National Toxicology Program
U.S. Department of Health and Human Services

Report on Carcinogens

Monograph on Cobalt and Cobalt Compounds That Release Cobalt Ions In Vivo

RoC MONOGRAPH 06

APRIL 2016

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Report on Carcinogens Monograph series began in 2012. Report on Carcinogens Monographs present the cancer hazard evaluations of environmental agents, substances, mixtures, or exposure circumstances (collectively referred to as "substances") under review for the Report on Carcinogens. The Report on Carcinogens is a congressionally mandated, science-based, public health document that provides a cumulative list of substances that pose a cancer hazard for people in the United States. Substances are reviewed for the Report on Carcinogens to (1) be a new listing, (2) reclassify the current listing status, or (3) be removed.

NTP evaluates cancer hazards by following a multistep process and using established criteria to review and integrate the scientific evidence from published human, experimental animal, and mechanistic studies. General instructions for the systematic review and evidence integration methods used in these evaluations are provided in the *Handbook for the Preparation of Report on Carcinogens Monographs*. The handbook's instructions are applied to a specific evaluation via a written protocol. The evaluation's approach as outlined in the protocol is guided by the nature, extent, and complexity of the published scientific information and tailored to address the key scientific issues and questions for determining whether the substance is a potential cancer hazard and should be listed in the Report on Carcinogens. Draft monographs undergo external peer review before they are finalized and published.

The Report on Carcinogens Monographs are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these evaluations are included in the Health Assessment and Workspace Collaborative. Information about the Report on Carcinogens is also available on the NTP website.

For questions about the monographs, please email NTP or call 984-287-3211.

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This report has been reformatted to meet new NTP publishing requirements; its content has not changed. The proposed substance profile is no longer part of the document because it is published in the 14th Report on Carcinogens.

About This Report

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Peer Review

Peer review of the Draft RoC Monograph on Cobalt and Certain Cobalt Compounds[1] was conducted by an ad hoc expert panel at a public meeting held July 22, 2015, in the Rodbell Auditorium at the National Institute of Environmental Health Sciences, David P. Rall Building, Research Triangle Park, NC (see http://ntp.niehs.nih.gov/go/38854 for materials, minutes, and panel recommendations from meeting). The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists, not as representatives of any institution, company, or governmental agency.

The charge to the Peer-Review Panel was as follows:

- Comment on the draft cancer evaluation component for cobalt and certain cobalt compounds, specifically, whether it was technically correct and clearly stated, whether the NTP had objectively presented and assessed the scientific evidence, and whether the scientific evidence was adequate for applying the RoC listing criteria.
- Comment on the draft profile for cobalt and certain cobalt compounds, specifically, whether the scientific justification presented in the profile supported the NTP's preliminary policy decision on the RoC listing status of the substance (available in the 14th edition of the Report on Carcinogens).

The Panel was asked to vote on the following questions:

- Whether the scientific information presented from human cancer studies supported the NTP's preliminary level of evidence conclusion of cobalt and cobalt compounds that release cobalt ions in vivo.[1]
- Whether the scientific information presented from studies in experimental animals supported the NTP's preliminary level of conclusion of cobalt and cobalt compounds that release cobalt ions in vivo.[1]
- Whether NTP's preliminary policy decision for 'cobalt and cobalt compounds that release cobalt ions in vivo'[1] in the RoC.

The RoC Monograph on Cobalt and Cobalt Compounds That Release Cobalt Ions In vivo has been revised based on NTP's review of the Panel's peer-review comments. The Peer-Review Panel Report, which captures the Panel recommendations for listing status of cobalt and cobalt compounds that release cobalt ions in vivo in the RoC and their scientific comments, and the NTP Response to the Peer-Review Report are available on the Peer-Review Meeting webpage for cobalt and certain cobalt compounds (http://ntp.niehs.nih.gov/go/38854).

[1]During the meeting the Panel recommended using the definition of "certain cobalt compounds," i.e., "cobalt compounds that release cobalt ions in vivo" in the listing rather than the word "certain."

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Abstract

Introduction: Cobalt is a transition element and a component of more than 70 naturally occurring minerals. It is often combined with other metals to make metal alloys or cemented carbides for a variety of medical or commercial applications; some cobalt compounds are used as pigments or in electronic devices. People can be exposed to cobalt or its compounds in workplaces that use or produce cobalt, from cobalt-containing orthopedic joint replacements that release cobalt ions from wear and tear, and from the environment. Exposure to cobalt from food and water is generally limited.

Methods: The National Toxicology Program (NTP) evaluated evidence for human exposure, cancer studies in humans and experimental animals, mechanisms of carcinogenesis, and other relevant information, including evaluating study quality, integrating the evidence across studies, and integrating evidence across data streams (mechanistic, animal, and human data). Using established criteria, NTP reached conclusions on the strength of evidence for the carcinogenicity of cobalt from cancer studies in experimental animals and humans, and the final listing recommendation was reached by applying the Report on Carcinogens (RoC) listing criteria to the body of evidence.

Results and Discussion: The physicochemical properties, toxicokinetics, mechanistic data, and other relevant data for cobalt and cobalt compounds were used to identify and compare the chemical and biological properties and events that are relevant to cobalt-induced carcinogenicity to determine if a group listing for cobalt and cobalt compounds that release cobalt ions in vivo was warranted.

Cancer studies in experimental animals: NTP concluded that there was sufficient evidence of carcinogenicity in animals based on its review of 16 rodent carcinogenicity studies. Exposure of experimental animals to cobalt metal or cobalt compounds (both water-soluble and poorly water-soluble compounds) caused tumors in rats and/or mice through several different routes of exposure and at several different tissue sites. Inhalation exposure to cobalt metal or cobalt sulfate caused lung tumors in rats and mice as well as tumors of the pancreas (male rats only), the adrenal gland (male and female rats for cobalt metal and female rats for cobalt sulfate), and the hematopoietic system (female rats exposed to cobalt metal only). Intratracheal instillation of cobalt oxide also caused lung tumors in rats. In addition, local injection of rats with cobalt or cobalt compounds at various anatomic locations caused tumors at the injection sites, including intraperitoneal or intramuscular injection of poorly water-soluble cobalt oxide, subcutaneous injection of water-soluble cobalt chloride, and intramuscular or intrathoracic injection of cobalt metal or nanoparticles.

Mechanistic data: The key events related to toxicity and carcinogenicity of cobalt and cobalt compounds are thought to include cellular uptake of cobalt, intracellular release of cobalt ions from particles, and immediate and downstream biological responses related to the proposed modes of action. These events are applicable to all cobalt forms that release cobalt ions in vivo, including water-soluble and poorly water-soluble particles. The biological responses include inhibition of DNA repair, genotoxicity, generation of reactive oxygen species resulting in oxidative damage, and stabilization of hypoxia-inducible factor 1α, a protein that increases the expression of genes that promote survival of cells that receive less oxygen.

Human cancer studies: NTP concluded that the data available from studies in humans were inadequate to evaluate the relationship between human cancer and exposure to cobalt and cobalt compounds that release cobalt ions in vivo. Although increased risks of lung cancer were found in most of the five cohort studies, it is unclear that the excess risks were due to exposure specifically to cobalt, because of potential confounding from exposure to known carcinogens or other study limitations.

NTP Hazard Conclusion and Significance: The conclusion of the cancer hazard evaluation was that cobalt and cobalt compounds that release cobalt ions in vivo should be listed as *reasonably anticipated to be human carcinogens* in the RoC. The Secretary of Health and Human Services approved the listing of cobalt compounds that release cobalt ions in vivo in the 14th RoC. The rationale for the listing was sufficient evidence of carcinogenicity from experimental animals and evidence from studies on mechanisms of carcinogenesis that indicate that the release of cobalt ions is a key event for cobalt-induced carcinogenicity.

Introduction and Methods

Cobalt is a naturally occurring element that is present in several different forms. Elemental cobalt is a hard, silvery grey metal that can combine with other elements, e.g., with oxygen (cobalt oxide), sulfur (cobalt sulfate) or arsenic (cobalt arsenide). The most common oxidation states of cobalt are +2 and +3; for most simple cobalt compounds, the valence is +2, designated as cobalt(II). Cobalt compounds can be organic or inorganic as well as water-soluble or -insoluble. Cobalt compounds are used in a variety of industrial applications and as a colorant for glass, ceramics, and paint, and as catalysts, as driers for inks and paints, and in feed supplements and batteries. Cobalt is used in alloys or composites, such as cobalt-tungsten carbide, and in cobalt-containing prosthetics. Cobalt nanoparticles are used in medical tests and treatments as well as in the textile and electronics industries.

Cobalt and cobalt compounds that release cobalt ions in vivo (collectively referred to as cobalt) was selected for review for possible listing in the Report on Carcinogens (RoC) based on evidence of widespread exposure and an adequate database of cancer studies to evaluate the potential carcinogenicity of cobalt. The listing does not include cobalt as part of the vitamin B_{12} molecule because of the stability of that molecule in biological fluids. Cancer and toxicological studies of forms of cobalt that have confounding exposures, such as cobalt alloys and radioactive forms of cobalt, were not included in the review of the cobalt compounds. Two cobalt-containing substances, 'cobalt sulfate' and 'cobalt-tungsten carbide: powders and hard metals,' are currently listed in the Report on Carcinogens (RoC) as *reasonably anticipated to be human carcinogens* (NTP 2014a; 2014c). Cobalt sulfate, which has been listed since 2004 based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP 2002), is included in the current review of cobalt as a class. Cobalt-tungsten carbide: powders and hard metals, which was first listed in 2011 based on limited evidence of carcinogenicity from studies in humans and supporting evidence from studies on mechanisms of carcinogenesis (NTP 2009) falls outside the review.

Monograph Contents

This RoC monograph on cobalt consists of the following components: (Part 1) the cancer evaluation component that reviews the relevant scientific information and assesses its quality, applies the RoC listing criteria to the scientific information, and recommends an RoC listing status for cobalt, and (Part 2), the draft substance or cancer hazard profile containing the NTP's preliminary listing recommendation, a summary of the scientific evidence considered key to reaching that recommendation, and data on properties, use, production, exposure, and Federal regulations and guidelines to reduce exposure to cobalt and cobalt compounds and cobalt compounds that release cobalt ions in vivo.

The methods for preparing the RoC monograph on cobalt are described in the "Cobalt Protocol" (NTP 2014b). The cancer evaluation component for cobalt provides information on the following topics that are relevant to understanding the relationship between exposure to cobalt compounds and cancer: Introduction and properties (Section 1), human exposure (Section 2), disposition and toxicokinetics (Section 3), human cancer studies (Section 4), studies in experimental animals (Section 5), mechanisms and other relevant effects (Section 6), and an overall cancer evaluation that provides a synthesis of Sections 1 through 6 and rationale for listing cobalt and cobalt

compounds and cobalt compounds that release cobalt ions in vivo as a class (Section 7). The information reviewed in Sections 3 through 7 (except for information on exposure and properties) must come from publicly available, peer-reviewed sources. The appendices in the RoC Monograph contain important supplementary information, including the literature search strategy, exposure-related information and regulations, clinical study description and/or quality tables for cancer studies in humans or experimental animals, and a discussion of the results from the genotoxicity studies.

Process for Preparation of the Cancer Hazard Evaluation Component

The process for preparing the cancer evaluation component of the monograph included approaches for obtaining public and scientific input and using systematic methods (e.g., standardized methods for identifying the literature [see Appendix A], inclusion/exclusion criteria, extraction of data and evaluation of study quality using specific guidelines, and assessment of the level of evidence for carcinogenicity using established criteria). [Links are provided within the document to the appendices, and specific tables or sections can be selected from the table of contents.]

The Office of the Report on Carcinogens (ORoC) followed the approaches outlined in the concept document, which discusses the scientific issues and questions relevant to the evaluation of the carcinogenicity of cobalt compounds, the scope and focus of the monograph, and the approaches to obtain scientific and public input to address the key scientific questions and issues for preparing the cancer evaluation component of the monograph. The ORoC presented the concept document for cobalt to the NTP Board of Scientific Counselors (BSC) at the April 17, 2014 meeting, which provided opportunity for written and oral public comments, after which the concept was finalized and cobalt was approved by the NTP Director as a candidate substance for review. The concept document is available on the RoC website (http://ntp.niehs.nih.gov/go/730697).

Key Scientific Questions and Issues Relevant for the Cancer Evaluation

The scientific issues in this review concern the evaluation of the topics mentioned earlier, including human exposure, disposition and toxicokinetics, cancer studies in humans and experimental animals, and mechanistic data. The key questions for each topic are as follows:

Questions Related to the Evaluation of Human Exposure Information

- How are people in the United States exposed to cobalt?
- How do we measure exposure?
- What are the non-occupational sources and levels of exposure?
- What are the occupational settings and levels of exposure?
- Has exposure changed over time?
- What federal regulations and guidelines limit exposure to cobalt?
- Are a significant number of people residing in the United States exposed to cobalt?

Questions Related to the Evaluation of Disposition and Toxicokinetics

- How is cobalt absorbed, distributed, metabolized, and excreted (ADME)?
- What, if any, are the qualitative and/or quantitative species or sex differences for ADME?
- What is known about the form of cobalt (particulate, ion) from ADME studies in exposed tissue, particularly in the lung?
- How can toxicokinetic models (if any) inform biological plausibility, interspecies extrapolation, or other mechanistic questions for cobalt?

Questions Related to the Evaluation of Human Cancer Studies

- Which epidemiologic studies should be included in the review?
- What are the methodological strengths and limitations of these studies?
- What are the potential confounders for cancer risk for the tumor sites of interest in these studies?
- Is there a credible association between exposure to cobalt and cancer?
- If so, can the relationship between cancer endpoints and exposure to cobalt be explained by chance, bias, or confounding?

Questions Related to the Evaluation of Cancer Studies in Experimental Animals

- What is the level of evidence (sufficient or not sufficient) of carcinogenicity of cobalt from animal studies?
- What are the methodological strengths and limitations of the studies?
- What are the tissue sites?

Questions Related to the Evaluation of Mechanistic Data and Other Relevant Data

- What are the genotoxic effects due to cobalt exposure? Does genotoxicity vary by cobalt compound?
- What are the cytotoxic or toxic effects of cobalt exposure? Does cytotoxicity or toxicity vary by cobalt compound?
- What are the major mechanistic modes of action for the carcinogenicity of cobalt?
    - What are the common key steps or mode(s) of action of toxicity or carcinogenicity across different cobalt compounds? What role and contribution does cobalt ion play in the proposed mechanism? What are the effects from exposure to particulate cobalt?
    - What factors influence biological or carcinogenic effects? How do particle size, solubility, and cellular uptake of a cobalt compound affect biological or carcinogenic effects?
    - Is there evidence that supports grouping cobalt and cobalt compounds that release cobalt ions in vivo together in the assessment?

Approach for Obtaining Scientific and Public Input

To help address the approach to identify a common mode of action involving the cobalt ion for cobalt compounds, additional scientific input was requested early in the review process to define the scope of the review, i.e., what cobalt compound(s) could reasonably be included in this evaluation? Based on input from several scientific experts at a Cobalt Information Group Meeting convened at NIEHS on October 7, 2014, the scope of the evaluation was recommended to include cobalt and cobalt compounds that release cobalt ions in vivo. Technical advisors for the review of cobalt are identified on the "CONTRIBUTORS" page.

Public comments on scientific issues were requested at several times prior to the development of the RoC monograph, including the request for information on the nomination, and the request for comment on the draft concept document, which outlined the rationale and approach for conducting the scientific review. In addition, the NTP posted its protocol for preparing the draft RoC monograph on cobalt for public input on the ORoC webpage for cobalt (http://ntp.niehs.nih.gov/go/730697) prior to the release of the draft monograph. Seven written public comments on cobalt have been received from the public as of the date on this document.

Methods for Writing the Cancer Evaluation Component of the Monograph

The procedures by which relevant literature was identified, data were systematically extracted and summarized, and the monograph was written, together with the processes for scientific review, quality assurance, and assessment and synthesis of data, are described below.

The preparation of the RoC monograph for cobalt began with development of a literature search strategy to obtain information relevant to the topics listed above for Sections 1 through 6 using search terms developed in collaboration with a reference librarian (see Protocol). The approximately 7,500 citations identified from these searches were uploaded to web-based systematic review software for evaluation by two separate reviewers using inclusion/exclusion criteria, and 484 references were selected for final inclusion in the monograph using these criteria.

Information for the relevant cancer and mechanistic sections was systematically extracted in tabular format and/or summarized in the text from studies selected for inclusion in the monograph. All sections of the monograph underwent scientific review and quality assurance (QA, i.e., assuring that all the relevant data and factual information extracted from the publications have been reported accurately) by a separate reviewer. Any discrepancies between the writer and the reviewer were resolved by mutual discussion in reference to the original data source.

Strengths, weaknesses, and study quality of the cancer studies for cobalt compounds in humans (see Appendix C) and experimental animals (see Appendix D) were assessed based on a series of a priori considerations (questions and guidelines for answering the questions), which are available in the protocol (available at http://ntp.niehs.nih.gov/go/730697). Two reviewers evaluated the quality of each study. Any disagreements between the two reviewers were resolved by mutual discussion or consultation with a third reviewer in reference to the original data source. Relevant genotoxicity and mechanistic studies were also assessed for their strengths and weaknesses.

RoC listing criteria (see text box) were applied to the available database of carcinogenicity data to assess the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of cobalt from studies in humans and the level of evidence (sufficient, not sufficient) from studies in experimental animals. The approach for synthesizing the evidence across studies and reaching a level of evidence conclusion was outlined in the protocol. The evaluation of the mechanistic data included a complete discussion and assessment of the strength of evidence for potential modes of action for cobalt-induced neoplasia, including those involving, e.g., cytotoxicity, genotoxicity, and oxidative stress. Mechanistic data are discussed across cobalt compounds. The RoC listing criteria were then applied to the body of knowledge (cancer studies in humans and experimental animals and mechanistic data) for cobalt and cobalt compounds that release cobalt ions in vivo to reach a listing recommendation.

RoC Listing Criteria

Known to Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated to Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded, OR

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset, OR

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

1. Chemical Identification and Properties

The candidate substance reviewed in this monograph is the class, "Cobalt and cobalt compounds that release cobalt ions in vivo."

Cobalt (Co) is a naturally occurring transition element with magnetic properties. It is the 33rd most abundant element and makes up approximately 0.0025% of the weight of Earth's crust. Cobalt is a component of more than 70 naturally occurring minerals including arsenides, sulfides, and oxides. The only stable and naturally occurring cobalt isotope is ^{59}Co. Metallic cobalt, Co(0), exists in two allotropic forms, hexagonal and cubic, which are stable at room temperature (ATSDR 2004; IARC 1991; WHO 2006). Cobalt predominantly occurs in two oxidation states, +2 (Co(II)) and +3 (Co(III)).

Cobalt compounds can be organic or inorganic as well as water soluble or insoluble. Water-soluble cobalt compounds dissolve in the fluids outside cells for cellular uptake, while particles of poorly soluble cobalt compounds can be taken up intact by cells and release ions within the cell (see Table 1-1). Of note, vitamin B_{12}, which is an essential cobalt-containing nutrient, does not meet the criteria for this review because it does not release cobalt ions in acidic gastric or lysosomal fluids and passes through the body intact while bound to specific carrier proteins (Neale 1990).

The available database on cobalt and cobalt compounds varies by cobalt form; however, there are carcinogenicity, genotoxicity, and toxicity studies on cobalt metal and of some water-soluble and poorly water-soluble compounds. Of note are the two NTP bioassay studies, one with a very soluble cobalt compound, cobalt sulfate (NTP 1998), and one with cobalt metal (NTP 2014d). Together, the carcinogenicity, genotoxicity, and other mechanistic information on these representative forms of cobalt inform the discussion in this document on cobalt and cobalt compounds that release cobalt ions in vivo.

1.1. Properties of Cobalt Metal and Cobalt Compounds, Both Soluble and Poorly Soluble

Table 1-1 presents physical and chemical properties (molecular weight, crystalline form, density or specific gravity, water solubility, and bioaccessibility) for cobalt and cobalt compounds for which animal or genotoxicity testing data are available or that are in commercial use greater than 100,000 pounds per year in the United States (per EPA Chemical Data Reporting rule). Additional cobalt compounds that do not meet either of these criteria are described in Table B-1. The physical and chemical properties are divided into three groups, including metals, soluble cobalt compounds, and poorly soluble cobalt compounds, to provide a framework for relating chemicals for which potential biological effects are unknown to chemicals for which biological effect data are available.

Table 1-1. Physical and Chemical Properties for Cobalt Metal and Representative Cobalt Compounds[a,b]

Name (+2 Valence Unless Otherwise Indicated)	CAS No.	Formula	Molecular Weight	Physical Form	Density or Specific Gravity	Solubility (Grams per 100 cc Cold Water)	Particle Size, µm (Surface Area, m^2/g)	Bioaccessiblity[c]
Metal								
Cobalt	7440-48-4	Co	58.9	Grey hexagonal or cubic metal	8.92	0.00029	7.2 (1.20)	100/100
Cobalt nanoparticles	7440-48-4	Co	58.9	–	–	–	–	–
Soluble cobalt compounds								
Sulfate heptahydrate	10026-24-1	$CoSO_4•7H_2O$	281.1	Red pink, monoclinic	1.95	60.4	942.0 (3.49)	100/100
Chloride	7646-79-9	$CoCl_2$	129.9	Blue hexagonal leaflets	3.36	45	458.0 (0.78)	100/100
Acetate (org.)	71-48-7	$Co(C_2H_2O_2)_2$	249.1	Red-violet, monoclinic	1.70	34.8	–	98/80
Nitrate	10141-05-6	CoN_2O_6	182.9	Red powder or crystals	2.49	67.0	–	96/100
Poorly soluble compounds								
(II) Oxide	1307-96-6	CoO	74.9	Green-brown cubic	6.45	0.00049	0.692 (4.79)	100/92.4
(II, III) Oxide	1308-06-1	Co_3O_4	240.8	Black, cubic	6.07	0.00016	–	2/2 (50%[d])
2-ethyl-hexanoate (org.)	136-52-7	$Co(C_8H_{15}O_2)_2$	173.7	Blue liquid (12% Co)	1.01	0.630	0.73 (ND)	100/100
Carbonate (org.)	513-79-1	$CoCO_3$	118.9	Red, trigonal	4.13	0.00114	1.834 (103.05)	100/100
Naphthenate (org.)	61789-51-3	$Co(C_{11}H_7O_2)_2$	401.3	Purple liquid (6% Co)	0.97	0.0293	0.70 (ND)	100/100
Hydroxide	21041-93-0	$Co(OH)_2$	93.0	Rose-red, rhomb	3.60	0.00032	–	95/98
Sulfide	1317-42-6	CoS	91.0	Reddish octahedral	5.45	0.00038	–	1/1
Oxalate (org.)	814-89-1	CoC_2O_4	147.0	White or reddish	3.02	0.00322	–	37/55
Propionate (org.)	1560-69-6	$Co(C_3H_5O_2)_2$	205.1	Reddish solid	–	7.49	–	91/94
Stearate (org.)	1002-88-6	$Co(C_{18}H_{35}O_2)_2$	625.9	Grey solid	–	0.00705	–	14/16

Sources: SciFinder (2015); PubChem Compounds Database (2015); ChemIDplus Database (2015); Cobalt Development Institute (CDI) Report (2006); Hazardous Substances Data Bank (2004; 2012); Stopford et al. (2003). Personal communication, CDI, July 21, 2015, and October 19, 2015.
org. = organic compound; all others are inorganic.
[a]Cobalt compounds selected for inclusion in the table include those with toxicological data or of commercial importance. All compounds contain Co(II) except where noted. Forms in italics have been tested for carcinogenicity, genetic toxicity, or have mechanistic data; org. = organic compound; all others are inorganic.
[b]Bioaccessibility usually assessed as % solubility in gastric/lysosomal fluids.
[c]() = Bioaccessibility assessed by release of cobalt ions into RPMI 1640 culture medium in the presence of canine alveolar macrophages after 2 weeks of culture (Kreyling et al. 1990).

1.2. Water Solubility and Bioaccessibility

Evaluation of toxicological and carcinogenic effects of cobalt compounds depends largely on the release of cobalt ions that can either be transported to and taken up at target sites or released within cells from particles (see Section 6, Mechanistic and Other Relevant Effects).

1.2.1. Water Solubility

Cobalt sulfate, chloride, and nitrate tend to be soluble in water, while oxides (including the mixed oxide, Co_3O_4), hydroxides, and sulfides tend to be poorly soluble or insoluble (Lison 2015). Organic cobalt compounds can be either soluble (e.g., cobalt(II) acetate) or insoluble (e.g., cobalt carbonate, cobalt(II) oxalate, cobalt propionate, cobalt stearate, cobalt naphthenate, cobalt 2-ethyl-hexanoate) (CDI 2006) (see Table 1-1). The water solubility of cobalt compounds is largely pH dependent, and cobalt is generally more mobile in acidic solutions than in alkaline solutions.

Co(0) metal nano- (reported particle size range = 20 nm to 500 nm) and microparticles (reported particle size range = 1.9 μm to 2.7 μm) dissolve in cell-free culture medium in a concentration- and time-dependent manner while cobalt(II,III) oxide particles (reported average particle size = 372 nm) are practically insoluble in water or culture medium (Ortega et al. 2014; Ponti et al. 2009; Sabbioni et al. 2014b). Smaller particles dissolve faster than larger particles (Kyono et al. 1992; Lison 2015).

1.2.2. Bioaccessibility

Solubilization of some water-insoluble compounds may be enhanced in biological fluids at low pH and in the presence of binding proteins (IARC 2006) (see below). Because in vivo bioavailability testing can be cost prohibitive and time consuming, solubility of compounds in artificial fluids (i.e., bioaccessibility) using synthetic equivalents of gastric and intestinal fluids (for ingestion exposure); alveolar, interstitial, and lysosomal fluids (for inhalation exposure); perspiration fluids (for dermal exposure); and synovial fluid (for metal joint prostheses), identified from exposure scenarios including manufacturing and use of alloy materials (Brock and Stopford 2003; Hillwalker and Anderson 2014; Stopford et al. 2003, personal communication from CDI to Dr. Ruth Lunn) can often be used as a surrogate for bioavailability. Cobalt metal, and several water-soluble compounds (e.g., cobalt sulfate heptahydrate, chloride, cobalt acetate) and poorly soluble compounds (e.g., cobalt(II) oxide, bis(2-ethyl-hexanoate), carbonate, naphthenate) were found to be soluble in biological fluids, suggesting that they release cobalt ions (see the right-hand column of Table 1-1 and Table B-1).

Some compounds that appear to be insoluble in these tests might actually be soluble in biological fluids in vivo. This is due to the inability of a simple model with an artificial biological fluid and a cobalt compound not providing for the potential equilibrium between cellular compartments such as the lysosomes with the cytoplasm and ultimately the extracellular fluid. Thus, additional assays that are more physiologically relevant because of the presence of lung cells are potentially more informative. For example, although very low values (≤2%) for bioavailability have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the test conditions reported in Table 1-1, other test conditions in the presence of lung cells have indicated that Co_3O_4 (cobalt (II,III) oxide) releases cobalt ions.

Kreyling et al. (1990) reported that cobalt ions dissolved from Co_3O_4 particles of different sizes were ultimately released into the culture medium (RPMI 1640) in the presence of canine alveolar macrophages with up to 50% solubilized from 0.3 μm particles after 2 weeks of culture (larger particles released 2% to 5% under the same conditions). The differences in findings between this study and the 2% solubility in gastric or lysosomal fluid may be due to the interaction of cobalt particles within cells as the soluble fraction of an initial particle mass of Co_3O_4 increased with time when the particles were taken up by alveolar macrophages in culture compared with the solubility in culture medium alone. Moreover, Ortega et al. (2014), found that intracellular concentrations of solubilized cobalt ions were similar for Co_3O_4 and cobalt chloride in human lung cells, suggesting that Co_3O_4 would release cobalt ions in vivo (see Section 6.1 for details). A similar result was reported for CoO nanoparticles, which increased intracellular cobalt ion concentration in human lung fibroblasts in culture in a concentration-dependent manner (Smith et al. 2014).

The intra- and inter-laboratory variability of bioaccessibility testing results for metals and metal compounds including cobalt powder and cobalt oxide in synthetic gastric, perspiration, lysosomal, and interstitial fluids was reported by Henderson et al. (2014), and the authors concluded that results demonstrated overall satisfactory within-laboratory variability. Relative standard deviation (RSD) values and associated threshold levels were used to assess sample-to-sample result variability (i.e., repeatability) and lab-to-lab result variability (i.e., reproducibility). Acceptable variability for this analysis was defined as RSD for repeatability <10% (per Wragg et al. 2011) and RSD for reproducibility <20% (per Ashley et al. 2012; and Wragg et al. 2011). Henderson et al. (2014) further noted that absolute bioaccessibility results in some biological fluids might vary between different laboratories.

Cobalt(II) ions released into solution can form complexes with organic or inorganic anions with equilibrium conditions determined by activity of electrons (Eh), activity of hydrogen ions (pH), and anion presence (Smith and Carson 1981). In general, lower pH generates higher free Co(II) concentrations in solution, and higher pH gives rise to cobalt-carbonate complex formation (WHO 2006). The in vivo concentration of free Co(II) ions is relatively low because these cations are complexed in the presence of physiological concentrations of phosphates and also bind nonspecifically to proteins such as albumin (Lison 2015).

1.3. Variability of Valence

As noted above, cobalt exists primarily as Co(II) and Co(III), and Co(II) is much more stable in aqueous solution (Nilsson et al. 1985; Paustenbach et al. 2013). Electron-donor ligands (e.g., NH_3) can stabilize Co(III) in aqueous solution (IARC 1991). In acid solution, Co(II) is the stable form in the absence of electron-donor ligands, and Co(III) ions are so unstable that they quickly reduce to Co(II), oxidizing water and liberating oxygen. In contrast, air or hydrogen peroxide can oxidize Co(II) to the Co(III) complex, which is more stable in alkaline solutions containing ammonium hydroxide or cyanide. This interconversion between Co(II) and Co(III) is important in the use of cobalt compounds as catalysts and paint driers (IARC 1991; Paustenbach et al. 2013).

Cobalt is present in its stable +2 valence state in the environment and in most commercially available cobalt compounds, with the exception of the mixed oxide (Co(II,III) or Co_3O_4) (IARC 1991; Paustenbach et al. 2013). Some simple salts of cobalt in its +3 valence state (e.g., Co_2O_3)

have been used commercially. Cobalt compounds of commercial and toxicological interest include cobalt metal, alloys, and composite materials; oxides (e.g., cobalt oxide and tetraoxide); and salts (e.g., cobalt(II) chloride, sulfide, and sulfate) (Lison 2015). Important salts of carboxylic acids include formate, acetate, citrate, naphthenate, linoleate, oleate, oxalate, resinate, stearate, succinate, sulfamate, and 2-ethylhexanoate. (See Table 1-1 and Table B-1.)

Cobalt can also exist in −1, +1, and +4 oxidation states (Nilsson et al. 1985). Cobalt is in its −1 state in cobalt carbonyls such as $[Co(CO)_4]H$ and in cobalt-nitrosyls, in its +1 state in some cobalt-cyanide complexes, and in its +4 state in compounds with cobalt bonded to fluoride or oxygen.

1.4. Summary

Cobalt metal particles have been found to be 100% bioaccessible (i.e., dissolving to release cobalt ions) in both artificial gastric and lysosomal fluids. The soluble compounds, cobalt(II) sulfate heptahydrate and cobalt(II) chloride, and the poorly soluble compounds, cobalt(II) oxide, cobalt bis(2-ethyl hexanoate), cobalt carbonate, and cobalt naphthenate, also were completely (or almost completely) soluble in the two acidic fluids. The metals and poorly soluble compounds tended to be less bioaccessible in neutral biological fluids, which is consistent with the pH dependence for releasing cobalt ions in solution. Although very low values (≤2%) for bioavailability in artificial gastric and lysosomal fluids have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the same test conditions, more informative tests (e.g., using more physiologically relevant conditions) in the presence of lung cells have shown higher bioavailability values for cobalt(II, III) oxide (i.e., Co_3O_4) in culture media in the presence of alveolar macrophages. Other studies have reported uptake of Co_3O_4 by lung cells, which suggests that compound would release ions in vivo.

2. Human Exposure

This section describes cobalt mining and production (Section 2.1); use (Section 2.2); recycling of electronic and electrical waste (Section 2.3); biomonitoring and environmental monitoring studies and methods to measure exposure to cobalt and cobalt compounds (Section 2.4); and potential exposure in the workplace (Section 2.5), from surgical implants (Section 2.6), from other sources such as food, consumer products, tobacco, and medical products (Section 2.7), and from the environmental exposure (Section 2.9). The material presented in Sections 2.1 through 2.8 is summarized in Section 2.9. Studies of cobalt alloys were not considered informative for either animal tumor studies or human carcinogenicity studies because they are not useful for evaluating potential carcinogenic effects from cobalt per se; cobalt alloys are a source of exposure to humans, and thus are discussed in this section.

2.1. Mining and Production

Cobalt is most often found in ores associated with copper or nickel, but may also be a by-product of zinc, lead, and platinum-group metals (CDI 2006; Davis 2000). Cobalt-containing ores often contain arsenic, such as safflorite, $CoAs_2$; skutterudite, $CoAs_3$; erythrite, $Co_3(AsO_4)_2 \cdot 8H_2O$; and glaucodot, CoAsS (ATSDR 2004; CDI 2006; Davis 2000). The largest cobalt reserves are in the Congo (Kinshasa), Australia, Cuba, Zambia, Canada, Russia, and New Caledonia (Shedd 2014b). Most U.S. cobalt deposits are in Minnesota, but other important deposits are in Alaska, California, Idaho, Missouri, Montana, and Oregon. Except for Idaho and Missouri, future production from these deposits would be as a by-product of another metal.

Except for a negligible amount of by-product cobalt produced as an intermediate product from mining and refining platinum-group metals ore, the United States did not refine cobalt in 2012 (Shedd 2014a). Since 2009, no cobalt has been sold from the National Defense Stockpile. In 2012, 2,160 metric tons of cobalt was recycled from scrap. Cobalt has not been mined in the United States in over 30 years (ATSDR 2004); however, a primary cobalt mine, mill, and refinery are currently being established in Idaho that will produce more than 1,500 tons of high-purity cobalt metal annually to capitalize on increasing cobalt demand driven in part by growth in “green” energy technology (e.g., rechargeable batteries for electric and hybrid electric vehicles or portable electronics applications (Farquharson 2015; Mining Technology Market Customer Insight 2015; Rufe 2010). Based on a presentation dated May 2015, preliminary work on the site has been completed (Formation Metals Inc. 2015).

Cobalt and several cobalt compounds are high-production-volume chemicals based on their production or importation into the United States in quantities of 1 million pounds or more per year. Table 2-1 shows U.S. cobalt and cobalt compound production volumes for 2012 that exceed 100,000 pounds per year; the highest United States production volume is for cobalt (7440-48-4) (23,384,002 lb.). Table 2-2 lists recent U.S. imports and exports of cobalt and cobalt compounds; the highest import value is for “unwrought cobalt excluding alloys, including powders” (16,151,599 lb.) and the highest export value is for “cobalt, wrought, and articles thereof” (4,841,750 lb.).

Table 2-1. U.S. Cobalt Compounds Production Volumes for 2012 Exceeding 100,000 Pounds per Year[a]

CAS Number[b]	Cobalt Compound	Quantity (lb.)[c]
7440-48-4	Cobalt	23,384,002
21041-93-0	Cobalt hydroxide ($Co(OH)_2$)	4,709,137
136-52-7	Cobalt 2-ethylhexanoate	4,294,523
1307-96-6	Cobalt oxide (CoO)	1,385,848
513-79-1	Cobalt carbonate	1,038,821
10124-43-3	Cobalt sulfate	1,000,000–10,000,000
10141-05-6	Cobalt nitrate	1,000,000–10,000,000
1308-06-1	Cobalt oxide (Co_3O_4)	1,000,000–10,000,000
1560-69-6	Cobalt propionate	1,000,000–10,000,000
71-48-7	Cobalt acetate	1,000,000–10,000,000
814-89-1	Cobalt oxalate	600,000
1317-42-6	Cobalt sulfide (CoS)	254,733
61789-52-4	Cobalt tallate	192,900
61789-51-3	Cobalt naphthenate	100,000–500,000

[a]Three cobalt compounds for which properties are reported in Table 1-1 are not listed in Table 2-1 because of the production level or lack of reported production data. Cobalt oxide (11104-61-3) production levels were 94,139 lb. in 2012. Cobalt sulfide (12013-10-4, CoS_2) and cobalt chloride (7646-79-9, $CoCl_2$) production levels for 2012 were withheld by the manufacturers.
[b]CAS# were identified from multiple sources: ChemIDplus Database (2015); EPA Chemical Data Reporting (2012); PubChem Compounds Database (2015); Ullmann's Encyclopedia of Industrial Chemistry (2012).
[c]EPA Chemical Data Reporting (2012). See reference list for specifics.

Table 2-2. U.S. Imports and Exports of Cobalt Compounds for 2013 (Converted from kg by NTP)

Cobalt-compound/Category	U.S. Imports (lb.)	U.S. Exports (lb.)
Cobalt acetates	342,918	520,996
Cobalt carbonates	1,193,856	–[a]
Cobalt chloride	215,661	14,304
Cobalt ores and concentrates	82,376	1,004,825
Cobalt oxides and hydroxides; commercial cobalt oxides	5,300,984	902,467
Cobalt sulfate	1,319,004	–[a]
Cobalt waste and scrap	1,549,151	1,557,515
Cobalt, wrought, and articles thereof	550,887	4,841,750
Other cobalt mattes and intermediate products of cobalt metallurgy; powders	1,992,434	–[a]
Unwrought cobalt alloys	2,132,331	–[a]
Unwrought cobalt excluding alloys, including powders	16,151,599	–[a]

Source: USITC (2014).
[a]No specific Schedule B code (i.e., 10-digit classification numbers administered and used by the U.S. Commerce Department to collect and publish statistics on physical goods exported from the United States to another country) was identified.

2.2. Use

Cobalt is used in numerous commercial, industrial, and military applications. On a global basis, the largest use of cobalt is in rechargeable battery electrodes; however, rechargeable battery production in the United States has been very limited (NIST 2005).

In 2012, the reported U.S. consumption of cobalt was approximately 8,420 metric tons (Shedd 2014a) for the uses shown below in Table 2-3.

Table 2-3. 2012 U.S. Consumption and Use Pattern for Cobalt

End Use	Consumption (Metric Tons Cobalt Content)	Percent of Total Consumption (%)
Superalloys	4,040	48
Chemical and ceramic	2,300	27.3
Cemented carbides	774	9.2
Other alloys[a]	699	8.3
Steels	548	6.5
Miscellaneous and unspecified	63	0.7

Source: Shedd (2014a).
[a]Includes magnetic, nonferrous, and wear-resistant alloys and welding materials.

The main uses of cobalt can be grouped into the following general categories: metallurgical; cemented carbides and bonded diamonds; chemicals; and electronics and "green" energy (CDI 2006). Cobalt nanoparticles are used for medical applications (e.g., sensors, MRI contrast enhancement, drug delivery); nanofibers and nanowires also are being used for industrial applications.

Metallurgical uses of cobalt include use in superalloys (Davis 2000; IARC 1991); magnetic alloys, low expansion alloys, nonferrous alloys, steels, coatings, and bone and dental prostheses (CDI 2006; Davis 2000; IARC 1991; Ohno 2010). Support structures for heart valves are also manufactured from cobalt alloys (IARC 1991).

Cemented tungsten carbides ("hard metals") are composites of tungsten carbide particles (either tungsten carbide alone or in combination with smaller amounts of other carbides) with metallic cobalt powder as a binder, pressed into a compact, solid form at high temperatures by a process called sintering (IARC 1991; NTP 2009). Cobalt is also used in **diamond tools** from steel with microdiamonds impregnated into a surface cobalt layer (CDI 2006; IARC 2006)).

Chemical uses of cobalt compounds include as pigments for glass, ceramics, and enamels, as driers for paints, varnishes, or lacquers, as catalysts, as adhesives and enamel frits (naphthenate, stearate, oxide), as trace mineral additives for animal diets, and in rechargeable batteries (see Section 2.3 (ATSDR 2004; CDI 2006; IARC 1991; WHO 2006) (see Table 2-4). Compounds of commercial importance are the oxides, hydroxide, chloride, sulfate, nitrate, phosphate, carbonate, acetate, oxalate, and other carboxylic acid derivatives (IARC 1991). A past use of

cobalt (as cobalt sulfate) was as an additive in some beers to increase the stability of the foam (NTP 1998).

Table 2-4. Chemical Uses for Representative Inorganic and Organic Cobalt Compounds

Use	Inorganic					Organic			
	Cl^-	OH^-	NO_3^-	O^{2-}	SO_4^{2-}	2-EH	$C_2H_3O_2^-$	CO_3^{2-}	Pro
Adhesives				X		X			
Animal diets			X	X	X		X	X	
Batteries		X	X						
Catalysts	X	X	X	X			X	X	
Driers		X		X		X	X		X
Pigments	X		X	X	X		X	X	

Sources: CDI (2006), Donaldson and Beyersmann (2012), Richardson and Meshri (2001).
Cl^- = chloride; OH^- = hydroxide; NO_3^- = nitrate; O^{2-} = oxide; SO_4^{2-} = sulfate; 2-EH = 2-ethyl-hexanoate; $C_2H_3O_2^-$ = acetate; CO_3^{2-} = carbonate, Pro = propionate.

Due to increased demand for portable rechargeable **electronic devices**, one of the fastest growth areas for cobalt use worldwide is in high-capacity, rechargeable batteries (CDI 2006; Davis 2000; Shedd 2014a). Cobalt is used in nickel-cadmium, nickel-metal hydride, and lithium-ion battery technologies. Applications for batteries containing cobalt compounds include portable computers, mobile telephones, camcorders, toys, power tools, and electric vehicles. Cobalt is also used in integrated circuit contacts and leads and in the production of semiconductors (CDI 2006; IARC 1991).

Cobalt is the key element in several forms of "**green" energy** technology applications including gas-to liquid (GTL) and oil desulfurization, coal-to liquid (CTL), clean coal, solar panels, wind and gas turbines, and fuel cells (Rufe 2010). Research is ongoing on use of cobalt-based catalysts in sunlight-driven water splitting to convert solar energy into electrical and chemical energy (Deng and Tüysüz 2014).

2.3. Recycling of Electronic and Electrical Waste

Electronic and electrical waste (i.e., e-waste) includes components of electrical and electronic equipment such as rechargeable batteries. Automobile rechargeable battery recycling is generally considered to be in its infancy, though more developed for nickel-metal hydride batteries than for lithium-ion batteries (Evarts 2013; Gaines 2014).

Recycling for Li-ion batteries is more difficult because these batteries have various active material chemistries (e.g., lithium cobalt oxide, lithium manganese oxide, lithium nickel manganese cobalt oxide, etc.), contain a wider variety of materials in each cell, are not currently subject to recycling regulations, and will not be ending their useful lives in large numbers for about 10 years (Cadex Eletronics Inc. 2015; Gaines 2014). Further, recent trends to reduce costs of battery manufacturing and to optimize performance (e.g., safety, durability, and output) have lead manufacturers to seek other non-cobalt-based constituents (e.g., iron phosphate, manganese spinel, and nickel manganese), which might reduce the economic incentive for recycling (Retriev Technologies 2015).

2.4. Biomonitoring and Environmental Monitoring for Cobalt

Information on biomonitoring and environmental monitoring for cobalt discussed below includes evidence of exposure (Section 2.4.1) and exposure surrogates and analytical methods (Section 2.4.2).

2.4.1. Evidence of Exposure

Evidence for widespread exposure to cobalt and cobalt compounds comes from biological monitoring data measuring cobalt levels in urine, blood, hair, nails, and tissues in individuals exposed to cobalt from occupational and non-occupational sources (see Table B-2 and Table B-3 for levels reported in these studies, source of exposure, and geographical location and Figure 2-1 and Figure 2-2). Several publications measured trace metals (e.g., heavy metals and essential metals) in tissue from cancer patients with a referent group or tissue. Several clinical surveys have compared levels of cobalt in cancer patients and non-cancer patients (see Table B-4). Several of the studies are of people residing in the United States, and thus demonstrate U.S. exposure. Data are reported for both a surrogate of recent (urine) and longer term (hair) exposure to cobalt.

Studies measuring cobalt in the urine of people exposed to cobalt from different sources indicate that the highest levels were generally seen in workers and patients with failed hip implants; with lower levels of exposure in patients with normal implants, people potentially exposed to cobalt from the environment, or in the general public (source of exposure unknown). (See Figure 2-1, which depicts the mean [or median] levels of urinary cobalt in these populations from the studies reported in Table B-2.) The geometric mean urinary cobalt concentration for the U.S. general public for the most recent National Health and Nutrition Examination Survey (NHANES) year (Centers for Disease Control and Prevention (CDC) and National Center for Health Statistics (NCHS) 2011) for which data are available is 0.326 μg/L; urinary cobalt measurements in the U.S. general public have remained consistent since 1999, with the geometric mean values ranging from 0.316 to 0.379 μg/L (CDC 2015).

Reported mean levels of cobalt in hair are highest among some workers and among patients with unstable hip implants (Figure 2-2). Cobalt levels in samples from patients with stable hip implants are next highest, with levels taken from people at risk of environmental exposure and the general public being the lowest. Measurements of cobalt in hair in the latter groups overlap significantly; while one study indicates that cobalt levels among environmentally exposed populations are similar to levels in workers.

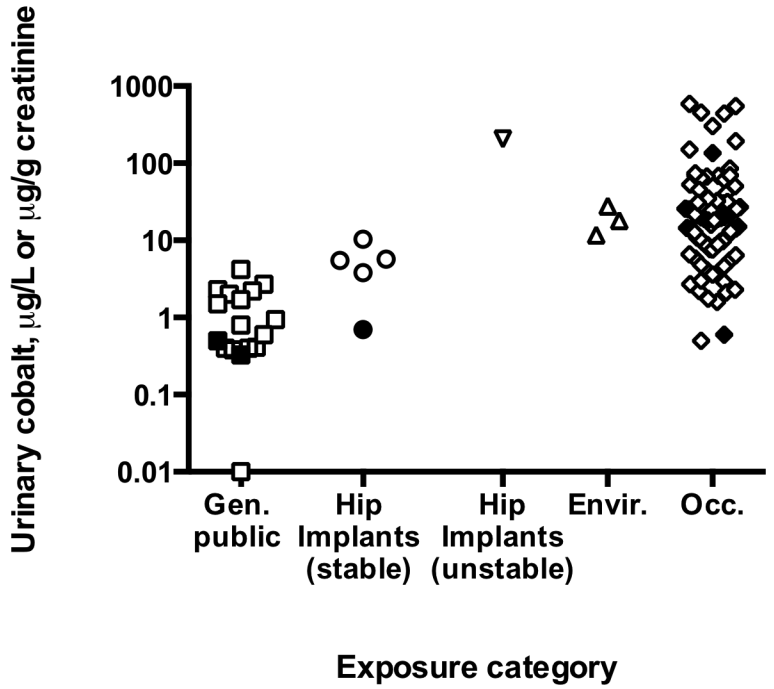

Figure 2-1. Cluster Graph of Urine Cobalt Levels from Different Sources of Exposure

Gen. public = general public exposure; Envir. = environmental exposure; Occ. = occupational exposure. Filled symbols = U.S. data; open symbols = non-U.S. data. Each graph point represents a different study (data are available in Table B-2).

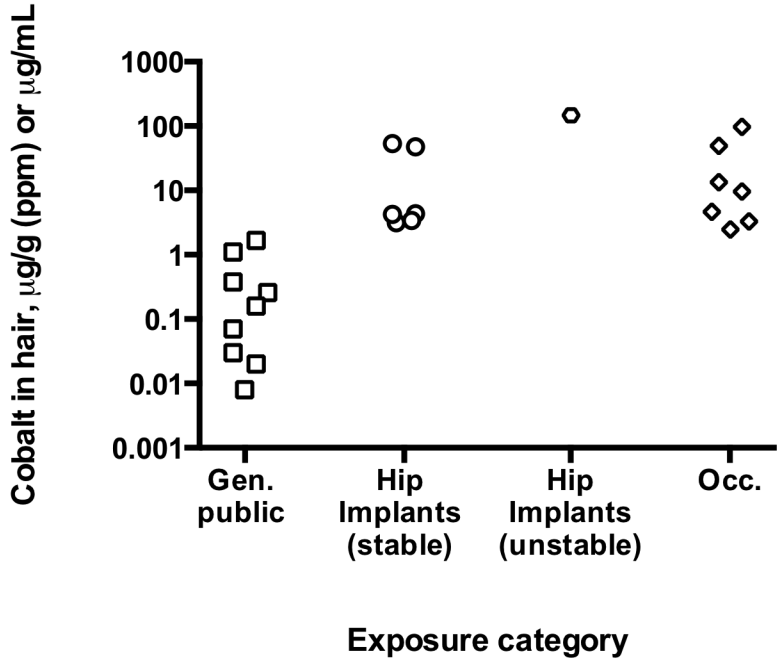

Figure 2-2. Cluster Graphs of Cobalt Levels in Hair

Gen. public = general public exposure; Occ. = occupational exposure. All data are from non-U.S. locations. Each graph point represents a different study (data are available in Table B-3).

2.4.2. Exposure Surrogates and Analytical Methods

Exposure Surrogates

Urinary cobalt is considered a good indicator of absorbed cobalt (IARC 2006; WHO 2006), especially from recent exposures (ATSDR 2004). Urinary and blood cobalt levels are more reflective of recent exposure for soluble compounds than less soluble compounds (ATSDR 2004). Although investigators have reported measurements of cobalt in whole blood, plasma, and serum, no consensus seems to exist for which of these provides the best relationship with levels of exposure to cobalt.

Because hair fixes trace elements in a permanent, chemically homogeneous matrix, hair samples reflect a time-integrated exposure (i.e., current and past exposure levels) over the previous few months, depending on the length of the hair sample (Suzuki and Yamamoto 1982) and hair metal contents provides a better estimate than blood in assessing the environmental risk to toxic metals for infrequent and highly variable exposures (Bax 1981; Petering et al. 1973). The average concentration of cobalt in hair is over 100 times greater than that in blood (Underwood 1977). Average metal concentration can be obtained by measuring bulk concentration from a length of hair equal to a few weeks' growth, by measuring the variation along the length of long hair equal to several months (Suzuki and Yamamoto 1982), or by taking periodic samples over time (Laker 1982).

Toenail clippings reflect time-integrated exposure occurring in the timeframe of 12 to 24 months prior to clipping, and thus are useful biomarkers of exposure when a single sample is assumed to represent long-term exposure (Fleckman 1985; He 2011). However, toenails generally provide larger samples and represent more distant past exposures because they take longer to grow out. Nails are considered to be relatively sheltered from environmental contaminants (relative to hair, which, though formed from the same keratinous tissue of nail, can be contaminated by dyeing, bleaching, and permanent waving). Toenails are also more convenient to collect and store than blood (Garland et al. 1993). However, nails can become contaminated through the use of nail polishes, some medications, and use of contaminated cutters to produce clippings (He 2011).

The source of exposure for urinary cobalt levels in the general public (see Figure 2-1) is unknown. Likewise, the source of exposure for the general public is unknown for the exposure surrogates (e.g., hair and nails).

Analytical Methods

Analytical methods for cobalt in biological materials include graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), differential pulse cathodic stripping voltammetry (DPCSV), and colorimetric determination (ATSDR 2004). Technical improvements using the Zeeman background correction in GF-AAS have increased specificity and lowered the background (see IUPAC guidelines in Cornelis et al. 1995). The colorimetric method generally has limited utility because it has poor sensitivity (Alessio and Dell'Orto 1988). The ICP-AES method is used by NIOSH for exposure to elements in blood and urine (NIOSH 1994b), and NHANES uses a related method of inductively coupled plasma-mass spectrometry (ICP-MS) for urine heavy metals. With the exception of the colorimetric method, these methods require wet (acid) digestion followed by flame ionization to liberate free cobalt ions for detection of total cobalt. Thus, in any biological sample, the original

form of the cobalt, whether inorganic cobalt or part of an organic molecule like vitamin B_{12}, cannot be determined with these methods (IARC 2006; WHO 2006).

The analytical method for air sampling (NIOSH Method 7027) involves collecting the sample on a 0.8 μm pore size cellulose ester membrane filter and analyzing the sample using a flame atomic absorption spectrophotometer. This is an elemental analysis and is not compound specific (NIOSH 1994a). For surface sampling, the analytical method (NIOSH Method 9102) involves collecting a wipe sample on a pre-packaged moist disposable towelette (e.g., Wash 'n Dri or ASTM equivalent per ASTM E1792-01) and analyzing the sample using ICP-AES. Likewise, this method also is an elemental analysis and is not compound specific (NIOSH 2003).

2.5. Characterization of Exposure in the Workplace

The primary route of occupational exposure to cobalt is via inhalation of dust, fumes, or mists or gaseous cobalt carbonyl; however, dermal contact with hard metals and cobalt salts can result in systemic uptake. Occupational exposure to cobalt occurs during (1) the refining of cobalt, (2) the production of cobalt powders, (3) use in the hard metal, diamond tool, and alloy industries (including the production and use of these cobalt-containing products), use to make chemicals, pigments, and electronics, and (4) in the recycling of electronics. Workers regenerating spent catalysts may also be exposed to cobalt sulfides. U.S. occupational exposure data are available for the following industries: metallurgical; cemented carbides and bonded diamonds; chemicals and pigments; and electronics, "green" energy, and recycling.

Occupational exposure has been documented by measurements of cobalt in ambient workplace air, in worker blood and urine, and in deceased worker lung tissue (ATSDR 2004; CDC 2013; IARC 1991; 2006). The NIOSH National Occupational Exposure Survey (NOES) estimated that approximately 386,500 workers were potentially exposed to cobalt and cobalt compounds (NIOSH 1990). The survey was conducted from 1981 to 1983, and the NOES database was last updated in July 1990.

Air levels for workplace for cobalt production, metallurgical uses of cobalt, cemented carbides (hard metals) and bonded diamonds, chemical and pigments, and electronics, "green" energy, and recycling are listed in Table 2-5. Exposure data for cobalt levels in urine and blood are listed in Table B-2, and levels in hair and nails are in Table B-3. The findings for these media are briefly summarized below.

Table 2-5. Workplace Air Levels of Cobalt

Exposure Scenario (Country)	Cobalt in Workplace Air Mean (Range) in μg/m^3	Reference(s)
Cobalt production		
Production of cobalt metal and cobalt salts (Belgium)	127.5 (2–7,700)	Swennen et al. (1993)
Production of cobalt salts (Russian Federation)	(0.05–50)	Talakin et al. (1991)
Nickel refining (Russian Federation)	Up to 4	Thomassen et al. (1999)
Production of cobalt metal and cobalt salts (Finland)	<100	Linna et al. (2003)

Exposure Scenario (Country)	Cobalt in Workplace Air Mean (Range) in µg/m^3	Reference(s)
Conversion of cobalt metal to cobalt oxide (South Africa)	9,900 (highest reported)	Coombs (1996)
Nickel refining (Norway)	<150[a]	(Grimsrud et al. 2005)
Metallurgical uses		
Metallurgical (United States)	ND–32,000[b]	Beaucham et al. (2014); Daniels et al. (1986); Decker (1991); Deitchman et al. (1994); Deng et al. (1990); Hervin and Reifschneider (1973); Kiefer et al. (1994); Marsh and Esmen (2007); McCleery et al. (2001); NIOSH (1972)
Production of Stellite, a cobalt-containing alloy (NR)	Several hundred µg/m^3	Simcox et al. (2000)
Production of Stellite, a cobalt-containing alloy (NR)	9	Kennedy et al. (1995)
Welding with Stellite, a cobalt-containing alloy (NR)	160	Ferri et al. (1994)
Cemented carbides (hard metals) and bonded diamonds		
Cemented carbides and bonded diamonds (United States)	ND–1,622.1	Bryant et al. (1987); Burr et al. (1988); Burr and Sinks (1988); Edmonds et al. (1981); Kerndt et al. (1986); McManus (1982); Sahakian et al. (2009); Salisbury and Seligman (1987); Tharr and Singal (1987)
Use of cobalt-containing diamond tools (Italy)	690 115 (with improved ventilation)	Ferdenzi et al. (1994)
Use of cobalt-containing diamond tools (NR)	(0.1–45)	van den Oever et al. (1990)
Chemicals and pigments		
Chemicals (United States)	ND–21	Almaguer (1987); Apol (1976); Burr et al. (2005); Chen et al. (2008); Durgam and Aristeguieta (2010); Hall (2003); Rosensteel et al. (1977); Zey (1985)
Painting porcelain plates with cobalt compounds (Denmark)	80 26 (after Danish surveillance program)	Christensen (1995); Christensen and Poulsen (1994); Poulsen et al. (1995)
Electronics, "green" energy, and recycling		
Electronics and "green" energy (United States)	ND–1.17	Beaucham et al. (2014); Thoburn and Larsen (1976)
Recycling batteries to recover cobalt (NR)	Up to 10	Hengstler et al. (2003)

Source: IARC (2006), http://www2a.cdc.gov/hhe/search.asp.
NR = Not reported.
[a]Reported as 0.15 mg/m^3. Among the 3,500 personal samples from the breathing zone taken, cobalt values above 50 mg/m^3 [50,000 µg/m^3] (3 measurements) were excluded.
[b]OSHA noted that this sample appeared to be tampered with. The next highest value was 21,000 µg/m^3.

2.5.1. Cobalt Production (Metals and Salts)

Cobalt concentrations in workplace air have been reported to range from 2 to 50,000 μg/m^3 from hydrometallurgical purification (to produce cobalt metal, cobalt oxide, and cobalt salt products), battery recycling (to recover cobalt for reuse), and cobalt compound (acetate, chloride, nitrate, and sulfate) production. Worker urinary cobalt for these facilities ranged from 1.6 to 2,038 μg/g creatinine (IARC 2006). The mean urinary and serum or blood cobalt levels reported in Table B-2 generally fall in the range of 10s or 100s of μg/L (or μg/g creatinine). Data for cobalt in hair and nails for cobalt production are limited, but one study listed in Table B-3 reported a mean level of almost 100 μg/g for hair compared with unexposed individuals in the same study with 0.38 μg/g.

Available data on emissions of cobalt from electrochemical production of cobalt (in nickel refining plants) indicate that exposure to cobalt is expected to be low. Based on analysis of nearly 3,500 personal breathing zone samples analyzed for cobalt at a Norwegian nickel refinery, the median 8-hour time-weighted arithmetic average exposures were less than 0.1 μg/m^3 (Grimsrud et al. 2005). A European report of processes to produce nickel and cobalt noted that total emissions of cobalt to air from grinding/leaching, solvent extraction, and final recovery or transformation were 0.9 kilograms per metric ton of cobalt produced (IPPC 2014).

2.5.2. Metallurgical-related Industries

Occupational exposure results from production and use (e.g., welding, grinding, and sharpening) of cobalt alloys. Concentrations of cobalt in workplace air of facilities producing and using Stellite have been reported to range from 9 to several hundred micrograms per cubic meter (IARC 2006). Urinary cobalt levels in the 10s of μg/g (reported as μg/mg but considered a typographical error) creatinine for a metallurgical site in the United States but no blood levels were identified for these activities.

U.S. cobalt occupational exposure level data available from NIOSH HETA surveys for metallurgical-related industries indicate the following: workplace air levels range from not detected to 32,000 μg/m^3; workplace arithmetic mean, median, or geometric mean urine levels range from 0.6 μg/L or μg/g creatinine to 50.4 μg/L or μg/g creatinine (it is generally accepted that 1 L of urine contains 1 g of creatinine); surface wipe levels range from 2.1 μg/100 cm^2 to 760 μg/100 cm^2; and the one reported value for cobalt in bulk samples of work materials was 0.08% (Beaucham et al. 2014; Daniels et al. 1986; Decker 1991; Deitchman et al. 1994; Deng et al. 1990; Hervin and Reifschneider 1973; Kiefer et al. 1994; Marsh and Esmen 2007; McCleery et al. 2001; NIOSH 1972).

2.5.3. Cemented Carbides and Bonded Diamonds

Exposure to cobalt can occur in hard-metal production, processing, and use and during the maintenance and re-sharpening of hard-metal tools and blades. Air levels of cobalt vary across different stages of the hard-metals manufacturing process, with levels for operations involving cobalt metal powder often reaching maximum levels between 1,000 and 10,000 μg/m^3 (NTP 2009). Continuous recycling of coolants used during the grinding of hard-metal tools after sintering and during maintenance and re-sharpening has been reported to increase concentrations of dissolved cobalt in the metal-working fluid, which can be a source of exposure to ionic cobalt

in aerosols from the coolants (IARC 2006). Wet grinding processes are reported to produce higher cobalt concentrations than dry grinding processes due to coolant mist emissions.

Diamond polishers inhale metallic cobalt, iron, and silica from the use of cobalt discs to polish diamond jewels. Cobalt concentrations in workplace air have been reported to range from 0.1 to 45 μg/m^3 in diamond jewel polishing and as high as 690 μg/m^3 in wood and stone cutting (air concentrations dropped to 115 μg/m^3 after implementation of ventilation system improvements in the wood and stone cutting factory) (IARC 2006).

A number of data points are available for cobalt in urine and blood or serum for these occupational exposures (see Table B-2). Most mean urinary cobalt values were between 1 and 100 μg/L or μg/g creatinine but some values up to 500 μg/L were reported for some operations involving cobalt powder. Blood cobalt generally falls in the range of 1 to 50 μg/L for exposures in these industries. The highest levels of blood cobalt were reported for a hard-metal manufacturing facility in Italy which also reported levels of approximately 50 μg/g for hair and toenails; other sites ranged down to 1 μg/g or less.

U.S. cobalt occupational exposure level data available from NIOSH Hazard Evaluation and Technical Assistance (HETA) surveys for cemented carbides and bonded diamonds indicate the following: workplace air levels range from not detected to approximately 1,620 μg/m^3; workplace arithmetic mean, median, or geometric mean urine levels range from 9.6 μg/L or μg/g creatinine to 27 μg/L or μg/g creatinine (it is generally accepted that 1 L of urine contains 1 g of creatinine); the one reported geometric mean blood cobalt level was 2.0 μg/L; surface wipe levels range from not detected to 4,400 μg/100 cm^2; skin (i.e., hand or neck) wipe levels range from 2 μg/sample to approximately 22,330 μg/sample (from charging operations in a cemented tungsten carbide plant); geometric mean exhaled breath condensate levels range from 5.5 μg/L to 6.2 μg/L; cobalt in bulk samples of work materials ranges from 0.033% to 8.97%; cobalt in settled dust samples from work areas ranges from 0.2% to 2% (Bryant et al. 1987; Burr et al. 1988; Burr and Sinks 1988; Edmonds et al. 1981; Kerndt et al. 1986; McManus 1982; Sahakian et al. 2009; Salisbury and Seligman 1987; Tharr and Singal 1987). One extreme value of 438,000 μg/m^3 was reported for weighing and mixing operations in a plant in the United States (Sprince et al. 1984).

2.5.4. Chemicals and Pigments

Cobalt concentrations in workplace air at Danish porcelain factories using cobalt-aluminate spinel or cobalt silicate dyes have been reported to exceed the Danish hygienic standard by 1.3- to 172-fold (Tüchsen et al. 1996) (see Section 4). Due to improvements made to workplace conditions in the 1982 to 1992 time period, concentrations of cobalt in workplace air decreased from 1,356 nmol/m^3 [80 μg/m^3] to 454 nmol/m^3 [26 μg/m^3] and worker urinary cobalt decreased from 100-fold to 10-fold above median concentration of controls (IARC 1991; 2006). Several studies have been published reporting urine and blood cobalt levels for pottery or plate painters in Denmark and cloisonne workers in Japan (see Table B-2). The mean urine levels were generally elevated, with levels in the 10s of μg/g creatinine for the pottery or plate painters, but <2 μg/L for the glaze workers in cloisonne production. Mean blood levels did not exceed 3 μg/L for any of the studies identified. No cobalt levels in hair or nails were identified for workers in these industries.

U.S. cobalt occupational exposure level data available from NIOSH HETA surveys for chemicals and pigments indicate the following: workplace air levels range from not detected to 21 μg/m^3; surface wipe levels range from not detected to 250 μg/100 cm^2; and cobalt in bulk samples of work materials ranges from less than 0.01% to 0.03% (Almaguer 1987; Apol 1976; Burr et al. 2005; Chen et al. 2008; Durgam and Aristeguieta 2010; Hall 2003; Kawamoto et al. 1999; Rosensteel et al. 1977; Zey 1985).

2.5.5. Electronics, "Green" Energy, and Recycling of Electronic and Electrical Waste

Recycling can be classified as either informal or formal. Informal e-waste recycling which is dismantling of end-of-life electronics by primitive techniques (e.g., mechanical shredding and open burning) can result in the release of cobalt and other toxic chemicals and generally occurs in developing countries such as China, India, Pakistan, Vietnam, Ghana, and Nigeria (Asante et al. 2012; Grant et al. 2013; Wang et al. 2009). Biomonitoring data from an informal e-waste recycling site in Ghana showed a geometric mean urinary cobalt level of 1.6 μg/L for e-waste recycling workers (Asante et al. 2012). Formal e-waste recycling involves the use of properly designed equipment to safely remove recoverable materials from obsolete electronics while protecting workers and the environment. Personal breathing zone (PBZ), blood, and urinary cobalt have been reported for three formal e-waste recycling sites in Sweden (Julander et al. 2014). PBZ data showed a geometric mean cobalt concentration of 0.066 μg/m^3 in the collected inhalable fraction and 0.041 μg/m^3 in the total dust fraction. Median blood cobalt reported for two sampling occasions were 0.081 μg/L (first occasion) and 0.073 μg/L (second occasion, significantly higher than in office workers, $p \leq 0.05$). Median urinary cobalt reported for two sampling occasions were 0.25 μg/L and 0.21 μg/L.

U.S. cobalt occupational exposure level data available from NIOSH HETA surveys for electronics, "green" energy, and recycling indicate the following: workplace air levels range from not detected to 1.17 μg/m^3; the one reported surface wipe level was reported as "detected" (level of detection = 0.02 μg/sample); and the one reported skin (i.e., hand or neck) wipe level was reported as "detected" (level of detection = 0.04 μg/sample) (Beaucham et al. 2014; Thoburn and Larsen 1976).

2.6. Surgical Implants

Patients receiving cobalt-containing surgical implants (e.g., orthopedic joint replacements, spinal system, dental implants, etc.) are potentially exposed to cobalt particles that are released from wear and/or corrosion of the implants. Release of metals from joint replacements (articulating surgical devices) has been characterized the most and lower levels of metals are released from non-articulating surgical devices (such as plates and screws) (Keegan et al. 2008). The total number of hip replacements in the United States has been variously reported as 120,000 per year (Polyzois et al. 2012) or 400,000 per year (Devlin et al. 2013; Frank 2012) with total knee replacements over 600,000 per year (Bernstein and Derman 2014).

Total hip implants consist of (1) femoral head attached to a stem that is inserted in the thigh bone (usually made of ceramic or metal) and (2) a socket or cup that is anchored in the pelvis, which can be made of metal, ceramic or polyethylene. Cobalt-chromium-molybdenum (CoCrMo) alloy is the predominant alloy used in metal-containing implants, e.g., metal on metal (MoM) implants

(both articulating surfaces are metal), polyethylene on metal or metal on ceramic implants); other metals such as nickel, tungsten, iron, aluminum, and titanium may also be used in implants. A MoM resurfacing hip prosthesis consists of a femoral head capped with a metal covering. MoM hip implants may release a greater number and smaller particles than other types of implants and their use is declining in the United States (Bradberry et al. 2014; Devlin et al. 2013).

Total knee replacement implants consists of (1) a metallic femoral component that attaches to the end of the femur, (2) a plastic articulating layer, and (3) a tibial component that permanently binds the articulating layer to the top of the tibia (KRC 2015). The most common metal components consist of either cobalt chrome or titanium (Novick 2013). Unlike some hip implants with metal-to-metal contact, knee implants are designed so that metal surfaces do not contact each other.

Blood, serum and urine concentrations of cobalt and chromium generally rise after implantation of MoM hip prosthesis; maximum levels are usually reached in the first year after operation and decline in subsequent years (Bradberry et al. 2014). A review of 43 studies with different MoM bearing found that mean blood levels of cobalt ranged from 0.9 to 3.4 µg/L in patients with well-functioning implants (Jantzen et al. 2013) (see Table B-1 for cobalt levels in blood, serum, urine from studies of hip implant patients and Figure 2-1 and Figure 2-2 for graphs of urine and hair levels). Only one study reported levels in hair following placement of the implants and not studies were identified that reported levels in nails for hip implants; levels in hair 6 months and 12 months after implant were higher in hair from patients with metal-on-metal (53.3 µg/g at 6 months and 47.4 µg/g at 12 months) compared to patients with metal-on-polyethylene hip implants (3.4 µg/g at 6 months and 4.2 µg/g at 12 months). Urine levels identified from studies of hip implants reported as stable or that did not specifically address stability ranged from ~0.7 to 12 µg/L) (see Figure 2-1 and Table B-2 and Table B-3). These differences might be explained by factors such as variations in implant design, differences in patient demographics, or differences in the time elapsed between surgery and sample collection (Schaffer et al. 1999) and a lack of information regarding stability or wear status of the implant.

One in eight total hip implants requires revision within 10 years, and 60% of those are due to wear-related complications (Bradberry et al. 2014). Release of metal (wear debris) from implants results from friction between the bearing surfaces and corrosion from non-moving parts, which is caused by body fluids contacting the metal surfaces or by formation of an electrochemical couple between different metal components (Sampson and Hart 2012). The Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom issued a safety alert that proposed a level of 7 µg/L cobalt in blood as an action level for further clinical investigation and action (MHRA 2012) and 10 µg/L in serum was proposed by the Mayo Clinic in the United States (Mayo Clinic 2015). Dunstan et al. (2005) also reported blood cobalt levels of 19 and 52 µg/L for two individuals with radiologically loose metal-on-metal hip implants. In rare cases, high levels of cobalt from failed implants may be associated with toxicity. A review of literature published since 1950 identified 18 case reports of hip implant patients with cobalt-associated systematic toxicity (such as cardio-, neuro-, or ocular toxicity) and found that the median cobalt blood levels were 506 µg/L; range = 353 to 6,521) among 10 patients with failed ceramic implants and 34.5 µg/L (range = 13.6 to 398.6) among 8 patients with MoM implants (Bradberry et al. 2014). Removal of a joint replacement device that is associated with high cobalt ion levels generally results in decreased cobalt ion levels as reported by Rodriguez de la Flor et al. (2013) for 11 hip implant patients before revision with mean serum cobalt of 25.8 µg/L, which decreased to

12.1 µg/L after revision surgery (see Table B-2). Only one study (Rodriguez de la Flor et al. 2013) was identified that reported mean levels in urine (~205 µg/l) and hair (47.1 µg/g) (see Figure 2-2, and Table B-2) for unstable hip implants and no data were identified for cobalt levels in nails.

2.7. Other Sources of Exposure: Food, Consumer and Other Medical Products and Tobacco

The general public is exposed to cobalt primarily through consumption of food and to a lesser degree through inhalation of ambient air and ingestion of drinking water; average daily cobalt intake from food has been reported to be 11 µg/day (ATSDR 2004; Lison 2015). Although this amount includes cobalt as part of both vitamin B_{12} and other cobalt compounds (ATSDR 2004), green, leafy vegetables and fresh cereals generally contain the most cobalt (IARC 1991), and these plant sources of cobalt do not contain vitamin B_{12}. No estimate for an average dietary intake of cobalt in the United States was identified. Reported values for cobalt content of foods can vary due to differences in environmental cobalt levels, analytical difficulties, and inadequate analytical techniques.

A past use of cobalt (as cobalt sulfate) was as an additive in some beers (NTP 1998), which was based on a U.S. patent (Thorn and Wrey 1958) for the use of cobaltous nitrate or cobaltous chloride to reduce the tendency for beer to gush or "overfoam" and to increase its foam stability. However, in 1963 to 1964 a form of cardiomyopathy was linked with consumption of beer containing cobalt (Alexander 1969), and in 1966 the FDA prohibited addition of cobaltous compounds to any human food, including beer, in the United States (see Regulations and Guidelines in Part 2, Cancer Hazard Profile).

Higher cobalt intake may result from consumption of over-the-counter or prescription vitamin and mineral preparations (e.g., cobalt chloride). In the 1970s, oral intake of cobalt chloride was used to increase red blood cell counts in anemic patients (but discontinued when enlarged thyroids and goiters were observed at higher doses). In the last decade, oral administration of cobalt chloride has been used to correct excessive estrogen production during female hormone replacement therapy (Lippi et al. 2005; Tvermoes et al. 2013; Unice et al. 2012).

Cobalt is present in consumer products including cleaners, detergents, and soaps (ATSDR 2004). The NLM Household Products Database listed 6 products containing cobalt as an ingredient: 1 nickel metal hydride battery (5% to 10% cobalt), 4 dishwasher detergents (2 powders and 2 semi-solid pouches containing powder), and 1 spray car wax product (HPD 2014).

Different brands of tobacco have been reported to contain cobalt ranging from <0.3 to 2.3 µg/g dry weight; 0.5% of the cobalt content is transferred to mainstream smoke (WHO 2006). Smokers with no occupational exposure have been reported to have a significantly higher mean urinary cobalt concentration (0.6 µg/L, SD = 0.6) than non-smokers (0.3 µg/L, SD = 0.1); cobalt concentrations in blood were the same (Alexandersson 1988; as cited in IARC 1991). However, examination of urinary cobalt levels between cigarette smoke-exposed and unexposed NHANES participants for survey years 1999 to 2004 indicates that there was no significant difference in urinary cobalt levels for smokers and non-smokers (unadjusted for creatinine) (Richter et al. 2009). Richter et al. (2009) noted that while cobalt deficiencies were not reported, smoking does interfere with vitamin B_{12} absorption.

2.8. Potential for Environmental Exposure

Information on potential for environmental exposure discussed below includes data for releases (Section 2.8.1), occurrence (Section 2.8.2), and exposure (Section 2.8.3).

2.8.1. Releases

Approximately 75,000 metric tons of cobalt enters the global environment annually (CDI 2006; Shedd 1993). Cobalt is released through the natural processes of rock weathering and biological extraction (i.e., biochemical processes of bacteria and other microorganisms that extract cobalt from rocks and soils). Figure 2-3 shows cobalt released from anthropogenic processes (i.e., burning of fossil fuels, metal production and use). Similar amounts come from natural (40,000 metric tons) and anthropogenic (35,000 metric tons) sources; the majority of the natural source contribution is from biochemical processes and the majority of the anthropogenic contribution is from metal production and use.

Cobalt's widespread use in numerous commercial, industrial (e.g., mining and extraction from ores), and military applications results in releases to the environment through various waste streams. According to the U.S. EPA Toxics Release Inventory (TRI), total reported on- and offsite release of cobalt and cobalt compounds was approximately 5.5 million pounds from 723 facilities in 2013 (USEPA 2014a; 2014b; 2014c). Calculations based on media-specific release data from TRI indicate that releases to land accounted for 82% of total releases, offsite disposal for 15%, and underground injection, air, and water for 1% each in 2013. The scenarios that generally contribute most to U.S. releases of cobalt and cobalt compounds as reported to EPA (USEPA 2014d) include gold, copper, and nickel ore mining, hazardous waste treatment and disposal, non-ferrous metal smelting and refining, fossil fuel electric power generation, and chemical operations (e.g., petrochemical manufacturing and synthetic dye and pigment manufacturing). Recycling of e-waste can result in releases to the environment (particularly from informal e-waste recycling; see Section 2.5.5). Other potential exposure scenarios (e.g., copper smelting) exist, but no air data were identified.

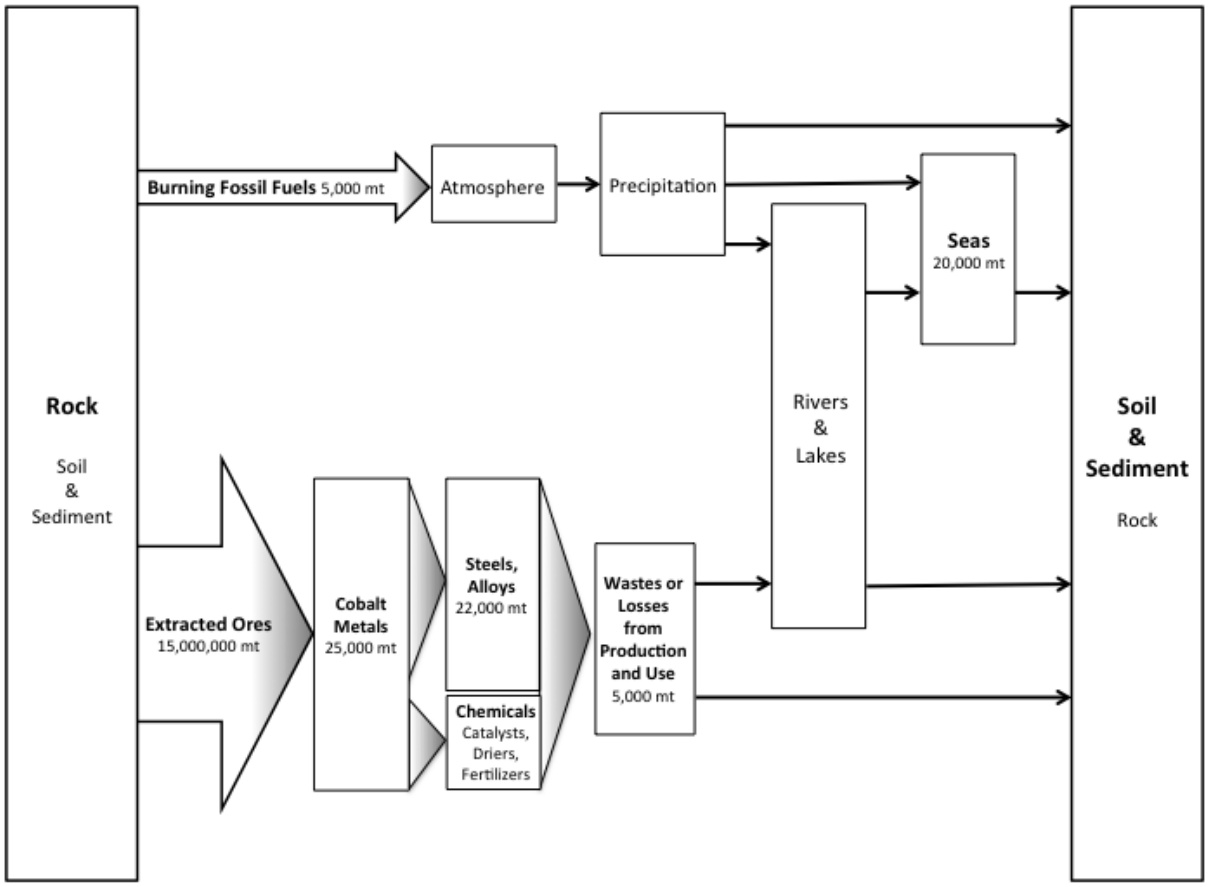

Figure 2-3. Flow of Cobalt Released from Anthropogenic Processes

Adapted from CDI (2006); Shedd (1993).

2.8.2. Occurrence

The average concentration of cobalt in ambient air in the United States has been reported to be approximately 0.4 ng/m^3 (ATSDR 2004). Levels can be orders of magnitude higher near source areas (e.g., near facilities processing cobalt-containing alloys, compounds, etc.). Sources of cobalt in the atmosphere can be natural (e.g., wind-blown continental dust, seawater spray, volcanoes, forest fires, and marine biogenic emissions), and anthropogenic (e.g., burning of fossil fuels, mining and smelting of cobalt-containing ores, hazardous waste treatment and disposal, etc.) (ATSDR 2004; USEPA 2012; 2014a)).

Median cobalt concentration in U.S. drinking water has been reported to be <2.0 µg/L; however, levels as high as 107 µg/L have been reported. It is unclear whether higher levels could indicate cobalt being picked up in distribution systems (ATSDR 2004). Cobalt concentrations have been reported to range from 0.01 to 4 µg/L in seawater and from 0.1 to 10 µg/L in freshwater and groundwater (IARC 2006).

Studies have reported cobalt soil concentrations ranging from 0.1 to 50 ppm. However, soils near ore deposits, phosphate rock, ore smelting facilities, soils contaminated by airport or highway

traffic, or other source areas may contain higher concentrations (e.g., soil cobalt concentrations as high as 12,700 ppm reported near hard-metal facilities) (IARC 2006). The soil concentration of cobalt available to be taken up by plants has been reported to range from 0.1 to 2 ppm (IARC 2006).

2.8.3. Exposure

Information on exposures to cobalt from environmental releases is limited, and no data for U.S. exposures were identified. Biomonitoring research has confirmed general public exposure to cobalt in scenarios including non-ferrous metal mining (see Figure 2-1). A study of metal exposure from mining and processing of non-ferrous metals in Katanga, Democratic Republic of Congo found that geometric mean urinary cobalt concentrations were 4.5-fold higher for adults and 6.6-fold higher for children in urban and rural communities near mines and metal smelters than in rural communities without mining or industrial activities (Cheyns et al. 2014).

2.9. Summary and Synthesis

Several lines of evidence indicate that a significant number of people living in the United States are exposed to cobalt and cobalt compounds. This evidence includes cobalt and several cobalt compounds being high-production-volume chemicals, widespread use in numerous commercial, industrial, and military applications, and biological monitoring data (i.e., urine, blood, hair, and nails) demonstrating exposure in occupationally and non-occupationally exposed populations. TRI data indicate that production- and use-related releases of cobalt and cobalt compounds have occurred at numerous industrial facilities in the United States.

Biomonitoring studies measuring cobalt in the urine of people exposed to cobalt from different sources indicate that the highest levels were generally seen for occupational exposures and unstable hip implants; lower cobalt levels were due to exposure from stable hip implants or the environment, or in the general public (source of exposure unknown). In general, levels of cobalt in blood (including whole blood, plasma, and serum), in hair, and in nails show a similar pattern to those for urinary cobalt levels.

The primary route of occupational exposure to cobalt is via inhalation of dust, fumes, mists containing cobalt, or gaseous cobalt carbonyl. Dermal contact with hard metal and cobalt salts can result in systemic uptake. Occupational exposure to cobalt occurs during (1) the refining of cobalt, (2) the production of cobalt powders, (3) use in the hard metal, diamond tool, and alloy industries (including the production and use of these cobalt-containing products), use to make chemicals, pigments, and electronics, and (4) in the recycling of electronics (more of a global than U.S. concern). Workers regenerating spent catalysts may also be exposed to cobalt sulfides. Occupational exposure has been documented by measurements of cobalt in ambient workplace air, worker blood and urine, and deceased worker lung tissue. U.S. occupational exposure data are available for the following industries: metallurgical; cemented carbides and bonded diamonds; chemicals and pigments; and electronics, “green” energy, and recycling.

Some of the highest levels of cobalt reported in blood or urine have been associated with failed medical devices (such as metallic hip implants containing cobalt alloys). Levels of cobalt reported in blood or urine from stable hip implants are lower than those reported for unstable hip implants and occupational exposures but higher than those reported for exposures from the environment or in the general public.

Although exposure to cobalt in the general public can occur via inhalation of ambient air and ingestion of drinking water, however, food has been reported to be the largest source of cobalt exposure to the general public. Higher cobalt intake may result from consumption of over-the-counter or prescription mineral preparations. Other sources of exposure to cobalt and cobalt compounds include some household consumer products, primarily dishwasher detergents and nickel metal hydride batteries.

3. Disposition and Toxicokinetics

Disposition and toxicokinetics refer to how a chemical can enter and leave the body, what happens to it once it is in the body, and the rates of these processes. Section 3.1 discusses the disposition of cobalt and cobalt compounds in humans and experimental animals, and toxicokinetic data are presented in Section 3.2. Disposition and toxicokinetic data are important because they describe various factors that affect the toxicity of a chemical. These factors include routes and rates of absorption, distribution, and retention; routes of elimination; and gender and/or species differences in these factors. The mechanistic implications of these data are discussed in Section 7.

3.1. Disposition

Disposition includes absorption, deposition, distribution, metabolism, retention, and excretion. The disposition of cobalt is affected by several factors including the chemical form, solubility, dose, particle size, route of exposure, nutritional status, and age of the species exposed. The primary exposure, distribution, and excretion pathways of cobalt are illustrated in Figure 3-1. Data derived from studies in humans are discussed in Section 3.1.1 while studies in experimental animals are discussed in Section 3.1.2.

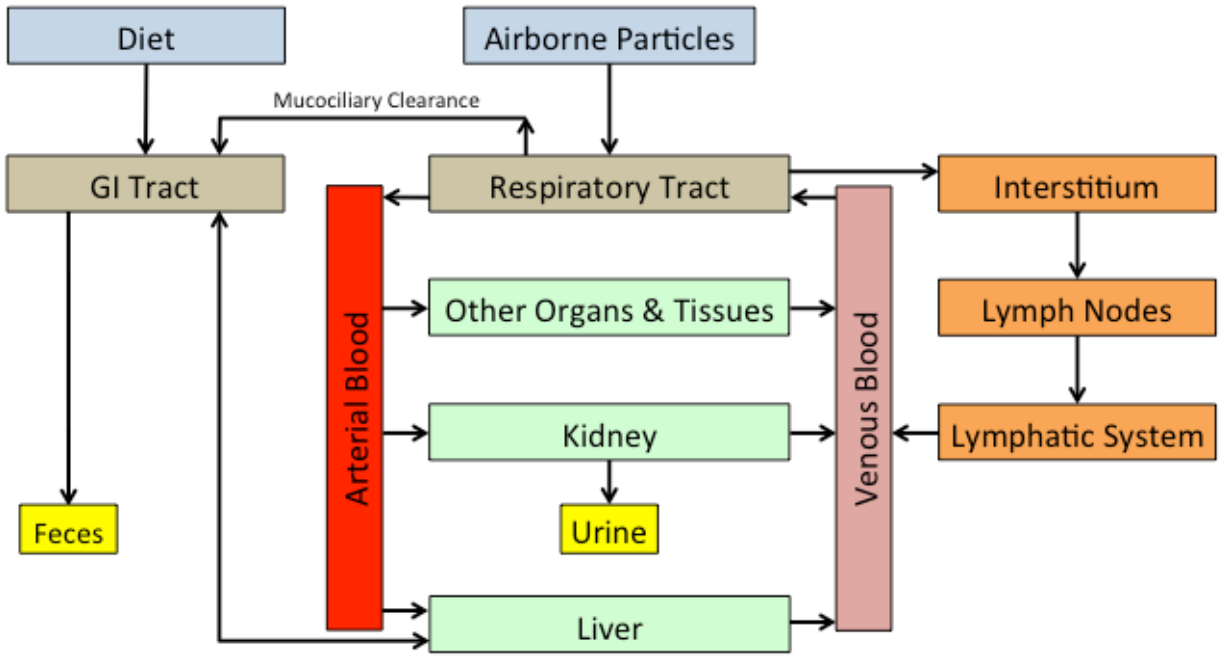

Figure 3-1. Cobalt Disposition

Source: Adapted from Keegan et al. (2008).

3.1.1. Humans

Dietary intake of cobalt has been reported as the largest source of exposure for the general population; an average daily intake of cobalt in Canada was reported as 11 µg/day (ATSDR 2004) (see Section 2.7). Most of the cobalt in the diet is inorganic with a very small fraction from

vitamin B_{12} (Lison 2015). The normal range of cobalt concentrations (nonoccupational exposure) in the blood and urine are about 0.1 to 0.5 µg/L and <2 µg/L, respectively (IARC 2006; Paustenbach et al. 2013) (see Section 2). About 90% to 95% of cobalt in blood is bound to serum albumin while the concentration of free cobalt is about 5% to 12% of the total cobalt concentration (Paustenbach et al. 2013; Simonsen et al. 2012). Letourneau et al. (1972) showed that a dose of vitamin B_{12} had no impact on retention of inorganic cobalt in humans. The total body burden of cobalt in humans is estimated as 1.1 to 1.5 mg with about 85% present in the vitamin B_{12} organometallic complex (Paustenbach et al. 2013; WHO 2006).

Absorption

Cobalt absorption from the gastrointestinal (GI) tract is highly variable, with reported values ranging from <5% to 97% (Holstein et al. 2015; IARC 2006; NTP 2014d; Paustenbach et al. 2013; Smith et al. 1972; WHO 2006). Unice et al. (2012) suggested a central tendency value of 25% for GI absorption of soluble inorganic cobalt while Unice et al. (2014) assumed GI absorption of 20% to 45% for aqueous forms and 10% to 25% for solid forms. Cobalt concentrations in whole blood increased 9 to 36 times above normal background concentrations in volunteers who ingested a liquid dietary supplement that contained cobalt chloride for up to 16 days (Tvermoes et al. 2013). Soluble cobalt compounds are better absorbed than insoluble forms (Christensen and Poulsen 1994; Christensen et al. 1993). For example, men and women volunteers who ingested tablets containing soluble cobalt chloride ($CoCl_2$) had approximately 10-fold higher concentrations of cobalt in blood and 50- to 90-fold higher concentrations in urine than when they ingested tablets containing insoluble cobalt oxide (Co_3O_4) (Christensen et al. 1993). Controlled studies in human volunteers also indicate that GI uptake is higher in women than in men with adjusted mean whole blood concentrations about two-fold higher in women (Christensen et al. 1993; Finley et al. 2013). The higher cobalt uptake in women may be due to a higher incidence of iron deficiency since cobalt absorption efficiency is higher in individuals with iron deficiency (31% to 71% compared to 18% to 44% in control subjects) (Sorbie et al. 1971; Valberg et al. 1969). Meltzer et al. (2010) reported that cobalt whole blood concentrations were significantly elevated in women with low serum ferritin concentrations compared to women with higher serum ferritin concentrations and in women with mild to moderate anemia compared to women with only slightly reduced hemoglobin. Low iron status was a prerequisite for high blood concentrations of cobalt; however, not everyone with low iron status had increased blood levels of cobalt. These data suggest that cobalt and iron may share a common gastrointestinal uptake mechanism that may be upregulated with anemia or iron deficiency (Paustenbach et al. 2013). Other nutritional factors may affect cobalt absorption due to the formation of complexes with certain organic anions (e.g., amino acids) present in foods.

Studies describing absorption of cobalt from the respiratory tract in humans are limited. Cobalt levels in blood and urine of workers generally increase in proportion to inhalation exposure levels to airborne cobalt dust and fumes, especially when workers were exposed to soluble cobalt-containing particles (IARC 2006; NTP 2014d). The pattern of urinary excretion of cobalt in workers exposed to less soluble cobalt oxide particles indicated a lower absorption rate and longer retention time in the lungs. Deposition in the respiratory tract primarily depends on particle size and breathing pattern (ATSDR 2004; WHO 2006). In general, particles larger than 2 µm tend to deposit in the upper respiratory tract due to higher airstream velocities and inertial impaction. These particles are readily cleared through mucociliary action and swallowed. Smaller particles escape inertial impaction and deposit in the bronchiolar or alveolar regions via

sedimentation and diffusion. Particles deposited in the respiratory tract may dissolve and be absorbed into the blood or undergo phagocytosis or endocytosis by macrophages. In addition, some nanoparticles can translocate rapidly from the lungs to the mediastinal lymph nodes and bloodstream (Luyts et al. 2013). Recent in vitro studies with human lung cells show that water-insoluble cobalt oxide particles (CoO or Co_3O_4) are readily taken up through endocytosis and are partially solubilized at the low pH within lysosomes while soluble cobalt salts utilize cellular transporters such as calcium channels or the divalent metal ion transporter to enter cells (Ortega et al. 2014; Papis et al. 2009; Sabbioni et al. 2014b; Smith et al. 2014). Controlled aerosol studies using human volunteers show that about half of the initial lung burden of inhaled cobalt oxide (Co_3O_4) particles may remain in the respiratory tract after six months (Bailey et al. 1989; Foster et al. 1989).

Dermal absorption of cobalt was demonstrated in two studies that measured increased cobalt concentrations in the urine of volunteers who immersed their hands in hard metal dust containing 5% to 15% cobalt for 90 minutes (Scansetti et al. 1994) or in a used coolant solution containing 1,600 mg/L cobalt for one hour (Linnainmaa and Kiilunen 1997). Cobalt also accumulated in the fingernails of three cobalt-sensitive patients after immersing a finger in a cobalt salt solution for 10 minutes/day for 2 weeks (Nielsen et al. 2000). In vitro percutaneous absorption studies were conducted with cobalt powder dispersed in synthetic sweat and applied to human skin mounted on Franz diffusion cells (Larese Filon et al. 2009; Larese Filon et al. 2007; Larese Filon et al. 2004). The mean permeation flux was 0.0123 $\mu g/cm^2/hr$, the lag time was 1.55 hr, and the permeation coefficient was 0.00037 cm/hr. Median cobalt concentrations in the receiving phase indicated that significantly more (~400 fold) cobalt penetrated damaged skin compared with intact skin (Larese Filon et al. 2009). Cobalt was detected in its ionic form in both the donor and the receiving phase. Significant amounts of cobalt also remained within the skin. These experiments showed that skin absorption was closely related to the capacity of synthetic sweat to oxidize metallic cobalt powder to soluble cobalt ions. No significant dermal absorption occurred when cobalt was dispersed in a saline solution (Larese Filon et al. 2004).

Distribution and Excretion

Numerous studies have shown that cobalt is found in blood, urine, hair, nails, and most other tissues. (See (1) Figure 2-1, Figure 2-2 or Table B-2 and Table B-3 for studies of cobalt levels in blood, urine, hair, and nails in specific exposed groups and the general population and (2) Table B-4 for cobalt levels reported in surrogate (hair and nails) or target tissues from cancer patients and referent groups, e.g., patients with other non-cancer diseases or healthy controls reported in several clinical studies.) In humans, inorganic cobalt is distributed to liver, kidney, heart, and spleen with lower concentrations found in bone, hair, lymph, brain, and pancreas (Paustenbach et al. 2013; WHO 2006). Cobalt chloride administered intravenously (i.v.) or orally to human volunteers was distributed primarily to the liver (Jansen et al. 1996; Smith et al. 1972). Whole body radioisotope scans (measured at various times up to 1,000 days) following i.v. administration of inorganic cobalt indicated that 10% to 30% (mean 20%) was found in the liver (Smith et al. 1972). Cobalt levels in plasma declined rapidly in this study due to rapid distribution to tissues and renal excretion; however, about 9% to 16% of the administered dose was retained with a half-life of about 800 days. Measurements of cobalt retention for up to 1,018 days indicated that about one fifth of the total body content was present in the liver. Cobalt can also transfer to human milk and across the placenta (Rudge et al. 2009; Wappelhorst et al. 2002). Most of the cobalt in plasma is bound to leukocytes or plasma proteins with a maximum

free fraction of 12%. Free cobalt is also taken up by red blood cells via a membrane transport pathway shared with calcium (Simonsen et al. 2011; Simonsen et al. 2012). Uptake of cobalt by red blood cells is practically irreversible because the ions bind to hemoglobin and are not extruded by the calcium pump. Thus, it has been speculated that cobalt partitions primarily into tissues with high calcium turnover and accumulates in tissues with slow turnover of cells although cobalt can be detected in most tissues. Although elevated concentrations of cobalt have been reported in the liver and kidney (oral or parental exposure) or lung (inhalation of insoluble particles), cobalt levels in the body do not appear to increase in any specific organ with age (IARC 2006; Lison 2015; Paustenbach et al. 2013).

Renal excretion of absorbed cobalt is rapid over the first days following exposure but is followed by a second, slower phase that lasts several weeks (IARC 2006; Simonsen et al. 2012). However, a small proportion (~10%) is retained in the tissues with a biological half-life of 2 to 15 years. Controlled experimental studies in humans indicate that 3% to 99% of an orally administered dose of cobalt is excreted in the feces and primarily represents unabsorbed cobalt (WHO 2006). Fecal elimination decreases as cobalt solubility increases. Following i.v. administration of cobalt chloride to 6 volunteers, fecal elimination accounted for about 2% to 12% of the administered dose while about 28% to 56% was eliminated in the urine after 8 days (Smith et al. 1972). Valberg et al. (1969) reported similar results in subjects administered cobalt by intramuscular injections and followed for 10 days (~6% excreted in feces and 58% in urine). Solubility and particle size affect elimination following inhalation exposure (WHO 2006). Clearance of cobalt particles from the lungs has been reported to follow three-phase kinetics (see Section 3.2.1). Large particles are rapidly cleared from the upper airways via the mucociliary pathway, swallowed, and eliminated in the feces. Urinary excretion of inhaled cobalt particles increases with time. Foster et al. (1989) reported that following inhalation of cobalt oxide (Co_3O_4) particles, about 17% was cleared mechanically to the gastrointestinal tract and eliminated in the feces within the first week. After 6 months, about 33% of the initial lung burden was eliminated in the urine and about 28% was eliminated in the feces.

3.1.2. Experimental Animals

The disposition of cobalt has been investigated in mice, rats, hamsters, guinea pigs, rabbits, dogs, miniature swine, and baboons and show some similarities with human studies. These data are briefly reviewed below. As in humans, cobalt as part of vitamin B_{12} is an essential micronutrient in experimental animals. However, cobalt deficiency has been described in ruminants (e.g., sheep, goats, and cattle) raised in areas with very low cobalt (Yamada 2013). Cobalt supplements were beneficial in these cases because cobalamin can be synthesized by gut bacteria and absorbed.

Absorption

Cobalt absorption in experimental animals is highly variable and depends on the chemical form of the compound, age of the animal, species, and nutritional status (Ayala-Fierro et al. 1999; NTP 2014d; WHO 2006). In rats, cobalt chloride was absorbed more efficiently from the gastrointestinal tract than insoluble cobalt oxide (Co_3O_4) (13% to 34% compared to 1% to 3%) (NTP 2014d). Gastrointestinal absorption of soluble cobalt compounds was lower in cows (1% to 2%) and guinea pigs (4% to 5%) compared with rats. Cobalt absorption was 3% to 15% greater

in young rats and guinea pigs than in adults (Naylor and Harrison 1995). As observed in humans, cobalt absorption was increased in iron-deficient rats (Thomson et al. 1971).

Inhalation studies of cobalt metal, cobalt oxides, or soluble cobalt salts in experimental animals show that dissolved cobalt is absorbed rapidly from the lungs while a small percentage is absorbed over several months (IARC 1991; 2006; Kyono et al. 1992; Leggett 2008; NTP 1998; 2014d). Cobalt particles are mechanically cleared by mucociliary action and swallowed or phagocytized by macrophages. The fraction of the remaining lung content of cobalt oxide (Co_3O_4) translocated to blood per day (i.e., dissolution of particles and absorption into the blood) varied according to particle size, particle surface area, species, and time (Andre et al. 1989; Bailey et al. 1989; Collier et al. 1989; Kreyling et al. 1991a; Patrick et al. 1989). Initially, translocation of the smaller particles (0.8 μm) ranged from about 0.4%/day in baboons to about 1.4%/day in HMT (inbred strain) rats. Initial translocation rates for the larger particles (1.7 μm) were lower in all species and ranged from about 0.2%/day in baboons to 0.6%/day in HMT rats (Bailey et al. 1989). Translocation rates for higher density Co_3O_4 particles were about a factor of 3 slower than for less dense particles (Bailey et al. 1989; Kreyling et al. 1991a). Translocation rates reported by Bailey et al. (1989) showed a variety of different forms with time, particularly for the smaller particles; this is discussed further in the following section (Figure 3-2). Translocation of cobalt from the lung to the blood also was significantly faster in younger rats compared with older rats (Collier et al. 1991).

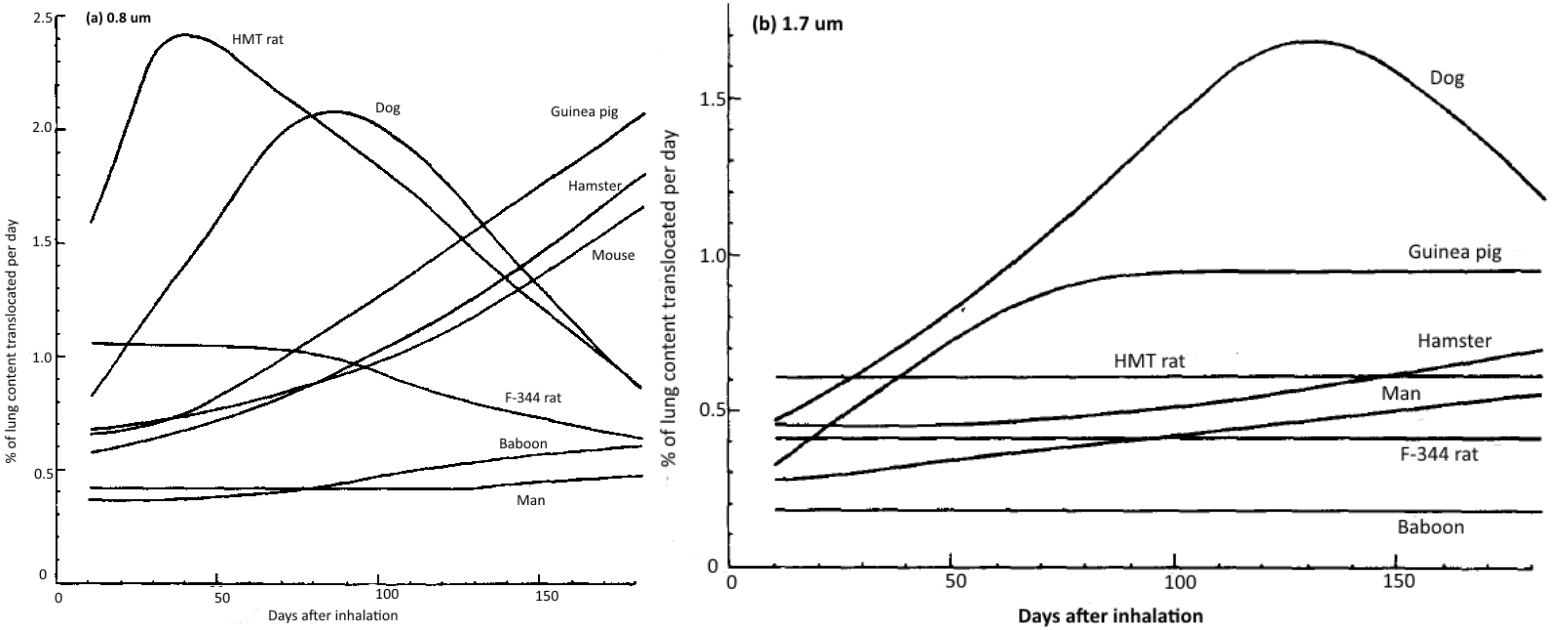

Figure 3-2. Rate of Translocation of Cobalt from Lung to Blood Following Inhalation of Cobalt Oxide Particles

Source: Bailey et al. (1989). Used with permission.

Dermal absorption of cobalt (applied as cobalt chloride) has been investigated in mice, guinea pigs, and hamsters (Inaba and Suzuki-Yasumoto 1979; Kusama et al. 1986; Lacy et al. 1996). Dermal absorption of cobalt applied to intact or acid-burned skin of mice was about 0.1% after one hour but increased to 25% to 50% when applied to skin damaged by incision, abrasion, or punctures (Kusama et al. 1986). In a similar study in guinea pigs, absorption of cobalt through intact skin was less than 1% while absorption through abraded skin was about 80% 3 hours after exposure (Inaba and Suzuki-Yasumoto 1979). Lacy et al. (1996) did not report the amount of cobalt absorbed through the intact skin of hamsters but reported that small amounts of cobalt were detected in urine 24 to 48 hours after application and that much of the metal was retained in the skin after 48 hours. These authors also reported that uptake of cobalt by keratinocytes exposed in vitro was about 5% of the dose.

Distribution and Excretion

Absorbed cobalt is distributed rapidly to all tissues in experimental animals and is similar to that in humans (NTP 2014d; WHO 2006). Edel et al. (1994) reported that tissue distribution depended on dose, route of administration (oral versus parenteral), and time. Following oral administration of cobalt compounds, the highest tissue concentrations generally occur in the liver and kidney with lower amounts in the heart, spleen, muscle, bone, brain, pancreas, lung, and gonads (Ayala-Fierro et al. 1999; Bourg et al. 1985; Clyne et al. 1988; Gregus and Klaassen 1986; Hollins and McCullough 1971; Thomas et al. 1976). Following single-dose parenteral administration, some studies reported that concentrations were initially highest in the liver and kidney but declined rapidly (Hollins and McCullough 1971; Thomas et al. 1976). However, Edel et al. (1994) reported higher concentrations in the lung, large intestine, kidney, liver, and spleen 24 hours after a single i.v. injection of cobalt chloride. One hundred days after a single i.p. injection, tissue distribution was affected by dose with higher concentrations in the spleen, pancreas, and bone following the lower dose but mainly in bone following higher doses with some accumulation in the heart.

Distribution of cobalt following inhalation exposure is similar to that observed for other routes with the exception of greater retention in the lung for both soluble and insoluble cobalt (Bailey et al. 1989; Bucher et al. 1990; Collier et al. 1991; Kerfoot et al. 1975; Kreyling et al. 1986; Kyono et al. 1992; NTP 2014d; Patrick et al. 1989; Patrick et al. 1994; Wehner and Craig 1972). Long-term retention of insoluble cobalt particles and soluble cobalt salts deposited in the lung shows wide interspecies variation and represents a potential continuing source of cobalt ion release (Bailey et al. 1989; Kreyling et al. 1991a; Patrick et al. 1994). In addition, some particles can translocate to the pulmonary interstitium where they are cleared from the lungs through the lymphatic system (Pauluhn 2009). Nanoparticles also may penetrate the alveolar membrane and distribute to extrapulmonary tissues via the circulation (Mo et al. 2008). The average size of the long-term retention component in humans is greater than in experimental animals (Bailey et al. 1989; Leggett 2008). Retention of insoluble cobalt oxide (Co_3O_4) particles (0.8 μm and 1.7 μm) after 90 and 180 days are shown in Table 3-1. These data show that lung retention is generally greater for larger particles than smaller particles and suggests temporal interspecies differences in the rate of particle dissolution and absorption. However, the percentage of total body cobalt content found in the lungs 30 and 180 days after exposure generally exceeded 90% in all species for both particle sizes. In spite of considerable clearance from the lung, very little accumulated in other tissues.

Table 3-1. Interspecies Comparison of Lung Retention of Cobalt Oxide (Co_3O_4)

Species/Strain	Lung Retention (%)[a]			
	90 Days		180 Days	
	0.8 µm	1.7 µm	0.8 µm	1.7 µm
Human	64	75	45	56
Baboon	55	55	26	37
Dog, beagle	27	45	5.5	12
Guinea pig	49	46	8.3	15
Rat, HMT (1985)	5.2	20	1.3	8.0
Rat, HMT (1986)	5.3	18	1.2	7.2
Rat, F-344	14	25	4.7	9.2
Rat, Sprague-Dawley	8	39	1.0	15
Hamster, Syrian golden	21	35	3.4	12
Mouse, CBA/H	15	ND	2.8	ND

Source: Bailey et al. (1989).
ND = no data.
[a]Calculated as the fraction of lung content (measured as activity of ^{57}Co) at 90 and 180 days relative to the lung activity at three days after inhalation. The amount retained after three days was thought to be representative of the amount deposited in long-term lung retention sites because, by this time, the rapid phase of mucociliary clearance should be complete.

Kreyling et al. (1991a) conducted a lung clearance study in baboons, dogs, and HMT rats using Co_3O_4 particles (0.9 µm diameter) that were chemically similar to those used by Bailey et al. (1989) but had a higher density (i.e., less porous) and a smaller specific surface area. In each species tested, the denser 0.9 µm particles had higher lung retention after 90 and 180 days than the more porous 0.8 µm particles.

Bailey et al. (1989) and Kreyling et al. (1991a) also applied a simple dissolution model to predict the diverse shapes of the time-dependent rate of cobalt translocation to blood from Co_3O_4 particles deposited in the lungs. This model was based on the assumption that the dissolution rate is proportional to the specific surface area of the particle (surface area per unit mass). Since the specific surface area increases as the particles dissolve, a high initial dissolution rate results in a rapid increase in specific surface area and, in turn, causes an increase in the dissolution rate with time. Thus, translocation will peak when another slow clearance mechanism is superimposed on particle dissolution. A small fraction of the dissolved cobalt will not immediately translocate to the blood but will be retained in the lungs and slowly released. The translocation rate was defined in terms of two parameters: (1) the initial fractional absorption rate and (2) the fraction of dissolved cobalt that is retained long-term in tissues (predicted as 1% to 10%). Although there were some discrepancies between the curves predicted by the model and the observed translocation rates (see Figure 3-2), overall, the model accounted remarkably well for the different forms of translocation rates by varying the fractional dissolution rate and the long-term retention fraction and suggested marked species differences in these parameters. The rate-determining step for translocation was intracellular particle dissolution.

In an attempt to better understand the basis for the interspecies differences in the rate of Co_3O_4 absorption, species differences in lung retention and translocation (absorption) of soluble cobalt

chloride also was investigated (Patrick et al. 1994). The mean fraction of cobalt retained in the lungs in the various test species administered cobalt chloride or cobalt nitrate (dog only) (expressed as percent of initial body content) ranged from about 0.13% (hamster) to 1.2% (dog, estimated value) after 100 days while the fraction retained in the whole body ranged from 0.35% (hamster) to 3.2% (dog). Lung retention by species declined in the following order: dog > HMT rat > guinea pig > baboon > F344 rat > hamster. These long-term retention values were lower than the predicted values of 1% to 10% used in the model (see previous paragraph). The mean fraction of cobalt retained in the lungs after 100 days in the various test species (expressed as percent of cobalt remaining in the body after 100 days) ranged from 11.8% (baboon) to 60% (HMT rat) with no significant accumulation in other organs with the exception of the trachea. However, relative concentrations in the trachea showed no significant interspecies differences. During the first week, 90% or more of the administered dose was cleared from the lung and was similar to the pattern observed for i.v.-injected $Co(NO_3)_2$ in the same species (Bailey et al. 1989; Patrick et al. 1994). These data suggest that interspecies differences in the time-dependent absorption rates (i.e., translocation of dissolved cobalt from the lung to the blood) for inhaled Co_3O_4 particles were not explained by differences in the fraction of dissolved cobalt retained long-term in lung tissue. Kreyling et al. (1991b) also found little interspecies variation in pH within alveolar macrophages; therefore, interspecies differences in translocation rates were not explained by differences in phagolysosomal pH. Alternative explanations for these interspecies differences could include a second long-term phase of lung retention as particles or as particle fragments (Patrick et al. 1994).

A recent inhalation study with rats and mice exposed to cobalt metal showed that cobalt concentrations increased with increasing exposure in all tissues examined; however, tissue burdens normalized to exposure levels did not increase with increasing exposure, with the exception of the liver (NTP 2014d). Cobalt tissue concentrations (µg Co/g tissue) in male and female rats showed the following order: lung > liver > kidney > femur > heart > serum > blood (NTP 2014d). Tissue cobalt burdens (µg Co/tissue) showed a similar order with the exceptions that liver accumulated more cobalt than the lung, and the heart accumulated more cobalt than the femur. At three weeks post-exposure in female rats, cobalt concentrations were markedly reduced in blood, serum, and lung (no data were available for other tissues). Tissue distribution in mice was similar to that observed in rats but concentrations in the femur and heart were similar to concentrations in blood and serum. These data from rodents exposed to cobalt by inhalation indicated that tissues tended to accumulate cobalt at concentrations greater than levels found in the blood and serum and that cobalt was distributed to extra-pulmonary tissues.

Cobalt excretion occurs rapidly with the majority of the administered dose eliminated within hours to a few days after exposure ceases (Gregus and Klaassen 1986; Paustenbach et al. 2013). Cobalt is excreted in the urine, feces, and bile with similar excretion patterns reported for all species studied (ATSDR 2004; NTP 2014d; WHO 2006). Most of the i.v.-injected dose of cobalt chloride (~73% to 75%) was eliminated in the urine while smaller amounts were excreted in the bile (2% to 5%) and feces (10% to 15%) (Ayala-Fierro et al. 1999; Gregus and Klaassen 1986). Soluble cobalt compounds are cleared from the lungs at a faster rate than less soluble compounds. The rate of urinary excretion correlates with the rate of translocation of cobalt from the lungs to the blood while fecal excretion rates correlate with the rate of mechanical clearance of cobalt particles from the lung (ATSDR 2004; WHO 2006). Following oral exposure, cobalt is primarily excreted in the feces but the rate decreases as cobalt particle solubility increases (WHO

2006). However, species and sex differences in cobalt excretion rates have been reported. Cobalt urinary excretion rates (µg/16 hr) in male rats were about two-fold higher than in females exposed to various concentrations of cobalt sulfate for 13 weeks (Bucher et al. 1990). In another study, mean urinary excretion rates of cobalt (administered as $CoCl_2$ solution to the lungs or inhaled as an aerosol) ranged from 0.002% of the initial body content per day in HMT rats to 0.026% per day in dogs (Patrick et al. 1994). Mean daily fecal excretion rates ranged from 0.0009% (dog) to 0.004% (HMT rat).

3.2. Toxicokinetics

Various toxicokinetic parameters of inorganic cobalt have been measured, and several pharmacokinetic models have been developed that describe cobalt disposition in the body (ATSDR 2004; Leggett 2008; Paustenbach et al. 2013; Unice et al. 2014; Unice et al. 2012). This section provides a brief review of toxicokinetic data in humans (Section 3.2.1) and laboratory animals (Section 3.2.2).

3.2.1. Humans

The kinetics of inhaled cobalt are determined by mechanical (mucociliary) clearance and by translocation to blood and the lymphatic system (Figure 3-1) (ATSDR 2004). Foster et al. (1989) calculated average translocation and mechanical clearance rates of inhaled cobalt oxide (Co_3O_4) particles in four human volunteers. The ratio of translocation to mechanical clearance was about 5:1 for particle sizes of 0.8 and 1.7 µm. Inhalation studies in workers and volunteers exposed to cobalt have shown that the elimination of poorly soluble cobalt metal or cobalt oxides (CoO or Co_3O_4) from the lungs is multiphasic with reported half-lives for the phases of 2 to 44 hours, 10 to 78 days, and years (Apostoli et al. 1994; Beleznay and Osvay 1994; Mosconi et al. 1994b; NTP 2014d; Newton and Rundo 1971; WHO 2006). The elimination pattern was independent of the degree of exposure. About 17% of the initial lung burden was eliminated within the first week while about 40% was retained at 6 months after exposure (Foster et al. 1989; WHO 2006). These elimination phases likely involve mucociliary clearance of cobalt particles from the tracheobronchial region, macrophage-mediated clearance of cobalt particles from the lungs, and long-term retention and clearance from the lung. The slower clearance with time likely reflects cobalt that is bound to cellular components in the lung (ATSDR 2004; Foster et al. 1989; Kreyling et al. 1986; WHO 2006). Studies in human volunteers administered cobalt chloride by i.v. injection also showed a multiphasic elimination pattern (Holstein et al. 2015; Jansen et al. 1996; Letourneau et al. 1972; Smith et al. 1972). These studies showed that 36% to 44% of the administered dose is cleared with a biological half-life of 6 to 12 hours, 45% to 56% is cleared with a biological half-life of 2 days to 60 days, and 9% to 11% is cleared with a biological half-life of 600 to 800 days (Paustenbach et al. 2013). Jansen et al. (1996) reported an apparent volume of distribution at steady state of 48 L that likely reflected inl accumulation in the liver (~50% of the administered dose).

Leggett (2008) developed a biokinetic model for inorganic cobalt that depicts recycling of cobalt between blood and four systemic tissues (liver, kidneys, skeleton, and other soft tissues) and transfer from blood to excretion pathways. The model assumes first-order kinetics, and parameter values are expressed as transfer coefficients (fractional transfers per day) that were largely derived from controlled human studies. Unice et al. (2014; 2012) further refined this model by incorporating different gastrointestinal absorption rates, adding compartments to

account for albumin-bound cobalt in intravascular and extravascular fluid, and accounting for additional parameters such as total blood volume, red blood cell age, and urinary excretion rates. The model was a reasonably good predictor of cobalt blood and urine concentrations measured in male and female volunteers who ingested a cobalt supplement for 16 days to 3 months (Tvermoes et al. 2013; Tvermoes et al. 2014; Unice et al. 2014).

3.2.2. Experimental Animals

Lung clearance kinetics of cobalt particles include both mechanical transport and translocation (Bailey et al. 1989; Kreyling et al. 1991a). Lung clearance of inhaled cobalt metal particles in rats and mice showed a well-defined two-phase elimination profile following 3-month or 2-year studies (NTP 2014d). The majority (>95% in rats and >82% in mice) of the deposited cobalt was cleared rapidly (half-life of 1 to 5 days) while the remainder was cleared more slowly (half-lives of ~20 to >400 days) depending on the concentration and study duration. Lung steady-state burdens were reached after approximately 6 months and were similar in rats and mice. Lung cobalt burdens were well below the levels that would cause lung overload. Other studies showed that interspecies differences in clearance patterns associated with mechanical transport and translocation were not correlated. Initial mechanical clearance rates were typically 10- to 20-fold greater in rodents than in other species, decreased monotonically with time, and were similar for different particle sizes. In contrast, interspecies differences in translocation rates varied by 3- to 10-fold, remained constant or increased and then decreased with time, and were affected by particle size (see Figure 3-2). Thus, in HMT rats, both rates were initially high, while in baboons and humans both rates were low. Mice, hamsters, and F344 rats had high rates of mechanical clearance but low to moderate rates of translocation while dogs had slow mechanical transport but rapid translocation.

Thomas et al. (1976) reported that the whole-body half-life of $^{60}CoCl_2$ administered by i.v. injection was longer in the mouse (495 days) than in the rat (309 days), monkey (183 days), or dog (180 days), but all were lower than values reported in humans (see Section 3.2.1). Other studies in rats and dogs showed multiphasic first-order elimination kinetics following oral, inhalation, or i.v. exposure (Table 3-2). These data indicate that soluble cobalt compounds are cleared faster than cobalt metal in rats and that the cobalt oxide particle clearance in dogs during the intermediate phase was proportional to particle size. Elimination of cobalt from the blood in the recent NTP (2014d) study also indicated rapid and slow clearance phases; however, it was not possible to fit the blood data to a two-compartment model due to the lack of early sampling times. However, cobalt elimination half-lives estimated from blood concentrations on the last day of exposure (2-week studies) and 3 weeks post-exposure were 9.2 to 11.1 days in female rats and 4.1 to 7.3 days in female mice.

Table 3-2. Elimination Half-lives for Cobalt Administered to Experimental Animals

Reference	Species: Exposure Route	Compound(s)	Elimination T½		
			Phase 1	Phase 2	Phase 3
Ayala-Fierro et al. (1999)	Male F344 rats: i.v.	$CoCl_2$	1.3 hr	4.3 hr	19 hr
Ayala-Fierro et al. (1999)	Male F344 rats: oral	$CoCl_2$	0.9 hr[a]	4.6 hr	22.9 hr

Reference	Species: Exposure Route	Compound(s)	Elimination T½		
			Phase 1	Phase 2	Phase 3
Menzel et al. (1989)	Male SD rats: inhalation	$CoCl_2$	1.8 hr	3.7–8.7 hr[b]	–
Kyono et al. (1992)	Male SD rats: inhalation	Co metal	52.8 hr[c] 52.8 hr[d]	156 hr[c] 172.8 hr[d]	–
Kreyling et al. (1986)	Male beagles: inhalation (endotracheal tube)	Co_3O_4 Co_3O_4 + CoO $Co(NO_3)_2$	0.5 d 1–4 d 0.8 d	6–80 d[e] 20–86 d[e] 27 d	300–380 d 340–440 d 400 d

– = No data.
[a]Absorption half-life.
[b]Calculated from elimination rate constants of 0.188 h^{-1} (single exposure) and 0.08 h^{-1} (repeat exposure).
[c]Lung.
[d]Blood.
[e]Half-lives were proportional to particle size.

3.3. Synthesis

Cobalt is absorbed from the GI tract, lungs, and skin and rapidly distributed throughout the body. Absorption from the gastrointestinal tract is highly variable and is affected by the chemical form, dose, age, formation of complexes with organic ions, and nutritional status. Soluble compounds are absorbed to a greater extent than poorly soluble forms. Current biokinetic models assume GI absorption of 20% to 45% for aqueous forms and 10% to 25% for solid forms. Studies in experimental animals indicate higher absorption in young rats and guinea pigs than in adults while studies in human volunteers indicate higher GI absorption in women than in men and may reflect iron status. Cobalt absorption from the GI tract is higher in iron deficient humans and experimental animals and suggests that cobalt and iron share a common uptake mechanism. Cobalt levels in blood and urine of workers generally increase in proportion to airborne concentrations. Although absorbed cobalt is distributed systemically, it does not accumulate in any specific organ with age. Translocation rates of cobalt from the lung to the blood show considerable interspecies variation with time and particle size with humans and baboons generally having lower rates than dogs or rodents, and the whole-body half-life of cobalt was longer in humans than in mouse, rat, monkey, or dog.

Cobalt excretion occurs rapidly with the majority of the administered dose eliminated within hours to a few days after exposure ceases. Cobalt is excreted in the urine, feces, and bile with similar excretion patterns reported for all species studied. Elimination in the feces primarily represents unabsorbed cobalt while absorbed cobalt is eliminated in the urine. Toxicokinetic studies indicate multiphasic elimination following inhalation of cobalt particles or i.v. injection of cobalt chloride and generally show shorter elimination half-lives in experimental animals compared to humans. Elimination half-lives reported for poorly soluble cobalt metal or cobalt oxide particles from human lung ranged from 2 to 44 hours, 10 to 78 days, and years. These elimination phases likely represent an initial rapid elimination from the tracheobronchial region via mucociliary clearance, macrophage-mediated clearance, and long-term retention and clearance. A similar pattern was reported in human volunteers given an i.v. injection of cobalt chloride with about 40% cleared with a half-life of 6 to 12 hours, 50% cleared with a half-life of 2 to 60 days, and 10% cleared with a half-life of 600 to 800 days.

4. Human Cancer Studies

Introduction

The objective of the cancer hazard evaluation of cobalt and cobalt compounds that release cobalt ions in vivo (hereinafter referred to as cobalt) is to reach a level of evidence conclusion (sufficient, limited, or inadequate) for the carcinogenicity of cobalt from studies in humans by applying the RoC listing criteria to the body of evidence.

In general, most of the human studies do not provide information on the type(s) of cobalt compounds to which the subjects were exposed.

The steps in the cancer hazard evaluation, including the location of the discussion of these steps in the document, are listed below.

1. Selection of the relevant literature included in the cancer evaluation (Section 4.1 and Cobalt Protocol (NTP 2014b)).
2. Description of the study methods and characteristics (Table C-1 through Table C-9) and evaluation of study quality and other elements related to the utility of the studies to inform the cancer hazard evaluation: Section 4.2 (cohort studies of lung cancer), Section 4.3 (case-control studies of esophageal, and other aerodigestive cancers (i.e., oral cavity, laryngeal, and pharyngeal cancers), and Table C-10 through Table C-12.
3. Cancer assessment: Lung (Section 4.2.3), esophagus (Section 4.3.3), and other cancers (Section 4.4).
4. Level of evidence conclusion for carcinogenicity (sufficient, limited, or inadequate) of cobalt from human studies (Section 4.5).

The cancer hazard evaluation of cobalt primarily focuses on cancers of the lung, the esophagus, and other aerodigestive cancers (i.e., oral cavity, laryngeal, and pharyngeal cancers) since these are the only tissue sites evaluated in multiple studies. (For rationale, see Protocol: Methods for Preparing the Draft Report on Carcinogens Monograph on Cobalt ["Cobalt Protocol"; NTP (2014b)] and Table 4-1). Because the occupational cohort studies primarily reported on lung cancer and the case-control studies reported on esophageal cancers and other aerodigestive cancers, this section is organized by study design (following the selection of literature): cohort studies and lung cancer are discussed in Section 4.2, case-control studies and esophageal cancer in Section 4.3, and aerodigestive and other cancers (reported in both case-control and cohort studies) in Section 4.4.

4.1. Selection of the Relevant Literature

Details of the procedures (such as the databases and literature search terms and screening methods) used to identify and select the primary studies and supporting literature for the human cancer evaluation are detailed in Appendix A and the cobalt protocol.

Primary epidemiologic studies were considered for the cancer evaluation if the study was (1) peer reviewed; (2) provided risk estimates (or sufficient information to calculate risk estimates) for cobalt and human cancer, and (3) provided exposure-specific analyses for cobalt at an

individual level, or based on the authors' report, cobalt exposure was probable or predominant in the population, job, or occupation under study.

Because cobalt can be released from hip and other joint implants, a preliminary literature search was also conducted to identify case reports and cohort studies of joint replacements or prosthetic devices. The case reports included at least 15 cases of malignant fibrous histiocytoma (12 cases reviewed by Hughes et al. 1987; Lucas et al. 2001; Min et al. 2008; Visuri et al. 2006), at least 5 cases of osteosarcoma (4 reviewed by Malcolm 1984; Visuri et al. 2006), at least 6 cases of other types of sarcoma (4 reviewed by Tayton 1980; van der List et al. 1988; Visuri et al. 2006), and at least 3 cases of non-Hodgkin or B-cell lymphoma (Cheuk et al. 2005; Dodion et al. 1983; McDonald 1981) occurring at the site of implantation of joint prosthetic devices (e.g., hip, knee, screws) containing cobalt alloys (primary cobalt-chromium). Case-reports of these types of cancer were also found among non-cobalt containing implants (reviewed by Visuri et al. 2006). The cohort studies (at least 16) were primarily record linkage studies conducted in Nordic countries, the United Kingdom, Austria and the United States, the majority of which did not provide information on the type of implants and most likely included patients with cobalt- and non-cobalt-containing implants. Two cohort studies (Visuri et al. 2010; Visuri et al. 1996) and patient series study (Visuri et al. 1996) reported on cancer risk among patients with McKee-Farrar implants, which contain a cobalt-chromium-molybdenum alloy. Overall, these studies were considered to be uninformative for evaluating effects due to cobalt because of study design (case reports have no comparison group), lack of specificity to cobalt (implants that are not made of cobalt or other metals present in cobalt-containing implants) and inadequate information on the extent of exposure to cobalt, and thus were excluded from the cancer assessment.

Studies of radioactive cobalt were also excluded, because of potential confounding from radioactivity. In general, cohort or case-control studies of populations with jobs, workplaces, or environmental exposures in which cobalt exposure may have occurred (e.g., studies of hard-metal workers) were excluded if a specific risk estimate for potential cobalt exposure alone was not reported.

Biomarker studies of cobalt and cancer were included if they were conducted within defined populations and provided risk estimates for cobalt levels and cancer. A series of clinical studies that compared cobalt and other metal levels in target tissues (such as tumors of different stages or normal tissue) or surrogates (e.g., hair, nails, blood) from cancer patients with a referent group (e.g., healthy humans, patients with cancer, other diseases) were identified and are summarized in Table B-2 (hair) and Table B-3 (tissues). For most studies, the source of the exposure was unknown, and it could not be distinguished whether metal levels could be a cause of cancer or whether the cancer process itself affected accumulation of cobalt in the tissue. Because these studies did not provide information to calculate an effect estimate, and most did not have defined methods for selecting the subjects, they are not included in the cancer hazard evaluation.

Environmental studies of cobalt and cancer were included if they were conducted within defined populations and provided risk estimates for cobalt levels and cancer. A total of four studies was identified, two of which investigated the relationship between cobalt in air to breast (Coyle et al. 2006) and lung (Coyle et al. 2005) cancer. The other two studies investigated the relationship between soil levels of cobalt and cancer (Kibblewhite et al. 1984; McKinley et al. 2013). None of the studies moved forward into the cancer hazard evaluation because they did not provide a

risk estimate (or sufficient information to calculate one) or exposure-specific analyses at the individual level.

4.2. Cohort Studies and Nested Case-control Studies Reporting on Lung Cancer

This section provides an overview of the cohort and nested case-control studies (Section 4.2.1), an overview of the adequacy of the studies to inform the cancer hazard evaluation (Section 4.2.2) and an assessment of the evidence from the studies on the association between cobalt exposure and lung cancer risk (Section 4.2.3).

4.2.1. Overview of the Methodologies and Study Characteristics

For each of the reviewed cohort studies, detailed data on study design, methods, and findings were systematically extracted from relevant publications, as described in the study protocol, and into Table 4-1, Table C-1 through Table C-7, and Table 4-2.

The available epidemiological studies that satisfy the criteria for consideration in the cancer evaluation consist of a series of occupational cohort or nested case-control studies conducted in five independent populations. These include a cohort of female Danish porcelain painters; a cohort of French electrochemical workers; and French cohorts of hard-metal workers, and stainless and alloyed steel workers; and Norwegian nickel refinery workers.

Tüchsen et al. (1996) reported on cancer incidence at multiple tissue sites among 1,394 female porcelain painters employed in underglazing departments of two porcelain plate factories in Denmark where cobalt-aluminate spinel and/or cobalt silicate was used, compared with top glaze decorators in a department in one of the factories without cobalt exposure.

Studies on the French electrochemical workers producing cobalt were reported in two publications. The first publication was on a historical mortality cohort and nested case-control study of lung cancer among 1,143 cobalt production workers in a French electrochemical plant (Mur et al. 1987). This study included workers who had been employed for at least one year between 1950 and 1980. At this plant, cobalt was produced from a cobalt chloride solution by etching roasted ore, neutralization, filtration, and electrolysis. The manufacturing process also included production of cobalt salts and oxides. The second publication was a re-analysis of the cohort (N = 1,148), incorporating revised case-ascertainment and an extended period of follow-up (Moulin et al. 1993). The electrochemical worker cohort analyses reported findings for trachea/bronchus/lung cancer, buccal cavity/pharynx, and larynx cancers (Mur et al. 1987); and bronchus/lung, buccal-cavity/pharynx, larynx, esophagus, and brain cancers (Moulin et al. 1993). Although both studied the same population, the original cohort is discussed because it contains additional information (e.g., a nested case-control analysis) not included in the update.

Two publications reported on overlapping populations of hard-metal workers. The first was a historical mortality cohort and nested case-control study of lung cancer among 7,459 workers at 10 hard-metal producing factories in France (Moulin et al. 1998) where activities also included powder metallurgy processes. The second was a sub-study of lung cancer among 2,860 workers in the largest hard-metal producing factory in France (the factory was included in the Moulin et al. (1998) study, with an additional year of follow-up included) which also produced magnets

and stainless steel with cobalt, and cobalt powders by calcination and reduction of cobalt hydroxide (Wild et al. 2000). This study also provided complete job histories.

A historical cohort and nested case-control study of stainless and alloyed steel workers and lung cancer conducted in one factory in France (N = 4,897), which produced and cast stainless and alloyed steel from cobalt, was also identified. Lastly, an incident nested case-control study of 213 cases of lung cancer among Norwegian nickel refinery workers was conducted to evaluate whether exposure to cobalt (and other metals) could explain the elevated risk of lung cancer in nickel workers.

In the two studies of electrochemical workers (Moulin et al. 1993; Mur et al. 1987), exposure was assessed based on company records, which grouped workers into general service, maintenance, and sodium production or cobalt production areas. Analysis was conducted for "ever employment" in the cobalt production workshop, or for exclusive employment in this area. Similarly, in the porcelain factories, exposure was based on company records, which grouped workers into those who worked in departments with and without cobalt exposure (Tüchsen et al. 1996). Exposure to cobalt in the hard-metal factories, and the stainless and alloyed steel factory was classified using a semi-quantitative job-exposure matrix (JEM) developed by experts; the nickel refinery workers were classified using this JEM which incorporated quantitative personal measurements from the breathing zone.

All of the cohort and nested case-control studies reported on lung cancer alone, or lung cancer and aerodigestive cancers, with only one of these reporting specifically about aerodigestive cancers (i.e., buccal cavity/pharynx, and larynx cancers) (Mur et al. 1987) in relation to cobalt exposure. Only one study reported on multiple sites in relation to cobalt (i.e., cervix, ovary, breast, and skin) (Tüchsen et al. 1996); thus, lung cancer is the only site with an adequate database to contribute to the cobalt and cancer assessment.

The description of study methods and characteristics of each study is included in Table C-1 through Table C-7.

Table 4-1. Cohort and Nested Case-control Studies of Exposure to Cobalt

Reference	Population	Design and Outcome (Cancer Sites)	Exposure: Cobalt Compounds, Assessment, Metrics
Tüchsen et al. (1996)	**Danish porcelain painters** 1943–1992 N = 1,394 female workers 874 exposed 520 unexposed	Cancer incidence cohort study (SIR); Danish cancer registry ICD-7: Lung (162.0, 162.1) and 16 other tissue/organ sites	Cobalt-aluminate spinel; cobalt silicate Company records Exposed: Ever employed in two plate underglazing factories Unexposed: workers employed in a cobalt-free department in one factory

Reference	Population	Design and Outcome (Cancer Sites)	Exposure: Cobalt Compounds, Assessment, Metrics
Mur et al. (1987) Moulin et al. (1993) (follow-up)	**French electrochemical workers** *Mur et al. (1987)* 1950–1980 N = 1,143 males *Moulin et al. (1993)* 1950–1988 N = 1,148 Number of cobalt production workers NR	Historical mortality cohort study (SMR) and nested case-control analysis (OR) *Mur et al. (1987)* ICD-8: All causes; trachea, bronchus, and lung (162); buccal cavity/pharynx/larynx (140–149, 161) *Moulin et al. (1993)* ICD-8: All causes; bronchus, lung (162); brain (191)	Production of cobalt, cobalt salts, and oxides. Company records classified workers exclusively employed in one of four work groups including cobalt production workshop *Mur et al. (1987)* Cohort analysis: Only or never employed in cobalt production Nested case-control analysis: Ever/never employed in cobalt production *Moulin et al. (1993)* Mortality SMR analysis Only vs. never employed in cobalt production
Moulin et al. (1998) (multi-plant) Wild et al. (2000) (sub-study of largest plant)	**French hard-metal workers** *Moulin et al. (1998)* 1945–1991 N = 7,459 men and women; 68 cases and 180 controls *Wild et al. (2000)* 1950–1992 N = 2,860 men and women Number of workers employed in cobalt production only NR	Nested case-control analysis (OR) and historical mortality cohort study (SMR) *Moulin et al. (1998)* ICD-8: Lung (162) *Wild et al. (2000)* ICD-8: Lung (162)	Production of magnets, stainless steel, and cobalt powders "Other" cobalt exposure may have included metallic and ionized cobalt Semi-quantitative JEM *Moulin et al. (1998)* Duration, intensity, and cumulative exposure *Wild et al. (2000)* Ever exposed
Moulin et al. (2000b)	**French stainless and alloyed steel worker cohort** 1968–1991 N = 4,897; 54 cases and 160 controls	Nested case-control analysis (OR) within a historical mortality cohort study ICD-8: Lung (162)	Steel production and casting of stainless steel, nickel, ferro-chromium, and other ferroalloys in which iron, chromium, nickel, and cobalt compounds are used Powder manufacture of metallic powders Semi-quantitative JEM Duration, intensity, and cumulative exposure
Grimsrud et al. (2005) (methods described in Grimsrud et al. 2002; Grimsrud et al. 2003)	**Nickel refinery worker cohort** 1952–1995 N = 5,389; 213 cases and 524 controls	Nested case-control analysis (OR) within an incidence cohort study; Norwegian Cancer Registry ICD NR: Lung	Cobalt present in raw materials and intermediates in refinery and produced electrolytically in an electrowinning process Breathing zone personal samples for cobalt and nickel JEM Quantitative cumulative exposure

4.2.2. Study Quality and Utility Evaluation

This section provides an overview of the adequacy of the cohort and nested case-control studies to inform the cancer hazard evaluation (see Appendix C for details on the assessment). This assessment considers factors related to study quality (potential for selection and attrition bias, information bias regarding exposure and outcome, and concern for inadequate analytical methods, selected reporting, and inadequate methods or information to evaluate confounding) and study sensitivity (e.g., such as adequate numbers of individuals exposed to substantial levels of cobalt). The ratings for each of these factors are provided in Table 4-2 and a detailed description of the rationale for the rating is provided in Appendix C.

No critical concerns for the potential for any of the biases (domains) were identified in the available studies; thus, each may have some utility for evaluating potential cancer hazards. All of the reported cohorts are relatively small or moderate sized and are, consequently, underpowered due to few exposed cases or deaths. With one exception (Grimsrud et al. 2005), the cohort or nested case-control studies included only very few cases exposed to cobalt alone, limiting their statistical power to evaluate a modest risk of lung cancer (if it exists) from cobalt. In addition, the level of exposure to cobalt alone in the cohort and nested studies was not defined with enough detail (excepting Grimsrud et al. 2005) to explore exposure-response relationships. Table 4-2 depicts the overall assessment of the ability to inform the cancer evaluation based on the overall utility of the studies, including potential for biases and study sensitivity.

The study of nickel refinery workers (Grimsrud et al. 2005) was considered to have the highest quality because it had adequate numbers of exposed cases, evaluated cancer incidence, incorporated quantitative assessments of exposure to cobalt, and had sufficient information on potential confounders and co-exposures to incorporate these factors into analyses. However, exposure to cobalt was highly correlated with nickel, which compromises the ability of the statistical models to disentangle effects from the two exposures.

The remaining studies were also considered to have low/moderate ability to inform the cancer hazard evaluation primarily because of more limited (semi-quantitative or qualitative) exposure assessments, potential bias, and/or lower sensitivity. The major concern in the studies of hard-metal workers (Moulin et al. 1998; Wild et al. 2000) and stainless-steel workers was potential confounding from potential co-exposure to other lung carcinogens; this was also the case, but to a lesser extent, for the electrochemical workers cohort. In the porcelain worker study (Tüchsen et al. 1996), subcohorts of workers employed prior to 1981 when biomonitoring began and exposure levels began to fall, would have contributed information about high exposures; however, only estimates for the entire cohort were reported, potentially diluting the effect. No relationship with duration of employment was found, but this was not reported by calendar period. In the electrochemical workers cohort, concerns arose about the changing source of outcome information from the first analysis (Mur et al. 1987) to the updated analysis (Moulin et al. 1993). The change from use of medical records to death certificates, in combination with a restriction to account for loss to follow-up in the foreign-born workers, reduced the estimate of the risk in the follow-up study. In general, potential bias from these studies was in the direction of the null, and they had limited sensitivity to detect an effect due to their small size or inadequate information regarding level of exposure.

Table 4-2. Bias and Quality Summary for Cohort and Nested Case-control Studies

Citation	Bias						Quality[a]	Utility[b]
	Selection	Exposure	Outcome	Confounding Methods	Adequacy of Analysis	Selective Reporting	Sensitivity	Integration
Porcelain painters Tüchsen et al. (1996)	++	++	+++	++	++	+++	+	++
Electrochemical workers Moulin et al. (1993) (with Mur et al. 1987)	++	++	++	+	+++	+++	+	+
Hard-metal workers Moulin et al. (1998)	++	++/+++	+++	+	+++	+++	++	++
Wild et al. (2000)	++	++/+++	+++	+	++	+++	++	++
Stainless and alloyed steel workers Moulin et al. (2000b)	+++	++	+++	+	+++	+++	++	++
Nickel refinery workers Grimsrud et al. (2005)	+++	+++	+++	++	+++	+++	+++	+++

[a]Levels of concern for bias and for study quality rating. Equal column width for types of bias does not imply they have equal weight (see appendix for description of terms): Scoring system: +++ = low/minimal concern or high quality; ++ = some concern or medium quality; + = major concern or low quality; 0 = critical concern.

[b]Utility of the study to inform the hazard evaluation (see Appendix C for description of terms) scoring system: ++++ = high utility; +++ = moderate utility; ++ = moderate/low utility; + = low utility; 0 = inadequate utility.

4.2.3. Cancer Assessment: Lung

The goal of the cancer assessment is to evaluate the evidence for the carcinogenicity of cobalt for lung cancer. The conclusions regarding the assessment of study utility are brought forward, and these are considered together with the evidence from the individual studies. Next, the evidence is integrated across studies to reach a level of evidence conclusion to determine whether there is credible evidence of an association between cobalt and lung cancer, and whether such an observed association could be explained by chance, bias, or confounding.

Several of the considerations developed by Hill (Hill 1965) are relevant to the evaluation of the level of evidence for this assessment, including the magnitude (strength) and consistency of any observed associations across studies, evidence of an exposure-response gradient, and temporality of exposure. The NTP listing recommendation is provided in Section 4.5.

Background Information

Lung cancer is the third most common cancer in the United States, making up 13.5% of all new cancers. The age-adjusted annual lung cancer rates (including trachea and bronchus) (per 100,000 males or females) in the United States from 2007 to 2011 (NCI 2015c) were approximately 72.2 (male) and 51.1 (female) for incidence; and 61.6 (male) and 38.5 (female) for mortality, with a 5-year survival rate of 16.8%. These data suggest that mortality and incidence data are approximately comparable for informing the cancer assessment. Rates for new lung and bronchus cancer cases have decreased on average 1.5% each year over the last 10 years; and death rates have decreased on average 1.8% each year from 2002 to 2011. Incidence trends and rates in European countries where all of the cohort studies were conducted are broadly similar (Ferlay et al. 2013). For example, in the European Union, lung cancer incidence per 100,000 males is 66.3, and mortality is 56.4.

Latencies for solid tumors such as lung cancer are generally estimated to exceed approximately 20 years but may vary considerably. Incidence rates of lung cancer generally increase after 50 years of age, and this cancer is most frequently diagnosed among people aged 65 to 74; the median age at diagnosis is 70. None of the studies of cobalt and lung cancer included in this review have indicated the sub-type(s) of lung cancer included in their analyses.

The single most important non-occupational risk factor for the development of lung cancer is smoking. Other risk factors of concern include exposure to arsenic, asbestos, cadmium, silica, chromates, nickel compounds, and polycyclic aromatic hydrocarbons, all of which are found in cobalt manufacturing processes.

Evidence from Individual Studies

Based on the study quality evaluation, all six cohort and/or nested case-control studies reporting on lung cancer and cobalt exposure were considered to have some utility for inclusion in the cancer assessment. The findings from the individual studies are discussed below and presented in Table 4-3. The available cohort and nested case-control studies of cobalt and lung cancer include a cohort of Danish female porcelain painters, a cohort of French electrochemical workers, a French multi-centric cohort of hard-metal factory workers, a related cohort of workers from the largest factory in the multi-centric French hard-metal factory cohort, a cohort of French stainless and alloyed steel workers, and a cohort of Norwegian nickel-refinery workers.

Table 4-3. Evidence from Cohort and Case-control Studies on Lung Cancer and Exposure to Cobalt

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
Tüchsen et al. (1996) Cohort Copenhagen, Denmark Factory 1: 1943–1992; Factory 2: 1962–1992	Danish porcelain painters. 1,394 total; 874 cobalt-exposed workers, 520 unexposed workers. Exposure assessment method: company records	Lung (162 and 162.1)				Employment in factories/departments with or without cobalt. Confounding: No control for smoking; however, smoking data on subset of workers suggests that smoking was not associated with exposure. Strengths: Population exposed primarily to cobalt compounds alone; only female population with data on cobalt. Limitations: Small number of exposed cases. Differential selection out of the cohort could have occurred as the authors mentioned that records of ill persons may have been removed potentially resulting in an underestimate of the true incidence of cancer.
		All exposed	8	SIR 2.35 (1.09–4.45)	Age, calendar year	
		Factory 1 exposed to cobalt silicate from 1972	3	1.6 (0.41–4.37)		
		Factory 2 exposed to cobalt-aluminate spinel dye thru 1988	5	3.25 (1.19–7.2)		
		Referents	7	1.99 (0.87–3.94)		
Mur et al. (1987) Cohort France 1950–1980	Electrochemical workers N = 1,143; number of cobalt production workers NR ~ 25% of current staff at time of publication Exposure assessment method: company records	Lung (162)				Exposure duration: 60% worked greater than 10 years; 75% hired before 1975. Confounding: Likely inadequate control for smoking; however, likely co-exposure to nickel and arsenic with no control for co-exposures. Strengths: Cobalt production workers exposed primarily to cobalt compounds. Limitations: Small number of exposed cases; high loss to follow-up (20%); potential for selection bias due to left truncation
		Only employed in cobalt production	4	SMR 4.66 (1.46–10.64)	Age, year of death	

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
Mur et al. (1987) Nested case-control France 1950–1980	Electrochemical plant workers Cases: 9; controls: 18 Exposure assessment method: company records	Lung (162) Ever worked in cobalt production	4	OR [4.0 (0.66–24.4)]	None	Confounding: Cases (deaths from lung cancer) were matched to controls (deaths from cause other than cancer) for year of birth, age at death, and smoking habits; smoking data on only 30% of the cohort; co-exposures to nickel and arsenic were not controlled. Strengths: Nested design reduces concern of potential confounding from lifestyle factors Limitations: Small numbers with limited information on exposures (only ever/never employment in cobalt production department); also, 46% of the cohort was hired prior to the start of follow-up which could induce a downward bias in the effect estimate due to over-prevalence of healthier workers (left truncation).
Moulin et al. (1993) Cohort France Extended follow-up of the Mur et al. (1987) study through 1988	Electrochemical workers Cohort 1: N = 1,148; Cohort II: N = 870; number of cobalt workers NR Exposure assessment method: company records	Lung (162) Exclusive employment in cobalt production, Cohort I	3	OR 0.85 (0.18–2.5)	Age	Confounding: No reported control for period effects, duration, or time since first exposure; no consideration of smoking; potential co-exposures to nickel and arsenic from its presence in cobalt ore not controlled. Strengths: Cobalt production workers exposed primarily to cobalt compounds. Limitations: Small number of exposed cases in overall or sub-cohort; low power to detect an effect; concern about outcome misclassification; potential for selection bias due to left truncation
		Exclusive employment in cobalt production, Cohort II	3	1.16 (0.24–3.4)		
		Ever worked in Cobalt production, Cohort I	4	0.88 (0.24–2.25)		

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
		Ever worked in cobalt production, Cohort II	4	1.18 (0.32–3.03)		
Moulin et al. (1998) Nested case-control France 1968–1991	Workers in all 10 hard-metal factories in France Cases: 61; controls: 180 Exposure assessment method: JEM	Lung (162)			Age	No information on actual exposure level or average exposure duration for the cohort. Confounding: Potential concern for exposure to other lung carcinogens, which were not controlled in the cobalt alone analyses. Strengths: Exposure-response analyses with multiple exposure metrics; JEM validated for atmospheric concentrations of cobalt; incident cohort reducing the potential for left truncation; internal analysis reducing the impact of the reported HWE; and lagged analysis. Limitations: Potential confounding by co-exposures classified only as "ever/never" in the JEM.
		Exposure level 2 to 9	15	OR 2.21 (0.99–4.9)		
		Exposure intensity trend	15	2.05 (0.94–4.45)		
		Exposure duration	15	2.2 (0.99–4.87)		
		Unweighted cumulative exposure	15	1.83 (0.86–3.91)		
		Frequency weighted cumulative exposure trend	15	2.03 (0.94–4.39)		
Wild et al. (2000) Cohort France 1968–1992	Hard-metal workers - Largest plant in France 2,216 men and 644 women Exposure assessment method: JEM	Lung (162)				No information on actual exposure level or average exposure duration for the cohort Confounding: Potential exposure to lung carcinogens which were not controlled in cobalt-only analyses. Strengths: Incident cohort; lagged analysis. Limitations: External analysis only presented; no exposure metrics except for ever/never provided.
		Cobalt except in hard metals	15	SMR 1.95 (1.09–3.22)	Age, sex	
Moulin et al. (2000b)		Lung (162)				

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
Nested case-control France 1968–1992	Stainless and alloyed steel workers Cases: 54 (17 cobalt exposed); controls: 162 (67 cobalt exposed) Exposure assessment method: JEM	Exposed, Crude	17	OR 0.64 (0.33–1.25)	–	No information on actual exposure level or average exposure duration for the cohort Confounding: Potential confounding from exposure to chromium and/or nickel, and iron; controlled for smoking Strengths: Semi-quantitative JEM; exposure metrics including duration and cumulative dose, frequency weighted and unweighted provided; HWE mitigated by use of internal analyses. Limitations: Known carcinogens had non-significant ORs <1.0, indicating that the study had low sensitivity to detect an effect.
		Exposed, known smoking status, Crude	12	0.62 (0.26–1.46)		
		Exposed, known smoking status, smoking adjusted	12	0.43 (0.16–1.14)		
		Exposed, known smoking status, PAH, silica, and smoking adjusted	12	0.44 (0.17–1.16)		
Grimsrud et al. (2005) Nested case-control Norway 1910–1995	Nickel refinery workers Cases: 213; controls: 525 Exposure assessment method: JEM	Lung				Exposure levels (μg/m^3): high (144–3,100); medium (29.7–142); low (0.31–29.5). Confounding: No multivariate estimates for the categorical variable (low, high, medium exposures) were possible due to collinearity with nickel. Continuous rise in OR controlled for smoking and co-exposures. Strengths: Quantitative cobalt levels reported based on measurements from the breathing zone; incident cases; internal analyses; relatively large number of cases compared to other cobalt studies. Limitations: Collinearity with nickel.
		Rise in OR per mg/m^3 × years, smoking adjusted	NR	OR 1.3 (0.9–1.8)	Smoking	
		Low (0.31–29.5 μg/m^3 × years	49	1.5 (0.6–3.8)		
		Med (29.7–142 μg/m^3 × years	73	2.4 (1–5.6)		

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
		High (144–3,100 μg/m^3 × years	82	2.9 (1.2–6.8)		
		Lung				
		Rise in OR per mg/m^3 × years, smoking and co-exposure adjusted	NR	0.7 (0.3–1.4)	Smoking, nickel, sulfuric acid mists, asbestos, arsenic	
		Lung				
		Cobalt electrolysis workshop, 0.03–2.2 yr	23	1.6 (0.8–3)	Smoking, and employment in other workshops	
		Cobalt electrolysis workshop, 2.3–11.8 yr	44	2.8 (1.5–5)		
		Cobalt electrolysis workshop 12–48 yr	62	5.1 (2.9–9.1)		

HWE = Healthy worker effect; HWSE = Healthy worker survival effect; JEM = job-exposure matrix/ NR = Exposure levels or duration not reported; OR = odds ratio; PAHs = polycyclic aromatic hydrocarbons.

Porcelain Painters

Tüchsen et al. (1996) reported a significantly increased risk of lung cancer in all exposed female workers compared with the Danish female population (SIR = 2.35, 95% CI = 1.01 to 4.62, based on 8 exposed cases). Factory-specific SIRs for lung cancer were also reported, indicating that Factory 1, where cobalt aluminate-spinel was replaced by cobalt silicate in 1972, had a non-significantly elevated SIR of 1.6 based on 3 exposed cases (no CI provided); and that Factory 2, where workers continued to be exposed to cobalt aluminate-spinel until 1989, had a significantly elevated SIR of 3.25 based on 5 exposed cases. In addition, the authors reported an elevated SIR of lung cancer in the referent group (SIR = 1.99, 95% CI = 0.80 to 4.11, 7 cases), similar in magnitude to that found in the exposed group.

This study had low sensitivity to detect an effect because of (1) small numbers of exposed cases in this relatively small cohort and (2) potentially combining workers with high and low exposures together, which could dilute any effect and bias the results towards the null. In addition, no lagged analyses were reported. A concern about differential selection also exists in this study. The authors suggested that removal of records of ill persons was known to take place in Danish manufacturing. The possibility of differential selection out of the cohort could have resulted in an underestimation of the true incidence of lung cancer in this study.

An elevated lung cancer SIR, similar in magnitude to that reported in the exposed group, was also observed in the referents; a comparison of the exposed departments with the reference department gave a relative risk ratio of 1.2 (95% CI = 0.4 to 3.8). The referents were reported to be top glaze decorators employed in a department without cobalt exposure. Data from a previous publication in this factory (Raffn et al. 1988) indicated an overlap of cobalt levels in referents and exposed individuals, suggesting that the referents in the Tüchsen et al. (1996) paper were not completely "unexposed." Limited information regarding smoking and its potential relationship with cobalt exposure was provided from two surveys of subsamples of workers (Prescott et al. 1992; Raffn et al. 1988). Based on a calculation of the weighted average of exposed and unexposed respondents from both studies taken over the total sample size of the two studies and disregarding the specific cobalt compound to which workers were exposed, the smoking rate is calculated to be 52% for exposed and 38% for referent women. The rate of smoking among exposed women is close to that of skilled Danish women taken in 1982 (47%) and 1987 (55%); and the rate of smoking in the referent group is similar to, but lower than, the rate in the general population of Danish women (43% and 42% in these two years). This suggests that there may be a non-smoking cause for the increased rate of lung cancer in the referent population, which might be due either to misclassification of cobalt exposure, or to another unmeasured confounder. It is also possible that cobalt-exposed workers are also exposed to the same unmeasured confounder, although data from the substudy indicates that levels of silica, nickel, and dust were very low based on air monitoring done in 1981 (Raffn et al. 1988). The porcelain painters cohort provides inconclusive evidence for a carcinogenic effect of cobalt and lung cancer because of the finding of similarly elevated levels of lung cancer among the referents.

The Tüchsen et al. (1996) study stands out from others in that it consists entirely of women. Christensen et al. (1993) conducted a cross-over study of oral administration of soluble and insoluble cobalt compounds and found that there are clear differences in biological levels by gender, with significantly higher urinary cobalt (higher uptake) levels and urinary excretion of cobalt in females compared with males.

Electrochemical Workers

Two publications reported on the same cohort of cobalt production workers in a French electrochemical plant (Moulin et al. 1993; Mur et al. 1987). Findings from both publications are reported because the methodologies employed in each differ in important ways that shed light on their interpretation; that is, the later paper (Moulin et al. 1993) is not simply an update of the earlier paper. The first paper reported a statistically significantly increased SMR for lung cancer among the workers employed in cobalt production only (SMR = 4.66, 95% CI = 1.46 to 10.64, based on 4 observed deaths) (Mur et al. 1987). There was large loss to follow-up and clear evidence of a healthy worker effect in the overall cohort (all-cause mortality SMR = 0.77 [95% CI = 0.67 to 0.88]), but not among cobalt production workers. However, in an internal matched analysis (matching variables were year of birth, age at death, and smoking habits), the percent of cases and controls matched on year of birth, age at death and smoking habits ever employed at cobalt production was provided, without estimated odds ratio or confidence interval. An unadjusted calculation computed by NTP = OR of 4.0 (95% CI = 0.7 to 24.4), indicating internal consistency with the reported SMR for those working only in cobalt production.

However, in an extension of the follow-up of the same cohort (Moulin et al. 1993) the SMR for lung cancer among French-born workers exclusively employed in cobalt production was 1.16, (95% CI = 0.24 to 3.40), based on 3 observed deaths. (Confidence in the SMR for the entire cohort is lower because of high loss to follow-up and strong healthy worker effect due to 24% foreign-born workers). In addition, Moulin et al. (1993) reported a discrepancy in the number of observed cases exclusively employed in cobalt production in the two analyses (e.g., Mur et al. (1987) [N = 4]; Moulin et al. (1993) [N = 3]) due to differences in the methods used to ascertain cause of death. The Mur et al. (1987) study used physicians' medical records, whereas Moulin et al. (1993) used death certificates for the years when they were available and, in the process, one exposed case was re-classified as non-diseased; furthermore, during the extended follow-up, no additional lung cancer cases were observed.

A further limitation of this study is its very weak consideration of risk factors for lung cancer, particularly smoking status, and possible co-exposures in the cobalt production process to nickel and arsenic. Mur et al. (1987) initially reported that smoking histories were available for 30% of workers, and the authors reported matching cases and controls on smoking status; however, no explanation was given regarding the methods of matching given the small percentage of workers with information on smoking status. Moulin et al. (1993) did not address smoking in the analysis but reported no excess of mortality from circulatory and respiratory diseases, suggesting that smoking is unlikely to be higher in this cohort than in the local French referent population.

Selection bias is somewhat of a concern in this cohort, as 46% of members were hired prior to the start of follow-up, which suggests that the cohort had a high proportion of healthy prevalent workers, which can bias the risk estimate downward (left truncation) (Applebaum et al. 2011).

The evidence from these electrochemical studies is inconclusive, based on the low sensitivity of the Moulin et al. (1993) study to detect an effect, the lack of exposure metrics in both studies, potential selection bias from left-truncation, and the inability to control for confounding. The changed outcome classification across the two analyses does not inspire confidence in the methods used in either study. The Mur et al. (1987) analysis was consistent across the internal and external analyses, reducing concerns about confounding from the HWE, however, selection bias due to left-truncation remains a concern.

French Hard-metal Worker Cohorts

The populations included in the two studies of cobalt exposure and lung cancer among hard-metal workers overlap, and both studies report either statistically significant elevated risks or borderline statistically significant risks of lung cancer among those exposed to cobalt without tungsten carbide. Moulin et al. (1998) first reported results from the multi-center study of 10 hard-metal factories in France. In the internal nested case-control analysis (Moulin et al. 1998), based on 15 exposed cases, a borderline statistically significant increased risk of lung cancer was associated with exposure to "cobalt alone or simultaneously with agents other than tungsten carbide" (levels 2 to 9) compared with little or no exposure (levels 0 or 1) (OR = 2.21, 95% CI = 0.99 to 4.90). Regarding the presence of an exposure-response relationship, Moulin et al. (1998) reported two-fold elevated trend tests (although not reaching statistical significance) based on 15 cases across levels of exposure (OR = 2.05, 95% CI = 0.94 to 4.45), levels of duration (2.20, 95% CI = 0.99 to 4.87), cumulative weighted (1.83, 95% CI = 0.86 to 3.91), and cumulative un-weighted doses (2.03, 95% CI = 0.94 to 4.39). Numbers of cases and category-specific OR estimates for levels or categories of duration or cumulative dose were not provided. Wild et al. (2000) added years of follow-up to the cohort from the largest factory included in the multi-center study and found a statistically significant elevated SMR of lung cancer among those exposed to "cobalt except in hard metals" based on the JEM (SMR = 1.95, 95% CI = 1.09 to 3.22). Wild et al. (2000), however, did not provide information on exposure-response relationships; and neither study provided an examination of latency.

Moulin et al. (1998) and Wild et al. (2000) both measured and addressed co-exposures to 9 workplace lung carcinogens and smoking in analyses for cobalt-tungsten carbide. In both studies, the JEM was used to assess exposure to other workplace carcinogens. Ever vs. never smoking was obtained through interviews with cohort members, and their colleagues and relatives in the Moulin et al. (1998) study and from occupational health department records in the Wild et al. (2000) study. However, in both studies, it is unclear whether the analyses of cobalt alone included models for adjusting for co-exposures to other carcinogens or smoking. In the Wild et al. (2000) study, exposure to any IARC carcinogen without considering exposure to cobalt-tungsten carbide was related to lung cancer (SMR = 2.05, 95% CI = 1.34 to 3.0).

Potential confounding from exposure to smoking is less of a concern in this study than potential confounding from exposure to other carcinogens. There is no evidence from data presented to indicate that exposure to cobalt alone and smoking was related. In addition, the low mortality from smoking-related disease suggests a limited potential for confounding, as smoking is unlikely to be more prevalent among the workers than in the overall population. In the French cohort, mortality from chronic bronchitis and emphysema was low (SMR = 0.4, 95% CI = 0.05 to 1.44) and there was no consistent mortality pattern for other smoking-related cancers (e.g., larynx, bladder, buccal cavity/pharynx, and esophagus). In addition, as internal analyses are usually assumed to be less affected by confounding from lifestyle factors (e.g., smoking) than SMRs, the OR estimate from the multivariate model reported by Moulin et al. (1998) in the internal analysis is likely to be the better estimate for cobalt and lung cancer from this cohort. Due to the lack of information about control of carcinogenic co-exposures, confidence in the finding is reduced.

Stainless and Alloyed Steel Cohort

No association between cobalt exposure and lung cancer was found in this study (Moulin et al. 2000b). In internal analyses of cobalt exposure based on the JEM in the stainless and alloyed steel plant, Moulin et al. (2000b) reported a crude OR of 0.64 (95% CI = 0.33 to 1.25), and an OR adjusted for PAHs and silica of 0.58 (95% CI = 0.29 to 1.17) based on 17 exposed cases and 67 controls in 10-year lagged analyses. Similar findings were found among those with known smoking habits (e.g., 12 cases and 36 controls). Moulin et al. (2000b) also reported significant decreasing trends in duration, and frequency un-weighted and weighted cumulative dose for workers with known smoking habits. (The overall cohort SMR for smoking and lung cancer was 5.37 [95% CI = 1.74 to 12.53] for those working less than 10 years). ORs adjusted for smoking were all less than 1.0 (Moulin et al. 2000b). It is likely that non-differential exposure misclassification was introduced into the exposure assessment because some job periods of cases or controls went back many decades, yet exposure was assessed based on memories of processes and exposures of current workers or reports in the literature, as historical exposure measurements were lacking. Models were reported controlling for PAHs and silica, none of which made any material difference; however, in the correlation matrix, neither of these was related to cobalt exposure. Exposure to nickel and/or chromium was related to cobalt exposure, although these exposures were not included in the cobalt model. However, these exposures were also not associated with lung cancer risk in these analyses.

In this study, chromium and/or nickel and asbestos, all lung carcinogens classified by RoC and IARC, were found to be unrelated to lung cancer, decreasing the confidence in this study and in the findings for cobalt. Only exposure to PAHs and silica were statistically significantly related to lung cancer along with increasing trends not confounded by smoking.

Misclassification of exposure in this study, its inability to control for the appropriate confounders correlated with cobalt, and the negative findings for lung cancer and other known lung carcinogens (e.g., nickel, chromium, asbestos) suggest little confidence in the evidence put forth in this study.

Norwegian Nickel Refinery Workers

The Grimsrud et al. (2005) cancer incidence study of nickel and lung cancer in a Norwegian nickel refinery was conducted to determine if cobalt or other potential carcinogens could explain the elevated risks of lung cancer in nickel workers. The authors reported that the cobalt variable could not be retained in the full model in its categorical form due to collinearity (all individuals exposed to nickel were also exposed to cobalt, although the correlation between cobalt and nickel was reported as r = 0.63); however, the positive exposure-response effect noted for the continuous cobalt variable adjusted only for smoking changed sign when smoking and co-exposures (nickel, arsenic, asbestos, and sulfuric acid mists) were controlled. The smoking-adjusted rise in OR per $mg/m^3 \times$ year was 1.3 (95% CI = 0.9 to 1.8), which was reduced to 0.7 (95% CI = 0.3 to 1.4) after adjustment for occupational co-exposures. The categorical ORs adjusted only for smoking were low exposure (0.31 to 29.5 $\mu g/m^3$) based on 49 cases, OR = 1.5 (95% CI = 0.6 to 3.8); medium exposure (29.7 to 142 $\mu g/m^3$) based on 73 cases, OR = 2.4 (95% CI = 1.0 to 5.6); and high exposure (144 to 3,100 $\mu g/m^3$) based on 82 cases, OR = 2.9 (95% CI = 1.2 to 6.8). No value for trend was reported for the smoking-adjusted variable. However, the fully adjusted model for this cobalt variable (including smoking as well as all co-exposures) could not be calculated due to collinearity.

The authors reported that cobalt levels typically amount to 4% to 15% of the total nickel levels, except in the cobalt electrolysis process where cobalt levels are triple the amount of nickel levels. This process is included in hydrometallurgical production, for which results are reported by duration of work. Strong gradients were found by duration of work in the hydrometallurgical production department with a 5-fold increase in the OR for 12 or more years (OR = 5.1, 95% CI = 2.9 to 9.1) based on 62 exposed cases, with the linear trend (per 10 years) (OR = 1.7, 95% CI = 1.4 to 2.1). However, no analyses were provided to help separate effects of exposure to cobalt and nickel.

Although the design of this study was of high quality, due to the collinearity with exposure to nickel, this study cannot separate out the effects of cobalt and nickel on lung cancer and thus the findings from the study are unclear.

Integration of Evidence across Studies

While almost all the cohort studies reported approximately a doubling of the risk of lung cancer mortality or incidence from exposure to various cobalt compounds, it is unclear that the excess lung cancer was due to exposure specifically to cobalt, because (1) it was not possible to rule out confounding by carcinogenic co-exposures, or (2) other complications prevented a clear interpretation of a cobalt effect (see Figure 4-1).

The Danish porcelain painters study showed similarly elevated risks of lung cancer in both the exposed and unexposed workers and could not control directly for smoking. Findings from the French electrochemical workers cohort were based on two papers analyzing the same cohort using different methods to ascertain cancer and publishing conflicting results—the first indicated a significantly elevated risk of lung cancer based on four exposed cases, and the second showed virtually no differences in risk of lung cancer among the exposed and unexposed workers based on three exposed cases in a subset of workers born in France. In two French studies of hard-metal workers, measures of cobalt exposures were likely mixed with other carcinogens and the methods did not clearly indicate whether these were controlled in the analyses. Although an exposure-response relationship between cobalt exposure and lung cancer was observed in the Norwegian nickel refinery workers study, risk estimates could not be calculated in models controlling for other co-exposures because nickel and cobalt were highly correlated. However, in this study a significant trend was reported with increasing duration of employment in workshops where cobalt concentrations tripled those of nickel, with control for employment in other workshops and smoking. Confounding by smoking was considered in each of the studies to varying degrees, and smoking either did not reduce the risk estimates materially when it was controlled or was unlikely to materially reduce the risk estimates in studies where there was only auxiliary information.

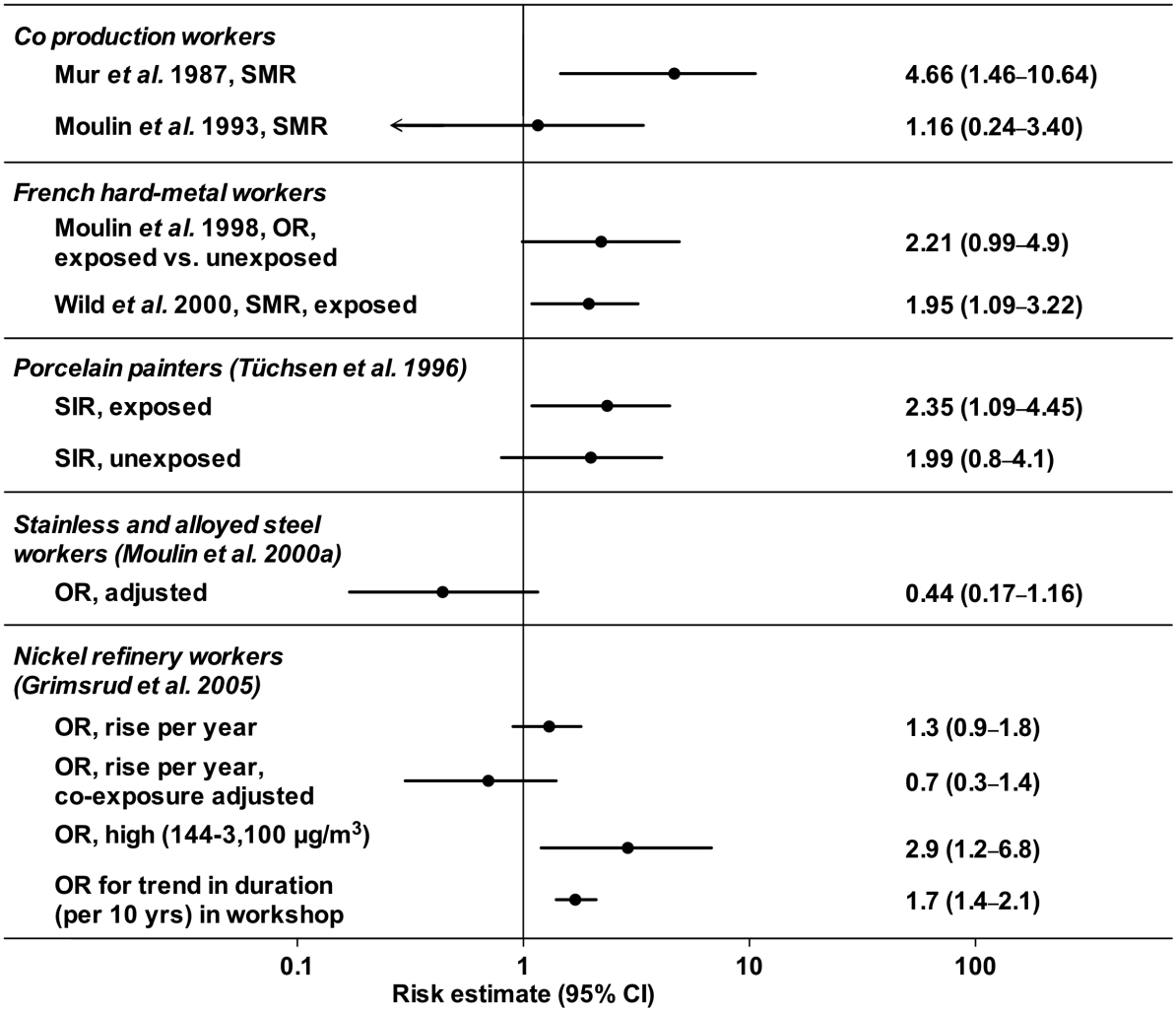

Figure 4-1. Forest Plot Showing Lung Cancer Risk Ratios (SIR, SMR, or OR as Noted) and 95% CI for Epidemiological Cohort Studies of Cobalt Exposure

4.3. Case-control Studies

This section provides an overview of the case-control studies (Section 4.3.1), an overview of the adequacy of the studies to inform the cancer hazard evaluation (Section 4.3.2) and an assessment of the evidence from the studies on the association between cobalt exposure and esophageal cancer risk (Section 4.3.3).

4.3.1. Overview of the Methodologies and Study Characteristics

The available epidemiological studies that satisfy the criteria for inclusion in the review consist of two population-based case-control studies of metals in biological tissues of cancer cases (lung, esophageal, oral cavity, and laryngeal cancers) and controls published in the literature between 1986 and 2012 (Table 4-4). Both of these studies (O'Rorke et al. 2012; Rogers et al. 1993) were initiated from an interest in the role of metals in the etiology of cancer, and specifically metals derived from nutritional sources. Detailed data on study design, methods, and findings were

systematically extracted from relevant publications, as described in the study protocol, into Table 4-5, Table C-8, Table C-9, and Table 4-6.

Table 4-4. Case-control Biomarker Studies of Exposure to Cobalt

Reference	Design and Population	Outcome	Exposure: Cobalt Compounds, Assessment, Metrics
Rogers et al. (1993)	Population-based case-control biomarker study Western WA state USA 1983–1987 501 cases (153 laryngeal, 73 esophageal, 359 oral cavity cancers)/434 controls	ICD-O Larynx (140.0–141.9) Esophagus (143.0–146.9) Oral cavity (148.0–150.9; 161.0–161.9)	Source and type of compounds unknown Cobalt levels in toenails measured Tertiles (ppm)
Rogers et al. (1993)	Population-based case-control biomarker study Ireland FINBAR* study 2002–2004	ICD not reported Esophagus Barrett's esophagus (metastatic precursor to esophageal cancer)	Source and type of compounds unknown Cobalt levels in toenails measured Tertiles (log transformed - cut points µg/g)

*FINBAR = Factors Influencing the Barrett's Adenocarcinoma Relationship.

4.3.2. Study Quality and Utility Evaluation

This section provides an overview of the adequacy of the cohort and nested case-control studies to inform the cancer hazard evaluation (see Appendix C for details on the assessment). This assessment considers factors related to study quality (potential for selection and attrition bias, information bias regarding exposure and outcome, and concern for inadequate analytical methods, selected reporting, and inadequate methods or information to evaluate confounding) and study sensitivity (e.g., such as adequate numbers of individuals exposed to substantial levels of cobalt). The ratings for each of these factors are provided in Table 4-5 and a detailed description of the rationale for the rating is provided in Appendix C.

Both of the case-control studies of cobalt in toenails have either low/minimal or some concern for most biases except for exposure assessment and sensitivity. Their overall low utility to inform the cancer hazard evaluation, however, is due to the potentially irrelevant window of exposure. Toenail clippings likely reflect an integrated exposure that occurred 12 to 18 months prior to clipping, and toenail samples were collected after cancer diagnosis in these studies. Many factors (including disease) can affect nail growth and metal deposition. The available studies (that evaluated cobalt levels and cancer stage [lung or laryngeal] are conflicting, thus it unclear whether the cancer process can affect cobalt levels in toenails Benderli Cihan et al. 2011; Klatka et al. 2011; Kuo et al. 2006). However, although exposure was assessed after the disease process began, in most cases it represents at least some pre-diagnosis exposure, but not pre-cancer exposure as the latency period of both esophageal cancer and Barrett's esophagus is of long duration (Butt and Kandel 2014). Rogers et al. (1993) conducted stratified analyses on tumor stage and time of diagnosis, which indicated no differences in cobalt levels, suggesting that reverse causality may not be a concern.

Table 4-5. Bias and Quality Summary for Case-control Studies

Citation	Bias[a]						Quality[a]	Utility[b]
	Selection	Exposure	Outcome	Confounding Methods	Adequacy of Analysis	Selective Reporting	Sensitivity	Integration
Rogers et al. (1993)	+++	+	+++	+++	+++	+++	+	+
O'Rorke et al. (2012)	++	+	+++	+++	+++	+++	+	+

[a]Levels of concern for bias and for study quality rating. Equal column width for types of bias does not imply they have equal weight (see appendix for description of terms): +++ = low/minimal concern or high quality; ++ = some concern or medium quality; + = major concern or low quality; 0 = critical concern.
[b]Utility of the study to inform the hazard evaluation (See appendix for description of terms): ++++ = high utility; +++ = moderate utility; ++ = moderate/low utility; + = low utility; 0 = inadequate utility.

4.3.3. Cancer Assessment: Esophageal Cancer

Background Information

Esophageal cancer is a relatively rare cancer, ranking as the eighteenth most common cancer in the United States, making up 1.1% of all new cancers. The age-adjusted annual rates of esophageal cancer (per 100,000 males or females) in the United States from 2007 to 2011 (NCI 2015b) were approximately 7.7 (male) and 1.8 (female) for incidence; and 7.5 (male) and 1.6 (female) for mortality, with a 5-year survival rate of 17.5%. Like lung cancer, these data suggest that mortality and incidence data are approximately comparable for informing the cancer assessment. Incidence trends and rates in European countries where all of the cohort studies were conducted are broadly similar (Ferlay et al. 2013); and in the European Union the annual incidence of esophageal cancer is 8.4 and the annual mortality rate is 7.0 (Cancer Research U.K. 2014). Evaluations of esophageal cancer risk factors have reported that sufficient evidence exists for x-and gamma-radiation, alcoholic beverages, betel quid, tobacco smoking, and smokeless tobacco; limited evidence exists for dry-cleaning, mate drinking, pickled vegetables, rubber production industry, tetrachloroethylene exposures, red and processed meats, and high temperature drinks. The sub-types of esophageal cancer, esophageal adenocarcinoma and, however, have distinct risk factors and trends. Esophageal adenocarcinoma, with risk factors being white race, increasing age, body fatness, and male gender, is the predominant histological type among men, while for women, esophageal squamous-cell cancer is more common, and rates are still increasing in several European countries. Unlike esophageal squamous-cell carcinoma, alcohol is not a risk factor for either Barrett's esophagus or for esophageal adenocarcinoma (Anderson et al. 2009; Freedman et al. 2011; Kubo et al. 2009); however, smoking is a risk factor for both subtypes and Barrett's esophagus (Cook et al. 2010).

Barrett's esophagus is a condition of intestinal metaplasia in which tissue that is similar to the lining of the intestine replaces the tissue lining the esophagus. The prevalence of Barrett's esophagus is estimated to be between 1.6% and 6.8% (Gilbert et al. 2011), although a more precise estimate is not possible as many patients are asymptomatic, and its natural history has been difficult to assess. Barrett's esophagus has an extended latency period prior to progressing to cancer (Butt and Kandel 2014). A recent meta-analysis of studies reports incidence rates for the development of esophageal cancer in nondysplastic Barrett's esophagus of 0.33% per year

and 0.19% for short-segment Barrett's esophagus (Desai et al. 2012). About 5% of patients with esophageal adenocarcinoma have a pre-cancer diagnosis of Barrett's esophagus (Corley et al. 2002); but its presence conveys a 30- to 40-fold increased risk of esophageal carcinoma (Sharma 2004). As incidence of esophageal adenocarcinoma has increased more than six-fold in the last decade, investigations of the risk factors for Barrett's esophagus have been of interest (Jemal et al. 2013). Barrett's esophagus incidence increases with age; the prevalence among non-Hispanic whites is 6.1% compared to 1.7% among Hispanics and 1.6% among blacks; and the male/female ratio is about 2:1 (Abrams et al. 2008), similar to esophageal cancer.

Evidence from Individual Studies

Both of the case-control studies (O'Rorke et al. 2012; Rogers et al. 1993) compared cobalt in toenails of cases of esophageal cancer and population-based controls. O'Rorke et al. (2012) limited their analysis to esophageal adenocarcinoma, while no histologic information was provided by Rogers et al. (1993), thus it is likely that the Rogers et al. (1993) study included both subtypes in unknown proportions. Findings are presented in Table 4-6.

Table 4-6. Evidence from Studies of Aerodigestive Cancers and Exposure to Cobalt

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
Rogers et al. (1993) Case-control Western WA state, USA 9/1/83–2/28/87	Population based study of aerodigestive cancers, USA Cases: N = 507; N = 153 laryngeal, N = 73 esophageal, N = 359 oral cavity cancers; Controls: N = 434 Exposure assessment method: personal monitoring	**Esophagus (143.0–146.9)**				**Exposure levels:** Tertiles of cobalt in toenails; highest level = 0.17 ppm **Confounding:** Cases and controls were matched on key likely confounders. No information provided about correlation of cobalt with other measured trace metal levels, and nutrients not correlated with cobalt were kept in the model because they resulted in ORs closer to the null. ORs for esophageal cancer were significantly elevated for iron and calcium **Strengths:** Population-based study; histologically confirmed cancers; cases and controls from same source population. **Limitations:** Not all samples reflect pre-diagnostic window of exposure. No USDA data available on cobalt levels in food as measured by a food frequency questionnaire. Single sample collected even though cobalt in toenails shown to have low reproducibility; window of exposure a concern with long latency cancer.
		<0.05	92	OR = 1.0	Age, sex, smoking (pack-years), alcohol (drink-years), beta-carotene (mg/day), energy intake (kcal/day), ascorbic acid (mg/day)	
		0.05–0.17	127	2.4 (0.8–7.2)		
		>0.17	66	9 (2.7–30)		
		Larynx (140.0–141.9)				
		<0.05	114	OR = 1.0	Age, sex, smoking (pack-years), energy intake (kcal/day), beta-carotene (mg/day), ascorbic acid (mg/day), alcohol (drink-years)	
		0.05–0.17	168	2 (1–3.8)		
		>0.17	62	1 (0.4–2.6)		
		Oral cavity cancer (148.0–150.9; 161.0–161.9)				
		<0.05	135	OR = 1.0	Age, sex, smoking (pack-years), alcohol (drink-years), energy intake (kcal/day), ascorbic acid (mg/day), beta-carotene (mg/day)	
		0.05–0.17	190	1.5 (0.9–2.6)		
		>0.17	92	1.9 (1–3.6)		
O'Rorke et al. (2012) Case-control	All Ireland population-based study of	**Esophageal cancer**				**Exposure levels:** Average (μg/g) ± SD: cases = 0.02 ± 0.06;
		< −5.4824	34	OR = 1.0		

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
All Ireland (Republic and Northern) 3/2002–12/2004	esophageal cancer and Barrett's esophagus Cases: N = 137 for esophageal cancer, N = 182 for Barrett's esophagus; Controls: N = 221 Exposure assessment method: personal monitoring	≥ −5.4824	39	1.06 (0.57–1.98)	Age, sex, GI reflux, education, *H. pylori* infection, location, smoking	controls = 0.02 ± 0.04. Range: cases = 0.002–0.60; controls = 0.002–0.47 **Confounding:** No correlation of cobalt levels with selenium, chromium, zinc, mercury, and cerium reported, nor were other metals included in models. **Strengths:** Population based; histologically confirmed cancer. **Limitations:** Differences in sources of cases and controls in N. Ireland and Rep. of Ireland may introduce some selection bias; low participation rate in controls, especially in Rep. of Ireland. Not all samples reflect pre-diagnostic window of exposure. Single sample collected even though cobalt in toenails shown to have low reproducibility; window of exposure a concern with long latency cancer.
		≥ −4.4705	52	1.54 (0.84–2.85)		
		Trend-test p value: 0.16				
				Esophageal cancer		
		< −5.4824	34	OR = 1.0	Age, sex	
		≥ −5.4824	39	1.13 (0.64–1.99)		
		≥ −4.4705	52	1.54 (0.9–2.68)		
		Trend-test p value: 0.11				
				Barrett's Esophagus		
		< −5.4824	55	OR = 1.0	Age, sex, GI reflux, *H. pylori* infection, smoking habits, energy intake, location	
		≥ −5.4824	54	1.08 (0.55–2.1)		
		≥ −4.4705	64	1.97 (1.01–3.85)		
		Trend-test p value: 0.05				
				Barrett's Esophagus		
		<5.4824	55	OR = 1.0	Age, sex	
		≥5.4824	54	0.97 (0.59–1.59)		
		≥4.4705	64	1.18 (0.72–1.93)		
		Trend-test p value: 0.5				
Mur et al. (1987)	Electrochemical workers	**Buccal cavity, pharynx, larynx (140–149, 161)**				

Reference, Study Design, Location, and Year	Population Description & Exposure Assessment Method	Exposure Category or Level	Exposed Cases/ Deaths	Risk Estimate (95% CI)	Co-variates Controlled	Comments, Strengths, and Weaknesses
Cohort France 1950–1980	N = 1,143; number of cobalt production workers NR ~ 25% of current staff at time of publication **Exposure assessment method:** company records	Employed only in cobalt Production	2	SMR 3.36 (0.29–10.3)	Age, year of death	Exposure duration: 60% worked greater than 10 years; 75% hired before 1975. **Confounding**: Likely inadequate control for smoking; however, likely co-exposure to nickel and arsenic with no control for co-exposures. **Strengths:** Cobalt production workers exposed primarily to cobalt compounds. **Limitations:** Small number of exposed cases; high loss to follow-up (20%); potential for selection bias due to left truncation

GI = gastrointestinal; HWE = healthy worker effect; HWSE = healthy worker survival effect; OR = odds ratio.

Western Washington State Study of Aerodigestive Cancers

Rogers et al. (1993) reported elevated odds ratio for esophageal cancer for those with the highest levels (≥0.17 ppm) of cobalt concentration in toenails compared to those with the lowest level (<0.05 ppm) of cobalt (OR = 9.0, 95% CI = 2.7 to 30.0). The OR was elevated but not significant for those with medium levels (0.05 to 0.17 ppm) of cobalt concentration compared to those with low levels (OR = 2.4, 95% CI = 0.8 to 7.2). The exposure-response test for trend was significant ($p < 0.001$). It is not possible to comment on the distribution of levels of cobalt in the cases compared to the controls, as cases and controls are combined across exposure levels.

Confounding from known risk factors for esophageal cancer can reasonably be ruled out, however, other metals measured and associated with esophageal cancer in this analysis, were not controlled for in the cobalt models, nor were additional data presented to show any relationship between cobalt levels and other metal levels. In this study, the risk of esophageal cancer was also associated with elevated levels of calcium and iron. Smoking and alcohol use were controlled in the multivariate models along with age and gender, energy intake, beta-carotene, and ascorbic acid; however, while cases were less educated than controls, this variable was not included in the model. Neither beta-carotene nor ascorbic acid confounded the relationships between cobalt and esophageal cancer, but the authors included these two nutrients in the logistic model as it reduced the ORs slightly, raising the concern that the model estimates might have been over-controlled, biasing them slightly towards the null. Co-exposures from other metals were not reported or considered in the analysis of cobalt, and no correlations among the metals were reported.

The source of the cobalt exposure is unknown. When cobalt in nail tissue was expressed as a continuous variable, there were no associations between nail concentration of cobalt and dietary intake of foods high in cobalt (e.g., meat) suggesting that diet does not explain the elevated levels of cobalt in cases. Although occupational histories using questionnaires were collected in this study, no exposure assessment or analyses were done specifically for exposure to cobalt.

Although the Rogers et al. (1993) study provides some evidence of an association, the analysis of a single sample of toenail clippings collected near the time of diagnosis, with no accompanying data on potential sources of cobalt from the environment or occupational exposure, limits the utility of the study. Based on data on reproducibility of measurements of metals in toenails, cobalt has low to intermediate within-person reliability, suggesting that a single sample is less than ideal. Measurements of nail cobalt reflect an integration of exposures that occurred 12 to 18 months prior to clipping, raising the question about whether cobalt levels sampled in toenails close to, and in many cases after cancer diagnosis, reflect the relevant period of exposure for long latency cancer. No differences in cobalt levels were found between those with early- or late-stage cancer nor between those who provided samples within 7 months or beyond 7 months of diagnosis, which helps reduce concerns regarding reverse causality.

FINBAR Study – Ireland

O'Rorke et al. (2012) reported a non-significant elevated risk of esophageal adenocarcinoma among those with the highest cobalt levels (OR = 1.54, 95% CI = 0.84 to 2.85). In addition, they reported a significantly increased risk of Barrett's esophagus among participants with higher toenail concentrations of cobalt (≥ −4.4705, log transformed values equivalent to ≥0.011 μg/g) (OR = 1.97, 95% CI = 1.01 to 3.85), with a significant ($p = 0.05$) linear test for trend. Both of the estimates were adjusted for age, sex, smoking, location (Northern Ireland or Republic of

Ireland), energy intake, gastro-esophageal reflux, and *H. pylori* infection. O'Rorke et al. (2012) reported no information regarding the correlation between dietary intake of cobalt and nail concentration. In this study, a 2-fold risk of Barrett's esophagus was also associated with higher toenail concentrations of zinc.

The major limitation of this study, similar to the Rogers et al. (1993) study, however, is the exposure assessment method, which is an analysis of a single sample of toenail clippings collected near the time of diagnosis, with no accompanying data on potential sources of cobalt from the environment or occupational exposure. Given the long latency period for both Barrett's esophagus and esophageal cancer, there is concern that a measurement reflecting integrated exposures 12 to 18 months in the past is relevant. Similar to the Rogers et al. (1993) study, co-exposures from other metals were not reported or considered in the analysis of cobalt, and no correlations among the metals were reported.

Integration of the Evidence across Studies

While these two well-conducted, population-based, case-control studies in Ireland and in Western Washington state reported relatively consistent findings, had adequate numbers of participants, used sound methodologies, and demonstrated exposure-response relationships, the key issue of temporality remains unaddressed. The dependence of these studies upon a single sample of toenails collected at the time of diagnosis meant that neither had complete or even adequate data on cobalt during the relevant windows of exposure throughout the natural history of the two conditions to definitely establish temporality.

4.4. Cancer Assessment: Other Types of Cancers

4.4.1. Other Aerodigestive Cancers – Oral Cavity, Pharyngeal, and Laryngeal Cancers

The available data to evaluate cobalt in relation to other aerodigestive cancers, specifically cancers of the oral cavity, pharynx, and larynx, consist of the electrochemical workers cohort study (Mur et al. 1987), and one population-based case-control biomarker study (Rogers et al. 1993). The first publication from the electrochemical workers cohort (Mur et al. 1987) provided an SMR for buccal cavity, pharyngeal, and laryngeal cancers for those working in cobalt production. Rogers et al. (1993) provided OR estimates of cobalt in toenails among incident laryngeal cancers and oral cavity cancers and controls and included exposure-response data as well. These are rare cancers (incidence 11.0 per 100,000 men and women for oral cavity cancer; and 3.3 per 100,000 men and women for laryngeal cancers) (NCI 2015a); and unlike lung and esophageal cancers, 5-year survival rates are much higher for oral cavity/pharyngeal and laryngeal cancers (62.7% and 60.0%, respectively), suggesting that mortality statistics are less useful for informing the cobalt and cancer assessment. Potential risk factors for these cancers include smoking and other tobacco use, alcohol (tobacco and alcohol together are worse than either alone), asbestos, and nickel.

The risk of death from buccal cavity, pharyngeal, and laryngeal cancer among electrochemical workers was SMR = 3.36 (95% CI = 0.29 to 10.29), based on 2 observed deaths (Mur et al. 1987).

Rogers et al. (1993) reported a borderline significantly elevated odds ratio for oral cavity cancer for the highest level (≥0.17 ppm) of cobalt concentration in toenails compared to the lowest level (<0.05 ppm) of cobalt (OR = 1.9, 95% CI = 1.0 to 3.6). The OR was elevated but not significant for those with medium levels (0.05 to 0.17 ppm) of cobalt concentration compared to those with low levels (OR = 1.5, 95% CI = 0.9 to 2.6). The exposure-response test for trend was not significant (p value not reported). The finding was present in both in situ/localized tumors and individuals with regional/distant tumors. In this study, diet was not found to be an explanation for the higher risks, and tobacco and alcohol levels were controlled in the analyses.

A borderline significantly elevated odds ratio for laryngeal cancer was reported for medium toenail levels (0.05 to 0.17 ppm) compared to the lowest level (<0.05 ppm) of cobalt (OR = 2.0, 95% CI = 1.0 to 3.8). However, the OR for the highest level of cobalt was 1.0 (95% CI = 0.4 to 2.6), with no indication of a trend in exposure response.

As with esophageal cancer, it is not possible to assess the actual exposure levels among cases and controls as they are combined at each concentration level. Because nails were collected after diagnosis, to address potential reverse causation, cases were stratified by stage at diagnosis (in situ/localized versus regional/distant) and by time from diagnosis to interview (<7 months vs. ≥7 months). No statistically significant differences in the odds ratios by time from diagnosis to interview or stage of disease were observed, which argues against reverse causation.

With respect to these aerodigestive cancers, information is inadequate to evaluate the association with exposure to cobalt based on findings from these two studies, one of which was underpowered (Mur et al. 1987) and one of which had critical concerns regarding exposure misclassification due to the use of a single sample of toenails collected at the time of diagnosis, which might not have been the relevant window of exposure (Rogers et al. 1993).

4.4.2. Other Types of Cancers

The available data to evaluate cobalt in relation to other types of cancers is inadequate as it was primarily limited to one cohort study reporting on multiple cancers (Tüchsen et al. 1996) and two studies reporting on brain cancer (Moulin et al. 1993; Tüchsen et al. 1996) (data not shown). Neither of the two studies had adequate numbers of exposed cases (2 cases or fewer) to evaluate brain cancer risk from exposure to cobalt. Among porcelain painters exposed to cobalt dyes, the authors reported that cervical cancer was elevated (SIR = 2.31, lower confidence limit >1.0) based on 12 exposed cases (Tüchsen et al. 1996). For other cancer sites with at least four cases, elevated SIRs (not statistically significant) were also observed for ovary and other skin, and the SIR was close to 1.0 for breast cancer.

4.5. NTP Listing Recommendation

The data available from studies in humans are inadequate to evaluate the relationship between human cancer and exposure to cobalt. While almost all the cohort studies reported approximately a doubling of the risk of lung cancer mortality or incidence from exposure to various cobalt compounds, it is unclear that the excess lung cancer was due to exposure specifically to cobalt, because (1) it was not possible to rule out confounding by carcinogenic co-exposures; or (2) other complications prevented a clear interpretation of a cobalt effect.

The relevant data for evaluation of exposure specifically to cobalt are from studies of five major cohorts of workers exposed to cobalt in Denmark (Tüchsen et al. 1996), France (Moulin et al. 2000b; Moulin et al. 1993; Moulin et al. 1998; Mur et al. 1987; Wild et al. 2000), Norway (Grimsrud et al. 2005), and two population based case-control studies of aerodigestive cancers: one in Ireland (O'Rorke et al. 2012) and the other in Washington State, United States (Rogers et al. 1993). The Danish study showed similarly elevated risks of lung cancer in both the exposed and unexposed workers and could not control directly for smoking. Findings from the French electrochemical workers cohort were based on two papers using different methods to ascertain cancer, which produced conflicting results—the first indicated a significantly elevated risk of lung cancer based on 4 exposed cases, and the second showed virtually no differences in risk of lung cancer among the exposed and unexposed workers based on 3 exposed cases in a subset of workers born in France. In two French studies of hard-metal workers, measures of cobalt exposures were likely mixed with other carcinogens and the methods did not clearly indicate whether these were controlled in the analyses. The Norwegian study attempted to control for other co-exposures and smoking, but nickel and cobalt were highly correlated and an estimate for the full model could not be produced. However, a significant trend was reported with increasing duration of employment in workshops where cobalt concentrations three times those of nickel was reported in this study, which controlled for employment in other workshops and smoking.

In addition to lung cancer, esophageal cancer was of interest. Increased risks of esophageal cancer were found in the two population-based case-control studies; however, cobalt exposure was assessed based on one sample of toenails collected at or after cancer diagnosis. Thus, it is unclear whether these cobalt levels reflect exposure to cobalt during the relevant time window necessary for the induction of cancer. The data were inadequate to evaluate cancer at other tissue sites.

5. Studies of Cancer in Experimental Animals

This section reviews and assesses the evidence from carcinogenicity studies in experimental animals exposed to cobalt and cobalt compounds that release cobalt ions in vivo (hereinafter referred to as cobalt). Cancer and co-carcinogen studies in experimental animals were identified using methods described in the protocol and literature search strategy document (http://ntp.niehs.nih.gov/go/730697). In all, 23 publications (16 carcinogenicity and 9 co-carcinogenicity studies) were identified that met the following inclusion criteria: reported on the presence or absence of neoplastic and related non-neoplastic lesion, had concurrent or historical control group, and had an observational duration of 12 months or greater for rats and mice or were co-carcinogen exposure studies (initiation/promotion and other co-carcinogen studies that isolate the effect of cobalt compound exposures). Some of these publications overlap since some co-carcinogenicity studies had a cobalt exposure alone group and a corresponding control as part of their design. Several studies were excluded from the review because they did not have concurrent controls or controls from a closely related study. These included Hopps et al. (1954), Delahant (1955), Gilman (1962), Nowak (1966), and Gunn et al. (1967). Studies of cobalt alloys cobalt composites, cobalt compounds containing other metals, and radioactive cobalt in experimental animals were not considered to be informative because of potential confounding by other carcinogens. The cobalt alloys that were tested in experimental animals also contained other metals shown to be carcinogenic in experiments such as nickel and chromium and thus it would not be possible to separate any effects due to cobalt from those due to the other metals [see ((See IARC 2006 for a review of studies of cobalt alloys.) for a review of studies of cobalt alloys].

This section is organized by the type of study, i.e., carcinogenicity (Section 5.1) and co-carcinogenicity (Section 5.2). For each of these study types, the monograph provides an overview of the available studies, assesses their quality, discusses the findings, and identifies potential treatment-related cancer sites (carcinogenicity studies only). The co-carcinogen studies are only briefly discussed because they do not contribute substantially to the evaluation of potential carcinogenicity. Section 5.3 provides a synthesis of the findings for the different types of cobalt compounds across the cancer sites. The level of evidence conclusion for the carcinogenicity of cobalt compounds that release cobalt ions in vivo as a class from studies in experimental animals is provided in Section 7, which provides the rationale for evaluating them as a class.

5.1. Carcinogenicity Studies

5.1.1. Overview of the Studies

Different forms of cobalt were tested in 16 carcinogenicity studies: cobalt metal or cobalt nanoparticles (6 studies); two soluble cobalt salts, cobalt sulfate heptahydrate (2 studies) and cobalt chloride (1 study); and two poorly soluble cobalt compounds, cobalt(II) oxide (6 studies) and cobalt sulfide (1 study); (see Table 5-1). Most carcinogenicity studies were conducted in rats, with three studies in mice, and one study in hamsters. Routes of administration included either administration through the respiratory tract (inhalation or intratracheal instillation) or by local injection (subcutaneous, intramuscular, intraperitoneal, intrapleural, or intrarenal). Three publications that did not have concurrent controls for all or part of their series of studies were

included in the evaluation because the authors either reported non-concurrent controls from other parts of their series of studies (Heath 1956; Shabaan et al. 1977) or authors reported non-concurrent controls from a previous study in the same laboratory (Heath and Daniel 1962).

Table 5-1. Overview of Cancer Studies in Experimental Animals Reviewed

Strain (Sex)	Substance	Route	Exposure Period/ Study Duration	Reference
Cobalt metal				
Rat F344/NTac (M&F)	Cobalt metal	Inhalation	2 yr/2 yr	NTP (2014d)
Mouse B6C3F$_1$ (M&F)	Cobalt metal	Inhalation	2 yr/2 yr	NTP (2014d)
Rat Sprague-Dawley (M)	Cobalt metal [nano] and Cobalt metal [bulk][a]	IM inj. SC inj.	Single dose/1 yr	Hansen et al. (2006)
Rat Sprague-Dawley (F)	Cobalt metal	Intrarenal inj.	Single dose/1 yr	Jasmin and Riopelle (1976)
Rat Hooded (F)	Cobalt metal	Intrapleural inj.	Single dose/2.3 yr	Heath and Daniel (1962)
Rat Hooded (M&F)	Cobalt metal	IM inj.	Single dose/lifespan	Heath (1956)
Soluble cobalt compounds				
Rat F344/N (M&F)	Cobalt sulfate heptahydrate	Inhalation	2 yr/2 yr	NTP (1998)
Mouse B6C3F$_1$ (M&F)	Cobalt sulfate heptahydrate	Inhalation	2 yr/2 yr	NTP (1998)
Rat Wistar (M)	Cobalt chloride	SC inj.	8–12 mo/8–12 mo	Shabaan et al. (1977)
Poorly soluble cobalt compounds				
Rat Sprague-Dawley (M&F)	Cobalt(II) oxide	Intratracheal instill.	1.5 yr/lifespan	Steinhoff and Mohr (1991)
Rat Sprague-Dawley (M&F)	Cobalt(II) oxide	IP inj.	6 mo/lifespan	Steinhoff and Mohr (1991)
Rat Sprague-Dawley (M)	Cobalt(II) oxide	SC inj.	730 day/lifespan	Steinhoff and Mohr (1991)
Rat Wistar (M&F)	Cobalt(II)oxide	IM inj.	Single dose/1.3 yr	Gilman and Ruckerbauer (1962)
Mouse Swiss (F)	Cobalt(II) oxide	IM inj.	Single dose/2 yr	Gilman and Ruckerbauer (1962)
Hamster Syrian Golden (M)	Cobalt(II) oxide	Inhalation	Lifespan/lifespan	Wehner et al. (1977)
Rat Sprague-Dawley (F)	Cobalt sulfide	Intrarenal inj.	Single dose/1 yr	Jasmin and Riopelle (1976)

M = male, F = female, instill. = instillation, inj. = injection, IP = intraperitoneal, IM = intramuscular, SC = subcutaneous, wk = week, yr = year.
[a]Both cobalt compounds tested in the same animal.

5.1.2. Study Quality Assessment

Each of these primary studies was systematically evaluated for its ability to inform the cancer hazard evaluation using a series of signaling questions related to the following study performance elements: population, exposure conditions, outcome assessment, potential confounding, and statistics and reporting (see Protocol for Preparing the RoC Monograph on Cobalt (NTP 2014b)). An overview of the quality evaluations for the carcinogenicity studies is shown in Table 5-2 and discussed below. Details of each study assessment and quality criteria on a study-by-study basis are reported in Appendix D.

No critical concerns for biases were identified in any of the 16 carcinogenicity studies and they were all considered to have some utility for the cancer hazard evaluation. The four NTP inhalation studies (cobalt metal and cobalt sulfate in rats and mice) were considered to be the most informative (high utility) because they used a sufficient number of experimental animals of both sexes for a near lifetime exposure duration and tested three dose levels along with an untreated control. Two inhalation/intratracheal instillation studies of exposure to cobalt(II) oxide (Steinhoff and Mohr 1991; Wehner et al. 1977) and three injection studies of cobalt metal or cobalt sulfide in two publications (Hansen et al. 2006; Steinhoff and Mohr 1991) were considered to have moderate utility. In general, most of the limitations of the studies were related to low sensitivity of the study to detect an effect, e.g., due to the use of a single dose, short study duration, or small numbers of animals. In the remaining seven injection studies (Gilman and Ruckerbauer 1962; Heath 1956; Heath and Daniel 1962; Jasmin and Riopelle 1976; Shabaan et al. 1977), there were major concerns for several potential biases; thus, these studies were considered to have lower utility. Most of these studies had low sensitivity or incomplete necropsies. Poor reporting of methods and results was also a common problem, and in some studies, there were concerns about potential confounding. Historical controls from a related study by the same authors were used in lieu of concurrent controls in one study (Heath and Daniel 1962). Overall, the major limitations in the studies with low and moderate utility were primarily (but not exclusively) due to low sensitivity and for these cases there is little concern that these limitations would decrease confidence in a positive finding.

Table 5-2. Overview of Experimental Animal Carcinogenicity Study Quality Evaluations

Study		Quality								Sensitivity				Overall Utility
		Controls	Historical Data	Ran-dom-ization	Purity	Dosing	Treatment-related Survival	Pathology	Confounding	Reporting & Analysis	Animal Model	Stat Power	Duration	
NTP (2014d) R	Cobalt metal	+++	Yes	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	High
NTP (2014d) M	Cobalt metal	+++	Yes	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	High
Hansen et al. (2006)[a]	Cobalt metal and nano	+++	No	NR	NR	++	+++	+++	++	++	++	+	+	Moderate
Jasmin and Riopelle (1976)[a]	Cobalt metal and sulfide	+++	No	NR	++	+	NR	++	++	++	++	++	+	Low
Heath and Daniel (1962)	Cobalt metal	+	Yes[b]	NR	++	+	NR	++	++	+	++	+	+++	Low
Heath (1956)	Cobalt metal	++	Yes[b]	NR	++	+	NR	++	++	+	++	+	+++	Low
NTP (1998) R	Cobalt sulfate	+++	Yes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	High
NTP (1998) M	Cobalt sulfate	+++	Yes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	High
Shabaan et al. (1977)	Cobalt chloride	++	Yes[b]	NR	NR	+	++	+	+	+	++	++	+	Low
Steinhoff and Mohr (1991)-(intratracheal)	Cobalt(II) oxide	+++	No	NR	++	++	++	++	++	++	+++	+++	+++	Moderate
Steinhoff and Mohr (1991) - (IP)	Cobalt(II) oxide	+++	No	NR	++	+	NR	++	++	++	+++	+	+++	Moderate
Steinhoff and Mohr (1991) - (SC)	Cobalt(II) oxide	+++	No	NR	++	++	NR	++	++	++	++	+	+++	Moderate

Study		Quality									Sensitivity			Overall Utility
		Controls	Historical Data	Ran-dom-ization	Purity	Dosing	Treatment-related Survival	Pathology	Confounding	Reporting & Analysis	Animal Model	Stat Power	Duration	
Gilman and Ruckerbauer (1962) R	Cobalt(II) oxide	+++	No	NR	+	+	++	++	+	+	+++	+	++	Low
Gilman and Ruckerbauer (1962) M	Cobalt(II) oxide	+++	No	NR	+	+	++	++	+	++	++	++	+++	Low
Wehner et al. (1977)	Cobalt(II) oxide	+++	No	NR	++	++	+++	+++	++	+	++	+	+++	Moderate

+++ = high quality/little to no concerns, ++ = moderate quality/moderate concerns, + = low quality/high concerns, 0 = inadequate, NR = not reported; M = mice; R = rats.
[a]Includes test results for two forms of cobalt, so considered two studies.
[b]Limited number of controls (less than 15) from an earlier study.

5.1.3. Assessment of Neoplastic Findings from Carcinogenicity Studies

Discussions of the findings from the 16 carcinogenicity studies grouped by site of tumor development are reported below and in Table 5-3, Table 5-4, and Table 5-5. The main neoplasm locations were the lung in inhalation and intratracheal studies (six studies) and injection sites in studies using various routes of injection (subcutaneous, intramuscular, intraperitoneal, intrapleural, and intrarenal). In addition, in some inhalation studies, some tumors were observed in sites distal from the site of administration. Findings for cobalt compounds across organ sites are discussed in Section 5.3.

Lung

Different types of cobalt compounds—cobalt metal (NTP 2014d), a soluble cobalt salt, cobalt sulfate heptahydrate (NTP 1998), and a poorly soluble cobalt compound, cobalt(II) oxide (Steinhoff and Mohr 1991)—caused lung neoplasms after exposure by inhalation or intratracheal instillation. Study results for six respiratory exposure studies are reported in Table 5-3 including two studies in mice, three studies in rats, and one study in hamsters. Four of these studies were high-quality, well-designed, and well-conducted studies (NTP 1998; 2014d) and all had either high (NTP 1998; 2014d) or moderate (Steinhoff and Mohr 1991; Wehner et al. 1977) utility for evaluating potential cancer hazards.

Four studies found strong evidence that cobalt (both cobalt metal and cobalt sulfate) causes lung tumors in both mice and rats (NTP 1998; 2014d). Significant dose-related increases were seen for alveolar/bronchiolar carcinoma and for alveolar/bronchiolar adenoma or carcinoma combined in all dose groups (low, 1.25 mg/m^3; medium, 2.5 mg/m^3; high, 5 mg/m^3) in male and female mice and rats exposed to cobalt metal by inhalation (NTP 2014d). The incidences of alveolar/bronchiolar adenoma were also significantly increased in rats and mice, although not always in all dose groups. The incidences of carcinoma were very high; when adjusted for intercurrent mortality, incidences in the high-dose groups were 81% for male rats, 69% for female rats, 94% for male mice, and 88% for female mice. In addition, dose-related significant increases in multiplicity (animals with more than one lung tumor) of carcinoma were also found for all dose groups in male and female mice and male rats and in the high-dose (5 mg/m^3) groups for female rats (NTP 2014d). Female rats also had, in all dose groups, non-significant increases in cystic keratinizing epithelioma, which is a benign squamous-cell neoplasm that can progress to squamous-cell carcinoma. Cystic keratinizing epithelioma (CKE) is considered to be exposure-related in females because it is very rare, and a single squamous-cell carcinoma was also observed in the high-dose group. In males, a single CKE was found in each of the low- and high-exposure groups and may have been exposure-related. Lesions of alveolar or bronchiolar epithelial hyperplasia, which can progress to neoplasms, was also significantly increased in both sexes of rats and mice in all dose levels tested, except for bronchiolar epithelium hyperplasia in mice, which were significantly increased in mid- and high-dose groups in females and high-dose group in males.

In the NTP (1998) inhalation studies of cobalt sulfate heptahydrate, significant dose-related increases were observed for alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma in male and female mice (high dose, 3.0 mg/m^3) and female rats (high and mid dose, 1.0 mg/m^3) and for alveolar/bronchiolar carcinoma or adenoma combined for male rats (high dose) (NTP 1998). A single squamous-cell carcinoma was also found in the mid- and high-dose groups of

female rats. Non-neoplastic lesions of alveolar or bronchiolar epithelial hyperplasia (considered pre-neoplastic) and metaplasia were also significantly increased in both sexes of rats, but not in mice.

The fifth study reported significant increases in lung neoplasms (alveolar/bronchiolar adenoma, benign squamous epithelial neoplasm, or alveolar/bronchiolar carcinoma combined) in male rats administered cobalt(II) oxide by intratracheal instillation (Steinhoff and Mohr 1991). Non-significant increases in lung neoplasms (alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma) were seen in females. There were significant increases in alveolar/bronchiolar proliferation (types of lesions not described) in both sexes combined. Histological examinations were performed on all high-dose group animals; in the low-dose group and untreated control group, only those organs with gross lesions suspected of having tumors and all respiratory tracts were examined, which could underestimate the incidence by not detecting microscopic neoplasms.

In the last study, lung tumors were not observed in hamsters exposed to cobalt(II) oxide by inhalation, although exposure did cause pneumoconiosis, which was evidenced by a variety of lesions including, e.g., interstitial pneumonitis, diffuse granulomatous pneumonia, fibrosis of alveolar septa, and bronchial and bronchiolar epithelial (basal cell) hyperplasia (Wehner et al. 1977). There was relatively poor survival among the cobalt-treated animals and the corresponding dust sham-treated controls, which may have limited the sensitivity to detect an effect. In addition, hamsters have been described as a less sensitive model for detecting lung tumors than rats or mice (McInnes et al. 2013; Steinhoff and Mohr 1991). (Findings not reported in Table 5-3 because no tumors were observed.)

Table 5-3. Lung Neoplasms and Non-neoplastic Lesions in Experimental Animals Exposed to Cobalt Compounds

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
NTP (2014d) Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Multiple alveolar/bronchiolar carcinoma**			Survival in exposed groups was similar to controls. Strengths: A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. Limitations: Decreases in body weight in mid and high dose rats. Other comments: Historical controls were limited (100 rats). Significantly increased non-neoplastic lesions: Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels Bronchiolar hyperplasia (pre-neoplastic) - all dose levels
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	6/50 (12%)*	
			2.5 mg/m³	16	14/50 (28%)**	
			5 mg/m³	16	30/50 (60%)**	
			Alveolar/bronchiolar carcinoma[a]			
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	16/50 (38%)***	
			2.5 mg/m³	16	34/50 (77%)***	
			5 mg/m³	16	36/50 (81%)***	
			Trend-test p value: 0.001			
			Multiple alveolar/bronchiolar adenoma			
			0 mg/m³	17	1/50 (2%)	
			1.25 mg/m³	20	3/50 (6%)	
			2.5 mg/m³	16	2/50 (4%)	
			5 mg/m³	16	6/50 (12%)	
			Alveolar/bronchiolar adenoma[a]			
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	10/50 (24%)*	
			2.5 mg/m³	16	10/50 (23%)*	
			5 mg/m³	16	14/50 (33%)***	
			Trend-test p value: 0.011			
			Alveolar/bronchiolar carcinoma or adenoma combined[a]			

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	25/50 (58%)***	
			2.5 mg/m³	16	39/50 (85%)***	
			5 mg/m³	16	44/50 (94%)***	
			Trend-test p value: 0.001			
			Cystic keratinizing epithelioma			
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	1/50 (2%)	
			2.5 mg/m³	16	0/50 (0%)	
			5 mg/m³	16	1/50 (2%)	
NTP (2014d) Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 µm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Multiple alveolar/bronchiolar carcinoma**			Survival was significantly decreased in the mid-dose group. Strengths: A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. Limitations: A significant decrease in survival of female rats and decreases in body weight in mid- and high-dose rats. Other comments: Historical controls were limited (100 rats).
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	4/50 (8%)	
			2.5 mg/m³	24	3/50 (6%)	
			5 mg/m³	25	18/50 (36%)**	
			Alveolar/bronchiolar carcinoma[a]			
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	9/50 (21%)***	
			2.5 mg/m³	24	17/50 (42%)***	
			5 mg/m³	25	30/50 (69%)***	
			Trend-test p value: 0.001			
			Multiple alveolar/bronchiolar adenoma			
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	1/50 (2%)	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			2.5 mg/m³	24	3/50 (6%)	Significantly increased non-neoplastic lesions: Alveolar hyperplasia (pre-neoplastic) - all dose levels. Bronchiolar hyperplasia (pre-neoplastic) - all dose levels
			5 mg/m³	25	4/50 (8%)	
			Alveolar/bronchiolar adenoma[a]			
			0 mg/m³	35	2/50 (5%)	
			1.25 mg/m³	26	7/50 (16%)	
			2.5 mg/m³	24	9/50 (22%)*	
			5 mg/m³	25	13/50 (31%)**	
			Trend-test p value: 0.002			
			Alveolar/bronchiolar carcinoma or adenoma combined[a]			
			0 mg/m³	35	2/50 (4%)	
			1.25 mg/m³	26	15/50 (35%)***	
			2.5 mg/m³	24	20/50 (49%)***	
			5 mg/m³	25	38/50 (86%)***	
			Trend-test p value: 0.001			
			Squamous cell carcinoma			
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	0/50 (0%)	
			2.5 mg/m³	24	0/50 (0%)	
			5 mg/m³	25	1/50 (2%)	
			Cystic keratinizing epithelioma[a]			
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	4/50 (10%)[i]	
			2.5 mg/m³	24	1/50 (3%)[i]	
			5 mg/m³	25	2/50 (5%)[i]	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
			Trend-test p value: 0.002			
NTP (2014d) Mouse ($B6C3F_1$/N) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Multiple alveolar/bronchiolar carcinoma**			**Survival** significantly decreased at 2.5 and 5 mg/m³. **Strengths:** A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** A significant decrease in survival of male mice and decrease in body weight in high dose mice **Significantly increased non-neoplastic lesions:** Alveolar/bronchiolar epithelium hyperplasia (pre-neoplastic) - all dose levels Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels Bronchiolar epithelium hyperplasia (pre-neoplastic) - high dose
			0 mg/m³	39	3/50 (6%)	
			1.25 mg/m³	31	18/49 (36%)**	
			2.5 mg/m³	29	24/50 (48%)**	
			5 mg/m³	25	36/50 (72%)**	
			Trend-test p value: 0.001			
			Alveolar/bronchiolar carcinoma[a]			
			0 mg/m³	39	11/50 (23%)	
			1.25 mg/m³	31	38/49 (79%)***	
			2.5 mg/m³	29	42/50 (88%)***	
			5 mg/m³	25	46/50 (94%)***	
			Trend-test p value: 0.001			
			Multiple alveolar/bronchiolar adenoma			
			0 mg/m³	39	0/50 (0%)	
			1.25 mg/m³	31	1/49 (2%)	
			2.5 mg/m³	29	1/50 (2%)	
			5 mg/m³	25	0/50 (0%)	
			Alveolar/bronchiolar adenoma[a]			
			0 mg/m³	39	7/50 (15%)	
			1.25 mg/m³	31	11/49 (25%)	
			2.5 mg/m³	29	15/50 (36%)*	
			5 mg/m³	25	3/50 (7%)	
			Alveolar/bronchiolar carcinoma or adenoma combined[a]			

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
			0 mg/m³	39	16/50 (33%)	
			1.25 mg/m³	31	41/49 (85%)***	
			2.5 mg/m³	29	43/50 (90%)***	
			5 mg/m³	25	47/50 (96%)***	
			Trend-test p value: 0.001			
NTP (2014d) Mouse (B6C3F$_1$/N) Female (5–6 wk old) 105 wk	Cobalt metal 98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Multiple alveolar/bronchiolar carcinoma**			**Survival** in exposed groups was similar to controls. **Strengths:** A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** Decrease in body weight in high **Significantly increased non-neoplastic lesions**: Alveolar/bronchiolar epithelium hyperplasia (pre-neoplastic) - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels; Bronchiolar epithelium hyperplasia (pre-
			0 mg/m³	36	1/49 (10%)	
			1.25 mg/m³	36	7/50 (50%)*	
			2.5 mg/m³	27	20/50 (76%)**	
			5 mg/m³	26	24/50 (86%)**	
			Trend-test p value: 0.001			
			Alveolar/bronchiolar carcinoma[a]			
			0 mg/m³	36	5/49 (11%)	
			1.25 mg/m³	36	25/50 (54%)***	
			2.5 mg/m³	27	38/50 (79%)***	
			5 mg/m³	26	43/50 (88%)***	
			Trend-test p value: 0.001			
			Multiple alveolar/bronchiolar adenoma			
			0 mg/m³	36	0/49 (0%)	
			1.25 mg/m³	36	1/50 (2%)	
			2.5 mg/m³	27	0/50 (0%)	
			5 mg/m³	26	1/50 (2%)	
			Alveolar/bronchiolar adenoma[a]			
			0 mg/m³	36	3/49 (7%)	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
			1.25 mg/m³	36	9/50 (20%)	neoplastic)—mid- and high-dose levels
			2.5 mg/m³	27	8/50 (19%)	
			5 mg/m³	26	10/50 (25%)*	
			Trend-test p value: 0.037			
			Alveolar/bronchiolar carcinoma or adenoma combined[a]			
			0 mg/m³	36	8/49 (18%)	
			1.25 mg/m³	36	30/50 (64%)***	
			2.5 mg/m³	27	41/50 (85%)***	
			5 mg/m³	26	45/50 (92%)***	
			Trend-test p value: 0.001			
NTP (1998) Rat (F344) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Alveolar/bronchiolar carcinoma[b]**			**Survival** in exposed groups was similar to controls. **Strengths:** A well-designed study in all factors **Limitations:** None. **Significantly increased non-neoplastic lesions:** Alveolar epithelium metaplasia - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels
			0 mg/m³	17	0/50 (0%)	
			0.3 mg/m³	15	0/50 (0%)	
			1.0 mg/m³	21	3/48 (11%)	
			3.0 mg/m³	15	1/50 (7%)	
			Alveolar/bronchiolar adenoma[b]			
			0 mg/m³	17	1/50 (2%)	
			0.3 mg/m³	15	4/50 (18%)	
			1.0 mg/m³	21	1/48 (2%)	
			3.0 mg/m³	15	6/50 (28%)	
			Alveolar/bronchiolar adenoma or carcinoma combined[b]			
			0 mg/m³	17	1/50 (2%)	
			0.3 mg/m³	15	4/50 (18%)	
			1.0 mg/m³	21	4/48 (13%)	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
			3.0 mg/m³	15	7/50 (34%)*	
			Trend-test p value: 0.032			
NTP (1998) Rat (F344) Female (6 wk old) 2 yr	Cobalt sulfate (99% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Alveolar/bronchiolar carcinoma[b]**			Survival in exposed groups was similar to controls. **Strengths:** A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** None. **Significantly increased non-neoplastic lesions**: Alveolar epithelium metaplasia - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - high dose; Alveolar epithelium hyperplasia, atypical (pre-neoplastic) - high dose
			0 mg/m³	28	0/50 (0%)	
			0.3 mg/m³	25	2/49 (8%)	
			1.0 mg/m³	26	6/50 (20%)*	
			3.0 mg/m³	30	6/50 (18%)*	
			Trend-test p value: 0.023			
			Alveolar/bronchiolar adenoma[b]			
			0 mg/m³	28	0/50 (0%)	
			0.3 mg/m³	25	1/49 (3%)	
			1.0 mg/m³	26	10/50 (36%)***	
			3.0 mg/m³	30	9/50 (30%)***	
			Trend-test p value: 0.001			
			Alveolar/bronchiolar adenoma or carcinoma combined[b]			
			0 mg/m³	28	0/50 (0%)	
			0.3 mg/m³	25	3/49 (11%)[c]	
			1.0 mg/m³	26	15/50 (51%)***[c]	
			3.0 mg/m³	30	15/50 (46%)***[c]	
			Trend-test p value: 0.001			
			Squamous cell carcinoma			
			0 mg/m³	28	0/50 (0%)	
			0.3 mg/m³	25	0/49 (0%)	
			1.0 mg/m³	26	1/50 (2%)	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			3.0 mg/m³	30	1/50 (2%)	
			Alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma combined[b]			
			0 mg/m³	28	0/50 (0%)	
			0.3 mg/m³	25	3/49 (11%)	
			1.0 mg/m³	26	16/50 (54%)***	
			3.0 mg/m³	30	16/50 (49%)***	
			Trend-test p value: 0.001			
NTP (1998) Mice ($B6C3F_1$) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure mass median aerodynamic diameter 1–3 µm	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Alveolar/bronchiolar carcinoma[b]**			**Survival** in exposed groups was similar to controls. **Strengths:** A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** None. **No significant increase in non-neoplastic lesions.**
			0 mg/m³	22	4/50 (13%)	
			0.3 mg/m³	31	5/50 (16%)	
			1.0 mg/m³	24	7/50 (25%)	
			3.0 mg/m³	20	11/50 (44%)*[d]	
			Trend-test p value: 0.006			
			Alveolar/bronchiolar adenoma[b]			
			0 mg/m³	22	9/50 (30%)	
			0.3 mg/m³	31	12/50 (31%)	
			1.0 mg/m³	24	13/50 (41%)	
			3.0 mg/m³	20	18/50 (55%)*[e]	
			Trend-test p value: 0.018			
			Alveolar/bronchiolar carcinoma or adenoma combined[b]			
			0 mg/m³	22	11/50 (36%)	
			0.3 mg/m³	31	14/50 (37%)	
			1.0 mg/m³	24	19/50 (57%)	
			3.0 mg/m³	20	28/50 (79%)***[f]	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N[+]) (%)	Comments
			Trend-test p value: 0.001			
NTP (1998) Mice ($B6C3F_1$) Female (6 wk old) 2 yr	Cobalt sulfate 99% pure mass median aerodynamic diameter 1–3 µm	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Alveolar/bronchiolar carcinoma[b]**			**Survival** in exposed groups was similar to controls. **Strengths:** A well-designed study in all factors and survival was similar to controls. **Limitations:** None. **No significant increase in non-neoplastic lesions.**
			0 mg/m³	34	1/50 (3%)	
			0.3 mg/m³	37	1/50 (3%)	
			1.0 mg/m³	32	4/50 (9%)	
			3.0 mg/m³	28	9/50 (25%)**[g]	
			Trend-test p value: 0.001			
			Alveolar/bronchiolar adenoma[b]			
			0 mg/m³	34	3/50 (9%)	
			0.3 mg/m³	37	6/50 (15%)	
			1.0 mg/m³	32	9/50 (25%)	
			3.0 mg/m³	28	10/50 (33%)*[h]	
			Trend-test p value: 0.024			
			Alveolar/bronchiolar carcinoma or adenoma combined[b]			
			0 mg/m³	34	4/50 (12%)	
			0.3 mg/m³	37	7/50 (18%)	
			1.0 mg/m³	32	13/50 (33%)*[i]	
			3.0 mg/m³	28	18/50 (50%)***[i]	
			Trend-test p value: 0.001			
Steinhoff and Mohr (1991) Rat (Sprague-Dawley) Male (10 wk old) life span	Cobalt(II) oxide "Chemically pure." 80% of particles were 5–40 µm)	Intratracheal instillation (dry particulate) 1 dose/2 wk × 18 doses, then 1 dose/4 weeks × 11 doses (up to	**Bronchioalveolar carcinoma**			**Survival** in exposed groups was similar as controls. **Strengths:** Two dose levels tested in a high number of both sexes of
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	3/50 (6%)[j]	
			Bronchioalveolar adenoma			

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
		30th dose), then 1 dose/2 weeks × 9 doses (total 39 doses)	0 mg/kg bw	NR	0/50 (0%)	rats for two years, with observations for the lifespan without any significant difference in survival compared to untreated controls. **Limitations:** Only the high-dose group received full necropsies. Details of the chemical and animal husbandry were not reported. **Significantly increased non-neoplastic lesions:** Bronchioalveolar proliferation - both dose levels.
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	2/50 (4%)	
			Bronchioalveolar adenomas or bronchioalveolar carcinomas combined			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	5/50 (10%)*	
			Benign squamous epithelial tumor			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	1/50 (2%)	
			10 mg/kg bw	NR	0/50 (0%)	
Steinhoff and Mohr (1991) Rat (Sprague-Dawley) Female (10 wk old) life span	Cobalt(II) oxide "Chemically pure" 80% of particles were 5–40 μm)	Intratracheal instillation (dry particulate) 1 dose/2 wk × 18 doses, then 1 dose/4 weeks × 11 doses (up to 30th dose), then 1 dose/2 weeks × 9 doses (total 39 doses)	**Bronchioalveolar carcinoma**			**Survival** in exposed groups was similar to controls. **Strengths:** Two dose levels tested in a high number of both sexes of rats for two years, with observations for the lifespan without any significant difference in survival compared to untreated controls. **Limitations:** Only the high-dose group received full necropsies. Details of the chemical and animal
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	1/50 (2%)	
			Bronchioalveolar adenoma			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	1/50 (2%)	
			10 mg/kg bw	NR	0/50 (0%)	
			Bronchioalveolar adenoma or bronchioalveolar carcinoma combined			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	1/50 (2%)	

Reference & Year, Animal, Study Duration	Substance, Purity, Size	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			10 mg/kg bw	NR	1/50 (2%)	husbandry were not reported. **Significantly increased non-neoplastic lesions:** Bronchioalveolar proliferation - both dose levels.

* = p value ≤ 0.05; ** = p value ≤ 0.01; *** = p value ≤ 0.001. NR = Not reported, wk = week, yr = year.
+ = Number of animals necropsied for NTP (2014d) and NTP (1998) (each group started with 50 animals per sex in the NTP studies) and is the number of animals at the beginning of the study for all other studies.
[a]Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[b]Adjusted percent incidence based on Kaplan-Meier estimated incidence at the end of the study after adjustment for intercurrent mortality.
[c]Increased over historical control levels with a mean of 7/650 and range of 0% to 4%.
[d]Increased over historical control levels with a mean of 75/947 and range of 0% to 16%.
[e]Increased over historical control levels with a mean of 141/947 and range of 6% to 36%.
[f]Increased over historical control levels with a mean of 205/947 and range of 10% to 42%.
[g]Increased over historical control levels with a mean of 38/939 and range of 0% to 12%.
[h]Increased over historical control levels with a mean of 61/939 and range of 0% to 14%.
[i]Increased over historical control levels with a mean of 97/939 and range of 0% to 16%.
[j]Includes adenocarcinoma (2) and bronchioalveolar adenocarcinoma (1).

Injection Sites (Subcutaneous, Intramuscular, Intraperitoneal, Intrapleural, and Intrarenal)

Exposure to several different cobalt forms (cobalt metal, cobalt chloride, and cobalt(II) oxide) by injection increased injection-site tumors in several studies in rats (Gilman and Ruckerbauer 1962; Hansen et al. 2006; Heath 1956; Heath and Daniel 1962; Shabaan et al. 1977; Steinhoff and Mohr 1991). However, no injection tumors were observed in other studies in rats (Hansen et al. 2006; Jasmin and Riopelle 1976) or in the only study in mice (Gilman and Ruckerbauer 1962). Differences in dose levels, sex, and inadequate statistical power could explain these different findings. These studies were considered to have moderate (Hansen et al. 2006; Steinhoff and Mohr 1991) or low utility (Gilman and Ruckerbauer 1962; Heath 1956; Heath and Daniel 1962; Jasmin and Riopelle 1976; Shabaan et al. 1977). However, many concerns for potential biases were related to sensitivity such as limited dosing regimens and statistical power and thus would not necessarily decrease confidence in positive findings. Many studies also had limited reporting, which in part may be typical of older studies (published in the 1950s to 1970s). The relevance of injection studies for evaluating carcinogenicity in humans is discussed in the synthesis (Section 5.3).

Injection of cobalt metal (nanoparticles or microparticles) caused significant increases in the incidences of various types of sarcoma in several studies. Hansen et al. (2006) directly compared potential carcinogenic effects of cobalt metal nanoparticles and larger size cobalt metal particles in rats. However, both sizes of particles were placed into the same animals; cobalt nanoparticles were administered intramuscularly, and bulk cobalt metal was administered subcutaneously. The study also used a similar design to test other materials (nickel, titanium dioxide, and silicon dioxide). Cobalt-treated animals were sacrificed at 6 and 8 months (due to mortality from tumors) and compared to controls, which were administered polyvinyl chloride (PVC) and sacrificed at 6 and 12 months. Local sarcomas developed around the site of the nanoparticles in one of four rats at the 6-month sacrifice and in five of six rats at the 8-month sacrifice. No tumors were observed around the injection site of the bulk cobalt metal at either sacrifice time, although a single lesion of local fibroblastic proliferation occurred in one of six rats sacrificed at 8 months. The short duration period of 8 months limited the ability to see if the fibroblastic proliferation caused by microparticles would progress into neoplasms. The study also had limited statistical power because of small numbers of animals in the exposed and control groups. With respect to the other materials, tumors were observed in animals after implantation (nanoparticles) or subcutaneous injection (bulk) with nickel but not with injections of titanium dioxide or silicon dioxide. The ratio of surface area to volume between the nickel/cobalt and other compounds was not significantly different, which suggests that the neoplasms were not mediated by physical events and thus supports that the carcinogenic effect is due to cobalt.

A series of studies in hooded rats (Heath 1956; Heath and Daniel 1962) that injected cobalt metal by different exposure routes rats reported sarcomas—rhabdomyofibrosarcoma (including in the heart, intercostal muscle), rhabdomyosarcoma, fibrosarcoma, or other sarcoma—at the site of injection, but not in the controls. The earlier study (Heath 1956) injected cobalt into male and female rats intramuscularly in the thigh and the later study injected cobalt into the intrathoracic region (Heath and Daniel 1962). The controls from the 1956 study were used for the 1962 study. Rhabdomyofibrosarcoma, especially cardiac rhabdomyofibrosarcoma, are very rare tumors. Evidence that the sarcomas were caused by a local carcinogenic effect—beyond the fact that they only developed at injection sites—was seen by their tissue of origin. The 1962 study was limited by poor survival at the beginning of the study (eight rats died within three days) caused by the

injections. Sarcomas originating from muscle tissue were only found in studies that injected cobalt metal by intramuscular injection (rhabdomyofibrosarcoma or rhabdomyosarcoma) or intrapleural injection (cardiac or intercostal muscle rhabdomyosarcoma). Relatively high incidences in sarcomas were observed in both studies although the studies had limited sensitivity because only a few animals were tested at only one dose.

In contrast, no neoplasms were reported in a study in which cobalt metal was injected directly into the kidney of female rats, (Jasmin and Riopelle 1976). Compared to the other injection-site studies that used a single dose, Jasmin and Riopelle used a lower dose (10 mg/rat) than those used in the studies that induced neoplasms (>20 mg/rat) (Gilman and Ruckerbauer 1962; Heath 1956; Heath and Daniel 1962), suggesting that the dose might have been too low; in addition the study duration was only 12 months. The purpose of this study was to evaluate kidney carcinogenicity.

Cobalt chloride was tested in only one study by subcutaneous injection in male rats (Shabaan et al. 1977) in two similar experiments, one that ended after 8 months and one that lasted for 12 months. Only the 12-month study included an untreated control, but it seems reasonable to use that control for the 8-month study, especially since no neoplasms developed in the controls at 12 months. In the 12-month experiment, fibrosarcomas were found in 8/11 survivors at both the subcutaneous injection sites (4) and at sites distant from the injection site (4). In the 8-month experiment, 6 of the 16 animals who were alive at the end of the observation period had tumors (Shabaan et al. 1977). (Animals who died before 8 or 12 months were not examined for tumors.) Due to poor reporting, it was not possible to differentiate between tumors that occurred at injection sites versus non-injection sites. The cobalt-exposed animals developed persistent hyperlipaemia, and mortality was high for the treated animals.

Cobalt(II) oxide was injected (i.p., s.c., i.m.) into rats in three studies (Gilman and Ruckerbauer 1962; Steinhoff and Mohr 1991) and into mice (i.m.) in one study (Gilman and Ruckerbauer 1962). All rat studies reported significant increases in local neoplasms, either sarcoma, histiocytoma, or both combined. Although few rats were used in the studies, more than 50% of the rats developed injection-site tumors. No treatment-related increase in neoplasms was found in the one study in mice. The number of animals was adequate in this study; however, only one dose was used (lower than the rat study) and there was little information on dose selection. There were some concerns about potential for confounding from the animal husbandry conditions and limited information on chemical purity in the studies in rats and mice by Gilman and Ruckerbauer (1962). However, no tumors were observed in mice, the controls, or rats and mice injected with thorium dioxide, thus arguing against any potential confounding.

Only one study tested cobalt sulfide, which was injected intrarenally into female rats (Jasmin and Riopelle 1976). No neoplasms were reported in this study; however, the doses used in this study may have been low since they were similar to the doses used in the study with cobalt metal that was also negative.

Table 5-4. Injection Site Neoplasms and Non-neoplastic Lesions in Experimental Animals Exposed to Cobalt Compounds

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
Hansen et al. (2006) Rat (Sprague-Dawley) Male (NR) 12 mo	Cobalt metal [bulk and nano]- Bulk metal particles: 6.5 mm diameter by 1 mm in height; surface area to mass ratio of 4.73; nanoparticles: 50–200 nm in size (average 120 nm); surface area to mass ratio of 50,000; PVC (bulk and nano)-bulk PVC, without additives: 10 mm in diameter by 1 mm in height; surface area to mass ratio of 4.2; PVC nanoparticles: 60–170 nm in size (average 130 nm); surface area to mass ratio of 50,000.)	Nano (dry particles): IM implant (left side of vertebra) Single dose Bulk (solid metal): SC implant (right side of vertebra) Single dose	**Fibroblastic proliferation 6 months**			4 animals (PVC control and treated) sacrificed at 6 months and the remaining 6 animals sacrificed at either 8 (treated) or 12 months (PVC controls). Treated animals sacrificed at 8 months due to mortality. **Strengths:** Tested multiple materials in addition to cobalt and thus able to provide information on whether effects were due to physical state. **Limitations:** Inert polyvinyl chloride particles were used as a negative control. Only a small number of males were tested at a single dose level. Short duration and unable to fully evaluate effects from cobalt bulk particles.
			0 cm^2	4	0	
			Nano 2 cm^2	4	2	
			Bulk 2 cm^2	4	0	
			Sarcoma 6 months			
			0 cm^2	4	0	
			Nano 2 cm^2	4	1	
			Bulk 2 cm^2	4	0	
			Fibroblastic proliferation 8 months			
			0 cm^2 (12 mo)	6	0/6 (0%)	
			Nano 2 cm^2	6	1/6 (16.7%)	
			Bulk 2 cm^2	6	1/6 (16.7%)	
			Sarcoma 8 months			
			0 cm^2 (12 mo)	6	0/6 (0%)	
			Nano 2 cm^2	6	5/6 (83.3%)[**]	
			Bulk 2 cm^2	6	0/6 (0%)	
Heath (1956) Rat (Hooded)	Cobalt metal	IM inj. (in fowl serum)	**Rhabdomyofibrosarcoma or sarcoma combined**			**Survival**: No data was given on the survival of untreated
			0 mg/rat	NR	0/10 (0%)	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
Male (2–3 mo old) life span	"Spectroscopically pure" Particle size: 3.5 × 3.5 µm to 17 × 12 µm)	Single dose	28 mg/rat	8	4/10 (40%)	controls. 2/10 treated males without tumors died before final sacrificed. **Strengths:** Observation duration was sufficient and both sexes were tested. **Limitations:** Incomplete reporting of many elements. Limited sensitivity due to only one dose level and few rats tested. Full necropsies were not reported.
Heath (1956) Rat (Hooded) Female (2–3 mo old) life span	Cobalt metal "Spectroscopically pure" Particle size: 3.5 × 3.5 µm to 17 × 12 µm)	Series I and Series II i.m. inj. (in fowl serum) Single dose	**Sarcoma (Rhabdomyofibrosarcoma or fibrosarcoma)**			**Survival**: No data were reported on the survival of untreated controls. For treated animals, 4/10 rats (Series I) and 0/10 (Series II) without tumors died before final sacrificed. **Strengths:** Observation duration was sufficient and both sexes were tested. **Limitations:** Incomplete reporting of many elements. Limited sensitivity due to only one dose level and few rats tested. Full necropsies were not reported. **Other comments:** Series I used a concurrent control, but Series II used the same controls, which was non-concurrent. 6/7 sarcoma in Series I and 2/5 in Series II were rhabdomyo-fibrosarcoma
			0 mg/rat	NR	0/10 (0%)	
			28 mg/rat Series I	6	5/10 (50%)	
			28 mg/rat Series II	10	7/10 (70%)	
Heath and Daniel (1962) Rat (Hooded) Female (2–3 mo old)	Cobalt metal Purity not reported,	Intrathoracic inj. (in serum) Single injection	**Mixed sarcoma intrathoracic region**			**Survival** was only reported for exposed rats, which was
			0 mg/dose[a]	NR	0/10 (0%)	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
28 months	Particle size: 3.5 × 3.5 μm to 17 × 12 μm)		28 mg/dose	11	4/12 (33%)	12/20 on day 3 and 11/20 after 11 months. **Strengths:** Observation duration was sufficient **Limitations:** Historical controls from Heath 1956 used because there was no concurrent control. Few animals were used, and full necropsies were not done, only skin tumors were histologically examined. Incomplete reporting of many elements. **Other comments:** 3 of 4 tumors originated in part from cardiac muscle, which are very rare.
Jasmin and Riopelle (1976) Rat (Sprague-Dawley) Female (120–140 g) 12 months	Cobalt metal NR	Intrarenal placement (in glycerin) Single dose	**Kidney neoplasm NOS**			**Survival** was not reported. **Strengths:** Moderate number of animals. **Limitations:** Only a single dose level, which was lower than other studies, was tested in only females. Incomplete reporting for many elements. Full necropsies were not performed, though the abdominal and thoracic cavities were examined.
			0 mg/rat	NR	0/16 (0%)	
			10 mg/rat	NR	0/18 (0%)	
Shabaan et al. (1977) Rat (Wistar) Male (4 wk old) 8 and 12 mo	Cobalt chloride NR	SC inj. (in saline) 1 dose/day × 5 days, then 9 days off, then	**Injection site and non-injection fibrosarcoma**			Treatment-related decrease in **survival;** 16/20 survived at 8 months and 11/20 survived at 12 months.
			0 mg/kg bw 12 mo	19	0/19 (0%)	
			40 mg/kg bw 8 mo	16	6/16 (30%)[**]	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N$^+$) (%)	Comments
		1 dose/day × 5 days (total 19 days)	40 mg/kg bw 12 mo	11	8/11 (40%)[***]	**Limitations:** Exposure resulted in persisent hyperlipaemia and high mortality. Animals dying before the end of observation period were not exmained for tumors. The tumors at injection sites and non-injection sites weren't clearly reported **Other comments:** No concurrent untreated controls used at 8 months, 12-month controls used as comparison group. Statistical testing (Fisher's Exact Test) reported by IARC.
Steinhoff and Mohr (1991) Rat (Sprague-Dawley) Male and Female (10 wk old) life span	Cobalt(II) oxide "Chemically pure," 80% of particles were 5–40 µm)	i.p. inj. (in saline) 1 dose/2 mo × 6 mo	**Sarcoma**			**Survival** was not reported. **Strengths:** Both sexes of rats were tested with a long duration of observation. **Limitations:** Incomplete reporting. Limited sensitivity because of few animals per group, only one dose level was tested, and exposure was for less than one year. Limited histological examination **Other comments:** Results were reported as combined for males and females.
			0 mg/kg	NR	1/20 (5%)	
			200 mg/kg	NR	3/20 (15%)[*]	
			Mesothelioma			
			0 mg/kg	NR	0/20 (0%)	
			200 mg/kg	NR	1/20 (5%)	
			Histiocytoma			
			0 mg/kg	NR	1/20 (5%)	
			200 mg/kg	NR	10/20 (50%)[**]	
Steinhoff and Mohr (1991) Rat (Sprague-Dawley) Male (10 wk old) life span	Cobalt oxide ("Chemically pure," 80% of particles were 5–40 µm)	s.c. inj. (in saline) 1 inj/day, 5 day/week × 730 days	**Histiocytoma or sarcoma combined**			**Survival** was not reported. **Strengths:** Duration of exposure and observation were sufficient. One dose level was tested, at two
			0 mg/kg/wk	NR	0/10 (0%)	
			0 mg/kg/wk	NR	0/10 (0%)	
			2 mg/kg × 5/wk	NR	5/10 (50%)[*]	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			10 mg/kg/wk	NR	4/10 (40%)[*]	intensity levels and two untreated control groups used. **Limitations:** Limited sensitivity due to few animals per group and only males tested. Limited histological examination. Incomplete reporting of many elements.
Gilman and Ruckerbauer (1962) Rat (Wistar) Male and female (2–3 mo old) 489 days	Cobalt oxide purity not reported, particle size was <5 μm	i.m. inj. (in aqueous suspension of penicillin G procaine) Single dose		**Sarcoma**		**Survival** was similar to control at 90 days. **Strengths:** The duration of observation was sufficient and both sexes were tested. **Limitations**: Limited sensitivity because only a single dose was given at one dose level and few animals per group were tested. Incomplete reporting for many elements. Animal bedding was periodically dusted with rotenone powder. **Other comments:** Results were reported as combined for males and females.
			0 mg/rat	10	0/10 (0%)	
			30 mg/rat	10	5/10 (50%)[*]	
Gilman and Ruckerbauer (1962)	Cobalt oxide purity not reported,	i.m. inj. (in aqueous suspension of		**Sarcoma**		**Survival** was similar to control at 90 days.
			0 mg/mouse	48	0/51 (0%)	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N[+]) (%)	Comments
Mouse (Swiss) Female (2–3 mo old) 751 days	particle size was <5 µm	penicillin G procaine) Single dose	20 mg/mouse	46	0/50 (0%)	**Strengths:** The duration of observation and the numbers of animals per group were sufficient. **Limitations:** Limited sensitivity due to only a single dose was given at one dose level, without a rationale, to females only. Half of the mice were survivors from a preliminary study who received unwashed cobalt, which was known to contain other toxic chemicals. Bedding was periodically dusted with rotenone powder. Incomplete reporting for many elements.
Jasmin and Riopelle (1976) Rat (Sprague-Dawley) Female (120–140 g) 12 months	Cobalt sulfide NR	Intrarenal placement (in glycerin) Single dose	**Kidney neoplasm NOS**			**Survival** was not reported. **Strengths:** Moderate number of rats per groups. **Limitations:** Limited sensitivity due to only a single dose level, which was lower than other studies and only females tested. Incomplete reporting. Full necropsies were not performed, though the abdominal and thoracic cavities were examined.
			0 mg/rat	NR	0/16 (0%)	
			10 mg/rat	NR	0/20 (0%)	

* = p value ≤ 0.05; ** = p value ≤ 0.01; *** = p value ≤ 0.001.
NR = Not reported, inj. = injection, i.p. = intraperitoneal, i.m. = intramuscular, s.c. = subcutaneous, wk = week, mo = month.
[+] = Number of animals at the beginning of the study, except for Hansen et al. (2006) and Heath and Daniel (1962), which used the number of animals that were examined at the time of sacrifice, 10 animals were originally assigned to each group (Hansen et al. 2006) or the number of animals that survived beyond day 4, 10 control and 20 exposed animals were originally assigned (Heath and Daniel 1962).
[ ] = Statistical significance calculated by NTP using Fisher's Exact Test.
[a]Historical control group from earlier study by the same author.

Other Neoplasms Including Those at Distal Sites

Several lines of evidence support systemic exposure of rats and mice to cobalt. Cobalt concentrations and burdens increased with increasing exposure concentrations in all studies in all tissues examined; however, tissue burdens normalized by exposure concentration showed increased levels only in the liver [(NTP 2014d); see Section 5.1.3]. In addition, neoplasms were observed at several organ sites (pancreas, hematopoietic system, and kidney distal to the route of administration.

Adrenal Gland

Neoplasms of the adrenal gland were reported in two inhalation studies that tested cobalt metal and cobalt sulfate (see Table 5-5) (NTP 1998; 2014d; Wehner et al. 1977). In the four NTP studies, cobalt metal and cobalt sulfate heptahydrate were each tested in both mice and rats, but adrenal gland neoplasms developed only in rats. One study reported a single adrenal gland neoplasm in hamsters exposed to cobalt(II) oxide (Wehner et al. 1977). There is a high background of adrenal tumors in the male rats in the two NTP studies. Adrenal gland neoplasms can develop because of damage to lungs that causes obstructive sequelae by causing systemic hypoxemia, leading to chronic stimulation of catecholamine release by the adrenal medulla and subsequent neoplastic development (NTP 2014d). Since inhalation of cobalt caused lesions in the lung that could cause obstruction (chronic inflammation), it is possible that the adrenal glands are not directly caused by systemic exposure to cobalt but could be a secondary response to lung damage. However, there is not enough evidence to differentiate between a direct or indirect cause of adrenal gland neoplasms from cobalt exposure.

The strongest evidence for a treatment-related effect comes from the rat studies with cobalt metal. Inhalation exposure to cobalt metal significantly increased bilateral malignant pheochromocytoma in the high-dose group (5 mg/m^3) and all malignant pheochromocytoma, malignant or benign pheochromocytoma combined, and benign pheochromocytoma in both the mid- (2.5 mg/m^3) and high-dose groups in male rats. In females, there was a significantly increased incidence of bilateral malignant pheochromocytoma as well as malignant pheochromocytoma overall at the high dose and malignant or benign pheochromocytoma combined, and bilateral benign pheochromocytoma as well as benign pheochromocytoma in both the mid- and high-dose groups (NTP 2014d). Hyperplasia of the adrenal gland was also significantly increased in females at mid and high doses and was significantly decreased in males in the mid- and high-dose groups.

Cobalt sulfate heptahydrate caused significant increases in malignant, benign, or complex adrenal neoplasms combined in both sexes, which were higher than historical controls (NTP 1998). However, increases were only significant in the high-dose (3 mg/m^3) group in females and the mid-dose (1 mg/m^3) group in males. Females had a significant trend of increasing tumor incidence with increasing dose for benign pheochromocytoma and all tumor types combined. Hyperplasia was significantly increased in females and the high-dose but was significantly decreased in the low-dose (0.3 mg/m^3) males.

Wehner et al. (1977) reported finding a single adrenal gland adenoma in the cortex of hamsters after inhalation of cobalt(II) oxide. Wehner et al. (1977) only tested one dose level, 10 mg/m^3, which was higher than those used in mice or rats in the two NTP studies. The significant increases in rats, but not mice or hamsters, could indicate a species difference in sensitivity to

developing adrenal gland tumors from cobalt exposure, especially considering hamsters received a higher dose level than the rats.

Distal Sites: Pancreatic Islet Cell, Hematopoietic System, and Kidney

Inhalation exposure to cobalt metal also caused other tumors at sites distant from the route of administration: pancreas in male rats and mononuclear-cell leukemia in female rats in the NTP inhalation bioassay of cobalt metal (Behl et al. 2015; NTP 2014d). A non-significant increase in the incidence of kidney tumors was observed in male rats. It is not clear whether the kidney tumors were treatment related. Tumors were not observed in the pancreas, kidney, or hematopoietic system of rats exposed to cobalt sulfate or mice exposed to either form of cobalt. Findings are presented in Table 5-5 and briefly summarized below.

Male rats exposed to cobalt metal were found to have a significant increase in the incidences of pancreatic islet-cell carcinoma or adenoma combined in both the mid- and high-dose groups and a significant positive dose-related trend was observed. A significant increase in the incidence of pancreatic adenoma was also observed in the mid-dose group in males. The non-significant increases in the incidence of pancreatic islet-cell carcinoma observed in female rats exceeded the historical controls for all routes of administration and thus might have been related to exposure. However, historical controls were limited as they were based on a dataset of only 100 Fischer 344/NTac rats from two NTP carcinogenicity studies. Significant increases in the incidence of mononuclear-cell leukemia were seen in females in all dose groups, which exceeded the limited historical controls for all exposure routes. In addition, time to first tumor was shorter in cobalt-exposed animals (117 to 590 days) compared to the concurrent control (663 days) albeit there was no pattern of decreasing duration with increasing dose and because of the limited historical control database, it is not known how much time to first tumor in untreated animals varies across studies. The incidence of mononuclear-cell leukemia was similar in male rats compared to the untreated controls.

The incidence of kidney neoplasms (adenoma or carcinoma combined) was higher (although not significantly so) in the low- and high-dose male rats compared to the concurrent controls and a significant trend was observed. The incidence exceeded the historical controls for all routes of administration, but the historical controls are limited as mentioned above. Four of the five neoplasms were adenomas. In analyses of standard and extended evaluations, a significant trend was observed; two of the seven neoplasms in the high-dose group were carcinomas. Kidney neoplasms are relatively rare, so non-significant increases may be related to cobalt exposure (NTP 2014d). No treatment-related non-neoplastic lesions were observed. Two studies injected cobalt sulfide or cobalt metal directly into the kidneys of female rats in one publication (Jasmin and Riopelle 1976). No kidney tumors or any other tumors were reported as being significantly increased. Only a single dose was given at one dose level and the dose was lower than that used in other injection studies.

Table 5-5. Other and Distal Site Neoplasms and Relevant Non-neoplastic Lesions in Experimental Animals Exposed to Cobalt Compounds

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
Adrenal gland						
NTP (2014d) Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Bilateral malignant pheochromocytoma**			**Survival** was similar to controls. **Strengths:** A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** Decreases in body weight in mid and high dose rats. **Other comments:** Historical controls were limited (100 rats). **No significantly increased non-neoplastic lesions.**
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	0/50 (0%)	
			2.5 mg/m³	16	0/50 (0%)	
			5 mg/m³	16	7/50 (14%)**	
			Malignant pheochromocytoma[a]			
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	2/50 (5%)	
			2.5 mg/m³	16	9/50 (21%)*	
			5 mg/m³	16	16/50 (39%)***	
			Trend-test p value: 0.001			
			Benign pheochromocytoma[a]			
			0 mg/m³	17	15/50 (36%)	
			1.25 mg/m³	20	23/50 (54%)	
			2.5 mg/m³	16	37/50 (81%)***	
			5 mg/m³	16	34/50 (76%)***	
			Trend-test p value: 0.001			
			Malignant or benign combined pheochromocytoma[a]			
			0 mg/m³	17	17/50 (40%)	
			1.25 mg/m³	20	23/50 (54%)	
			2.5 mg/m³	16	38/50 (83%)***	
			5 mg/m³	16	41/50 (91%)***	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^{+}) (%)	Comments
			Trend-test p value: 0.001			
NTP (2014d) Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal 98% pure, mass median aerodynamic diameter 1–3 µm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Bilateral malignant pheochromocytoma**			**Survival** was significantly decreased in the mid-dose group. **Strengths:** A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** A significant decrease in survival of female rats. Decreases in body weight in mid- and high-dose rats. **Other comments:** Historical controls were limited, as Fischer 344/NTac rats (100 rats). **Significantly increased non-neoplastic lesions:** Hyperplasia - low and medium
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	1/50 (2%)	
			2.5 mg/m³	24	1/49 (2%)	
			5 mg/m³	25	4/50 (8%)*	
			Malignant pheochromocytoma[a]			
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	2/50 (5%)	
			2.5 mg/m³	24	3/49 (8%)	
			5 mg/m³	25	11/50 (27%)***	
			Trend-test p value: 0.001			
			Bilateral benign pheochromocytoma			
			0 mg/m³	35	2/50 (4%)	
			1.25 mg/m³	26	4/50 (8%)	
			2.5 mg/m³	24	8/49 (16%)*	
			5 mg/m³	25	19/50 (38%)**	
			Benign pheochromocytoma[a]			
			0 mg/m³	35	6/50 (14%)	
			1.25 mg/m³	26	12/50 (27%)	
			2.5 mg/m³	24	22/49 (52%)***	
			5 mg/m³	25	36/50 (81%)***	
			Trend-test p value: 0.001			
			Malignant or benign combined pheochromocytoma[a]			

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N[+]) (%)	Comments
			0 mg/m³	35	6/50 (14%)	
			1.25 mg/m³	26	13/50 (29%)	
			2.5 mg/m³	24	23/49 (55%)***	
			5 mg/m³	25	40/50 (89%)***	
			Trend-test p value: 0.001			
NTP (1998) Rat (F344) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure)	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Benign pheochromocytoma[b]**			**Survival** was similar to controls. **Strengths:** A well-designed study in all factors and survival was similar to controls such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** None. **No significantly increased non-neoplastic lesions.**
			0 mg/m³	17	14/50 (51%)	
			0.3 mg/m³	15	19/50 (70%)	
			1.0 mg/m³	21	23/48 (72%)	
			3.0 mg/m³	15	20/50 (71%)	
			Malignant, benign, or complex pheochromocytoma combined[b]			
			0 mg/m³	17	15/50 (52%)	
			0.3 mg/m³	15	19/50 (70%)	
			1.0 mg/m³	21	25/48 (74%)*[c]	
			3.0 mg/m³	15	20/50 (71%)	
NTP (1998) Rat (F344) Female (6 wk old) 2 yr	Cobalt sulfate 99% pure	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	**Benign pheochromocytoma[b]**			**Survival** was similar to controls. **Strengths**: A well-designed study in all factors such as such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** None **Significantly increased non-neoplastic lesions**: Adrenal gland:
			0 mg/m³	28	2/48 (5%)	
			0.3 mg/m³	25	1/49 (3%)	
			1.0 mg/m³	26	3/50 (9%)	
			3.0 mg/m³	30	8/48 (26%)*	
			Trend-test p value: 0.004			
			Malignant, benign, or complex combined[b]			
			0 mg/m³	28	2/48 (4%)	
			0.3 mg/m³	25	1/49 (2%)	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			1.0 mg/m³	26	4/50 (8%)	hyperplasia - high dose.
			3.0 mg/m³	30	10/48 (21%)*[d]	
			Trend-test p value: 0.001			
Wehner et al. (1977) Hamster (Syrian Golden, random bred ENG:ELA) Male (2 mo old) Lifespan	Cobalt(II) oxide Purity not reported Median diameter of particles 0.14 µm, Median mass diameter 0.45 µm Geometric standard deviation 1.9 µm	Inhalation (dry particulate) 7 hr/day, 5 days/wk × lifespan		**Adenoma (cortex)**		**Survival** in exposed group is similar to control **Strengths:** Duration of exposure and observation were sufficient. **Limitations:** Incomplete reporting. Low sensitivity because of relatively poor survival of both exposed and controls, only a single dose level was tested with no justification for choosing that level. **Other comments:** The study looked at cobalt's effect on cigarette smoke, but a cobalt oxide only group was tested. Cobalt-exposed hamsters developed pneumoconiosis.
			0 µg/L	NR	0/51 (0%)	
			10.1 µg/L	NR	1/50 (2%)	
Pancreas						
NTP (2014d) Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk		**Carcinoma[a]**		**Survival** was similar to controls. **Strengths:** A well-designed study in all factors such as long
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	1/50 (3%)	
			2.5 mg/m³	16	5/48 (13%)[e]	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^{+}) (%)	Comments
			5 mg/m³	16	6/49 (15%)[e]	observation period, sufficient dose levels, adequate number of animals. **Limitations:** Decreases in body weight in mid- and high-dose rats. **Other comments:** Historical controls were limited (100 rats). **No significantly increased non-neoplastic lesions.**
			Trend-test p value: 0.021			
				Adenoma[a]		
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	1/50 (3%)	
			2.5 mg/m³	16	6/48 (15%)*	
			5 mg/m³	16	3/49 (8%)	
				Carcinoma or adenoma combined[a]		
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	2/50 (5%)	
			2.5 mg/m³	16	10/48 (25%)*[e]	
			5 mg/m³	16	9/49 (23%)*[e]	
			Trend-test p value: 0.002			
NTP (2014d) Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk		**Carcinoma[a]**		**Survival** was significantly decreased in the mid-dose group. **Strengths:** A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** A significant decrease in survival of female rats. Decreases in body weight in mid- and high-dose rats.
			0 mg/m³	35	1/50 (2%)	
			1.25 mg/m³	26	0/50 (0%)	
			2.5 mg/m³	24	0/50 (0%)	
			5 mg/m³	25	3/50 (7%)[f]	
				Adenoma		
			0 mg/m³	35	0/50 (0%)	
			1.25 mg/m³	26	0/50 (0%)	
			2.5 mg/m³	24	0/50 (0%)	
			5 mg/m³	25	1/50 (2%)	
				Carcinoma or adenoma combined[a]		

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^+) (%)	Comments
			0 mg/m³	35	1/50 (2%)	**Other comments:** Historical controls were limited (100 rats). **No significantly increased non-neoplastic lesions**
			1.25 mg/m³	26	0/50 (0%)	
			2.5 mg/m³	24	0/50 (0%)	
			5 mg/m³	25	3/50 (7%)[f]	
Hematopoietic system						
NTP (2014d) Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Mononuclear cell leukemia[a]**			**Survival** was similar to controls. **Strengths:** A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** None. **Other comments:** Historical controls were limited (100 rats). **No significantly increased non-neoplastic lesions.**
			0 mg/m³	17	21/50 (49%)	
			1.25 mg/m³	20	25/50 (58%)	
			2.5 mg/m³	16	22/50 (50%)	
			5 mg/m³	16	22/50 (48%)	
NTP (2014d) Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Mononuclear cell leukemia[a]**			**Survival** was significantly decreased in the mid-dose group. **Strengths:** A well-designed study in almost
			0 mg/m³	35	16/50 (36%)	
			1.25 mg/m³	26	29/50 (62%)**[g]	
			2.5 mg/m³	24	28/50 (61%)*[g]	

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^{+}) (%)	Comments
			5 mg/m³	25	27/50 (59%)*[g]	all factors such as long observation period, sufficient dose levels, adequate number of animals. **Limitations:** A significant decrease in survival of female rats. Decreases in body weight in mid- and high-dose rats. **Other comments:** Historical controls were limited (100 rats). **No significantly increased non-neoplastic lesions**
Kidney						
NTP (2014d) Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	**Tubule adenoma[a]**			**Survival** was similar to controls. **Strengths:** A well-designed study in all factors. **Limitations:** Decreases in body weight in mid- and high-dose rats. **Other comments:** Historical controls were limited (100 rats.) **No significantly increased non-neoplastic lesions**
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	1/50 (3%)[h]	
			2.5 mg/m³	16	0/50 (0%)	
			5 mg/m³	16	3/50 (8%)[h]	
			Tubule carcinoma or adenoma[a]			
			0 mg/m³	17	0/50 (0%)	
			1.25 mg/m³	20	1/50 (3%)[h]	
			2.5 mg/m³	16	0/50 (0%)	
			5 mg/m³	16	4/50 (10%)[h]	
			Trend-test p value: 0.018			
			Tubule carcinoma or adenoma[ai]			

Reference & Year, Animal, Study Duration	Substance & Purity	Dosing Regimen	Dose Levels	# Animals at Sacrifice	Tumor Incidence (n/N^{+}) (%)	Comments
			0 mg/m³	17	3/50 (8%)	
			1.25 mg/m³	20	1/50 (3%)	
			2.5 mg/m³	16	1/50 (2%)	
			5 mg/m³	16	7/50 (17%)	
			Trend-test p value: 0.023			

*p value < 0.05; **p value < 0.01; ***p value < 0.01.
[+] = Number of animals necropsied for NTP (2014d) and NTP (1998) (each group started with 50 animals per sex in the NTP studies) and is the number of animals at the beginning of the study for all other studies.
NR = Not reported, M = male, F = female, hr = hour, wk = week, mo = month, yr = year.
[a]Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[b]Adjusted percent incidence based on Kaplen-Meier estimated incidence at the end of the study after adjustment for intercurrent mortality.
[c]Increased over historical control levels with a mean of 176/623 and range of 8% to 50%.
[d]Increased over historical control levels with a mean of 39/608 and range of 2% to 14%.
[e]Increased over historical control levels with a mean of 2/100 and range of 0% to 4%.
[f]Increased over historical control levels with a mean of 1/100 and range of 0% to 2%.
[g]Increased over historical control levels with a mean of 35/100 and range of 32% to 38%.
[h]Increased over historical control levels with a mean of 1/100 and range of 0% to 2%.
[i]Analyzed by standard and extended evaluation. In a standard evaluation a single section of each kidney is examined histologically, while in an extended evaluation, three to four additional sections, taken at 1 mm intervals are examined histologically.

5.2. Co-carcinogenicity Studies

5.2.1. Overview of the Studies

Nine co-carcinogen studies were identified that tested soluble compounds, including four studies using cobalt chloride (Finogenova 1973; Kasirsky et al. 1965; O'Hara et al. 1971; Zeller 1975) and three studies using sodium cobaltinitrite (O'Hara et al. 1971; Orzechowski et al. 1964; Thompson et al. 1965); and a poorly soluble compound, cobalt(II) oxide, in two studies (Steinhoff and Mohr 1991; Wehner et al. 1977) (see Table 5-6). Most co-carcinogen studies were conducted in mice, though two studies were conducted in rats (Steinhoff and Mohr 1991; Zeller 1975) and one study conducted in hamsters (Wehner et al. 1977). Almost all of the co-carcinogen studies used dermal exposure to methylcholanthrene as the known carcinogen, with Zeller using subcutaneous injections of diethylnitrosamine, Steinhoff and Mohr using intratracheal instillation of benzo[*a*]pyrene, and Wehner using inhalation exposure to cigarette smoke. Methylcholanthrene induced skin tumors, while diethylnitrosamine induced liver and nasal tumors, benzo[*a*]pyrene induced lung tumors, and cigarette smoke increased incidences of total malignant or total benign neoplasms. Cobalt compounds were administered by intraperitoneal injection in all but four studies, which used subcutaneous injection (Zeller 1975), drinking water (Thompson et al. 1965), inhalation (Wehner et al. 1977), and intratracheal instillation (Steinhoff and Mohr 1991) as routes of exposure.

Table 5-6. Overview of Co-carcinogenicity Studies in Experimental Animals Reviewed

Strain (Sex)	Substance	Route	Co-carcinogen & Route	Exposure Period/Study Duration	Reference
Rat Wistar (M&F)	Cobalt chloride	SC inj.	diethylnitrosamine SC inj.	43 wk/lifespan	Zeller (1975)
Mouse CBAxC57B$_1$ (F)	Cobalt chloride	IP inj.	methylcholanthrene dermal	8 wk/8 wk	Finogenova (1973)
Mouse CF-1 (M&F)	Cobalt chloride	IP inj.	methylcholanthrene dermal	5 wk/17 wk	O'Hara et al. (1971)
Mouse CF-1 (M&F)	Cobalt chloride	IP inj.	methylcholanthrene dermal	10 wk/10 wk	Kasirsky et al. (1965)
Mouse CF-1 (M&F)	Sodium cobaltinitrite	IP inj.	methylcholanthrene dermal	5 wk/17 wk	O'Hara et al. (1971)
Mouse CF-1 (M&F)	Sodium cobaltinitrite	Drinking water	methylcholanthrene dermal	11 wk/11 wk	Thompson et al. (1965)
Mouse CF-1 (M&F)	Sodium cobaltinitrite	IP inj.	methylcholanthrene dermal	72 days/75 days	Orzechowski et al. (1964)
Rat Sprague-Dawley (F)	Cobalt(II) oxide	Intratracheal instill.	benzo[*a*]pyrene intratracheal instill.	47 wk/lifespan	Steinhoff and Mohr (1991)
Hamster Syrian Golden (M)	Cobalt(II) oxide	Inhalation	cigarette smoke inhalation	Lifespan/lifespan	Wehner et al. (1977)

M = male, F = female, instill. = instillation, inj. = injection, IP = intraperitoneal, IM = intramuscular, SC = subcutaneous, wk = week, yr = year.

5.2.2. Overview of the Assessment of Study Quality and Utility

Each of these primary studies was systematically evaluated for its ability to inform the cancer hazard similar to that described for the carcinogenicity studies in Section 5.1.2. O'Hara et al. (1971) conducted two co-carcinogenicity studies (one using cobalt chloride and the other using sodium cobaltinitrite) that were considered inadequate for evaluation of the carcinogenicity of cobalt, because the authors did not test the influence of cobalt on tumor formation, as cobalt was not administered until after neoplasms were already detectable. No critical concerns were identified in the remaining studies although they were considered to be of low quality. Finogenova (1973) did not report neoplasm incidences but did report neoplasm onset and latency. The other studies had poor reporting of duration, survival, and results, as they were not reported for each gender, but had combined data for both sexes. The study quality assessment is discussed in Appendix D. All co-carcinogenicity studies were categorically restricted to being ranked no higher than “low” for the utility to inform the carcinogenicity evaluation. This restriction was applied to account for the indirect measure of carcinogenicity that co-carcinogenicity studies provide.

5.2.3. Assessment of Findings from Co-carcinogenicity Studies

Co-carcinogenicity studies are also divided by site of neoplasm development into skin, lung, liver, nasal neoplasms, and neoplasms of unspecified location. Only one co-carcinogen study demonstrated an increased incidence of lung neoplasms from cobalt (cobalt(II) oxide), while three studies showed no effect from cobalt (cobalt chloride and cobalt(II) oxide) and three studies reported a decrease in neoplastic incidence with the additional exposure to cobalt compounds (cobalt chloride and sodium cobaltinitrite).

Skin

Four co-carcinogenicity studies of cobalt and methylcholanthrene were reviewed (Finogenova 1973; Kasirsky et al. 1965; O'Hara et al. 1971; Orzechowski et al. 1964; Thompson et al. 1965). In all of the studies, methylcholanthrene was applied dermally to mice and either sodium cobaltinitrite or cobalt chloride was administered in drinking water or by i.p injection. All studies reported skin squamous-cell carcinoma (Finogenova was translated from Russian and was reported as skin cancer NOS). Skin tumor incidences were reduced by co-administration of cobalt in three of the four studies (Kasirsky et al. 1965; Orzechowski et al. 1964; Thompson et al. 1965). In the fourth study, no differences were seen in the onset or latency of neoplasm development for either skin “cancer NOS” or papilloma from the addition of cobalt chloride (Finogenova 1973). The authors didn’t report any tumor incidences.

Lung

Two co-carcinogenicity studies used either inhalation or intratracheal instillation as the route of exposure for both the cobalt compound and the known carcinogen (Steinhoff and Mohr 1991; Wehner et al. 1977). Steinhoff and Mohr (1991) administered benzo[*a*]pyrene and cobalt(II) oxide to female rats by intratracheal instillation. The addition of cobalt(II) oxide increased the incidence of squamous-cell carcinoma of the lung (Steinhoff and Mohr 1991). An adenocarcinoma was also reported in the group exposed to both compounds, but not in the group exposed to just benzo[*a*]pyrene. However, the incidence of adenocarcinoma was not significantly increased by cobalt(II) oxide. Wehner exposed male hamsters to cigarette smoke and cobalt(II) oxide by inhalation (Wehner et al. 1977). No significant change in tumor incidence from the

addition of cobalt(II) oxide was reported, but the locations of the neoplasms were not clearly reported.

Liver and Nose

Only one co-carcinogen study reported neoplasms of the liver and nose (Zeller 1975). In this study, the known carcinogen, diethylnitrosamine, was subcutaneously injected together with cobalt chloride into male and female rats. Diethylnitrosamine induced neoplasms of the nose (esthesioneuroepithelioma, poorly differentiated carcinoma NOS, and squamous-cell carcinoma) and liver (hepatoma NOS, hepatocellular carcinoma, and cholangioma), but the addition of cobalt chloride had no effect on the incidences.

Locations of Unspecified Neoplastic or Non-neoplastic Lesions

Only one co-carcinogen study reported neoplasms that were not specified as to their location or even their histological type (Wehner et al. 1977). Significant decreases in the incidences of neoplasms in cigarette smoke-exposed groups were seen with the addition of cobalt oxide. Groups that were exposed to cobalt and cigarette smoke also had significantly lower body weights than those exposed to just cigarette smoke, which might account for the lower neoplasm incidence. This co-carcinogen study included a cobalt(II) oxide alone group, which did not show a significant increase in neoplasm incidence above that of untreated controls.

5.3. Synthesis of the Findings across Studies

Strengths of the available dataset include testing of cobalt compounds with different properties such as particle versus salt and poorly soluble vs. readily soluble compounds. For some compounds, several studies were available including robust studies with high utility for evaluating carcinogenicity; importantly these include inhalation studies on both a water-soluble (cobalt sulfate) and poorly soluble species (cobalt metal). For other cobalt compounds, there were few studies, some of which were of more limited utility. The overall results for the carcinogenicity studies are summarized by cobalt compound in Table 5-7.

In general, the injection studies were less robust than the inhalation studies. Occupational exposure to cobalt compounds usually occurs by inhalation and not by injection. However, the injection route may be relevant to human exposure, in that cobalt is used in many types of surgical implant materials. The interpretation of the carcinogenicity of the injection studies is limited because many different types of particles or metals, including substances that are considered to be relatively inert, have induced tumors in rats (IARC 2006). Nevertheless, Hansen et al. (2006) found that implantation of some substances (e.g., titanium dioxide and silicon dioxide) did not induce neoplasms and these materials had the same physical characteristics (i.e., surface to volume ratio) as those material that did (cobalt and nickel) tumors, which suggests that the tumors were due to carcinogenic properties of cobalt and not just to a reaction to any physical implant. Further, neoplasms developed at the injection sites when exposed to a soluble cobalt compound, cobalt chloride, indicating a cobalt-specific, rather than a particle-specific effect (Shabaan et al. 1977). Overall, the injection studies are considered to provide supporting evidence for the carcinogenicity of cobalt.

Most of the neoplasms induced by cobalt compounds occur at the site of administration. Lung tumors are only seen in inhalation or intratracheal instillation studies and tissue sarcoma developed in the local tissue at the sites of injection. Both the lung tumors from inhalation and

tissue sarcomas from injections were caused by different cobalt forms including cobalt metal, a poorly soluble compound (cobalt(II) oxide) and two water-soluble compounds (cobalt sulfate for lung tumors and cobalt chloride for injection tumors). In addition, cobalt metal induced several types of tumors distal from the site of administration that were not caused by the other cobalt species (with the possible exception of adrenal tumors from cobalt sulfate), although most of the cobalt compounds were not adequately tested in models to evaluate these sites.

The most widely studied form of cobalt was cobalt metal. Lung tumors were observed in rats and mice in both sexes after inhalation exposure (NTP 1998; 2014d), and injection-site sarcomas (primarily rhabdomyofibrosarcoma, fibrosarcoma or sarcoma) were observed in male and female rats in several studies injecting cobalt metal by different methods (i.m. or intrathoracic) (Heath 1956; Heath and Daniel 1962). In addition, inhalation exposure to cobalt metal also increased the incidences of adrenal gland tumors and tumors at distal sites—mononuclear-cell leukemia and pancreas, and possibly kidney tumors (NTP 2014d). Cobalt metal nanoparticles, when administered by i.m. injection, caused sarcoma in male rats; however, no inhalation studies were identified (Hansen et al. 2006).

Similarly, a poorly soluble cobalt compound (cobalt(II) oxide) caused both lung neoplasms (after intratracheal instillation) in male rats and sarcoma and histiocytoma in several studies of male and/or female rats after injection by various methods (s.c., i.m., i.p.) (Gilman and Ruckerbauer 1962; Steinhoff and Mohr 1991). Inhalation exposure to cobalt(II) oxide did not increase the incidences of lung tumors in Syrian golden hamsters, but the hamster is a less sensitive model for evaluating lung carcinogenicity (McInnes et al. 2013; Steinhoff and Mohr 1991) than the rat or mouse. No tumors were observed in the only study of another poorly soluble cobalt compound, cobalt sulfide, after intrarenal injection, but there were concerns about the dose level in that study (Jasmin and Riopelle 1976).

Finally, consistent findings are also found for soluble cobalt salts. Inhalation exposure to cobalt sulfate heptahydrate caused lung tumors in rats and mice and adrenal tumors in female rats. Adrenal gland tumors were also induced by exposure to cobalt sulfate (NTP 1998). Although no injection studies were identified that tested cobalt sulfate heptahydrate, a subcutaneous study of cobalt chloride provided suggestive evidence that cobalt causes fibrosarcoma at the site of administration and possibly at sites distant from the sites of administration; however, the confidence in the evidence is reduced somewhat because of possible inadequate reporting or procedures (Shabaan et al. 1977).

Co-carcinogenicity studies overall provided little if any support for the co-carcinogenicity of cobalt compounds. One study reported that cobalt enhanced carcinogenicity, but the remaining co-carcinogenicity studies reported either no effect or a decrease in carcinogenicity with co-exposure to cobalt.

Table 5-7. Overall Results of Carcinogenicity Studies in Experimental Animals Sorted by Cobalt Compound

Substance	Strain (Sex)	Route	Exposure Period/Study Duration	Results	Reference
Cobalt metal					
Cobalt metal	Rat F344/NTac (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F Squamous-cell tumors (primarily cystic keratinizing epithelioma), F; [Equivocal] M Mononuclear-cell leukemia, F Adrenal gland Benign and malignant pheochromocytoma, M&F Pancreas Islet-cell adenoma or carcinoma, M; [Equivocal: carcinoma] F Kidney Adenoma or carcinoma combined, [Equivocal] M	NTP (2014d)
Cobalt metal	Mouse $B6C3F_1$/N (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F	NTP (2014d)
Cobalt metal [Nano][a]	Rat Sprague-Dawley (M)	i.m. inj.	Single dose/1 yr	Injection site Sarcoma, M	Hansen et al. (2006)
Cobalt metal [Bulk][a]	Rat Sprague-Dawley (M)	s.c. inj.	Single dose/1 yr	No increased incidence in tumors Fibroblastic proliferation (non-neoplasia)	Hansen et al. (2006)
Cobalt metal	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/1 yr	No increased incidence in tumors	Jasmin and Riopelle (1976)
Cobalt metal	Rat Hooded (F)	Intrathoracic	Single dose/2.3 yr	Injection-site sarcoma [including rhabdomyosarcoma of cardiac and intercostal muscle, mixed	Heath and Daniel (1962)
Cobalt metal	Rat Hooded (M&F)	i.m. inj.	Single dose/lifespan	Injection-site sarcoma [rhabdomyofibrosarcoma, M&F; sarcoma M; fibrosarcoma F	Heath (1956)
Soluble cobalt compounds					
Cobalt sulfate heptahydrate	Rat F344/N (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F Adrenal Benign or malignant pheochromocytoma, F	NTP (1998)

Substance	Strain (Sex)	Route	Exposure Period/Study Duration	Results	Reference
Cobalt sulfate heptahydrate	Mouse $B6C3F_1$ (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F	NTP (1998)
Cobalt chloride	Rat Wistar (M)	s.c. inj.	8–12 mo/8–12 mo	Injection site Fibrosarcoma, M Non-injection site Fibrosarcoma. M	Shabaan et al. (1977)
Poorly soluble cobalt compounds					
Cobalt oxide	Rat Sprague-Dawley (M&F)	Intratracheal instill.	1.5 yr/lifespan	Lung Alveolar/bronchiolar carcinoma, benign squamous epithelial neoplasm, or alveolar/bronchiolar adenoma combined M	Steinhoff and Mohr (1991)
Cobalt oxide	Rat Sprague-Dawley (M&F)	i.p. inj.	6 mo/lifespan	Injection site Histiocytoma and sarcoma, M&F	Steinhoff and Mohr (1991)
Cobalt oxide	Rat Sprague-Dawley (M)	s.c. inj.	730 day/ lifespan	Injection site Histiocytoma and sarcoma, M	Steinhoff and Mohr (1991)
Cobalt oxide	Rat Wistar (M&F)	i.m. inj.	Single dose/1.3 yr	Injection site Sarcoma, M&F	Gilman and Ruckerbauer (1962)
Cobalt oxide	Mouse Swiss (F)	i.m. inj.	Single dose/2 yr	No increased incidence in tumors	Gilman and Ruckerbauer (1962)
Cobalt oxide	Hamster Syrian Golden (M)	Inhalation	Lifespan/ lifespan	No increased incidence in tumors	Wehner et al. (1977)
Cobalt sulfide	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/1 yr	No increased incidence in tumors	Jasmin and Riopelle (1976)

F = female; inj. = injection; instill. = instillation; i.m. = intramuscular; i.p. = intraperitoneal; M = male; mo = month; s.c. = subcutaneous; wk = week; yr = year.

[a]Cobalt bulk and nanoparticle tested in the same animal in the Hansen et al. (2006) study.

6. Mechanistic and Other Relevant Effects

Cobalt particles and ions induce similar biological effects in vivo (e.g., respiratory and inflammatory responses in both experimental animals and humans and carcinogenic effects in experimental animals) and in vitro (e.g., cytotoxicity, genotoxicity, and at high concentrations, necrosis with an inflammatory response). This section discusses the relative role of cobalt particles and ions in cobalt toxicity (Section 6.1), several proposed modes of action for cobalt carcinogenicity (Section 6.2), other biological responses (Section 6.3), and a synthesis (Section 6.4). Although the mechanism(s) of action for the reported cobalt-induced carcinogenic effects are not completely understood, the experimental support for several possible modes of action, including genotoxicity, is reviewed below. Studies on genotoxicity and cell transformation of cobalt and cobalt compounds are reviewed in Appendix E.

6.1. Cobalt Particles and Cobalt Ions

Studies with toxic metals in general, and cobalt specifically, show that solubility and particle size can play an important role in metal-induced toxicity, genotoxicity, and carcinogenicity (Smith et al. 2014). The main cobalt compounds studied for toxicological effects (including both micro- and nanoparticles) are metallic cobalt (Co(0)), cobalt(II) oxide (CoO), cobalt(II,III) oxide (Co_3O_4), and various cobalt(II) salts (e.g., cobalt sulfate, cobalt chloride) (Beyersmann and Hartwig 2008; Lison 2015; Ortega et al. 2014; Sabbioni et al. 2014b; Smith et al. 2014). Many cobalt(II) salts are readily soluble in water and biological fluids (see Section 1).

Several in vitro studies that specifically compared the cellular uptake and/or molecular and cellular effects (e.g., cytotoxicity, genetic toxicity, reactive oxygen species [ROS] production) of cobalt ions and particles (i.e., cobalt metal nanoparticles or cobalt(II) oxide micro or nanoparticles are shown in Table 6-1. In vitro studies generally show that cobalt nanoparticles are more toxic than cobalt microparticles due to increased surface reactivity resulting from a higher surface area/volume ratio (Mo et al. 2008; Peters et al. 2007; Simonsen et al. 2012; Zhang et al. 2000). In addition, relatively soluble cobalt particles (e.g., cobalt metal) are generally more cytotoxic and genotoxic than cobalt ions (Peters et al. 2007; Ponti et al. 2009; Sabbioni et al. 2014b) and cobalt ions are generally more cytotoxic than cobalt particles with low solubility (e.g., cobalt oxides) (Table 6-1) (Ortega et al. 2014; Papis et al. 2009; Smith et al. 2014). NTP (2009) previously reviewed cobalt-tungsten carbide powders and hard-metals and reported that cobalt-tungsten carbide particles were more cytotoxic and/or genotoxic than cobalt powder when tested in vivo (rat lung) or in vitro in mammalian cells. The greater toxicity of cobalt-tungsten carbide was attributed to a synergistic effect between the particles of cobalt and tungsten carbide that resulted in enhanced production of ROS. Synergistic toxicity in vitro was also reported for cobalt with zinc (Bresson et al. 2013) and cobalt with nickel (Patel et al. 2012) but not with chromium (Allen et al. 1997).

Table 6-1. In Vitro Mechanistic Data Comparing Effects of Cobalt Nanoparticles, Microparticles, and Ions

Reference	Cobalt Form (Size, nm) and Cell Types	Cytotoxicity	Genotoxicity[a]	ROS	Cellular Uptake
Sabbioni et al. (1994a; 1994b)	Co NP (3.4) Co MP (2,200) $CoCl_2$ Balb/3T3 mouse fibroblasts	IC50 µg/mL Time / Co MP / Co NP / Co^{2+} 4 h / 12 / 19.5 / 47 12 h / 10 / 10 / 22 24 h / 11 / 10 / 10 48 h / 10 / 9.9 / 10	Relative amount of Co incorporated into the DNA was Co MP > Co NP > cobalt ions. Cell transformation: Co MP > Co NP; negative for Co^{2+}	Dose-dependent increase in ROS production by Co NP > Co MP; Co^{2+} did not induce significant increase. All forms induced lipid peroxidation: Co NP > Co MP > Co^{2+}	Co uptake was dose dependent but significantly higher for NP and MP than for cobalt ions. Maximum uptake at 4 hours post-exposure.
Ortega et al. (2014)	Co_3O_4 MP (100–400) $CoCl_2$ BEAS-2B human lung	Co_3O_4 / $CoCl_2$ IC25 µg/mL: 50 / 2.9 IC50: 170 / 4.4 IC75: 600 / 6.5	No data	No data	Co_3O_4 particles entered cells via endocytosis and released cobalt ions within lysosomes over long periods of time and were responsible for toxicity.
Smith et al. (2014)	CoO MP (270–3,560) $CoCl_2$ WTHBF-6 human lung fibroblasts	Both forms induced concentration-dependent increase in cytotoxicity; however, similar levels of cytotoxicity at intracellular cobalt levels <1,000 µM while cobalt ions were more cytotoxic than particulate Co at higher levels.	Chromosome aberrations (similar effect for particulate and soluble forms).	No data	Both particulate and soluble Co induced a concentration-dependent increase in intracellular cobalt ion levels. Particle-cell contact was required for uptake of CoO.
Alarifi et al. (2013)	Co_3O_4 NP (21) $CoCl_2$ HepG2 human hepatocarcinoma cells	Both forms induced concentration-dependent increase in cytotoxicity, but particulate Co was more cytotoxic than soluble Co.	DNA damage (comet assay, NP were more potent than soluble form)	Particles induced ROS and oxidative stress. Effects were lower for cobalt ions.	No data

Reference	Cobalt Form (Size, nm) and Cell Types	Cytotoxicity	Genotoxicity[a]	ROS	Cellular Uptake
Horie et al. (2012)	CoO NP (>10) $CoCl_2$ HaCaT human keratinocytes A549 human lung carcinoma cells	Both forms induced similar concentration-dependent increase in cytotoxicity in both cell types.	No data	No increase in intracellular ROS in cells treated with cobalt ions or particles.	No data
Papis et al. (2009)	Co_3O_4 NP (45) $CoCl_2$ HepG2 and ECV-304 human cell lines	Both forms induced concentration-dependent increase in cytotoxicity, but cobalt ions were more toxic. HepG2 cells not as sensitive as ECV-304 cells.	No data	Particles but not ions induced dose-dependent increase in ROS production in both cell lines. HepG2 cells less sensitive.	No data
Limbach et al. (2007)	Co_3O_4 NP (20–75) Co_3O_4/silica NP Cobalt salt A549 human lung adenocarcinoma epithelial cells	No data	No data	Release of ROS was up to 8 times higher for particles than cobalt ions.	No data
Nyga et al. (2015)	CoNP (2–60) $CoCl_2$ U937 human monocytic cell line, peripheral blood mononuclear cells, and alveolar macrophages	NPs induced a concentration-dependent reduction in all three monocytic cell lines (prevented by co-incubation with ascorbic acid). $CoCl_2$ at comparable concentrations (50–350 μM) was not cytotoxic.	No data	NPs induced ROS in a concentration-dependent manner in all cell lines (prevented by both ascorbic acid and glutathione). $CoCl_2$ did not significantly increase ROS.	No data
Annangi et al. (2014)	CoNP (30.7 ± 20.2) Ogg1+/+ and Ogg1−/− mouse embryo fibroblasts (MEF)	NPs induced dose-dependent cytotoxicity in wild-type and knockout MEF cells (more toxic to knockout cells).	Sub-toxic doses for 12 weeks induced cell transformation (knockout cells were more sensitive).	Acute and subchronic exposure induced ROS. Greater toxicity in knockout cells attributed to increased sensitivity to oxidative damage.	Dose-dependent increase in cellular uptake of CoNPs in wild-type and knockout cells.

Reference	Cobalt Form (Size, nm) and Cell Types	Cytotoxicity	Genotoxicity[a]	ROS	Cellular Uptake
Horev-Azaria et al. (2011)	Co NP (10–50) $CoCl_2$ A549, NCIH441, Caco-2, HepG2 (human lung, colorectal, liver); MDCK (dog kidney); murine dendritic cells	NPs and ions induced dose-dependent cytotoxicity. NPs were generally more toxic. Ion sensitivity: A549 > MDCK > NCIH441 > Caco-2 > HepG2 > DC; NP sensitivity: A549 = MDCK = NCIH441 = Caco-2 > DC > HepG2. Toxicity of NP aggregates attributed to extracellular cobalt ion dissolution (34%–44% at 48 and 72 hrs).	No data	No data	No data
Ponti et al. (2009)	Co NP (20–500) $CoCl_2$ Balb/3T3 mouse fibroblasts	Dose-dependent cytotoxicity for both forms (higher for particles at 2 and 24 h but overlapping at 72 h).	Co NP induced DNA damage, MN, and cell transformation; $CoCl_2$ induced DNA damage only.	No data	No data
Kwon et al. (2009)	Co NP (30) $CoSO_4$ RAW 264.7 murine macrophages	NPs and ions induced dose-dependent cytotoxicity.	No data	No data	NP toxicity likely resulted from cellular uptake rather than extracellular dissolution.
Colognato et al. (2008)	Co NP (100–500) $CoCl_2$ Human peripheral blood leukocytes	Co NP and cobalt ions induced dose-related cytotoxic effects (decrease in the cytokinesis-block proliferation index (CBPI). CBPI was slightly higher for ions at 10^{-5} M but similar toxicity at $>2 \times 10^{-5}$ M	Cobalt ions induced clear trend in increase of MN frequency while Co NP were less effective; MN response varied with donor. DNA damage with NP only (comet assay, short incubation time). No MN observed at non-cytotoxic concentrations.	No data	NP readily taken up by cells. Cells exposed to cobalt ions showed only slight or no change in intracellular cobalt compared to baseline levels.

Reference	Cobalt Form (Size, nm) and Cell Types	Cytotoxicity	Genotoxicity[a]	ROS	Cellular Uptake
Peters et al. (2007)	Co NP (28) $CoCl_2$ Human dermal microvascular endothelial cells	Concentration-dependent effect (greater effect for NP than ions)	No data	Co NP induced strong concentration-dependent increase in ROS, cobalt ions induced less ROS and was concentration independent.	NP readily taken up by cells and stored in vacuoles. Pro-inflammatory activation after exposure to Co NP was attributed to intercellular release of cobalt ions.

MP = microparticles (diameter > 100 nm), NP = nanoparticles (diameter < 100 nm).
[a]Genotoxicity also includes data for related effects (e.g., cell transformation assay) that do not necessarily measure a specific genotoxic endpoint.

Ortega et al. (2014) reported that although cobalt ions were more cytotoxic than poorly soluble Co_3O_4 particles, human lung cells exposed to the IC_{25} (inhibitory concentration at which the ATP content was reduced by 25% compared to non-exposed cells) of cobalt chloride (2.9 µg/mL) or Co_3O_4 (50 µg/mL) had similar intracellular concentrations of solubilized cobalt (6.5 fg/cell for Co_3O_4 compared to 5.4 fg/cell for cobalt chloride) (Figure 6-1). Smith et al. (2014) also reported that at intracellular cobalt concentrations less than 1,000 µM, the cytotoxic effects of cobalt chloride and CoO to human lung fibroblasts were similar while cobalt chloride was more cytotoxic than CoO at intracellular concentrations greater than 1,000 µM. Horie et al. (2012) studied a variety of metal oxide nanoparticles and concluded that cellular influences (cell viability and oxidative stress) of metal oxide nanoparticles were most dependent on metal ion release (i.e., effects were greater for soluble particles compared to insoluble particles). In addition, Auffan et al. (2009) reported that chemically stable nanoparticles did not have significant cellular toxicity while nanoparticles that could be oxidized, reduced, or dissolved were cytotoxic and genotoxic. Thus, the available data indicate that intracellular cobalt ions are the primary toxic form, and it is likely that the mode of action for systemic toxicity is related to cobalt ions (Ortega et al. 2014; Paustenbach et al. 2013; Simonsen et al. 2012; Smith et al. 2014).

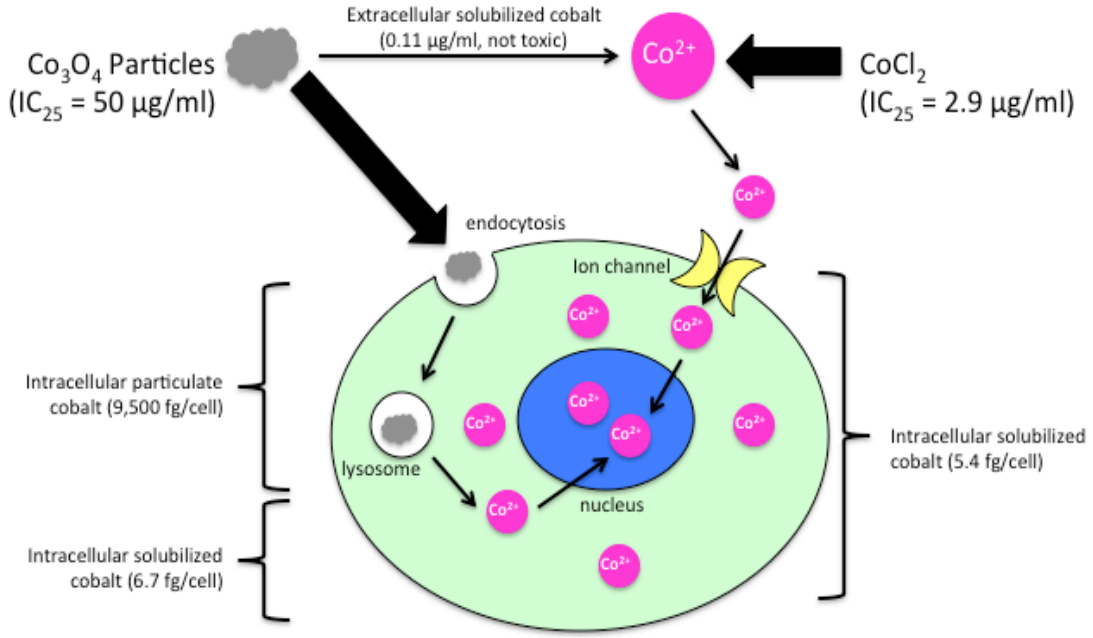

Figure 6-1. Cellular Uptake of Cobalt Particles and Ions

Source: Ortega et al. (2014).

Because of similar physical/chemical properties, cobalt ions compete with essential divalent metal ions (e.g., calcium, copper, zinc, iron, manganese, and magnesium) for absorption, specific receptor activation, and ion channel transport (Paustenbach et al. 2013). For example, cobalt absorption is increased in humans and animals with iron deficiency suggesting that these metals share a common uptake mechanism (Thomson et al. 1971). Further, cobalt ions have the same size and charge as zinc ions; therefore, both ions bind to the same types of ligands (e.g., oxygen, nitrogen, and sulfur groups of biomolecules) (Beyersmann and Hartwig 2008). The bioavailability of cobalt ions in vivo is limited because of extensive binding (90% to 95%) to

serum proteins (e.g., albumin, α_2-macroglobulin) (Paustenbach et al. 2013; Simonsen et al. 2012). Thus, the concentration of free, ionized cobalt in serum is about 5% to 12% of the total cobalt concentration (Simonsen et al. 2012). However, Heath et al. (1969) demonstrated that myoblasts exposed to cobalt-bound protein complexes (primarily globulin and albumin), but not to cobalt chloride, developed cytological alterations (e.g., enlarged hyperchromatic nucleoli, chromocenters, and nuclei) in actively growing cultures that were similar to those seen in pre-malignant myoblasts in vivo. In contrast, myoblasts exposed to cobalt chloride were either killed or showed no cytological abnormalities when exposed to sublethal concentrations.

Differences in toxicity reported for cobalt particles and ions may be partially explained by differences in cellular uptake mechanisms (see Figure 6-1). Cobalt ions first saturate binding sites in the extracellular milieu and on cell surfaces and, after saturation, are actively transported inside the cell via metal ion transport systems such as calcium channels or divalent metal ion transporters (Garrick et al. 2003; Sabbioni et al. 2014b; Simonsen et al. 2012; Smith et al. 2014). However, current knowledge of the molecular mechanisms of cobalt ion-specific transporters is very limited (Guskov and Eshaghi 2012). In contrast, particulate cobalt is transported into cells by phagocytosis/endocytosis. However, nanoparticles are not as readily phagocytized by alveolar macrophages as larger particles and also may enter the systemic circulation by penetrating through the alveolar membrane (Mo et al. 2008).

Studies with low-solubility cobalt oxide (CoO or Co_3O_4) particles show that these particles readily enter cells through endocytosis via a clathrin-mediated pathway (called a Trojan-horse type mechanism) and are partially solubilized in the low pH environment within the lysosomes (Limbach et al. 2007; Ortega et al. 2014; Papis et al. 2009; Smith et al. 2014). Although the intracellular solubilized cobalt content was small compared to the intracellular particulate content, the data suggest that the solubilized fraction was responsible for the overall toxicity to human lung cells (Ortega et al. 2014; Smith et al. 2014).

Endocytosis of Co_3O_4 particles was a more efficient uptake pathway compared to the specific transport or ionic pumps involved with uptake of cobalt ions (Ortega et al. 2014). These studies also demonstrated that concentrations of extracellular solubilized cobalt were too low to induce cytotoxicity and that particle-to-cell contact was necessary to generate high intracellular cobalt levels. Further, cobalt particles taken up by lung cells can lead to long-term intracellular release of toxic metal ions. Similarly, cobalt metal nanoparticles are internalized by phagocytosis and endocytosis and spread rapidly to the cytoplasm, cellular organelles, and nucleus where they release cobalt ions (Ponti et al. 2009; Sabbioni et al. 2014b). However, one study reported that the toxic effects of aggregated cobalt metal nanoparticles in vitro were attributed to extracellular release of cobalt ions from particle dissolution (Horev-Azaria et al. 2011) while another study reported that extracellular release of cobalt ions had no effect on cell viability (Nyga et al. 2015).

Sabbioni et al. (2014b) also reported that the intracellular distribution of cobalt in Balb/3T3 cells was different following exposure to cobalt nanoparticles compared to cobalt ions. Cells exposed to cobalt nanoparticles had a higher nuclear fraction and a lower cytoplasmic fraction than cells exposed to cobalt ions. The amount of cobalt bound to DNA was significantly greater in cells exposed to cobalt microparticles than nanoparticles but was the lowest in cells exposed to cobalt ions (tested concentrations were 10 and 100 μM for 4 hours). Intracellular distribution studies in primary rhabdomyosarcoma induced by intramuscular injection of metallic cobalt also reported that most of the total cellular content of cobalt was associated with the nuclear fraction and was

bound by components of the nucleoplasm, chromatin, and nucleoli (Heath and Webb 1967; Webb et al. 1972).

The in vivo toxicity and carcinogenicity of soluble cobalt sulfate heptahydrate and cobalt metal particles from the NTP (1998; 2014d) bioassays were recently compared (Behl et al. 2015). The findings supported the possibility of a common underlying mechanism of cobalt toxicity irrespective of the form of cobalt exposure based on the following: (1) common sites of carcinogenicity (lung and adrenal gland) and a similar spectrum of nonneoplastic, inflammatory, fibrotic and proliferative lesions in the upper respiratory tract following subchronic and chronic exposure; (2) similar mutation spectrum in the K-*ras* oncogene in lung tumors; (3) toxicity in common extra-pulmonary sites; and (4) similar clinical findings. Possible explanations for the reported differences between cobalt particles and ions may involve a synergistic effect between the particles and the transition metal on reactive oxygen species (ROS) release and/or differences in intracellular cobalt accumulation and distribution (Peters et al. 2007; Sabbioni et al. 2014b; Smith et al. 2014).

6.2. Proposed Modes of Action of Cobalt Carcinogenicity

Similar cytotoxic, genotoxic, and carcinogenic effects have been described for soluble and particulate forms of cobalt. Three major mechanisms have been identified that are applicable for the majority of carcinogenic metal compounds (Angelé-Martínez et al. 2014; Beyersmann and Hartwig 2008; Koedrith and Seo 2011). These include (1) oxidative stress, (2) DNA repair modulation, and (3) disturbances of signal transduction pathways that affect cell growth and differentiation. Modes of action most likely involved in cobalt-induced carcinogenesis are consistent with these general mechanisms and include: (1) genotoxicity and inhibition of DNA repair, (2) induction of reactive oxygen species (ROS) and oxidative damage, and (3) induction of hypoxia-like responses by activating hypoxia-inducible factors (HIFs) (see Figure 6-2) (De Boeck et al. 2003a; Green et al. 2013; Lison et al. 2001; Magaye et al. 2012; Simonsen et al. 2011; Simonsen et al. 2012; Smith et al. 2014).

In addition to HIFs, signaling pathways, receptors, and transcription factors that are potentially relevant to carcinogenesis and are affected by cobalt include MAPKs, AP-1, P13K/Akt, and NF-κB (Davidson et al. 2015; Lee et al. 2012; Leonard et al. 2004; Mates et al. 2010; Valko et al. 2006). Dysregulation of these signaling pathways alters expression of genes that mediate cell growth, proliferation, differentiation, inflammation, invasion, angiogenesis, metastasis, apoptosis, and transformation and have been implicated in a variety of cancers (Davidson et al. 2015). In addition, there is some evidence that cobalt may also has epigenetic effects such as histone modifications that can lead to altered gene expression (e.g., tumor suppressor gene silencing and oncogene activation) and genomic instability; however, epigenetic effects of cobalt have not been as extensively studied as some other carcinogenic metals and are not further reviewed (Broberg et al. 2015; Davidson et al. 2015; Li et al. 2009). The experimental evidence for the proposed modes of action is briefly reviewed below.

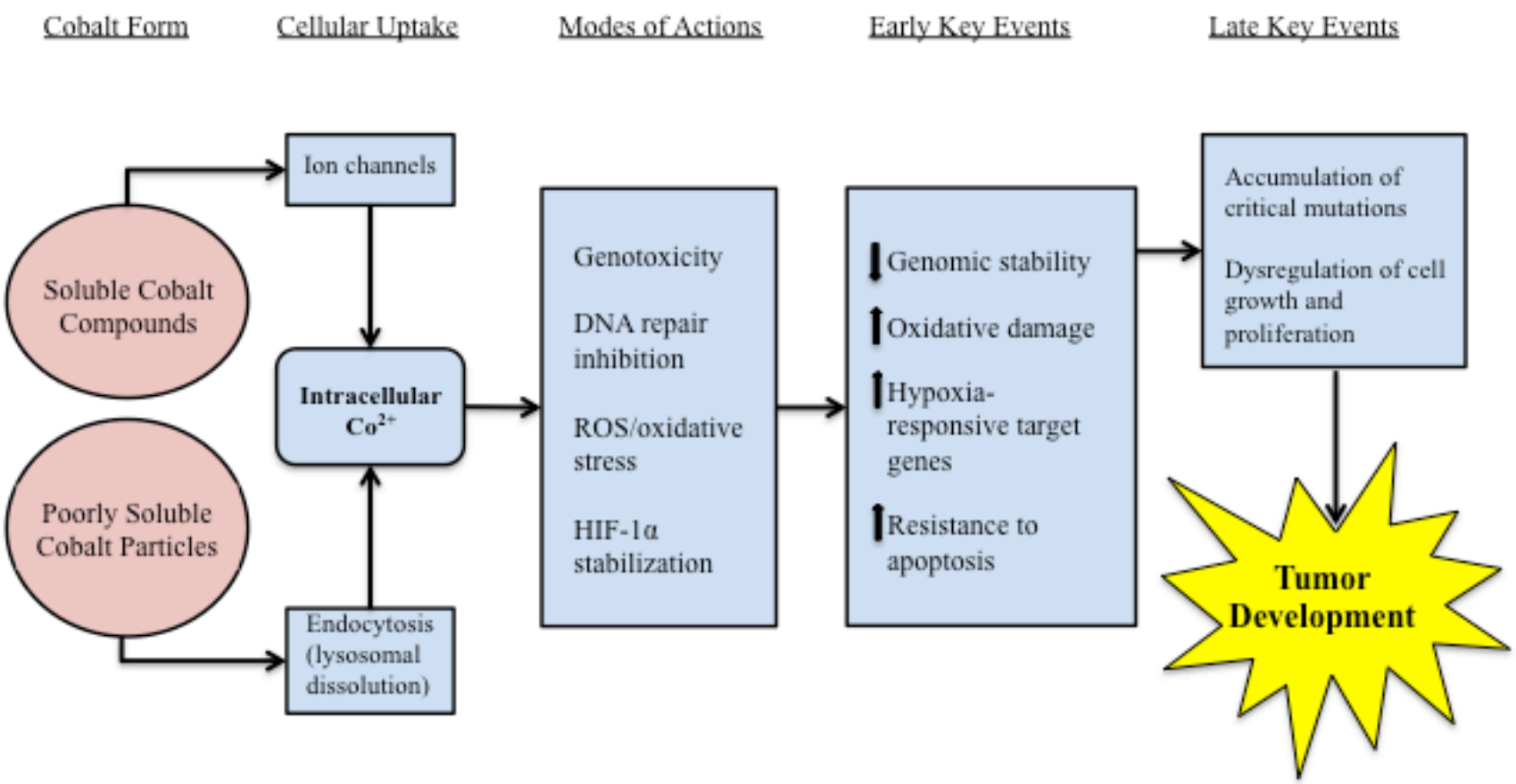

Figure 6-2. Proposed Modes of Action of Cobalt Carcinogenicity

Adapted from Beyersmann and Hartwig (2008) and De Boeck et al. (2003a).

6.2.1. Genotoxicity, Inhibition of DNA Repair, and Related Key Events

This section addresses genotoxicity and related biological adverse effects or key events (e.g., cell transformation, cell-cycle arrest) that are possibly relevant to the mode of action of cobalt-induced carcinogenicity. Genotoxicity (e.g., DNA reactivity, mutagenicity, chromosomal damage, enzyme-mediated effects on DNA damage or repair) are well recognized as key events associated with carcinogenesis (Guyton et al. 2009).

Overview of Genotoxicity Findings

The genotoxic and related effects for cobalt metal and soluble and insoluble cobalt compounds are reviewed in Appendix E and briefly summarized here (see Table 6-2). Increases in DNA strand breaks, sister chromatid exchange, micronuclei, aneuploidy, chromosomal aberrations, and DNA-protein crosslinks were reported in mammalian cells in vitro following exposure to cobalt and cobalt compounds. These data provide evidence that cobalt mainly causes clastogenic effects and DNA damage. Cobalt compounds were mostly non-mutagenic in bacterial assays and mutagenicity data in mammalian cells were conflicting. The positive genotoxic effects were reported for a variety of cobalt compounds, including water-soluble salts (chloride, sulfate, nitrate), poorly water-soluble cobalt compounds (oxide, sulfide, metal, nanoparticles) and a water-soluble organic cobalt compound (acetate). Although the number of available in vivo studies was limited, they indicated that cobalt chloride induced genotoxic effects including aneuploidy in the bone marrow and testes of male hamsters and chromosomal damage and micronucleus formation in mouse bone marrow; cobalt acetate caused oxidative DNA damage in rat kidney, liver, and lung (Kasprzak et al. 1994). Dose-dependent responses were reported in some of these studies, supporting the evidence for some types of genotoxicity in vivo.

Some recent in vitro studies are consistent with the earlier data and show that cobalt ions and particles induce genotoxic effects in human and animal cells, but the studies also compared effects and relative potency of cobalt ions and particles (Table 6-1) (Alarifi et al. 2013; Colognato et al. 2008; Patel et al. 2012; Ponti et al. 2009; Smith et al. 2014). Smith et al. (2014) compared the effect of CoO particles with cobalt chloride and reported similar genotoxic effects (primarily chromatid lesions); however, particle-to-cell contact was required to induce genotoxicity from CoO. Soluble cobalt also induced cell-cycle arrest at a much lower intracellular cobalt concentration than CoO. Alarifi et al. (2013) compared Co_3O_4 nanoparticles and cobalt chloride and reported that both forms caused DNA damage in human HepG2 cells, but the nanoparticles were more potent. Two studies investigated the genetic effects of metallic cobalt nanoparticles in Balb/3T3 mouse fibroblast (Patel et al. 2012) and human leukocytes (Colognato et al. 2008). Cobalt nanoparticles induced DNA strand breaks, micronuclei, and cell transformation in mouse fibroblasts and DNA damage in human leukocytes. Cobalt ions had no effect in human leukocytes but induced DNA damage in mouse fibroblasts. Ponti et al. (2009) reported that cobalt chloride induced double-strand breaks in human lung epithelial cells and that the effects were increased with co-exposure to nickel chloride.

Potential molecular mechanisms for cobalt-induced genotoxicity (primarily clastogenic effects) include (1) a direct effect of cobalt(II) ions to induce oxidative damage to DNA through a Fenton-like mechanism (see Section 6.2.2), and (2) an indirect effect of cobalt(II) ions to inhibit repair of DNA damage caused by endogenous events or induced by other agents (IARC 2006; Lison 2015). These mechanisms are discussed below.

Inhibition of DNA Repair

Evidence for cobalt-induced inhibition of DNA repair comes from several studies that show exposure to cobalt enhances the genotoxic effects of some mutagens and that cobalt modifies the catalytic activity of DNA repair proteins (Beyersmann and Hartwig 1992; 2008; IARC 2006). It is thought that interaction with DNA repair proteins, transcription factors, and tumor suppressors may be more relevant for metal-mediated carcinogenesis than direct binding to DNA (Beyersmann and Hartwig 2008; Koedrith and Seo 2011). Possible mechanisms include substitution of cobalt ions for zinc ions resulting in proteins with modified catalytic activity (e.g., p53 tumor suppressor protein and zinc finger domains of DNA repair proteins) or substitution of cobalt for magnesium in DNA polymerases or topoisomerases (Asmuss et al. 2000; Baldwin et al. 2004; Beyersmann and Hartwig 2008; Hartwig 1998; Hartwig et al. 1991; Kasten et al. 1997; Kopera et al. 2004; Witkiewicz-Kucharczyk and Bal 2006). The DNA binding capacity of p53 protein can be modulated by cobalt(II) ions (Adámik et al. 2015; Lee et al. 2001; Méplan et al. 2000; Palecek et al. 1999). In addition to cell-cycle arrest and apoptosis, p53 and its downstream genes also regulate DNA excision repair pathways, including repair of oxidative damage (Smith and Seo 2002). Kasten et al. (1997) reported that non-cytotoxic doses of cobalt enhanced DNA damage caused by ultraviolet radiation in human fibroblasts by inhibiting both the incision and polymerization steps of nucleotide excision repair. Kopera et al. (2004) and Asmuss et al. (2000) showed that cobalt reduced the DNA-binding ability of xeroderma pigmentosum group A (XPA) protein (a zinc finger protein involved in nucleotide excision repair). Further, poly(ADP-ribose)polymerase (PARP), a DNA strand break repair protein also was inhibited by cobalt (Hartwig et al. 2002). Unrepaired genotoxicity can contribute to accumulation of critical mutations and dysregulation of cell growth and proliferation that can lead to cancer.

Key Events

In addition to DNA and chromosome damage and inhibition of DNA repair, cobalt also causes other effects that can contribute to malignant transformation, genomic instability, and survival of damaged cells. There is some evidence that cobalt decreases the cell's resistance to apoptosis (i.e., avoidance of cell death). Green et al. (2013) reported that normal human cell lines (IMR90 fibroblasts and primary bronchial epithelial cells) and a lung cancer cell line (H460) treated with cobalt had several times higher accumulation, less efficient activation of p53, and a delayed and weaker caspase activation compared to cells treated with nickel. This facilitates cell survival and proliferation of damaged cells (e.g., such as cells with cobalt-induced chromosomal damage).

Cell transformation assays measure induction of phenotypic alterations characteristic of tumorigenic cells, which could be caused by genotoxic or non-genotoxic mechanisms. Overall, the available studies provide strong evidence that different forms of cobalt can induce cellular transformation; however, cobalt particles were generally more effective than cobalt ions. Some studies suggested that cell transformation was related in part to ROS production or decreases in DNA repair of oxidative DNA damage, which can lead to genotoxicity and these studies are briefly reviewed here.

Cobalt metal particles, water-soluble cobalt compounds (cobalt chloride, cobalt sulfate, cobalt acetate), and water-insoluble cobalt compounds (cobalt sulfides) induced cell transformation in different types of rodent cells (C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), or BALB/3T3 cells) (Abbracchio et al. 1982; Annangi et al. 2014; Casto et al. 1979; Costa et al. 1982; Doran et al. 1998; Kerckaert et al. 1996; Ponti et al. 2009; Sabbioni et al.

2014a; Sighinolfi et al. 2014). A few studies of either cobalt chloride (Ponti et al. 2009; Sabbioni et al. 2014a) or cobalt metal (Doran et al. 1998) were negative, which could be due to differences in experimental conditions such as the types of cells, because positive findings were found for the same cobalt forms using other experimental conditions. Sabbioni et al. (2014a) reported that Type III foci in Balb/3TC cells induced by cobalt nanoparticles and microparticles were inhibited by ascorbic acid and suggested that the response was dependent on ROS production and lipid peroxidation. Another study found that mouse fibroblast cells without a DNA base-pair excision gene (8-oxoguanine glycosylate, $Ogg1^{-/-}$) were more sensitive to cobalt nanoparticle-induced cellular transformation (after 12 weeks of exposure to sub-toxic doses) compared to wild type cells ($Ogg1^{+/+}$) (Annangi et al. 2014). Ogg is involved in the repair of 8-oxoguanine and thus, this study supports the role of oxidative DNA damage in cobalt carcinogencitiy. Oxidative stress is discussed in more detail in the following section.

Table 6-2. Summary Assessment of Genotoxicity and Related Effects for Cobalt Compounds[a]

Endpoint (Test System)	Cobalt Chloride		Cobalt Sulfate	Cobalt Nitrate	Cobalt(II) Oxide	Cobalt(II,III) Oxide	Cobalt Acetate		Cobalt Metal		Cobalt Sulfide	Cobalt Nanoparticles
	In Vitro[b]	In Vivo	In Vitro[b]	In Vitro[c]	In Vitro[c]	In Vitro[c]	In Vitro[c]	In Vivo	In Vitro[b]	In Vivo	In Vitro[c]	In Vitro[c]
Mutation												
Mutation (prokaryotes)	(−)		(−)						(−)			
Mutation (eukaryotes)	±			+							±	−
Chromosomal damage/cytogenetic effects												
Chromosomal aberrations	+	+	+	−	±		−					
Micronucleus induction	±	+							+	−		
Recombination	+			+								+
Gene conversion	(+)											
Aneuploidy	+	+	+									
Sister chromatid exchange	+											
DNA damage and repair												
DNA damage/ strand breaks or bases	+		+	+		+		+	+		+	+
DNA repair inhibition	+						+		+			
Binding/cross-links												
DNA-protein crosslinks	+		+						+			+
DNA-protein binding inhibition	+			+								

Sources: IARC (2006) review and additional primary references as described in tables and text.
+ = positive; (+) = mostly positive evidence; ± = mixed results; (−) = mostly negative evidence; − = negative.
[a]No column is included for in vivo results if none were identified.
[b]Results shown are for −S9; test +S9 was negative.
[c]Results shown are for −S9; not tested with the addition of metabolic activation (S9).

6.2.2. Oxidative Stress and Damage

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) induce oxidative and nitrative stress and are recognized as key contributors to carcinogenesis (Mates et al. 2010). Redox-active transition metals (e.g., iron, zinc, copper, chromium, cobalt, nickel, manganese) have been shown to produce oxidative stress through redox reactions in vivo and in mammalian cells in vitro (Beyersmann and Hartwig 2008; Jomova and Valko 2011; Kasprzak 2002; Koedrith and Seo 2011; Valko et al. 2005; Valko et al. 2006). Oxidative stress has been demonstrated to be one of the principal injury mechanisms through which metal and metal oxide nanoparticles induce adverse health effects (Zhang et al. 2012a). In addition, cobalt nanoparticles that are translocated from the lungs to the blood may directly or indirectly activate peripheral blood neutrophils to release ROS, RNS, and pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-12, MIP-2, and TNF-α) (Mo et al. 2008). Excessive or inappropriate neutrophil activation is recognized as a potential cause of tissue damage. Increased formation of reactive ROS/RNS can overwhelm body antioxidant defenses leading to oxidative stress and damage to lipids, proteins, and DNA (Jomova and Valko 2011; Petit et al. 2005; Romero et al. 2014; Valko et al. 2005).

Mechanisms of ROS

Direct interactions between cobalt metal or ions and oxygen or lipids can generate ROS. High concentrations (10 mg/mL) of aqueous suspensions of Co(0) metal particles can react with dissolved oxygen to generate hydrogen peroxide and hydroxyl radicals in the presence of superoxide dismutase (SOD) as illustrated below in Figure 6-3 (reactions 1–3) (Jomova and Valko 2011; Lee et al. 2012; Leonard et al. 1998). The hydroxyl radical was not generated when catalase, a hydrogen peroxide scavenger, was added. Cobalt(II) ions alone did not generate significant amounts of hydroxyl radicals from hydrogen peroxide except when bound to certain endogenous chelators such as glutathione and anserine (Figure 6-3, reaction 4) (Leonard et al. 1998; Mao et al. 1996; Shi et al. 1993). Glutathione and anserine normally function as antioxidants; however, these data suggest that a cobalt(II)-mediated switch to pro-oxidants may occur and cause cellular damage (Valko et al. 2005). Cobalt(II) ions also are capable of reacting with lipid hydroperoxides to generate free radicals in the presence of proper chelating agents (Shi et al. 1993). Hydroxyl radicals and lipid hydroperoxide-derived free radicals are considered important intermediates in oxidative stress-induced genetic damage and as mediators of tumor initiation and promotion (Barrera 2012; Shi et al. 1993; Vaca et al. 1988). Thus, under certain conditions, both cobalt metal and cobalt ions are capable of generating ROS through Fenton-like reactions (reactions 3 and 4) with the potential to increase oxidative stress and cellular injury through DNA damage, protein modification, induction of oncogene expression, and nuclear transcription factor activation.

$$Co + O_2 \longrightarrow Co(I) + O_2^{\bullet -} \longrightarrow Co(I) - OO^{\bullet} \quad (1)$$

$$Co(I) - OO^{\bullet} \xrightarrow{SOD} Co(I) + H_2O_2 \quad (2)$$

$$Co(I) + H_2O_2 \longrightarrow Co(II) + {}^{\bullet}OH + OH^{-} \quad (3)$$

$$[Co(II\text{-chelate}] + H_2O_2 \longrightarrow [Co(III)\text{-chelate}] + {}^{\bullet}OH + OH^{-} \quad (4)$$

Figure 6-3. ROS Generation by Cobalt Metal or Ion Interaction with Oxygen or Lipids

Evidence for Cobalt-induced Oxidative Stress

Petit et al. (2005) reported that cobalt ions induced time- and dose-dependent protein oxidation in human U937 macrophages that was inhibited by glutathione. In addition to generating DNA damage, ROS also activate stress-response genes and redox-sensitive transcription factors (e.g., NF-κB, AP1, p53, Nrf2) (Beyersmann and Hartwig 2008; Klaunig et al. 2010; Valko et al. 2005; Valko et al. 2006). Although high levels of ROS may lead to apoptosis or necrosis, low or transient increases in ROS may lead to increased cell proliferation through altered growth factor and oncogene expression (Klaunig et al. 2010). Dysregulation of stress response and redox-sensitive transcription factors have been linked to carcinogenesis because of their role in regulating DNA repair, inflammation, cell proliferation, differentiation, angiogenesis, and apoptosis. Thus, depending on the dose and the extent and timing of interference, ROS may initiate tumor development by mutagenesis and/or promote tumor growth by dysregulation of cell growth and proliferation (Beyersmann and Hartwig 2008; Davidson et al. 2015; Klaunig et al. 2010; Valko et al. 2005; Valko et al. 2006).

Both cobalt ions and cobalt metal can catalyze the formation of ROS in vivo and in vitro (Alarifi et al. 2013; Annangi et al. 2014; Chattopadhyay et al. 2015; Dick et al. 2003; Hanna et al. 1992; Kadiiska et al. 1989; Kawanishi et al. 1989; 1994; Kotake-Nara and Saida 2007; Lewis et al. 1991; 1992; Limbach et al. 2007; Moorhouse et al. 1985; Papis et al. 2009; Patel et al. 2012; Peters et al. 2007; Pourahmad et al. 2003; Qiao et al. 2009; Scharf et al. 2014; Zou et al. 2001). Cobalt sulfate heptahydrate and cobalt(II) acetate (PubChem 2015) were strongly active in the antioxidant response element signaling pathway (Nrf2/ARE assay) in human hepatocellular carcinoma (HepG2) cells (Shukla et al. 2012). Cobalt chloride-induced apoptosis in rat pheochromocytoma (PC12) cells was attributed to ROS formation (Pulido and Parrish 2003; Zou et al. 2001). Treatment with antioxidants suppressed ROS formation and blocked apoptosis. Annangi et al. (2014) reported that oxidative stress exacerbated the acquisition of a cancer-like phenotype as indicated by greater sensitivity of *Ogg* knockout mouse embryonic fibroblasts compared to wild-type cells. Scharf et al. (2014) conducted a proteomic analysis of periprosthetic tissues collected from joint replacement patients during surgery and reported that cobalt ions induced oxidative damage to proteins involved in the cellular redox system, metabolism, molecular transport, cellular motility, cell signaling, and organelle function. Dick et al. (2003) reported evidence for a role of ROS in the toxic and inflammatory effects in rat lung following intratracheal instillation of Co_3O_4, and Lewis et al. (1991; 1992) reported evidence of oxidative stress in hamster lung following exposure to cobalt ions in vivo and in vitro. Evidence of oxidative stress included decreased levels of reduced glutathione, increased levels of oxidized glutathione, and increased activity of the pentose phosphate pathway. Simultaneous incubation with hydrogen peroxide potentiated cobalt-induced increases in levels of oxidized glutathione and pentose phosphate pathway activity. Although the data suggested that oxidation of glutathione occurred as an early event in cobalt-induced lung toxicity, the data did not indicate that glutathione oxidation related directly to the observed toxicity. Thus, oxidative effects that occur at sites other than the glutathione system might mediate cobalt toxicity.

Oxidative Stress and DNA Damage

As mentioned above, one of the likely mechanisms for cobalt particles and ions to induce genetic damage is through ROS and oxidative stress. Several types of DNA damage are associated with ROS including single- and double-strand breaks, base modifications, deoxyribose modification, and DNA cross-linking (Klaunig et al. 2010). If not repaired prior to DNA replication, DNA

damage can lead to cell death, mutations, replication errors, and genomic instability. Kasprzak et al. (1994) reported oxidative damage to DNA in the liver, kidney, and lung of rats injected with cobalt ions.

Two studies, using different cobalt forms, evaluated K-*ras* mutations (Figure 6-4) in cobalt-induced lung neoplasms of B6C3F$_1$ mice (cobalt metal and cobalt sulfate heptahydrate) or F344/NTac rats (cobalt metal only). Rodents were exposed by inhalation (Hong et al. 2015; NTP 1998; 2014a). Both studies found a higher frequency of G to T transversions in codon 12 of the K-*ras* gene in cobalt-induced neoplasms compared to spontaneous lung neoplasms from historical control or other laboratory control rodents. In contrast, the predominant type of K-*ras* mutation observed in spontaneous lung tumors from historical control mice was G to A transitions. No K-*ras* mutations were observed in spontaneous lung tumors in the concurrent or historical control rats. K-*ras* G to T transversion mutations are associated with the production of 8-hydroxydeoxyguanosine, an oxidative DNA lesion that is formed when ROS reacts with deoxyguanosine (Itsara et al. 2014; Klaunig et al. 2010). This is also consistent with the mutation pattern observed in bacteria (i.e., results were correlated with ability of the tester strain to detect mutational events at G:C base pairs) (Hong et al. 2015) G to T transversions are also the most common type of mutation observed in human lung tumors in the *p53* gene (Harty et al. 1996). These studies suggest that oxidative DNA damage may play a role in cobalt-mediated lung tumorigenicity.

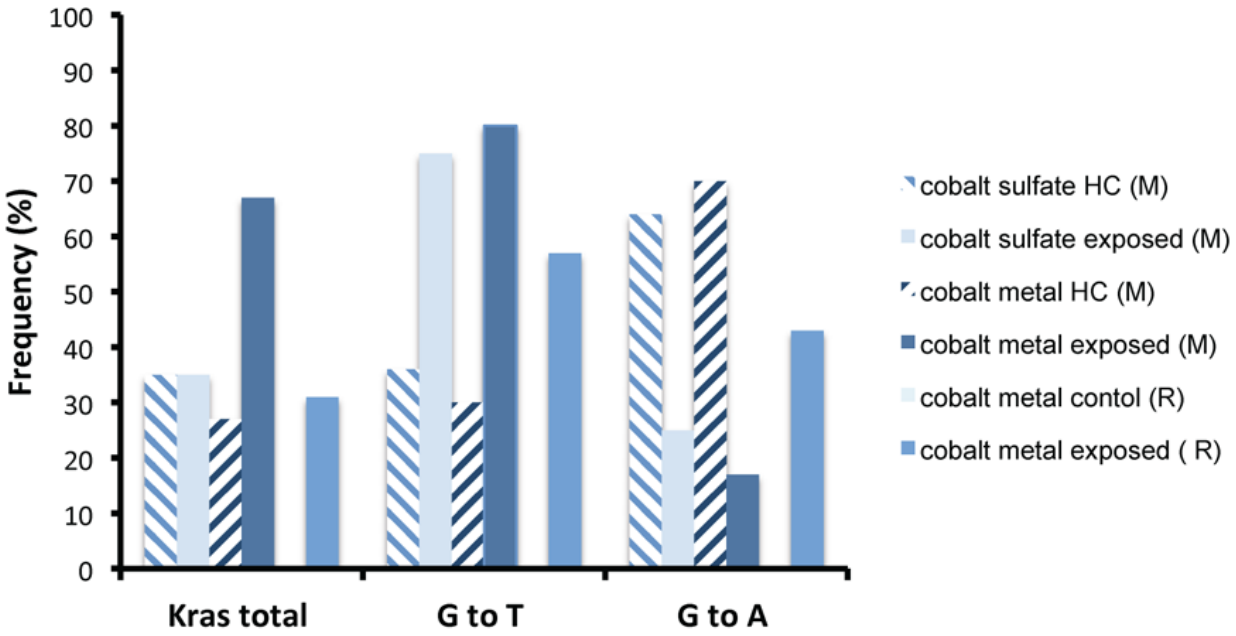

Figure 6-4. K-*ras* Mutations in Lung Tumors from Cobalt-exposed and Non-exposed Rodents

Sources: Hong et al. (2015); NTP (1998; 2014a).
HC = historical control; M = mouse; R = rat.
Frequency of K-ras mutations from lung tumors in mice or rats exposed to cobalt metal or cobalt sulfate and spontaneous tumors. Total K-ras is the incidences of any K-ras mutation detected in all samples and includes mutations in codon 12, 13, and 61. G to T and G to A is the frequency of these specific mutations occurring in codon 12 only. i.e., the total number of K-ras mutations in codon 12 is the denominator.

One argument against the oxidative-stress hypothesis of metal-induced carcinogenesis is that high, cytotoxic doses of metals (e.g., mM range) are often required to induce oxidative damage while much lower doses induce tumors (Beyersmann and Hartwig 2008; Paustenbach et al. 2013). However, as mentioned above, G to T transversions in mouse and rat lung tumors induced by cobalt sulfate and/or cobalt metal are characteristic of oxidative damage. Further, sub-toxic doses of cobalt nanoparticles induced oxidative stress and cell transformation in mouse embryo fibroblasts (Annangi et al. 2014; Sighinolfi et al. 2014) and oxidative stress and DNA damage in human lung epithelial (A549) cells (Wan et al. 2012). It has been suggested that oxidative stress is not the sole cause of cobalt-induced carcinogenicity but may contribute in a potentiating manner (Beyersmann and Hartwig 2008).

6.2.3. HIF Stabilization and Hypoxia Mimicry

Oxygen homeostasis in mammals is tightly regulated in order to provide sufficient oxygen levels to body tissues and cells while minimizing production of ROS (Bracken et al. 2003). HIFs are heterodimers composed of a labile α subunit and a stable β subunit and are the primary transcriptional regulators that mediate the cellular response to hypoxia (Befani et al. 2013; Davidson et al. 2015; Forooghian et al. 2007; Galanis et al. 2008; Salnikow et al. 2004; Zhang et al. 2014). The α subunit is post-translationally regulated by oxygen and is rarely detectable at normal oxygen tension, while the β subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed. There are three known isoforms of the α subunit (HIF-1α, HIF-2α, and HIF-3α) in humans and mammals; however, the HIF-1α subunit is the most widely studied (Bracken et al. 2003; Forooghian et al. 2007; Wolff et al. 2013; Zhang et al. 2014). HIF-1α and HIF-2α share significant structural homology and function and are expressed in multiple tissues and cell types in response to hypoxia (Befani et al. 2013). Less is known about HIF-3α; however, it is a transcriptional target of HIF-1α (Tanaka et al. 2009). Most studies with cobalt investigated its effects on HIF-1α; however, Befani et al. (2013) reported that cobalt stimulated HIF-1-dependent gene expression in two human liver cancer cell lines but inhibited HIF-2-dependent gene expression. Therefore, the following discussion is limited to HIF-1α.

Cobalt-induced HIF Stabilization

HIF-1 overexpression and enhanced transcriptional activity are linked to cancer initiation and progression. There is strong experimental support that cobalt is a potent inducer of HIF-1α activation. Cobalt metal particles, cobalt chloride, and cobalt sulfate heptahydrate promote a hypoxia-like state in vivo and in vitro, even with normal molecular oxygen pressure, by stabilizing HIF-1α (Beyersmann and Hartwig 2008; Galán-Cobo et al. 2013; Galanis et al. 2009; Gao et al. 2013; Maxwell and Salnikow 2004; Nyga et al. 2015; Qiao et al. 2009; Saini et al. 2010a; Saini et al. 2010b; Xia et al. 2009). Further, Wang and Semenza (1995) demonstrated that HIF-1 induction either from hypoxia or cobalt chloride treatment was indistinguishable with respect to DNA binding specificity and contacts with target DNA sequences.

Evidence for cobalt-induced HIF-1 stabilization has been demonstrated in several human cell lines, including cancer cell lines (Ardyanto et al. 2008; Fu et al. 2009; Wang and Semenza 1995). Cobalt chloride-induced hypoxia also increased the invasiveness of one primary breast cancer cell line (Fu et al. 2009). Human A549 lung adenocarcinoma cells exposed to cobalt chloride overexpressed Cap43, a hypoxia-regulated gene (Salnikow et al. 2000). Increased expression of

Cap43 was reported in tumors and serum of lung cancer patients compared to adjacent normal tissues and may be predictive of tumor angiogenesis and poor prognosis (Azuma et al. 2012; Wang et al. 2012). Permenter et al. (2013) investigated gene expression and intracellular protein abundance in two rat liver cell lines exposed to cobalt chloride. Many genes, proteins, and pathways were modulated, which were mainly due to induction of a hypoxia-like response and oxidative stress. These data were consistent with gene expression profiling in hypoxia signaling in human hepatocellular carcinoma (Hep3B) cells exposed to cobalt chloride (Vengellur et al. 2005). Cobalt nanoparticles and ions also induced a time-dependent increase in HIF-target genes and expression of proinflammatory cytokines in the U937 human monocytic cell line (Nyga et al. 2015).

Under normal oxygen conditions, the iron-containing oxygen-sensing enzymes (prolyl hydroxylases) and an asparagine hydroxylase that hydroxylate specific proline or asparagine residues in the HIF-1α subunits (Maxwell and Salnikow 2004). Hydroxylated HIF-1α binds to a multiprotein complex that contains the von Hippel-Lindau (VHL) tumor suppressor. VHL acts as part of a ubiquitin ligase complex resulting in rapid ubiquitination and proteolysis of HIF-1α. Under hypoxic conditions, HIF-1α subunits are not hydroxylated, and consequently the protein is stabilized and translocates to the nucleus where it binds with a HIF-1β subunit. The response to hypoxia includes increased red blood cell production, blood vessel growth and increased blood supply to tissues, and increased anaerobic metabolism. Cobalt affects the function of several genes and enzymes responsible for posttranslational modification of HIF-1α such as prolyl hydroxylases and VHL (Davidson et al. 2015). Possible mechanisms by which cobalt ions activate HIF-1 include replacing iron in the regulatory prolyl hydroxylases or depleting intracellular ascorbate (a cofactor for prolyl hydroxylase activity), thus, deactivating these enzymes (Davidson et al. 2015; Maxwell and Salnikow 2004; Qiao et al. 2009; Salnikow et al. 2004). Kang et al. (2006) reported that metal-induced (cobalt or nickel) HIF-1α stabilization was reversed in human lung carcinoma A549 cells when the cells were treated simultaneously with iron and metal ions. Oxidative stress does not appear to be a primary mechanism of cobalt-induced HIF activation. Salnikow et al. (2000) found that while cobalt and nickel produced oxidative stress in A549 cells, activation of HIF-1-dependent genes was independent of ROS formation. Although the mitochondria are a main target of cobalt toxicity and generate ROS that trigger hypoxia-induced transcription, cobalt activates hypoxia-induced transcription via a mitochondria-independent mechanism (Chandel et al. 1998; Karovic et al. 2007). In a study with rat hepatocytes, lysosomes were shown to be the source of ROS formation with redox transition metals (including cobalt), while the mitochondria were the source of ROS formation for non-redox or poor redox cycling transition metals (Pourahmad et al. 2003). Nyga et al. (2015) also reported evidence that HIF-1α stabilization in human macrophages treated with cobalt metal nanoparticles or cobalt ions occurred via an ROS-independent pathway.

Hypoxia Mimicry

HIF-1α is present in almost all human and animal cells and its activation has a central role in the transcriptional regulation of more than 100 hypoxia-responsive genes (including genes encoding for multiple angiogenic growth factors (e.g., VEGF), erythropoietin synthesis, endothelin, glucose transporters, inflammatory factors, and regulation of apoptosis and cell proliferation) that allow for cell survival at low oxygen pressure (Beyersmann and Hartwig 2008; Gao et al. 2013; Greim et al. 2009; Saini et al. 2010a; Saini et al. 2010b; Simonsen et al. 2012; Wang and Semenza 1995). The evidence suggests that HIF-1α is a major regulator of the adaptation of

cancer cells to hypoxia and may contribute to tumor development and progression by decreasing both repair and removal of mutated cells, selecting for cells with genetic instability, reducing *p53* transcriptional activity, evading growth arrest checkpoints, and inducing apoptosis resistance (Ardyanto et al. 2008; Greim et al. 2009; Hammond and Giaccia 2005; Lee et al. 2001; Maxwell and Salnikow 2004).

HIF-1α overexpression, stabilization, and transcriptional activation is found in more than 70% of human cancers (e.g., breast, ovarian, cervical, prostate, brain, lung, head and neck) and is associated with poor clinical outcomes (Cheng et al. 2013; Galanis et al. 2009; Galanis et al. 2008; Maxwell and Salnikow 2004; Paul et al. 2004). Greim et al. (2009) also identified hypoxia and HIF activation as a relevant mechanism for pheochromocytoma in rats. Further evidence for a role of HIF-1 in cancer is as follows: (1) enhanced glycolytic and angiogenic activities are hallmarks of many tumors and are consequences of HIF-1 activation, (2) immunolabelling for HIF-1α subunits confirms there is a common activation in solid tumors, (3) genetic studies comparing tumor growth with and without HIF-1 have generally shown that tumors without specific HIF subunits have decreased vascularization and growth, (4) a number of pathways implicated in cancer progression increase activation of the HIF-1 pathway in normoxia and hypoxia, and (5) as described above, the VHL tumor suppressor protein is required to regulate HIF-1 (Maxwell and Salnikow 2004). VHL loss of function results in constitutive HIF activation and an increased risk of developing cancer and is evident in people with VHL disease. VHL disease is a hereditary cancer syndrome that is caused by inactivation of the VHL protein (Ben-Skowronek and Kozaczuk 2015). This disease is characterized by the development of multiple vascular tumors including pheochromocytomas, pancreatic islet cell tumors, renal cell tumors, retinal and central nervous system hemangioblastomas, and others. Loss of the VHL protein results in elevated levels of HIF and leads to increased production of VEGF, platelet-derived growth factor (PDGF), transforming growth factor α (TGF-α) and other hypoxia-responsive transcripts that promote cell growth and angiogenesis. HIF also contributes to overproduction of tyrosine hydroxylase and catecholamines in pheochromocytomas.

6.3. Other Biological Effects

In addition to the biological effects discussed in Section 6.2 and illustrated in Figure 6-2, different forms of cobalt ions induce similar biological effects that may or may not be related to carcinogenicity. The effects of chronic exposure to cobalt and cobalt compounds on the respiratory system in humans and experimental animals are well documented (ATSDR 2004; IARC 1991; 2006). Effects include a spectrum of inflammatory and proliferative changes including respiratory irritation, diminished pulmonary function, asthma, alveolar epithelial hyperplasia and metaplasia, squamous metaplasia, and interstitial fibrosis. Respiratory effects have been observed in workers employed in cobalt refineries, hard-metal workers, diamond polishers, and ceramic dish painters.

Potential mechanisms for these endpoints might be some of the modes of action discussed in Section 6.2 as well as other cobalt-related biological responses. Oxidative damage and inflammatory events are characteristics of fibrosing alveolitis (hard-metal lung disease) and lung cancer and there is some evidence that lung fibrosis is a risk factor for lung cancer (IARC 2006). Lung injury may be due in part to cobalt-induced apoptosis which is primarily mediated via loss of mitochondrial membrane potential and release of cytochrome c and apoptosis-inducing factor (AIF) (Araya et al. 2002; Battaglia et al. 2009; Karovic et al. 2007; Pulido and Parrish 2003).

Cobalt also competes with essential divalent metal ions (e.g., calcium, copper, zinc, iron, manganese, and magnesium) for absorption, specific receptor activation, and ion channel transport (Paustenbach et al. 2013) and interferes with a number of iron-containing proteins such as the iron regulatory protein 1 (IRP1)/iron response element (IRE) system and various iron-sulfur cluster proteins that are important for maintaining iron homeostasis, energy production, metabolism, gene expression, DNA/RNA processing and repair, and defense against oxidative stress (Davidson et al. 2015; Lee et al. 2006; Sheftel et al. 2010). Thus, disruption of iron homeostasis could potentially lead to numerous adverse health effects, including cancer.

6.4. Synthesis

Cobalt metal and several cobalt compounds induce similar carcinogenic effects in experimental animals. The mechanisms of cobalt-induced neoplasms are not completely understood but the available data provide strong support that intracellular cobalt ions are the principal toxic entity. Cobalt ions are actively transported inside the cell via metal ion transport systems while cobalt particles with low solubility are readily taken up by cells via endocytosis. Once inside the cell, cobalt particles are partially solubilized at the low pH within lysosomes and release cobalt ions that can react with various cytoplasmic and nuclear proteins and lipids and possibly DNA. Mechanistic data provide strong support that inhibition of DNA repair, oxidative stress, and activation of HIF-1α likely contribute to cobalt-induced neoplastic development and progression. All of these mechanisms are relevant to humans.

7. Overall Cancer Evaluation and NTP Listing Recommendation

This section brings forward and integrates the evaluations of the human, animal, and mechanistic and other relevant data, applies the RoC listing criteria, and reaches an NTP listing recommendation.

NTP Listing Recommendation

"Cobalt and cobalt compounds that release cobalt ions in vivo" are reasonably anticipated to be human carcinogens based on sufficient evidence from studies in experimental animals and supporting mechanistic data. Mechanistic data indicate that the release of cobalt ions in vivo (whether from soluble or poorly water-soluble compounds and particles) is a key event for cobalt-induced carcinogenicity.

Mechanistic data (discussed in Section 6) formed the basis for the approach for grouping cobalt and cobalt compounds that release cobalt ions in vivo as a class (Section 7.1). The scientific data supporting the conclusion of sufficient evidence of cobalt and cobalt compounds that release cobalt ions in vivo from studies in experimental animals is discussed in Section 7.2, and the conclusions from the cancer studies in human studies is briefly summarized in Section 7.3.

7.1. Cobalt and Cobalt Compounds That Release Cobalt Ion In Vivo as a Class

Chemical grouping describes a general approach for considering more than one chemical at the same time for hazard assessment or regulatory purposes. Chemicals whose physicochemical and/or toxicological properties are likely to be similar or follow a consistent pattern, usually as a result of structural similarity, may be considered as a group, or category of substances (ECHA 2009; OECD 2014). One of the primary advantages of grouping is that every chemical within the group does not necessarily require testing for every endpoint. Where scientifically justifiable, chemicals and endpoints that have been tested can be used to fill in the data gaps for the untested chemicals and endpoints. Obviously, only a limited number of cobalt compounds have been tested for one or more of the endpoints evaluated in this monograph. Therefore, a group approach is proposed, and the following sections are based on data reviewed in the previous sections of this document that are relevant to the proposed group listing.

Mechanistic data informed the approach for grouping cobalt and cobalt compounds that release cobalt ions in vivo as a class. The key events involve cellular uptake of cobalt, intracellular release of cobalt ions from particles, intracellular concentrations and distribution, immediate and downstream molecular effects (discussed below and illustrated in Figure 6-1), and tumor formation. Thus, physicochemical properties, toxicokinetics, mechanistic data and other relevant data were used to identify and compare the chemical and biological properties and events that were relevant to cobalt-induced carcinogenicity to determine if a group listing for cobalt and cobalt compounds that release cobalt ions in vivo was warranted. These endpoints are compared for several cobalt compounds in Section 7.1.4 and Table 7-1 and discussed in more detail in the sections below.

- Physicochemical properties and toxicokinetics (Section 7.1.1)
- Overview of the major modes of action (7.1.2)
- Toxicological effects related to a common functional group (i.e., the cobalt ion) (Section 7.1.3)
- Overall synthesis (Section 7.1.4).

7.1.1. Physicochemical Properties and Toxicokinetics

Physicochemical properties and toxicokinetic data for cobalt metal and various cobalt compounds were presented in Sections 1 and 3. Solubility, particle size, bioavailability, and cellular uptake and retention affect toxicity. These data show the following general rank order for aqueous solubility: cobalt(II) salts > cobalt metal > cobalt oxides. Bioaccessibility, defined as the availability of a metal for absorption when dissolved in artificial body fluids, is often used as an in vitro surrogate for bioavailability testing (Stopford et al. 2003). Bioaccessibility measurements showed the same general rank order as aqueous solubility at near neutral pH but, in acidic solutions associated with lysosomes (pH 4.5) or gastric fluid (pH 1.5), bioaccessibility was 100% or near 100% for cobalt metal and several cobalt compounds tested including water-soluble and several poorly soluble compounds indicating that they release cobalt ions in solution (see Table 1-1). Although very low values (≤2%) for bioavailability in artificial gastric and lysosomal fluids have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the same test conditions, other, more informative (e.g., physiologically relevant) test conditions in the presence of lung cells (Section 1.2.2) have found higher bioavailability values for cobalt(II,III) oxide (i.e., Co_3O_4) in culture media with alveolar macrophages and other studies have reported its uptake by lung cells, which suggests that Co_3O_4 would release ions in vivo.

As discussed in Section 3, a number of factors affect cobalt absorption. This is reflected by the fact that absorption of cobalt compounds following oral exposure varies widely but soluble forms are better absorbed than insoluble forms. Inhalation studies also indicate better absorption and shorter retention in the respiratory tract of soluble forms compared to insoluble forms. Thus, cobalt particles with low solubility (e.g., cobalt oxides) are retained in the lungs for long periods and represent a continuing source of exposure. Although cobalt metal has low aqueous solubility, NTP's chronic inhalation study showed that lung clearance in rats and mice was similar to that observed for soluble cobalt sulfate heptahydrate. Cobalt concentrations and tissue burdens increased with increasing exposure concentrations in all tissues examined, indicating systemic exposure; however, normalized tissue burdens increased only in the liver.

Although soluble cobalt compounds are better absorbed, cellular uptake mechanisms for particles also are important (see Section 6.1). Thus, cellular uptake of poorly soluble cobalt particles via endocytosis/phagocytosis can result in intracellular dissolution within the lysosomes and release of cobalt ions. In vitro studies of cobalt metal and cobalt oxide particles generally show that intracellular cobalt ion release is responsible for toxicity as opposed to extracellular dissolution. These studies demonstrated that direct particle contact with the cultured cells was required for cellular uptake and intracellular ion release and toxicity, while cells that were exposed only to extracellular ions dissolved from the particles were not affected. In contrast, cobalt ions readily form complexes with proteins and low molecular weight components and must first saturate binding sites in the extracellular milieu and on cell surfaces before entering the cell via metal ion

transport systems. Solubility, particle size, and particle surface area also affect elimination from the body. Elimination of cobalt particles and ions is multiphasic with fast, intermediate, and slow phases; however, soluble compounds are cleared faster with a smaller fraction of the dose retained long term.

7.1.2. Mechanistic and Other Relevant Data

Although the mechanisms of cobalt-induced carcinogenicity are not completely understood, three biologically plausible modes-of-action have been identified and were reviewed in Section 6. These include (1) genotoxicity and inhibition of DNA repair, (2) ROS and oxidative damage, and (3) stabilization of HIF-1α. Cobalt ions can replace zinc ions in the zinc finger domains of DNA repair proteins, thus altering their catalytic activity, and in vitro assays consistently show genotoxic effects (primarily clastogenic) in mammalian cells exposed to a wide range of cobalt compounds. Cobalt is a redox-active transition metal and in vitro studies show that cobalt particles and ions can induce ROS in mammalian cells with cobalt metal and cobalt oxide particles having a greater effect than ions. Evidence of oxidative stress and oxidative damage also were shown in in vivo studies. Finally, HIF-1α stabilization is well established for cobalt. Although most studies used cobalt chloride to promote a hypoxia-like state, cobalt metal nanoparticles were also shown to have this effect. HIF-1α plays a central role in the transcriptional regulation of more than 100 hypoxia-responsive genes and is a major regulator of the adaptation of cancer cells to hypoxia. Although there were some differences in the degree of toxicity or biological response among cobalt metal particles, cobalt oxide particles, and cobalt ions the modes of action are relevant for all of these cobalt forms.

7.1.3. Toxicological Effects and Key Events

In vivo studies in humans and experimental animals consistently show that cobalt and cobalt compounds induce a similar spectrum of inflammatory, fibrotic, and proliferative lesions in the upper respiratory tract. Toxicological effects of cobalt are attributed primarily to the cobalt ion; however, in vitro studies indicate that direct toxic effects of cobalt particles also contribute. Relevant toxic effects reviewed in this document include carcinogenicity in humans and experimental animals, genetic and related effects (in vitro and in vivo), oxidative stress (in vitro and in vivo), and cytotoxicity (in vitro). Although not completely understood, cellular uptake mechanisms and intracellular release of cobalt ions and their distribution are important factors.

Cobalt metal and cobalt compounds exhibited similar carcinogenic effects in animals and similar genotoxic and cytotoxic effects in vitro. Inhalation studies with cobalt sulfate or cobalt metal primarily induced lung tumors (although tumors distal to the lung were found for cobalt metal) while injection-site tumors were induced following subcutaneous, intraperitoneal, intramuscular, or intratracheal administration of various cobalt particles and compounds. In vitro assays show that cobalt metal and cobalt compounds induce genetic damage and inhibit DNA repair. In vivo genotoxicity data were mostly conducted with cobalt chloride and were positive for aneuploidy, micronucleus formation, and chromosomal aberrations; cobalt acetate caused DNA damage in the lung and several other tissues. In vitro cytotoxicity assays were consistent in reporting dose-related effects for cobalt metal particles, cobalt oxide particles, and cobalt ions. In general, metallic cobalt particles induced cytotoxicity, ROS formation, genotoxicity, and carcinogenicity to a greater extent than cobalt ions while cobalt oxide particles with low solubility were less cytotoxic than cobalt ions but induced higher levels of ROS (see Table 6-1). Many studies (both

in vitro and in vivo) have reported evidence that cobalt induces oxidative stress, particularly when complexed with endogenous chelators such as glutathione or anserine. In addition, mutations in lung tumors induced by cobalt sulfate or cobalt metal included G to T transversions that are characteristic of oxidative damage.

7.1.4. Overall Synthesis

Several biological endpoints were identified from physicochemical, toxicological, and mechanistic data for cobalt metal, cobalt chloride, cobalt sulfate, and cobalt oxides (CoO and Co_3O_4). These cobalt forms were the most studied and included both soluble and poorly soluble forms (see Table 7-1 for synthesis of available information). Data for two cobalt oxides, CoO and Co_3O_4, were combined because both are poorly water-soluble, but are bioavailable (under more informative, physiologically relevant, test conditions in the presence of lung cells; see Sections 1.2.2 and 7.1.1) since they enter cells by endocytosis and release Co ions in the lysosomes, and induced similar biological effects (e.g., genotoxicity and cytotoxicity). Although data was not available for all endpoints for each oxide, overall the mechanistic data support the inclusion of both oxides in the class of cobalt compounds that release ions in vivo.

Symbols (i.e., −, +) in Table 7-1 are used to indicate the overall evaluation for the various endpoints and cobalt forms. These data provide justification for the proposed group approach and are consistent with the OECD (2014) and ECHA (2009) guidelines for chemical grouping. Thus, biological properties of cobalt compounds that are not included in this table may be inferred by comparing with an analogous cobalt compound within the table.

Table 7-1. Comparison of Chemical and Biological Properties of Cobalt Metal and Cobalt Compounds

Endpoint	Soluble Cobalt Salts	Cobalt Metal	Poorly Soluble Cobalt Compounds
	$CoCl_2$ and/or $CoSO_4$	Particles	CoO and/or Co_3O_4
Bioaccessibility			
Lysosomal fluid	+	+	+[a]
Gastric fluid	+	+	+[b]
Cellular uptake	+	+	+[c]
Cytotoxicity	+	+	+[c]
ROS	+[d]	+	+[e]
HIF-1α stabilization	+	+	+[f]
DNA repair inhibition	+[d]	+	ND
Genotoxicity in vitro	+	+	+[g]
Genotoxicity in vivo	+[d]	−	ND
Animal carcinogenicity	+[h]	+	+[i]

ND = No data, + = positive, − = negative.
[a]CoO = 92.4% (Stopford et al. 2003); Co_3O_4 = 50% by release of cobalt ions into RPMI 1640 culture medium in the presence of canine alveolar macrophages after 2 weeks of culture (Kreyling et al. 1990).
[b]CoO = 100% (Stopford et al. 2003); Co_3O_4 = ND.
[c]CoO = + (Smith et al. 2014); Co_3O_4 = + (Ortega et al. 2014).
[d]$CoCl_2$ = +; $CoSO_4$ = ND.
[e]CoO = + (Chattopadhyay et al. 2015); Co_3O_4 = + (Alarifi et al. 2013).
[f]CoO = ND; Co_3O_4 = + (Ortega et al. 2014).
[g]CoO = ± for chromosomal aberrations (Horie et al. 2012); Co_3O_4 = + for DNA damage (Alarifi et al. 2013; Kain et al. 2012).
[h]$CoCl_2$ = + by local injection; $CoSO_4$ = + by inhalation.
[i]CoO = + by intratracheal, intraperitoneal, and subcutaneous injection; Co_3O_4 = ND.

7.2. Evidence of Carcinogenicity from Studies in Experimental Animals

There is sufficient evidence for the carcinogenicity of cobalt and cobalt compounds that release cobalt ions in vivo (collectively referred to as cobalt) in experimental animals based on increased incidence of malignant and/or a combination of malignant and benign neoplasms at several tissue sites in rats and mice by different routes of exposure. Inhalation exposure to cobalt caused dose-related increases in the incidence of lung neoplasms (mainly alveolar/bronchiolar adenoma and carcinoma) in male and female mice and rats, adrenal gland (benign and malignant pheochromocytoma) in male and female rats, hematopoietic system (mononuclear-cell leukemia) in female rats, and pancreas (islet-cell adenoma or carcinoma combined) in male rats. (Evidence is insufficient to differentiate between a direct and indirect cause of adrenal gland neoplasms from cobalt exposure.) Tumors of the pancreas (islet-cell carcinoma) in female rats and kidney (adenoma or carcinoma combined) in male rats may have been related to exposure to cobalt metal. The increased tissue levels of cobalt reported in treated animals support the likelihood that the tumors (e.g., mononuclear-cell leukemia and pancreatic cancers) observed distal from the site of exposure resulted from systemic exposure to cobalt. Injection-site tumors (such as sarcoma, histiocytoma, rhabdomyofibrosarcoma, or fibrosarcoma) were observed in rats exposed to

different forms of cobalt by parenteral administration (such as intramuscular, subcutaneous, intraperitoneal injection).

Both lung and injection-site tumors were induced in rodents by different forms of cobalt, including cobalt metal, and soluble (e.g., cobalt sulfate or cobalt chloride) and poorly soluble cobalt compounds (cobalt(II) oxide). Data are summarized in Table 7-2. A comparison of the inhalation studies conducted by NTP of cobalt metal and cobalt sulfate suggests that cobalt metal was more toxic and carcinogenic at a similar cobalt concentration as evidenced by the incidence and spectrum of lung neoplasms and the extent of systemic lesions. This is consistent with mechanistic studies showing that cobalt metal has a greater effect on ROS than cobalt ions.

Table 7-2. Carcinogenic Effects of Cobalt Metal and Cobalt Compounds in Experimental Animals

Animal Neoplasms	Soluble Cobalt Salts		Cobalt Metal	Poorly Soluble Cobalt Compounds
	$CoCl_2$	$CoSO_4$	Particles	CoO
Lung	ND	+	+	+
Adrenal gland	ND	+	+	ND
Pancreatic islet cell	ND	−	+	ND
Mononuclear cell leukemia	ND	−	+	ND
Kidney	ND	−	±	ND
Injection site sarcomas	+	ND	+	+

ND = no data; + = positive; − = negative; ± = equivocal.

7.3. Evidence of Carcinogenicity from Studies in Humans

There is inadequate evidence from studies in humans to evaluate the association between exposure to cobalt and cobalt compounds that release cobalt ions in vivo and cancer. The data relevant to the evaluation were from studies of five independent cohorts of workers in various industries that focused on lung cancer and two case-population case-control studies (see Section 3) Although almost all the cohort studies reported approximately a doubling of the risk of lung cancer from exposure to various cobalt compounds, it is unclear that the excess risks were due to exposure specifically to cobalt, because of potential confounding from exposure to known lung carcinogens or other limitations (such as concerns about unexposed groups) which complicate the interpretation of the results. In addition, the studies had limited sensitivity to detect a true risk because of small number of cases, crude exposure assessment, or concern about healthy worker related effects.

Increased risks of esophageal cancer were found in the two population-based case-control studies; however, cobalt exposure was assessed in a single sample of toenail clippings taken at or several months after diagnosis of esophageal cancer. Based on data on reproducibility of measurements of metals in toenails, cobalt has low to intermediate within-person reliability, suggesting that a single sample is less than ideal. Measurements of nail cobalt reflect integrated exposures that occurred 12 to 18 months prior to clipping, raising the question about whether cobalt levels taken in toenails close to, and in many cases after cancer diagnosis, reflect the relevant period of exposure for long latency cancer.

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Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	absorption, distribution, metabolism, and excretion
ANOVA	analysis of variance
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
BDL	below detection limit
CA	chromosomal aberration
CASRN	Chemical Abstracts Service registry number
CDC	Centers for Disease Control and Prevention
CDR	Chemical Data Reporting Rule
CI	confidence interval
CIN	chromosomal instability
cm^2	centimeters squared
cm^3	centimeters cubed (mL)
DLMI	dominant lethal mutation index
DLMR	dominant lethal mutation rate
DNA	deoxyribonucleic acid
dw	drinking water
EPA	Environmental Protection Agency
EQ	exposure quartiles model
EUSES	European Union System for the Evaluation of Substances
Exp.	exposed
F	female
FDA	Food and Drug Administration
FR	*Federal Register*
ft	feet
FTE	full-time equivalent
FU	follow-up
g	gram
G	guanine

GC/MS	gas chromatography/mass spectroscopy
GI	gastrointestinal
GM	geometric mean
Hb	hemoglobin
HETA	Health Hazard Evaluation and Technical Assistance
HHE	Health Hazard Evaluation
HHS	Department of Health and Human Services
HIC	highest ineffective concentration
HID	highest ineffective dose
HPLC	high-performance liquid chromatography
hr	hour
HWE	healthy worker effect
HWSE	healthy worker survival effect
I	inconclusive
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
ICD-7, -8, -9	International Classification of Diseases, Seventh, Eighth or Ninth Revision
ICD-O	International Classification of Diseases for Oncology
IDLH	immediately dangerous to life and health
in	inch
inj.	injection
JEM	job-exposure matrix
kg	kilogram
L	liter
LEC	lowest effective concentration
LED	lowest effective dose
LOD	limit of detection
Log K_{ow}	logarithm of octanol/water partition coefficient
M	male
m^3	cubic meter
MCL	maximum contaminant level

mg	milligram
mL	milliliter
MN	micronuclei
mol	mole
MS	mass spectrometry
N	number
NA	not available; not applicable
NCE	normochromatic erythrocyte
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
ND	not detected; not determined; not done
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NI	no information
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NOES	National Occupational Exposure Survey
NOS	not otherwise specified
NPL	National Priorities List
NR	not reported; none reported
ns	not specified
NS	not significant
NT	not tested
NTP	National Toxicology Program
OHAT	Office of Health Assessment and Translation
OR	odds ratio
OSHA	Occupational Safety and Health Administration
P	probability
p value	the statistical probability that a given finding would occur by chance compared with the known distribution of possible findings
p.o.	per os (oral administration)

PBZ	personal breathing zone
PCE	polychromatic erythrocyte
PEL	permissible exposure limit
ppm	parts per million
ppt	parts per trillion
QSAR	quantitative structure-activity relationship
R	estimated daily production of adducts
r	correlation coefficient
RAHC	Reasonably anticipated to be a human carcinogen
RBC	red blood cell
REL	recommended exposure limit
RNS	reactive nitrogen species
RoC	Report on Carcinogens
ROS	reactive oxygen species
RQ	reportable quantity
RR	relative risk
RTG	relative total growth
s.c.	subcutaneous
SAFE	significance analysis of function and expression
SCE	sister-chromatid exchange
SD	standard deviation
SEER	Surveillance, Epidemiology, and End Results Program, NCI
SIC	Standard Industrial Classification
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOCMI	synthetic organic chemical manufacturing industry
SRR	standardized rate ratio, standardized relative risk
SSB	single-strand break
STS	soft tissue sarcoma
TDS	Total Diet Study
TLV-TWA	threshold limit value time-weighted average
t_{max}	time to maximum concentration in plasma
TMD	tail moment dispersion coefficient

TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TSFE	time since first employment
UDS	unscheduled DNA synthesis
UK	United Kingdom
US	United States
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
wk	week
wt%	weight percent
yr	year or years
µg	microgram

Glossary

Ames assay: The Ames *Salmonella*/microsome mutagenicity assay is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations.

Analysis bias: A bias arising from inappropriate data assumptions, models, or statistical methods used to evaluate findings, exposure-response relationships, latency, or confounding.

Aneuploidy: An abnormality involving a chromosome number that is not an exact multiple of the haploid number (one chromosome set is incomplete).

Apoptosis: Cell deletion by fragmentation into membrane-bound particles, which are phagocytosed by other cells.

Arabinose resistance: The L-arabinose resistance test with *Salmonella typhimurium* (Ara test) is a forward mutation assay that selects a single phenotypic change (from L-arabinose sensitivity to L-arabinose resistance) in a unique tester strain (an araD mutant).

Aroclor 1254-induced liver: Liver tissue treated with the polychlorinated biphenyl mixture Aroclor 1254 used as a source of S9 fraction for mutagenic and genotoxic effects testing.

Ascertainment bias: Systematic failure to represent equally all classes of cases or persons supposed to be represented in a sample.

Attrition bias: Systematic differences between **comparison groups** in withdrawals or exclusions of **participants** from the results of a study.

Biexponential process: A process of drug (or xenobiotic) clearance with two phases with different rates. The first phase often involves rapid distribution of a drug to peripheral tissues, while the second phase represents clearance mechanisms that eliminate the drug from the body. (See "Two-compartment pharmacokinetic model.")

Boiling point: The boiling point of the anhydrous substance at atmospheric pressure (101.3 kPa) unless a different pressure is stated. If the substance decomposes below or at the boiling point, this is noted (dec). The temperature is rounded off to the nearest °C.

Chemical Data Reporting Rule: Chemical Data Reporting (CDR) is the new name for Inventory Update Reporting (IUR). The purpose of Chemical Data Reporting is to collect quality screening-level, exposure-related information on chemical substances and to make that information available for use by the U.S. Environmental Protection Agency (EPA) and, to the extent possible, to the public. The IUR/CDR data are used to support risk screening, assessment, priority setting and management activities and constitute the most comprehensive source of basic screening-level, exposure-related information on chemicals available to EPA. The required frequency of reporting currently is once every four years.

Co-exposures: substances to which study participants are exposed that can potentially confound the relationship between the exposure and disease.

Cochran-Armitage trend test: A statistical test used in categorical data analysis when the aim is to assess for the presence of an association between a variable with two categories and a

variable with *k* categories. It modifies the chi-square test to incorporate a suspected ordering in the effects of the *k* categories of the second variable.

Comet assay: The comet assay evaluates DNA damage by measuring DNA migration in single cells using gel electrophoresis. Migration of DNA is directly related to DNA strand length: the smaller the strands (produced by breaks in the DNA, i.e., damage), the further the DNA will migrate from the nucleus in an electric field.

Confounding bias and potential confounders: A bias arising when the comparison groups under study (e.g., exposed versus unexposed, or the cases versus controls) have different background risks of disease (Pearce et al. 2007), in effect mixing the association of interest with the effects of other factors. Potential confounders can include any co-exposures or risk factors associated with both the exposure and the disease, and that are not part of the disease pathway.

Conversion factor: A numerical factor used to multiply or divide a quantity when converting from one system of units to another.

Critical temperature: The temperature at and above which a gas cannot be liquefied, no matter how much pressure is applied.

Differential misclassification bias: A bias that arises when the probability of being misclassified differs across groups of study subjects. The effect(s) of such misclassification can vary from an overestimation to an underestimation of the true value.

Differential selection: Selective pressure for self-renewal. Gene mutations that confer a growth or survival advantage on the cells that express them will be selectively enriched in the genome of tumors.

Disposition: The description of absorption, distribution, metabolism, and excretion of a chemical in the body.

Dominant lethal mutation assay: The dominant lethal assay identifies germ cell mutagens by measuring the ability of a chemical to penetrate gonadal tissue and produce embryonic death due to chromosomal breakage in parent germ cells.

Ecological study: A study in which the units of analysis are populations or groups of people rather than individuals.

ELISA assay: Enzyme-linked immunosorbent assay; a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody.

Epigenetic mechanisms: Changes in gene function that do not involve a change in DNA sequence but are nevertheless mitotically and/or meiotically heritable. Examples include DNA methylation, alternative splicing of gene transcripts, and assembly of immunoglobulin genes in cells of the immune system.

Exposure-response gradient: Describes the change in effect caused by differing levels of exposure (or doses) to a chemical or substance.

FDA Good Laboratory Practice Regulations: A quality system codified by the U.S. Food and Drug Administration that prescribes operating procedures for conducting nonclinical laboratory studies that support or are intended to support applications for research or marketing permits for products regulated by the Food and Drug Administration.

Fisher's exact test: The test for association in a two-by-two table that is based on the exact hypergeometric distribution of the frequencies within the table.

Follow-up: Observation over a period of time of a person, group, or initially defined population whose appropriate characteristics have been assessed to observe changes in health status or health-related variables.

Genomic instability: An increased propensity for genomic alterations that often occurs in cancer cells. During the process of cell division (mitosis) the inaccurate duplication of the genome in parent cells or the improper distribution of genomic material between daughter cells can result from genomic instability.

Genotoxic: The property of a chemical or agent that can cause DNA or chromosomal damage.

Healthy worker hire effect: Initial selection of healthy individuals at time of hire so that their disease risks differ from the disease risks in the source (general) population.

Healthy worker survival effect: A continuing selection process such that those who remain employed tend to be healthier than those who leave employment.

Henry's Law constant: The ratio of the aqueous-phase concentration of a chemical to its equilibrium partial pressure in the gas phase. The larger the Henry's law constant the less soluble it is (i.e., greater tendency for vapor phase). The relationship is defined for a constant temperature, e.g., 25°C.

Information bias: A bias arising from measurement error. Information bias is also referred to as observational bias and misclassification (see differential and non-differential misclassification bias). When any exposure, **covariate**, or outcome variable is subject to measurement error, a different quality or accuracy of information between comparison groups can occur.

Integration of scientific evidence across studies: The final step in the cancer assessment that assigns greater weight to the most informative studies to reach a preliminary listing recommendation.

Job exposure matrix (JEM): A tool used to assess exposure to potential health hazards in occupational epidemiologic studies by converting coded occupational data (usually job titles) into a matrix of possible levels of exposures to potentially harmful agents, reducing the need to assess each individual's exposure in detail.

Lagging: Statistical methods that weight exposure times in order to account for prolonged induction and latency periods, particularly in occupational epidemiology studies.

Latency and prolonged induction: The induction period is the time required for a cause to lead to the disease process (regardless of symptoms); the latent period is the time between the exposure and clinical manifestation of the disease. Especially important when considering cancer outcomes.

Left truncation: This bias can occur when workers hired before the start of the study, and thus exposed and at risk for disease, do not remain observable at the start of follow-up. The remaining prevalent workers may be healthier and not representative of all workers hired before the start of the study.

Melting point: The melting point of the substance at atmospheric pressure (101.3 kPa). When there is a significant difference between the melting point and the freezing point, a range is given. In case of hydrated substances (i.e., those with crystal water), the apparent melting point is given. If the substance decomposes at or below its melting point, this is noted (dec). The temperature is rounded off to the nearest °C.

Metaplasia: A change of cells to a form that does not normally occur in the tissue in which it is found.

Methemoglobin: A form of hemoglobin found in the blood in small amounts. Unlike normal hemoglobin, methemoglobin cannot carry oxygen. Injury or certain drugs, chemicals, or foods may cause a higher-than-normal amount of methemoglobin to be made. This causes a condition called methemoglobinemia.

Micronuclei: Small nuclear-like bodies separate from, and additional to, the main nucleus of a cell, produced during the telophase of mitosis or meiosis by lagging chromosomes or chromosome fragments derived from spontaneous or experimentally induced chromosomal structural changes.

Miscible: A physical characteristic of a liquid that forms one liquid phase with another liquid (e.g., water) when they are mixed in any proportion.

Molecular weight: The molecular weight of a substance is the weight in atomic mass units of all the atoms in a given formula. The value is rounded to the nearest tenth.

Mutagenic: Capable of inducing genetic mutation, e.g., a genotoxic substance or agent that can induce or increase the frequency of mutation in the DNA of an organism.

Mutations: A change in the structure of a gene, resulting from the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes. The genetic variant can be transmitted to subsequent generations.

National Health and Nutrition Examination Survey: A program of studies designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations.

Nondifferential misclassification bias: Arises when all classes, groups, or categories of a variable (whether exposure, outcome, or covariate) have the same error rate or probability of being misclassified for all study subjects. In the case of binary or dichotomous variables nondifferential misclassification would usually result in an ‘underestimation’ of the hypothesized relationship between exposure and outcome.

Normochromatic erythrocyte: A mature erythrocyte that lacks ribosomes and can be distinguished from immature, polychromatic erythrocytes by stains selective for RNA.

Octanol/water partition coefficient (log K_{ow}): A measure of the equilibrium concentration of a compound between octanol and water.

One-compartment model: A pharmacokinetic modeling approach that models the entire body as a single compartment into which a drug is added by a rapid single dose, or bolus. It is assumed that the drug concentration is uniform in the body compartment at all times and is eliminated by a first order process that is described by a first order rate constant.

Personal breathing zone: A sampling area as close as practical to an employee's nose and mouth, (i.e., in a hemisphere forward of the shoulders within a radius of approximately nine inches) so that it does not interfere with work performance or safety of the employee.

Personal protective equipment: Specialized clothing or equipment, worn by an employee to minimize exposure to a variety of hazards. Examples of PPE include such items as gloves, foot and eye protection, protective hearing devices (earplugs, muffs) hard hats, respirators, and full body suits.

Plate incorporation: A commonly used procedure for performing a bacterial reverse mutation test. Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. In the plate-incorporation method, these suspensions are mixed with an overlay agar and plated immediately onto minimal medium. After two or three days of incubation, revertant colonies are counted and compared with the number of spontaneous revertant colonies on solvent control plates.

Point emission: A release that can be identified with a single discharge source or attributed to a specific physical location.

Poly-3 trend test: A survival-adjusted statistical test that takes survival differences into account by modifying the denominator in the numerical (quantal) estimate of lesion incidence to reflect more closely the total number of animal years at risk.

Proto-oncogene: A gene involved in normal cell growth. Mutations (changes) in a proto-oncogene may cause it to become an oncogene, which can cause the growth of cancer cells.

Proxy: A substitute authorized to act for the study participant. Often this is a spouse or other family member who may consent to be interviewed, offering information about the participant.

P_{trend}: Level of statistical significance of a change over time in a group selected to represent a larger population.

QUOSA: A collection of scientific literature management software and services for researchers and information professionals in the life sciences and related scientific and medical areas designed to retrieve, organize, and analyze full-text articles and documents.

Recall bias: A bias arising from systematic error in the accuracy or completeness of "recalled" by study participants regarding past events, and usually arises in the context of retrospective case-control interviews or questionnaires. The concern is that those with the disease may search their memories more thoroughly than unaffected controls to try to recall exposure to various causal factors. This bias is often differential and biases towards an overestimate of effect.

Reverse causality: May arise in case-control studies when exposure is measured after disease diagnosis, as the concern is that symptoms or early manifestations of the disease may affect the measured exposure; this is particularly of concern in studies using biomarkers of effect.

Right truncation: For right truncated data, only participants or person-time under observation up to a given date are included. Right truncation results in limiting person-time to values that are limited below the given date. Truncation is similar to but distinct from the concept statistical censoring. A truncated sample is similar to an underlying sample with all values outside the bounds entirely omitted, with no count of participants or person-time omitted kept. Alternatively, with statistical censoring, the value of the bound exceeded is known and documented.

Selection bias: An error in choosing the individuals or groups to take part in a study. Ideally, the subjects in a study should be very similar to one another and to the larger population from which they are drawn (for example, all individuals with the same disease or condition). If there are important differences, the results of the study may not be valid, and bias can be introduced in either direction.

Selective reporting: Selective reporting occurs when the effect estimate for a measurement (of exposure or disease) was selected from among analyses using several measurement instruments, reflecting the most favorable result or subcategories.

Sensitivity: The proportion of truly diseased persons in the screened population who are identified as diseased by the screening test; or the probability of correctly diagnosing a true case with the test.

Sister-chromatid exchange: The exchange during mitosis of homologous genetic material between sister chromatids; increased as a result of inordinate chromosomal fragility due to genetic or environmental factors.

Solubility: The ability of a substance to dissolve in another substance and form a solution. The Report on Carcinogens uses the following definitions (and concentration ranges) for degrees of solubility: (1) *miscible* (see definition), (2) *freely soluble*- capable of being dissolved in a specified solvent to a high degree (>1,000 g/L), (3) *soluble*- capable of being dissolved in a specified solvent (10–1,000 g/L), (4) *slightly soluble*- capable of being dissolved in a specified solvent to a limited degree (1–10 g/L), and (5) practically insoluble- incapable of dissolving to any significant extent in a specified solvent (<1 g/L).

Specific gravity: The ratio of the density of a material to the density of a standard material, such as water at a specific temperature; when two temperatures are specified, the first is the temperature of the material and the second is the temperature of water.

Specificity: The proportion of truly nondiseased persons who are so identified by the screening test; or the probability of correctly identifying a non-diseased person with the test.

Spot test: Qualitative assay in which a small amount of test chemical is added directly to a selective agar medium plate seeded with the test organism, e.g., *Salmonella*. As the chemical diffuses into the agar, a concentration gradient is formed. A mutagenic chemical will give rise to a ring of revertant colonies surrounding the area where the chemical was applied; if the chemical is toxic, a zone of growth inhibition will also be observed.

Study sensitivity: The ability of a study to detect an effect (if it exists) which would include a large number of exposed cases; evidence of substantial exposure (e.g., level, duration, frequency, or probability) during an appropriate window; an adequate range in exposure levels or duration allowing for evaluation of exposure-response relationships; and an adequate length of follow-up.

Study utility: The overall utility of a study is based on consideration of the potential for bias (i.e., study quality) and study sensitivity. Serious concerns about study quality will result in lower utility of the study; a high-quality study with low sensitivity could also have low utility.

Surrogate exposure data: Ideally, a study would provide multiple quantitative metrics of each individual's exposure to the substance of interest. However, a surrogate metric correlated with exposure may be used instead of, or in addition to exposure data.

Time-weighted average: The average exposure concentration of a chemical measured over a period of time (not an instantaneous concentration).

Toxicokinetics: The mathematical description (toxicokinetic models) of the time course of disposition of a chemical in the body.

Transitions: DNA nucleotide substitution mutation in which a purine base is substituted for another purine base (adenine → guanine or guanine → adenine) or a pyrimidine base for another pyrimidine base (cytosine → thymine or thymine → cytosine).

Transversions: DNA nucleotide substitution mutation in which a purine base (adenine or guanine) is substituted for a pyrimidine base (cytosine or thymine) or vice versa.

Two-compartment pharmacokinetic model: A two-compartment pharmacokinetic model resolves the body into a central compartment and a peripheral compartment. The central compartment generally comprises tissues that are highly perfused such as heart, lungs, kidneys, liver, and brain. The peripheral compartment comprises less well-perfused tissues such as muscle, fat, and skin. A two-compartment model assumes that, following drug administration into the central compartment, the drug distributes between that compartment and the peripheral compartment. However, the drug does not achieve instantaneous distribution (i.e., equilibrium), between the two compartments. After a time interval (t), distribution equilibrium is achieved between the central and peripheral compartments, and elimination of the drug is assumed to occur from the central compartment.

Type-I error: The error of rejecting a true null hypothesis, i.e., declaring that a difference exists when it does not.

Type-II error: The error of failing to reject a false null hypothesis, i.e., declaring that a difference does not exist when in fact it does.

Vapor density, relative: A value that indicates how many times a gas (or vapor) is heavier than air at the same temperature. If the substance is a liquid or solid, the value applies only to the vapor formed from the boiling liquid.

Vapor pressure: The pressure of the vapor over a liquid (and some solids) at equilibrium, usually expressed as mm Hg at a specific temperature (°C).

Appendix A. Literature Search Strategy

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Figure

This document identifies the data sources, search terms, and search strategies that were used to identify literature for the draft monograph on cobalt and certain cobalt compounds (hereafter referred to as 'cobalt'). The literature search strategy used for cobalt involved several approaches designed to identify potentially useful information for the broad range of topics covered by a Report on Carcinogens (RoC) monograph, as listed below.

- Properties and Human Exposure (focusing on the U.S. population)
- Disposition (ADME) and Toxicokinetics
- Human Cancer Studies
- Studies of Cancer in Experimental Animals
- Mechanistic Data and Other Relevant Effects
  - Genetic and Related Effects
  - Mechanistic Considerations

The methods for identifying the relevant literature for the draft cobalt monograph including (1) the search strategy, (2) updating the literature search, and (3) review of citations using web-based systematic review software are illustrated in Figure A-1 and discussed below. The detailed literature search strategy, including all database sources, and exclusion/inclusion criteria, are available at http://ntp.niehs.nih.gov/go/730697.

A.1. Search Strategies

Relevant literature is identified using search terms, data sources, and strategies as discussed below.

(1) General data search: This search covers a broad range of general data sources for information relevant to many or all of the wide range of monograph topics pertaining to cobalt.

(2) Exposure-related data search: This search covers a broad range of potential sources for exposure-related information and physical-chemical properties.

(3) Database searches in PubMed, Scopus, and Web of Science: The majority of the primary literature used to draft the cobalt monograph was identified from searches of these three extensive databases available through the NIEHS Library. Searches for cobalt were combined with the search terms for each of the monograph topics listed above to create the specific literature searches.

(4) Searches for human cancer studies are somewhat unique because they involve the identification of search terms for exposure scenarios that might result in exposure of people to cobalt. For cobalt, these exposure-related search terms were based on uses of cobalt identified from the EPA's TRI database and the Chemical Data Report rule website.

(5) QUOSA library of occupational case-control studies search of the QUOSA-based library of more than 6,000 occupational case-control studies, approximately 95% of which are currently available as searchable full-text pdfs, was conducted using the "cobalt."

(6) Secondary sources: Citations identified from authoritative reviews or from primary references located by literature search, together with publications citing key papers identified using the Web of Science, "Cited Reference Search," were also added.

A.2. Updating the Literature Search

The literature searches will be updated prior to submitting the draft monograph for peer review and prior to finalizing the monograph. Monthly search alerts for cobalt searches were created in PubMed, Scopus, and Web of Science, and the results of these searches from the closing date of the initial search will be downloaded for review.

A.3. Review of Citations Using Web-based Systematic Review Software

Citations retrieved from literature searches were uploaded to web-based systematic review software and screened using inclusion and exclusion criteria. Multi-level reviews of the literature were conducted, with initial reviews (Level 1) based on titles and abstracts only to identify citations that could be excluded and to assign the included literature to one or more monograph topics; subsequent reviews (Level 2) for literature assigned to the various monograph topics (Exposure, ADME & TK, Human cancer studies, etc.) were based on full-text (i.e., PDFs) of the papers and were carried out by the writer and scientific reviewer for each monograph section. Two reviewers, at least one of whom is a member of the ORoC at NIEHS, participated at each level of review.

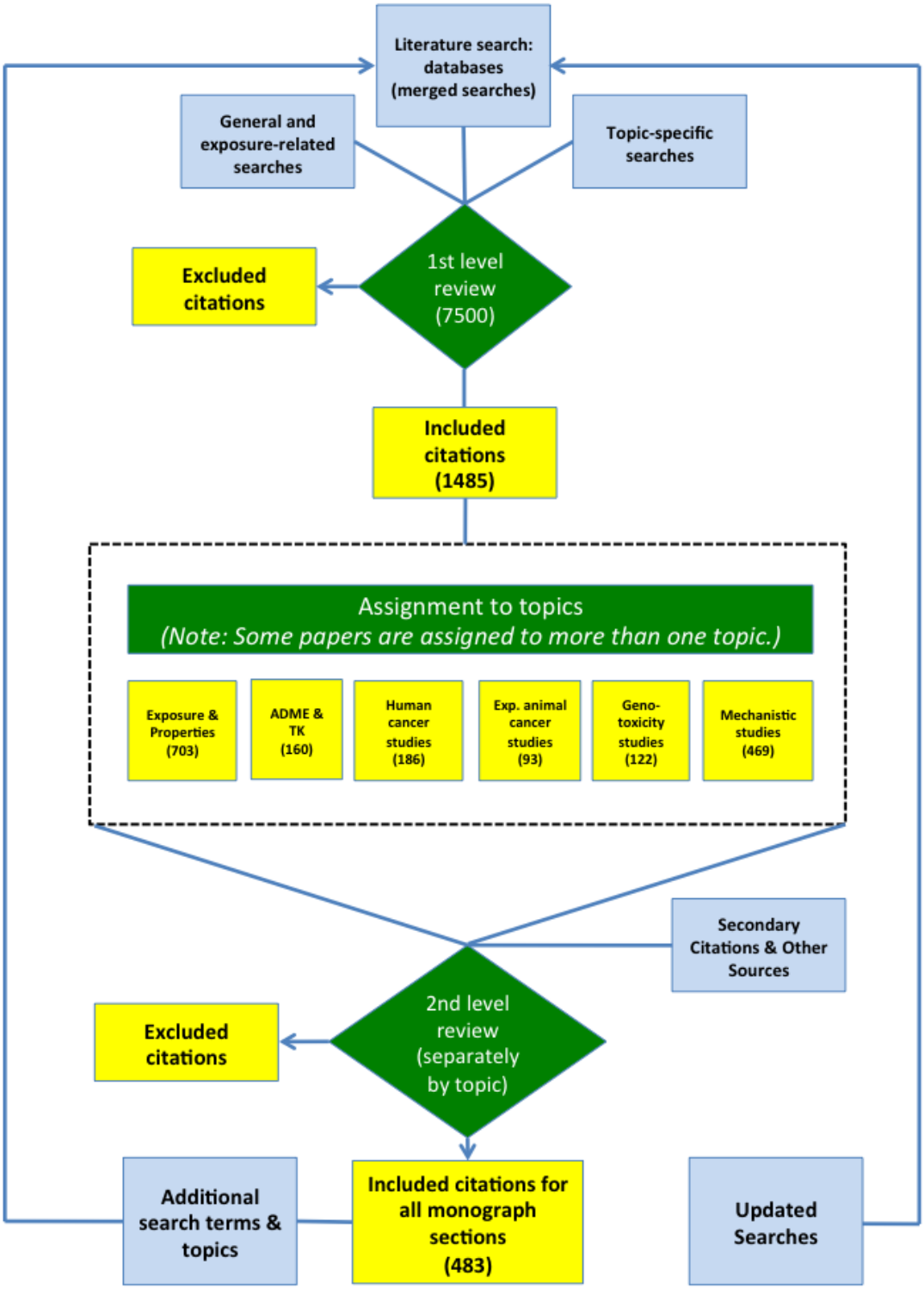

Figure A-1. Literature Search Strategy and Review

Appendix B. Chemical Properties and Exposure-related Information, Clinical Surveys and Studies, and Regulations

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Tables

This appendix reports chemical properties for cobalt compounds not included in Table 1-1 because they did not meet either of the criteria for inclusion in the table (i.e., no animal or genotoxicity testing data are available and they are in commercial use greater than 100,000 pounds per year in the United States (per EPA Chemical Data Reporting rule) exposure information for cobalt levels in urine and blood (Table B-1), in hair and nails (Table B-2), and in tissues from cancer patients (Table B-3). The regulations and guidelines that are likely to decrease human exposure to cobalt and cobalt compounds are reported in Section B.3.

B.1. Chemical Properties

The chemical forms of cobalt listed in Table B-1 below were not included in Table 1-1 because they did not meet either of the two criteria for inclusion in that table (i.e., availability of animal or genotoxicity testing data for these compounds or in commercial use greater than 100,000 pounds per year in the United States (per the EPA Chemical Data Reporting rule)). For many of these cobalt compounds listed in the table below, solubility in water and bioaccessibility (based on % solubility in gastric/lysosomal fluids) are not particularly correlated. Most of the compounds are fairly bioaccessible but not always soluble in water. For example, as shown in Table B-1, cobalt trihydroxide and cobalt borate propionate are both bioaccessible but the trioxide is insoluble in water while the borate propionate is water soluble.

Table B-1. Physical and Chemical Properties for Additional Chemical Forms of Cobalt

Name	CAS No.	Formula	Molecular Weight	Physical Form	Solubility (Grams per 100 cc Cold Water)	Bioaccessibility (% Solubility in Gastric/Lysosomal Fluids)
Hydroxide oxide	12016-80-7	CoOOH	91.9	Solid	0.00007	21/42
Trihydroxide	1307-86-4	$Co(OH)_3$	110.0	Pasty liquid	0.00013	65/80
Lithium dioxide	12190-79-3	CoO_2Li	97.9	Solid	0.00003	26/4
Isononanoate	84255-52-7	$Co(C_9H_{17}O_2)_2$	373.4	Liquid	0.705	91/89
Neodecanoate	27253-31-2	$Co(C_{10}H_{19}O_2)_2$	401.5	Liquid	0.0772	83/72
Acetyl acetonate	14024-48-7	$Co(C_5H_7O_2)_2$	257.2	Solid	0.516	100/96
Borate propionate	91782-61-5	$BO_3(CoC_3H_5O_2)_3$	454.8	Solid	3.33	89/88
Borate 2-ethylhexanoate	91782-60-4	$BO_3(CoC_8H_{15}O_2)_3$	665.3	Solid	0.994	88/92
Borate neodecanoate	68457-13-6	$BO_3(CoC_{10}H_{19}O_2)_3$	749.5	Pasty liquid	0.190	100/87
Tallate	61789-52-4	–	–	Pasty liquid	–	62/48
Resinate	68956-82-1	–	–	Solid	0.00335	9/14

B.2. Exposure

The values for urine cobalt listed below are in Table B-2 are also illustrated in Figure 2-1 in Section 2. Values identified for the following exposure groups are listed in the table for: (1) general population, (2) environmental exposure, (3) occupational exposure, (4) medical (hip) implants functioning normally, and (5) medical (hip) implants that have failed. Values for cobalt in hair and nails are reported in Table B-3 and also illustrated in Figure 2-2 for (1) the general population, (2) environmental exposure, (3) occupational exposure, and (4) hip implants.

Table B-2. Values for Urine and Blood Cobalt Levels (Means or Medians) in the United States and Other Countries[a]

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
General Public				
Bibi et al. (2016) *Pakistan*	Control group that never drank arsenic-contaminated water from 48 total subjects, including children (10–15 yr) and 2 groups of adults (25–35 yr; 40–50 yr)	Control (N not reported)	***Mean (SE) Units NR***[h] ***0.07*** ± 0.01	***Blood Mean (SE) Units NR*** 0.08 ± 0.03
Bradberry et al. (2014) *United Kingdom*	Normal ranges for UK	–	–	*Blood or serum* <0.6
Centers for Disease Control and Prevention (CDC) and National Center for Health Statistics (NCHS) (2011) *United States*	–	(2,504)	***0.326***[b,c] *GM*	–
Dunstan et al. (2005) *England*	Controls for patients receiving a hip implant	Controls (4)	Mean (95% CI) ***0.6*** *(0.40–0.74)*	*Blood Mean* (95% CI) 0.69 (0.43–0.89)
De Boeck et al. (2000) Belgium, Norway, Finland (cobalt refineries), Sweden, England (hard-metal plants)	Matched controls from 2 cobalt refineries and two hard metal-producing plants	Controls (27)	GM ± SD ***1.7*** ± 1.6	–
Nemery et al. (1992) *Belgium*	Workers in workshops in the diamond polishing industry (sawing diamonds or drawing jewelry) not thought to be exposed to cobalt (controls for occupational study)	(48)	***2.3*** ± 1.8	–

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Angerer (1989) (as reported in IARC 1991) Germany	NR	(NR)	***0.01*** (NR)	*Blood (range)* 0.2–1.3
Alexandersson (1988) *Sweden*	Workers not exposed to cobalt (controls for occupation study)	(25)	[***0.4*** (0.1–2.2)]	*Blood* [0.5 (0.1–1.2)]
Christensen and Mikkelsen (1986) Not specified	Porcelain workers without exposure to cobalt (controls for occupational study)	(46)	µg/g creatinine[e,f] [**0.8** (0.04–10.7)]	*Blood* 0.24 (0.05–0.6)
Hartung (1986) (as reported in IARC 1991) *Not specified*	–	(NR)	–	Serum 0.1 (NR)
Ichikawa et al. (1985) *Not specified* Japan	Office workers (controls for occupational study)	(20)	***2.0*** ± 1.0	*Blood* 1.9 ± 1.1
Lewis et al. (1985) (as reported in IARC 1991) *Not specified*	–	–	–	*Serum* 0.28 (NR)
Scansetti et al. (1985) *Italy*	"White collar" workers (control for occupational study)	(NR)	***0.41*** ± 0.22	–
Andersen and Høgetveit (1984) (as reported in IARC 1991) *Not specified*	NR	(NR)	–	*Plasma* 0.15 ± 0.07

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Mikkelsen et al. (1984) (as cited in IARC 1991) *Not specified*	–	(NR)	***0.94*** (0.05–13.8)	–
Ostapczuk et al. (1983) (as reported in IARC 1991) Not specified	NR	(NR)	–	*Blood* 0.09 ± 0.02
Kasperek et al. (1981) (as reported in IARC 1991) *Not specified*	NR	(NR)	–	*Plasma* 0.195 ± 0.015
Schumacher-Wittkopf and Angerer (1981) (as reported in IARC 1991) *Not specified*	NR	(NR)	***0.38*** (0.1–0.75)	–
Alexandersson and Swensson (1979) (as reported in IARC 1991) Not specified	NR	(NR)	–	*Blood* 0.5 ± 0.1
Versieck et al. (1978) (as reported in IARC 1991) *Not specified*	NR	(NR)	–	*Serum* 0.108 ± 0.06
Hip Implants (stable)				

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Savarino et al. (2014) *Not reported; authors from Italy*	Metal on metal hip resurfacing (HR, 2013 and 2014 studies) and total hip replacement patients (THR, 2013 study) Not clear if HR patients overlap in the two studies	Controls (48) THR mean 121 mo (16) HR mean 105 mo (25) HR *follow-up* 2 yr (14) 5 yr (19) 9 yr (22)	–	0.3 (0.1–0.5) 0.7 (0.3–1.6) 1.2 (0.3–2.5) 1.2 ± 0.6 1.1 ± 0.3 0.90 ± 0.12
Sidaginamale et al. (2013) *United Kingdom*	Metal on metal hip arthroplasty (total hip or articulating surface replacement) patients; tested for cobalt levels at various times after implant (mean times reported)	Controls (3,042) *Implant #1* 32 mo (416) *Implant #2* 55.4 mo (165) *Implant #2* 66 mo (467)	–	*Serum*[d] 0.5 (0.3–6.7) 2.99 (0.20–228) 2.29 (0.65–190) 2.63 (0.37–204)
Zeh et al. (2007); Zeh et al. (2009) *Not reported; prostheses from Germany and procedure carried out in Germany*	Metal-on-metal artificial lumbar disc implant patients followed up at different times post operation	Controls (5) Patients; avg.; range follow-up (10) 14.8; 11–22 mo 36.7; 32–43.1 mo	–	*Serum* 0.72 ± 0.76 4.75 ± 2.71 1.89 ± 1.54
Witzleb et al. (2006) *Not reported; replacements were from UK and Switzerland*	Hip resurfacing arthroplasty and metal on metal total hip replacement (THR) patients	Controls (130) *Hip resurfacing* 3 mo (56) 24 mo (23) *THR (24 mo)* bilateral (3) unilateral (34)	–	*Serum*[d] 0.25 (NR) 2.17 (NR) 4.28 (NR) 3.18 (NR) 1.70 (NR)

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Dunstan et al. (2005) *England*	Patients receiving a metal-on-metal hip implant because of bone cancer; 10 survivors with samples, 5 of which retained their original implants (3 metal-on-metal, 2 metal-on-polypropylene); 5 converted to metal-on-polyethylene	Type of stable implant Metal-on-polyethylene (2) Metal-on-metal (3) Converted from metal-on-metal to metal-on-polypropylene (5)	Mean (95% CI) 1.0 (NR) 12.2 (3.6–18.0) 2.88 (1.3–7.8)	*Blood* Mean (95% CI) 0.48 (0.5–0.5) 1.97 (1.1–2.4) 0.65 (0.3–1.1)
Adami et al. (2003) *Italy*	Metal-on-metal total hip replacement patients	 Controls (15) Patients (15)	–	*Blood* 0.3 ± 0.1 4.1 ± 1.5
Lhotka et al. (2003) *Not reported; hip manufacturers are European*	Metal-on-metal hip replacement patients 131 implant #1 128 implant # 2 Patients divided into 5 groups and sampled at different time periods (mo) post operations	 Controls (31) *Implant #1* Immediate PO (24) 3–6 mo (27) 12–15 mo (27)) 35–38 mo (28) 42–48 mo (25) *Implant #2* Immediate PO (24) 3–6 mo (25) 12–15 mo (27) 35–38 mo (26) 42–48 mo (26)	–	*Blood* 0.7 ± 0.45 3.23 ± 2.0 10.9 ± 3.2 23.3 ± 6.9 36.6 ± 10.2 17.0 ± 15.7 8.1 ± 4.9 14.8 ± 6.4 33.6 ± 17.1 17.4 ± 7.4 27.7 ± 18.0
Schaffer et al. (1999) Not reported; authors from Austria and prostheses from Austria	Metal-on-metal total hip replacement (76 patients). Cobalt measured at different times in subsets of the population post operation	 Controls (26) Patients 1-yr PO (22) 2-yr PO (25) 3-yr PO (29)	 **0.4**[d] (0.1–1.1) **5.5**[d] (0.2–13.2) **5.7** (estimated from graph) **10.3** (estimated from graph)	*Blood*[d] 1.1 (0.6–2.0) 1.5 (0.3–5.5) 2.1 (estimated from graph) 2.0 (estimated from graph)

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Hennig et al. (1992) (as cited in Schaffer et al. 1999) *Not specified*	Metal-on-metal total hip replacement patients	(10)	**3.8**[d] (2.1–286.2)	–
Sunderman et al. (1989) *United States*	Metal-on-polyethylene hip (N = 21) Pre and post implantation NB: The pre-operative group included patients receiving either Ti-Al-V or Co-Cr prostheses and knee implants	 Controls (42) Patients (Time sampled) 6–120 wk PO (NR)	µg/g creatinine[e] **0.5** ± 0.4 (SE) **0.7** ± 0.3	Serum 0.05 ± 0.01 (SE) 0.13 ± 0.05
Medical implants (unstable)				
Bradberry et al. (2014) *Not reported; authors from UK*	Failed hip replacement patients (18) with systemic toxicity (e.g., neuro-ocular toxicity, cardiotoxicity, thyroid toxicity)	Total with levels (17) Metal-on metal (8) Ceramic (9)	–	Median peak 398 (14–6,521) 34.5 (13.6–399) 506 (353–6,521)
Rodriguez de la Flor et al. (2013) *Spain*	Hip implants requiring revision surgery	Before revision (11) After revision (11)	**205.6** ± 310.6 44.3 ± 94.9	Serum 25.8 ± 40.6 13.1 ± 29.3
Dunstan et al. (2005) *England*	Patients receiving a metal-on-metal hip implant because of bone cancer; 10 survivors with samples, 5 of which retained their original implants	Radiologically loose hip implants (2)	Mean (95% CI) 205 (140, 270)	Mean (95% CI) 35.5 (19, 52)
Environmental exposure				

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Cheyns et al. (2014) *D.R. Congo*	Adults and children (<14 yrs) living in urban and rural communities close to metal mining and/or refining plants or villages near a lake receiving effluents from metal refining plants	 *Controls* Children (24) Adults (57) *Lake* Children (13) Adults (47) *Polluted communities* Children (32) Adults (79)	µg/g creatinine[e] Mean[b] (25%–75% CI) 4.2 (2.6–7.2) 2.7 (1.3–5.3) 14.0 (7.5–21.0) 9.4 (4.0–19.8) **27.9** (13.4–62.7) **11.7** (7.1–22.0)	–
Moreno et al. (2010) *Mexico*	Urine cobalt levels in children in the Taxco mining area of Southern Mexico Reference values were reported in the literature for children from other countries	Reference Children (35)	**2.2** (NR) **18**[d] (3–47)	–
Occupational exposure				
Cobalt production: Refining				
Lantin et al. (2011) *Belgium*	Cobalt refinery workers	 Cobalt production (249)	µg/g creatinine[f] **3.90**[e] (0.3–204)	Blood 1.0 (<0.5–32.0)
De Boeck et al. (2000) Belgium, Norway, Finland (cobalt refineries), Sweden, England (hard metal plants)	Workers exposed to cobalt dust from 2 refineries and hard metal dust from two hard metal-producing plants, location not specified	 Co refinery workers (24) Hard metal workers (29)	GM ± SD **21.5** ± 2.1 **19.9** ± 2.4	–
Thomassen et al. (1999) *Russia*	Nickel refinery workers	Roasting (25) Anode casting [old] (20) Anode casting [new] (11) Electrorefining [old] (23) Electrorefining [new] (10) Rinsing [old] (18) Rinsing [new] (12))	**5.8** ± 5.7 **8.4** ± 9.0 **14** ± 37 **2.9** ± 4.8 **2.7** ± 2.4 **8.7** ± 11 **16** ± 19	–

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Cobalt production: Cobalt salts or oxide				
Coombs (1996) *South Africa*	Cobalt oxide workers, pre- and post-environmental and exposure controls (plant converts cobalt metal to cobalt oxide)	Pre-controls (43 samples across 8 job titles) Post-controls (91 samples across 12 job titles)	**454.6** µg/g creatinine[e] **68.5**[i]	–
Swennen et al. (1993) *Belgium*	Production of cobalt powder, oxides, and salts	Day and shift Mon; pre-shift (82) Mon; post-shift (82) Fri; pre-shift (82) Fri; post-shift (82)	µg/g creatinine[b,f] **21.1** (0.3–488) **52.9** (2.7–2245) **40.6** (0.9–1288) **69.8** (1.6–2038)	*Blood* 9.7 (2.0–120) 11.0 (2.0–120) 11.2 (2.0–110) 12.7 (2.0–120)
Angerer et al. (1985) *Germany*	Foundry workers using cobalt as a powder (4 groups) or salt (3 groups)	All 7 groups (40)	Range of means **18.9–438.4**	Blood 4.9–47.9
Metallurgical				
Beaucham et al. (2014) *(State NR), United States*	Metallurgical workers at an orthopedic implant manufacturing company	(21)	**0.6**[d] (0.3–2.0)	–
Deng et al. (1990) *Michigan, United States*	Metallurgical Site visit; 261 urine samples from 39 workers; up to 7 specimens /person per day	Monday Pre-shift (33) Post-shift (32) Tuesday Pre-shift (34) Post-shift (34) Wednesday Pre-shift (36) Post-shift (36) Thursday Pre-shift (35) Post-shift (0)	µg Co/mg creatinine[g] **21.3** ± 28.6 **44.3** ± 65.7 **26.4** ± 32.1 **50.4** ± 71.7 **36.3** ± 56.7 **50.2** ± 78.0 **40.8** ± 58.6 ND	–
Cemented carbides (hard meals) and bonded diamonds (diamond abrasives)				

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]	
Sahakian et al. (2009)	Cemented carbides and bonded diamonds		µg Co/g creatinine GM[b] (95% CI)	*Blood* GM[b] (95% CI)	
Alabama, United States	Site visit at three cemented tungsten manufacturing facilities; urine collected post shift near end of work week	All plants (84) Areas with air levels > recommended exposure limits	**9.6** (7.1–12.8)	2.0 (1.5–2.5)	
		Reclamation (7)	**25.2** (8.7–73.2)	4.0 (1.2–13.2)	
		Powder mixing (5)	**14.5** (2.5–84.9)	3.7 (0.6–23.6)	
		Milling (5)	**134.7** (96–189)	15.6 (11.2–22)	
		Spray drying (2)	**20.0** (0.9–438)	3.2 (1.8–5.8)	
		Pressing (10)	**30.3** (14.9–61.8)	3.7 (2.6–5.2)	
		Shaping (4)	**25.7** (5.0–133.5)	3.3 (2.2–5.1)	
Kraus et al. (2001)	Hard-metals production		µg/g creatinine[e]		–
Germany	workers (87) in different workshops	Forming (23)	**13.5** (0.8–106.4)		
		Pressing (30)	**5.5** (0.4–35.9)		
		Heavy alloy production (3)	**1.6** (1.1–2.2)		
		Powder processing (14)	**28.5** (0.8–227.8)		
		WC production (4)	**2.1** (0.3–5.7)		
		Sintering (6)	**4.1** (0.3–9.6)		
		Grinding (5)	**2.2** (0.2–6.0)		
		Maintenance (2)	**3.0** (1.3–4.7)		
Linnainmaa and Kiilunen (1997)	Hard metal workers;	(131)	**14.2** (0.5–160)		–
Finland	manufacturing or sharpening blades at 16 workplaces				
Ferdenzi et al. (1994)	Production of diamond cutting	Pre- & post-workplace modifications (Friday end of shift)			–
Italy	wheels workers (20)	1988 (NR)	**550**		
		1991 (15)	**85**		

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Mosconi et al. (1994a) *Province of Bergamo, Italy*	Diamond abrasive production and hard metal production	People exposed (314; numbers not reported for subgroups)		–
		Diamond abrasive production		
		Mold-filling	**587** (39–2,100)	
		Sintering	**193** (102–390)	
		Grinding	**151** (34–520)	
		Mechanical-working	**67.2** (14–165)	
		Hard-metals exposures		
		Grinding	**31.5** (0.8–730)	
		Tool production	**19.4** (0.8–100)	
		Hard metal alloy filling	**4.8** (0.8–18)	
		Other	2.85 (0.8–72)	
Sabbioni et al. (1994a) *Italy*	Hard metal workers (251) in four locations; three were hard metal mfg/tool production; Pavia produced diamond wheels			*Blood*
		Bergamo (88)	**303.6** ± 837.5	45.6 ± 66.9
		Milan (24 urine, 20 blood)	**13.9** ± 9.7	5.06 ± 4.37
		Pavia- powder mixing (23)	**61.1** ± 58.2	NR
		Turin (28)	**32.5** ± 35.7	NR
Suardi et al. (1994) *Italy*	Diamond abrasive production and grinding activities workers (159)		µg/g creatinine[f]	–
		Producer (6)	**50.17** ± 24.30	
		Grinder (87)	**10.89** ± 15.23	
		Hard-metal form grinder (9)	**7.67** ± 5.94	
		Others (76)	4.55 ± 7.31	
		Total (178)	9.17 ± 14.79	
Nemery et al. (1992) *Belgium*	Diamond polishers using cobalt-containing disks workers in 10 workshops (194); 5 with low Co exposure and 5 with high exposure	Exposure category		–
		Low (73)	**9.0** ± 7.2	
		High (86)	**25.2** ± 23.8	

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]
Burr and Sinks (1988) *Michigan, United States*	Cemented carbides and bonded diamonds Site visit; 149 urine samples from 24 tool manufacturing workers (up to 7 specimens/person per day)	Monday Pre-shift (19) Post-shift (20) Tuesday Pre-shift (21) Post-shift (23) Wednesday Pre-shift (20) Post-shift (23) Thursday Pre-shift (21) Post-shift (0)	Creatinine adjusted **10.8** ± 7.0 **19.0** ± 14.8 **18.3** ± 20.3 **25.1** ± 21.4 **15.0** ± 15.4 **27.0** ± 21.9 **22.1** ± 26.2 ND	–
NIOSH (1987) *South Carolina, United States*	Cemented carbides and bonded diamonds Site visit for grinding of tungsten carbide tools (post-sintering process)	 Pre-shift (10) Post-shift (10) Change from pre-to post-shift (10)	µg/g creatinine[e] [**10.5**] (4.7–19.0) [**18.1**] (8.4–27.7) [**7.6**] (1.4–15.1)	–
Kusaka (1996); Kusaka et al. (1986) *Asia*	Hard metal asthma patients (8)	(4)	(1–29)	*Blood* (2.8–4.2)
Posma and Dijstelberger (1985) *The Netherlands*	Hard metal production workers	6 subgroups (27) Sawing (3) Pressing/mixing (4) Grinding (10) Sintering (3)	µg/g creatinine[e] **64.3** (45–102) **45.1** (31–56) **25.5** (5.8–39) **6.4** (2.5–11.1)	Blood 18.3 (9.6–32) 11.5 (10.4–12.9) 8.6 (4.0–14.6) 2.0 (<0.3–4.4)
Pellet et al. (1984) (reported in IARC 1991) *Not specified*	Hard metal production workers	Co powder production (6) Pre-sintered WC (15) Hard metal use (7)	**35.1** **9.6** **11.7**	–
Chemicals and pigments: Pottery or plate painting or cloisonné				

Reference Location	Study Population	Exposure Group (# People or Samples)	Urine Cobalt (µg/L)[a]	Serum Plasma, or Blood Cobalt (µg/L)[a]	
Christensen and Poulsen (1994); Raffn et al. (1988); Christensen and Mikkelsen (1986) *Denmark*	Adult female pottery painters at two factories	10 yr surveillance date 1982 (46) 1984 (49) 1989 (145) 1991 (107) Type of Co exposure soluble cobalt (46) slightly soluble cobalt (15) Exposure timing/1982 (46) After 6 wk vacation 4 wk after returning to work	µg/g creatinine[e,f] [**69.5**] [**21.7**] [**12.7**] [**9.8**] [**4.8** (<0.1–26.2)] [**77.0** (2.2–848.0)]	*Blood*[g] 2.16 ± 3.72 0.63 ± 0.27 [0.5 ± 0.3] [2.2 ± 3.7]	
Arai et al. (1994) *Japan*	Cloisonne workers	Glaze workers (49)	**1.75** ± 2.81	*Blood* 1.5 ± 0.9	
Other or unspecified					
Afridi et al. (2009) *Pakistan*	Steel mill workers	Non-exposed (75) Quality control (35) Production (56)	**1.5** ± 0.4 **2.3** ± 0.4 **3.6** ± 0.6	1.2 ± 0.3 2.4 ± 0.6 3.9 ± 0.8	
Scansetti et al. (1998) *Italy*	Not specified	Monday (6) Thursday (6)	**13.2** ± 9.8 **30.9** ± 21.9		–
Chadwick et al. (1997) *United Kingdom*	Thermal spraying workers (34) at 6 worksites in the industrial processes	Grit blasting (5) Plasma spraying (89) Detonation gun spraying (27)	µg/g creatinine[d,e,f] **6.6** (0.16–11.0) **0.5** (0.16–139) **8.9** (0.73–34.1)		–
Meecham and Humphrey (1991) *Not reported; authors from UK*	Unspecified occupational exposure to Co	(1)	NA	234	

Urine values are plotted in Figure 2-1; values plotted for the general public or unexposed controls are shown in ***bold italic*** while those for other exposures are in **bold**).
Co = cobalt; NF = no studies found, NR = not reported in IARC or primary reference; PO = post-operation; WC = tungsten carbide.
[a]Mean ± standard deviation or range () unless stated otherwise. Studies are arranged within groups by most recent to oldest by publication date.
[b]Geometric mean.
[c]Reported urinary cobalt concentration is the geometric mean for the most recent (2011-2012) National Health and Nutrition Examination (NHANES) survey year for which data are available. Urinary cobalt data ranged from 0.316 to 0.379 µg/L for 1999 to 2012 (CDC 2015).
[d]Median.

[e]It is generally accepted that 1 L of urine contains 1 g creatinine.
[f]Value reported by authors as µg/mmol creatinine or µmol/mmol creatinine; converted to µg/g creatinine using the following conversion factor: 1 mol creatinine = 113.1 g creatinine; nmol/L blood converted to µg/L using the MW of cobalt = 58.9.
[g]Values were reported in units of µg/mg creatinine; however, µg/mg creatinine appears to be a typographical error. All other HHEs reported urine values in either µg/L or µg/g creatinine.
[h]Not graphed because units not reported by authors.
[i]Values reported are weighted means across the 8 or 12 job titles.

Table B-3. Values for Hair and Nail Cobalt Levels in the United States and Other Countries[a]

Reference Location	Study Population	Exposure Group, Number of People or Samples (N)	Hair Concentration, µg/g[b]	Nails Concentration, µg/g[b]
General Population				
Carneiro et al. (2011) Brazil	Healthy male and female urban students 12–18 years of age from 9 public schools in Porto Alegre	(126)	**0.008** ± 0.007	0.08 ± 0.1
Dongarrà et al. (2011) Sicily	Students 11–13 years of age	Overall (136) Males (38) Females (98)	0.19 ± 0.33 **0.26** ± 0.51 **0.16** ± 0.22	–
Elenge et al. (2011) Province of Katanga, Congo	General, non-industrialized population (medical students with no occupational history of exposure to metals) in the copper-belt.	109	Mean (5 to 95 percentile) **1.67** (0.8–2.02)	–
González-Muñoz et al. (2010) Spain	Normotensive and hypertensive postmenopausal women	 Normotensive (12) Hypertensive (14)	Median **0.02** (0.01–0.03) **0.03** (0.02–0.3)	–
Bergomi et al. (2002) Emilia-Romagna region, No. Italy	Randomly sampled controls for an ALS study enrolled in the Italian National Health Service	Controls (40)	–	Median (25–75 percentile) 0.018 (0.009–0.041)
Campbell et al. (1988) UK	Healthy controls (hospital staff, volunteers) on no medication	Controls (160)	µg/mL **0.07** ± 0.02	–

Reference Location	Study Population	Exposure Group, Number of People or Samples (N)	Hair Concentration, µg/g[b]	Nails Concentration, µg/g[b]
Kanabrocki et al. (1979) Unknown—U.S. institutions	General population - details NR	Females (9) Males (11)	–	0.07 (0.02–0.15) 0.04 (0.01–0.15)
Environmental				
Bibi et al. (2016) Lahore district, Punjab province, Pakistan	Persons of various ages from 3 sites near industrial areas used for agricultural purposes ranked as high, medium, and low exposure and controls living far from arsenic-contaminated regions	Risk area Low risk (12) Medium risk (12) High risk (12) Control (12)	–	 0.59 ± 0.02[c] 0.53 ± 0.04 0.32 ± 0.07 0.22 ± 0.07 p = 0.00
Occupational				
Afridi et al. (2009) Pakistan	Steel mill workers and non-exposed males 25–55 years of age	Production (56) Quality control (35) Control (75)	**4.67** ± 0.8 **2.48** ± 0.5 **1.1** ± 0.2	–
Sabbioni et al. (1994a) Bergamo, Milan, Pavia, and Turin, Italy	Hard metal workers (male and female) from four plants; three were hard metal manufacturing/tool production; hair and nail data not available from the diamond workers	Bergamo; hair (90); toenails (92) Milan; hair (22); toenails (23) Turin; hair (28); toenails (NR)	**49.09** ± 114.2 **9.6** ± 10.7 **13.4** ± 25.3	53.8 ± 107.2 18.9 ± 27.3
Bencko et al. (1986) Czech Republic	Nickel (Ni) and Co production workers, and age-matched healthy workers unexposed to Co	Exposed to Co (30) Exposed to Ni (33) Unexposed (27)	**96.8** ± 59.7 **3.3** ± 2.0 **0.38** ± 0.27	–
Hip Implants				
Rodriguez de la Flor et al. (2013) Spain	Patients with metal-on-metal resurfacing arthroplasty before and after revision surgery	Before revision surgery (i.e., unstable) (11) After revision surgery (11)	**147.4** ± 233.3 47.11 ± 74.20 p = 0.249	–

Reference Location	Study Population	Exposure Group, Number of People or Samples (N)	Hair Concentration, µg/g[b]	Nails Concentration, µg/g[b]
Liu et al. (2011) China	Patients with metal-on-metal hip resurfacing arthroplasty Compared with patients with metal-on-polyethylene hip arthroplasty	Metal-on-metal (22) Preop 6 mos post-op 12 mos post-op	 **4.4** ± 2.13 **53.3** ± 11.84 **47.4** ± 10.0 p = 0.0000	–
		Polyethylene bearings (22) Preop 6 mo post-op 12 mo post-op	 **3.2** ± 2.42 **3.4** ± 1.69 **4.2** ± 2.46 p = 0.3371	–

Hair values are plotted in Figure 2-2; values plotted for the general public or unexposed controls are shown in ***bold italic*** while those for other exposures are in **bold**.
NR = not reported.
[a]Reported values are means ± standard deviations or ranges () except where noted (e.g., medians or percentiles). Studies are arranged from most recent to oldest by publication date.
[b]Other units noted in table.
[c]No units were reported by the authors in this paper, but Bibi et al. (2016) reported results for arsenic for the same population as µg/g hair and nails.

B.3. Clinical Surveys and Studies

Several publications were identified that measured trace metals (such as heavy metals and essential metals) in tissue (such as tumors of different stages or normal tissue) or surrogates (e.g., hair, nails, blood) from cancer patients with a referent group (e.g., healthy humans, other diseases) or referent tissue (e.g., non-tumor from the same or different subjects). Because this information may inform several other sections (such as exposure, disposition, and toxicokinetics), these studies are discussed in below and are cross-referenced in the other sections.

For most studies, the source of the exposure is unknown with the exception of the study (reported in a series of publications) of copper smelter workers exposed to cobalt and other metals (Gerhardsson et al. 1985; Gerhardsson and Nordberg 1993; Gerhardsson et al. 1984). The studies varied in design and reporting quality. The source (i.e., underlying population) and methods for selecting the "cases" and "controls" were unclear. Three studies were hospital-based case-control studies with defined populations (Benderli Cihan et al. 2011; Kuo et al. 2006; Zhu et al. 2011), one of which included patients with other lung diseases as the referent group (Kuo et al. 2006); however, none calculated a risk estimate for exposure to cobalt and cancer. Most studies were conducted in Asia or in countries in the Middle East; few studies were conducted in Europe.

Findings from the studies are briefly discussed below: Section B.3.1 discusses the studies of patients with cancers of the lung and larynx, which have been identified as cancer sites of interest, and Section B.3.2 discusses studies of patients with cancer or tumors at other tissue sites (breast, brain, colon, leukemia, and thyroid).

B.3.1. Studies of Lung or Laryngeal Cancer Patients

Table B-4 describes the findings from five studies that measured cobalt in lung tissues and two studies that measured cobalt in non-target (e.g., surrogate) tissues of lung cancer patients (living or deceased) and referents (healthy controls or living or deceased patients with lung disease or other cancers). In the only study of workers likely to be highly exposed to metals, Gerhardsson et al. (1993; 1985; 1984) reported cobalt levels in lung tissue from deceased copper smelter workers. Cobalt levels were higher (although not significantly so) in lung tissue from workers who died of lung cancer compared to rural referents who died of other causes (primarily cardiovascular disease). However, cobalt levels were also significantly higher among all workers who died of other cancers compared to the referents, and similar relationships were reported between workers exposed to other metals and referents. Thus, this study can only provide evidence to support exposure to cobalt and not whether exposure to cobalt was associated with lung cancer.

Of the two clinic or hospital-based studies that measured cobalt in lung tissues from cases with lung cancer and referents, lung-tissue cobalt levels were similar between the two groups in the study using referents who died of other cancers (Adachi et al. 1991) but were significantly lower in the study using living patients with lung disease as the referents (De Palma et al. 2008). Cobalt levels did not differ significantly between tumor and non-tumor tissues from the same patients in two studies (De Palma et al. 2008; Zhang et al. 2012a) or by stage of lung cancer (I/II vs. III) in a

study by Kuo et al. (2006). Due to the choice of diseased referents in all of these studies, each had limited sensitivity to detect effects of cobalt on lung cancer.

The two lung cancer studies measuring cobalt in surrogate tissues of cases and non-diseased referents, a hospital-based case-control study in Turkey (Benderli Cihan et al. 2011) and a case-referent study in Pakistan (Qayyum and Shah 2014), had more defined methods for participant selection. Both studies found significantly higher levels of cobalt in hair and/or nails among cases compared to matched controls (Benderli Cihan et al. 2011) or volunteer referents (Qayyum and Shah 2014). Benderli Cihan et al. (2011) reported that cobalt levels in both nails and hair decreased with increasing cancer stage.

There were two small studies of laryngeal cancer, a Polish study investigating cobalt in normal and laryngeal tissue in cases (Klatka et al. 2011), and an Italian study measuring cobalt in tissue and plasma in cases and plasma in "normal males" (Collecchi et al. 1986). Both studies found higher cobalt levels in the laryngeal tumor tissue than the non-tumor tissues in the same patient. In addition, Klatka et al. (2011) reported higher cobalt levels in stage 4 tumors compared to stage 3 tumors. The findings by stage and by tissue type suggest that the carcinogenesis process may alter metal balances. Levels were significantly higher in laryngeal tissues among Polish patients from rural regions compared to those from urban areas suggesting the possibility of a role for environmental exposure to cobalt (Klatka et al. 2011). The Italian study found significantly higher levels of cobalt in the plasma from laryngeal cancer patients compared to the non-diseased referent group; however, selection of the cases and healthy subjects was not defined. No association between laryngeal cancer and cobalt concentration in toenails was found in a population-based case-control study of aerodigestive cancers from Washington state, United States (see Human Cancer Studies, Section 4).

B.3.2. Other Cancers

Nine clinical studies were identified that measured cobalt level in target tissues (N = 3) (e.g., same organ as cancer) or surrogate tissue (N = 6) (e.g., serum, urine, and nails) of cancer patients and referents (see Table B-5). In addition to these studies, the occupational study of copper smelter workers discussed above for lung cancer (Gerhardsson et al. 1993; Gerhardsson et al. 1985; Gerhardsson et al. 1984), measured cobalt in liver and kidney tissues. In contrast to the findings for lung tissues, cobalt concentrations in liver and kidney tissue were similar among deceased workers as the rural referents (Gerhardsson et al. 1984).

Two clinical studies measured cobalt in target tissues in tumor and non-tumor tissues; compared to non-tumor tissue, one small study (4 individuals) found levels higher in the tumor tissue (thyroid, Reddy et al. 2002)and the other study found lower levels in the tumor tissue (colon polyps, Alimonti et al. 2008). In the latter study, cobalt levels were similar in tissues from controls as the non-tumor tissue from the lung cancer patients. In a study using breast biopsies (Kanias et al. 1994), cobalt levels were two-fold higher (although not statistically significant) in individuals with fibroadenoma than with fibrocystic disease.

Three of the six studies that measured cobalt in surrogate tissue (hair, urine, serum) found statistically higher levels in cancer patients than "healthy" or "normal" subjects; two studies measuring hair in either all cancer patients (Pasha et al. 2007) or stage III breast cancer (Benderli Cihan et al. 2011) and one study measuring serum in liver cancer cases (Yin 1990). Two studies of leukemia found non-significantly higher levels of cobalt compared to healthy subjects, one

measuring cobalt in serum in acute leukemia patients (Demir et al. 2011) and the other measuring cobalt in urine of childhood leukemia patients (Zhu et al. 2011). In the sixth study, cobalt concentrations were similar from brain cancer patients and "healthy humans" (Arslan et al. 2011).

B.3.3. Synthesis

Overall, several studies found statistically significantly higher levels of cobalt in surrogate tissues (hair, nails, urine, or serum) from patients with several different types of cancer including all cancers (Pasha et al. 2007), cancer of the lung (Benderli Cihan et al. 2011; Qayyum and Shah 2014), larynx (Collecchi et al. 1986), liver (Yin 1990), or breast (Benderli Cihan et al. 2011) compared to healthy controls. However, except for lung cancer, there was only one study per specific cancer site. Findings were less consistent in studies measuring cobalt levels in target tissues, as the referent groups included people with or who had died from other cancers or diseases rather than healthy controls, which complicates their interpretation. In other studies of lung or breast cancer, there were no significant differences in cobalt levels between the cancer patient and referent group (lung cancer, Adachi et al. 1991; De Palma et al. 2008); breast cancer (Kanias et al. 1994) or levels were higher in the referent group (lung disease) compared to lung cancer patients (Kuo et al. 2006). In a series of studies (Gerhardsson et al. 1993; Gerhardsson et al. 1985; Gerhardsson et al. 1984), cobalt levels were higher in lung tissues (but not liver or kidney) from cancer cases from deceased cobalt-exposed workers compared to the same type of tissue from the rural referent group who died from other causes.

Studies comparing cobalt levels in tumor and non-tumor tissue (from the same or different subjects) or by cancer stage were conflicting and were limited by only one or two studies available for each type of cancer. Higher levels of cobalt were found in tumors of the larynx (Collecchi et al. 1986; Klatka et al. 2011) and thyroid (Reddy et al. (2002) than non-tumor tissue; however, lower levels of cobalt were found in colon polyps (significant Alimonti et al. 2008) or lung tumors (Zhang et al. 2012b) although not significantly so) than the corresponding normal tissue. For cancer stage, higher levels of cobalt were found in tissues in more advanced cancers for laryngeal cancer; while for lung cancer, cobalt levels were similar across stage when measured in lung tissue but decreased with increasing cancer stage when measured in nails and hair.

None of the studies were able to distinguish whether metal levels could be a cause of cancer or whether the cancer process itself affects metal balances, although the focus of some studies was on this latter concern. There are several limitations of these studies that make interpretation of results difficult. Co-exposures with cobalt are present, and cobalt concentrations are correlated with other metals in the positive studies; most studies include very few subjects; and there is inadequate information on how cases and referents were selected. In general, more information was provided on cases than referents, although whether certain cases were selected by convenience, or according to a systematic protocol was not clear.

Table B-4. Findings from Studies That Measured Cobalt in Tissues (Means or Medians) of Lung and Larynx Cancer Patients and Referents

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
Adachi et al. (1991)	Japanese clinical survey Autopsies from deaths (men and women) due to lung cancer and other cancers from the same medical center	Lung cancer Lung tissue	224 1,715	Lung cancer Other cases	0.33 ± 1.49 0.27 ± 0.41	Dried and digested; atomic absorption spectrophotometer (AAS)	NS
Benderli Cihan and Öztürk Yildirim (2011)	Turkish hospital-based case-control study Male non-small cell lung cancer (NSCLC; stage IIIB) and controls from the same geographical region using similar inclusion/criteria; similar age and ethnic background; all smokers	Lung cancer Hair	67 74	NSCLC Controls	[0.0031 ± 0.011]* [0.0004 ± 0.0005]	3 g; inductively coupled plasma mass spectrometry (ICP-MS)	$p < 0.05$
De Palma et al. (2008)	Italian clinical study NSCLC and controls (men and women) undergoing pulmonary resection (lung metastasis from other cancers and lung disease) from the same hospital; smoking higher in cases	Lung cancer Lung biopsies	45 45 8	NSCLC (non-tumor tissue) NSCLC (tumor) Controls	0.07 (0.05–0.11) 0.05 (0.01–0.10) 0.04 (0.02–0.18)	Dried and digested; ICP-MS; standard	NS No differences in cobalt levels in non-tumor tissue in occupationally exposed (to metals) vs. non-exposed subjects and in smokers vs. non-smokers
Gerhardsson et al. (1985); Gerhardsson and Nordberg (1993);	Swedish male smelter workers and rural and urban referents (deaths)	Lung and other cancers Lung tissue	7 24	workers/lung Cancer Workers/all cancer	[0.015] [0.016]**	Freeze dried; irradiated; neutron activation analysis (NAA)	Mean exposure duration 31.2 ± 8.4 yr Metal concentrations did not differ in

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
Gerhardsson et al. (1984)			29	Workers/ cardiovascular	[0.016]***		smokers vs. non-smokers
			12	Workers/other causes	[0.016]*		Authors state cobalt levels did not decline with time after exposure had ceased
			65	All workers	[0.015]***		
			14	Rural referents	[0.007]		
Kuo et al. (2006)	Taiwanese hospital-based case-control study; 1994–1998	Lung cancer Lung tissue	57	Lung cancer cases	0.18 ± 0.03*	Dried and digested; AAS; standard references	Cases were older and smoked more than controls
			40	Controls (lung disease)	0.25 ± 0.06		Cobalt levels were similar in non-smokers and smokers
	Cases (82% men) had primary lung cancer presenting at a veteran's hospital. Controls (81% men) had lung disease presenting at the veteran's hospital and 2 other teaching hospitals.		25	Adenocarcinoma	0.11 ± 0.01		
			35	Squamous-cell carcinoma	0.23 ± 0.04		
			39	Stage I/II	0.20 ± 0.04		
			21	Stage I/III	0.15 ± 0.02		
Qayyum and Shah (2014)	Pakistan case-referent study: lung patients and controls	Lung cancer Scalp hair	56	Cases	10.77 ± 1.599*	Hair (3g); nails (1g) dried; flame atomic absorption spectrophotometry (FAAS); 3 subsamples/ sample; standard references	Cases were more likely to be male and smoked more than controls
			54	controls	6.787 ± 0.873		
	Newly diagnosed patients from medical center and matched volunteer controls from same localities	Lung cancer Nails	56	Cases	51.36 ± 10.47*		Cobalt levels and variables- stage (nails & hair): decreasing 1 to 3
			54	Controls	45.38 ± 7.491		Inconsistent patterns between nails and hair for other variables such as sex, location, smoking
Zhang et al. (2012b)	Chinese case-series (clinical)	Lung cancer	30	Malignant tumor/lung cancer	[0.00012 ± 0.00005]	Dried, powder and digested;	Number of subjects and whether the

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
	Malignant and normal tissue from lung cancer patients in regions with high lung cancer incidence	Lung tissue and tumor	30	Normal tissue/lung cancer	[0.00025 ± 0.00016]	ICP-MS; standard references, spiked entire process; blanks to test for contamination	tumor and non-tumor tissue is from same subject not clear p = 0.051
Collecchi et al. (1986)	Italian clinical study Males without known exposure to arsenic and cobalt with laryngeal carcinoma and "normal males"	Larynx cancer Larynx tissue	15	Malignant tissue/laryngeal cancer	0.069 ± 0.007**	Radioactive NAA; standard references	Population undefined; spiked samples
			15	Non-malignant tissue/laryngeal cancer	0.040 ± 0.007		
		Plasma	15	Laryngeal cancer	18.27 ± 2.10*** ng/mL		
			11	Controls	0.73 ± 0.10		
Klatka et al. (2011)	Polish clinical survey Male laryngeal cancer patients: tumor and normal tissue from the same patient	Larynx cancer Larynx tissue	43	Laryngeal carcinoma	0.031 ± 0.0375	Digested; plasma optical emission spectrometry (ICP-OES); separate tissue dried for calibration validated with reference material	–
			43	Non-tumor tissue	0.017 ± 0.013		
			29	Stage 3 tumor	0.025 ± 0.034*		
			14	Stage 4 tumor	0.044 ± 0.043		
			19	Rural regions	0.046 ± 0.050*		
			24	Urban regions	0.019 ± 0.017		

Table B-5. Findings from Studies That Measured Cobalt in Tissues (Means or Medians) of Cancer Patients and Referents

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
Pasha et al. (2007)	Pakistan clinical survey; 2001 to 2003 Men and women cancer patients from two hospitals (15 to 93 yr) and normal donors from same region	All cancers Hair	111	Cancer patients	24.6 ± 1.5*	Flame atomic absorption spectrophotometry (FAAS); 3 samples/person; reference	Normal donors matched for age group. Types of cancer not reported Cobalt levels correlated with other metals such as cadmium and chromium
			113	Normal donors	6.10 ± 0.36		
Gerhardsson et al. (1993); Gerhardsson et al. (1985); Gerhardsson et al. (1984)	Swedish retried copper smelter workers and 8 rural referents Tissue from deceased subjects who died of cancer and other causes	All cancers Liver	10	Workers/cancer	[0.012]	Freeze dried; irradiated; neutron activation analysis (NAA)	47 workers were retired for 0 to 10 years and 18 workers were retired for 11 to 23 years; mean retirement = 7.2 ± 5.9 yr Mean exposure duration 31.2 ± 8.4 yr Metal concentrations did not differ in smokers vs. non-smokers
			8	Workers/ cardiovascular	[0.011]		
			2	Workers/other causes	[0.015]		
			20	All workers	[0.011]		
			8	Rural referents	[0.016]		
		All cancers Kidney	10	Workers/cancer	[0.003]		
			8	Workers/ cardiovascular	[0.003]		
			3	Workers/other causes	[0.006]		
			21	All workers	[0.003]		
			8	Rural referents	[0.001]		
Kanias et al. (1994)	Greek clinical survey Women (23) undergoing biopsy because of	Breast breast tissue	17	Fibrocystic disease	0.051 ± 0.045	Samples and standards irradiated;	Differences in cobalt levels between disease
			6	Fibroadenoma	0.10 ± 0.17		

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
	mammography or clinical findings with fibrocystic disease or fibroadenoma tumor		NR	Fibroadenoma & fibrocystic disease (same sample)	0.027 ± 0.025	radioactive count Corresponding to standards	groups not Significant Correlation of cobalt with scandium in fibroadenoma and with zinc in combined fibroadenoma & fibrocystic disease
Benderli Cihan et al. (2011)	Turkish clinical study Breast cancer from one hospital and volunteers or employees at the hospital (same age)	Breast (stage III) Hair	52	Cancer patients	0.664 ± 0.566*	3 g; ICP-MS	Cobalt was correlated with several other heavy metals in cancer patients
			52	Healthy humans	0.269 ± 0.390		
Arslan et al. (2011)	Turkey clinical survey Patient with malignant glial tumors operated from one clinical center and healthy humans.	Brain Serum	22	Cancer patients	0.04 ± 0.03 (µg/dL)	Frozen; atomic absorption spectrophotometer (AAS)	No information on healthy humans NS
			22	Healthy humans	0.03 ± 0.03 (µg/dL)		
Alimonti et al. (2008)	Italian clinical survey Male and female patients with colorectal polyps and control group from same hospital	Colorectal polyps Colorectal tissue	17	Tumor/polyps	[0.019 ± 0.016]*	Dried; digested samples, mass spectrometry; internal standards	No information about control group Sign differences between normal vs. polyps sign but not controls vs. normal or polyps
			17	Normal tissue/polyps	[0.04 ± 0.02]		
			15	Normal tissue/controls	[0.03 ± 0.016]		
			42	Leukemia cases	0.20 ± 0.17 (µg/dL)	Frozen; AAS	

Reference	Population	Cancer Tissue	Number of Subjects	Category	Co Levels (µg/g Dry Tissue)	Exposure Methods	Comments
Demir et al. (2011)	Turkey case-referent study Male and female newly diagnosed cases of acute leukemia from one clinical center and healthy subjects with similar distribution of sex, socioeconomics, and food habits	Acute leukemia (AML/ALL) Serum	40	Controls	0.11 ± 0.06		No information on source of controls. Not statistically Significant
Zhu et al. (2011)	Chinese case-control; 2007–2008 Newly diagnosed male and female cases (71) of childhood leukemia (15 yr or less) at a hospital and sex- and age-matched controls	Childhood leukemia Urine	71	Childhood leukemia cases	0.98 (0.57–2.28) ng/mg creatinine	Inductively coupled plasma mass spectrometry (ICP-MS)	NS
			113	Controls	0.77 (0.44–1.44) ng/mg		
Yin (1990)	Chinese clinical survey Male and female liver cancer cases (930) and age-matched healthy adults selected from the same hospital	Liver cancer Serum	30	Liver cancer cases	0.0085 ± 0.0017 ppm*	ICP-AES	Cobalt levels correlated with other metals
			30	Healthy adults	0.0035 ± 0.0012 ppm		
Reddy et al. (2002)	Indian thyroid samples Normal thyroid, adenoma, carcinoma samples from four subjects from pathology dept.	Thyroid cancer Thyroid tissue	NR	Carcinoma	17.9 ± 2	Freeze-dried converted to powder with standards, particle induced X-ray emission technique (PIXE)	No information on subjects. Not clear if different types of tissues are from same person
			NR	Adenoma	11.6 ± 1.2		
			NR	Normal thyroid	11.3 ± 1.2		

B.4. Regulations and Guidelines

B.4.1. Regulations

B.4.1.1. Coast Guard, Department of Homeland Security

Minimum requirements have been established for safe transport of cobalt naphthenate in solvent naphtha on ships and barges.

B.4.1.2. Department of Transportation (DOT)

Numerous cobalt compounds are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

B.4.1.3. Environmental Protection Agency (EPA)

B.4.1.3.1. Clean Air Act

National Emission Standards for Hazardous Air Pollutants: Cobalt compounds are listed as hazardous air pollutants.

B.4.1.3.2. Clean Water Act

Cobalt discharge limits are imposed for numerous processes during the production of cobalt at secondary cobalt facilities processing tungsten carbide scrap raw materials.

Discharge limits for cobalt are imposed for numerous processes during the production of cobalt at primary cobalt facilities; for numerous processes during the production of batteries; and for numerous processes during the production of cobalt salts.

Discharge limits for cobalt are imposed for wastewater discharges from centralized waste treatment facilities except discharges and activities exempted in 40 CFR 437.1(b), (c), and 40 CFR 421, Subpart AC.

Cobaltous bromide, formate, and sulfamate are designated as hazardous substances.

B.4.1.3.3. Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 1,000 lb. for cobaltous bromide, formate, and sulfamate.

B.4.1.3.4. Emergency Planning and Community Right-to-Know Act

Toxics Release Inventory: Cobalt and cobalt compounds are listed substances subject to reporting requirements.

Reportable quantity (RQ) = 100 lb. for cobalt, ((2,2′-(1,2-ethanediylbis (nitrilomethylidyne)) bis(6-fluorophenolato))(2-)-N,N′,O,O′)- (also called fluomine); = 10 lb. for cobalt carbonyl.

Threshold planning quantity (TPQ) = 100 lb. for fluomine (solids in powder form with particle size <100 μm or solution or molten form); = 10,000 lb. for all other forms of fluomine; = 10 lb. for cobalt carbonyl (solids in powder form with particle size <100 μm or solution or molten form); = 10,000 lb. for all other forms of cobalt carbonyl.

B.4.1.3.5. Federal Insecticide, Fungicide, and Rodenticide Act

Boiled linseed oil (containing no more than 0.33% manganese naphthenate and no more than 0.33% cobalt naphthenate) is exempt from the requirement of a tolerance when used as a coating agent for *S*-ethyl hexahydro-1*H*-azepine-1-carbothioate. No more than 15% of the pesticide formulation may consist of boiled linseed oil, and this exemption is limited to use on rice before edible parts form.

B.4.1.4. Food and Drug Administration (FDA)

Cobaltous salts are prohibited from use in human food.

All drugs containing cobalt salts (except radioactive forms of cobalt and its salts and cobalamin and its derivatives) have been withdrawn from the market because they were found to be unsafe or not effective, and they may not be compounded.

Chromium-cobalt-aluminum oxide used as a color additive for linear polyethylene surgical sutures used in general surgery must comprise no more than 2% by weight of the suture material, not migrate to surrounding tissue, and conform to labeling requirements in 21 CFR 70.25.

Chromium cobalt-aluminum oxide may be used as a color additive in contact lenses in amounts not to exceed the minimum reasonably required to accomplish the intended coloring effect.

Ferric ammonium ferrocyanide and ferric ferrocyanide used to color externally applied drugs (including those for use in the area of the eye) must not contain more than 200 ppm cobalt (as Co) and conform to labeling requirements in 21 CFR 70.25.

21 CFR 369 contains recommended drug labeling statements for over-the-counter cobalt preparations containing ≥0.5 mg cobalt as a cobalt salt per dosage unit and which recommend administration rates of ≥0.5 mg per dose and ≥2 mg per 24-hour period.

An approved new drug application is required for marketing cobalt preparations intended for use by man.

21 CFR 872, 874, and 888 identify class designations (Class I, II, or III) of various cobalt-containing dental prosthetic device alloys, cobalt-chromium-alloy-based facial prosthetics, and cobalt-chromium-molybdenum orthopedic devices that determine the type of premarketing submission or application required for FDA clearance to market.

Cobalt naphthenate may be used in quantities that do not exceed those reasonably required as an accelerator in the production of cross-linked polyester resins used as articles or components of articles intended for repeated use in contact with food.

Cobalt aluminate may be safely used as a colorant in the manufacture of articles or components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding of food at levels not to exceed 5% by weight of all polymers except in resinous and polymeric coatings complying with 21 CFR 175.300, melamine-formaldehyde resins in molded articles complying with 21 CFR 177.1460, xylene-formaldehyde resins complying with 21 CFR 175.380, ethylene-vinyl acetate copolymers complying with 21 CFR 177.1350, and urea-formaldehyde resins in molded articles complying with 21 CFR 177.1900.

B.4.1.5. Occupational Safety and Health Administration (OSHA)

This legally enforceable PEL was adopted from the 1968 ACGIH TLV-TWA shortly after OSHA was established; it may not reflect the most recent scientific evidence and may not adequately protect worker health.

Permissible exposure limit (PEL) (8-h TWA) = 0.1 mg/m^3 for cobalt metal, dust, and fume (as Co).

B.4.2. Guidelines

B.4.2.1. American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value-time-weighted average (TLV-TWA) = 0.02 mg/m^3 for cobalt and inorganic compounds; = 0.1 mg/m^3 for cobalt carbonyl and cobalt hydrocarbonyl.

Biological exposure index (BEI) = 15 μg/L for cobalt in urine for cobalt and inorganic compounds, including cobalt oxides but not combined with tungsten carbide for end of shift at end of workweek.

B.4.2.2. Consumer Product Safety Commission (CPSC)

The CPSC has issued guidance regarding the potential hazards of specific cobalt- or cobalt-compound-containing art and craft materials (e.g., glazes, glass colorants, paints, toners, pigments, and dyes) and specific precautions to take when using them.

B.4.2.3. Environmental Protection Agency (EPA)

Regional Screening Levels (formerly Preliminary Remediation Goals): residential soil = 23 mg/kg; industrial soil = 350 mg/kg; residential air = 0.00031 $\mu g/m^3$; industrial air = 0.0014 $\mu g/m^3$; tap water = 6 μg/L.

B.4.2.4. National Institute for Occupational Safety and Health (NIOSH)

Recommended exposure limit (REL) (10-h TWA) = 0.05 mg/m^3 for cemented tungsten carbide containing >2% Co (as Co); = 0.05 mg/m^3 for cobalt metal dust and fume (as Co); = 0.1 mg/m^3 for cobalt carbonyl (as Co) and cobalt hydrocarbonyl (as Co).

Immediately dangerous to life and health (IDLH) limit = 20 mg/m^3 for cobalt metal dust and fume (as Co).

Appendix C. Human Cancer Study Tables

Table of Contents

Tables

This appendix contains background information related to the cancer assessment on cobalt and certain cobalt compounds in humans including detailed (1) data information on study design, methods, and findings for human cancer studies (Table C-1 through Table C-9) and (2) detailed information on the quality assessment of the individual studies (Table C-10, Table C-11, and Table C-12).

C.1. Methodologies and Study Characteristics

The data from several cohort studies, which include four nested case-control studies and two case-control studies on esophageal and head and neck cancers, were systematically extracted from relevant publications and are summarized in the tables below (Table C-1 through Table C-9). Some of the studies were conducted on overlapping populations. The cohort studies are organized by occupational group and listed in chronological order (earliest studies first) similar to Table 4-1.

Table C-1. Study Description and Methodologies of Cohort Studies: Tüchsen et al. (1996)

Field	Description
Reference	Tüchsen et al. (1996) Tüchsen F, Jensen MV, Villadsen E, Lynge E. Incidence of lung cancer among cobalt-exposed women. Scand J Work Environ Health. 1996 Dec;22(6):444–50. PubMed PMID: 9000312.
Study-design type	Cohort
Location and enrollment dates	Copenhagen, Denmark; Jan 1, 1943 (Factory 1) or Jan 1, 1962 (Factory 2) – Dec 31, 1992
Population description	Danish women porcelain plate workers.
Eligibility criteria	All women employed at any time in two underglaze porcelain plate departments (Factory 1 and Factory 2); and all female top glaze decorators in a department without cobalt exposure (Factory 1).
Cohort details	Population size: 1,394 total; 874 cobalt-exposed workers, 520 unexposed workers. Loss-to-follow-up: 13 (0.92%) Referent Group: External (SIR); also calculated SIR for unexposed workers.
Outcome data source	Followed for death and emigration using data in the Central Population Register and the municipal population registers. Cancer cases identified by linkage to Danish Cancer Register (ICD-7).
Exposure assessment	Company records
Exposure assessment notes	Exposure to cobalt-aluminate spinel and/or cobalt silicate at 2 factories. Detailed information on work history; exposure monitoring data was reported for air and urine from the 1980s which was not used in the exposure assessment; calendar period was adjusted for in analysis.
Exposure-level	Employment in factories/departments with or without cobalt
Co-exposures	Nickel, silica

Field	Description
Analysis methods and control for confounding	Analytical methods: Personnel files for permanently ill persons may have been removed in earlier years, potentially resulting in an underestimate of incidence. Covariates: Age Confounder consideration: Calculation of expected number of cancer cases took 5-year age groups and calendar periods in consideration. No HWE, no control for other variables; unclear if calendar period was controlled

Table C-2. Study Description and Methodologies of Cohort Studies: Mur et al. (1987)

Field	Description			
Reference	Mur et al. (1987) Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. Am J Ind Med. 1987;11(1):75–81. PubMed PMID: 3812499.			
Study-design type	Cohort and nested case control study			
Location and enrollment dates	France; 1950–1980			
Population description	**Male electrochemical workers** including cobalt production workers			
Eligibility criteria	N = 1,143. All men employed for at least one year at a cobalt production plant producing cobalt, cobalt salt and oxides, and sodium between 1950 and 1980; hired between 1900 and 1979.			
Cohort details	*Population size:* N = 1,143; number of cobalt production workers not reported but ~25% of current staff at publication *Loss-to-follow-up:* 17.9% for cobalt production workers; 75% hired before 1975. *Referent Group:* Internal and external comparing cohort mortality to male mortality in France			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	9	NR	All lung cancer cases from cohort
	Controls	18	NR	Two controls/case were matched on year of birth and age at death and "smoking habits" (undefined); controls were selected from among those dying of conditions other than cancer.
Outcome data source	Vital status ascertained by registry offices in the birth places of Frenchmen, and at embassies and consulates for foreign-born. Cause of death (ICD-8) ascertained by physicians and medical records. 80% of causes of death determined and classified.			
Exposure assessment	Company records			
Exposure assessment notes	Job histories grouped according to employment in general service, maintenance, sodium or cobalt production. Only included those with exclusive employment in any of these departments. No Co levels reported, nor were prior measurements available.			
Exposure-level	60% worked greater than 10 years; 75% hired before 1975			

Field	Description
Co-exposures	Arsenic, nickel
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* age, year of death SMR all-cause mortality = 0.77 ($p < 0.01$); no methods to control HWE; all-cause mortality for cobalt production = SMR 1.29 (0.86–1.87). Analytical method: matched case-control study *Covariates:* None *Confounder consideration:* Cases (deaths from lung cancer) were matched to controls (deaths from cause other than cancer) for year of birth, age at death, and smoking habits

Table C-3. Study Description and Methodologies of Cohort Studies: Moulin et al. (1993)

Field	Description
Reference	Moulin et al. (1993) Moulin JJ, Wild P, Mur JM, Fournier-Betz M, Mercier-Gallay M. A mortality study of cobalt production workers: an extension of the follow-up. Am J Ind Med. 1993 Feb;23(2):281–8. PubMed PMID: 8427256.
Study-design type	Cohort
Location and enrollment dates	France; Extended follow-up of the Mur et al. (1987) study through 1988
Population description	**Male electrochemical plant workers including cobalt production workers**
Eligibility criteria	All men employed for at least one year at a cobalt production plant producing cobalt, cobalt salt and oxides, and sodium between 1950 and 1988; hired between 1900 and 1979. Cohort I included all workers excluding person years of foreign-born workers over 75 years of age; Cohort II included only French-born workers.
Cohort details	*Population size:* Cohort 1 – N = 1148; Cohort II – N = 870; number of cobalt workers NR *Loss-to-follow-up:* Unknown cause of death 1% for all French born workers; Overall, no cause of death for 11.7% Cohort I; 9.7% in Cohort II; or 11% unknown cause of death overall. Loss to follow-up for cobalt production workers was not reported. *Referent Group:* External comparison with French male mortality rates
Outcome data source	Used death certificates from the French National Institute for Medical Research and Health files for deaths 1968–1988 for French born; cause of death prior to 1968 was ascertained from physicians and hospital records; for foreigners, cause of death ascertained from embassies and consulates.
Exposure assessment	Company records
Exposure assessment notes	Job histories grouped according to employment in general service, maintenance, sodium or cobalt production. Either "ever" or "only" employment in any of these departments. No Co levels reported, nor were prior measurements available.
Exposure-level	NR, but likely similar to Mur et al. (1987)
Co-exposures	Nickel, arsenic
Analysis methods and control for confounding	*Analytical methods:* Restriction to French-born reduced the power to detect effect, yet mitigated concerns about attrition bias. *Covariates:* age *Confounder consideration:* No reported control for period effects, duration, or and time since first exposure

Table C-4. Study Description and Methodologies of Nested Case Control Studies: Moulin et al. (1998)

Field	Description			
Reference	Moulin et al. (1998) Moulin JJ, Wild P, Romazini S, Lasfargues G, Peltier A, Bozec C, Deguerry P, Pellet F, Perdrix A. Lung cancer risk in hard-metal workers. Am J Epidemiol. 1998 Aug 1;148(3):241–8. PubMed PMID: 9690360.			
Study-design type	Nested Case-Control			
Location and enrollment dates	FRANCE; January 1, 1968–December 31, 1991.			
Population description	**Male and female French hard-metal workers**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	61	97%	All cohort workers who died of lung cancer
	Controls	180	98%	Three controls/case sampled from among those at risk - i.e., who were under FU and alive on the date the case died and had completed 3 mos of employment. Controls matched for gender and date of birth ±6 mos of the case.
Exposure assessment	JEM			
Exposure assessment notes	Semi-quantitative (JEM) exposure assessment based on administrative records and interviews with colleagues; 320 job periods assigned estimates of exposure to cobalt and tungsten carbide - Intensity score from 0 (no exposure) to 9 (highest exposure level); frequency score of <10%, 10%–50%, and >50% of work time. 744 historical atmospheric concentrations of cobalt were used to validate matrix scores, but no concentrations were included from Co powder production area.			
Exposure-level	NR			
Co-exposures	Employment in maintenance shop, PAHs, asbestos, silica, certain chromium compounds, certain nickel compounds, arsenic compounds, cadmium compounds, nitrosamines, benzene, tungsten carbide			
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* unclear which variables were controlled in the multivariate analysis for cobalt alone *Confounder consideration:* mentioned the full list of IARC carcinogens, but did not indicate if these were controlled in the cobalt alone analyses			

Table C-5. Study Description and Methodologies of Cohort Studies: Wild et al. (2000)

Field	Description
Reference	Wild et al. (2000) Wild P, Perdrix A, Romazini S, Moulin JJ, Pellet F. Lung cancer mortality in a site producing hard metals. Occup Environ Med. 2000 Aug;57(8):568–73. PubMed PMID: 10896965.
Study-design type	Cohort

Field	Description
Location and enrollment dates	France; January 1968–December 1992
Population description	Hard metal workers in the largest such factory in France (included in the Moulin et al. 1998 paper).
Eligibility criteria	Subjects who had worked at least 3 months between January 1, 1950 and June 30, 1992, and were still alive by January 1, 1968. 80% of cohort were hired prior to 1970. Mean follow-up 18.6 years
Cohort details	*Population size:* 2,216 men and 644 women *Loss-to-follow-up:* 20.2%; Foreign-born workers terminated before 1968 censored and considered lost to follow-up *Referent Group:* External analysis using "local death rates" as comparison.
Outcome data source	Vital status ascertained by registry offices of birthplaces and computer database of all deaths in France starting in 1978. Cause of death obtained by matching the file of dead subjects with the national file of causes of death from 1968, coded to ICD-8 of disease before 1978, and to ICD-9 for disease after 1978; 96% of causes could be retrieved.
Exposure assessment	JEM
Exposure assessment notes	Semi-quantitative (JEM) exposure assessment based on administrative records and interviews with colleagues; 320 job periods assigned estimates of exposure to cobalt and tungsten carbide - Intensity score from 0 (no exposure) to 9 (highest exposure level); frequency score of <10%, 10%–50%, and >50% of work time. Ever or only employment in the "powder production workshop" was also used as an indicator of potential exposure to cobalt.
Exposure-level	NR
Co-exposures	PAHs, certain chromium compounds, certain nickel compounds, silica, cobalt-tungsten carbide, asbestos, arsenic compounds, cadmium compounds, nitrosamines, benzene
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* Age, unclear if these are crude estimates *Confounder consideration:* conducted separate smoking analyses

Table C-6. Study Description and Methodologies of Cohort Studies: Moulin et al. (2000a)

Field	Description		
Reference	Moulin et al. (2000a) Moulin JJ, Clavel T, Roy D, Dananche B, Marquis N, Fevotte J, Fontana JM. 2000. Risk of lung cancer in workers producing stainless steel and metallic alloys. Int Arch Occup Environ Health 73(3): 171–180. PMID 10787132		
Study-design type	Nested Case-Control		
Location and enrollment dates	France; January 1, 1968–December 31, 1992		
Population description	**Male and female workers in a French factory producing stainless and alloyed steel.**		
	Population size	*Response rates*	*Source*

Field				
Case-control description and eligibility criteria	Cases	54 (17 Co-exposed)	NR	All workers who died from lung cancer determined thru death certificate and medical record matching process.
	Controls	162 (67 Co-exposed)	NR	3 controls / case sampled from those under follow-up at the date of death, had completed 1 year of employment, and known to be alive on this date, same gender, and DOB within 6 months of deceased case.
Exposure assessment	JEM			
Exposure assessment notes	Semi-quantitative JEM had 5 levels of exposure no exposure, occasional, and low, medium, and high exposure. Frequency was coded as 10% to 100% of working time; low, medium, and high probability of accuracy of intensity and frequency codes was included. Increasing exposure levels, duration of exposure, and cumulative dose (frequency weighted and unweighted)			
Exposure-level	NR			
Co-exposures	Iron, acid mists, PAHs, asbestos, silica, chromium and/or nickel			
Analysis methods and control for confounding	*Analytical methods:* Analyses were lagged. *Covariates:* PAHs, age, gender, silica, smoking ever/never *Confounder consideration:* Co correlated in a reported matrix with Chromium and/or Nickel, and Iron, but neither of these were included in the multivariate analysis			

Table C-7. Study Description and Methodologies of Cohort Studies: Grimsrud et al. (2005)

Field				
Reference	Grimsrud et al. (2005) Grimsrud TK, Berge SR, Haldorsen T, Andersen A. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel? Epidemiology. 2005 Mar;16(2):146–54. PubMed PMID: 15703528.			
Study-design type	Nested Case-Control			
Location and enrollment dates	Norway; 1910–1995			
Population description	**Norwegian nickel refinery workers**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	213	NR	lung cancers diagnosed from 1952–1995 and in the Cancer Registry of Norway during this time.

Field				Description
	Controls	525	NR	3 controls / case randomly drawn among cohort members at risk at the time of dx (incidence density sampling), free of lung CA, and born within 24 months of the case's DOB, and matched by gender. Controls drawn in a 1:1 ratio for cases diagnosed before 1970.
Exposure assessment	JEM			
Exposure assessment notes	A semi-quantitative JEM was developed for various species of nickel based on 5,900 personal measurements; this was supplemented with 3,500 personal samples from the breathing zone for cobalt.			
Exposure-level	In μg/m^3: High (144–3,100); Medium (29.7–142); Low (0.31–29.5)			
Co-exposures	Nickel, arsenic, asbestos, sulfuric acid mists			
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* smoking *Confounder consideration:* No multivariate estimates were possible due to collinearity with nickel.			

Table C-8. Study Description and Methodologies of Case-control Studies: Rogers et al. (1993)

Field				Description
Reference	Rogers et al. (1993) Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. A case-control study of element levels and cancer of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev. 1993 Jul-Aug;2(4):305–12. PubMed PMID: 8348053.			
Study-design type	Case-Control			
Location and enrollment dates	Western WA state, USA; 9/1/83–2/28/87			
Population description	**Population based randomly selected controls and cases from SEER**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	N = 507; N = 153 laryngeal, N = 73 esophageal, N = 359 oral cavity cancers	52.8% providing toenail samples	Laryngeal, esophageal, or oral cavity cancers of epithelial origin identified from local SEER registry with positive histological findings; some cases confirmed by cytology and followed with attending physician.
	Controls	N = 434	66.4% providing toenail samples	Controls from same area as cases selected by random digit dialing and frequency matched by sex and age in 5-year intervals of cases.

Field	Description
Exposure assessment	Personal monitoring
Exposure assessment notes	Dietary sources of trace elements of cobalt, iron, calcium, zinc, chromium explored; toenails collected and cleaned; personnel blinded to case status; formation of nail matrix takes 8–24 mos; median time from dx to interview was 6.5 mos, so samples likely to represent prediagnostic levels; qx data on occupation collected but not reported.
Exposure-level	Tertiles of cobalt in toenails; highest level 0.17 ppm
Co-exposures	Iron, calcium, zinc, chromium
Analysis methods and control for confounding	*Analytical methods:* "Exposed cases" in this table refers to both cases and controls combined; exposed cases alone NR. *Covariates:* age, alcohol (drink years), ascorbic acid mg/day, beta-carotene, mg/day, energy intake, kcal/day, sex, smoking (pack-years). Correlations between cobalt and other measured metals not provided; and models for cobalt did not include other metal levels. *Confounder consideration:* Nutrients in the model did not greatly confound the relationship between exposure and disease, but inclusion resulted in ORs closer to the null. ORs for Esophageal cancer significantly elevated for iron and calcium.

Table C-9. Study Description and Methodologies of Case-control Studies: O'Rorke et al. (2012)

Field				
Reference	O'Rorke et al. (2012) O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, FINBAR Study Group. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. Int J Cancer. 2012 Oct 15;131(8):1882–91. PubMed PMID: 22262413.			
Study-design type	Case-control study			
Location and enrollment dates	All Ireland (Republic and Northern); 3/2002–12/2004			
Population description	**Population based cases and controls**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	N = 137 esophageal cancer; N = 182 Barrett's esophagus	Esophageal CA = 38.6%; Barrett's esophagus = 66.9	No. Ireland: Esophageal cases (≤85 yrs) identified from electronic path records from all path labs. Rep of Ireland: cases identified from the main referral hospitals diagnosing and treating esophageal CA. Pathology review and histologically confirmation; excluding in situ CA. Barrett's esophagus - pts with ≥3 cm of Barrett's mucosa at endoscopy or biopsy showed specialized intestinal metaplasia. Pts with dysplasia on histology excluded.

Field	Description			
	Controls	221	35.5	Adults (35 to 84) without history of esophageal or other gastrointestinal cancer or known diagnosis of Barrett's esophagus; frequency matched by sex and age (5 yrs). Selected at random from general practitioner (GP) list (No. Ireland) and from 4 GP practices (2 rural and 2 urban) in Dublin and Cork areas that reflected the distribution of the Rep. Ireland cases
Exposure assessment	Personal monitoring			
Exposure assessment notes	Cobalt level in toenails; tertile cutpoints of log(e) transformed Co based on control distribution. Questionnaire for demographics, lifestyle habits, diet, manual/non-manual occupation, and medical history; anthropometric measurements; personnel blinded as to case status.			
Exposure-level	Average (μg/g) ± SD: cases – 0.02 ± 0.06; controls – 0.02 ± 0.04. Range: cases 0.002–0.60; controls – 0.002–0.47			
Co-exposures	Selenium, iron, chromium, zinc measured			
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* GI reflux, H. pylori infection, age, education, energy intake, location, sex, smoking, smoking habits *Confounder consideration:* Unadjusted model almost identical results to the age and sex adjusted model; other metals measured included selenium, chromium, zinc, mercury, cerium. No correlation with cobalt reported. Not in models.			

C.2. Assessment of Study Quality, Sensitivity, and Utility of Human Studies of Cobalt

This appendix provides (1) an assessment of the study quality, sensitivity, and utility of the human studies to inform the cancer hazard evaluation, and (2) study quality and utility summaries for cohort studies and for case-control studies. Each primary study was systematically evaluated for its utility to inform the cancer hazard identification using core, signaling and follow-up questions outlined in the protocol (NTP 2014b) for five domains of study quality (selection bias, methods to evaluate potential confounding, exposure misclassification, outcome misclassification, selective reporting, and quality of the analysis) and one domain for study sensitivity. Two reviewers evaluated study quality and utility and differences were resolved by reference to the original publication and discussion.

For each domain, the following terms were used to rate the potential for bias and/or quality:

- *Low/minimal concerns*: Information from study designs and methodologies indicate that they are close to the ideal study characteristics and that the potential for bias is unlikely or minimal, recognizing general limitations of observational studies. [+++ high quality]
- *Some concerns*: Study designs or methodologies are less than ideal, indicating possible bias. [++ medium quality]

- *Major concerns*: Study designs or methodologies suggest that the potential for a specific type of bias is likely albeit depending on the direction and distortion of the potential bias, the study may have some limited utility. [+ low quality]
- *Critical concern:* Distortion of bias would make study findings unreliable for cancer hazard identification. [0 rating]
- *No information*: The information in the study is inadequate to evaluate the level of concern for the domain.

In addition, when adequate information was available, an assessment was made whether a bias was likely to be differential (systematic) or non-differential and the predicted direction of the bias (towards or away from the null; over or underestimate of the effect estimate). The impact of the potential bias or confounding on the study findings is discussed in the cancer hazard assessment (see Sections 4.2.3, 4.3.3).

Based on the overall evaluation, studies were broadly grouped according to their ability to inform the cancer hazard evaluation based on the above characteristics, as follows:

- High (low/minimal concerns for most potential biases, high or moderate sensitivity rating)
- Moderate (low/minimal or some concerns for most potential biases, high or moderate sensitivity rating)
- Moderate/low (some to major concerns for several potential biases, sensitivity rating varies)
- Low (major concerns for several potential biases, sensitivity rating varies)
- Inadequate (critical concerns for any bias, sensitivity rating varies)

The overall study judgment is not meant to be an algorithm that sums up the ratings across domains. The quality of the exposure assessment and potential for exposure misclassification and potential confounding was given considerable weight in ranking the studies. In addition, studies with high probability of systematic (i.e., differential) biases were rated low.

Guidelines and characteristics of the ratings specific for each domain as well as the overall study utility are provided in the cobalt protocol. The assessment (rating and rationale for the rating) of the study quality and sensitivity domain for each study and the overall study evaluation are summarized in the following sections. The studies in each table are ordered by study design, with cohort and nested case-control studies first, followed by case-control studies, and then by publication date of the first study publication.

Selection bias and evaluation of methods used to address potential confounding (Table C-10)

Information bias: exposure and outcome misclassification (Table C-11)

Selective reporting and analysis bias (Table C-12)

Study sensitivity, quality and utility of cohort and nested case-control studies (Table 4-2)

Study sensitivity, quality, and utility of case-control studies (Table 4-5)

C.2.1. Selection Bias and Evaluation of Methods Used to Address Potential Confounding

C.2.1.1. Cohort and Nested Case-control Studies

In three of the four nested case-control studies in the lung cancer cohort studies, the potential for selection bias was thought to be low (Grimsrud et al. 2005; Moulin et al. 2000b; Moulin et al. 1998), as all studies appropriately selected and matched cases and controls on relevant variables. The fourth nested case-control study, i.e., the study of electrochemical workers by Mur et al. (1987), did not provide information on methods of selection and matching.

The loss to follow-up was large in the cohort studies of electrochemical and hard-metal workers (Moulin et al. 1993; Moulin et al. 1998; Mur et al. 1987; Wild et al. 2000), but there was no evidence presented to assess whether the loss was related to exposure. In these studies, the largest losses were due to the inability to find death records for foreign-born workers (15%–21%). Except for Mur et al. (1987), follow-up for these workers ended at the last date of employment (Moulin et al. 1998; Wild et al. 2000); or analyses were restricted to non-foreign-born workers (Moulin et al. 1993). Differential selection out of the porcelain workers cohort (Tüchsen et al. 1996) could have occurred as the authors mentioned that records of ill persons may have been removed potentially resulting in an underestimate of the true incidence of cancer.

Evidence of a healthy worker effect (HWE) based on external analyses showing statistically significant decreases in all-cause mortality rates was present in Moulin et al. (1998), Mur et al. (1987) and Moulin et al. (2000b) studies. Internal analyses, however, were conducted that have the effect of minimizing HWE, although no adjustment in any analysis was made for time since hire. In the electrochemical workers (Moulin et al. 1993; Mur et al. 1987) while HWE was evident in the full cohort, it was not apparent among cobalt only workers.

In the electrochemical workers cohort (Moulin et al. 1993) 46% of cohort members had been hired prior to the start of follow-up, with the likely effect of inducing a downward bias in the effect estimate. Such left censoring can result in a cohort with healthier prevalent hires who have remained working from earlier periods of exposure. The Moulin et al. (2000b) study indirectly addressed the healthy worker survival effect (HWSE) indicating that cases and controls in the nested study were matched on age and were reported to have similar distributions for the date of hire, suggesting there was little concern for HWSE.

C.2.1.2. Case-control Studies

Both of the population-based case-control studies of cobalt in toenails report sufficient information to evaluate whether selection of participants is related to exposure and disease. Selection bias is unlikely in the Rogers et al. (1993) study, which ascertained all cases of aerodigestive cancers (e.g., oral cavity, esophageal, and laryngeal cancer) from the Western Washington state SEER cancer registry and used random-digit dialing to identify controls in a defined area.

In the FINBAR study, there is some concern that selection bias may been operating in the selection of cases and controls from the Republic of Ireland in this study (O'Rorke et al. 2012). Cases were identified from the “main” hospitals involved in the diagnosis and treatment of esophageal cancer including the national referral center for esophageal cancer. In contrast, Republic of Ireland controls were selected at random from two urban and two rural general

practices, purportedly reflecting the urban/rural distribution of esophageal cancer cases in the Republic. However, smoking rates among the controls suggest that participating controls may not be fully representative of the case population or of those who did not submit toenail samples. Current smoking was higher in the cases, as expected, but the level of smoking in the controls was lower than that of the general population (16% of controls returning toenail samples, and 17.7% in all controls; 23.6% of males 55 years and over). Among those not returning toenail samples, the proportion of current smokers was higher (27%).

Participation rates were low in both studies, especially when combined with the reduced percentage of those returning toenail samples. In the Rogers et al. (1993) study, the proportion of all eligible cases who returned usable toenails was 52.8%, and for controls 66.4%. This proportion was 36% for esophageal, 63.5% for laryngeal, and 54.5% for oral cancers. However, the distribution of risk factors (alcohol consumption and tobacco smoking) was consistent with what is known about risk factors for aerodigestive cancers and argues against systematic selection bias. In the O'Rorke et al. (2012) study, the participation rate (including those who submitted toenails) was 38.6% in cases and 35.5% in controls.

C.2.2. Evaluation of Methods to Address Confounding

This section addresses whether the studies used appropriate methods to control confounding or provided relevant data to evaluate potential confounding. The final evaluation of whether confounding bias can explain the results of each study is discussed in the cancer assessment sections (Sections 4.2.2 and 4.3.2 and 4.4).

C.2.2.1. Cohort and Nested Case-control Studies

Most of the cohort and nested case-control studies conducted age-, sex-, and calendar year- or period-standardized comparisons in external analyses (SMR or SIR) and, in some cases, restricted internal analyses to men (when there were small numbers of women workers) in internal analyses. All studies provided information about or directly controlled for smoking; however, the quality of these data ranged widely. Some conducted sub-studies of smoking habits of some proportion of the workers (ranging from <30% to 70%) (Moulin et al. 1993; Mur et al. 1987; Tüchsen et al. 1996); others categorized workers as “ever-never” smokers (Moulin et al. 2000b), and others were able to incorporate detailed information on former and current smokers and their level of smoking (Grimsrud et al. 2005). While smoking is a strong risk factor for lung cancer, there was no evidence of smoking being strongly associated with cobalt exposure in any of the studies.

Regarding co-exposures, studies among the hard-metal workers (Moulin et al. 1998; Wild et al. 2000), stainless and alloyed steel workers (Moulin et al. 2000a) and nickel refinery workers (Grimsrud et al. 2005) assessed co-exposures to several known IARC carcinogens; however, in none was information on co-exposures either sufficiently reported, or controlled. Based on communications with the author (Dr. Wild) it is unlikely that co-exposures were controlled in the hard-metal studies. Furthermore, exposure to cobalt (not in the presence of tungsten carbide) ranged from exposure to pure cobalt in cobalt powder production workshops to mixed exposures, with potential exposure to lung carcinogens in the other production workshops. In the stainless and alloyed steel workers (Moulin et al. 2000a) no control was indicated for metals most closely correlated with cobalt. In the Grimsrud et al. (2005) study, data were available to evaluate the role of cobalt on lung cancer controlling for a number of other carcinogens. Their focus was to

understand the confounding effect of co-exposures with nickel (correlation, r = 0.63); however, they were not able to separate the effects of cobalt from nickel as all nickel workers were exposed to cobalt. The electrochemical worker study (Moulin et al. 1993; Mur et al. 1987) and the porcelain painters (Tüchsen et al. 1996) did not collect information on co-exposures, although an earlier paper on this cohort provided information about low levels of nickel (<10% of Danish occupational limit of 0.1 mg/m^3), silica (no detectable concentrations), and dust (average of 7.6 mg/m^3) measured in 1981 prior to changes in practices that reduced air levels of cobalt somewhat. The electrochemical workers cohort may have been exposed to arsenic, which is added during the production process, and nickel and arsenic are contained in cobalt ore, but no measurements of these were taken.

C.2.2.2. Case-control Studies

Methods used to control for potential confounding in the two biomarker studies are adequate overall. While neither author reported correlations among the multiple metals analyzed in these studies, Rogers et al. (1993) also investigated the risk of cancer for iron, zinc, chromium, and calcium and provided ORs for each in relation to each cancer; however, these ORs were not controlled for presence of the other elements. Similarly, O'Rorke et al. (2012) also measured included selenium, chromium, zinc, mercury, and cerium; however, no correlation with cobalt was indicated, nor were these other metals included in the cobalt models. Both studies frequency matched controls to cases within 5-year age bands and sex strata; and both collected information on a wide range of risk factors and potential confounders. Both studies reported the distributions of potential confounders among cases and controls and provided clear descriptions of their process for multivariate analysis. Neither study reported on cobalt levels according to the occupational or dietary data collected.

Table C-10. Selection Bias and Evaluation of Methods Used to Address Potential Confounding in Human Studies of Cobalt

Study	Selection Bias	Methods Used to Address Potential Confounding
Tüchsen et al. (1996)	*Rating*: **++;** Direction ↓ *Rationale:* No HWE was evident; only external analyses comparing cancer incidence in the Danish population. Differential selection out of the cohort could have occurred as the authors mentioned that records of ill persons may have been removed potentially resulting in an underestimate of the true incidence of cancer.	*Rating;* **++;** ↑ *Rationale*: No internal or statistical analysis controlling for smoking or potential carcinogens; however, minimal exposure to occupational co-exposures indicated from measurements in 1981 and smoking data on subset of the population

Study	Selection Bias	Methods Used to Address Potential Confounding
Mur et al. (1987) Moulin et al. (1993)	*Rating:* ++ (Mur for case-control analysis); ++ (Moulin et al. 1993; French nationals analysis); ↓ *Rationale*: HWE present based on significant decreases in all-cause mortality rates (Mur et al. 1987) in the entire cohort, but not for workers exposed only to cobalt. High loss to follow-up in both studies due to foreign-born workers, with no information provided to assess if the loss was related to exposure. Moulin et al. (1993) provided a restricted analysis for French nationals in addition to a whole-cohort analysis, to mitigate a potential bias. However, 46% of the cohort was hired prior to the start of follow-up (left truncation), which could induce a downward bias in the effect estimate.	*Rating;* +; ↑ *Rationale*: Mur et al. (1987) reported smoking data from admin records available from 30% of the cohort (Mur et al. 1987), although cases and controls were reported as matched on smoking status with no report on methods of matching. Moulin et al. (1993) did not adjust for smoking. Potential exposure to nickel and arsenic from cobalt ore and/or processing methods, however, no analysis conducted controlling for potential carcinogens. Internal analysis (nested case-control) available in the Mur et al. (1987) study helps minimize potential confounding.
Moulin et al. (1998)	*Rating: ++ for nested case-control analysis;* ↓ *Rationale:* High loss to follow-up largely due to foreign-born workers who were right censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that loss was related to exposure. Concerns with statistically significant decreases in all-cause mortality rates (HWE) mitigated by nested case-control analysis. Left truncation not an issue in this incidence cohort.	*Rating;* **+;** ↑ Rationale: Ever vs. never smoking data collected, and ever/never data on co-exposures in the JEM. Unlikely if models for cobalt alone included smoking and co-exposures (based on communication with author). Little information provided on cobalt co-exposures. Internal analyses may help reduce potential confounding from lifestyle factors. "Other cobalt exposures" were a mix of cobalt exposures from pure cobalt alone to other cobalt (not tungsten carbide) production activities with exposure to other carcinogens and not well defined.
Wild et al. (2000)	*Rating:* ++; ↓ *Rationale:* High loss to follow-up largely due to foreign-born workers who were censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that the loss was related to exposure. No case-control analysis of cobalt alone provided. Left truncation not an issue in this incidence cohort.	*Rating;* **+;** ↑ *Rationale:* Smoking data abstracted from factory health records and for earlier smoking from former colleagues. Co-exposures to several IARC carcinogens likely (no correlations provided) and assessed (ever/never) from JEM exposure. Unlikely that smoking and co-exposures were included in estimate for cobalt alone exposure. See Moulin et al. (1998) also.

Study	Selection Bias	Methods Used to Address Potential Confounding
Moulin et al. (2000a)	*Rating:* +++ *Rationale:* HWE effect based on significant decreases in all-cause mortality rates in external analysis; cross-sectional cohort, internal analyses may have mitigated potential HWE bias. Nested case-control study of living controls matched on age at selection with the case; similar as to their distribution of year of hire, suggesting that it is unlikely that the HWSE was operating.	*Rating:* ++; ↑ *Rationale:* Statistical analysis controlled for smoking (ever-never) for 71% of subjects and some co-exposures (levels assigned from JEM). However, the models did not control for co-exposure to other metals (nickel/chromium and iron), which were correlated with exposure to cobalt, although not to lung cancer in these data.
Grimsrud et al. (2005)	*Rating:* +++ *Rationale:* No information about whether HWE was present in the original cohort, but only cases who were identified from 1970 onwards were included; controls were matched to cases by gender and age and at risk at the time of the case diagnosis and were similar with respect to year of first employment, reducing the concern with HWSE. However, some concern remains given that sick workers hired at the same dates as healthy workers could move from higher to lower exposure groups. No analysis was conducted to account for this possibility. Participation rates were 94% for both cases and controls.	*Rating:* ++; ↑ Statistical analyses controlled for smoking (5 categories) and exposure to lung carcinogens (arsenic, asbestos, sulfuric acid mists and nickel). Due to the high correlation between cobalt and nickel exposure, categorical levels of cobalt variable could not be retained in the fully adjusted model. Only smoking adjusted exposure response estimates were available.
Rogers et al. (1993)	*Rating:* +++ *Rationale:* Ascertained all cases of aerodigestive cancer from the Western Washington State SEER cancer registry and used random digit dialing to identify controls in the same defined area as controls.	*Rating:* +++ *Rationale:* Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol and tobacco use among cases and controls. Differences in education, not occupation reported. Food frequency questionnaire based on usual dietary habits 10 years prior to the interview. Other metals measured included zinc, chromium, iron, and calcium; however, no correlations were shown with cobalt, nor were these metals included in models.

Study	Selection Bias	Methods Used to Address Potential Confounding
O'Rorke et al. (2012)	*Rating:* ++ *Rationale:* Methods for case and control selection vary by location. Northern Ireland cases identified systematically from electronic pathology records across the country; cases in the Republic were identified from "main" hospitals involved in the diagnosis and treatment of esophageal cancer. Potential for cases not referred to the major referral centers to be excluded. Very low participation rates and lower smoking rates in controls suggests the potential for selection bias is present due to lifestyle factors related to esophageal cancer.	*Rating:* +++ *Rationale:* Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol, manual labor, and tobacco use among cases and controls. Dietary intake based on food frequency questionnaire assessing diet and alcohol use 5 years previously. Other metals measured included selenium, chromium, zinc, mercury, and cerium; however, no correlation with cobalt was indicated, nor were these other metals included in the models for cobalt.

C.2.3. Information Bias: Exposure Assessment and Disease Endpoints

C.2.3.1. Cohort and Nested Case-control Studies

The exposure assessment evoking the most confidence is that of Grimsrud et al. (2005), which incorporated 3,500 personal breathing zone samples of cobalt air concentrations into the JEM which had been developed for an analysis of exposure to types of nickel using time- and department-specific exposure estimates. Non-differential misclassification may be possible for earlier decades of exposure based on limited data for those years. The study of nickel workers is followed by the hard-metal studies in which semi-quantitative assessments were conducted with validation for cobalt in non-production areas (Moulin et al. 1998; Wild et al. 2000), and then by the stainless and alloyed steel workers cohort (Moulin et al. 2000a), which also used a non-validated semi-quantitative JEM. Studies using only qualitative assessments warranted the lowest confidence in the exposure assessment (Moulin et al. 1993; Mur et al. 1987; Tüchsen et al. 1996).

Semi-quantitative categories of exposure based on job-exposure or job-task exposure matrices with estimates of exposure ranks or levels, which do not allow for the estimation of the risk per unit of exposure, were used in the hard-metal (Moulin et al. 1998; Wild et al. 2000) and stainless and alloyed steel worker studies (Moulin et al. 2000a). Strengths of the assessments are that they were based on expert opinion, were job-period specific, and incorporated information on frequency, intensity, duration, or probability. The hard-metal exposure assessment was considered to be somewhat higher quality than that used in the stainless and alloyed steel cohort, as their JEM was validated by historical exposure measurements, but these were not specific for cobalt (the focus of these studies was for exposure to cobalt-tungsten carbide hard metals thus less information is available for cobalt alone.) While the Moulin et al. (2000a) study was specifically designed to measure cobalt and other co-exposures in the JEM, there was little information provided on past cobalt exposure.

The most concern about exposure misclassification existed primarily for studies in which cobalt exposure was simply defined as employment in particular workshops (Moulin et al. 1993; Mur et al. 1987), or in factory departments (Tüchsen et al. 1996). Previously published data from the

porcelain painters (Raffn et al. 1988) indicated an overlap of cobalt levels in referents and exposed individuals, suggesting that the referents in the Tüchsen et al. (1996) paper may not have been "unexposed." In addition, exposure assessment in these studies did not differentiate workers according to exposure level. Potential misclassification of exposure would arise from lack of information on job tasks, use, and exposure conditions.

None of these occupational cohort studies provided information on the use of protective measures in workplace, nor was there any indication that such measures were taken into consideration in the job exposure matrices, nor were changes in hygiene practices over time when reported (e.g., Tüchsen et al. 1996) incorporated into the analyses as proxies for such changes.

In all the studies, the potential for exposure misclassification was generally considered to be non-differential, and would most likely bias towards the null, reducing the power to detect an effect. In subgroup and trend analyses, (specifically, in the Grimsrud et al. (2005), Moulin et al. (1998), and Moulin et al. (2000a) studies) exposure misclassification between exposure groups would most likely attenuate any exposure-response relationships.

C.2.3.2. Case-control Studies

Both population-based studies were conducted to determine the relationship of low levels of metals derived primarily from dietary sources to esophageal cancer, Barrett's esophagus, and other aero-digestive cancers. However, a major concern about information bias in exposure assessment exists for both studies because the window of exposure implied by measuring metals in nails for cancer outcomes may not be appropriate. Nail samples were collected a median of 6.5 months after diagnosis in the Rogers et al. (1993) study, at or near the time of study enrollment in both studies. O'Rorke et al. (2012) reported that 76.7% of esophageal cancer cases had habitually clipped their toenails prior to admission to hospital. Toenail clippings likely reflect an integrated exposure that occurred 12 to 18 months prior to clipping (Fleckman 1985). Given that Barrett's esophagus, esophageal and aerodigestive cancers have long latency periods, clippings that reflect at most an exposure window 12 months prior to diagnosis may or may not reflect ongoing exposure related to the development of cancer. In addition, several known factors can influence nail growth and possibly affect the time window of exposure represented by the sample. For example, nail growth is faster during pregnancy, and in warmer climates (Fleckman 1997). Certain medical conditions have been shown to increase or decrease the rate of nail growth, and age and sex affect nail growth with faster nail growth in men, and declining rates with increasing age (Dawber and Baran 1987; Fleckman 1985). Trace element deposition in nails may be influenced by several factors including those that are correlated with cancer (e.g., immobilization, decreased circulation, malnutrition, weight loss, age, gender, changes in diet and smoking and alcohol consumption) (Hunter et al. 1990; Slotnick and Nriagu 2006). Furthermore, reproducibility of cobalt specifically, in toenails from multiple samples over time has been reported to have intermediate to high within-person variability suggesting that sampling at any one point in time may not reflect long-term exposure (Garland et al. 1993). Another concern is whether the cancer process itself could alter deposition of cobalt in the nails, resulting in reverse causality. Two studies of cobalt levels by cancer stage in patients with lung cancer (Benderli Cihan et al. 2011; Kuo et al. 2006) and one with laryngeal cancer (Klatka et al. 2011) show contradictory findings, with no difference, increasing, and decreasing cobalt levels with increasing stage of disease. Rogers stratified cases by stage at diagnosis (in situ, localized versus

regional, and distant), and by the time from diagnosis to interview (which was either <7 months or >7 months); there were no significant differences by stage or by time from diagnosis to interview, suggesting that reverse causality was not operating in this study.

C.2.4. Information Bias – Disease Endpoints

C.2.4.1. Cohort and Nested Case-control Studies

Overall, the incidence and mortality measures used in the cohort studies were likely to distinguish between the presence and absence of cancer and reliably distinguish one cancer from another. Two cohorts, the Danish porcelain painters cohort (Tüchsen et al. 1996) and the Norwegian nickel refinery workers nested case-control study (Grimsrud et al. 2005), were based on incident cases of lung cancer obtained through linkage with the Danish Cancer Registry and the Norwegian Cancer Registry, respectively.

The remaining cohorts, with the exception of the first analysis of the electrochemical workers (Mur et al. 1987) were based on mortality data obtained primarily from death certificates (Moulin et al. 2000a; Moulin et al. 1993; Moulin et al. 1998; Wild et al. 2000). For lung cancer results and cancers with similarly low survival rates (lung and esophageal cancer 5-year survival rates, 16.8% and 17.8%, respectively), mortality data adequately reflect incidence.

Concerns regarding disease misclassification primarily existed in the cobalt production worker studies (Moulin et al. 1993; Mur et al. 1987). In the Mur et al. (1987) study, the cause of death was ascertained by physician interviews and medical records; in the Moulin et al. (1993) update, a decision had been made a priori to use the cause of death indicated on the death certificate, regardless of whether lung cancer was indicated in the medical record. As a result, one of the four exposed cases of lung cancer was dropped. While death certificate data are usually preferred over medical records, they are more likely to result in both missing cases and misclassification as compared to cancer registry data used in incidence studies, in which cancers are histologically confirmed. In neither of these studies was cancer histologically confirmed.

C.2.4.2. Case-control Studies

Both of the biomarker studies appear to be able to reliably distinguish between the presence and absence of the cancer outcome, suggesting low/minimal concern for information bias of the disease endpoints. Cancer diagnoses were based on histological findings in both studies with follow-up cytology for some cases (Rogers et al. 1993) or review by study pathologists (O'Rorke et al. 2012).

Table C-11. Information Bias – Exposure Assessment and Disease Endpoints in Human Studies of Cobalt

Study	Exposure Misclassification	Outcome Misclassification
Tüchsen et al. (1996)	*Rating*: ++, Direction ↓ *Rationale:* Exposure designated by employment in a department considered to include exposure to cobalt or not; calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level, or duration	*Rating;* +++ *Rationale:* Incident cases of lung cancer obtained through linkage with the Danish Cancer Registry

Study	Exposure Misclassification	Outcome Misclassification
Mur et al. (1987) Moulin et al. (1993)	*Rating:* ++, ↓ *Rationale:* Exposure was assigned based on job location in various workshops including cobalt production, calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level, or duration.	*Rating;* ++ Some evidence that lung cancer cases may have been missed. Cause of death ascertained by physician interviews and medical records (Mur et al. 1987), and only by death certificates in the re-analysis (Moulin et al. 1993), resulting in one of four cases being dropped. Mortality may have missed cases certain cases with better survival (e.g., laryngeal, oral cavity and pharyngeal cancers), which were first reported by Mur et al. (1987), but not by Moulin et al. (1993).
Moulin et al. (1998)	*Rating:* ++ to +++, ↓ *Rationale*: JEM based on expert judgment and limited data from records. Intensity scores (0–9), and frequency of work time exposed (<10%, 10%–50%, >50%). JEM exposure scores for cobalt validated but did not include cobalt powder production areas.	*Rating;* +++ *Rationale*: Mortality data are adequate for lung cancer, which has a low survival rate.
(Wild et al. 2000)	*Rating:* ++ to +++, ↓ *Rationale:* Similar JEM as Moulin et al. (1998). As the focus of the study was on hard metals, exposures within other workshops (i.e., cobalt powder production) were assessed in less detail or were not precise as those for cobalt-tungsten carbide.	*Rating;* +++ *Rationale*: Mortality data are adequate for lung cancer, which has a low survival rate.
Moulin et al. (2000a)	*Rating:* ++, ↓ *Rationale:* JEM for specific job periods based on expert's subjective quantification which was based on interviews with former and current workers in each workplace and on measurements in other French factories and published results from the literature. No airborne exposure level measurements used to validate these judgments and little information on past exposures. Categories of exposure were based on frequency, intensity, duration, and probability.	*Rating;* +++ *Rationale*: Mortality data, which is adequate for lung cancer that has a low survival rate

Study	Exposure Misclassification	Outcome Misclassification
Grimsrud et al. (2005)	*Rating:* +++, ↓ *Rationale:* Quantitative JEM developed for nickel analysis using time- and department-specific exposure estimates plus a cobalt surrogate intensity measurement based on estimated levels of time- and department-specific periods from 3,500 personal samples of cobalt air concentrations from the breathing zone. Low level of concern for non-differential exposure misclassification as monitoring did not begin until 1973 and personnel files carried some degree of uncertainty concerning the earlier decades.	*Rating;* +++ *Rationale:* Incident cases of lung cancer obtained through linkage with the Norwegian Cancer Registry.
Rogers et al. (1993)	*Rating:* +; direction not known *Rationale:* Unlike other elements investigated, cobalt levels in food measured by a food frequency questionnaire were not available from USDA, Window of exposure implied by a single measurement of cobalt in nails (12–18 months exposure) for cancer outcomes may not be valid for induction of esophageal cancer. Single sample of toenails shown to have low to intermediate reproducibility for cobalt (Garland et al. 1993).	*Rating;* +++ *Rationale*: Cases identified through the local SEER cancer registry; diagnosis based on a positive histological finding or a positive cytology with follow-up to the attending physician to confirm the diagnosis. Potential controls with a history of any cancer were excluded.
O'Rorke et al. (2012)	*Rating:* +; direction not known *Rationale:* Window of exposure implied by a single measurement of cobalt in nails (12–18 months exposure) for cancer outcomes may not be valid for cancer induction of esophageal cancer or Barrett's esophagus; single sample of toenails shown to have low to intermediate reproducibility for cobalt (Garland et al. 1993).	*Rating;* +++ *Rationale*: Esophageal adenocarcinoma cases had a histologic confirmation of adenocarcinoma within the esophagus and excluded in situ cancers. Available clinical and histologic records (surgical and radiological reports) were reviewed by 3 authors and a pathologist to confirm location of the tumor in the esophagus. Potential controls had no history of esophageal or any gastrointestinal cancer or Barrett's esophagus.

C.2.5. Selective Reporting and Analysis Bias in Human Studies of Cobalt

C.2.5.1. Cohort and Nested Case-control Studies of Cobalt

There is little evidence of selective reporting in any of the cohort studies. For the hard-metal studies, the focus of the analysis was not on cobalt alone; thus, few analyses were presented. For the porcelain painter and electrochemical worker studies, additional information regarding exposure and the cohort might have been reported, but this is more a problem of reduced quality of reporting than of selective reporting.

The analysis of the nickel refinery workers (Grimsrud et al. 2005) is the strongest in terms of its methods, assumptions, and statistical analysis, using categorical and continuous variables were reported and methods of model fitting described. The nested case-control analyses of stainless-

steel workers (Moulin et al. 2000a) and hard-metal workers (Moulin et al. 1998) were also considered adequate and used appropriate models to evaluate exposure-response relationships.

For the hard-metal cohorts, both Moulin et al. (1998) and Wild et al. (2000) lagged exposure indices 10 years to account for disease latency. However, in contrast to Moulin et al. (1998), Wild et al. (2000) did not report detailed analyses for cobalt without hard metal, and lagged exposure indices only for the workshop analysis of hard metals, but not the analysis based on the JEM, on which the cobalt SMR was based.

C.2.5.2. Case-control Studies

In both the Rogers et al. (1993) and the O'Rorke et al. (2012) studies, concerns are low/minimal that the study does not provide results for all relevant measures and participants that would bias its interpretation.

Concerns with analysis bias were low/minimal in both of these studies, although O'Rorke et al. (2012) provided somewhat more detail about the assumptions and methods of their analyses. The O'Rorke et al. (2012) study, after log transforming the toenail element concentrations, used a backwards elimination approach using multivariate logistic regression investigating the association between tertiles of toenail trace element concentrations and the risk of esophageal cancer. Rogers et al. (1993) reported a categorical analysis using unconditional logistic regression to calculate ORs as estimates of the relative risk for each cancer, adjusting for the effects of potentially confounding factors. The primary table in the Rogers et al. (1993) study combined cases and controls, so it was not possible to ascertain the distribution of cases and controls across tertiles of cobalt.

Table C-12. Selective Reporting and Analysis Bias in Human Studies of Cobalt

Study	Selective Reporting	Analysis
Tüchsen et al. (1996)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* ++ *Rationale:* SMR external analysis only conducted; no internal analyses conducted to account for potential confounding, or induction period. No exposure-response analyses.
Mur et al. (1987) Moulin et al. (1993)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Conducted cobalt-specific external and internal analyses and did not report exposure-response analyses or particular methods for analysis of the data (Mur); Moulin restricted analyses to French-born workers exposed to cobalt exclusively, to minimize bias from selection or confounding. Unclear whether adjustments for censoring or recent exposures (lagging) were considered to address the large number of electrochemical workers who worked fewer than 10 years, which would address potential bias from inclusion of short-term workers with potentially lower exposures.

Study	Selective Reporting	Analysis
Moulin et al. (1998)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Conducted internal cobalt analysis with trend analyses for exposure to cobalt alone by levels of intensity, duration, and cumulative (weighted or unweighted) doses using a fit of the ranks of the recoded variables as a test for trend.
Wild et al. (2000)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* ++ *Rationale:* Conducted external SMR analysis only for cobalt alone with little documentation of what the models included
Moulin et al. (2000b)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Methods, assumptions, and statistical analysis are described in detail. Analyses using categorical and continuous variables were reported and methods of model fitting described
Grimsrud et al. (2005)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Methods, assumptions, and statistical analysis are described in detail. Analyses using categorical and continuous variables were reported and methods of model fitting described; and an indicator variable was included to denote first employment prior to 1930 when exposure assessments were not as reliable
Rogers et al. (1993)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Categorical analyses using unconditional logistic regression to calculate ORs as estimates of the relative risk of each cancer, adjusting for the effects of potentially confounding factors.
O'Rorke et al. (2012)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Backwards elimination approach using multivariate logistic regression investigating the association between tertiles of toenail trace element concentrations (log transformed) and the risk of esophageal cancer

C.2.6. Study Sensitivity, Quality, and Utility of Cohort and Nested Case-control Studies

C.2.6.1. Cohort and Nested Case-control Studies

Factors that influence the ability of a study to detect an effect (if present) include the sample size; the exposure prevalence; the range, level, duration, window, or route of exposure; and the length of follow-up in cohort studies. Studies with greater sensitivity to detect an effect are more informative for the evaluation, and an investigation of study sensitivity can help explain heterogeneity across studies. All studies with the exception of the nickel refinery workers (Grimsrud et al. 2005) study (204 workers) observed small numbers of exposed cases of lung cancer: 3 and 4 among the electrochemical workers (Moulin et al. 1993); 15 among hard-metal workers (Moulin et al. 1998; Wild et al. 2000); 8 among porcelain painters (Tüchsen et al. 1996) and 17 among the stainless and alloyed steel workers (Moulin et al. 2000b).

Except for Grimsrud et al. (2005), all of the studies are limited with regards to the level or range of exposures, either because these were not reported nor included in the analyses (Moulin et al. 1993; Moulin et al. 1998; Mur et al. 1987; Tüchsen et al. 1996; Wild et al. 2000).

The sensitivity of the porcelain painters study to detect any effect may have been limited by potentially combining workers with high and low exposures together, diluting any effect. Tüchsen et al. (1996) reported that high levels of cobalt aluminate-spinel dust were measured in 1954 (170 particles (0.5 to 5 $\mu g/cm^{-3}$) and in 1967 (150 particles); and that levels of cobalt silicate, which began to be used in both factories in 1981, initially exceeded the hygienic standard for all measurements in the range from 1.3 to 172 times (as reported by Tüchsen et al. 1996). While there were no analyses to differentiate high and low exposure levels of the two types of cobalt compounds, overall, there is information that levels of any cobalt compound changed from high to low from 1982 to 1984 and leveled off through 1990. Thus, combining low- and high-exposure workers could decrease the ability to detect an effect.

No information on exposure levels was reported for the electrochemical workers (Moulin et al. 1993; Mur et al. 1987), hard-metal workers (Moulin et al. 1998; Wild et al. 2000), or the stainless and alloyed steel workers (Moulin et al. 2000a). Moulin et al. (1998) (hard-metal workers) and Moulin et al. (2000a) (stainless and alloyed steel workers) analyzed trends across duration of exposure and un-weighted and frequency-weighted cumulative dose information, but did not provide the numbers of exposed cases across categories of these variables. Thus, it is difficult to know how many workers were exposed to higher levels or longer durations of exposure.

Most studies had sufficient follow-up time to allow for a cancer induction period. The hard-metal, stainless and alloyed steel, and nickel refinery studies (Grimsrud et al. 2005; Moulin et al. 2000b; Moulin et al. 1998; Wild et al. 2000) lagged analyses to discount years after initial exposure and prior to diagnosis.

C.2.6.2. Case-control Studies

The sensitivity of these case-control studies to detect effects at high and low levels is somewhat limited, given the likely low levels of cobalt in these "non-exposed" populations. Both case-control studies report low levels of cobalt in toenails, reported as categorical variables with tertile cut points (μg/g) of cobalt concentrations: O'Rorke et al. (2012) (Ireland): <0.004, 0.004 to <0.011, and ≥0.011; Rogers et al. (1993) (Western Washington state U.S.A.): <0.05, 0.05 to 0.17, and >0.17 μg/g. The range of levels in the O'Rorke et al. (2012) study are 0.002 to 0.60 μg/g (mean = 0.02 ± 0.06); Rogers et al. (1993) did not report ranges, means, or SDs. Cobalt levels in toenails from a general population sample (the Nurses' Health Study (Garland et al. 1993)) were comparable to these levels (mean ± SD = 0.042 ± 0.023).

With respect to the differences between the levels of cobalt reported in these two papers, a U.S. Geologic Survey professional paper (Shacklette and Boerngen 1984) reported that soils of the Pacific Northwest generally have high concentrations of cobalt; however, studies of soils in Ireland and the United Kingdom have reported low or deficient soil cobalt levels in several areas (Lark et al. 2013). These studies support the differences seen in exposure distributions for these two populations and suggest that environmental exposures in different geographical areas with different metal composition may influence population levels of cobalt.

Appendix D. Cancer Studies in Experimental Animals

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Tables

D.1. Study Quality

D.1.1. Evaluation Methods

Each primary study was systematically evaluated for its ability to inform the cancer hazard evaluation using a series of signaling questions related to the following study performance elements: population, exposure conditions, outcome assessment, potential confounding, and statistics and reporting (see NTP 2014b). The response for answering the signaling question of whether there is a potential bias or limitation is based on a comparison of the study element with that of the "ideal" study for a specific endpoint and exposure to the candidate substance. Guidelines for the ideal study are provided in the protocol. Two reviewers evaluated each study and differences were resolved by reference to the original publication and consultation with a third reviewer.

D.1.2. Study Quality and Sensitivity Questions and Responses

The following questions were used to evaluate study quality and sensitivity; the questions are grouped according to the study performance element. A short description (typically one to two words) of the questions is provided in the study quality tables in Section D.2 (e.g., controls for the question on concurrent controls below).

D.1.2.1. Population (Selection of Study Animals)

- Are there concerns that the concurrent control group was not adequate for evaluating the study?
- Are historical control data reported?
- Are there concerns that the study design did not include randomization of animals to dose groups or take appropriate steps to ensure that dose groups are identical except for dosing status?

D.1.2.2. Quality of the Exposure

- Are there concerns that the chemical characterization and dose formulations (e.g., confirmation, homogeneity, purity, solubility, and stability) and delivery of the chemical (actual vs. desired dose) are not adequate for attributing any neoplastic effects to the substance?
- Are there concerns that the dosing regimen (dose selection and dose groups, or other factors) or the exposure duration are either not (1) adequate for detecting a neoplastic effect or (2) for attributing any tumor effects to the substance?
- Are there concerns that survival or body weight change(s) over time for treatment and/or control groups could affect attributing the study findings to the exposure?

D.1.2.3. Quality of the Endpoint Assessment

- Are there concerns that the methods to assess tumor outcome and the pathology procedures (necropsy, histology, or diagnosis) are not adequate for attributing the effects to the exposure?

D.1.2.4. Potential for Confounding

- Are there concerns about the potential for confounding?

D.1.2.5. Analysis and Reporting

- Are there concerns that reporting of the data and statistical analysis are inadequate for evaluating the results? Are there concerns that different types of tumors are not accurately combined in the analysis?

D.1.2.6. Sensitivity

- Are there concerns about the animal model (source, species, strain, or sex) that could affect study interpretation?
- Are there concerns that the study does not have adequate statistical power (number of animals per exposure and control group) to detect a neoplastic effect, if present? Are there concerns that survival-related effects or high mortality due to poor husbandry conditions have decreased statistical power?
- Are there concerns that the study duration (observation period) is not adequate to detect a neoplastic effect, if present?

For each question, the following terms were used to rate the potential for bias and/or quality:

- Minimal concerns: Information from study designs and methodologies indicate that they are close to the ideal study characteristics and that the potential for bias is unlikely or minimal (+++).
- Some concerns: Study designs or methodologies are less than ideal, indicating possible bias (++).
- Major concerns: Study designs or methodologies suggest that the potential for a specific type of bias is likely (+).
- Inadequate: Study designs or methodologies suggest that the bias is critical and would make the study not informative for cancer hazard evaluation.
- No information: The information is inadequate to evaluate the level of concern.

D.2. Overall Assessment of Study Utility

An overall assessment of study utility is based on consideration of both the potential for bias (i.e., limitations) and consideration of study sensitivity, and the studies are broadly grouped into the four categories below. Studies having critical concerns for important issues will generally be considered to be inadequate to inform the evaluation. It should also be noted that some concerns about a study element (such as inadequate observation and/or exposure period and statistical power) would decrease the sensitivity of a study to detect an effect; however, if despite these limitations positive findings were described, these studies would inform a cancer assessment. Some studies, such as co-carcinogen studies, have less utility for determining whether a substance is a cancer hazard but may provide utility regarding mechanism of action or other issues and thus utility would be rated based on the purpose of the study.

- High (low/minimal concerns for most potential biases)

- Moderate (low/minimal or some concerns for most potential biases)
- Low (major concerns for several potential biases)
- Inadequate (critical concerns for some potential bias)

D.3. Study Quality Assessment and Study Quality Tables: Carcinogenicity Studies

The following tables contain the study quality assessment (rating and rationale for the rating) for each study. The studies are organized by study type, and then by metal type or compound, followed by route of exposure. For summary tables of study quality across all studies, see Table 5-2 (carcinogenicity) and Table D-24 (co-carcinogenicity).

Table D-1. NTP (2014d) (Rats): Cobalt Metal/Powder; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	Yes Historical controls were reported.
Randomization	+++ Random allocation was done.
Exposure	
Chemical purity	+++ The cobalt was 98% pure and tested for stability.
Dosing regimen	+++ Dose levels were based on the 3-month studies, and three dose levels were tested.
Survival	++ A significant decrease in survival of females and significant decrease in body weight of both sexes was reported.
Pathology	+++ Full necropsies were performed and a quality assurance program was in place to verify the histopathological evaluations.
Confounding	+++ Little to no sources of confounding. Animal husbandry and disease surveillance were well conducted and chemical purity was tested by a third party.
Reporting and analysis	+++ Full details were reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+++ Large number of animals used.

Study Utility Domain Question	Rating Rationale
Study duration	+++ Near lifespan duration of 2 years was used. Although survival rates were low in both control and exposed groups at the end of the study, survival did not decline after ~80 weeks and there was ≥90% probability of survival at 75 weeks. Thus, there is minimal concern that the low survival rates at the end of the study limited the sensitivity to observed treatment-related cancer effects.

Overall utility: High
A well-designed study in all factors, but with a significant decrease in survival of female rats.

Table D-2. NTP (2014d) (Mice): Cobalt Metal/Powder; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	Yes Historical controls were reported.
Randomization	+++ Random allocation was done.
Exposure	
Chemical purity	+++ The cobalt was 98% pure and tested for stability.
Dosing regimen	+++ Dose levels were based on the 3-month studies, and three dose levels were tested.
Survival	++ A significant decrease in survival of males and significant decrease in body weight of both sexes was seen.
Pathology	+++ Full necropsies were performed and a quality assurance program is in place to verify the histopathological evaluations.
Confounding	+++ Few to no sources of confounding. Animal husbandry and disease surveillance were well conducted and chemical purity was tested by a third party.
Reporting and analysis	+++ Full details were reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	+++ Large number of animals used.
Study duration	+++ Near lifespan duration of 2 years was used.

Overall utility: High
A well-designed study in all factors, but with a significant decrease in survival of male mice.

Table D-3. Hansen et al. (2006): Cobalt Metal/Powder; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Untreated controls were not included but PVC particles, which are assumed to be inert, were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information on the chemical purity was reported, only information on the particle size of the bulk metal and the surface to mass ratios of the bulk metal and nanoparticles.
Dosing regimen	++ Only a single dose and single dose level was given, but the dose level was not reported. Particles were reported to cover a specific area of tissue; continuous exposure to cobalt nanoparticles.
Survival	+++ Survival was lower than PVC controls after 8 months, but it was due to moribund tumor growth.
Pathology	+++ Complete necropsies were performed.
Confounding	++ Details of animal husbandry were not reported and neither was chemical purity; however, it was stated that animals were looked after in accordance with European standard requirements and that animals were observed daily for clinical abnormalities. The negative controls underwent the same procedures of implanting particles.
Reporting and analysis	++ Neither the chemical purity, dose level, age of rats, nor statistical analysis were reported.
Sensitivity	
Animal model	++ Only male rats were tested, so sex differences can't be determined.
Statistical power	+ Few animals per group were tested. Two time points were used to sacrifice animals, which reduced the effective number of animals at each sacrifice and for each reported incidence, thus lowering the statistical power of each individual time point.
Study duration	+ Treated animals were sacrified at 6 and 8 months; animal sacrificed at 8 rather than 12 months because of tumor growth from expousre to cobalt nanoparticles. Controls were sacrificed at 6 and 12 months. Duration may not be adquate for evaluating cobalt metal.

Overall utility: Moderate

The longest duration of observation was 8 months (due to tumors induced by cobalt nanoparticles) and two forms of cobalt metal were tested in the same individual rat, bulk metal particles and nanoparticles. Duration may not be adequate for evaluating cobalt metal. Complete necropsies were performed. Inert polyvinyl chloride particles were used as a negative control. Only a small number of males were tested with a single dose level, though dose was never fully reported. Poor reporting of chemical purity and animal husbandry.

Table D-4. Jasmin and Riopelle (1976): Cobalt Sulfide or Cobalt Metal; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent vehicle controls were used.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The chemical purity was reported as "reagent grade."
Dosing regimen	+ Only a single dose was given at one dose level without a reported basis for that level. Dose was lower than that used in other injection studies.
Survival	NR No survival information was reported.
Pathology	++ The level of necropsy was not full: in addition to the kidney, study looked for metastases in the abdomen and thorax, but not the entire body.
Confounding	++ No information about animal husbandry, including disease surveillance, was reported and chemical purity was only reported as "reagent grade."
Reporting and analysis	++ Details were not reported for animal husbandry or disease surveillance and chemical purity reported was limited as it was reported as "reagent grade." Surival information was not reported and number of animals at sacrifice is unclear.
Sensitivity	
Animal model	++ Only non-transgenic female rats were tested.
Statistical power	++ A moderate number of animals per group were used and survival was not reported.
Study duration	+ 12 months.

Overall utility: Low
A moderate number of rats per group was used; however, sensitivity was limited by short observation period, use of only a single dose level, which was lower than that used in other studies and and testing in only females. No information on animal husbandry, including disease surveillance; chemical purity or stability, and number of animals at sacrifice were poorly reported. Full necropsies were not performed, though the abdominal and thoracic cavities were examined.

Table D-5. Heath and Daniel (1962): Cobalt Metal/Powder; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+ There was no concurrent control, but there was a historical control from Heath 1954, which was cited in the paper as the reason for not having a concurrent control.

Study Utility Domain Question	Rating Rationale
Historical data	Yes (limited) Controls from an earlier studies were used in place of concurrent controls.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ Only stated as “spectroscopically pure.”
Dosing regimen	+ Only one dose was given at one dose level and the number of injections was not reported but assumed to be single injection. Rationale for dose not provided.
Survival	NR Unable to determine if there were treatment-related survial effects since survival of controls from the 1956 study was not reported. Survival in exposed group was low, with 8 out of 20 rats dying only days after the injection; however, survival was good after initial deaths; deaths may have been due to technical difficulties with the intrapleural injections.
Pathology	++ Only looked at the injection site; full necropsies were not reported.
Confounding	++ No information regarding animal husbandry conditions and disease surveillance were reported, but chemical purity was reported as “spectroscopically” pure.
Reporting and analysis	+ No animal husbandry or necropsy methods were reported and chemical purity was reported only as “spectroscopically pure.”
Sensitivity	
Animal model	++ Only non-transgenic female rats were tested.
Statistical power	+ Small numbers of animals. Survival was low due to deaths caused within the first 3 days from difficulties with the intrapleural injection.
Study duration	+++ 28 months.

Overall utility: Low
The duration of observation was over two years long, but exposure was only a single dose. There was no concurrent control, but there was a historical control. No statistics were done, a low number of animals was used, and necropsies were not done; only skin tumors were histologically examined. No information was reported about chemical purity or stability, or animal husbandry.

Table D-6. Heath (1956): Cobalt Metal/Powder; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	++ Untreated controls were used in the first series of experiments, but not in the second series; however, untreated controls from the first series could be used as historical or non-concurrent controls for the second series of studies. The survival of untreated controls was not reported.

Study Utility Domain Question	Rating Rationale
Historical data	Yes (limited) The untreated controls from the first series of studies was used as a historical control for the second series of studies.
Randomization	NR No information about randomization was reported.
Exposure	
Chemical purity	++ Purity was reported as "spectroscopically pure."
Dosing regimen	+ Only one dose level was used with no explanation as to why that level was chosen. The duration of treatment was not reported, but was assumed to be a single dose.
Survival	NR No data reported for untreated controls; two of 10 males, 4 of 10 females (Series I) and 0/10 females (Series II) without tumors died before sacrificed.
Pathology	++ No methods reported, but it was stated that no other tumors besides local tumors were found, though a metastasis to the lymph nodes were found, suggesting necropsies were performed, but the extent of the necropsies is not known.
Confounding	++ No information regarding animal husbandry conditions and disease surveillance were reported, but chemical purity was reported as "spectroscopically" pure.
Reporting and analysis	+ No reporting of animal husbandry, disease surveillance, randomization, treatment duration, necropsy methods, or survival for untreated controls.
Sensitivity	
Animal model	++ Both male and female rats were used in the first series of studies, but only females were tested in the second series.
Statistical power	+ Small numbers of animals were tested.
Study duration	+++ Duration of observation was near the animals' expected lifespan.

Overall utility: Low
Observation duration was sufficient and both sexes were tested. However, there was no reporting of animal husbandry, necropsy methods, or chemical stability. Only one dose level was tested, and a small number of male and female rats was used. Full necropsies were not reported to have been performed.

Table D-7. NTP (1998) (Rats): Cobalt Sulfate; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	Yes Historical controls were reported.

Study Utility Domain Question	Rating Rationale
Randomization	+++ Random allocation was done.
Exposure	
Chemical purity	+++ The cobalt was 99% pure and tested for stability.
Dosing regimen	+++ Dose levels were based on 16-day and 13-week studies, and three dose levels were tested.
Survival	+++ Survival was high and not affected by cobalt exposure.
Pathology	+++ Full necropsies were performed and a quality assurance program was in place to verify the histopathological evaluations.
Confounding	+++ Little to no sources of confounding. Animal husbandry and disease surveillance were well conducted and chemical purity was tested by a third party.
Reporting and analysis	+++ Full details were reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+++ Large number of animals used.
Study duration	+++ Near lifespan duration of 2 years was used.

Overall utility: High
A well-designed study in all factors and survival was similar to controls.

Table D-8. NTP (1998) (Mice): Cobalt Sulfate; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	Yes Historical controls were reported.
Randomization	+++ Random allocation was done.
Exposure	
Chemical purity	+++ The cobalt was 99% pure and tested for stability.
Dosing regimen	+++ Dose levels were based on 16-day and 13-week studies, and three dose levels were tested.
Survival	+++ Survival was high and not affected by cobalt exposure.

Study Utility Domain Question	Rating Rationale
Pathology	+++ Full necropsies were performed and a quality assurence program is in place to verify the histopathological evaluations.
Confounding	+++ Little to no sources of confounding. Animal husbandry and disease survalence were well-conducted and chemical purity was tested by a third party.
Reporting and analysis	+++ Full details were reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	+++ Large number of animals used.
Study duration	+++ Near lifespan duration of 2 years was used.

Overall utility: High
A well-designed study in all factors and survival was similar to controls.

Table D-9. Shabaan et al. (1977): Cobalt Chloride; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	++ Untreated controls were not reported for the 8-month study, but were for the 12-month study and could be used for the 8-month study as a non-concurrent control.
Historical data	Yes (limited) There were untreated controls that were sacrificed at the 12-month time point that could serve as non-concurrent or historical controls for the 8-month study. Fewer neoplasms would be expected at 8 months than at 12 months and no neoplasms were found at 12 months, so using 12-month untreated controls seems justified.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+ Only one dose tested and no basis given for choosing that level. Animals were treated 19 days. Treated animals developed persisent hyperlipaemia
Survival	++ Mortality of exposed rats was high compared to controls; 11/20 survived at 12 months and 16/20 survived at 8 months
Pathology	+ Only survivors were necropsied; those that died before 8 months or 12 months were not examined.

Study Utility Domain Question	Rating Rationale
Confounding	+ No information about chemical purity or disease surveillance was reported and animal husbandry was poorly reported.
Reporting and analysis	+ Untreated controls were not clearly reported, neither was animal husbandry. Chemical purity and the rationale for the single dose level used was not reported. Statistics were not calculated. The route of exposure was reported as s.c. injection, but the tumors developed in sites outside of the reported injection sites (central abdominal wall) and the authors didn't differentiate which sites were injection sites and which were non-injection sites.
Sensitivity	
Animal model	++ Only male non-transgenic rats were tested.
Statistical power	++ A reasonable number of animals tested, but there was a significant decrease in survival of the exposed rats.
Study duration	+ 8 months (experiment 1) or 12 months (experiment 2). The 8-month study would not normally meet inclusion criteria, but since tumors were induced it was included.

Overall utility: Low
Exposure was only for 19 days and animals that did not survive to the end of the experiments were not necropsied and there was a significant decrease in survival of exposed rats, so the studies may underestimate the true results. Only a single injection was given to male rats; females were not tested. The tumors and injection sites were not clearly reported and tumor sites were not designated as injection site or non-injection sites.

Table D-10. Steinhoff and Mohr (1991): Cobalt Oxide; Intratracheal

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Dose levels were randomly verified by gravimetric measurements taken several times during the study.
Dosing regimen	++ Two dose levels were given every 2 or 4 weeks for close to 2 years (39 doses).
Survival	++ Data are not provided, but it was stated in the results that there were “no appreciable differences.”

Study Utility Domain Question	Rating Rationale
Pathology	++ Only the high-dose group was fully necropsied. Histological examinations were done on organs with gross lesions suspected of having tumors and all respiratory tracts in the low-dose group or controls.
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+++ Large number of animals were used; survival was reported as similar to controls.
Study duration	+++ Observation duration was for lifespan.

Overall utility: Moderate
Two dose levels were tested in a high number of both sexes of rats for two years, with treatment and observations for the lifespan without any significant difference in survival compared to untreated controls. However, only the high-dose group received full necropsies. Details of the chemical and animal husbandry were not reported.

Table D-11. Steinhoff and Mohr (1991): Cobalt Oxide; Injection (IP)

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
Dosing regimen	+ One dose level was given every 2 months for 6 months with no explanation as to what the dose level was based on.
Survival	NR No survival information was reported.
Pathology	++ All organs and tissues suspected of having tumors and all tumors in the injection region were evaluated by histological examination.

Study Utility Domain Question	Rating Rationale
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+ There were only 10 animals per sex per group tested and survival was not reported.
Study duration	+++ Observation duration was for lifespan.

Overall utility: Moderate
Both sexes of rats were tested with a long duration of observation. However, reporting of animal husbandry, including disease surveillance and chemical purity was poor and survival was not reported. There was a small number of animals per group, only one dose level was tested, exposure was for less than one year and histological examination was only done on organs with gross tumors, all of which would limit the sensitivity to detect an effect.

Table D-12. Steinhoff and Mohr (1991): Cobalt Oxide; Injection (SC)

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
Dosing regimen	++ Only a single dose level was tested, but it was given at two intensities, either weekly or daily (1/5 the level) for 2 years. There was no reported basis for that dose level.
Survival	NR No survival information was reported.
Pathology	++ All organs and tissues suspected of having tumors and all tumors in the injection region were evaluated by histological examination.
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Animal husbandry was described, but disease surveillance was not reported.

Study Utility Domain Question	Rating Rationale
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
Sensitivity	
Animal model	++ Only male rats were tested.
Statistical power	+ There were only 10 animals per group and survival was not reported.
Study duration	+++ Observation duration was for lifespan.

Overall utility: Moderate
Duration of exposure and observation were sufficient; one dose level was tested, but it was tested at two intensity levels. However, few animals per group were used and only included males; histological exam was only done on organs with gross tumors, which would limit the senstivity to detect an effect. Reporting of animal husbandry, including disease surveillance, and chemical purity were poor and no survival data was provided.

Table D-13. Gilman and Ruckerbauer (1962) (Rats): Cobalt Oxide; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls (vehicle-aqueous suspension of pencillin G procaine) were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was “washed” to remove water soluble impurities and was <5 μm particle size. Cobalt was administered in an aqueous suspension of pencillin G procaine.
Dosing regimen	+ Only a single dose given at one dose level, but preliminary tests using unwashed unwashed particles, which contained an unknown water-soluble toxin that had killed many mice. Rats tolerated dose.
Survival	++ No survival-related effects at 90 days, only time period reported.
Pathology	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
Confounding	+ Animals and bedding periodically dusted with rotenone powder; not clear if the same rats were used from the preliminary experiment using unwashed cobalt.
Reporting and analysis	+ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis. Tumor incidences were reported as both sexes combined.

Study Utility Domain Question	Rating Rationale
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+ Only 10 animals were tested and they were reported as both sexes combined.
Study duration	++ 1.3 years.

Overall utility: Low
The duration of observation was sufficient and both sexes were tested. However, only a single dose was given at one dose level with results reported as both sexes combined; few animals per group were tested. Reporting was poor and lacked information on chemical purity or stability, animal husbandry, and necropsy methods. Animal bedding was periodically dusted with rotenone powder and half of the exposed group had been administered unwashed cobalt, which was known to contain additional chemicals.

Table D-14. Gilman and Ruckerbauer (1962) (Mice): Cobalt Oxide; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls (vehicle-aqueuous suspension of pencillin G procaine) were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was “washed” to remove water soluble impurities and was <5 μm particle size. Cobalt was administered in an aqueous suspension in pencillin G procaine.
Dosing regimen	+ Only a single dose given at one dose level, but preliminary tests using unwashed particles, which contained an unknown water-soluble toxin that had killed many animals, was reported. Half the animals died in this study between 2nd and 6th day.
Survival	++ No treatment-related survival effects at 90 days, only time period reported.
Pathology	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
Confounding	+ Animals and bedding periodically dusted with rotenone powder. Half of the animals were given washed particles and the other half were survivors of animals given unwashed particles in a preliminary experiment, which contained an unknown water-soluble toxin that had killed many animals.
Reporting and analysis	++ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis.
Sensitivity	

Study Utility Domain Question	Rating Rationale
Animal model	++ Only non-transgenic female mice were tested.
Statistical power	++ A large number of animals were tested; however, survival was only reported for 90 days.
Study duration	+++ 2 years.

Overall utility: Low
The duration of observation and the numbers of animals per group were sufficient. Survival was only reported for 90 days. However, only a single dose was given (without rationale for level), to females only; half of them received unwashed cobalt, which was known to contain other chemicals. Reporting was poor and lacked chemical purity or stability, animal husbandry, and necropsy methods, though metastasis was reported suggesting necropsies were performed. Bedding was periodically dusted with rotenone powder.

Table D-15. Wehner et al. (1977): Cobalt Oxide; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent untreated controls (sham-smoked) were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ There was no information on chemical purity or stability of the cobalt.
Dosing regimen	++ Single dose level with no justification for choosing that level, but administered for life.
Survival	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls although surival was low in both groups.
Pathology	+++ Detailed necropsies were performed.
Confounding	++ Animal conditions were partly reported, but chemical purity and disease surveillance were not reported.
Reporting and analysis	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as “carcinoma” or “polyp” without saying what tissue they originated from is meaningless.
Sensitivity	
Animal model	++ Only male hamsters were tested. Hamsters are less senstive for evaluating lung tumors.

Study Utility Domain Question	Rating Rationale
Statistical power	+ A large number of animals was used; however, statstical power was reduced by poor survival (fewer than 10 animals were alive at 18 months) in both cobalt-exposed and the corresponding control animals (IARC 1991).
Study duration	+++ Duration of treatment and observation was for the animals' lifespans.

Overall utility: Moderate
Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported. Only a single dose level was tested (with no justification for choosing that dose level) in a large number of male hamsters. There was relatively poor survival in both exposed and control groups.

D.3.1. Study Quality Tables: Co-carcinogen Studies

Table D-16. Finogenova (1973): Cobalt Chloride; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	++ Two dose levels (10-fold apart from each other) of cobalt were tested and were given twice a week for only 8 weeks, but this is a co-carcinogen study.
Survival	NR No survival information was reported.
Pathology	++ Only local skin tumors were reported and described as to histologic grade. Full necropsies were not conducted. Because it's a co-carcinogen study and tumors from the known carcinogen are what is of interest and the types of induced tumors are already known or expected, lack of a full necropsy is less critical than for a carcinogenicity study.
Confounding	NR No information about chemical purity or animal husbandry, including disease surveillance, was reported.
Reporting and analysis	+ Very poor reporting. There is no chemical purity, stability, or homogeneity reported and no information on animal husbandry disease surveillance, duration of observation, extent of necropsy, survival, or tumor incidence (only tumor latency was reported).
Sensitivity	
Animal model	++ Only females of non-transgenic mice were tested.

Study Utility Domain Question	Rating Rationale
Statistical power	+++ Large number of animals used, but survival was not reported.
Study duration	++ The duration of observation was unreported, but was at least 24 weeks, however, this is a co-carcinogen study.
Assay utility	+ Co-Carcinogen study

Overall utility: Low
All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. A large number of female animals per group were given two dose levels. However, there was very poor reporting. Incidence was not reported, only tumor latency and onset were reported. Survival, chemical purity or stability, animal husbandry, duration of observation, and extent of necropsy were not reported.

Table D-17. Kasirsky et al. (1965): Cobalt Chloride; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	++ A single dose level of the known carcinogen and four dose levels of cobalt chloride were given for about 10 weeks, though the exact duration of exposure was not clearly reported.
Survival	+ Survival was only reported for three dose levels of cobalt chloride in the first trial, but not in any groups of the second trial or the carcinogen alone control of the first trial. The high cobalt chloride dose level of the first trial reported conflicting survival between Table 1 and the summary at the end of the paper.
Pathology	+ No necropsies were performed, just measurment of tumor size by external examination and histological exam of excised tumors.
Confounding	++ Very few details were reported about animal husbandry and no information was reported about chemical purity.
Reporting and analysis	++ Results were not reported per sex and the duration was not clearly reported. Survival for all groups, chemical purity, and animal husbandry were not reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.

Study Utility Domain Question	Rating Rationale
Statistical power	++ Sufficient number of animals of each sex, but results were not reported as per sex. Survival was not clearly reported.
Study duration	++ It was not clearly reported, but is thought to have been at least 72 days, though this is a co-carcinogen study.
Assay utility	+ Co-carcinogen study

Overall utility: Low
All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Neither duration, survival, chemical purity, stability or animal hubandry were clearly reported. Results were not reported per sex. A full necropsy was not conducted, so this study is only relevant to skin tumors induced by the carcinogen.

Table D-18. O'Hara et al. (1971): Cobalt Chloride; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ There was no untreated group and no cobalt alone group, but it's similar to a co-carcinogen study and did have a known carcinogen alone group.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	Inadequate Cobalt was administered after tumors had already started to develop; this is more like a tumor treatment study. Only two very similar dose levels were tested: 50 and 60 mg/kg.
Survival	+++ The high dose level caused an increase in mortality, however low survival was caused by tumors. Statistical analysis was not done on survival.
Pathology	++ No full necropsies were reported; only local skin tumors were reported.
Confounding	++ Very few details reported about animal husbandry and no information reported about chemical characteristics.
Reporting and analysis	++ Chemical purity or stability or disease surveillance methods were not reported and animal husbandry conditions were poorly reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.

Study Utility Domain Question	Rating Rationale
Statistical power	+++ The high-dose level group had a decrease in survival, but statistics were not calculated for survival and the low survival was caused by tumors.
Study duration	++ 17 weeks, but this is a co-carcinogen study and neoplasms were induced before cobalt exposure started.
Assay utility	Inadequate Co-carcinogen study

Overall utility: Inadequate
This study has little utility for evaluation because the cobalt was not administered until after tumors had developed. No necropsies were performed; it only looked at local tumors. Only two, closely spaced dose levels were tested. A good number of mice per group was used. Chemical purity or stability and animal husbandry or disease surveillance were not reported. All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity.

Table D-19. Zeller (1975): Cobalt Chloride; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ A cobalt chloride alone group was said to have been tested, but no results of that group were reported. The reported control was the known carcinogen alone, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+ Only one dose level of cobalt, given for 43 weeks, was tested with no rationale for choosing that dose level.
Survival	+++ There was no significant effect on survival from cobalt.
Pathology	++ Pathology procedures not described but histopathological evaluations were done on the respiratory tract and the liver.
Confounding	NR No information on animal husbandry conditions, disease surveillance, or chemical purity were reported.
Reporting and analysis	+ Poor reporting of chemical purity or stability, animal husbandry, or necropsy methods. Tumor incidences were reported as both sexes combined, making it impossible to examine sex differences.
Sensitivity	

Study Utility Domain Question	Rating Rationale
Animal model	++ Both sexes of non-transgenic rats were tested; however, tumor incidences were reported as both sexes combined.
Statistical power	+ Only 12 animals per group per sex were tested, but tumor incidences were reported as both sexes combined.
Study duration	+++ Duration of treatment was 43 weeks and duration of observation was for the animals' lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low
All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. The study had a long duration of observation. However, poor reporting of chemical purity/stability, animal husbandry, necropsy methods, or necropsy with histological descriptions only of the respiratory tract and liver. Only one dose level was tested, without a reported rationale, on a small number of males and females, with tumor incidences reported as both sexes combined.

Table D-20. Orzechowski et al. (1964): Sodium Cobaltinitrite; Injection

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+++ Six dose levels were given over 72 days and maximum dose level was based on toxicity in preliminary studies.
Survival	+++ Survival was high and similar to known carcinogen only controls.
Pathology	+ Necropsies were not done, only histological examination of tumors were done.
Confounding	++ Very few details reported about animal husbandry and no information was reported about chemical purity.
Reporting and analysis	+ No chemical purity or animal husbandry were reported. Tumor incidences were reported as both sexes combined.

Study Utility Domain Question	Rating Rationale
Sensitivity	
Animal model	++ Both sexes of non-transgenic mice were used, though tumor incidence were only reported as both sexes combined.
Statistical power	+++ Large number of animals per group and experiments were conducted in triplicate and survival was high and similar to controls.
Study duration	++ A very short duration of 75 days was used, but it's a co-carcinogenicity study and tumors were induced.
Assay utility	+ Co-carcinogen study

Overall utility: Low
All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Tumor incidences were reported as both sexes combined. No information on chemical purity or stability or animal husbandry were reported. Six dose levels were tested, which were based on preliminary studies and a high number of animals per group was used, with experiments conducted in triplicate. Necropsies were not performed; histological examination was conducted only on tumors.

Table D-21. Thompson et al. (1965): Sodium Cobaltinitrite; Drinking Water

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+++ Three dose levels were tested. The duration of treatment was not reported, but assumed to be about 11 weeks, but this is a co-carcinogen study.
Survival	NR No survival information was reported.
Pathology	+ No full necropsies were performed, just histology of the tumors and hematology measurements.
Confounding	++ Very little information about animal husbandry and no information about chemical purity or disease surveillance.

Study Utility Domain Question	Rating Rationale
Reporting and analysis	++ Nothing was reported for chemical purity or stability, disease surveillance, survival, or duration of treatment. Dosing regimen and duration of observation were not clearly reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	+++ Large number of animals per group, but survival was not reported.
Study duration	++ Not clearly reported, assumed to be about 11 weeks, but this is a co-carcinogen study.
Assay utility	+ Co-carcinogen study

Overall utility: Low
All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Both sexes of mice were tested with three dose levels. Nothing was reported for chemical purity or stability, survival, duration of treatment, and animal husbandry and the dosing regimen were not clearly reported. Full necropsies were not done; histology was only performed on tumors.

Table D-22. Steinhoff and Mohr (1991): Cobalt Oxide; Intratracheal

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ No untreated control was used, but a benzo[*a*]pyrene-only control was included, which is consistent with a co-carcinogenicity study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
Dosing regimen	+ Only a single dose level was used and given for 47 weeks for cobalt and 13 wk for benzo[*a*]pyrene.
Survival	NR No survival information was reported.
Pathology	++ Only organs with gross lesions and the respiratory tract were examined histologically.
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure." Animal husbandry was described, but disease surveillance was not reported.

Study Utility Domain Question	Rating Rationale
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical and animal husbandry.
Sensitivity	
Animal model	++ Only female rats were tested.
Statistical power	+++ A large number of animals were used and survival was similar to controls.
Study duration	++ Treatment with cobalt was for 47 weeks, benzo[*a*]pyrene was given for 13 wk, while observation was lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low
All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. One dose level was tested on a high number of females for almost a year, with observations for their lifespan. However, only partial necropsies were performed. Details of the chemical, animal husbandry, and survival were not reported.

Table D-23. Wehner et al. (1977): Cobalt Oxide; Inhalation

Study Utility Domain Question	Rating Rationale
Selection of animals	
Controls	+++ Controls were cigarette smoke alone.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ There was no information on chemical purity or stability of the cobalt and the composition of the cigarettes is complex and variable. However, information on the type of research cigarette (Kentucky IRI research cigarettes) was provided.
Dosing regimen	++ Single dose level with no justification for choosing that level, but administered for life.
Survival	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls.
Pathology	+++ Detailed necropsies were performed.
Confounding	+ Chemical purity and disease surveillance were not reported. The uncertainty of the composition of the cigarettes may contribute to confounding.

Study Utility Domain Question	Rating Rationale
Reporting and analysis	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as “carcinoma” or “polyp” without saying from which tissue they originated is meaningless.
Sensitivity	
Animal model	++ Only male hamsters were tested.
Statistical power	+++ A large number of animals were used and survival was similar to controls.
Study duration	+++ Duration of treatment and observation was for the animals' lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported. Only a single dose level was tested (with no rationale for choosing that dose level).

Table D-24. Overview of Experimental Animal Co-carcinogenicity Study Quality Evaluations

Study	Metal	Controls	Historical Data	Random-ization	Purity	Dosing	Survival	Pathology	Con-founding	Reporting & Analysis	Animal Model	Stats	Duration	Assay Utility	Overall Utility
Finogenova (1973)	**Cobalt chloride**	+++	**No**	**NR**	**NR**	++	**NR**	++	**NR**	+	++	+++	++	+	**Low**
Kasirsky et al. (1965)	**Cobalt chloride**	+++	**No**	**NR**	**NR**	++	+	+	++	++	+++	++	++	+	**Low**
O'Hara et al. (1971)	**Cobalt chloride Sodium cobaltinitrite**	+++	**No**	**NR**	**NR**	**0**	+++	++	++	++	+++	+++	++	**0**	**Inadequate**
Zeller (1975)	**Cobalt chloride**	+++	**No**	**NR**	**NR**	+	+++	++	**NR**	+	++	+	+++	+	**Low**
Orzechowski et al. (1964)	**Sodium cobaltinitrite**	+++	**No**	**NR**	**NR**	+++	+++	+	++	+	++	+++	++	+	**Low**
Thompson et al. (1965)	**Sodium cobaltinitrite**	+++	**No**	**NR**	**NR**	+++	**NR**	+	++	++	+++	+++	++	+	**Low**
Steinhoff and Mohr (1991)	**Cobalt oxide**	+++	**No**	**NR**	++	+	**NR**	++	++	++	++	+++	++	+	**Low**
Wehner et al. (1977)	**Cobalt oxide**	+++	**No**	**NR**	+	++	+++	+++	+	+	++	+++	+++	+	**Low**

+++ = high quality/little to no concerns, ++ = moderate quality/moderate concerns, + = low quality/high concerns, 0 = inadequate, NR = not reported.

Appendix E. Genotoxicity and Cellular Transformation

Tables of Contents

Tables

This section describes the assessment of studies evaluating genetic and related effects from exposure to cobalt compounds and provides the background for the discussion of genotoxicity as a possible mode of action for cobalt-induced carcinogenicity (see Section 6). The genotoxicity data summarized in Section 6.2.1 and Table 6-2 are described more fully below.

Cobalt metal and several cobalt compounds have been tested in short-term assays to evaluate mutagenicity, DNA damage, and other potential genotoxic effects. These compounds include several forms of cobalt: (1) the water-soluble salts cobalt chloride (and its hexahydrate), cobalt sulfate (and its heptahydrate), and cobalt nitrate (and its hexahydrate); (2) a water-insoluble compound, cobalt oxide; (3) an organic water-soluble compound, cobalt acetate; (4) cobalt metal and (5) the cobalt particles: cobalt sulfide(s) and cobalt nanoparticles. Most of the genotoxicity studies identified reported on tests using cobalt chloride (or its hexahydrate). The specific cobalt compound form (i.e., hydrate) tested is indicated when provided by the study authors. The oxidation state of the cobalt salts, oxide and acetate compounds in this section is +2 (cobalt(II)), unless indicated otherwise.

A discussion of the genotoxic effects of certain cobalt compounds reported for in vitro and in vivo assays are presented below and a compilation of studies is provided in tabular form for each section. An overall summary call for genetic and related effects is provided for the compounds by endpoint in Table 6-2; the calls are based on the integration of the evidence from an authoritative review (namely IARC 2006) and several primary studies published since the IARC review.

E.1. In Vitro Mutagenicity and DNA-damage Studies of Cobalt Compounds in Bacteria

Cobalt metal and two water-soluble cobalt salts (cobalt chloride and its hexahydrate, cobalt sulfate heptahydrate) were tested for mutagenicity in prokaryotic (bacterial) systems (see Table E-1 for study details and sources not provided in text). Results for mutagenicity are mostly negative for cobalt compounds in bacterial tester strains; however, there were some positive results in a few studies that detect mutations involving GC base pairs, although the evidence was not entirely consistent.

Cobalt chloride was reported to be mutagenic in only some of the studies in three *Salmonella* strains (TA97, TA98, and TA1537) (Pagano and Zeiger 1992; Wong 1988) but not in other strains (TA100, TA102, TA1535, TA1538, TA2637). It was also mutagenic in one of several studies in *Escherichia coli* (Ogawa et al. 1999) but not *Bacillus subtilis*. Cobalt sulfate heptahydrate was positive in TA100 but not in TA98 and TA1537. A growth inhibition assay for cobalt chloride in *B. subtilis* had mixed results in two studies; however, the test that showed growth inhibition (positive results) was conducted using preincubation, which is a more sensitive assay that the standard plate incorporation assay. Cobalt metal was positive in the *Salmonella* strains in which it was tested (TA98 and TA100) but not in *E. coli*.

Positive results in a particular set of *Salmonella typhimurium* and *E. coli* bacterial tester strains can suggest very specific types of mutations. Tester strains TA100, TA102 and TA1535 are generally considered indicators of base-pair substitution. The frameshift mutation detected by *S. typhimurium* strain TA98 is a disruption of a dinucleotide run of $(CG)_4$ residues, while TA100 detects base-pair mutations in a codon for proline (GGG sequence) in the *histidine G46* gene.

Reverse mutations at the trpE ochre (TAA) codon can be identified by positive results in the *E. coli* WP2 *uvrA*/pKM101 strain. The results for these strains in the studies identified in this review are not strong; however, sequencing of the *supF* tRNA mutational reporter gene in bacteria exposed to cobalt chloride showed that both frameshift and base-pair substitution occurred at G:C base pairs (Ogawa et al. 1999).

Overall, the results for cobalt-induced bacterial mutagenicity are considered mostly negative without the addition of S9 and are completely negative in all assays with S9. The negative results for the assays that included the addition of S9 (and which were positive without S9) may be due to the presence of radical-scavenging enzymes in the mixture, which could eliminate a mutagenic effect; alternatively, proteins in the S9 mixture could bind the cobalt ions, rendering them ineffective as a mutagen.

Some studies reported anti-mutagenic effects. Potent anti-mutagenic effects were observed for cobalt chloride on reverse mutations induced by 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) in *Salmonella* strains TA98 and TA1538. Cobalt chloride hexahydrate showed an anti-mutagenic effect (inhibition of spontaneous mutation) when tested in *B. subtilis* (Inoue et al. 1981).

Table E-1. In Vitro Mutagenicity and DNA-damage Studies of Cobalt Compounds in Bacteria

Compound	Reference	LED/HID	Results −S9	Results +S9	Comments and Conclusions
Reverse mutation/ Salmonella typhimurium/TA100					
Cobalt chloride	Ogawa et al. (1986)*	NR	−		Negative −S9
Cobalt chloride hexahydrate	Tso and Fung (1981)* Arlauskas et al. (1985)*	23,800 µg/mL NR	− −		Negative −S9 in two studies
Cobalt sulfate heptahydrate	Zeiger et al. (1992), NTP (1998)	100 µg/plate	+	(+)	Positive −S9; weak positive −+S9
Cobalt metal	NTP (2014d)	500 µg/plate −S9 7,500 µg/plate +S9	(+)	−	Weak positive −S9; negative +S9
Reverse mutation/Salmonella typhimurium/TA102					
Cobalt chloride	Wong (1988)*	40 µg/mL [approx. 100 µg/plate]	−	−	Negative ±S9
Reverse mutation/Salmonella typhimurium/TA1535					
Cobalt chloride	Arlauskas et al. (1985)* Wong (1988)*	NR 40 µg/mL	− −	 −	Negative −S9 in 2 of 2 studies; negative +S9
Cobalt sulfate heptahydrate	Zeiger et al. (1992) NTP (1998)	10,000 µg/plate	−	−	Negative ±S9
Reverse mutation/Salmonella typhimurium/TA97					
Cobalt chloride	Pagano and Zeiger (1992)*	13 µg/mL [approx. 32 µg/plate]	+		Positive −S9; Preincubation assay (generally more sensitive than standard plate incorporation assay)
Reverse mutation/Salmonella typhimurium/TA98					
Cobalt chloride	Arlauskas et al. (1985)* Ogawa et al. (1986)* Wong (1988)*	NR NR 40 µg/mL [approx. 100 µg/plate]	− − +	 −	Positive −S9 in 1 of 3 studies; negative +S9
Cobalt chloride hexahydrate	Mochizuki and Kada (1982)*	20 µg/mL	−		Anti-mutagenic effect on Trp-P-1-induced reverse mutations; same effect in TA1538, so independent of plasmid pKM101 (which is in TA98 but not TA1538).

Compound	Reference	LED/HID	Results		Comments and Conclusions
			−S9	+S9	
Cobalt sulfate heptahydrate	Zeiger et al. (1992) NTP (1998)	10,000 µg/plate	−	−	Negative ±S9
Cobalt metal	NTP (2014d)	100 µg/plate −S9 7,500 µg/plate +S9	+	−	Positive −S9 (weak effect, not well-correlated with dose); negative +S9
Reverse mutation/Salmonella typhimurium/TA1537					
Cobalt chloride	Arlauskas et al. (1985)* Ogawa et al. (1986)* Wong (1988)*	NR 130,000 µg/plate 40 µg/mL [approx. 100 µg/plate]	− − +	 −	Positive −S9 in 1 of 3 studies; negative +S9 [note: recommended maximum dose for assay is generally 5,000 to 6,000 µg/plate, depending on toxicity]
Reverse mutation/Salmonella typhimurium/TA1538					
Cobalt chloride	Arlauskas et al. (1985)*	NR	−		Negative −S9
Cobalt chloride hexahydrate	Mochizuki and Kada (1982)*	20 µg/mL	−		Anti-mutagenic effect on Trp-P-1-induced reverse mutations; same effect in both strains, independent of plasmid pKM101, which is in TA98 but not TA1538
Reverse mutation/Salmonella typhimurium/TA2637					
Cobalt chloride	Ogawa et al. (1986)*	130,000 µg/plate	−		Negative −S9; strain detects bulky DNA adduct formation
Mutation/Escherichia coli strain WP2 uvrA/pKM101					
Cobalt chloride hexahydrate	Arlauskas et al. (1985)*	NR	−		Negative −S9
Cobalt chloride hexahydrate	Kada and Kanematsu (1978)* Leitao et al. (1993)*	20 µg/mL 50 µg/mL	− −		Negative −S9 Induced anti-mutagenic effect (inhibition of mutagenesis induced by N-methyl-N′-nitrosoguanidine) in two studies
Cobalt metal	NTP (2014d)	450 µg/plate	−	−	Negative ±S9
Mutation/ Escherichia coli strain SY1032/pKY241 supF tRNA locus					
Cobalt chloride	Ogawa et al. (1999)*	2.6 µg/mL	+		Positive −S9
Prophage induction/Escherichia coli					
Cobalt chloride	Rossman et al. (1984)*	415 µg/mL [approx. 1,037 µg/plate]	−		Negative −S9

Compound	Reference	LED/HID	Results −S9	Results +S9	Comments and Conclusions
Reverse mutation/Bacillus subtilis strain NIG 1125					
Cobalt chloride hexahydrate	Inoue et al. (1981)*	30 µg/mL	−		Anti-mutagenic effect (inhibition of spontaneous mutation)
Growth inhibition/Bacillus subtilis rec strain strains H17					
Cobalt chloride	Nishioka (1975)*	325 µg/plate	−		Positive −S9 in 1 of 2 studies; positive study used 'cold preincubation' procedure
	Kanematsu et al. (1980)*	325 µg/plate		+	

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weak positive, − = negative.

E.2. Genotoxicity Studies of Cobalt Compounds in Non-mammalian Eukaryotes

Cobalt water-soluble compounds (cobalt chloride, cobalt nitrate, and cobalt sulfate) and nanoparticles were tested for mutations, DNA damage and chromosomal damage in numerous studies in non-mammalian eukaryotes. Mostly positive effects were observed in yeast, plants, insects, nematodes, and zebrafish, for genotoxic activity of a variety of cobalt compounds for the evaluated endpoints. These include mutation (cobalt chloride and cobalt nitrate hexahydrate), gene conversion (cobalt chloride), DNA damage (cobalt chloride, cobalt nitrate hexahydrate, and cobalt sulfate), chromosomal aberration (cobalt chloride and cobalt sulfate) and aneuploidy (cobalt sulfate). Recombination was reported as treatment-related in *Drosophila* studies on cobalt chloride, cobalt nitrate hexahydrate, and cobalt nanoparticles. None of these studies reported using the addition of a metabolic activation mixture (S9). The results of the genotoxicity studies of cobalt compounds tested in non-mammalian eukaryotes are described below and are summarized in Table E-2.

In fungi, cobalt chloride treatment resulted in induction of gene mutation and conversion in several assays. In the yeast *Saccharomyces cerevisiae*, cobalt chloride was at least weakly mutagenic in five of eight studies identified (Egilsson et al. 1979; Kharab and Singh 1985; 1987; Prazmo et al. 1975; Putrament et al. 1977), depending on the type of mutation. Respiratory deficiency mutations are consistently positive, while others, such as for the *ilv* gene, were negative; the significance of this difference is not clear. Gene conversion was observed as at least weakly positive at the *trp* locus in the yeast *S. cerevisiae* D7 in all three of the studies reported (Fukunaga et al. 1982; Kharab and Singh 1985; Singh 1983).

Studies on tissues from two plants reported DNA damage due to exposure to cobalt chloride for *Allium cepa* bulbs (Yildiz et al. 2009) and cobalt nitrate hexahydrate treatment of *Zea mays* seedlings (Erturk et al. 2013). Chromosomal aberrations were reported after cobalt chloride exposure in the Yildiz et al. (2009) study and also in an earlier *A. cepa* study with cobalt sulfate (Gori and Zucconi 1957), which also reported the induction of aneuploidy.

Cobalt soluble salts (cobalt chloride and cobalt nitrate hexahydrate) caused somatic mutation and/or recombination in *Drosophila melanogaster* fruit flies strain *mwh/flr3* (Ogawa et al. 1994; Vales et al. 2013; Yesilada 2001). In the study of cobalt nanoparticles, Vales et al. (2013) used the somatic mutation wing spot assay with strain *mwh/TM3* to distinguish somatic mutations from recombination that suggested that the genotoxic effect for the *mwh/flr3* is not due to mutation (see study details in Table E-2).

Fish have been used to assess genotoxic effects of cobalt compounds. Direct DNA damage was reported in sperm from exposed male zebrafish (*Danio rerio*) for both cobalt chloride and cobalt sulfate, showing a dose-dependent increase for both compounds.

Table E-2. Genotoxicity Studies of Cobalt Compounds in Non-mammalian Eukaryotes

Compound	Reference	LED/HID		Results (−S9)[a]	Comments and Conclusions
FUNGI (Yeast)					
Mutation/Saccharomyces cerevisiae					
Cobalt chloride	Prazmo et al. (1975)*	'Petite' mutation	260 µg/mL	+	Some positive results for mutation in yeast, especially respiratory deficiency type
	Egilsson et al. (1979)*	SBTD-2B, respiratory deficiency	640 µg/mL	(+)	
	Putrament et al. (1977)*	Respiratory deficiency	520 µg/mL	+	
	Putrament et al. (1977)*	Strain 197/2d	520 µg/mL	−	
		Erythromycin-resistant mutation/ilv mutation DL7:			
	Fukunaga et al. (1982)*	1,300 µg/mL		−	
	Singh (1983)*	13,000 µg/ml		−	
	Kharab and Singh (1985)*	3,000 µg/mL		(+)	
	Kharab and Singh (1987)*	'Petite' mutation/DL7 respiratory deficiency: 750 µg/mL		+	
Gene conversion (trp)/Saccharomyces cerevisiae D7					
Cobalt chloride	Fukunaga et al. (1982)*	1,300 µg/mL		+	Positive for 3 of 3 studies
	Singh (1983)*	13,000 µg/mL		(+)	
	Kharab and Singh (1985)*	1,500 µg/mL		(+)	
PLANTS (Onion or corn)					
DNA damage/Allium cepa or Zea mays					
Cobalt chloride	Yildiz et al. (2009)	5.5 ppm		+	Positive in comet assay in Allium cepa bulbs
Cobalt nitrate hexahydrate	Erturk et al. (2013)	5 mM		+	Genomic template instability increases with cobalt exposure levels in Zea mays seedlings
Chromosomal aberrations/Allium cepa					
Cobalt chloride	Yildiz et al. (2009)	5.5 ppm		+	Positive in anaphase-telophase chromosome aberration assay in Allium cepa bulbs
Cobalt sulfate	Gori and Zucconi (1957)*	3 µg/mL		+	Positive results

Compound	Reference	LED/HID	Results (−S9)[a]	Comments and Conclusions
Aneuploidy/Allium cepa				
Cobalt sulfate	Gori and Zucconi (1957)*	15 µg/mL	+	Positive results/ dosed 5 d, then water 3d
INSECTS (Drosophila melanogaster; fruit fly)				
Mutation or mitotic recombination/ wing spot test				
Cobalt chloride	Ogawa et al. (1994)*	*mwh/flr* 260 µg/mL	+	Positive results for single and total mutant spots at high doses (10 mM) of ionic cobalt indicate CoCl2 is more genotoxic than nanoparticles in this assay (see below).
	Ogawa et al. (1994)*	*mwh/TM3* 1,040 µg/mL	−	
	Vales et al. (2013)	*mwh/flr*^{3} wings		
		small spots 10 mM	+	
		large spots 10 mM	i	
		twin spots 10 mM	i	
		total 10 mM	+	
Cobalt nitrate hexahydrate	Yesilada (2001)*	Strain *mwh/flr*^{3} wings		Positive effects; additional details were not provided in review paper
		Mutations, chromosomal deletion, nondisjunction 291 µg/mL	+	
		Mitotic recombination 2,910 µg/mL	+	
Cobalt nanoparticles	Vales et al. (2013)	*mwh/flr*^{3} wings		Dose-dependent induction of small, but not large, spots indicates slow progression of nanoparticles to reach the wing imaginal disks. Single mutant spots result from both somatic mutation and somatic recombination; twin spots only result from somatic mutation. Results negative for this assay, suggesting that effect for experiment above is due to somatic recombination and <u>not</u> mutation.
		small spots 1 mM	+	
		large spots 10 mM	−	
		twin spots 10 mM	i	
		total 5 mM	+	
		mwh/TM3 wings		
		small spots 1 mM	i	
		large spots 10 mM	−	
		total 10 mM	i	

Compound	Reference	LED/HID	Results (−S9)[a]	Comments and Conclusions
FISH (Danio rerio; zebrafish)				
DNA damage				
Cobalt chloride	Reinardy et al. (2013)	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water
Cobalt sulfate	Reinardy et al. (2013)	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, − = negative, i = inconclusive.
[a]No studies reported testing with +S9.

E.3. In Vitro Studies of Genotoxicity of Cobalt Compounds in Mammalian Cells

Cobalt compounds have been evaluated for genotoxic effects in mammalian cells in vitro in rodent (mouse, hamster, and rat) and human cells. In general, no major species differences (with one possible exception) were observed, albeit not all types of effects were tested in cells from all species. Although most studies tested cobalt chloride, a greater number of different cobalt compounds were tested compared to the other experimental systems including another soluble cobalt salt (cobalt sulfate), an organic water-soluble cobalt compound and insoluble cobalt forms including cobalt metal, cobalt nanoparticles, and cobalt sulfide particles.

Overall, there is strong evidence that all types of cobalt compounds are clastogenic and induced DNA damage in both human and animal cells and most of the compounds (except cobalt metal) caused cellular transformation in animal cell lines (see Section E.7). There is also some evidence that a soluble cobalt compound (cobalt chloride, which was the only compound tested for most of the endpoints) induces sister chromatid exchange and aneuploidy. However, mixed results were reported for mutagenicity in animal cells for a variety of cobalt compounds and chromosomal aberrations in human cells. Cobalt nanoparticles caused micronuclei in both rodent and human cells; however, findings for other compounds differ by species with positive findings for cobalt chloride and cobalt metal in human cells and negative findings for cobalt chloride in rodent cells.

All of the described studies in mammalian cells were performed without the addition of exogenous S9 metabolic activation mixture. Results for the in vitro studies in mammalian cells are discussed below and genotoxic and related effects are summarized in Table E-3 and Table E-4.

E.3.1. Rodent Cells

Rodent cells were tested in vitro for genotoxicity (mutagenicity, DNA strand breaks, sister chromatid exchange, and micronuclei) and related effects (i.e., cellular transformation and apoptosis) with soluble cobalt salts (cobalt chloride) and some relatively insoluble forms or particles (cobalt oxide, cobalt sulfides and particles, cobalt metal and nanoparticles) (see Table E-3).

There is strong evidence that different types of cobalt compounds (both soluble and relatively insoluble forms) cause DNA damage. Positive results were reported for cobalt chloride, cobalt metal, and cobalt nanoparticles in BALB/3T3 cells (Anard et al. 1997; Ponti et al. 2009); cobalt chloride and cobalt sulfides in Chinese hamster ovary (CHO) cells (Hamilton-Koch et al. 1986; Robison et al. 1982), as well as in rat neuronal PC12 cell mitochondria (Wang et al. 2000). The only negative study reported using a different type of assay (nucleoid sedimentation) in CHO cells to test cobalt chloride (Hamilton-Koch et al. 1986).

Cobalt assays for mutagenicity generally gave negative results; conflicting results may be due in part to the type of loci evaluated in the various assays. Cobalt chloride and cobalt sulfide caused mutations in studies using V79 Chinese hamster fibroblast cells *Hprt* locus (Hartwig et al. 1990; Miyaki et al. 1979) and for the transgenic G12 *Gpt*, but not for the normal fibroblast *Gpt* locus (Kitahara et al. 1996). Both assays testing cobalt chloride hexahydrate were negative, but they

tested different gene loci, one at the *Tk* locus of mouse lymphoma L5178Y cells (Amacher and Paillet 1980) and the 8AG locus of V79 cells (Yokoiyama et al. 1990). Thus, the disparity of results may be due to the specific locus tested in these assays; the *Hprt* locus was positive in the two studies where it was evaluated for cobalt chloride, while the other assays looked at different loci. Mixed results were also reported for cobalt sulfide tested in a Chinese hamster transgenic cell line; the *Gpt* locus for G10 was negative while the G12 strain tested positive. These cells lines have different *Gpt* locus insertion sites and differ in their response to clastogens. When compared with G10, the G12 strain has a lower spontaneous mutant frequency (30 compared with 100 per million cells) and is highly sensitive to insoluble metal (nickel) compounds, with mutant induction of 20 to 30 fold for G12, compared with only 2 to 3 times the number of spontaneous mutants induced for G10 (Klein et al. 1994).

In cytogenetic assays, cobalt chloride induced sister chromatid exchange (SCE) in mouse macrophage-like cells (Andersen 1983). SCE involves double-strand DNA breaks and is induced by agents that form DNA adducts or interfere with DNA replication and/or repair. Cobalt nanoparticles (Ponti et al. 2009), but not cobalt chloride (Ponti et al. 2009; Suzuki et al. 1993), caused micronucleus induction.

E.3.2. Human Cells

Human cells were tested in vitro for genotoxicity (DNA strand breaks, sister chromatid exchange, micronuclei, chromosomal aberrations, and aneuploidy) with soluble cobalt salts (cobalt acetate, cobalt chloride, cobalt nitrate, and some relatively insoluble forms or particles (cobalt oxide, cobalt metal, and nanoparticles) (see Table E-3).

There is strong evidence that different types of cobalt compounds caused DNA damage after exposure in vitro in human cells, similar to that of the rodent cells described previously. DNA damage, such as strand breaks, was reported after cobalt chloride treatment in assays in several human cell lines including diploid fibroblasts, mononuclear leukocytes, HepG2 cells, H460 lung epithelial cells, and T-cells (Alarifi et al. 2013; Caicedo et al. 2007; Davies et al. 2005; De Boeck et al. 1998; Hamilton-Koch et al. 1986; Hartwig et al. 1990; McLean et al. 1982; Patel et al. 2012). Negative results were reported in studies that used different techniques like nucleoid sedimentation (Hamilton-Koch et al. 1986) or different cell types like peripheral blood leukocytes (Colognato et al. 2008). Interestingly, T-cells did not show DNA damage in the comet assay for cobalt chloride but did for cobalt nanoparticles in the same study (Jiang et al. 2012). Treatment with cobalt metal also gave very strong positive results for lymphocytes, mononuclear leukocytes, and normal fetal fibroblasts (Anard et al. 1997; De Boeck et al. 1998; De Boeck et al. 2003b; Qiao and Ma 2013; Van Goethem et al. 1997). Cobalt nanoparticles and cobalt oxide nanoparticles gave positive results in all identified studies for lymphocytes, HepG2 cells, A549 lung epithelial cells, and bronchial BEAS-2B bronchial cells (Alarifi et al. 2013; Cavallo et al. 2015; Colognato et al. 2008; Jiang et al. 2012; Kain et al. 2012; Wan et al. 2012).

Evidence that cobalt compounds cause chromosomal damage comes primarily from studies using human lymphocytes or lung fibroblast cells. Both soluble (cobalt chloride) and insoluble (cobalt metal and cobalt nanoparticles) cobalt forms induced micronucleus formation (Colognato et al. 2008; De Boeck et al. 2003b; Miller et al. 2001; Van Goethem et al. 1997). Chromosomal aberrations were evaluated after exposure to various forms of cobalt, with mixed results possibly related to cell type or exposure level and not compound solubility. Cobalt chloride hexahydrate

and cobalt oxide were positive for aberrations in lung fibroblast cells (Figgitt et al. 2010; Smith et al. 2014); however, exposure to cobalt oxide, cobalt acetate tetrahydrate, and cobalt nitrate did not induce chromosomal aberrations in lymphocytes, diploid fibroblasts or mononuclear leukocytes (Paton and Allison 1972; Voroshilin et al. 1978). These results appear to be related to cell type but not compound solubility, although intracellular soluble cobalt has been shown to be more cytotoxic than particulate cobalt in human lung fibroblasts at levels above 1 mM. For example, the relative survival for 1.7 mM cobalt chloride treated cells was 29% but was 55% survival for the same concentration of cobalt oxide (Smith et al. 2014). Regarding the negative results in the study by Paton and Allison, the top dose of 0.015 μg/mL cobalt nitrate to treat fibroblasts may have been too low to see an effect in the assay.

Cobalt chloride induced sister chromatid exchange in lymphocytes (Andersen 1983) as well as aneuploidy in lymphocytes and primary fibroblasts (Figgitt et al. 2010; Resende de Souza Nazareth 1976).

Table E-3. In Vitro Studies of Genotoxic Effects of Cobalt Compounds in Mammalian Cells

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
RODENT CELLS				
Mutation/V79 Chinese hamster lung fibroblasts (V79) or Mouse lymphoma (MOLY), hamster transgenic cell line or BALB/3T3 mouse cells				
Cobalt chloride				V79—mixed results
	Miyaki et al. (1979)*	26 μg/mL	(+)	*Hprt* locus
	Hartwig et al. (1990)*	13 μg/mL	+	*Hprt* locus
	Kitahara et al. (1996)*	13 μg/mL	−	*Gpt* locus
	Kitahara et al. (1996)*	6.5 μg/mL	+	Transgenic G12, *Gpt* locus
Cobalt chloride hexahydrate	Yokoiyama et al. (1990)*	2 μg/mL	−	V79-8AG locus, negative results
	Amacher and Paillet (1980)*	57.11 μg/mL	−	MOLY L5178Y cells, *Tk* locus, negative results
Cobalt sulfide (CoS_2 and CO_3S_4) particles				Chinese hamster transgenic cell lines (derived from V79)
	Kitahara et al. (1996)*	1 μg/mL	−	G10, *Gpt* locus, negative results
		0.5 μg/mL	+	G12, *Gpt* locus, positive results
DNA damage/strand breaks/alkaline elution, sucrose gradient, or Q-PCR/3T3 mouse cells, Chinese Hamster Ovary (CHO) cells, BALB/3T3 cells/neuronal cell mitochondria				
Cobalt chloride	Hamilton-Koch et al. (1986)*	260 μg/mL (ASG)	+	CHO cells—positive using alkaline sucrose gradient but not
	Hamilton-Koch et al. (1986)*	1,300 μg/mL (NS)	−	nucleoid sedimentation in the same study
	Ponti et al. (2009)	1 μM	+	Positive in BALB/3T3 cells—2 hr. exposure to sub-toxic dose
	Wang et al. (2000)	100 μM	+	Positive in rat neuronal PC12 cell mitochondria
Cobalt (metal)	Anard et al. (1997)*	1 μg/mL	+	BALB/3T3 mouse cells—positive for alkaline elution; used purified DNA
Cobalt sulfides (CoS_2 and CO_3S_4) particles	Robison et al. (1982)*	10 μg/mL	+	CHO cells—Positive using sucrose gradient
Cobalt metal nanoparticles	Ponti et al. (2009)	1 μM	+	Positive in BALB/3T3 cells—2 hr. exposure to sub-toxic dose
Micronucleus formation/mouse cells				
Cobalt chloride	Ponti et al. (2009)	10 μM	−	Negative for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
Cobalt chloride hexahydrate	Suzuki et al. (1993)*	50 μg/mL	−	Negative for MN induction in BALB/c mouse bone marrow

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cobalt metal nanoparticles	Ponti et al. (2009)	1 μM	+	Positive for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
Sister chromatid exchange/mouse macrophage-like cells P388D$_1$				
Cobalt chloride	Andersen (1983)*	13 μg/mL	+	Positive for SCE induction
HUMAN CELLS				
DNA damage - strand breaks/several cell types/alkaline elution, alkali-labile sites, or comet assay				
Cobalt chloride				Mostly positive results
	McLean et al. (1982)*	6.5 μg/mL	+	White blood cells—fluorescence analysis of DNA unwinding
				Diploid fibroblasts
	Hamilton-Koch et al. (1986)*	650 μg/mL	+	Alkaline sucrose gradient
	Hamilton-Koch et al. (1986)*	1,300 μg/mL	+	Nick translation
	Hamilton-Koch et al. (1986)*	1,300 μg/mL	−	Nucleoid sedimentation
	Hartwig et al. (1990)*	65 μg/mL	+	Nucleoid sedimentation
	De Boeck et al. (1998)*	0.3 μg/mL	+	Mononuclear leukocytes—comet assay
	Alarifi et al. (2013)	10 μg/mL	+	HepG2 hepatocarcinoma cells (24 hr)—comet assay
	Colognato et al. (2008)	100 μM	−	Negative in peripheral blood leukocytes—comet assay
	Patel et al. (2012)	150 μM	+	Damage in H460 lung epithelial cells—comet assay
	Caicedo et al. (2007)	5 mM	+	Damage in CD4+ T-cells obtained from lymphoma Jurkat cell line
	Davies et al. (2005)	0.84 μM	+	Damage for artificial spiked fluids—comet assay
	Jiang et al. (2012)	30 μM	−	Negative for DNA damage on T-cells—comet assay
Cobalt chloride hexahydrate	Anard et al. (1997)*	25 μg/mL	−	Negative for lymphocytes using alkaline elution
Cobalt metal	Anard et al. (1997)*	3.0 μg/mL	+	Positive for lymphocytes using alkaline elution
	Anard et al. (1997)*	4.5 μg/mL	+	Positive in several studies, for mononuclear leukocytes for DNA
	Van Goethem et al. (1997)*	0.6 μg/mL	+	single-strand breaks and alkali-labile sites, and alkaline comet
	De Boeck et al. (1998)*	0.3 μg/mL	+	assay
	De Boeck et al. (2003b)*	0.6 μg/mL	+	
	Qiao and Ma (2013)	5 μM	+	Positive for normal fetal fibroblast cells in single cell array assay
Cobalt nanoparticles	Colognato et al. (2008)	50 μM	+	Positive in peripheral blood leukocytes—comet assay
	Wan et al. (2012)	5 μg/ml	+	Positive in exposed A549 lung epithelial cells—comet assay
	Jiang et al. (2012)	3 μM	+	Positive for DNA damage in T-cells—comet assay

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cobalt oxide (Co_3O_4) nanoparticles	Kain et al. (2012)	20 µg/mL	+	Positive in A549 lung cell line (DNA breaks)
	Kain et al. (2012)	20 µg/mL	+	Positive in BEAS-2B lung cell (DNA breaks and oxidative damage)
	Alarifi et al. (2013)	5 µg/mL	+	Positive in HepG2 hepatocarcinoma cells (24 hr)—comet assay
	Cavallo et al. (2015)	A549		Positive in alveolar A549 and bronchial BEAS-2B cells, for both
		20 µg/mL	+	direct and oxidative damage—comet assay
		BEAS-2B	+	
		40 µg/mL (direct)	+	
		5 µg/mL (oxidative)	+	
Micronucleus formation/ binucleates, cytochalasin-B assay/ lymphocytes or osteoblast-like cell line				
Cobalt chloride	Colognato et al. (2008)	40 µM	+	Positive in peripheral blood leukocytes, with clear trend for increase, high variability in response of donors
Cobalt metal	Van Goethem et al. (1997)*	0.6 µg/mL	+	Positive for micronuclei induction in three studies with different cell types
	Miller et al. (2001)*	0.75 µg/ml	+	
	De Boeck et al. (2003b)*	3 µg/mL	+	
Cobalt nanoparticles	Colognato et al. (2008)	40 µM	+	Increase in peripheral blood leukocytes, high variability among donors, less effective than cobalt chloride in same study
Chromosomal aberrations/ lung fibroblast cells or lymphocytes				
Cobalt chloride hexahydrate	Fairhall et al. (1949)	1.3 ppb	+	Induced significant increase of total aberrations in primary fibroblasts
	Smith et al. (2014)	50 µM	+	In WTHBF-lung fibroblast cells—soluble cobalt induces more cytotoxicity and cell cycle arrest than particulate (cobalt oxide, see below) but both produced similar levels of genotoxicity; chromosomal damage significant ($p < 0.05$).
Cobalt nitrate	Paton and Allison (1972)*	0.015 µg/mL	−	Negative in diploid fibroblasts WI38 (derived from embryonic lung
		0.15 µg/mL	−	tissue) and MRC-5 (derived from fetal lung tissue) toxic dose Negative in mononuclear leukocytes (toxic dose)
Cobalt oxide (CoO)	Smith et al. (2014)	0.5 µg/mL	+	Lung fibroblast cells—significant chromosome damage at $p < 0.05$
Cobalt acetate tetrahydrate	Voroshilin et al. (1978)*	0.6 µg/mL	−	Negative in lymphocytes
Sister chromatid exchange (SCE)/lymphocytes				
Cobalt chloride	Andersen (1983)*	1.3 µg/mL	+	Positive for SCE in lymphocytes

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Aneuploidy/ lymphocytes				
Cobalt chloride	Resende de Souza Nazareth (1976)*	3.7 µg/mL	+	Positive for aneuploidy in lymphocytes
Cobalt chloride hexahydrate	Figgitt et al. (2010)	25 ppb	+	Induced significant increase in aneuploidy in primary fibroblasts

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weakly positive, − = negative.
[a]No studies reported testing with +S9.

Table E-4. In Vitro Studies of Other (Genotoxic-related) Effects of Cobalt Compounds in Mammalian Cells

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cell transformation/C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), or BALB/3T3 cells				
Cobalt chloride	Doran et al. (1998)* Ponti et al. (2009)	5 µg/mL 70 µM	+ −	Positive in C3H10T1/2 mouse fibroblast cells Negative in BALB/3T3 cells (72 hr exposure)
Cobalt sulfate monohydrate	Kerckaert et al. (1996)*	0.125 µg/mL	+	Positive in SHE cells
Cobalt acetate	Casto et al. (1979)*	0.2 mM (approx. 35.4 µg/mL)	+	Positive for cell transformation enhancement by simian adenovirus SA7/ SHE cells
Cobalt metal	Doran et al. (1998)*	500 µg/mL	−	Negative in C3H10T1/2 mouse fibroblast cells, even at high exposure
Cobalt metal nanoparticles	Ponti et al. (2009) Sighinolfi et al. (2014) Annangi et al. (2014)	7 µM 10 µM 0.05 µg/ml	+ + +	Positive in BALB/3T3 cells (72 hr exposure) Positive in BALB/3T3 cells (72 hr exposure) Cell transformation in mouse embryo fibroblasts after 12-wk exposure to sub-toxic dose; $Ogg1^{+/+}$ and $Ogg1^{-/-}$ with knockout cells more sensitive
Cobalt sulfide (CoS, amorphous)	Abbracchio et al. (1982)* and Costa et al. (1982)*	10 µg/mL	(+)	Positive in Syrian hamster embryo cells
Cobalt sulfide (CoS_2, crystalline)	Abbracchio et al. (1982)* and Costa et al. (1982)*	1 µg/mL	+	Positive in Syrian hamster embryo cells

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose; + = positive; (+) = weakly positive; − = negative.
[a]No studies reported testing with +S9.

E.4. Protein Binding and DNA Repair Inhibition by Cobalt Compounds

Protein binding and DNA repair inhibition due to exposure to several cobalt compounds (including cobalt chloride, cobalt sulfate, cobalt nitrate, cobalt acetate, cobalt metal, and cobalt nanoparticles) have been evaluated; the available studies are summarized in Table E-5 and discussed in Section 6.3 on potential mechanisms of carcinogenesis. Protein binding is important in the consideration of genotoxicity assay results for cobalt compounds because cobalt binding in vivo, e.g., to serum proteins, could render it less effective than when tested in vitro for the same endpoint.

E.5. In Vivo Genotoxicity Studies of Cobalt Compounds in Rodents

Several studies tested different type of cobalt compounds, including a water-soluble salt (cobalt chloride), cobalt acetate, and cobalt metal for genotoxic effects in vivo. The available data suggests that cobalt compounds are clastogenic and can induce DNA and chromosomal damage, i.e., micronucleus formation, chromosomal aberrations, and aneuploidy, as shown by results reported for in vitro assays. The results of the in vivo studies are discussed below and summarized in Table E-6.

DNA damage was observed after i.p. exposure to cobalt acetate in the Fischer rat, with strongest results observed for cells from the kidney, liver, then lung (Kasprzak et al. 1994). In two studies, cobalt chloride exposure in vivo induced micronucleus formation in mouse bone marrow after i.p. injection (Rasgele et al. 2013; Suzuki et al. 1993) but results were negative in a third study of cobalt metal in murine peripheral blood lymphocytes after inhalation exposure (NTP 2014d). Route of exposure and tissue type varied between these studies; one or both of these factors may be the cause of the disparate results. Dose-dependent increases in chromosomal breaks and aberrations were reported in Swiss mouse bone marrow after oral exposure to a single dose of cobalt chloride in the test animals (Palit et al. 1991a; Palit et al. 1991b; 1991c; 1991d; as cited in WHO 2006). Aneuploidy was observed in hamster bone marrow and testes after i.p. injection of either cobalt chloride or cobalt chloride hexahydrate (Farah 1983).

Table E-5. Studies of Nucleic Acid and Protein Binding and DNA Damage/Repair Inhibition of Cobalt Compounds

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Binding/crosslinks				
Cobalt chloride	Wedrychowski et al. (1986)*	130 µg/mL	+	Positive for DNA-protein crosslinks/ in rat Novikoff ascites hepatoma cells
	Palecek et al. (1999)*	>13 µg/mL 78 µg/mL	+ (full) +	Inhibition of p53 protein-DNA binding for consensus sequence and supercoiled DNA
Cobalt chloride hexahydrate	Sabbioni et al. (2014b)	10 µM	+	Radiolabeled cobalt binding to DNA (4 hr exposure) much lower (0.0019 ng/10^6 cells) than for microparticles or nanoparticles (see below)
Cobalt sulfate	Lloyd et al. (1998)	20 µM	+	Salmon sperm DNA generative cross-links, induced single (not double) strand DNA damage
Cobalt metal ion	Bal et al. (2013)	NTS 110 µM A 90 µM B 11 µM	+	Cobalt binds to human serum albumin at three sites: N-terminal site (NTS), A and B; they had different affinities—site B was the strongest
Cobalt nanoparticles	Sabbioni et al. (2014b)	10 µM	+	Radiolabeled cobalt binding to DNA (4 hr exposure) Binding 1.7 ng/10^6 cells
Cobalt microparticles	Sabbioni et al. (2014b)	10 µM	+	Radiolabeled cobalt binding to DNA (4 hr exposure) Binding 9.2 ng/10^6 cells
Cobalt ions (see comments)	Alipázaga et al. (2008)	1.0 mM	+	Cobalt binds to O_2 in presence of glycylglycylhistidine, directly forming adducts; cobalt was prepared from cobalt carbonate reaction with perchloric acid
Inhibition of DNA repair				
Cobalt chloride hexahydrate	Hartwig et al. (1991) and Kasten et al. (1997)*	12 µg/mL (incision and polymerization step) 48 µg/mL (ligation step)	+ −	Positive for Inhibition of nucleotide excision repair of UV-induced DNA damage, alkaline unwinding/ repair VH16 fibroblasts; data shown for Kasten et al. (1997) (same research group, subsequent publication)
Cobalt chloride hexahydrate	Kasten et al. (1997)*	86 µg/mL (incision step)	+	Inhibition of UV-induced cyclobutane pyrimidine dimers, alkaline unwinding + T4 endonuclease V/VH16 fibroblasts

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cobalt (metal)	De Boeck et al. (1998)*	1.2 µg/mL (with 5.5 µg/mL MMS post-treatment)	+	Positive for DNA repair inhibition, alkaline Comet assay/ human mononuclear leukocytes
		1.2 µg/mL (with 5.5 µg/mL MMS co-exposure)	+	
Cobalt acetate	Snyder et al. (1989)*	100 µg/mL	+	Inhibition of repair of UV-induced pyrimidine dimers, nucleoid sedimentation in HeLa S-3 cells
Inhibition/inactivation of protein				
Cobalt chloride	Asmuss et al. (2000)*	6.5 µg/mL (XPA) 130 µg/mL (Fpg)	+ −	Positive for inhibition of xeroderma pigmentosum group A (XPA) protein (with Zn finger domain) binding to UV-irradiated oligonucleotide [XPA is a zinc finger protein involved in nucleotide excision repair, but no effect on bacterial Fpg protein (Zn finger domain)]
Cobalt nitrate hexahydrate	Kopera et al. (2004)	10 µM	+	Reported substitution for zinc in the zinc finger derived from the DNA report protein XPA

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, − = negative.
[a]No studies reported testing with +S9.

Table E-6. In Vivo Genotoxicity Studies of Cobalt Compounds in Rodents

Compound	Species/Sex/#	Reference	Exposure	Results	Comments and Conclusions
DNA damage					
Cobalt acetate	F344/CR rat male and female/12 per group	Kasprzak et al. (1994)	i.p. one dose 50 or 100 µmol 2 or 10 d	Kidney + Liver + Lung +	Damage in kidney > liver > lung cells; retention of cobalt in kidney and liver, less in lung
Micronucleus formation/peripheral blood lymphocytes					
Cobalt metal	B6C3F1/N mouse/male and female	NTP (2014a)	Inhalation 3 months 10 mg/m^3	−	Negative for micronuclei induction in peripheral blood lymphocytes

Compound	Species/Sex/#	Reference	Exposure	Results	Comments and Conclusions
Micronucleus formation/bone marrow					
Cobalt chloride	Swiss albino mouse/male/5 per group	Rasgele et al. (2013)	i.p. 24 hr 22.5 mg/kg 48 hr 11.2 mg/kg	 + +	Significant increase in micronucleated polychromatic erythrocytes; no toxicity observed; distilled water control
Cobalt chloride hexahydrate	BALB/c AnNCrj mouse/male	Suzuki et al. (1993)*	i.p. 50 mg/kg bw	+; also enhanced formation with other mutagens	Micronuclei enhanced, compared with other mutagens used (20 mg/kg DMH, 50 mg/kg benzo(a)pyrene, and 200 mg/kg 2-napthylamine)
Chromosomal breaks and chromosomal aberrations (bone marrow)					
Cobalt chloride	Swiss mouse/male	Palit et al. (1991a; 1991b; 1991c; 1991d)**	Oral Single dose 4.96 to 19.8 mg/kg	+	Dose response increase in chromosomal damage
Aneuploidy, pseudodiploidy, and hyperploidy/bone marrow and testes (meiosis 1)					
Cobalt chloride	Hamster	Farah (1983)*	i.p. 400 mg/kg bw total dose	+ (bone marrow and testes)	Exposure is total dose over 9 days
Cobalt chloride hexahydrate	Hamster	Farah (1983)*	i.p. 400 mg/kg bw total dose	+ (bone marrow and testes)	Exposure is total dose over 9 days

*As cited by IARC (2006), ** as cited by WHO (CICAD) (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, − = negative.

E.6. Genotoxicity Studies of Occupational Exposure to Cobalt

The available database for evaluating occupational exposure to cobalt and genetic effects is inadequate because of the paucity of studies, exposure to other genotoxic agents, or small numbers of exposed workers. Two studies reported by IARC are not reviewed because they were not specific for cobalt exposure and a more recent study of occupational exposure was identified; however, a study of Brazilian copper smelter workers is also not reviewed because blood cobalt levels were similar among unexposed controls (De Olivera et al. 2012). Two studies are reviewed below, only one of which is of cobalt workers (De Boeck et al. 2000); however, the second study is briefly reviewed (Hengstler et al. 2003) because it conducted multivariate analyses (see Table E-7).

De Boeck et al. (2000) measured 8-hydroxydeoxyguanosine, DNA damage (comet assay), and micronuclei in lymphocytes from workers at cobalt refinery facilities in Belgium, Norway, and Finland, cobalt hard workers, and unexposed controls from the same plants. All genetic markers were similar between the cobalt-exposed workers and non-exposed workers. Limitations of the study were small numbers of workers and the measurement of damage at least 40 hours after exposure.

In the second study, DNA strand breaks were measured in 78 German workers producing or recycling cadmium or cadmium products. In analysis considering only exposure to single metals, DNA single-strand breaks correlated better with cobalt concentrations (measured in air and urine) than cadmium concentrations (air and blood). Logistic analysis evaluating all variables as well as interactions between metals found that the increases in DNA strand breaks were explained by cobalt (air), cadmium (air), cadmium (blood), and interaction between lead and cobalt in the air (Hengstler et al. 2003).

Table E-7. Genotoxicity Studies of Occupational Exposure to Cobalt

Endpoint	Population	Reference	Exposure Assessment	Results	Comments and Conclusions
DNA damage Cobalt in air and urine	Workers in 10 facilities producing or recycling cadmium or its products in Germany (N = 78; 62 men and 16 women)	Hengstler et al. (2003)	Cobalt meas in air (range): 0–10 μg/m^3	+	Co-exposures to cadmium and lead contribute to DNA strand breaks in logistic regression models.
			Cobalt in urine – to air	R = 0.453 p = 0.000	
			Cobalt in urine – to air (normalized to creatinine)	R = 0.504 p = 0.000	DNA single strand breaks correlated with cobalt conc in air ($p < 0.001$, R = 0.401).

Endpoint	Population	Reference	Exposure Assessment	Results	Comments and Conclusions
DNA damage 8-Hydroxy-deoxyguanosine (8-OHdG) Micronuclei	Refinery workers (three facilities—in Belgium, Norway, and Finland) exposed to cobalt (analysis = 24); workers exposed to hard metal plants in one plant (analysis = 29) and unexposed workers from the four facilitates (analysis = 27) Workers and exposed workers from one plant were excluded from the analysis because of older population and higher 8-OHdG	De Boeck et al. (2000)	Cobalt in urine (µg cobalt per gram creatinine) Level (SD; range): Controls: 1.7 (1.6; 0.6–5.5) Exposed: 21.5 (2.1 (5.0–82.5)	− (all three endpoints)	Negative for DNA damage measured in lymphocytes—comet assay; cobalt levels were measured in urine Exposure equivalent to 20 µg/m^3 of cobalt in air Exposure assessment from samples on Friday, and genetic damage assessed from samples the following Monday

R = correlation coefficient.

E.7. Cell Transformation

Cobalt compounds caused cellular transformation, which may be related to genotoxicity but is not a genotoxic effect per se (see Table E-8). The Syrian hamster embryo (SHE) transformation assay identifies non-genotoxic carcinogens with 80% to 90% accuracy and detection for genotoxic carcinogens is even higher (Benigni et al. 2015). Cell-transformation assays in SHE and other cell lines were positive for three soluble cobalt compounds (cobalt chloride, cobalt sulfate monohydrate, and cobalt acetate) (Casto et al. 1979; Doran et al. 1998; Kerckaert et al. 1996; Ponti et al. 2009), cobalt nanoparticles (Annangi et al. 2014; Ponti et al. 2009; Sighinolfi et al. 2014), cobalt microparticles (Sabbioni et al. 2014a), and cobalt sulfide (Abbracchio et al. 1982; Costa et al. 1982), but negative for cobalt metal (Doran et al. 1998).

Table E-8. In Vitro Studies of Other (Genotoxic-related) Effects of Cobalt Compounds in Mammalian Cells

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cell transformation/C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), BALB/3T3 cells				
Cobalt chloride	Doran et al. (1998)*	5 µg/mL	+	Positive in C3H10T1/2 mouse fibroblast cells
	Ponti et al. (2009)	70 µM	−	Negative in BALB/3T3 cells (72 hr exposure)
Cobalt sulfate monohydrate	Kerckaert et al. (1996)*	0.125 µg/mL	+	Positive in SHE cells
Cobalt acetate	Casto et al. (1979)*	0.2 mM (approx. 35.4 µg/mL)	+	Positive for cell transformation enhancement by simian adenovirus SA7/ SHE cells
Cobalt metal	Doran et al. (1998)*	500 µg/mL	−	Negative in C3H10T1/2 mouse fibroblast cells, even at high exposure

Compound	Reference	Concentration (LED or HID)	Results (−S9)[a]	Comments and Conclusions
Cobalt nanoparticles	Ponti et al. (2009)	7 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Sabbioni et al. (2014a)	5 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Sighinolfi et al. (2014)	10 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Annangi et al. (2014)	0.05 µg/ml	+	Cell transformation, mouse embryo fibroblasts, 12-wk exposure to sub-toxic dose; $Ogg1^{+/+}$ and $Ogg1^{-/-}$ with knockout cells more sensitive
Cobalt microparticles	Sabbioni et al. (2014a)	1 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
Cobalt sulfide (CoS, amorphous)	Abbracchio et al. (1982)* and Costa et al. (1982)*	10 µg/mL	(+)	Positive in Syrian hamster embryo cells
Cobalt sulfide (CoS_2, crystalline)	Abbracchio et al. (1982)* and Costa et al. (1982)*	1 µg/mL	+	Positive in Syrian hamster embryo cells

*As cited by IARC (2006).
LED/HID = lowest effective dose/highest ineffective dose; NR = not reported; + = positive; (+) = weakly positive; − = negative.
[a]No studies reported testing with +S9.

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