliferation). On the basis of transcriptional and proteomic comparisons and Ingenuity Pathway Analysis (IPA), sodium arsenate and antimony(III) potassium tartrate were reported to share virtually the same signalling pathways and upstream regulators (Phillips et al., 2020). Of the merged top five pathways enriched for genes modulated by sodium arsenate and antimony(III) potassium tartrate in SIK cells, four were identical (Nrf2-mediated oxidative stress, glucocorticoid receptor signalling, xenobiotic metabolism signalling, and the γ-glutamyl cycle). Proteomic analysis also predicted the involvement of the Nrf2-mediated oxidative stress response as one of the most-affected pathways, and of the top five predicted upstream transcriptional regulators, three (Myc, EFNA4, and ROCK2) were affected by both sodium arsenate and antimony(III) potassium tartrate (Phillips et al., 2020).

Kawata et al. (2007) used DNA microarrays to classify the toxic effects of six heavy metals, including antimony, compared with those of the "model chemicals" 2,3-dimethoxy-1,4-naphthoquinone, phenol, and *N*-nitrosodimethylamine. Specific gene alterations in HepG2 cells and hierarchical clustering revealed that the effects of antimony were very similar to those of 2,3-dimethoxy-1,4-naphthoquinone, which has been reported to be an inducer of ROS and associated with the increased expression of genes, such as CCNB2 and UBE2C, involved in cell proliferative responses (Kawata et al., 2007).

Modulation of gene expression in the human monocytic THP-1 cell line by treatment with sodium stibogluconate (200 µg/mL) was measured by Affymetrix human DNA microarray and further validated by real-time PCR. The results showed that sodium stibogluconate induced the upregulation of endothelin receptor type B (EDNRB) (G-protein signalling coupled to the second messenger inositol trisphosphate), HMOX1, glutamate-cysteine ligase modifier subunit (GCLM) (GSH biosynthesis pathway in response to oxidative stress), melanoma antigen family B2 (MAGEB2) (protein binding), and 5′-nucleotidase, cytosolic II (NT5C2) gene expression, and downregulation of mannose receptor, C type 1 (MRC1) (receptor-mediated endocytosis) and selenoprotein W, 1 (SEPW1) gene expression (El Fadili et al., 2008). Induction of HMOX1 by sodium stibogluconate was also observed in primary human monocyte-derived macrophages (El Fadili et al., 2008). HMOX1 is a potent inducer of vascular endothelial growth factor, a critical factor that governs tumour angiogenesis, and has been recognized as a promoter of tumour metastasis (Cherrington et al., 2000).

(ii) Evaluation of high-throughput in vitro toxicity screening data

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 131 was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Antimony was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2022).

The ToxCast/Tox21 high-throughput screening results are presented based on the assays that have been mapped to the key characteristics (Reisfeld et al., 2022). The detailed results are available in supplementary information for this volume (Annex 4, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, web only, available from: https://publications.iarc.fr/618). Here, for brevity, assays for which there is a positive "hit call" are referred to as "active" assays. A summary of these results is given below as the number of active assays (without any caution flags)/total number of key characteristic-related assays for the chemical.

The results for the four trivalent antimony compounds screened are: (i) acetic acid, antimony(III) salt: 0/111; (ii) antimony(III) potassium tartrate trihydrate: 17/117; (iii) antimony(III) chloride: 13/170; and (iv) antimony(III) potassium tartrate hydrate: 6/38.

The active assays that appear for one or more of the compounds are as follows:

Key characteristic 2, "is genotoxic" (three assays): (i) DT40, a single-readout assay that uses DT40 (a chicken lymphoblast cell line); (ii) H2AX_HTRF_CHO_Agonist, a single-readout assay that uses the CHO-K1 cell line; and (iii) p53_BLA_p3, a single-readout assay that uses HCT 116 (a human intestinal cell line).

Key characteristic 5, "induces oxidative stress" (two assays): (i) HSE_BLA_Agonist, a single-readout assay that uses HeLa (a human cervix cell line); and (ii) ARE_BLA_Agonist, a single-readout assay that uses HepG2 (a human liver cell line).

Kay characteristic 8, "modulates receptor-mediated effects" (10 assays): (i) PPARd_BLA_Antagonist, a single-readout assay that uses HEK-293T (a human kidney cell line); (ii) PR_BLA_Antagonist, a single-readout assay that uses transfected HEK-293T cells; (iii) RORg_LUC_CHO_Antagonist, a single-readout assay that uses CHO-K1 cells; (iv) TR_LUC_GH3_Antagonist, a single-readout assay that uses GH3 (a rat pituitary gland cell line); (v) FXR_BLA_Antagonist, a single-readout assay that

uses HEK-293T cells; (vi) AR_BLA_Antagonist, a single-readout assay that uses HEK-293T cells; (vii) AR_LUC_MDAKB2_Antagonist, a single-readout assay that uses MDA-kb2 (a human breast cell line); (viii) ERR, a single-readout assay that uses ERR-HEK-293T (a human kidney cell line); and (ix) PGC_ERR, a single-readout assay that uses PGC/ERR HEK-293T (a human kidney cell line).

Key characteristic 10, "alters cell proliferation, cell death, or nutrient supply" (four assays): (i) TR_LUC_GH3_Antagonist, a single-readout assay that uses GH3 (a rat pituitary gland cell line); (ii) AP1_BLA_Agonist, a single-readout assay that uses ME-180 (a human cervix cell line); (iii) AR_LUC_MDAKB2_Antagonist, a single-readout assay that uses MDA-kb2 (a human breast cell line); and (iv) RORg_LUC_CHO_Antagonist, a single-readout assay that uses the CHO-K1 cell line.

For the pentavalent antimony compound antimony(V) sulfide, the assay results were as follows: 0/38 (no active assays for any key characteristic).

Overall, for trivalent antimony compounds, the results were uninformative, except for key characteristic 8, "modulates receptor-mediated effects".

For pentavalent antimony compounds, the results were uninformative for all key characteristics.

5. Summary of Data Reported

5.1 Exposure characterization

The following agents are considered in this monograph: the trivalent antimony compounds antimony(III) oxide, antimony(III) chloride, antimony(III) potassium tartrate, antimony(III) sulfide, antimony(III) ions, antimony(III) hydride; and the pentavalent antimony compounds antimony(V) oxide, meglumine antimoniate(V), and antimony(V) in sodium stibogluconate.

The major antimony form that is mined is antimony(III) sulfide, a High Production Volume chemical. Primary product compounds are typically antimony(III) oxide, antimony metal, or sodium antimonate, with other antimony products synthesized from those forms. Antimony compounds are mainly used in flame retardants, lead–acid batteries, lead alloys, plastics (as catalysts and stabilizers), glass and ceramics, as a lubricant in brake pads and clutch discs, and as an ammunition primer in explosives. Antimony(III) potassium tartrate has been widely replaced with meglumine antimoniate(V) and sodium stibogluconate in the treatment of leishmaniasis.

Releases of antimony into the environment occur from natural processes, such as the weathering of rocks, soil runoff, wind-blown dust, volcanic eruptions, sea spray, and forest fires, and from anthropogenic activities, including the mining and processing of ores, metal production, and other related industrial processes.

Inhalation of airborne dust is considered the primary route of occupational exposure to antimony; skin exposure and inadvertent ingestion may also occur. Occupational exposure may arise in various industrial sectors, such as smelting, production of antimony compounds and of other metals, glass manufacture, textile production, battery manufacture, and electronic and electrical waste processing. Workers may be exposed to multiple antimony compounds in the workplace and may also have co-exposure to other agents. Non-occupational exposures are typically relatively lower, arising from exposure to contaminated water, air, and soil, and use of consumer products, tobacco, and electronic cigarette liquids. Estimated dietary intakes are generally well below the current WHO-derived tolerable daily intake values. Both occupational and environmental exposure guidelines exist for antimony, as do reference values for

concentrations in urine and blood. Exposure assessments involving detailed characterization of antimony speciation and co-occurrence of other agents such as arsenic and lead, notably in airborne dusts, are limited.

5.2 Cancer in humans

The available body of evidence on antimony and cancers in humans comprised four occupational cohort studies, including one in glassblowers, two in antimony-smelter workers, and one in tin-smelter workers, as well as nine cohort, case–cohort, case–case, and case–control studies in the general population. The three most informative of these were the studies of lung cancer mortality in smelter workers, which consistently observed elevated standardized mortality ratios for workers exposed to antimony. In one of these studies, an elevated standardized mortality ratio among antimony or maintenance workers was observed, but not among those in other job categories. In another of the studies, standardized mortality ratios increased according to duration of employment. The most recent study found that excess lung cancer mortality risk increased with increasing weighted cumulative exposure to antimony.

The major limitation in these studies was the inability to control for exposure to arsenic (classified in IARC Group 1, *carcinogenic to humans*), which is present in both antimony and tin smelting. In all three studies, there was little evidence that co-exposure to arsenic was a convincing explanation for the observed associations between antimony and lung cancer among smelter workers. Nonetheless, although these studies observed a positive association between antimony exposure and lung cancer mortality, the possibility of confounding by exposure to arsenic or other known lung carcinogens could not be fully excluded.

Studies on other cancers were considered only minimally informative, too few in number, and without consistent evidence to contribute to the evaluation. Occupational exposure to antimony among smelter workers was considered to be exposure to trivalent antimony. All the general-population studies using biomarkers or estimates of air pollution considered antimony exposure without regard to chemical form. No epidemiological studies were identified that specifically evaluated exposure to pentavalent antimony.

5.3 Cancer in experimental animals

5.3.1 Trivalent antimony

Treatment with antimony(III) oxide caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms, in two species or in both sexes of a single species, in well-conducted studies that complied with Good Laboratory Practice.

Antimony(III) oxide was administered by inhalation (whole-body exposure) in one well-conducted study that complied with Good Laboratory Practice in male and female B6C3F$_1$/N mice. Antimony(III) oxide caused an increase in the incidence of bronchioloalveolar carcinoma in males and females, of fibrous histiocytoma of the skin and of histiocytoma or fibrosarcoma (combined) of the skin in males, and of malignant lymphoma (all organs) in females.

Antimony(III) oxide was administered by inhalation (whole-body exposure) in one well-conducted study that complied with Good Laboratory Practice in male and female Wistar rats. In females, antimony(III) oxide caused an increase in the incidence of cystic keratinizing epithelioma or squamous cell carcinoma (combined) of the lung, and of benign or malignant pheochromocytoma (combined) of the adrenal medulla.

Antimony(III) oxide administered by inhalation (whole-body exposure) in one study in female

Wistar rats caused an increase in the incidence of squamous cell carcinoma, scirrhous adenocarcinoma, and bronchioloalveolar adenoma or carcinoma (combined) of the lung.

Antimony(III) oxide administered by inhalation (whole-body exposure) in one study in female Fischer 344 (CDF) rats caused an increase in the incidence of lung scirrhous adenocarcinoma.

5.3.2 Pentavalent antimony

No data on pentavalent antimony were available to the Working Group.

5.4 Mechanistic evidence

Humans are primarily exposed occupationally to antimony via inhalation. The available data on the absorption and distribution of antimony in humans are limited. Antimony has been detected in the lung, blood, and urine of workers occupationally exposed to antimony in the air. Antimony particles accumulate in the lung of exposed humans and persist for long periods of time (from months to years). The available data in humans indicate that trivalent or pentavalent antimony is eliminated in the urine, regardless of the route of exposure. Ingested trivalent antimony is also excreted into the bile.

Inhalation studies with trivalent antimony in rodents indicate that antimony blood concentrations and lung burden increase with increasing levels of exposure. Pulmonary clearance of antimony is lung burden-dependent, and antimony remains in the lung for a long period of time. Sparse data from studies in experimental animals indicate that absorption of trivalent antimony is poor, whereas absorption of pentavalent antimony is rapid via the gastrointestinal tract. The distribution of antimony is similar for different routes of exposure. Trivalent or pentavalent antimony can accumulate in erythrocytes. The excretion of trivalent or pentavalent antimony is via the urine in rodent studies. The elimination of trivalent antimony from the blood or lung is generally biphasic, with a rapid and a slow phase.

The metabolism of antimony primarily involves the conversion of the valence state from +5 to +3.

5.4.1 Trivalent antimony

There is consistent and coherent evidence that trivalent antimony exhibits key characteristics of carcinogens.

Trivalent antimony is genotoxic. In exposed humans, there is suggestive evidence of genotoxicity in two of four studies of antimony exposure. There is consistent and coherent evidence in human primary cells in multiple studies and in human cell lines that trivalent antimony induces DNA damage, chromosomal aberration, micronucleus formation, and/or increased frequency of sister-chromatid exchange. Although the findings for the genotoxic potential of trivalent antimony were mixed in mice and rats in vivo, DNA damage and micronucleus formation were increased in mice exposed by inhalation. Trivalent antimony was genotoxic in hamster cell lines in several studies but gave negative results in one study in mouse embryonic stem cells. Findings for trivalent antimony were mixed in non-mammalian systems, including yeast and bacteria.

Trivalent antimony induces oxidative stress. The few available studies in exposed humans were either uninformative or did not suggest effects of trivalent antimony on oxidative stress parameters. However, there is consistent and coherent evidence in experimental systems that trivalent antimony induces oxidative stress, including findings of increased levels of reactive oxygen species, in human cell lines in several studies. One study in human primary cells gave negative results for oxidative DNA damage. Trivalent antimony also altered oxidative stress parameters in non-human mammalian systems in vivo and in vitro in multiple studies.

Trivalent antimony induces chronic inflammation. Suggestive evidence in exposed humans is provided by two studies in workers exposed to trivalent antimony, which reported radiographic evidence of pneumoconiosis. There is consistent and coherent evidence in experimental systems that trivalent antimony induces chronic inflammation. Multiple studies in mice and rats showed that subacute to chronic exposure via different routes caused chronic inflammation in multiple organs, including the lung. One study showed that intraperitoneal or intratracheal exposure to trivalent antimony caused non-collagenous pneumoconiosis in rats.

Trivalent antimony alters cell proliferation, cell death, or nutrient supply. One study in workers exposed to trivalent antimony reported negative results for this key characteristic; however, there is consistent and coherent evidence in experimental systems that trivalent antimony alters cell proliferation, cell death, or nutrient supply. Multiple studies showed that trivalent antimony induced increased cell proliferation in human cell lines. Tumour size increased in athymic nude mice injected with human cancer cells and exposed to antimony in a few studies. Trivalent antimony also induced increased cell proliferation and hyperplasia, including in the lung and bone marrow, after inhalation exposure in mice and rats in multiple studies.

There is suggestive evidence that trivalent antimony alters DNA repair or causes genomic instability. Human exposure to antimony was associated with an increased frequency of mosaic loss of chromosome Y in one study. Trivalent antimony decreased the capacity for DNA repair in human cell lines in a few studies but had no effect in vivo in rats in one study. Trivalent antimony decreased the capacity for DNA repair in hamster cells and yeast (single studies) but gave negative results in mouse cells in one study.

There is suggestive evidence in three human population studies that antimony exposure induces epigenetic effects. In two cohort studies, changes in DNA methylation were shown to be associated with antimony exposure. In a cohort study, antimony levels in urine samples were associated with decreased miRNAs in plasma. Trivalent antimony induced some epigenetic-related effects in human cell lines in a few studies and in mouse cells in one study. There were no studies in non-human mammals in vivo.

There is suggestive evidence that antimony is immunosuppressive on the basis of two studies of antimony exposure in human populations. Trivalent antimony also caused cellular depletion of the thymus in mice in one study.

There is suggestive evidence in experimental systems that trivalent antimony modulates receptor-mediated effects. Two studies in pregnant women exposed to antimony showed decreased thyroid hormone levels, but a third study in men found no antimony-associated effects on reproductive hormones. Trivalent antimony altered signalling pathways for androgen receptor, epidermal growth factor receptor, and/or miR-132-3P/SREBP1 receptor in human cell lines in multiple studies.

For other key characteristics of carcinogens, there is a paucity of data.

5.4.2 Pentavalent antimony

For pentavalent antimony, the mechanistic evidence is suggestive. There is suggestive evidence that pentavalent antimony is genotoxic. It gave mixed results in human primary cells in two studies. However, pentavalent antimony induced DNA damage and micronucleus formation in mice exposed by intraperitoneal injection in several studies. Findings for genotoxicity in bacteria were mostly negative. There is suggestive evidence that pentavalent antimony induces oxidative stress in rodent systems in vivo and in vitro in multiple studies, including oxidative DNA damage in vivo in mice in one study. For other key characteristics of carcinogens, there is a paucity of data.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of trivalent antimony. Positive associations have been observed between exposure to trivalent antimony and cancer of the lung.

There is *inadequate evidence* in humans regarding the carcinogenicity of pentavalent antimony.

6.2 Cancer in experimental animals

Trivalent antimony

There is *sufficient evidence* in experimental animals for the carcinogenicity of antimony(III) oxide.

Pentavalent antimony

There is *inadequate evidence* in experimental animals regarding the carcinogenicity of pentavalent antimony.

6.3 Mechanistic evidence

There is *strong evidence* in human primary cells and in experimental systems that trivalent antimony exhibits key characteristics of carcinogens.

For pentavalent antimony, there is *limited* mechanistic evidence.

6.4 Overall evaluation

Trivalent antimony is *probably carcinogenic to humans (Group 2A).*

Pentavalent antimony is *not classifiable as to its carcinogenicity to humans (Group 3).*

6.5 Rationale

The Group 2A evaluation for trivalent antimony is based on *limited evidence* for cancer in humans, *sufficient evidence* for cancer in experimental animals, and *strong* mechanistic evidence. There is *limited evidence* that exposure to trivalent antimony causes lung cancer in humans. Among the available human cancer studies, three occupational cohort studies among antimony- or tin-smelter workers were found to be most relevant for assessing trivalent antimony's association with cancer. A positive association was observed between trivalent antimony exposure and risk of lung cancer in all three studies, but confounding by exposure to arsenic or other known lung carcinogens could not be ruled out as an explanation for the findings. For all other cancer sites, the evidence is *inadequate*, because the studies were few in number, minimally informative, or did not show consistent positive associations. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms caused by antimony(III) oxide, in two species or in both sexes of a single species, in a well-conducted study that complied with Good Laboratory Practice. There is *strong evidence* of key characteristics of carcinogens in human primary cells; trivalent antimony is genotoxic. There is also *strong evidence* of key characteristics of carcinogens in experimental systems; trivalent antimony induces oxidative stress, induces chronic inflammation, and alters cell proliferation, cell death, or nutrient supply.

The evidence is *inadequate* regarding whether exposure to pentavalent antimony causes cancer in humans and in experimental animals because no studies were available. There is *limited evidence* in experimental systems that pentavalent antimony is genotoxic and induces oxidative stress.

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WEAPONS-GRADE TUNGSTEN (WITH NICKEL AND COBALT) ALLOY

1. Exposure Characterization

1.1 Identification of the agent

This monograph focuses on alloys containing tungsten, nickel, and cobalt that are used in the production of military weapons. Such alloys typically contain approximately 91–93% tungsten, 3–5% nickel, and 2–4% cobalt (Andrew et al., 1991, as cited in Miller et al., 2004), and are sometimes referred to as "tungsten heavy alloys" or "weapons-grade tungsten alloys". Other sources have defined these alloys more broadly in terms of the percentages of the constituent metals (tungsten, nickel, and cobalt). The Working Group defined the name of the agent under evaluation in the present monograph as "weapons-grade tungsten (with nickel and cobalt) alloy" (WGTA). [The Working Group noted that, by definition, co-exposure to tungsten, nickel, and cobalt is a feature of exposure to this agent; however, as there are limited data available for the WGTAs, exposure details for the individual elements contained in the alloys are presented when considered relevant.]

Tungsten, a relatively rare metal, has several unique chemical and physical properties that make it suitable for use in lighting, aerospace, electronic, radiation shielding, and military applications. These properties include a high density (19.3 g/mL), high melting point (3410 °C, the highest melting point of all metals), low vapour pressure, high tensile strength at temperatures greater than 1650 °C, and high corrosion resistance (US EPA, 2017; Wasel & Freeman, 2018; Michaux, 2021). It is also a good conductor of electricity (Wasel & Freeman, 2018) and has the lowest coefficient of expansion of all the metals (van der Voet et al., 2007). However, one drawback to tungsten is its low ductility (or brittleness) at room temperature; therefore, it is often combined with other elements to create alloys that have the physical and chemical properties needed for various applications (Vergara et al., 2016).

In general, tungsten alloys (including tungsten heavy alloys) are only slightly less dense than pure tungsten (16–18 g/mL compared with 19.3 g/mL). They also have high tensile strengths (1000–1700 MPa) and ductility (10–30%), and are easily machinable (Upadhyaya, 2001). Tungsten heavy alloys that are used in many military applications typically contain 90–98% tungsten by weight, with two or more transition metals such as nickel, iron, copper, and/or cobalt (van der Voet et al., 2007). The use of iron in place of cobalt enhances the ductility and strength of the alloy (van der Voet et al., 2007; Dinçer et al., 2015). One commercially available WGTA contains 91.1% tungsten, 6% nickel, and 2.9% cobalt (Kalinich et al., 2005). [The Working Group acknowledged the lack of information on

the physical and chemical properties of specific WGTAs in the literature.]

1.2 Production and use

1.2.1 Production processes and volumes

WGTAs are typically produced using the liquid-phase sintering method. In this process, elemental powders of tungsten, nickel, and cobalt are blended, isostatically compressed into desired shapes, and then sintered at high temperatures (~1500 °C) under a protective atmosphere. The sintering temperatures are lower than the melting point of tungsten, but higher than the melting points of the alloying metals. To prevent intermetallic compounds, such as WCo_3, from forming, post-sintering water quenching is performed (Upadhyaya, 2001; Ogundipe et al., 2006). The composite that is formed contains two phases: a tungsten phase, which consists of almost-pure tungsten grains approximately 30–35 μm in size, and a binder phase, which surrounds the tungsten phase and contains dissolved tungsten in a matrix of nickel and cobalt (Ogundipe et al., 2006; Sunwoo et al., 2006). The quantities and compositions of the alloying metals determine the amount of tungsten present in the binder phase (Ogundipe et al., 2006).

Overall, the production of WGTAs accounts for only a small proportion of the global demand for tungsten, because more than 60% of tungsten is used for the production of cemented carbides (Michaux, 2021). Tungsten alloys have been used to replace lead and depleted uranium in munitions since the 1990s (Zoroddu et al., 2018). [However, the Working Group acknowledged that details regarding the production volumes of the WGTAs and of the munitions containing them are not readily available in the published literature.] In general, the demand for tungsten is closely tied to the global economy, and global production of tungsten for use in the construction, metalworking, mining, and oil and gas industries has increased from approximately 26 000 tonnes in 1994 to 84 000 tonnes in 2020 (United States Geological Survey, 1996, 2021). China is the world leader in tungsten production, producing more than 69 000 tonnes [more than 82% of the world's production] in 2020 (United States Geological Survey, 2021), followed by Viet Nam and the Russian Federation (accounting for [5.1%] and [2.6%] of global tungsten production, respectively).

1.2.2 Uses

The search for more "environmentally friendly" alternatives to depleted uranium and lead in munitions led to the investigation and use of tungsten alloys in several armour-penetrating and small-calibre munitions during the 1990s (Zoroddu et al., 2018). At the time, given the limited data available, tungsten was considered relatively inert; therefore, it was perceived to be less environmentally toxic than depleted uranium or lead (Ogundipe et al., 2006). Tungsten–nylon munitions largely replaced the use of lead bullets in the USA from 1999 until concerns about flight instability affecting munition performance halted their production in 2003 (Clausen & Korte, 2009). However, only the WGTAs were deemed to be suitable replacements for depleted uranium in kinetic energy penetrators. Designed to pierce armour, these munitions do not explode, but instead are propelled at high speeds and rely on their mass to penetrate, damage, and destroy enemy targets (Machado, 2011). Therefore, with densities similar to depleted uranium and desirable mechanical properties, WGTAs were used by the militaries of many nations to replace depleted uranium in kinetic energy penetrators, guided missiles, and other types of armour-piercing munitions (Kalinich et al., 2005, 2008; van der Voet et al., 2007; Dinçer et al., 2015). [The Working Group acknowledged that information on the use of munitions containing WGTAs by

specific countries is lacking. In addition, other non-munition-related uses for the WGTAs were not available to the Working Group.]

1.3 Detection and quantification

1.3.1 Air

Exposure monitoring of WGTAs in air is achieved by measuring air concentrations of each individual element: tungsten, nickel, and cobalt. Table 1.1 summarizes examples of analytical methods used for the measurement of tungsten and nickel. For details on the measurement of cobalt in air, refer to Table 1.5 in the monograph on Cobalt metal (without tungsten carbide) and some cobalt compounds, in the present volume.

The recommended technique of the United States National Institute for Occupational Safety and Health (NIOSH), Method No. 7074, for the measurement of tungsten in air uses flame atomic absorption spectrometry (AAS). This method involves the collection of air samples using cellulose ester filters followed by acid digestion, and has analytical limits of detection (LODs) of 50 and 125 µg for soluble and insoluble forms of tungsten per sample, respectively (ATSDR, 2005a).

As described in United States Occupational Safety and Health Administration (OSHA) Method ID-213, air concentrations of tungsten can also be measured using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (OSHA, 1994). Before ICP-AES analyses, samples are collected using mixed cellulose ester filters and subjected to acidification. This method has an LOD of 0.34 mg/m^3 (US EPA, 2017).

Common methods for the measurement of nickel and cobalt in air samples include flame or graphite furnace AAS, ICP-AES, and inductively coupled plasma mass spectrometry (ICP-MS) (ATSDR, 2004, 2005b). Samples are typically collected on glass or quartz fibre filters and undergo acid or microwave-assisted digestion (US EPA, 1999d). For both nickel and cobalt, reported LODs are lower for methods using graphite furnace AAS and ICP-MS than for flame AAS and ICP-AES. As shown in Table 1.1, reported sample LODs for nickel range from 0.02 ng/m^3 using ICP-MS (US EPA, 1999c) to 3.1 ng/m^3 using ICP-AES. For cobalt, reported LODs range from 0.01 ng/m^3 using ICP-MS (US EPA, 1999c) to 2.2 µg/m^3 using flame AAS (US EPA, 1999a).

The presence of WGTA particles in air can be determined using scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDXA), energy-dispersive X-ray fluorescence, and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). These methods are described further in Section 1.3.4 of the present monograph.

1.3.2 Water

Exposure monitoring for WGTAs in water is also achieved by measuring concentrations of each individual element: tungsten, nickel, and cobalt. For analytical methods for tungsten and nickel measurement, see Table 1.1 in the present monograph, and for analytical methods for cobalt measurement, see Table 1.5 in the monograph on Cobalt metal (without tungsten carbide) and some cobalt compounds, in the present volume.

The most sensitive analytical method for the measurement of concentrations of metals in water is ICP-MS. ICP-MS is a multi-element analytical technique, which typically requires the filtration and acidification of samples before analysis and offers LODs in the sub or low parts-per-billion range for most metals (US EPA, 2014). Specifically, LODs for tungsten are 0.4–5 µg/L, depending on the species being studied (Bednar et al., 2007), and LODs as low as 0.5 µg/L have been reported for nickel (ATSDR, 2005b).

Table 1.1 Representative methods for the detection and quantification of tungsten and nickel

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Tungsten				
Air	Collection on cellulose ester membrane filter, acid digestion, drying, addition of sodium hydroxide and sodium sulfate, and dilution	Flame AAS (NIOSH Method 7074)	Soluble tungsten, 50 µg; insoluble tungsten, 125 µg	ATSDR (2005a) NIOSH (1994a)
Air	Collection on mixed-cellulose ester filter and acidification	ICP-AES (OSHA Method ID-213)	0.34 mg/m^3	US EPA (2017) OSHA (1994)
Water	Filtration and acid digestion using US EPA SW-846 Method 3050 (modified)	ICP-MS	NR	Clausen & Korte (2009)
Water	Filtration and acid digestion	High-performance liquid chromatography ICP-MS (SW-846 US EPA Method 6020B, modified)	0.1–5 µg/L, depending on the species	Bednar et al. (2007)
Soil	Microwave-assisted acid digestion using modified version of US EPA SW-846 Method 3051A	ICP-MS (SW-846 US EPA Method 6020B, modified)	NR	Clausen & Korte (2009)
Urine	Dilution and acidification	ICP-MS with dynamic cell reaction technology	0.018 µg/L	CDC (2019)
Urine	Dilution and acidification	ICP-AES (NIOSH Method 8310)	50 µg/L	ATSDR (2005a) NIOSH (1994b)
Blood	Samples typically wet ashed with nitric acid and then diluted	ICP-MS	Plasma, 0.01 µg/L; serum, 0.04 µg/L; and blood, 0.2 µg/L	ATSDR (2005a)
Nickel				
Air	Collection on glass or quartz fibre filter, acid or microwave digestion; microwave preferred	AAS (US EPA Methods IO-3.1 and IO-3.2)	1.1 ng/m^3 using flame AAS, 0.1 ng/m^3 using graphite furnace AAS	ATSDR (2005b) US EPA (1999a)
Air	Collection on glass or quartz fibre filter, hot acid or microwave digestion (US EPA Method IO-3.1)	ICP-AES (US EPA Method IO-3.4)	3.1 ng/m^3	ATSDR (2005b) US EPA (1999b)
Air	Collection on glass or quartz fibre filter; microwave or hot acid digestion (US EPA Method IO-3.1)	ICP-MS (US EPA Method IO-3.5)	0.02 ng/m^3	ATSDR (2005b) US EPA (1999c)
Water	Filtration and acid digestion	ICP-MS (US EPA Method 200.8)	0.5 µg/L	ATSDR (2005b) US EPA (1994)
Water	Filtration and acid digestion	ICP-MS (US EPA Method 6020B)	[NR, but generally < 0.1 µg/L for metals]	US EPA (2014)

Table 1.1 (continued)

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Soil	Microwave-assisted acid digestion and filtration (US EPA SW-846 Method 3051A)	ICP-MS (US EPA Method 6020B)	[NR, but generally < 0.1 µg/L for metals]	US EPA (2014)
Urine	Dilution and acidification	ICP-MS	0.31 µg/L	CDC (2018)
Blood	Acid digestion using mixture of nitric, perchloric, and sulfuric acids	ICP-AES (NIOSH 8005)	1 µg/100 g of blood	NIOSH (1994c) ATSDR (2005b)

AAS, atomic absorption spectroscopy; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OSHA, Occupational Safety and Health Administration; US EPA, United States Environmental Protection Agency.

1.3.3 Soil

As shown in Table 1.1 and in Table 1.5 in the monograph on Cobalt metal (without tungsten carbide) and some cobalt compounds, in the present volume, concentrations of tungsten, nickel, and cobalt, in the sub and low parts-per-billion range, have also been measured in soil using ICP-MS (US EPA, 2014). Collected soil samples are typically prepared for analysis using microwave-assisted acid digestion (US EPA, 2007). For tungsten, modifications to the standard United States Environmental Protection Agency (US EPA) soil preparation protocol are required to maintain tungsten in a soluble form. These modifications include the addition of phosphoric acid to the digestion process and changes to the rinse solution (Clausen & Korte, 2009). Samples are then filtered and analysed using ICP-MS (US EPA, 2014).

1.3.4 Munitions fragments

There are currently no analytical methods that can be used to determine the composition of embedded metal fragments in the body. However, the composition of surgically excised metal fragments from war-related injuries can be determined using a variety of different analytical techniques, including SEM-EDXA, energy-dispersive X-ray fluorescence, and LA-ICP-MS (Centeno et al., 2014). SEM-EDXA can be used to define the elemental compositions and physical appearances of metal fragments, and energy-dispersive X-ray fluorescence is a non-destructive technique that permits qualitative and semi-quantitative elemental analysis of a fragment's surface. LA-ICP-MS allows for semiquantitative elemental composition analysis of the entire fragment, without requiring digestion of the specimen (Centeno et al., 2014).

1.3.5 Human biomarkers

There are no biomarkers of human exposure that are specific to WGTAs. However, concentrations of tungsten, nickel, and cobalt can each be measured in biological samples, including blood and urine, using ICP-MS; for tungsten and nickel, see Table 1.1, and for cobalt, see Table 1.6 in the monograph on Cobalt metal (without tungsten carbide) and some cobalt compounds, in the present volume. In biological monitoring programmes and large population studies, urine is often the preferred matrix because sample collection is easy and non-invasive (Smolders et al., 2009). Analysis of urine typically involves dilution of the sample in 2% nitric acid, and detection limits range from 0.1 to 1 ppb for most metals (Centeno et al., 2014). For example, detection limits in urine as low as 0.018, 0.31, and 0.023 µg/L for tungsten, nickel, and cobalt, respectively, have been reported (CDC, 2018, 2019).

To assess the distribution of solubilized metals in soft tissue after the removal of a metal fragment from a war-related injury, candidate methodologies including SEM-EDXA, X-ray photoelectron spectroscopy, and LA-ICP-MS have been investigated (Centeno et al., 2014; Smith et al., 2021). However, a recent study comparing these methods suggested that LA-ICP-MS is the only method that is sufficiently sensitive to detect trace elements in tissue samples (Smith et al., 2021).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

WGTAs do not occur naturally in the environment. No data on environmental exposures that may occur from the production and use of WGTAs in military weapons were available to the Working Group. However, studies measuring concentrations of tungsten, nickel, and cobalt

in the environment resulting from hard-metal production and the deposition of metal particles in soil resulting from the firing of other tungsten-containing munitions can provide some insight into potential environmental exposure pathways.

In general, background concentrations of tungsten in soil are relatively low, being reported to average around 1 mg/kg (Zoroddu et al., 2018), and tungsten is not detected in most ground and surface waters (ATSDR, 2005a). However, elevated concentrations of tungsten, as well as cobalt, have been found in soil and water samples in communities surrounding hard-metal industries where tungsten and cobalt powders have been used. For example, Fallon (Nevada), USA, is a community known to have elevated background surface dust concentrations of tungsten and cobalt [< 10 and 15 mg/kg, respectively]. Spatial patterns and high peak concentrations of tungsten and cobalt [934 and 98 mg/kg, respectively] measured in environmental surface dust samples from the surrounding community have suggested the local hard-metal industry to be the point source (Sheppard et al., 2007). [The Working Group considered that although the local hard-metal industry was primarily involved in the production of tungsten carbides and not the WGTAs in question, these findings suggest that air, soil, and groundwater contamination may occur in communities surrounding hard-metal industries that produce tungsten alloys.]

Elevated tungsten concentrations, orders of magnitude higher than background levels, have also been found in soil and groundwater samples from military firing ranges (Clausen & Korte, 2009; Bostick et al., 2018). For example, studies have found tungsten concentrations in soil to be as high as 2080 mg/kg (Clausen & Korte, 2009) and 5500 mg/kg (Bostick et al., 2018). Although tungsten concentrations decrease rapidly with increasing soil depth, elevated tungsten concentrations have also been detected in pore-water samples collected from lysimeters installed in berm areas, and in groundwater well samples collected 30 feet below the surface, at concentrations ranging from 0.001–400 to 0.001–0.56 mg/L, respectively (Clausen & Korte, 2009). In comparison, background tungsten concentrations in surface water samples were reported to be below the detection limit of 0.0002 mg/L. However, these studies were conducted at military firing ranges for small arms where tungsten–nylon munitions were primarily used. [The Working Group acknowledged that soil and water tungsten concentrations related to firing of WGTA munitions would probably differ. Although these studies may not be representative of all settings in which the agent could be released, they have shed light on the environmental fate of tungsten deposited in soil, showing that over time (and especially in areas with high acidity) tungsten in soil can solubilize and migrate into groundwater.] Additional research suggests that tungsten deposited in soil rapidly oxidizes to hexavalent tungsten(VI) species, the majority being polytungstates and/or polyoxometalates (Bostick et al., 2018). Polyoxometalates are considered to be weakly retained in soil, thereby increasing tungsten's mobility and solubility in the environment, and potentially leading to long-range transport of tungsten in the soil and subsequent groundwater contamination (Bostick et al., 2018).

1.4.2 Occupational exposure

The main route of occupational exposure to metals contained in WGTAs is via the respiratory tract. Exposure to metal particulates and aerosols via inhalation can occur during the production or firing, or result from the impact, of military munitions that contain the agent. In addition, it is possible that service members injured as a result of the impact of WGTA munitions may experience ongoing systemic metal exposure because of retained WGTA fragments in tissue.

(a) Exposure via inhalation

There are no published studies specifically examining exposure to the agent among workers who are involved in the production of WGTAs. However, several studies have investigated tungsten, nickel, and cobalt exposure in workers involved in the production of tungsten carbides (Kennedy et al., 2017; Westberg et al., 2017). The manufacturing process for tungsten carbides is similar to that for WGTAs as it involves the blending, pressing, forming, and sintering of metal powders. A study in Sweden reviewed historical personal and area air concentrations of tungsten, nickel, and cobalt for workers producing primarily tungsten carbides from 1970 to 2012 (Westberg et al., 2017). Area air concentrations of tungsten were not clearly reported, but area air concentrations of cobalt and nickel ranged from 0.0001 to 2.8 mg/m^3 (Westberg et al., 2017). Workers involved in pressing and powder and roll production had the highest metal exposure. [The Working Group noted that these findings are from tungsten-carbide production facilities and that exposure concentrations related to the production of WGTAs probably differ.]

Inhalation exposure of military personnel to aerosols of metals can result from the firing and resulting impact of WGTAs into targets. An exposure study conducted by the United States Army Research Laboratory measured concentrations of metal aerosols in the breathing zones of range personnel. Two experiments involving 120 mm KEW-A2 penetrators, containing WGTA, being fired against steel plates were conducted each day over a 6-day period (Gunasekar & Stanek, 2011). Although specific metal aerosol concentrations were not published, metal aerosol concentrations in the personal breathing zones of range personnel were reported to be 10 times lower than established threshold limit values during a 9-hour work shift. [However, the Working Group noted that these aerosol concentrations were measured in a controlled study environment that had existing engineering controls (i.e. proper ventilation), and it is unclear whether such engineering controls would be feasible in a combat situation.]

The firing of WGTA munitions that are used to perforate armoured vehicles can also result in the aerosolization of fine metal particles upon impact. When kinetic energy penetrators perforate a target, aerosol debris is generated at the front and rear of the target because of target and rod erosion (Machado, 2011). In a simulation experiment, Gold et al. (2007) used gravimetric analyses and scanning electron microscopy to assess potential exposure to aerosolized metal particulates of crew members riding in an Abrams tank breached by a kinetic energy penetrator containing WGTA. Although specific concentrations of tungsten, nickel, and cobalt in breathing zone air samples were not reported, the initial total concentrations of inhalable aerosols (can be breathed into the nose or mouth) and respirable aerosols (can penetrate beyond the terminal bronchioles into the gas–exchange region of the lungs) measured in the crew compartment were 6–12 and 5 g/m^3, respectively. At the end of the 2-hour test period, concentrations were in the milligrams-per-cubic-metre range. [The Working Group noted that similar aerosol concentrations may be found in combat situations, especially if vehicle ventilation systems fail, but that it is unclear how often such a scenario would occur.]

Machado et al. (2010) conducted a similar experiment, passing a subscale kinetic energy penetrator containing WGTA through steel plates in an encapsulated environment to characterize the aerosol particulates produced, and assess the probable resulting respiratory and inflammatory health effects. Particulate specimens, captured using cascade impactors, were analysed using gravimetric methods to determine total particulate weights and concentrations, while inductively coupled plasma optical emission spectrometry was used to assess the respirable

concentrations of each metal. Most of the aerosol generated consisted of nano-sized particles (with a size ranging from 1 to 100 nm), with overall total and total respirable particulate concentrations of 116 and 80 mg/m^3, respectively. Iron from the steel plates was found to be predominant in the air samples, with concentrations of 51.90 and 40.20 mg/m^3 for total and respirable particulates, respectively. Total and respirable particulate concentrations were 3.7 and 1.9 mg/m^3 for tungsten, 0.741 and 0.537 mg/m^3 for nickel, and 0.415 and 0.415 mg/m^3 for cobalt.

(b) Fragment-related exposures

Individuals with war-related injuries may have embedded fragments containing WGTAs in tissue, resulting in long-term exposure to metal ions from the retained fragments. Estimates suggest that more than 40 000 United States military veterans may have an embedded fragment injury; however, most of these injuries are reported to be from contact with improvised explosive devices (Gaitens et al., 2017). The number of embedded-fragment injuries related specifically to WGTA munitions is unknown, but such injuries are considered to be relatively rare, because no published reports were available relating to removal of WGTA fragments from an individual.

As a result of toxicity concerns related to the use of WGTAs, a United States Department of Defense policy in 2007 mandated that all surgically removed fragments must be sent for laboratory analysis (Centeno et al., 2014; Kalinich & Kasper, 2016). Quantitative chemical analyses of more than 800 fragments surgically removed from 344 United States military personnel over a 6-year period revealed that only one fragment contained tungsten (Centeno et al., 2014). This fragment also contained trace concentrations of lead and titanium, but no detectable concentrations of nickel or cobalt. SEM-EDXA analysis of adherent tissue accompanying the fragment showed the presence of single tungsten particles, with a size range of 1–2.5 µm, that were non-uniformly distributed throughout the tissue. (Centeno et al., 2014). [The Working Group noted that the availability and use of WGTA munitions varies among countries and therefore WGTA exposures in wounded United States military veterans may not be representative of other military populations.]

Most embedded fragments (often multiple) from war-related injuries are not excised because of the risk of surgical morbidity (Centeno et al., 2014; Gaitens et al., 2016, 2017). Therefore, on the basis of evidence from animal and human studies, which suggests that metal ions can be released from retained fragments over time, permitting their entry into the systemic circulation, biomonitoring of metal concentrations in urine has been used to gain insight into fragment-related exposures (Gaitens et al., 2016, 2017). In a study of more than 500 United States military veterans who reported having embedded fragments from war-related injuries, urine concentrations of 14 different metals frequently found in fragments were measured using ICP-MS (Gaitens et al., 2017). Results were reported as micrograms of metal per gram of creatinine (µg/g creatinine) and compared with reference values from unexposed populations. Among veterans, mean concentrations in the urine were reported to be 0.23 µg/g creatinine (range, 0.003–2.70) for tungsten, 2.42 µg/g creatinine (range, 0.02–76.25) for nickel, and 0.58 µg/g creatinine (range, 0.07–22.31) for cobalt. While urinary concentrations of tungsten, nickel, and cobalt were higher than the established reference values in approximately 12%, 5%, and 9% of samples, respectively, these elevations could not be directly attributed to the presence of a WGTA fragment, as other potential sources of metal exposure must be considered. The composition of a fragment can only be confirmed if it is removed and analysed.

1.4.3 Exposure of the general population

The general population is not likely to be exposed to WGTAs except when living in close proximity to locations where WGTA munitions are manufactured or have been fired. [However, the Working Group acknowledged that there was a lack of available data to enable the characterization of exposure to the agent.]

1.5 Regulations and guidelines

In 2007, a memorandum from the United States Secretary of Defense encouraged munition developers and researchers to consider using alternative materials to WGTAs in munitions production (Magness et al., 2014). The memorandum was written after the results of a study in rats showed rapid development of rhabdomyosarcomas in rats implanted with WGTA pellets (Kalinich et al., 2005). However, to date, there are no known bans, in the USA or elsewhere, on the use of WGTAs in military munitions.

There are no health and safety regulations or guidelines specific to WGTAs, although they do exist for the specific elements that they contain. [The Working Group noted that regulations and guidelines for tungsten, nickel, and cobalt are limited in applicability since they are not specific to WGTAs; however, in the absence of specific regulations and guidelines for WGTAs, they may provide useful information.] Table 1.2 and Table 1.3 summarize guidelines and regulatory limit values for tungsten and nickel. Guidelines and regulatory limit values for cobalt are described in Section 1.5 and occupational exposure limits in some countries are summarized in Table S1.15 in the monograph on Cobalt metal (without tungsten carbide) and some cobalt compounds in the present volume.

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 Epidemiological studies of cancer in humans

No epidemiological studies of cancer in humans exposed to WGTAs were available to the Working Group.

1.6.2 Mechanistic studies in humans

(a) Exposure assessment methods

The Working Group identified one mechanistic study for which a critical appraisal of exposure assessment methods was undertaken (De Hauteclocque et al., 2002; see also Table S1.4; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

In a case report of a single hard-metal industry worker who developed asthma, De Hauteclocque et al. (2002) assessed exposure to tungsten and other metals using occupational history and bronchoalveolar lavage (BAL) fluid. Urinary concentrations of nickel and cobalt, but not tungsten, were also measured.

(b) Critical review of exposure assessment

Urine is not always a preferred medium for the assessment of internal concentrations of metals (e.g. lead), and the exact period of exposure indicated by the urine sample depends on the biological half-life of the metal being investigated.

The case report by De Hauteclocque et al. (2002) provided a detailed, qualitative narrative history for exposure to hard metal dust. This exposure included tungsten carbide, which is not the agent evaluated in the present monograph. The case report did not clearly describe whether the worker was exposed to WGTAs. Biological measurements of tungsten were conducted after

Table 1.2 Occupational exposure limits for tungsten in different countries

Countries	Limit values (mg/m³)			
	Soluble tungsten and its compounds		Insoluble tungsten and its compounds	
	8-hour	Short-term	8-hour	Short-term
Austria[a], Denmark	1	2	5	10
Australia, Belgium[b], Canada (Ontario), Canada (Quebec), Ireland[c], Republic of Korea, Spain, USA – NIOSH[b], United Kingdom	1	3	5	10
New Zealand	1	–	5	10
Finland, Israel[d], Poland, Singapore, Switzerland[c]	1	–	5	–
Sweden	5	–	5	–
China[b]	–	–	5	10
Norway, USA – OSHA[e]	–	–	5	–

NIOSH, National Institute for Occupation Safety and Health; OSHA, Occupational Safety and Health Administration.

[a] Values for inhalable aerosol.

[b] 15-minute averages for short-term limit values.

[c] 15-minute reference period for short-term limit values.

[d] Values for inhalable fraction.

[e] For construction and maritime industries only (OSHA, 2021).

Adapted from IFA (2022a).

Table 1.3 Occupational exposure limits for nickel and nickel compounds in different countries

Countries	Limit values (mg/m³)									
	Nickel, metal		Nickel, metal, total dust		Nickel, metal and compounds		Nickel compounds, insoluble		Nickel compounds, soluble	
	8-hour	Short-term	8-hour	Short-term	8-hour	Short-term	8-hour	Short-term	8-hour	Short-term
Australia, Canada (Ontario)[a], China, France, Republic of Korea, Singapore, Spain, USA – OSHA	–	–	1	–	–	–	–	–	–	–
Ireland, Sweden, Switzerland[a]	–	–	0.5	–	–	–	–	–	–	–
Austria	–	–	0.5	2	–	–	–	–	–	–
Belgium	1	–	1	–	–	–	–	–	–	–
Canada (Quebec)	1	–	1	–	–	–	1	–	0.1	–
Denmark	–	–	0.05	0.1			0.01	0.02[b]	0.05	0.1[b]
Finland[c]	0.01	–	–	–	–	–	–	–	–	–
Germany	0.006[d,e]	0.048[b,d,e]	–	–	0.03[d]	0.24[b,d]	–	–	–	–
Hungary	–	–	0.1	0.1	–	–	–	–	–	–
Israel	1.5	–	–	–	–	–	0.2	–	0.1	–
Japan	1	–	–	–	–	–	0.1[f]	–	0.01	–
Latvia	–	–	0.05	–	–	–	–	–	–	–
New Zealand[d]	–	–	0.005	–	–	–	–	–	–	–
Norway	–	–	–	–	0.05	–				
Romania[b]	–	–	–	–	0.1	0.5	–	–	–	–
USA – NIOSH	–	–	0.015	–	–	–	–	–	–	–

NIOSH, National Institute for Occupation Safety and Health; OSHA, Occupational Safety and Health Administration.

[a] Values for inhalable aerosol.

[b] 15-minute average for short-term limit values.

[c] Calculated as nickel.

[d] Values for respirable fraction.

[e] An assessment based on the occupational exposure limit for nickel metal can be made if only metal is present.

[f] Values for total dust as nickel.

Adapted from IFA (2022b).

onset of the outcome. Co-exposure to cobalt, chromium, and nickel was described.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Groups of 10 or 20 male B6C3F$_1$ mice [age not reported, purchased at age 3–4 weeks] were given a single intramuscular implantation of four pellets fabricated from WGTA (91.1% tungsten, 6% nickel, and 2.9% cobalt), tantalum (negative control), or nickel (positive control) (1 mm diameter × 2 mm length, designated as groups at the higher dose), with two pellets inserted into each quadricep, and followed for up to 24 months (Emond et al., 2015a). Groups of 20 male mice were given a single implantation of two pellets of test alloy plus two pellets of tantalum per mouse and followed for 24 months (designated as groups at the lower dose). Groups of 10 or 20 male mice followed for 24 months served as surgical-sham controls. Groups of 10 mice were killed at 1, 3, 6, and 12 months (groups at the higher dose), while groups of 20 mice were killed at 24 months (at the lower and higher dose, and the surgical-sham groups). Median survival age for the groups implanted with pellets of WGTA at the lower and higher dose, nickel at the lower and higher dose, and tantalum was 92.5, 72.5, 83.5, 61.5, and 99.5 weeks, respectively. Gain of body weight (bw) of mice in the group at the higher dose of WGTA was slower than that of controls but did not become statistically significant until week 32 post-implantation. Complete necropsies and histopathological evaluations were performed.

In mice implanted with WGTA, there was a significant increase in the incidence of invasive sarcoma (identified as rhabdomyosarcoma) at sites of implantation in mice killed at 6, 12, and 24 months (in the groups at both the lower and higher dose) post-implantation [P = 0.0015, $P < 0.0001$, and $P = 0.0042$, respectively, assessed by Fisher exact test] compared with tantalum-implanted or surgical-sham controls. At 24 months, there was a significant positive trend [$P < 0.001$, Cochran–Armitage test] in the incidence of rhabdomyosarcoma for the groups implanted with WGTA. No surgical-sham controls or tantalum-implanted mice developed tumours, and all mice implanted with nickel developed rhabdomyosarcomas starting 3 months post-implantation. Neither the nickel- nor the WGTA-induced tumours metastasized to regional or distant sites.

[The Working Group noted that the duration of exposure was adequate, and that the quality of analytical techniques and statistical analyses was good. However, there was no group of mice implanted with tungsten only, statistical analysis for tumour incidence was not provided, the potential role of inflammatory components was not addressed properly (e.g. there was no investigation regarding the involvement of myofibroblasts, among other factors), and there were low numbers of mice per group.]

3.2 Rat

Four groups of 46 or 36 male Fischer 344 rats (age, 9 weeks) were implanted intramuscularly with 20 pellets (1 mm diameter × 2 mm length), split between each hind leg, of tantalum (negative control; purity, 99.95%; n = 46), WGTA (91.1% tungsten, 6.0% nickel, and 2.9% cobalt) (4 WGTA-based munitions pellets and 16 tantalum pellets in the group at the lower dose; 20 WGTA pellets in the group at the higher dose; both n = 46), or nickel (positive control; purity, 99.995% metallic

Table 3.1 Studies of carcinogenicity with weapons-grade tungsten (with nickel and cobalt) alloy in mice and rats

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) NR (purchased at age 3–4 wk) Up to 24 mo (104 wk) Emond et al. (2015a)	Intramuscular implantation WGTA, (91.1% W, 6% Ni, and 2.9% Co) None Pellet(s): 0 (sham control) or 4 Ta (control) for 6 mo, 4 WGTA for 6 mo 0 (sham control) or 4 Ta (control) for 12 mo, 4 WGTA for 12 mo 0 (sham control) or 4 Ta (control) for 24 mo, 2 WGTA + 2 Ta for 24 mo (lower dose), 4 WGTA for 24 mo (higher dose) 1× 10, 10, 10, 10, 20, 20, 20 NR, NR, NR, NR, 10 [sham control, read from Fig. 1] or 7 [Ta control, read from Fig. 1], 5 [read from Fig. 1], 1 [read from Fig. 1]	*Implantation site:* rhabdomyosarcoma 0/10 (control, 6 mo), 7/10* (WGTA, 6 mo) 0/10 (control, 12 mo), 9/10** (WGTA, 12 mo) 0/20 (control, 24 mo)[a], 7/20*** (WGTA, lower dose, 24 mo), 16/20** (WGTA, higher dose, 24 mo)	*[$P = 0.0015$, Fisher exact test] **[$P < 0.0001$, Fisher exact test] ***[$P = 0.0042$, Fisher exact test]; versus respective sham control, [a][$P < 0.001$, Cochran–Armitage trend test]; incidence in Ta-implanted (control) mice or sham controls, and consequently statistical comparisons with WGTA-implanted groups, was identical	Principal strengths: adequate duration of study, good quality of analytical techniques, and appropriate statistical analysis, except for tumour incidence (NR) Principal limitations: small number of mice per group, lack of a group implanted with W only, lack of statistical analysis for tumour incidence, and inflammatory components were not addressed properly (e.g. no investigation of myofibroblast involvement). Other comments: controls were sham, negative implantation (Ta pellets used), and positive implantation (Ni pellets used), with 10–20 mice per group, that were observed at 1, 3, 6, 12 ($n = 10$ per group), or 24 mo ($n = 20$ per group). Similar to sham controls, no Ta-implanted mice developed tumours (incidence was thus the same). All Ni-implanted mice developed rhabdomyosarcomas starting 3 mo post-implantation. Pellets were implanted in quadriceps; cylindrical pellets (1 mm in diameter × 2 mm in length). Tumours (rhabdomyosarcomas at implantation site) were not metastatic, unlike those observed in studies carried out in F344 rats (Kalinich et al., 2005; Schuster et al., 2012).

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344 (M) 9 wk Up to ~6 mo post-implantation Kalinich et al. (2005)	Intramuscular implantation WGTA (91.1% W, 6.0% Ni, and 2.9% Co) None Pellet(s): 20 Ta (negative control), 4 WGTA + 16 Ta (lower dose), 20 WGTA (higher dose), 20 Ni (positive control) 1× 46, 46, 46, 36 46 at 52 wk [read from Fig. 1], 0 at 38 wk [read from Fig. 1], 0 at 27 wk [read from Fig. 1], 0 at 30 wk [read from Fig. 1]	*Implantation site:* rhabdomyosarcoma 0/46, 46/46*, 46/46*, 36/36*	*[$P < 0.0001$, Fisher exact test]; versus negative control	Principal strengths: well-conducted study, adequate number of rats per group, appropriate statistical analysis, except for tumour incidence (NR). Principal limitations: lack of a group implanted with W only, lack of statistical analysis for tumour incidence. Other comments: intramuscular implantations of pellets on the gastrocnemius muscle of each leg; cylindrical pellets (1 mm in diameter × 2 mm in length). Study duration originally for 24 mo; however, because of the rapid tumour development, no WGTA- or Ni-implanted rat survived much past 6 mo post-implantation. Purity: Ni, 99.995% and Ta, 99.95% Ta. Since rats did not survive for more than 6 mo, the surveillance data included rats designated to be killed at 12, 18, and 24 mo who died earlier. Rapid tumour formation at the implantation site in 100% of the rats, with lung metastasis. The rate of tumour formation correlated with pellet number. Tumours developed rapidly in WGTA-implanted rats as well as in Ni-implanted group (positive control). Palpable tumours were apparent as early as 14 wk post-implantation in some rats at the WGTA higher dose; the tumours were aggressive and fast growing (with metastasis in the lung), necessitating killing of the rats upon becoming moribund several weeks later. Histopathological and immunohistochemical data (use of anti-desmin polyclonal antibody) supported a diagnosis of a pleomorphic rhabdomyosarcoma.

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344 (M) 8 wk Up to 24 mo Schuster et al. (2012)	Intramuscular implantation WGTA (91.1 wt% W, 6.0 wt% Ni, and 2.9 wt% Co) None Pellet(s): 20 Ta (negative control), 8 WGTA + 12 Ta (for up to 6 mo), 8 WGTA + 12 Ta (for up to 12 mo) 1× 50, 15, 15 NR	*Implantation site:* rhabdomyosarcoma 0/50, 10/15*, 15/15*	*[$P < 0.0001$, Fisher exact test]	Principal strengths: adequate duration of study, the control groups were adequate (Ta-implanted and untreated controls), adequate number of negative control rats, well-defined adverse effects (tumours and metastasis), appropriate data analysis, except for tumour incidence (NR). Principal limitations: lack of statistical analysis for tumour incidence, small number of rats in the WGTA-implanted groups, no information on number of surviving rats, and the inflammatory components were not addressed properly (e.g. no investigation of myofibroblasts involvement). Other comments: pellets were inserted into the hind legs; cylindrical pellets (1 mm in diameter × 2 mm in length). Mean survival time of the WGTA-treated rats, 36 ± 5.5 wk. Between 6 and 12 mo, 15/15 (100%) of the WGTA-treated rats developed aggressive tumours at the site of implantation and were killed; in 5/15 rats (33%), tumours were found in both legs; thus not all pellets resulted in tumours. 50% of the rhabdomyosarcoma-bearing rats developed metastasis to several organs, mainly the lungs. No rhabdomyosarcomas observed in W-treated and untreated control groups.

Co, cobalt; M, male; mo, month; Ni, nickel; NR, not reported; sham, surgical-sham; Ta, tantalum; W, tungsten; WGTA, weapons-grade tungsten (with nickel and cobalt) alloy; wk, week, wt, weight.

nickel; n = 36), and followed for up to 52 weeks (Kalinich et al., 2005). Because of rapid tumour development, the observation times for rats implanted with WGTA or nickel were only 1, 3, and 6 months. All rats in these groups were killed in a moribund condition before 38 weeks, whereas all rats implanted with tantalum were still alive at 52 weeks. Mean survival for the group implanted with WGTA at the higher dose was significantly lower (21.8 ± 2.1 weeks) than that for the groups implanted with WGTA at the lower dose (27.0 ± 4.6 weeks) or with nickel (25.4 ± 2.1 weeks). Complete necropsies and analyses of histopathology and immunohistochemistry (using an anti-desmin polyclonal rabbit antibody that indicates muscle origin) were performed.

At approximately 16–20 weeks post-implantation, tumours were observed at implantation sites in rats implanted with WGTA or nickel pellets, with palpable tumours detected as early as 14 weeks post-implantation in some rats in the group implanted with WGTA at the higher dose. The tumours were aggressive and fast-growing, necessitating killing of the rats several weeks later. Histopathological examination and immunohistochemical staining identified neoplastic cells that were strongly positive for desmin, suggesting a skeletal muscle origin. At 6 months, all rats implanted with WGTA at the lower (46/46 [$P < 0.0001$, Fisher exact test]) and higher dose (46/46 [$P < 0.0001$, Fisher exact test]) showed rhabdomyosarcoma at the implantation site, whereas none of the 46 tantalum-implanted controls developed tumours. All rats implanted with nickel also developed rhabdomyosarcoma (36/36), although not as rapidly as with WGTA. Tumours metastasized to the lung in WGTA-implanted rats [although the incidence of rats with metastases was not reported], whereas none of the nickel-implanted rats showed signs of metastases. [The Working Group noted this was a well-conducted study, with an adequate number of mice per group. However, there was no group implanted with tungsten only, statistical analysis for tumour incidence was not provided, and the role of inflammatory components was not addressed properly (e.g. there was no investigation of the involvement of myofibroblasts, among other factors).]

Groups of 15 or 50 male Fischer 344 rats (age, 8 weeks) were implanted with 20 pellets (1 mm diameter × 2 mm length; pellets were implanted in a circular pattern and placed approximately 1.5 mm apart), split between each hind leg, of WGTA (91.1% tungsten, 6.0% nickel, and 2.9% cobalt; 4 WGTA pellets and 6 tantalum pellets per leg) or tungsten (4 tungsten pellets and 6 tantalum pellets per leg in the group at the lower dose; 10 tungsten pellets per leg in the group at the higher dose; purity, 100%), and followed for up to 12 months (Schuster et al., 2012). Groups of 15 rats were killed at 3, 6, and 12 months for each implantation group. In addition, four groups of rats were observed for up to 24 months: 50 untreated (controls), 50 implanted with tantalum (negative controls), and 50 implanted with tungsten at the lower or higher dose. Mean survival for the WGTA-implanted group was 36 ± 5.5 weeks, which was comparable to that observed for the group implanted with WGTA at the lower dose in the study by Kalinich et al. (2005). Complete necropsies and histopathological evaluations were performed.

Histopathological examination of all grossly visible tumours was performed and showed that rats implanted with WGTA developed rhabdomyosarcomas at the site of implantation at 6 months (10/15 [$P < 0.0001$, Fisher exact test]) and 12 months (15/15 [$P < 0.0001$, Fisher exact test]) post-implantation, whereas none of the 50 tantalum-implanted negative controls or 50 untreated controls developed tumours after 24 months. Half of the rhabdomyosarcoma-bearing rats had metastases to multiple organs, including the lung, liver, prostate, and lymph nodes. In the group implanted with tungsten at the higher dose, 3/14 rats developed sebaceous adenomas

that surrounded the implanted pellets; these did not progress further over a 22-month period. None of the rats implanted with tungsten at the lower or higher dose developed rhabdomyosarcoma (0/50) (Schuster et al., 2012). [The Working Group noted this was a well-conducted study with adequate duration and control groups; however, only the mean survival time of rats exposed to WGTA was reported. Survival rates at the end of the experiment for controls and exposed rats, and the statistical analysis for tumour incidence, were not reported, the numbers of rats in WGTA-implanted groups were low, and the role of inflammatory components was not addressed properly (e.g. there was no investigation of the involvement of myofibroblasts, among other factors).]

In a study reported in a doctoral dissertation, Shinn (2012) investigated whether positron emission tomography-computed tomography (PET-CT) imaging offers greater sensitivity for the identification of metabolic changes (including inflammation and cell proliferation rates) and primary-stage tumours in muscle tissue surrounding embedded fragments of WGTA when compared with X-rays. Two groups of 17 male Fischer 344 rats (age, 8 weeks) [2 rats per group serving as back-up replacements] were implanted with WGTA (91.1% tungsten, 6% nickel, and 2.9% cobalt) or tantalum (purity, 99.95%; negative controls) pellets (2 pellets/rat, cylinders of 1 mm diameter × 2 mm length, spaced approximately 1.5 mm apart on the lateral side of the right hind leg), and followed for up to 16 weeks (Shinn, 2012). A third group of 2 rats served as sham controls. A series of 1–5 X-rays and ^{18}F-fluoro-2-deoxy-D-glucose PET-CT scans were performed on each rat over 16 weeks (weeks 1, 7, 10, 13, and 16 post-surgery). At scheduled intervals and at 16 weeks post-implantation, the rats were killed. Histopathological examination of tissue around the implants and immunohistochemical staining for desmin and MyoD1 (two biomarkers of myogenic origin, used for the diagnosis of rhabdomyosarcoma) were performed at each time point, as well as measurement of metal concentrations in the urine. The locations of the metal pellets and increased changes in ^{18}F-fluoro-2-deoxy-D-glucose uptake around the pellets were captured on PET-CT images over the 16 weeks. Significant differences in uptake were observed between WGTA-implanted rats and negative controls, with a sensitivity of 86% and specificity of 100%.

WGTA-implanted rats had no visible or palpable tumours at 16 weeks post-implantation; however, they all had histological findings of an invasive disease process: typical skeletal muscle fibres abnormalities, and positive staining for desmin overexpression at 13 weeks post-implantation and for MyoD1 at 7 weeks post-implantation. At 16 weeks, all 15 rats implanted with WGTA developed malignant invasion of fibres of the skeletal muscle, whereas none was reported in the tantalum-implanted negative controls. [The Working Group noted the short duration of observation, the small number of rats per group, and limited reporting and interpretation of the data (e.g. the cellular changes reported by the author were consistent with those observed in malignant tumours and the lesions were not labelled as tumours by the author). Therefore, the Working Group judged the study inadequate for the evaluation of the carcinogenicity of WGTA in experimental animals.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of WGTA has been assessed in one study in male mice treated by intramuscular implantation (Emond et al., 2015a) and in two studies in male rats treated by intramuscular implantation (Kalinich et al., 2005; Schuster et al., 2012).

In one study in male B6C3F$_1$ mice exposed to WGTA by intramuscular implantation, there

was a significant increase in the incidence of rhabdomyosarcoma at the implantation site at 6, 12, and 24 months post-implantation when compared with tantalum-implanted or surgical-sham controls (Emond et al., 2015a).

In one well-conducted study in male Fischer 344 rats exposed to WGTA by intramuscular implantation, WGTA at the lower and higher doses caused a significant increase in the incidence of rhabdomyosarcoma at implantation sites when compared with negative controls (Kalinich et al., 2005). In another study in male Fischer 344 rats exposed to WGTA by intramuscular implantation, WGTA caused a significant increase in the incidence of rhabdomyosarcoma at the implantation site at 6 and 12 months post-implantation when compared with negative controls (Schuster et al., 2012).

One study in male Fischer 344 rats exposed to WGTA by intramuscular implantation was considered inadequate for the evaluation of the carcinogenicity of WGTA in experimental animals (Shinn, 2012).

4. Mechanistic Evidence

Studies evaluating absorption, distribution, metabolism, and excretion, and mechanistic evidence for WGTA were performed using either WGTA or simulated WGTA (simulated forms of WGTAs are typically prepared as mixtures of metal microparticles in the same weight ratios as the parent WGTAs). Neither the alloy pellets nor the metal particles had any surface treatment or coating. The pellets were made with tungsten microparticles sintered with nickel and cobalt. Sintering was not reported in the simulated preparations. [The Working Group noted that the effects of sintering with regards to ion release are not known.] WGTAs are named according to the chemical symbols for the component metal elements followed by the percentage of each metal by weight; for example, WNiCo (97–2–1) for a WGTA containing (by weight) 97% tungsten, 2% nickel, and 1% cobalt. Simulated WGTAs are named using an "s" prefix followed by the chemical symbols for the metals and the percentages (by weight) of the metals in the mixture, separated by forward slashes; for example, sW/Ni/Co (92/5/3).

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Relevant human exposure to WGTA can result through embedded shrapnel in the body, and/or inhalation of aerosolized particles generated at high temperatures, such as when WGTA hits hard surfaces.

(a) Exposed humans

Only one study has evaluated the exposure of humans to WGTA (Gaitens et al., 2017). This cross-sectional study reported measurements of concentrations of 14 metals in the urine as part of a large case series of United States military veterans with embedded metal fragments resulting from traumatic injury (see Section 1.4.2(b)). [The Working Group noted that none of the veterans had known exposure to the specific tungsten alloy and therefore excluded the study from the evaluation of WGTA.]

(b) Human cells in vitro

No data were available to the Working Group.

4.1.2 Experimental systems

(a) Non-human mammals in vivo

[The Working Group noted that the available data on the absorption, distribution, metabolism, and excretion of WGTAs were limited to absorption (after intramuscular implantation), distribution, and excretion in rodent models.

No data on absorption, distribution, metabolism, and excretion after exposure via the skin or respiratory tract, or on the metabolism of WGTAs after exposure by any route, were available to the Working Group.]

Intramuscular implantation of WGTA pellets consisting of WNiCo (91–6–3) has been used to study metal distribution in serum of male Fischer 344 rats (Kalinich et al., 2008) and multiple organs/tissues of male B6C3F$_1$ mice and male Fischer 344 rats (Emond et al., 2015a; Vergara et al., 2016), and excretion in the urine of male Fischer 344 rats (Kalinich et al., 2008; Schuster et al., 2012; Shinn, 2012) and male B6C3F$_1$ mice (Emond et al., 2015a). [No data on absorption, distribution, metabolism, and excretion in female rodents were available to the Working Group.] Absorption, distribution, metabolism, and excretion of the individual components – tungsten (ICRP, 1981), nickel (WHO, 2000), and cobalt (see the first monograph in the present volume) – have been summarized elsewhere, and are only discussed here in comparison with the same metal in a WGTA.

In rodents with WNiCo (91–6–3) pellets implanted in the muscle, tungsten, nickel, and cobalt were absorbed and distributed systemically (in serum and organs or tissues) and excreted in the urine. [No data on excretion via other routes, such as in the faeces or milk, were available to the Working Group.] High concentrations of these metals were reported in the kidney, spleen, and liver compared with other tissues. Concentrations of tungsten in tissues (kidney, liver, spleen, testes, muscle, and femur, but not brain) and in the urine increased over time until 6–12 months after implantation (Emond et al., 2015a; Vergara et al., 2016). The concentration of tungsten in kidney 6 months post-implantation (at the higher dose) was increased by 7-fold compared with 1 month post-implantation (Vergara et al., 2016). In muscle, only tungsten increased in concentration over time until 6 months (at the higher dose), whereas concentrations of cobalt and nickel decreased between 1 and 6 months post-implantation (Vergara et al., 2016). Compared with tungsten, concentrations of cobalt and nickel in tissues were relatively stable over time (at 1, 3, and 6 months), with notable increases in nickel concentrations in the brain (higher dose) and cobalt concentrations in the liver 6 months post-implantation (Vergara et al., 2016). All three metals in the pellets (tungsten, nickel, and cobalt) have been shown to cross the blood–brain and blood–testes barriers; Emond et al. (2015a) demonstrated that tungsten passes the blood–testes barrier, and Vergara et al. (2016) demonstrated that tungsten, nickel, and cobalt cross the blood–brain and blood–testes barriers. Garrick et al. (2003) showed that cobalt metal binds to a divalent metal transporter 1 isoform (–IRE DMT1), which is predominantly expressed in neuronal cells (Garrick et al., 2003). Soluble tungsten can cross the blood–brain barrier and the placenta (McInturf et al., 2008, 2011, both in US EPA, 2015).

Exposure to WNiCo (91–6–3) caused an increase in metal concentrations in the tissues and urine when compared with WNiFe (97–2–1) or (91–7–2) (Schuster et al., 2012; Emond et al., 2015a). [Although WNiFe alloys are not the agent under evaluation, the Working Group noted that they are informative as control compounds for comparison with WGTAs.] This effect cannot be explained by small differences in the proportions of tungsten and nickel in WNiFe and WGTA pellets, because when the percentage of tungsten or nickel in the WGTA pellets was the same or lower than that in the WNiFe pellets, the concentrations of tungsten and nickel in tissue and urine samples were higher by several fold in groups exposed to WGTA than in groups exposed to WNiFe. It should be noted that the pellets are not homogeneous mixtures of three metals but are composed of tungsten microparticles embedded in a matrix with nickel, cobalt, or iron, and some tungsten (Schuster et al., 2012). [The Working Group noted that tungsten microparticle sizes

were not reported, and that particle sizes could not be estimated from the transmission electron microscopic images because particles were sectioned at various planes and angles.] Higher fractions of nickel and cobalt or iron are present in the matrix than in the whole pellets. After implantation, erosion occurred mostly in the matrix, and WGTA pellets were more deeply eroded than those made of WNiFe. Furthermore, a layer of metal oxide was observed on the surface of WNiFe pellets, but not on the surface of WGTA pellets, and may have functioned as a protective layer against further erosion (and metal release from the WNiFe pellets) (Schuster et al., 2012).

$B6C3F_1$ mice were surgically implanted with pellets composed of one or two metals – tungsten, nickel, and/or cobalt – and tantalum as an inert metal component. Of the cobalt-containing pellets tested (each contained 2.9% cobalt: cobalt with tantalum, tungsten with cobalt and tantalum with 91.1% tungsten, and nickel with cobalt and tantalum with 6% nickel), significant and consistent increases in cobalt concentrations (in the brain, kidney, liver, spleen, femur, testes, serum, and urine; for most of the measured time points: 1, 3, 6, 12, and 24 months) were only measured in the mice exposed to tungsten with cobalt and tantalum (Emond et al., 2015b). [The Working Group noted that the results suggest that the release of cobalt is enhanced by tungsten, but not by nickel.]

(b) Non-human mammalian cells in vitro

No data were available to the Working Group.

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 WGTA is genotoxic; induces oxidative stress; induces chronic inflammation; is immunosuppressive; induces epigenetic alterations; causes immortalization; or alters cell proliferation, cell death, or nutrient supply. No data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

See Table 4.1.

WGTA pellets and simulated WGTAs increased DNA strand breaks, micronucleus formation, and sister-chromatid exchange in various cultured human cells.

In human primary skeletal muscle cells (HSkMC) in vitro, pellets made of WNiCo (97–2–1) and WNiCo (91–6–3) (207 mm^2 particle surface area/75 cm^2 tissue culture flask), but not WNiFe (97–2–1) or inert tantalum, caused a significant increase in the frequency of DNA strand breaks, as measured by comet assay, after 24 hours of exposure (Harris et al., 2015). [The Working Group noted that the pellets were not cytotoxic at this exposure level based on a Toxilight adenylate kinase assay, but that histological examination had identified regions of dead and dying (apoptotic) cells surrounding the WGTA pellets.]

In an immortalized, non-tumourigenic, human osteosarcoma (HOS) cell line, sW/Ni/Co (92/5/3) caused significant increases in the frequency of DNA strand breaks (at a concentration of ≥ 50 µg/mL, 1-hour exposure, or 5 µg/mL, 24-hour exposure; alkaline elution test), micronucleus formation (at a concentration of ≥ 25 µg/mL, 1-hour exposure, or 5 µg/mL, 24-hour exposure), and sister-chromatid exchange (5 µg/mL dose only) (Miller et al., 2001, 2002). [On the basis of the cytotoxicity reported and other end-points evaluated by the two publications, the Working Group

Table 4.1 Genotoxic effects of weapons-grade tungsten (with nickel and cobalt) alloy or the simulated alloy in human and non-human mammalian cells in vitro

End-point	Tissue, cell type or line	WNiCo or sW/Ni/Co (% of each metal)	Results	Exposure concentration or range, and duration	Comments	Reference
DNA strand breaks (alkaline comet assay)	Primary human skeletal muscle, HSkMC cells	WNiCo (97–2–1) WNiCo (91–6–3), 7 mo after production WNiCo (91–6–3), 39 mo after production WNiCo (91–6–3), 67 mo after production	+ + – +	~207 mm^2 particle surface area per 75 cm^2 tissue culture flask growth area (24-h exposure)	No change in cell viability as measured by Toxilight adenylate kinase assay kit, but histologically there were regions of dead/dying cells surrounding the WNiCo (91–6–3) and WNiCo (97–2–1) pellets. WNiCo (91–6–3) was stored for 7, 39, or 67 mo before testing. [The Working Group noted that storage conditions were not specified.] WNiCo (97–2–1) was not stored. Effect of WNiCo (91–6–3) > WNiCo (97–2–1).	Harris et al. (2015)
DNA strand breaks (rapid alkaline elution test)	Human osteosarcoma, HOS cells (TE85, clone F-5)	sW/Ni/Co (92/5/3)	+	≥ 25 µg/mL (1-h exposure)	50 µg/mL was the lowest exposure concentration at which the response was greater than or equal to that of the positive control (25 µg/mL was also statistically significant at the lowest exposure concentration). Decreased plating efficiency was used for cytotoxicity measurements (> 90% cell viability after 24-h exposure to 50 µg/mL). Reported both particle size and purity. Positive control included.	Miller et al. (2001)
DNA strand breaks (rapid alkaline elution test)	Human osteosarcoma, HOS cells	sW/Ni/Co (92/5/3)	+	5 µg/mL (24-h exposure)	[5 mg/mL was reported in the study, but the Working Group determined that such a high in vitro concentration was most likely a reporting error.]	Miller et al. (2002)

Table 4.1 (continued)

End-point	Tissue, cell type or line	WNiCo or sW/Ni/Co (% of each metal)	Results	Exposure concentration or range, and duration	Comments	Reference
Micronucleus formation (micronucleus assay)	Human osteosarcoma, HOS cells	sW/Ni/Co (92/5/3)	+	≥ 25 µg/mL (1-h exposure)	25 µg/mL was the lowest exposure concentration. Decreased plating efficiency was used for cytotoxicity measurements (> 90% cell viability after 24-h exposure to 50 µg/mL). Reported both particle size and purity. Positive control included.	Miller et al. (2001)
Micronucleus formation (micronucleus assay) Sister-chromatid exchange	Human osteosarcoma, HOS cells	sW/Ni/Co (92/5/3)	+ +	5 µg/mL (24-h exposure) 5 µg/mL (24-h exposure)	Micronuclei measured in binucleate cells. [5 mg/mL was reported in the study, but the Working Group determined that such a high in vitro concentration was most likely a reporting error.]	Miller et al. (2002)
DNA strand breaks (modified alkaline comet assay)	Rat skeletal muscle myoblast, L6-C11 cell line	WNiCo (91–6–3) WNiCo (91–6–3) WNiCo (97–2–1) WNiCo (97–2–1)	+ (with *fpg*) + (without *fpg*) – (with *fpg*) – (without *fpg*) – (ratio of DNA damage levels detected with/without *fpg*) The difference in the DNA damage levels detected with/without *fpg* (*fpg*-sensitive) was not statistically significant.	"Small quantities of particles" with ~207 mm^2 particle surface area in 0.5 mL volume (24-h exposure)	No clear description of concentrations used was reported. Cytotoxicity was indicated histologically by regions of dead/dying cells surrounding the WNiCo (91–6–3) and WNiCo (97–2–1) pellets. No quantitative measurements of cell viability reported. [The Working Group noted that the microscopic images of this study resemble those in Harris et al. (2015), which reported no significant changes in cell viability for WNiCo-exposed samples based on an adenylate kinase assay.]	Harris et al. (2011)

+, positive; –, negative; Co, cobalt; *fpg*, formamidopyrimidine-DNA glycosylase; mo, month; Ni, nickel; sW/Ni/Co, simulated weapons-grade tungsten (with nickel and cobalt) alloy (followed by the percentage of each metal by weight); W, tungsten; WNiCo, weapons-grade tungsten (with nickel and cobalt) alloy (followed by the percentage of each metal by weight).

determined that the reported concentration of 5 mg/mL in Miller et al. (2002) was probably 5 µg/mL.] Median diameters (d_{50}) of particles were 1–4 µm for cobalt, 3–5 µm for nickel, and 1–3 µm for tungsten. [The Working Group noted that exposure to the simulated WGTA caused minimal cytotoxicity at the 50 µg/mL dose. Also, injection of the HOS (TE85, clone F-5) cells into 128 (Miller et al., 2001) and 82 (Miller et al., 2002) athymic nude mice (strain/breed not reported) did not cause any tumour development up to 6 months after injection. However, in a study by Lauvrak et al. (2013), the incidence of tumours after injection of HOS (CRL-1543) cells into NOD/SCID/IL2r gamma^{null} (NSG) mice was 12/12 mice within 10–20 days post-injection.]

[The lack of effect of WNiFe, compared with WGTA, is probably caused by decreased metal release after the formation of a metal oxide layer on the surface of WNiFe (but not WGTA) pellets (Schuster et al., 2012). Miller et al. (2001) pointed out that the induction of DNA strand breaks by simulated WGTA was more than the sum of the induction of DNA strand breaks by the individual metals at concentrations matching those in the simulated WGTA.]

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

See Table 4.1.

In a rat skeletal-muscle myoblast cell line (L6.C11), there was a significant increase in the incidence of DNA strand breaks (as measured by modified comet assay) after exposure to WNiCo (91–6–3), but not WNiCo (97–2–1) or WNiFe (97–2–1), for 24 hours (Harris et al., 2011) [see also Section 4.2.2(b)(ii)]. Cytotoxicity was indicated histologically by regions of dead and dying cells surrounding the WNiCo (91–6–3) and WNiCo (97–2–1) pellets. [The Working Group noted that the concentrations used were not clearly described. In addition, cell viability measurements were not reported, and microscopic images resembled those reported in Harris et al. (2015).]

[The Working Group noted that muscle implantation of WNiCo (92–6–3) caused rhabdomyosarcoma in Fischer 344 rats (Kalinich et al., 2005; Schuster et al., 2012) and B6C3F$_1$ mice (Emond et al., 2015a). No bioassay evidence for WNiCo (97–2–1) was available to the Working Group. The Working Group also noted the possibility of species differences related to exposure to WNiCo (97–2–1), which gave positive results for genotoxicity in human cells.]

4.2.2 Induces oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

Oxidative stress was suggested by changes in urinary metabolite concentrations, as measured by ^{1}H nuclear magnetic resonance spectroscopic profiling, in male Sprague-Dawley rats injected intraperitoneally with 0.5 mL sW/Ni/Co (91/5/4) at one of two doses (Tyagi et al., 2014). The lower doses were reported to be one tenth to one fifth of the median lethal dose (LD_{50}; per kg bw), while the higher doses were two to four fifths of the LD_{50}. [The Working Group noted that it was not clear if the LD_{50} values were for individual metals or for the combined simulated WGTA mixture, and LD_{50} values were not reported.] Urinary concentrations of *N*-methylnicotinamide were increased significantly 8 hours after injection of the higher dose and decreased significantly 72 hours after injection of the lower dose. Creatinine and choline levels were increased significantly 8–120 hours after injection of the higher or lower dose. Not all changes were significant at all time points, and the measured

metabolites could be linked to different metabolic pathways (including glutathione), or enzymes related to oxidative stress (Tyagi et al., 2014). [The Working Group noted other possible causes of the observed increases, which were not explored and therefore cannot be ruled out.]

(ii) Non-human mammalian cells in vitro

Concentrations of reactive oxygen species (ROS) were increased significantly in a rat skeletal muscle myoblast cell line (L6.C11) as early as 1 hour after exposure to WNiCo (91–6–3) and WNiCo (97–2–1), but not WNiFe (97–2–1) (Harris et al., 2011). The increase in ROS was greatest for WNiCo (91–6–3), followed by WNiCo (97–2–1), and then WNiFe (97–2–1), which was also the same ranking for cytotoxicity. After exposure to WNiCo (91–6–3) for 24 hours, there was a significant increase in the frequency of DNA strand breaks, as measured by modified comet assay with DNA-formamidopyrimidine glycosylase enzymatic cleavage. However, the difference in incidence measured with or without DNA-formamidopyrimidine glycosylase enzymatic cleavage was not statistically significant. In line with the observed changes in ROS, exposure to WNiCo (97–2–1) and WNiFe (97–2–1) with DNA-formamidopyrimidine glycosylase enzymatic cleavage did not increase DNA damage (Harris et al., 2011) (see Table 4.1).

In primary pulmonary macrophages collected from BAL fluid obtained from male Sprague-Dawley rats, exposure to 12.5–200 µg/mL of sW/Ni/Co (92/5/3) caused a significant increase in intracellular ROS and reactive nitrogen species (measured together and including hydrogen peroxide, peroxyl radical, nitric oxide, and peroxynitrite anion) 1 hour after exposure compared with controls (Roedel et al., 2012). Exposure to concentrations of 100 µg/mL or greater caused a significant decrease in cell viability. Median particle sizes were 1–5 µm for tungsten, 3–7 µm for nickel, and 1.6 µm for cobalt.

4.2.3 Evidence relevant to other key characteristics of carcinogens

(a) Induces chronic inflammation

(i) Exposed humans

A case report of occupational asthma (an indicator of a chronic inflammatory response) in a French hard-metal industry worker evaluated the occupational history and the effects of exposure to tungsten on the lower respiratory system (via BAL). There was co-exposure to multiple metals, including tungsten carbide, nickel, cobalt, and chromium. Mechanistic assessments (allergic reactions) were carried out for nickel and cobalt, but not for tungsten (De Hauteclocque et al., 2002). [The Working Group noted that the assessment was not specific to tungsten alloys and excluded the study from the evaluation of WGTA (see Table S1.4; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).]

(ii) Human cells in vitro

In one study of human hepatoma (HepG2) cells in vitro, exposure to sW/Ni/Co (92/5/3) (5–50 µg/mL) caused a significant increase in the expression of 6/13 stress gene promoters/response elements after 48 hours, as measured by CAT-Tox (L) (chloramphenicol acetyl transferase-toxicity (L)iver) reporter assay (Miller et al., 2004). Two of the genes were related to immune function: NFκBRE (serving as a binding site for the transcription factor NFκB, also known as NFKB1) and CRE (serving as a binding site for CREB protein, which is activated by pro-inflammatory signals and various transcription factors, and also known as Cre recombinase) (Wen et al., 2010). [The Working Group noted that the cell viability was greater than 85% at ~5 µM.]

(iii) Experimental systems

While no studies directly evaluating chronic inflammation were available to the Working Group, acute changes in biomarkers of inflammation and immune activation were reported in vivo (Roedel et al., 2012). Pulmonary inflammation was shown histologically by increased numbers of inflammatory cells (including neutrophils) in the lung tissues of male Sprague-Dawley rats 24 hours after intratracheal instillation of sW/Ni/Co (92/5/3) at 20 and 40 mg/kg bw, compared with controls instilled with saline or tungsten alone (Roedel et al., 2012). Acute inflammation was also indicated by significant increases in numbers of neutrophils, and levels of Cinc-1, Cinc-3, Tnfb, Il1b, and albumin proteins in the BAL fluid of treated rats, compared with controls. In addition, quantification of messenger RNA (mRNA) of several genes related to inflammation indicated significant differences in lung tissue and cells from BAL fluid of treated rats compared with controls (Roedel et al., 2012) (see also Section 4.2.4).

Male Fischer 344 rats were chronically exposed to WNiCo (91–6–3) at a lower (4 pellets/rat) or higher (20 pellets/rat) dose administered by intramuscular implantation for 3 months (i.e. those animals that did not die or were not killed early due to incidence of rhabdomyosarcoma). Both treatment groups showed a significant increase in peripheral blood counts for total leukocytes, neutrophils, lymphocytes, and monocytes (but not eosinophils or basophils) compared with controls implanted with tantalum pellets, and a significant increase in spleen weights in the group at the higher dose (Kalinich et al., 2005). In the group at the higher dose, significant increases in neutrophil counts and spleen weights were observed by 1 month compared with controls (Kalinich et al., 2005). In killed rats, counts of total leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils, and spleen weights were increased significantly in the group at the higher dose, while neutrophil counts were increased significantly in the group at the lower dose. However, in one study by Shinn (2012), no significant changes in haematology parameters, i.e. total leukocytes or any specific immune or inflammatory cells (lymphocytes, monocytes, or granulocytes), were observed in rats treated with the same WGTA (or tantalum) pellets up to 16 weeks post-implantation.

(b) Is immunosuppressive

Experimental systems

In Fischer 344 male rats, chronic exposure to WNiCo (91–6–3) (20 pellets/rat) administered by intramuscular implantation for up to 5 months caused a significant decrease in thymus-to-body-weight ratio (Kalinich et al., 2005).

In primary pulmonary macrophages collected from BAL fluid of untreated male Sprague-Dawley rats, exposure to sW/Ni/Co (92/5/3) (25–200 µg/mL) caused a significant decrease in phagocytosis of (non-opsonized) zymosan particles after 1 hour (Roedel et al., 2012). Exposure concentrations of ≥ 100 µg/mL caused a significant decrease in cell viability. Phagocytosis was measured using a CytoSelect 96-well phagocytosis assay and was also observed by microscopy for a subset of cells.

(c) Induces epigenetic alterations

Epigenetic changes induced by exposure to sW/Ni/Co (91/6/3) were studied in four cell types in vitro (two of human and two of mouse origin): human embryonic kidney (HEK-293) and neuroepithelioma (SK-N-MC) cell lines, and mouse hippocampal primary neuronal cell cultures and a myoblast (C2C12) cell line (Verma et al., 2011). Exposure times were 1 week for the mouse primary neuronal cell cultures and 1 day for the other cell types. sW/Ni/Co (91/6/3) was cytotoxic at concentrations greater than 50 µg/mL. Exposure to simulated WGTA (183.9 µg/mL) caused a significant decrease in

the phosphorylation of serine 10 on histone 3 in primary neuronal cell cultures and C2C12 cells, and significant decreases were measured in all four cell types after exposure to cobalt alone (58.9 µg/mL). Exposure to simulated WGTA and cobalt alone also caused a significant decrease in the acetylation of histone 3 in C2C12 cells and in all cell types except primary neuronal cell cultures, respectively. Trimethylation of lysine 4 on histone 3 was not affected by exposure to simulated WGTA or cobalt. Additionally, exposure to nickel did not induce any of these epigenetic changes in the four cell types studied. Further investigation of histone modifications induced by exposure to simulated WGTA and cobalt in C2C12 cells showed that an intracellular calcium chelator (but not an extracellular calcium chelator or calcium channel blocker) reversed simulated WGTA- and cobalt-induced hypophosphorylation of serine 10 on histone 3, suggesting that this epigenetic effect may be due to increased concentrations of intracellular calcium or changes in intracellular calcium dynamics (Verma et al., 2011).

Histone modification was further supported by the study of Harris et al. (2011), which showed that exposure to WNiCo (91–6–3) caused significant upregulation in mRNA levels of histone methylase, histone acetylase, and histone deacetylase in rat L6-C11 cells but induced an increase in protein levels of histone 2A only. Quantification of mRNA expression revealed a significant increase in histone deacetylase mRNA, but not histone methylase or histone acetylase mRNA, after exposure to WNiCo (97–2–1). Exposure to WNiCo (97–2–1) caused a greater increase in histone 2A protein concentrations than did exposure to WNiCo (91–6–3).

(d) Causes immortalization

In HOS cells cultured in vitro, exposure to sW/Ni/Co (92/5/3) at a non-cytotoxic concentration of 50 µg/mL for 24 hours caused a significant increase in the frequency of neoplastic transformation (with increased anchorage-independent cell growth in soft agar) and invasion (through Matrigel) over a 5-week period in vitro, compared with untreated cells. Untreated HOS cells are not tumourigenic; subcutaneous injection of these cells did not cause any tumours in 128 female athymic nude mice after 6 months. In contrast, HOS cells transformed with sW/Ni/Co (92/5/3) caused adenocarcinoma in 6 out of 12 mice within 4 weeks. Transformation of HOS cells with sW/Ni/Co (92/5/3) caused a significant increase in the expression of K-*ras* oncogene mRNA, and the adenocarcinomas gave positive results for cytokeratin (an epithelial cell marker) and negative for vimentin (a mesenchymal cell marker) (Miller et al., 2001). [The Working Group noted that this suggested that there was no epithelial–mesenchymal transition.] Exposure of HOS cells to sW/Ni/Co (92/5/3) at a non-cytotoxic concentration of 10 µg/mL for 24 hours also caused a significant increase in the frequency of neoplastic transformation (with increased anchorage-independent cell growth in soft agar) in vitro compared with untreated cells. Injection of the untreated cells did not cause any tumours in 82 athymic nude mice after 6 months. In contrast, sW/Ni/Co (92/5/3)-transformed cells caused adenocarcinoma in 8 out of 20 mice within 4 weeks (Miller et al., 2002). [The Working Group noted that the metal that contributed to the transformation of HOS cells in vitro was probably nickel, because nickel (but not tungsten or cobalt) at a concentration equivalent to that in sW/Ni/Co (92/5/3) induced a small but significant increase in transformation frequency in vitro in the study by Miller et al. (2001). Nickel also increased the transformation frequency in Miller et al. (2002), but nickel-transformed cells did not consistently cause tumours in the athymic nude mice, with tumour incidence reported to be 0/12 in Miller et al. (2001) and 6/20 in Miller et al. (2002).]

4.2.4 Multiple characteristics identified via microarray or omics

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

See Table 4.2.

Exposure to WNiCo (91–6–3) (stored for 7, 39, or 67 months) for 24 hours induced changes in many pathways related to key characteristics in human primary HSkMC cells cultured in vitro. [The Working Group noted that freshly made WNiCo (91–6–3) was not tested.] On the basis of annotation enrichment analysis, there was increased expression of mRNA transcripts relevant to immune/pro-inflammatory responses and cytokine–cytokine receptor interactions, the oxidative stress response (including NRF2), components of the glutathione system, antioxidant enzymes, the response to hypoxia, increased glycolysis and angiogenesis, and decreased cell death (increased expression of mRNA transcripts associated with negative regulation of apoptosis including anti-apoptosis, wound healing, and regulation of cell proliferation). Decreased expression of mRNA transcripts for immunoglobulins, muscle-specific proteins and contractile fibres, the actin cytoskeleton, and cell differentiation-associated genes indicated that immune function and muscle tissue differentiation were decreased (Harris et al., 2015). Exposure of HSkMC cells to WNiCo (97–2–1) in vitro caused transcriptional changes that were similar to those caused by exposure to WNiCo (91–6–3), albeit in fewer genes. [The Working Group noted that decreased differentiation is critical to carcinogenesis because, when they do not terminally differentiate, cells may maintain the ability to multiply and/or proliferate, which increases their likelihood of transformation.] Caspase-3 activity was not altered in HSkMC cells exposed to WNiCo (97–2–1), WNiCo (91–6–3), or WNiFe (97–2–1) in vitro for 24 hours (Harris et al., 2015). [The Working Group noted that this is consistent with gene expression, indicating anti-apoptotic effects. Moreover, the Working Group noted possible species differences, because human cells were less sensitive to the effects of WNiCo (91–6–3) than rat L6.C11 cells in vitro (based on measurement of caspase-3 activity and the number of genes with altered expression), to which the authors refer in their previous study (Harris et al., 2011) (see also Section 4.2.4(b)(ii) and Table 4.3).]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

In addition to the evaluation of immune/pro-inflammatory responses by assessment of phagocytosis, concentrations of cytokines in BAL fluid, and lung histology (see also Section 4.2.4), cells collected from BAL fluid from male Sprague-Dawley rats exposed to sW/Ni/Co (92/5/3) (20 mg/kg bw) by intratracheal instillation were assessed by Rat Stress and Toxicity PathwayFinder RT^2 Profiler PCR Array (expression of 84 genes was assessed). Significant differences in mRNA transcript expression compared with cells from control rats exposed to saline were related to signalling pathways involved in apoptosis (increased NFkbia and decreased Casp1 expression), growth arrest and senescence (increased Cdkn1a and Ddit3), cell proliferation (decreased E2f1), and inflammation (increased Il6, Mip-1a, Mip-1b, Il1b, Tnfb, and Nos2/iNOS, and GM-CSF, and decreased Il18) (Roedel et al., 2012). In lung tissue collected from rats exposed to sW/Ni/Co (92/5/3) via intratracheal instillation (i.e. they did not undergo the BAL procedure), significant differences in mRNA transcript expression were only observed for two genes, with increased expression of Il6 and decreased expression of cytochrome P450, family 2, subfamily A, polypeptide 3 (CYP2A3)

Table 4.2 Microarray and omics results for multiple key characteristics after exposure to weapons-grade tungsten (with nickel and cobalt) alloy or the simulated alloy in human cells in vitro

End-point	Platform	Results	Relevant KCs[a]	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Expression of 13 stress gene promoter/ response elements	CAT-Tox (L) reporter assay (ELISA)	Increased induction of gene promoters related to metal sequestration (hMTIIA), oxidative stress and immune function (FOS and NFκBRE), DNA/protein damage (P53RE and HSP70), and the binding site for CREB (CRE)	KCs 2,3, 5, and 6	Human hepatoma, 13 HepG2-derived cell lines transfected with gene promoter-CAT fusion constructs (CAT-Tox (L) reporters)	sW/Ni/Co (92–5–3), 5–50 μg/mL, 48-h exposure	Cell viability was > 85% at ~5 μM. CREB is involved in the differentiation of T lymphocytes, among other functions.	Miller et al. (2004)

Table 4.2 (continued)

End-point	Platform	Results	Relevant KCs[a]	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Transcriptomics	Microarray slides (Agilent human gene expression microarray kit 4×44K; Design ID 026652)	WNiCo (91–6–3) induced decreased expression of mRNA transcripts encoding muscle-specific proteins and contractile fibres, components of the actin cytoskeleton, cell differentiation-associated proteins, and immunoglobulins WNiCo (91–6–3) and WNiCo (97–2–1) induced increased expression of mRNA transcripts encoding components of the glycolic and pentose phosphate pathways, response to hypoxia, immune response, cytokine–cytokine receptor interactions, anti-apoptotic pathways, wound healing, and regulation of cell proliferation WNiCo (91–6–3) induced increased expression of mRNA transcripts encoding components of the oxidative stress response (including NRF2), glutathione metabolic system, antioxidant enzymes (SOD1, SOD2, PRDX1, SRXN1, and NQO1), pro-inflammatory response, and angiogenesis (VEGFA)	KCs 5, 6, 7, and 10	Primary human skeletal muscle, HSkMC cells	WNiCo (91–6–3), WNiCo (97–2–1), ~207 mm^2 particle surface area per 75 cm^2 tissue culture flask growth area, 24-h exposure	The effects of WNiCo (91–6–3) and WNiCo (97–2–1) were similar qualitatively, but exposure to WNiCo (97–2–1) altered the expression of fewer genes. WNiCo (91–6–3) was stored for 7, 39, or 67 mo before testing. [The Working Group noted that storage conditions were not provided.] Longer storage time caused a decrease in the number of genes with altered expression. WNiCo (97–2–1) was not stored.	Harris et al. (2015)

CAT-Tox (L), chloramphenicol acetyl transferase-toxicity (L)iver; Co, cobalt; CREB (CRE), cAMP response element; ELISA, enzyme-linked immunosorbent assay; FOS, Fos proto-oncogene, AP-1 transcription factor subunit; hMTIIA, human metallothioneinIIA; KC, key characteristic; mo, month; mRNA, messenger RNA; NFκBRE, nuclear factor kappa (B site) response element; Ni, nickel; p53RE, 53 kDa protein tumour suppressor response element; PRDX1, peroxiredoxin; SOD, superoxide dismutase; SRXN1, sulfiredoxin; sW/Ni/Co, simulated weapons-grade tungsten (with nickel and cobalt) alloy (followed by the percentage of each metal by weight); VEGFA, vascular endothelial growth factor; W, tungsten; WNiCo, weapons-grade tungsten (with nickel and cobalt) alloy (followed by the percentage of each metal by weight).

[a] Key characteristic 2, is genotoxic; key characteristic 3, alters DNA repair or causes genomic instability; key characteristic 5, induces oxidative stress; key characteristic 6, induces chronic inflammation; key characteristic 7, is immunosuppressive; key characteristic 10, alters cell proliferation, cell death, or nutrient supply.

Table 4.3 Microarray and omics results for multiple key characteristics after exposure to weapons-grade tungsten (with nickel and cobalt) alloy or the simulated alloy in non-human mammals in vivo and non-human mammalian cells in vitro

End-point	Platform	Results	Relevant KCs[a]	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Gene expression	RT-PCR array (Rat Stress and Toxicity PathwayFinder RT^2 Profiler PCR Array (84 genes), QIAGEN)	BAL cells with altered expression of mRNAs encoding proteins related to apoptosis signalling (↑ Nfkbia and ↓ Casp1), growth arrest and senescence (↑ Cdkn1a and Ddit3), heat shock (↑ Hspa1a), inflammation (↑ Il6, Mip-1a, Mip-1b, GM-CSF, Il1b, Tnfb, and Nos2/iNOS, and ↓ Il18), and cell proliferation (↓ E2f1) Lung tissue with 2/84 genes assayed with differential expression (↑ Il6 and ↓ CYP2A3)	KCs 5, 6, and 10	Primary BAL cells and lung tissue	sW/Ni/Co (92–5–3), 20 mg/kg bw, intratracheal instillation of male SD rats, 24-h exposure		Roedel et al. (2012)
Metabolomics	^{1}H NMR spectroscopy	↓ NMN (lower dose at 8 and 24 h), ↑ NMN (higher dose at 8, 24, and 72 h), ↑ choline (lower dose at 8 h, higher dose at 24, 72, and 120 h), ↑ creatinine (lower dose at 8 and 24 h, higher dose at 24 and 72 h), and ↓ gut flora metabolite (hippurate) (lower dose at 72 h, higher dose at 24, 72, and 120 h) Altered metabolic pathways (at 24 h) were TCA cycle; alanine, aspartate, and glutamate (amino acid) metabolism; butanoate metabolism; and glyoxylate and dicarboxylate (carbohydrate) metabolism	KCs 5 and 10	Urine	sW/Ni/Co (91/5/4), 0.5 mL intraperitoneal injection of male SD rats (lower and higher dose), 8, 24, 72, and 120-h exposure	The Working Group noted that NMN changes may be due to oxidative stress in the liver. Lower dose was one tenth of LD_{50} per kg bw. Higher dose was two fifths to four fifths of the LD_{50} per kg bw.	Tyagi et al. (2014)

Table 4.3 (continued)

End-point	Platform	Results	Relevant KCs[a]	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Transcriptomics	Microarray (Rat Genome 230 2.0 whole-genome oligonucleotide arrays, Affymetrix, Inc.)	↑ Expression of cell cycle-related genes; ↓ expression of muscle development and differentiation genes Top 3 KEGG pathways altered (adherens junction, p53 signalling pathway, and cell cycle) ↑ Expression of genes common to cancer including sarcoma (CCND1, CDKN2A, MDM2, CDK4, and MAPK3) ↑ Expression of genes on chromosomal cytoband 7q22 including MDM2 (binds/inhibits p53), CDK4 (G1 phase progression), WiF1 (inhibits Wnt signalling), OS9 (amplified in osteosarcoma), SLC35E3, and XRCC6 (DNA repair double-strand breaks)	KCs 3 and 10	Tumours (rhabdomycosarcomas)	Intramuscular WNiCo (91–6–3), 4 pellets per leg + 6 Ta pellets per leg (10 pellets per leg total) per male F344 rat		Schuster et al. (2012)

Table 4.3 (continued)

End-point	Platform	Results	Relevant KCs[a]	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Transcriptomics	Microarray slides (prepared from a set of rat AROS V3.0 oligonucleotides (Eurofins MWG Operon, Alabama, USA) by the Functional Genomics Facility at the School of Biosciences, University of Birmingham, UK)	5 functional areas affected WNiCo (9–6–3) altered 4 areas (induced ↓ expression of mRNA transcripts encoding components of muscle structure/function; induced ↑ expression of mRNA transcripts encoding protein components of carbohydrate metabolism/glycolysis, DNA damage/stress response, and apoptosis/cell death) WNiCo (97–2–1) altered 3 areas (induced ↓ expression of mRNA transcripts encoding protein components of muscle structure/function; induced ↑ expression of mRNA transcripts encoding proteins involved in DNA organization/regulation and carbohydrate metabolism/glycolysis)	KCs 2,3, 4, 5, and 10	Rat skeletal muscle myoblasts, L6-C11 cell line	WNiCo (91–6–3) and WNiCo (97–2–1), "Small quantities of particles" with ~207 mm^2 particle surface area in 0.5 mL volume, 24- or 48-h exposure	No clear description of concentrations used was reported. No quantitative measurements of cell viability reported.	Harris et al. (2011)

Table 4.3 (continued)

End-point	Platform	Results	Relevant KCs	Tissue, cell type or line	Test article, exposure concentration or range, and duration	Comments	Reference
Transcriptomics	Microarray (RatRef-12 v1.0 Expression and MouseRef-8 v2.0 Expression BeadChips, Illumina); qRT-PCR for validation of selected genes	↑ FN3K expression, significantly altered compared with control (Ta) exposure in rat L6-C11 cells ↑ Fos expression, but not significantly altered compared with control.	KCs 5 and 10	Rat skeletal muscle myoblasts, L6-C11 cell line Mouse skeletal muscle myoblasts, C2C12 cell line	sW/Ni/Co (91/6/3), 10 µg/mL, 24-h exposure		Bardack et al. (2014)

↓, decreased; ↑, increased; BAL, bronchoalveolar lavage; bw, body weight; Co, cobalt; CYP2A3, cytochrome P450, family 2, subfamily A, polypeptide 3; ^{1}H NMR, ^{1}H nuclear magnetic resonance; KC, key characteristic; KEGG, Kyoto Encyclopaedia of Genes and Genomes; LD_{50}, median lethal dose; mRNA, messenger RNA; Ni, nickel; NMN, *N*-methyl nicotinamide; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; SD, Sprague-Dawley; sW/Ni/Co, simulated weapons-grade tungsten (with nickel and cobalt) alloy; Ta, tantalum; TCA, tricarboxylic acid; W, tungsten.

[a] Key characteristic 2, is genotoxic; key characteristic 3, alters DNA repair or causes genomic instability; key characteristic 4, induces epigenetic alterations; key characteristic 5, induces oxidative stress; key characteristic 6, induces chronic inflammation; key characteristic 7, is immunosuppressive; key characteristic 10, alters cell proliferation, cell death, or nutrient supply.

(Roedel et al., 2012). Histological assessment of protein expression in lung tissue by immunohistochemical analysis also showed that CYP2A3 protein expression was decreased. [The Working Group noted that differences in CYP2A3 mRNA expression are related to metabolism but are unlikely to be related to oxidative stress.]

Metabolomics analysis of urine samples collected from rats after intraperitoneal injection of sW/Ni/Co (91/5/4) (see Section 4.2.2 for doses) showed alterations in nutrient supply (Tyagi et al., 2014). Significant changes in energy metabolism (including the tricarboxylic acid cycle, amino acid metabolism, and carbohydrate metabolism) were observed in the urinary metabolomics profile 24 hours after exposure.

Schuster et al. (2012) performed microarray analysis to identify altered mRNA transcript expression in rhabdomyosarcomas from male Fischer 344 rats exposed to WNiCo (91–6–3) pellets by intramuscular implantation. Exposure to WNiCo (91–6–3) caused a significant increase in the expression of cell cycle-related genes and decreased expression of genes involved in muscle development and differentiation (see Table 4.3).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

As in the study by Harris et al. (2015) that used human primary HSkMC cells, transcriptomics analysis of rat L6.C11 cells treated with WNiCo (97–2–1) or WNiCo (91–6–3) showed increased carbohydrate metabolism (of enriched functional groups), and increased expression of mRNA transcripts associated with glycolysis and response to hypoxia (Harris et al., 2011). [The Working Group noted that this suggested that WGTA drives cells towards anaerobic glycolysis, also known as the Warburg effect.] Exposure to WNiCo (97–2–1) and WNiCo (91–6–3) also caused a significant decrease in the expression of mRNA transcripts of genes related to cell differentiation, specifically genes involved in muscle structure and function (Harris et al., 2011). [The Working Group noted that exposure to WGTA caused rhabdomyosarcoma in both mice (Emond et al., 2015a) and rats (Kalinich et al., 2005; Schuster et al., 2012) (see Section 3), and that evidence of blocked differentiation of muscle cells was observed in rhabdomyosarcoma in humans (Sirri et al., 2003).] In addition, exposure of rat L6.C11 cells to WNiCo (91–6–3), WNiCo (97–2–1), and WNiFe (97–2–1) caused a significant decrease in (pro-apoptotic) caspase-3 activity after 24 hours (Harris et al., 2011, 2015), but this was not observed in human primary HSkMC cells (Harris et al., 2015). [The Working Group noted possible species differences since human cells were less sensitive to the effects of WNiCo (91–6–3) than rat L6.C11 cells on the basis of caspase-3 activity and the number of genes with altered expression as reported in Harris et al. (2011), to which the authors refer.] Increased glycolysis was suggested by a significant increase in the expression of fructosamine 3 kinase (Fn3k) mRNA transcripts in rat L6.C11 cells treated with sW/Ni/Co (91/6/3) at 10 µg/mL for 24 hours, as assessed by microarray analysis and quantitative real-time polymerase chain reaction validation (Bardack et al., 2014). Fn3k can de-glycate Nrf2 (also known as Nfe2l2), which activates its oncogenic function, although how glycation affects the function of other Fn3k-sensitive proteins (e.g. translation factors, heat shock proteins, and histones) is not clear (Sanghvi et al., 2019). Fos expression, which is relevant to oxidative stress, was also increased in rat L6.C11 cells exposed to sW/Ni/Co (91/6/3) in vitro, but was not significantly different compared with controls exposed to tantalum, which is inert (Bardack et al., 2014).

Adams et al. (2015) treated rat pheochromocytoma (PC-12) cells with sW/Ni/Co (55/33/17), which was composed of a mixture of soluble ionic metal salts (from sodium tungstate, nickel acetate, and cobalt acetate) based on their predominant oxidation states and metal ratios in the leachate of WNiCo (91–6–3) alloy, in cell culture media for 24 hours. Microarray experiments using

Affymetrix GeneChip Rat Genome 230 2.0 chips showed that expression of only a few mRNA transcripts was altered by exposure to sW/Ni/Co (55/33/17) at concentrations of ~3 μg/mL for tungsten, 2 μg/mL for nickel, and 1 μg/mL for cobalt. Pathway enrichment analysis could not be performed because too few genes had altered expression.

4.2.5 Other adverse effects

Although not directly measured, the gut microbiome is a potential target of sW/Ni/Co. Exposure of rats to sW/Ni/Co (91/5/4) by intraperitoneal injection caused significant alterations in gut flora metabolites (decreased hippurate) and butanoate metabolism, as assessed by profiling of urinary metabolites (Tyagi et al., 2014). Butanoate metabolites include short-chain fatty acids and alcohols, which are typically produced by fermentation in the intestine. Both nickel and cobalt have been shown to alter the diversity and composition of the gut microbiome in rats exposed to these metals by gavage (Richardson et al., 2018).

5. Summary of Data Reported

5.1 Exposure characterization

Weapons-grade tungsten (with nickel and cobalt) alloys (WGTAs) typically contain 91–93% tungsten, 3–5% nickel, and 2–4% cobalt. During the 1990s, the search for alternatives to lead and depleted uranium resulted in the investigation and use of these tungsten alloys in kinetic energy penetrators, guided missiles, and other types of armour-piercing munitions.

Inhalation is probably the primary route of exposure for occupationally exposed populations. Occupational exposure to the elements contained in WGTA may occur during the manufacture and production of munitions. In addition, military personnel and civilians can be exposed to metal aerosols generated during the firing of WGTA munitions and on impact with their targets. Individuals with ammunition-related injuries may have retained embedded fragments containing the WGTA, which may lead to long-term exposure to metal ions released from the fragments. There are few studies in the published literature that quantify exposures for these different scenarios.

No regulations were identified to date concerning exposure to WGTAs; however, exposure limits and guidelines for its individual elemental constituents (tungsten, nickel, and cobalt) do exist.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with WGTA caused an increase in the incidence of malignant neoplasms in two species.

WGTA administered by intramuscular implantation in one study in male B6C3F$_1$ mice caused an increase in the incidence of rhabdomyosarcoma at the implantation site.

In two studies, WGTA administered by intramuscular implantation in male Fischer 344 rats caused an increase in the incidence of rhabdomyosarcoma at the implantation site.

5.4 Mechanistic evidence

Data on absorption, distribution, and excretion were available from studies on intramuscular implantation of WGTA pellets in rodents. Tungsten, nickel, and cobalt in the implanted WGTA were absorbed, distributed systemically (including to the brain and testes), and excreted in the urine. Concentrations of these metals

were higher in the kidney, spleen, and liver than in other tissues. Tungsten concentrations in the tissues and urine increased over the course of 1, 6, and 12 months after implantation, while nickel and cobalt tissue concentrations stabilized over 1 to 6 months, with noticeable increases in cobalt concentrations in the liver and nickel in the brain. Nickel and cobalt were shown to be more readily released from WGTA than was tungsten.

Overall, the mechanistic evidence is suggestive but sparse, based on only a few available studies in experimental systems. There was one study in exposed humans that was uninformative to the evaluation because the exposure was not to WGTA. Evidence was suggestive for the key characteristics "is genotoxic", "induces epigenetic alterations", "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply" in experimental systems.

There is suggestive evidence that WGTA is genotoxic based on induction of DNA damage and micronucleus formation by WGTA and/or simulated WGTA (sW/Ni/Co) in vitro. WNiCo (91–6–3) and WNiCo (97–2–1) at non-cytotoxic concentrations increased the frequency of DNA strand breaks in human primary skeletal muscle cells in multiple experiments in one study. WNiCo (91–6–3), but not WNiCo (97–2–1), increased the frequency of DNA strand breaks in a rat skeletal muscle cell line (L6-C11) in one study. Furthermore, sW/Ni/Co (92/5/3) induced DNA strand breaks and micronucleus formation in a human osteosarcoma cell line (HOS) in multiple experiments in two studies. No genotoxicity studies were available in non-human mammals in vivo or in non-mammalian systems.

Multiple lines of evidence suggest that WGTA induces oxidative stress. WNiCo (91–6–3) and WNiCo (97–2–1) increased levels of reactive oxygen species in rat L6-C11 cells, and in vitro exposure to sW/Ni/Co (92/5/3) increased levels of reactive oxygen species and reactive nitrogen species in rat pulmonary macrophages. Intraperitoneal injection of rats with sW/Ni/Co (91/5/4) caused increased levels of urinary metabolites indirectly associated with oxidative stress. Additionally, sW/Ni/Co (92/5/3) increased protein levels of FOS and NFKB1 (also known as NFκBRE) in a human cell line (HepG2).

WGTA-induced epigenetic alterations are suggested on the basis of histone modifications in vitro in two studies. Exposure to WNiCo (91–6–3) decreased phosphorylation of serine 3 on histone 3 in mouse primary neural cells and myoblast (C2C12) cells, and decreased acetylation of histone 3 in C2C12 cells. Trimethylation of lysine 4 on histone 3 was unaffected in either cell type. In rat L6-C11 cells, WNiCo (91–6–3) increased messenger RNA (mRNA) levels of histone methylase, histone acetylase, and histone deacetylase, while WNiCo (97–2–1) increased mRNA levels of histone deacetylase only. No in vitro or in vivo studies regarding other epigenetic changes were available.

Indirect evidence suggests that WGTA alters cell proliferation, cell death, or nutrient supply. Transcriptomic data from human primary skeletal muscle cells treated with WNiCo (91–6–3) and WNiCo (97–2–1), and from BALF cells collected from rats instilled intratracheally with sW/Ni/Co (92/5/3), showed changes in mRNA levels associated with anti-apoptosis, decreased wound healing, regulation of cell proliferation, differentiation, and altered cell growth arrest and senescence. WNiCo (91–6–3) and WNiCo (97–2–1) both decreased caspase 3 activity in rat cells, but not in human primary skeletal muscle cells, which is consistent with the gene expression changes indicating an anti-apoptotic effect. Altered nutrient supply was suggested on the basis of increased mRNA levels associated with glycolysis and angiogenesis in rat BALF cells, and changes in several metabolic pathways in the urine of rats injected intraperitoneally with sW/Ni/Co (91/5/4).

Indirect evidence suggests that WGTA is immunosuppressive. Thymus-to-body-weight ratio

Swas decreased in rats 3 months after intramuscular implantation of WNiCo (91–6–3). The phagocytic capacity of rat pulmonary macrophages was decreased by in vitro exposure to sW/Ni/Co (92/5/3). In addition, WNiCo (91–6–3) decreased mRNA levels of genes related to the immune response in human primary skeletal muscle cells.

WGTA caused immortalization, but only sW/Ni/Co was tested and not the alloy. Untreated immortalized HOS cells were non-tumourigenic in nude mice. However, HOS cells exposed to non-cytotoxic concentrations of sW/Ni/Co (92/5/3) for 24 hours became capable of anchorage-independent growth in soft agar and invasion into Matrigel, indicating neoplastic transformation. Injection of the transformed HOS cells into athymic nude mice caused adenocarcinomas, confirming that the cells were tumourigenic.

The effects of WGTA with regard to chronic inflammation are unclear. Studies with WNiCo (in rats) and sW/Ni/Co (in human cell lines and rats in vivo) showed evidence of pro-inflammatory responses, including increased peripheral neutrophils, lymphocytes, monocytes, and eosinophils in chronically exposed rats, but these findings are not necessarily associated with chronic tissue inflammation. Regarding the key characteristics "is electrophilic or can be metabolically activated to an electrophile", "alters DNA repair or causes genomic instability", or "modulates receptor-mediated effects", no data were available to the Working Group.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of weapons-grade tungsten (with nickel and cobalt) alloy.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of weapons-grade tungsten (with nickel and cobalt) alloy.

6.3 Mechanistic evidence

There is *limited evidence* that weapons-grade tungsten (with nickel and cobalt) alloy exhibits key characteristics of carcinogens.

6.4 Overall evaluation

Weapons-grade tungsten (with nickel and cobalt) alloy is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for weapons-grade tungsten (with nickel and cobalt) alloy is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* in experimental animals for the carcinogenicity of weapons-grade tungsten (with nickel and cobalt) alloy is based on an increase in the incidence of malignant neoplasms in two species. There is *limited evidence* in experimental systems that weapons-grade tungsten (with nickel and cobalt) alloy is genotoxic; causes epigenetic alterations; induces oxidative stress; is immunosuppressive; and alters cell proliferation, cell death, or nutrient supply. The evidence regarding cancer in humans is *inadequate* because no studies were available.

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LIST OF ABBREVIATIONS

AAS	atomic absorption spectrometry
ACB	albumin-cobalt-binding (assay)
ADM	adrenomedullin
AM	arithmetic mean
ARNT	aryl hydrocarbon receptor nuclear translocator
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BKMR	Bayesian kernel machine regression
BMI	body mass index
BrdU	5-bromo-2′-deoxyuridine
BSA	bovine serum albumin
bw	body weight
CAS	Chemical Abstracts Service
CAT	catalase
CI	confidence interval
CNS	central nervous system
CYP2A3	cytochrome P450, family 2, subfamily A, polypeptide 3
DEN	diethylnitrosamine
EGF	epidermal growth factor
ENU	ethylnitrosourea
EPO	erythropoietin
ER	estrogen receptor
ET	endothelin
ETAAS	electrothermal atomic absorption spectrometry
EU	European Union
e-waste	electronic and/or electrical waste
FAAS	flame atomic absorption spectrometry
FN3K	fructosamine 3 kinase
Fpg	formamidopyrimidine DNA glycosylate
FT4	free thyroxine
GLP	Good Laboratory Practice
GM	geometric mean
GPT	guanine phosphoribosyltransferase
GPX	glutathione peroxidase

GSD	geometric standard deviation
GSH	glutathione
GST	glutathione *S*-transferase
Hb	haemoglobin
HIF	hypoxia-inducible factor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMOX1	haem oxygenase 1
HMT	Harwell Mouth Tumour
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HR	hazard ratio
HSkMC	human skeletal muscle cells
HUVEC	human umbilical vein endothelial cell
8-I	8-isoprostane
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma-atomic emission spectroscopy
ICP-MS	inductively coupled plasma-mass spectrometry
Ig	immunoglobulin
INAA	instrumental neutron activation analysis
iNOS	inducible nitric oxide synthase
IQR	interquartile range
JEM	job-exposure matrix
LA-ICP-MS	laser ablation inductively coupled plasma-mass spectrometry
LASSO	least absolute shrinkage and selection operator
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LINE	long interspersed element
LOD	limit of detection
LOQ	limit of quantification
m^6A	methylation of the adenosine base at the N^6 position of mRNA
MDA	malondialdehyde
MDE	mean dietary exposure
MEF	mouse embryonic fibroblast
miRNA	microRNA
mLOY	mosaic loss of chromosome Y
MMAD	mass median aerodynamic diameter
MoM	metal-on-metal
mRNA	messenger RNA
NAC	*N*-acetylcysteine
NATA	National Air Toxics Assessment
NC	not calculated
NER	nucleotide excision repair
NF-κB	nuclear factor-kappa B
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NOS	not otherwise specified
NP	nanoparticle
NTP	National Toxicology Program
NTS	N-terminal binding site
OGG1	8-oxoguanine DNA glycosylase

8-OHdG	8-hydroxy-2′-deoxyguanosine
OR	odds ratio
8-oxo-dG	8-oxo-2′deoxyguanosine
8-oxo-dGTPase	8-oxo-2′-deoxyguanosine-5′-triphosphatase
OSHA	Occupational Safety and Health Administration
p53CON	p53 consensus binding site sequence
PAH	polycyclic aromatic hydrocarbon
PBL	peripheral blood leukocyte
PBMC	peripheral blood mononuclear cell
PCA	principal component analysis
PCE	polychromatic erythrocyte
PCNA	proliferating cell nuclear antigen
PCOS	polycystic ovary syndrome
PEG	primary exposure group
PEL	permissible exposure limit
PET	polyethylene terephthalate
PI3K	phosphatidylinositol 3-kinase
PM	particulate matter
PPAR	peroxisome proliferator-activated receptor
PR	progesterone receptor
ROS	reactive oxygen species
RR	relative risk
SD	standard deviation
SEM-EDXA	scanning electron microscopy with energy-dispersive X-ray analysis
SIK	spontaneously immortalized keratinocyte
SIR	standardized incidence ratio
SIRT1	sirtuin 1
SMR	standardized mortality ratio
SOD	superoxide dismutase
T_{90}	theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation
TAK1	TGFβ-activated kinase 1
TDI	tolerable daily intake
TDS	total diet study
TNFα	tumour necrosis factor alpha
TWA	time-weighted average
US EPA	United States Environmental Protection Agency
UV	ultraviolet
VEGF	vascular endothelial growth factor
WC-Co	cobalt with tungsten carbide
WGTA	weapons-grade tungsten (with nickel and cobalt) alloy
WHO	World Health Organization
WNiFe	weapons-grade tungsten (with nickel and iron) alloy
WQS	weighted quantile sum
XPA	xeroderma pigmentosum complementation group A
XPAzf	XPA zinc finger sequence

ANNEX 1. SUPPLEMENTARY MATERIAL FOR SECTION 1, EXPOSURE CHARACTERIZATION

These supplementary web-only tables are available from: https://www.publications.iarc.fr/618.

Please report any errors to imo@iarc.who.int.

Cobalt metal (without tungsten carbide) and some cobalt compounds

Table S1.3	Global production of cobalt from mining, by country
Table S1.4	Global production of cobalt in refineries, by country
Table S1.10	Distribution of air concentrations of cobalt in industrial sectors, 1996–2016, Italy
Table S1.11	Distribution of air concentrations of cobalt by occupational group in industrial sectors, 1996–2016, Italy
Table S1.15	Occupational exposure limits of cobalt and its compounds in different countries
Table S1.16	Environmental regulations and guidelines for cobalt
Table S1.17	Biomonitoring guidance and reference values for cobalt

The following tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited:

Table S1.18	Exposure assessment review and critique for epidemiological studies on cancer and exposure to cobalt metal (without tungsten carbide) and some cobalt compounds

Trivalent and pentavalent antimony

The following tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited.

Weapons-grade tungsten (with cobalt and nickel) alloy

This table was produced in draft form by the Working Group and was subsequently fact-checked but not edited.

ANNEX 2. SUPPLEMENTARY MATERIAL FOR SECTION 2, CANCER IN HUMANS

These supplementary web-only tables are available from: https://www.publications.iarc.fr/618.

Please report any errors to imo@iarc.who.int.

Cobalt metal (without tungsten carbide) and some cobalt compounds

Table S2.6 Epidemiological studies on cancer of other sites, including all sites combined, and exposure to cobalt

ANNEX 3. SUPPLEMENTARY MATERIAL FOR SECTION 4, MECHANISTIC EVIDENCE

These supplementary web-only tables are available from: https://www.publications.iarc.fr/618.

Please report any errors to imo@iarc.who.int.

Cobalt metal (without tungsten carbide) and some cobalt compounds

ANNEX 4. SUPPLEMENTARY MATERIAL FOR SECTION 4, EVALUATION OF HIGH-THROUGHPUT IN VITRO TOXICITY SCREENING DATA

These supplementary web-only tables (available from: https://www.publications.iarc.fr/618) contain summaries of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for those chemicals evaluated in the present volume that have been tested in high-throughput screening assays performed by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health. The results were generated by the Working Group using the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) available from https://gitlab.com/i1650/kc-hits.git (Reisfeld et al., 2022), using the US EPA Toxicity Forecaster (ToxCast) assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for *IARC Monographs* Volume 131. Data were available for cobalt metal (without tungsten carbide) and some cobalt compounds, and trivalent and pentavalent antimony, but not for weapons-grade tungsten (with cobalt and nickel) alloy.

Please report any errors to imo@iarc.who.int.

Cobalt metal (without tungsten carbide) and some cobalt compounds

1. Cobalt(II) sulfate heptahydrate: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens

Trivalent and pentavalent antimony

1. Acetic acid, antimony(III) salt: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens

2. Antimony potassium(III) tartrate trihydrate: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
3. Antimony(III) trichloride: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
4. Antimony(V) sulfide: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
5. Antimony(III) potassium tartrate hydrate: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
6. Triphenylstibine(III): ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens

Reference

Reisfeld B, de Conti A, El Ghissassi F, Benbrahim-Tallaa L, Gwinn W, Grosse Y, et al. (2022). kc-hits: a tool to aid in the evaluation and classification of chemical carcinogens. *Bioinformatics*. 38(10):2961–2. doi:10.1093/bioinformatics/btac189 PMID:35561175

SUMMARY OF FINAL EVALUATIONS

Summary of final evaluations for Volume 131

Agent	Evidence stream			Overall evaluation
	Cancer in humans	Cancer in experimental animals	Mechanistic evidence	
Cobalt metal (without tungsten carbide or other metal alloys)	*Inadequate*	*Sufficient*	*Strong*[c]	Group 2A
Soluble cobalt(II) salts	*Inadequate*	*Sufficient*[a] *Limited*[b]	*Strong*[c]	Group 2A
Cobalt(II) oxide	*Inadequate*	*Sufficient*	*Limited*	Group 2B
Cobalt(II,III) oxide	*Inadequate*	*Inadequate*	*Limited*	Group 3
Cobalt(II) sulfide	*Inadequate*	*Limited*	*Inadequate*[c]	Group 3
Other cobalt(II) compounds	*Inadequate*	*Inadequate*	*Inadequate*	Group 3
Trivalent antimony	*Limited*	*Sufficient*[c]	*Strong*	Group 2A
Pentavalent antimony	*Inadequate*	*Inadequate*	*Limited*	Group 3
Weapons-grade tungsten (with nickel and cobalt) alloy	*Inadequate*	*Sufficient*	*Limited*	Group 2B

[a] There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt(II) sulfate.
[b] There is *limited evidence* in experimental animals for the carcinogenicity of cobalt(II) chloride.
[c] There is *sufficient evidence* in experimental animals for the carcinogenicity of antimony trioxide.

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of nine agents: cobalt metal (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, cobalt(II) oxide, cobalt(II,III) oxide, cobalt(II) sulfide, other cobalt(II) compounds, trivalent antimony, pentavalent antimony, and weapons-grade tungsten (with nickel and cobalt) alloy.

Cobalt is used in the manufacture of cutting and grinding tools, in pigments, paints, coloured glass, medical implants, and electroplating, and in lithium-ion battery production. Occupational exposure is expected to occur during cobalt refining and production of cobalt compounds and dental materials, use of diamond–cobalt tools, plate painting with cobalt pigments, manufacture of nickel–hydrogen batteries, hard-metal production, and electronic-waste recycling. The general population is exposed via food, air, tobacco smoke, and medical implants.

Antimony is used in flame retardants, lead–acid batteries and alloys, plastics, brake pads, clutch discs, glass, ceramics, and as a primer in explosives. Some pentavalent antimony compounds are used to treat leishmaniasis. Workers can be exposed during smelting, manufacture of antimony compounds, glass, textiles, and batteries, and electrical-waste processing. Non-occupational exposures occur via water, air, soil, consumer products, and tobacco.

Weapons-grade tungsten (with nickel and cobalt) alloy is used in armour-penetrating munitions. Occupational exposure can occur during munitions production, and military personnel and civilians can be exposed to metal aerosols generated during firing or impact, or via injuries with retained embedded fragments.

An *IARC Monographs* Working Group reviewed evidence from cancer studies in humans (available mainly for cobalt and antimony), cancer bioassays in experimental animals, and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- Cobalt metal (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, and trivalent antimony are *probably carcinogenic to humans (Group 2A)*;
- Cobalt(II) oxide and weapons-grade tungsten (with nickel and cobalt) alloy are *possibly carcinogenic to humans (Group 2B)*;
- Cobalt(II,III) oxide, cobalt(II) sulfide, other cobalt(II) compounds, and pentavalent antimony were each evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)*.

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