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

Peer-Review Draft:
Report on Carcinogens Monograph on Cobalt and Certain Cobalt Compounds

June 5, 2015

Office of the Report on Carcinogens
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
U.S. Department of Health and Human Services

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of identified substances (i) that either are *known to be human carcinogens* or are *reasonably anticipated to be human carcinogens* and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of HHS, has delegated responsibility for preparation of the RoC to the NTP, which prepares the report with assistance from other Federal health and regulatory agencies and nongovernmental institutions. The most recent RoC, the 13th Edition (2014), is available at http://ntp.niehs.nih.gov/go/roc13.

Nominations for (1) listing a new substance, (2) reclassifying the listing status for a substance already listed, or (3) removing a substance already listed in the RoC are evaluated in a scientific review process (http://ntp.niehs.nih.gov/go/rocprocess) with multiple opportunities for scientific and public input and using established listing criteria (http://ntp.niehs.nih.gov/go/15209). A list of candidate substances under consideration for listing in (or delisting from) the RoC can be obtained by accessing http://ntp.niehs.nih.gov/go/37893.

Background 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 certain cobalt compounds' 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. "Certain" refers to those cobalt compounds that release cobalt ions *in vivo*, which does not include cobalt as part of the vitamin B_{12} molecule because of the stability of that molecule in biological fluids. Cancer and toxicology 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 cobalt and certain 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 2014d, 2014a). Cobalt sulfate, which has been listed since 2004 based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP 2002b), 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 draft 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 certain cobalt compounds, and (Part 2, which will be drafted with input from the internal review group) the draft substance 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 certain cobalt compounds.

The methods for preparing the draft RoC monograph on cobalt and certain cobalt compounds are described in the "Cobalt and Certain Cobalt Compounds Protocol"(NTP 2014c). The cancer evaluation component for cobalt and certain cobalt compounds 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 certain cobalt compounds 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, such as the literature search strategy, study quality tables, and study descriptions and results for some sections.

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 and certain 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 draft 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 and cobalt compounds?
- 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 and cobalt compounds?

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 certain cobalt compounds 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 certain 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, 2104, the scope of the evaluation was defined as "cobalt and certain cobalt compounds," where "certain" refers to those cobalt compounds that release cobalt ions in biological fluids. Technical advisors for the review of cobalt and certain cobalt compounds are identified on the "CONTRIBUTORS" page.

Public comments on scientific issues were requested at several times prior to the development of the draft 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 and certain cobalt compounds for public input on the ORoC webpage for cobalt and certain cobalt compounds (http://ntp.niehs.nih.gov/go/730697) prior to the release of the draft monograph. One written public comment on cobalt (in response to the request for information on the nomination) has 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 draft 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 and certain cobalt compounds 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 7,150 citations identified from these searches were uploaded to web-based systematic review software for evaluation by two separate reviewers using inclusion/exclusion criteria, and 465 references were selected for final inclusion in the draft monograph using these criteria.

Information for the relevant cancer and mechanistic sections was systematically extracted in tabular format and/or summarized in the text, following specific procedures developed by ORoC, 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

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.

applied to the available database of carcinogenicity data to assess the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of cobalt and certain cobalt compounds 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 certain cobalt compounds to reach a listing recommendation.

Contributors

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Part 1

Draft Cancer Hazard Evaluation

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1 Chemical identification and properties

The candidate substance being reviewed in this monograph is “Cobalt and Certain Cobalt Compounds.” “Certain” refers to those cobalt compounds that release cobalt ions *in vivo*. The available database on cobalt and cobalt compounds vary by cobalt form; however, there are carcinogenicity, genotoxicity and toxicity studies on cobalt metal and of some water-soluble and poorly 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 2014b). Together, the carcinogenicity, genotoxicity, and other mechanistic information on these representative forms of cobalt inform the discussion in this document on cobalt and certain cobalt compounds. 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 “certain cobalt compounds” 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). Cancer and toxicity studies of some forms of cobalt, such as cobalt alloys and radioactive forms of cobalt, are excluded from this review because of confounding exposures.

Cobalt (Co) is a naturally occurring transition element with magnetic properties. It is the 33rd most abundant element and comprises 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 (WHO 2006, ATSDR 2004, IARC 1991). Cobalt predominantly occurs in two oxidation states, +2 (Co(II)) and +3 (Co(III)).

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). 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.

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 be transported to and taken up at target sites or released within cells from particles (see Section 6). Cobalt sulfates, chlorides, and nitrates 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) (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- and microparticles dissolve in cell-free culture medium in a concentration- and time-dependent manner while cobalt oxide particles are practically insoluble in water or culture medium (Ortega *et al.* 2014, Sabbioni *et al.* 2014, Ponti *et al.* 2009). Smaller particles dissolve faster than larger particles (Lison 2015, Kyono *et al.* 1992).

While water solubility represents a measure of a compound's tendency to release ions available for biological uptake, 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). The bioavailability (i.e., extent of systemic absorption) of a metal species is determined by its solubility in biological fluids (Brock and Stopford 2003, Stopford *et al.* 2003). For metals like cobalt, with several species with different valence states having dissimilar solubility characteristics existing in different compounds, *in vivo* bioavailability testing can be cost-prohibitive and time consuming. Therefore, in lieu of *in vivo* testing, measured solubility of compounds in artificial fluids (i.e., bioaccessibility) can be used as a surrogate for bioavailability.

Cobalt metal, and several water-soluble compounds (cobalt sulfate heptahydrate and chloride) and poorly soluble compounds (cobalt oxide, bis(2-ethyl-hexanoate), carbonate, and naphthenate) were found to be soluble in biological fluids, suggesting that they release cobalt ions (see the right-hand column of Table 1-1). These bioaccessibility studies for cobalt compounds have been performed 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 drawing with soft pastels and manufacturing and use of alloy materials (Hillwalker and Anderson 2014, Brock and Stopford 2003, Stopford *et al.* 2003). These studies indicate that species of cobalt compound, particle size, surface area, and pH of the surrogate fluid can impact cobalt solubility (Stopford *et al.* 2003). In a study of 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, results demonstrated overall satisfactory within-laboratory variability; however, absolute bioaccessibility results in some biological fluids may vary between different laboratories (Henderson *et al.* 2014).

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 precipitate in the presence of physiological concentrations of phosphates and nonspecifically bind to proteins such as albumin (Lison 2015).

Table 1-1. Physical and chemical properties for cobalt metal and representative cobalt compounds[a]

Name (+2 valence unless otherwise indicated)	CAS No.	Formula	Molecular weight	Physical form	Density or specific gravity	Solubility (grams per 100 cc cold water)	Bioaccessiblity (% solubility in gastric/lysosomal fluids)
Metal							
Cobalt	7440-48-4	Co	58.9	Grey hexagonal or cubic metal	8.92	.000875	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, monocl.	1.95	60.4	100/100
Chloride	7646-79-9	$CoCl_2$	129.9	Blue hexagonal leaflets	3.36	45	100/100
Acetate (org.)	71-48-7	$Co(C_2H_2O_2)_2$	249.1	Red-violet, monocl.	1.70	s	–
Nitrate	10141-05-6	CoN_2O_6	182.9	Red powder or crystals	2.49	s	–
Poorly soluble compounds							
Oxide	1307-96-6	CoO	74.9	Green-brown cubic	6.45	i	100/92.4
(II, III) Oxide	1308-06-1	Co_3O_4	240.8	Black, cubic	6.07	i	
2-ethyl-hexanoate (org.)	136-52-7	$Co(C_8H_{15}O_2)_2$	173.7	Blue liquid (12% Co)	1.01	–	100/100
Carbonate (org.)	513-79-1	$CoCO_3$	118.9	Red, trigonal	4.13	i	100/100
Naphthenate (org.)	61789-51-3	$Co(C_{11}H_7O_2)_2$	401.3	Purple liquid (6% Co)	0.97	–	100/100
Hydroxide	21041-93-0	$Co(OH)_2$	93.0	Rose-red, rhomb	3.60	0.00032	–
Sulfide	1317-42-6	CoS	91.0	Reddish octahedral	5.45	0.00038	–
Oxalate (org.)	814-89-1	CoC_2O_4	147.0	White or reddish	3.02	i	–
Propionate (org.)	1560-69-6	$Co(C_3H_5O_2)_2$	205.1	–	–	–	–
Stearate (org.)	1002-88-6	$Co(C_{18}H_{35}O_2)_2$	625.9	–	–	–	–

Sources: SciFinder; PubChem Compounds Database; ChemIDplus Database; Cobalt Development Institute (CDI) Report (2006); Hazardous Substances Data Bank (HSDB); Stopford *et al.* (2003).
org. = organic compound; all others are inorganic.
[a]Cobalt forms or compounds tested for carcinogenicity or genetic toxicity, or for possible mechanisms of action are italicized.

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 (Paustenbach *et al.* 2013, Nilsson *et al.* 1985). 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) is so unstable that it quickly reduces to Co(II), oxidizing water and liberating oxygen. In contrast, air or hydrogen peroxide can oxidize Co(II) to the more stable Co(III) complex 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, and in biological reactions involving vitamin B_{12} (Paustenbach *et al.* 2013, IARC 1991).

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) (Paustenbach *et al.* 2013, IARC 1991). 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.

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

Both cobalt metal, as particles or nanoparticles, have been found to be 100% bioaccessible (i.e., dissolving to release cobalt ions) in 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.

2 Human Exposure

This section describes cobalt mining and production (Section 2.1); use (Section 2.2); biomonitoring and exposure to cobalt and cobalt compounds (Section 2.3); exposure in the workplace (Section 2.4); potential exposure from other sources such as food, consumer products, tobacco, and medical products (Section 2.5); and potential for environmental exposure (Section 2.6). The material presented in Sections 2.1 through 2.6 is summarized in Section 2.7. Although studies of cobalt alloys were not considered informative for either animal tumor studies or human carcinogenicity studies because they are not specific for exposure only to cobalt, humans can be exposed to cobalt from producing or using these compounds, and thus those exposures 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, $Co_3(AsO_4)_2{\bullet}8H_2O$; and glaucodot, CoAsS (CDI 2006, ATSDR 2004, Davis 2000). The largest cobalt reserves are in the Congo (Kinshasa), Australia, Cuba, Zambia, Canada, Russia, and New Caledonia (Shedd 2014a). 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 2014b). 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 and 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; EPA Chemical Data Reporting (2012); PubChem Compounds Database; 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 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 2014b) 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 2014b).
[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), and nanofibers and nanowires also are being used for industrial applications.

Uses for cobalt compounds with their reported levels of U.S. production are listed in Table 2-1 above. Table 2-4 lists uses for several cobalt compounds for which no production information was identified.

2.2.1 Metallurgical uses

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

2.2.2 Cemented carbides and bonded diamonds

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 (NTP 2009, IARC 1991). Cobalt is also used in diamond tools from steel with microdiamonds impregnated into a surface cobalt layer (CDI 2006, IARC 2006).

2.2.3 Chemical uses

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 2.2.4) (CDI 2006, WHO 2006, ATSDR 2004, IARC 1991) (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. Uses for representative inorganic and organic cobalt compounds

	Inorganic					Organic			
Use	**Chloride**	**Hydroxide**	**Nitrate**	**Oxides**	**Sulfate**	**2-ethyl-hexanoate**	**Acetate**	**Carbonate**	**Propionate**
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.

2.2.4 Electronics and "green" energy

Due to increased demand for portable rechargeable electronic devices, one of the fastest growth areas for cobalt use is in high-capacity, rechargeable batteries (Shedd 2014b, CDI 2006, Davis 2000). Cobalt is used in nickel-cadmium (Ni-Cd), nickel-metal hydride (Ni-MH), and lithium-ion (Li-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 (see Section 2.2.2), 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 Biomonitoring for cobalt

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 Appendix B, Tables B-1 and B-2 for levels reported in these studies, source of exposure, and geographical location, and Figures 2.1 and 2.2). Several of the studies are of people residing in the United States, and thus demonstrate U.S. exposure.

2.3.1 Evidence of exposure

Urine

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 failed hip implants; with lower levels from exposure from normal implants, 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 Appendix B, Table B-1.) The geometric mean urinary cobalt concentration for the U.S. general public for the most recent (2011-2012) National Health and Nutrition Examination Survey (NHANES) year 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 a geometric mean value of 0.316 to 0.379 µg/L (CDC 2015).

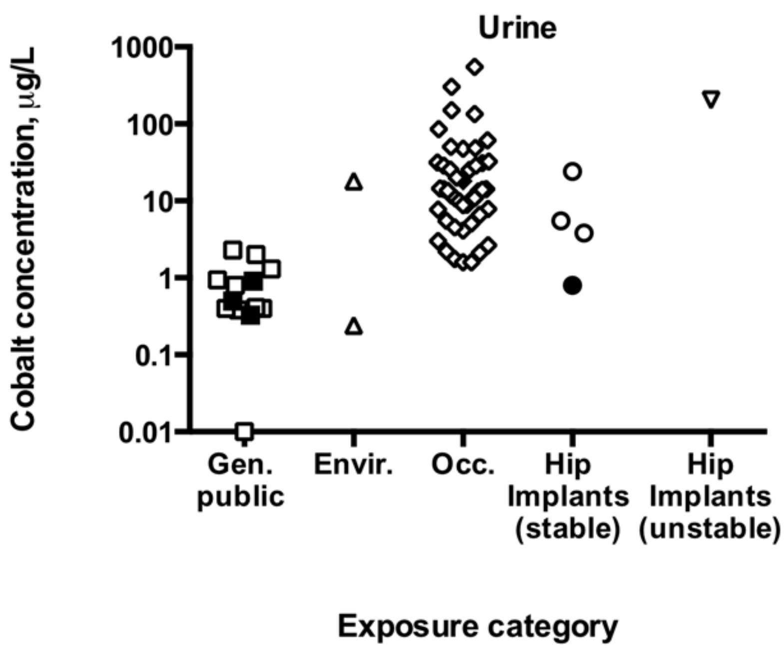


Figure 2-1. Cluster graph of urine cobalt levels

Filled symbols = U.S. data; open symbols = non-U.S. data.

Hair

Reported mean levels of cobalt in hair are highest among 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 populations at risk of environmental exposure and in 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.

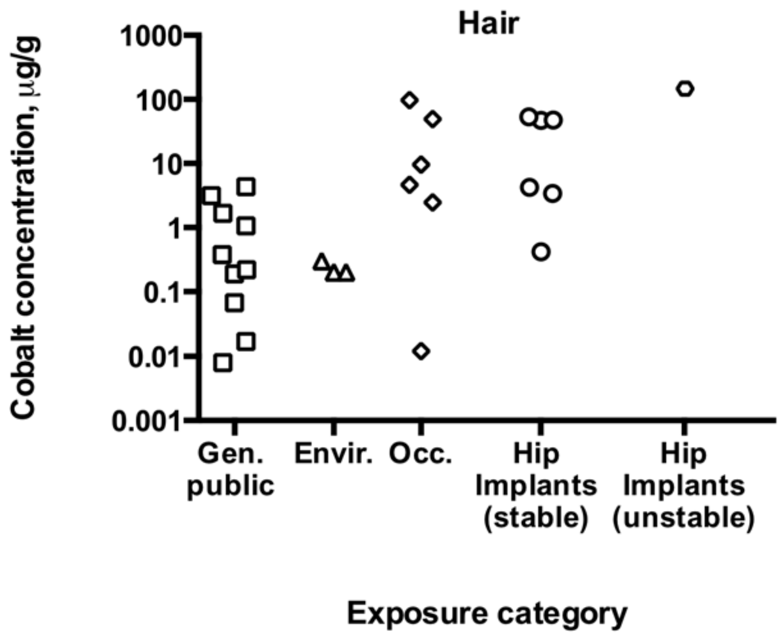


Figure 2-2. Cluster graphs of cobalt levels in hair

Filled symbols = U.S. data; open symbols = non-U.S. data.

2.3.2 Methods

Urine

Urinary cobalt is considered a good indicator of absorbed cobalt (IARC 2006, WHO 2006), especially from recent exposures (ATSDR 2004). Urinary cobalt levels are more reflective of recent exposure for soluble compounds than less soluble compounds (ATSDR 2004).

Blood

Blood cobalt levels are also more reflective of recent exposure for soluble compounds than less soluble compounds (ATSDR 2004). Although measurements of cobalt in whole blood, plasma, and serum have been reported by investigators, no consensus seems to exist for which of these provides the best relationship with levels of exposure to cobalt.

Hair

Although cobalt concentrations in hair provide a potential biomarker for cobalt exposure over time, the source of exposure in the studies is not known. 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).

Nails

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 (He 2011, Fleckman 1985). 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).

Tissue

Several publications measure 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 Appendix B, Table B-3).

Analytical methods

Analytical methods for cobalt in biological materials include graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma-atomic emisson spectrometry (ICP-AES), differential pulse cathodic stripping voltammetry (DPCSV), and colorimetric determination (ATSDR 2004). 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 1994), 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).

2.4 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 the refining of cobalt; during the production of cobalt powder; during the production and use of cobalt alloys; during hard metal production, processing, and use; during the maintenance and re-sharpening of hard metal tools and blades; during the manufacture and use of cobalt-containing diamond tools; and during the use of cobalt-containing pigments and driers. Workers regenerating spent catalysts may also be exposed to cobalt sulfides.

Occupational exposure has been documented by measurements of cobalt in ambient workplace air, in worker blood and urine, and in deceased worker lung tissue (CDC 2013, IARC 2006, ATSDR 2004, IARC 1991). 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. Table 2-5 reports workplace air levels of cobalt for various industrial uses of cobalt or cobalt compounds.

Several exposure scenarios arise from the use of cobalt and cobalt compounds in numerous commercial, industrial, and military applications. The scenarios that generally contribute most to U.S. releases of cobalt and cobalt compounds as reported to EPA (TRI 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). Other potential exposure scenarios (e.g., copper smelting) exist, but no air data were identified.

Table 2-5. Workplace air levels of cobalt

Exposure scenario (Country)	Cobalt in workplace air mean (range) in μg/m^3	Reference
Use of cobalt-containing diamond tools (NR)	(0.1–45)	(van den Oever *et al.* 1990)
Use of cobalt-containing diamond tools (Italy)	690 115 (with improved ventilation)	(Ferdenzi *et al.* 1994)
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)
Painting porcelain plates with cobalt compounds (Denmark)	80 26 (after Danish surveillance program)	(Christensen 1995, Poulsen *et al.* 1995, Christensen and Poulsen 1994)
Production of cobalt metal and cobalt salts (Belgium)	127.5 (2–7,700)	(Swennen *et al.* 1993)
Recycling batteries to recover cobalt (NR)	Up to 10	(Hengstler *et al.* 2003)
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)
Conversion of cobalt metal to cobalt oxide (South Africa)	9,900 (highest reported)	(Coombs 1996)
Nickel refining (Norway)	< 150[a]	(Grimsrud *et al.* 2005)

Source: (IARC 2006).
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.

2.4.1 Hard-metal production, processing, and use

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). 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). 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.

2.4.2 Cobalt-containing diamond tools and cobalt alloys

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).

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).

2.4.3 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 2006, 1991).

2.4.4 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, battery recycling, and cobalt compound (acetate, chloride, nitrate, oxide, and sulfate) production. Worker urinary cobalt for these facilities ranged from 1.6 to 2,038 μg/g creatinine (IARC 2006).

2.5 Surgical implants

Surgical implantation (e.g., orthopedic joint replacements) can result in exposure to cobalt. 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). Blood cobalt ion concentrations generally increase by 5- to 10-fold from preoperative to postoperative levels (Polyzois *et al.* 2012) (see Table B-1 for cobalt levels in blood from individuals with implants). Metal-on-metal (MoM) implants are reportedly associated with blood cobalt less than 4 μg/L and urine levels less than 7.5 μg/L (Jantzen *et al.* 2013, Sampson and Hart 2012).

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). All hip implants that contain metal components contain cobalt as part of cobalt-chromium-molybdenum alloys (Devlin *et al.* 2013, Sampson and Hart 2012). Release of metal from implants results from both wear and corrosion, 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). Implants that have failed because of excessive wear or corrosion have been associated with systemic cobalt toxicity in some cases, and cobalt levels in some of these individuals have been reported. Blood levels associated with toxicity may be related to the type of implant; levels were higher among 10 patients with failed ceramic prosthesis (median blood concentration = 506 μg/L; range = 353 to 6,521) compared with eight patients with toxicity from implanted MoM devices (median 34.5 μg/L range = 13.6 to 398.6) (Bradberry *et al.* (2014)) Peak blood cobalt concentrations were > 250 μg/L. 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 as an action level for further clinical investigation and action (MHRA 2012) and at 10 μg/L in the United States by the Mayo Clinic (Mayo Clinic 2015).

2.6 Other sources of exposure: Food, consumer and other medical products and tobacco

Exposure to cobalt in the general population also occurs via inhalation of ambient air and ingestion of drinking water; however, food is the largest source of cobalt exposure in the general population (ATSDR 2004). Daily cobalt intake from food has been reported to range from 5 to 50 μg/day (Lison 2015). Green, leafy vegetables and fresh cereals contain the most cobalt, and dairy products, refined cereals, and sugar contain the least (IARC 1991) (see Table 2-6). Cobalt compounds were added to beer in the past, but this use has been discontinued. Reported values for cobalt content of foods can vary due to differences in environmental cobalt levels, analytical difficulties, and inadequate analytical techniques.

Table 2-6. Cobalt content of some foods

Food	Cobalt content (micrograms per 100 grams of food)
Green, leafy vegetables	20–60
Organ meats	15–25
Muscle meats	7–12
Dairy products, refined cereals, sugar	1–3

Sources: (Briggs and Wahlqvist 1998, IARC 1991).

Higher cobalt intake may result from consumption of over-the-counter or prescription vitamin and mineral preparations (e.g., cobalt chloride or vitamin B_{12} formulations). 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 (Tvermoes *et al.* 2013, Unice *et al.* 2012, Lippi *et al.* 2005).

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.* noted that while cobalt deficiencies were not reported, smoking does interfere with vitamin B_{12} absorption.

2.7 Potential for environmental exposure

Information on potential for environmental exposure discussed below includes data for releases (Section 2.1.7), occurrence (Section 2.7.2), and exposure (Section 2.7.3).

2.7.1 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.

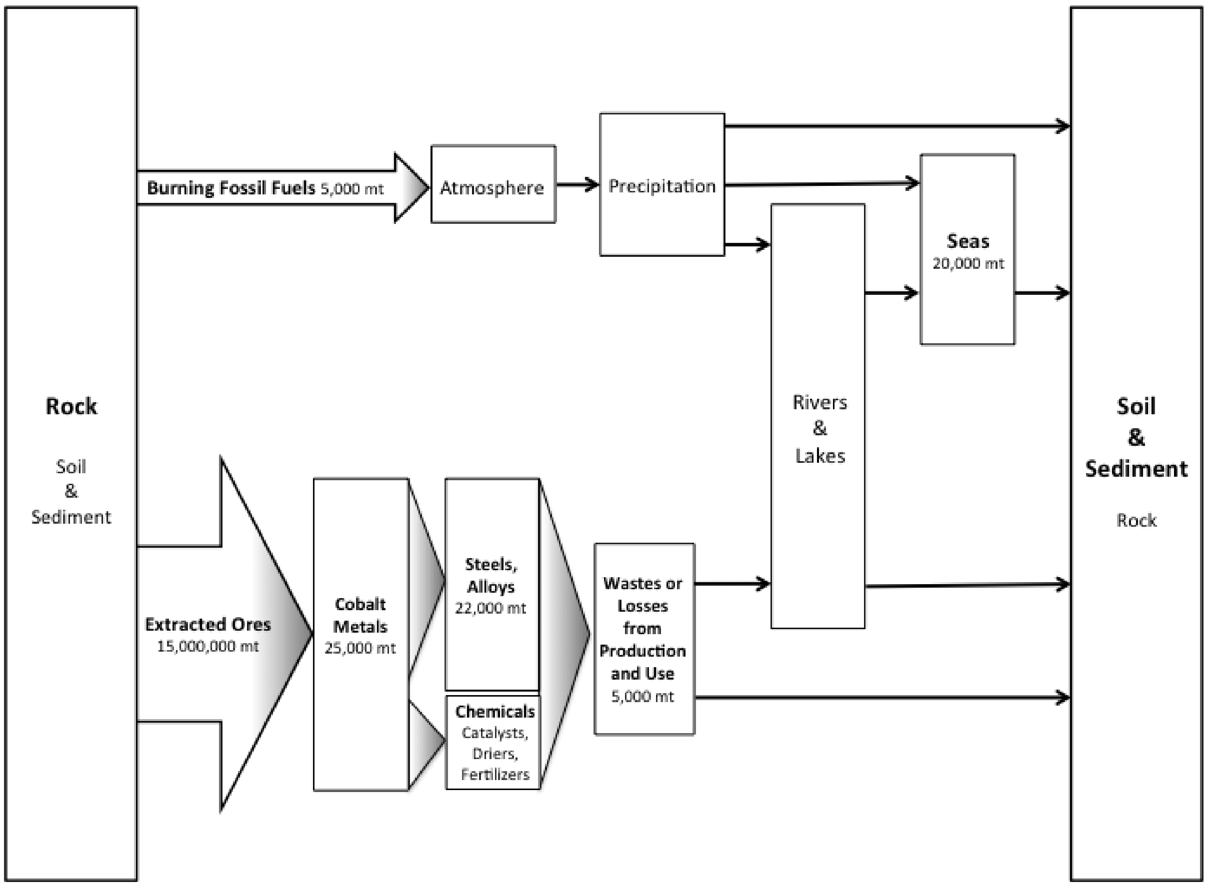


Figure 2-3. Flow of cobalt released from anthropogenic processes

Adapted from (CDI 2006, Shedd 1993).

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 TRI, total reported on- and offsite release of cobalt and cobalt compounds was approximately 5.5 million pounds from 723 facilities in 2013 (TRI 2014c, 2014b, 2014a). 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.

2.7.2 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.) (TRI 2014a, EPA 2012, ATSDR 2004).

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).

2.7.3 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).

2.8 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 of gaseous cobalt carbonyl. Dermal contact with hard metal and cobalt salts can result in systemic uptake. Occupational exposure to cobalt occurs during refining of cobalt; production of cobalt powder; the production and use of cobalt alloys; hard-metal production, processing, and use; maintenance and re-sharpening of hard-metal tools and blades; manufacture and use of cobalt-containing diamond tools; and use of cobalt-containing pigments and driers. Occupational exposure has been documented by measurements of cobalt in ambient workplace air, worker blood and urine, and deceased worker lung tissue.

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 generally less than those reported for unstable hip implants and occupational exposures but more 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, for the majority of the general public the primary source of cobalt exposure is food; daily cobalt intake from food has been reported to range from 5 to 50 μg/day. 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.

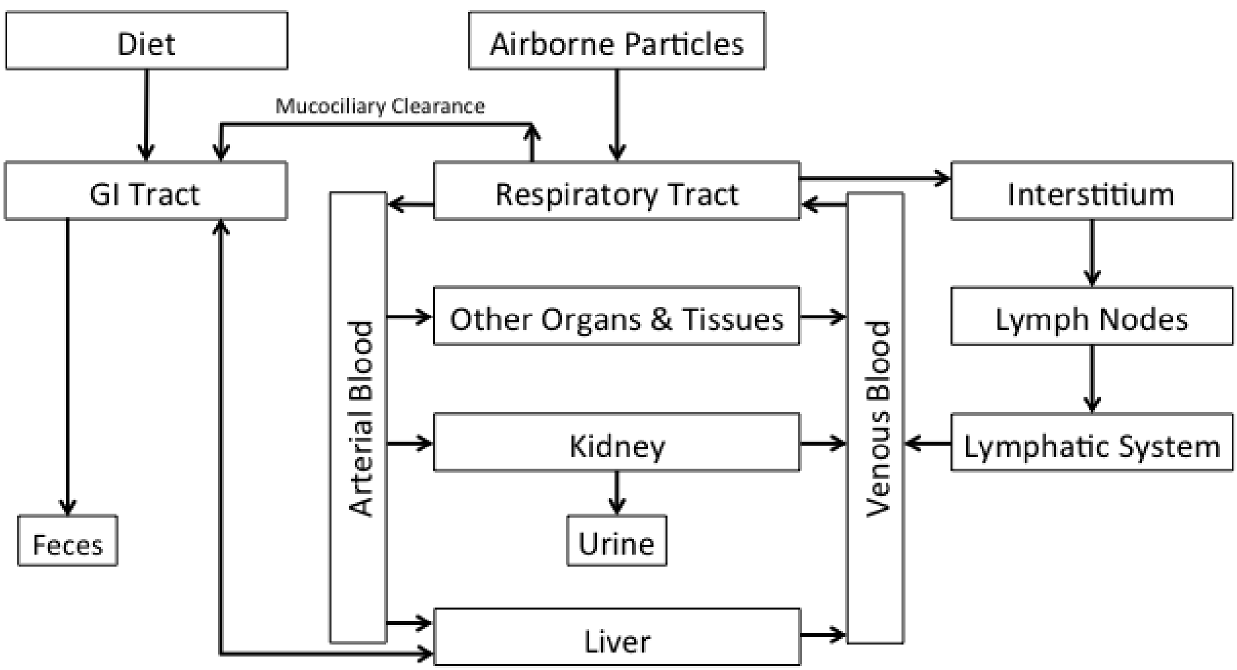


Figure 3-1. Cobalt disposition

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

Humans

The normal dietary intake of cobalt ranges from about 5 to 50 μg/day, most of which 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 (Paustenbach *et al.* 2013, IARC 2006) (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, NTP 2014b, Paustenbach *et al.* 2013, IARC 2006, WHO 2006, Smith *et al.* 1972). 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 that 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.* 1993a). 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.* 1993a). 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 (Finley *et al.* 2013, Christensen *et al.* 1993a). 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 (NTP 2014b, IARC 2006). 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 (WHO 2006, ATSDR 2004). 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 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, Sabbioni *et al.* 2014, Smith *et al.* 2014, Papis *et al.* 2009). 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

Cobalt occurs in most tissues of non-occupationally exposed people because it is a component of vitamin B_{12}. 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 levels measured in blood and urine from various exposed populations compared to control populations are discussed in Section 2-3 and summarized in Figure 2-1 and Table B-1 In addition, several case-referent studies compared cobalt tissue levels in patients dying from cancer with patients dying from other causes (see Appendix B). 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.* 2012, Simonsen *et al.* 2011). 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 turn over of cells. 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 (Lison 2015, Paustenbach *et al.* 2013, IARC 2006).

Renal excretion of absorbed cobalt is rapid over the first days but is followed by a second, slower phase that lasts several weeks (Simonsen *et al.* 2012, IARC 2006). 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 intramuscular injections of cobalt 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.

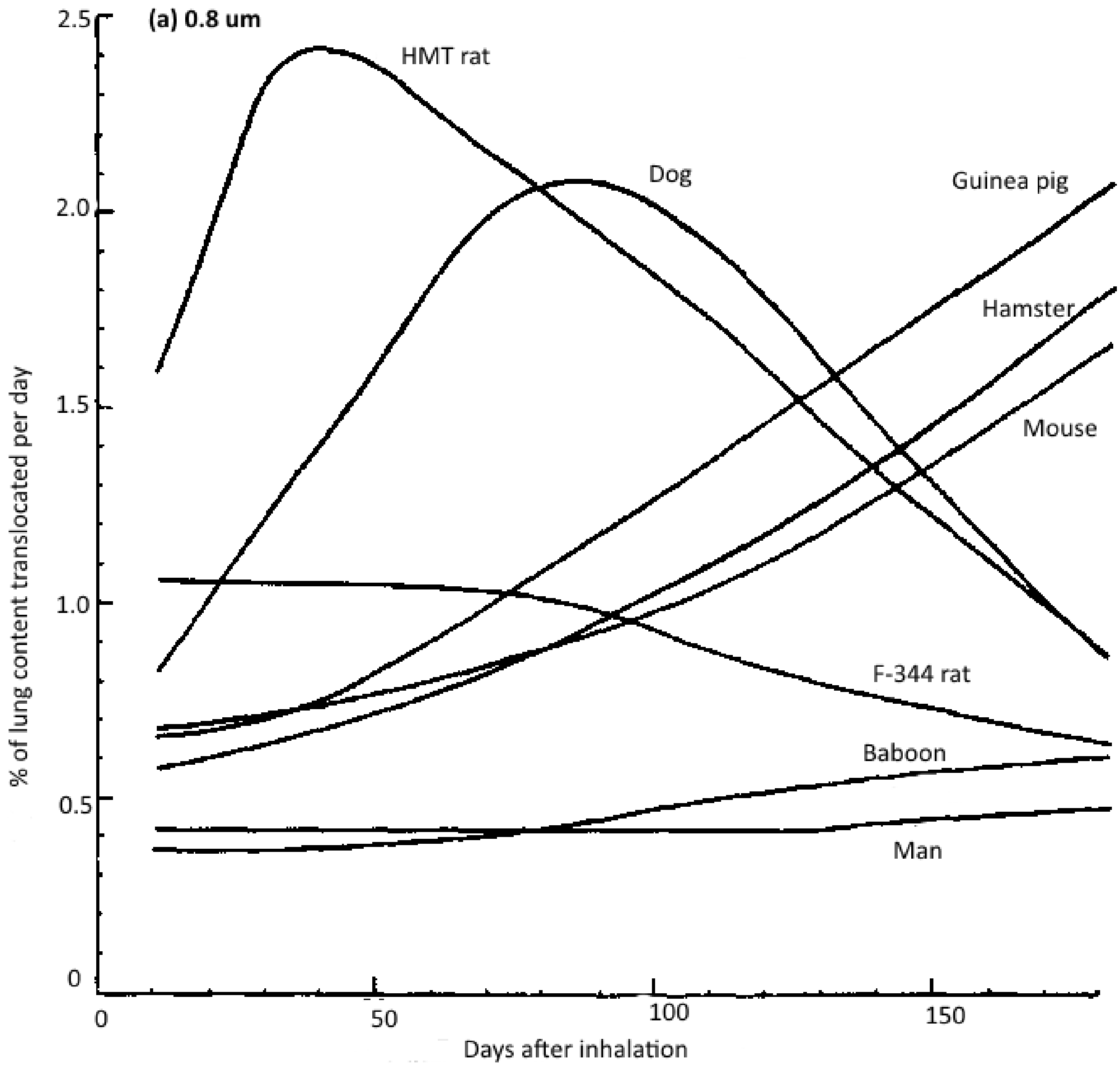
3.1.1 Experimental animals

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 (NTP 2014b, WHO 2006, Ayala-Fierro *et al.* 1999). 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 2014b). Gastrointestinal absorption of soluble cobalt compounds was much 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 (NTP 2014b, Leggett 2008, IARC 2006, NTP 1998, Kyono *et al.* 1992, IARC 1991). 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 (Kreyling *et al.* 1991a, Andre *et al.* 1989, Bailey *et al.* 1989, Collier *et al.* 1989, 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 the HMT (inbred strain) of 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 (Kreyling *et al.* 1991a, Bailey *et al.* 1989). 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).


(a) 0.8 um
HMT rat
Dog
Guinea pig
Hamster
Mouse
F-344 rat
Baboon
Man
% of lung content translocated per day
2.5
2.0
1.5
1.0
0.5
0
0
50
100
150
Days after inhalation

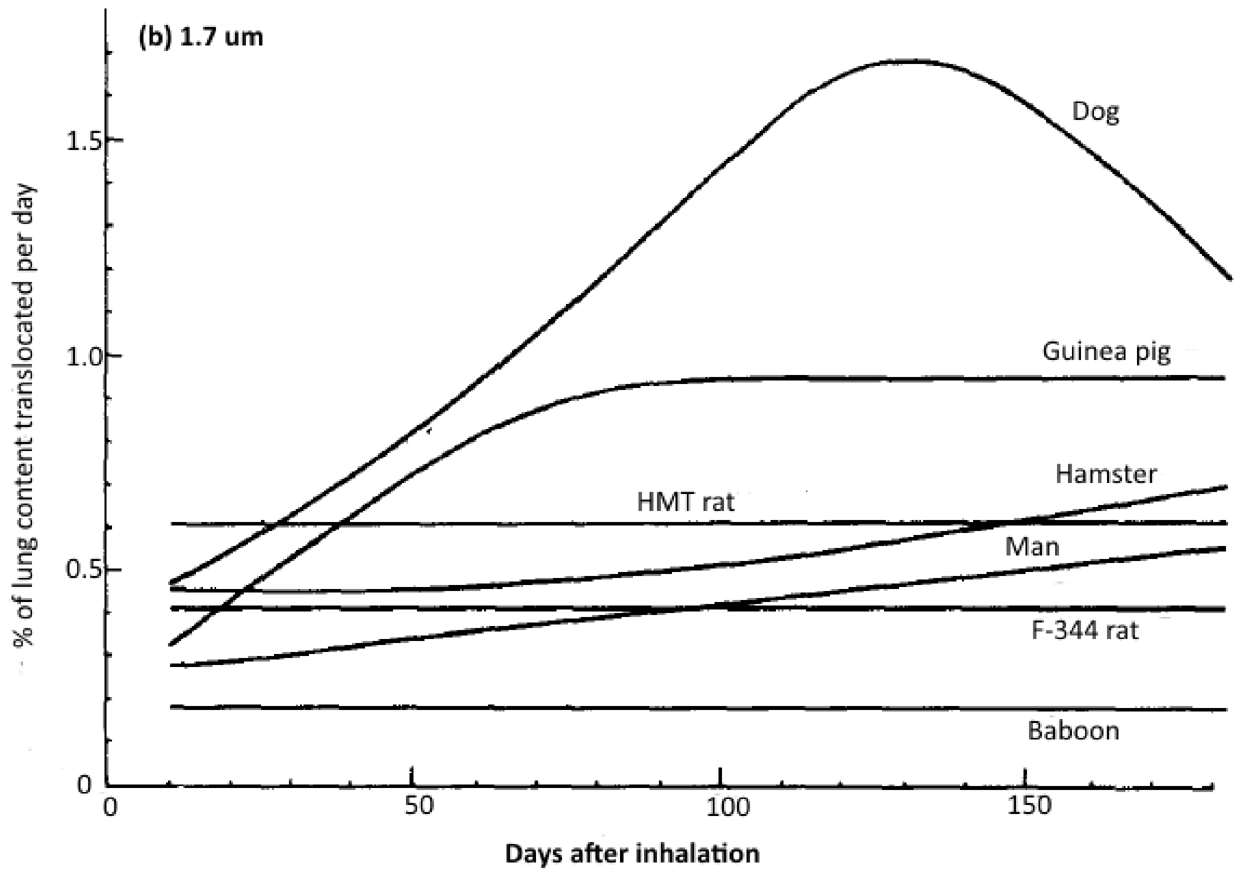


Figure 3-2. Rate of translocation of cobalt from lung to blood following inhalation of cobalt oxide particles

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 (Lacy *et al.* 1996, Kusama *et al.* 1986, Inaba and Suzuki-Yasumoto 1979). 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 2014b, 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, Clyne *et al.* 1988, Gregus and Klaassen 1986, Bourg *et al.* 1985, Thomas *et al.* 1976, Hollins and McCullough 1971). Following single-dose parenteral administration, some studies reported that concentrations were initially highest in the liver and kidney but declined rapidly (Thomas *et al.* 1976, Hollins and McCullough 1971). 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 (NTP 2014b, Patrick *et al.* 1994, Kyono *et al.* 1992, Collier *et al.* 1991, Bucher *et al.* 1990, Bailey *et al.* 1989, Patrick *et al.* 1989, Kreyling *et al.* 1986, Kerfoot *et al.* 1975, 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 (Patrick *et al.* 1994, Kreyling *et al.* 1991a, Bailey *et al.* 1989). 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 (Leggett 2008, Bailey *et al.* 1989). 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)

	Lung retention (%)[a]			
	90 days		180 days	
Species/strain	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.* 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 (Patrick *et al.* 1994, Bailey *et al.* 1989). 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, normalized tissue burdens did not increase with increasing exposure (NTP 2014b). Cobalt tissue concentrations (μg Co/g tissue) in male and female rats showed the following order: lung > liver > kidney > femur > heart > serum > blood (NTP 2014b). 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 data in mice were similar to that observed in rats but concentrations in the femur and heart were similar to concentrations in blood and serum. These data 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 (Paustenbach *et al.* 2013, Gregus and Klaassen 1986). Cobalt is excreted in the urine, feces, and bile with similar excretion patterns reported for all species studied (NTP 2014b, WHO 2006, ATSDR 2004). 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 (WHO 2006, ATSDR 2004). 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 (Unice *et al.* 2014, Paustenbach *et al.* 2013, Unice *et al.* 2012, Leggett 2008, ATSDR 2004). 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 is 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 insoluble cobalt metal or cobalt oxides (CoO or Co_3O_4) from the lungs is multiphasic with reported half-lives of 2 to 44 hours, 10 to 78 days, and years (NTP 2014b, WHO 2006, Mosconi *et al.* 1994a, Apostoli *et al.* 1994, Beleznay and Osvay 1994, Newton and Rundo 1971). 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 (WHO 2006, Foster *et al.* 1989). 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 (WHO 2006, ATSDR 2004, Foster *et al.* 1989, Kreyling *et al.* 1986). Studies in human volunteers administered cobalt chloride by i.v. injection also show a multiphasic elimination pattern (Holstein *et al.* 2015, Jansen *et al.* 1996, Letourneau *et al.* 1972, Smith *et al.* 1972). These studies show 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 initial 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 humans 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.* 2014, Unice *et al.* 2014, Tvermoes *et al.* 2013).

3.2.2 Experimental animals

Lung clearance kinetics of cobalt particles include both mechanical transport and translocation (Kreyling *et al.* 1991a, Bailey *et al.* 1989). 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 2014b). 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 (2014b) 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

			Elimination T½		
Reference	**Species: exposure route**	**Compound(s)**	**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[a] hr	4.6 hr	22.9 hr
(Menzel *et al.* 1989)	Male SD rats: inhalation	$CoCl_2$	1.8 hr	3.7–8.7[b] hr	−
(Kyono *et al.* 1992)	Male SD rats: inhalation	Co metal	52.8[c] hr 52.8[d] hr	156[c] hr 172.8[d] hr	−
(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[e] d 20−86[e] d 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.

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4 Human Cancer Studies

Introduction

The objective of the cancer hazard evaluation of cobalt and certain cobalt compounds (hereafter referred to as cobalt) is to reach a preliminary 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 2014c]).
2. Description of the study methods and characteristics (Appendix C.1, Tables C-1a-i) 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 Appendix C.2, Tables C-2a to C-2c).
3. Cancer assessment: Lung (Section 4.2.3), esophagus (Section 4.3.3), and other cancers (Section 4.4).
4. Preliminary recommendation for the level of evidence of 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 that multiple studies evaluated. (For rationale, see Protocol: Methods for Preparing the Draft Report on Carcinogens Monograph on Cobalt ["Cobalt Protocol"; NTP 2014c] and Tables 4-1 and 4-4). Because the occupational 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) are discussed 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 certain cobalt compounds and human cancer, and (3) provided exposure-specific analyses for cobalt or certain cobalt compounds at an individual level, or based on the authors' report, cobalt exposure was probable or predominant in the population, job, or occupation under study. Studies of hip implants and prosthetic devices made from cobalt alloys and of radioactive cobalt were excluded, as the extent of exposure to cobalt is often unclear and because of potential confounding from the other compounds in the alloy (which are often metals) or 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 cobalt exposure alone was not reported as noted above.

Biomarker studies of cobalt and cancer were included if they were conducted within defined populations and provided risk estimates of cobalt levels and cancer. A series of clinical studies that compared cobalt levels in hair of patients with cancer and referent groups or in tissues (collected from autopsies) were identified and are summarized in tabular format in Appendix B, Tables B-2 (hair) and B-3 (tissues). These studies did not provide information to calculate an effect estimate, and most did not have defined methods for selecting the subjects and are not included in the cancer hazard evaluation. Most of these studies measured numerous trace and heavy metals.

Environmental studies of cobalt and cancer were included if they were conducted within defined populations and provided risk estimates of cobalt levels and cancer. A total of four studies was identified, two of which investigated the relationship of cobalt in air to breast (Coyle *et al.* 2006) and lung (Coyle *et al.* 2005) cancer. The other two investigated the relationship between soil levels of cobalt and cancer (McKinley *et al.* 2013, Kibblewhite *et al.* 1984). 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, Tables C-1a-g in Appendix C, and Table 4-2 in Section 4.2.2.

The available epidemiologic 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, 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 included two publications. The first publication was on an 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 by etching roasted ore, followed by neutralization, filtration, and electrolysis of the cobalt chloride solution. 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 an overlapping population 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 relationship 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 Appendix C, Tables C-1a-g.

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
(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/never Nested case-control analysis: Ever/never employed in cobalt production *Moulin* et al. *1993* Mortality SMR analysis Only/never employed
(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 cobalt-exposed only workers 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.* 2000a)	**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

Reference	Population	Design and outcome (cancer sites)	Exposure: Cobalt compounds, assessment, metrics
			Semi-quantitative JEM Duration, intensity, and cumulative exposure
(Grimsrud *et al.* 2005) (methods described in Grimsrud *et al.* 2003, Grimsrud *et al.* 2002)	**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 cohorts reported 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

	Bias						Quality[a]	Utility[b]
Citation	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.* (2000a)	+++	++	+++	+	+++	+++	++	++
Nickel refinery workers Grimsrud *et al.* (2005)	+++	+++	+++	++	+++	+++	+++	+++

NI = no information.

[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.

4.2.3 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 preliminary 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 guidelines developed by Austin Bradford 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 preliminary 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 (SEER 2015a) 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 of 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, French multi-centric cohort of hard-metal factory workers, a related cohort of workers from the largest

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. 1394 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:** Calculation of expected number of cancer cases took five year age groups and calendar periods in consideration. No HWE **Strengths:** Population exposed primarily to cobalt compounds alone; only female population with data on cobalt. No Healthy Worker Effect apparent in cohort. **Limitations:** Small number of exposed cases. Sporadic info on smoking, and no control for smoking.
		All exposed	8	SIR 2.35 (1.09–4.45)	Age	
		Factory 1 (CoSilicate from 1972)	3	1.6 (0.41–4.37)		
		Factory 2 (CoAlSpin 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=1143; number of cobalt production workers NR ~ 25% of current staff at time of publication **Exposure assessment method:** company records	**Lung (162)**				60% worked greater than 10 years; 75% hired before 1975 **Confounding:** 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). **Strengths:** Cobalt production workers exposed primarily to cobalt compounds; estimates of cancer risk among those exclusively employed in cobalt production. **Limitations:** Small number of exposed cases; high loss to follow-up (20%); smoking history only known on 30% of cases; co-exposure to nickel and arsenic; significant HWE in this cross-sectional cohort; no adjustments for
		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
						HWE or HWSE.
(Mur *et al.* 1987) Nested case-control France 1950–1980	Electrochemical plant workers Cases: 9; Controls: 18 **Exposure assessment method:** company records	**Lung (162)**				60% worked greater than 10 years; 75% hired before 1975 **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 **Strengths:** Nested study reduces concern with strong HWE in this cohort. **Limitations:** Small numbers with limited information on exposures (only ever/never employment in cobalt production department); no description of control selection given only 30% had smoking data. No information on differences in cases and controls by date of hire or last employment to assess potential HWSE.
		Ever worked in Co production	4	OR [4.0 (0.66–24.4)]	None	
(Moulin *et al.* 1993) Cohort France Extended followup of the Mur 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)**				NR, but likely similar to Mur 1987 **Confounding:** No reported control for period effects, duration, or and time since first exposure **Strengths:** Electrochemical plant workers exposed to cobalt compounds, with exposure estimates separated for workers in cobalt production. **Limitations:** Small number of exposed cases in overall or sub-cohort; concern about outcome misclassification; no consideration of smoking; potential co-exposures to nickel and arsenic. All cause mortality for the full
		Exclusive Cobalt production, Cohort I	3	OR 0.85 (0.18–2.5)	Age	
		Exclusive Cobalt Production, Cohort II	3	1.16 (0.24–3.4)		
		Ever worked in Cobalt production,	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
		Cohort I				cohort was SMR = 0.85 (upper 95% CI = 0.95) due to large loss among foreign-born; no internal analysis, nor any adjustments for HWE or HWSE in this cross-sectional cohort.
		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)**				NR **Confounding:** mentioned the full list of IARC carcinogens, but did not indicate if these were controlled in the cobalt alone analyses **Strengths:** Data available for cobalt without tungsten carbide; provided estimates for intensity, duration, and cumulative exposure (both weighted and unweighted); 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)	Unclear which variables were controlled in the multivariate analysis for cobalt alone	
		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)		

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
(Wild *et al.* 2000) Cohort France 1968–1992	Hard metal workers - Largest plant in France 2216 men and 644 women **Exposure assessment method:** JEM	**Lung (162)**				NR **Confounding:** conducted separate smoking analyses **Strengths:** Incident cohort; ability to control for co-exposures and smoking; no HWE; lagged analysis. **Limitations:** Unclear if co-exposures or smoking are controlled in the estimates for Co; external analysis only presented; no exposure metrics except for ever/never shown.
		Cobalt except in hard metals	15	SMR 1.95 (1.09–3.22)	Unclear if these are crude estimates, Age	
(Moulin *et al.* 2000a) Nested case-control France 1968–1992	Stainless and alloyed steel workers Cases: 54 (17 Co exposed); Controls: 162 (67 Co exposed) **Exposure assessment method:** JEM	**Lung (162)**				NR **Confounding:** Co correlated in a reported matrix with Chromium and/or Nickel, and Iron, but neither of these were included in the multivariate analysis **Strengths:** Semi-quantitative JEM; exposure metrics including duration and cumulative dose, frequency weighted and unweighted; HWE mitigated by use of internal analyses; controlled for smoking; reported information to assist in evaluating that healthy worker survival bias was unlikely. **Limitations:** Models did not control for substances
		Exposed, Crude	17	OR 0.64 (0.33–1.25)	Smoking ever/never, age, PAHs, silica, gender	
		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)		

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
		Exposed, known smoking status, PAH, silica, and smoking adjusted	12	0.44 (0.17–1.16)		related to Cobalt in the correlation analysis. Known carcinogens had non-significant ORs < 1.0, indicating that the study had low sensitivity to detect an effect.
(Grimsrud *et al.* 2005) Nested case-control Norway 1910–1995	Nickel refinery workers Cases: 213; Controls: 525 **Exposure assessment method:** JEM	**Lung**				In μg/m^3: High (144–3,100); Medium (29.7–142); Low (0.31–29.5) **Confounding:** No multivariate estimates for the categorical variable were able to be estimated due to collinearity with nickel. **Strengths:** Quantitative cobalt levels reported based on measurements from the breathing zone; incident cases; internal analyses; relatively large number of cases compared to other Co studies. **Limitations:** Confounded by exposure to nickel; collinearity was such that no estimates controlled for other co-exposures was possible.
		Rise in OR per (mg/m^3) × years, Crude	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)		
		High (144–3,100 μg/m^3 × years	82	2.9 (1.2–6.8)		
		Lung				
		Rise in OR per (mg/m^3) × years, Crude	NR	0.7 (0.3–1.4)	Smoking, nickel, sulfuric acid mists, asbestos,	

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
					arsenic	
		Lung				
		Co electrolysis workshop, 0.03–2.2 yrs	23	1.6 (0.8–3)	Smoking, employment in other workshops	
		Co electrolysis workshop, 2.3–11.8 yrs	44	2.8 (1.5–5)		
		Co electrolysis workshop	62	5.1 (2.9–9.1)		

NR = Exposure levels or duration not reported.

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.

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*. 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*. (1993a) 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 a cohort of cobalt production workers in a French electrochemical plant (Moulin *et al.* 1993, Mur *et al.* 1987). Both findings are reported because the updated follow-up (Moulin *et al.* 1993) not only introduced different case ascertainment methods than the earlier analyses of the cohort (Mur *et al.* 1987), but also restricted analyses to account for large loss to follow-up among foreign-born workers. 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*.). There was large loss to follow-up and clear evidence of a healthy worker effect (HWE) (all-cause mortality SMR = 0.77 [95% CI = 0.67 to 0.88]). However, in an internal matched analysis (matching variables were year of birth, age at death, and smoking habits), no estimated odds ratio or confidence interval was reported, but a crude calculation based on reported numbers of exposed and unexposed cases and controls indicated an OR of 4.0 (95% CI = 0.7 to 24.4), indicating internal consistency. However, in the follow-up of the 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. (The SMR for the entire cohort has lower confidence because of high loss to follow-up and strong healthy worker effect among foreign-born workers). In addition, Moulin *et al*. reported a discrepancy in the number of observed cases exclusively employed in cobalt production in the two analyses (e.g., Mur *et al.* [N = 4]; Moulin *et al.* [N = 3]) due to differences in the methods used to ascertain cause of death. The Mur *et al.* 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*. initially reported that smoking histories were available for 30% of workers, and the authors matched cases and controls on smoking status; based on their table, a crude OR of 4.0 was calculated. No explanation was given in that paper regarding the methods of matching given the small percentage of workers with information on smoking status. However, Moulin *et al*. 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.

The evidence from these electrochemical studies are inconclusive, based on the low sensitivity of the Moulin *et al*. study to detect an effect, the lack of exposure metrics in both studies, 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*. analysis was consistent across the internal and external analyses, and was able to apply corrections to address the HWE. The Moulin *et al.* analysis reduced power dramatically to detect an effect.

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 (levels 2 to 9) to “cobalt alone or simultaneously with agents other than tungsten carbide” 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*. 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*., 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*. study and from occupational health department records in the Wild *et al.* 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.* 2000a). In internal analyses of cobalt exposure based on the JEM in the stainless and alloyed steel plant, Moulin *et al*. reported a crude OR of 0.64 (95% CI = 0.33 to 1.25), and an OR adjusted for PAHs and silica OR 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*. (2000a) 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*.). 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 were not included in the cobalt model. However, these exposures were also not associated with lung cancer risk in these data.

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 × 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 μg/m^3) based on 49 cases, OR = 1.5 (95% CI = 0.6 to 3.8); medium exposure (29.7 to 142 μg/m^3) based on 73 cases, OR = 2.4 (95% CI = 1.0 to 5.6); and high exposure (144 to 3,100 μg/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 typically amounts to 4% to 15% of the total nickel, 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.

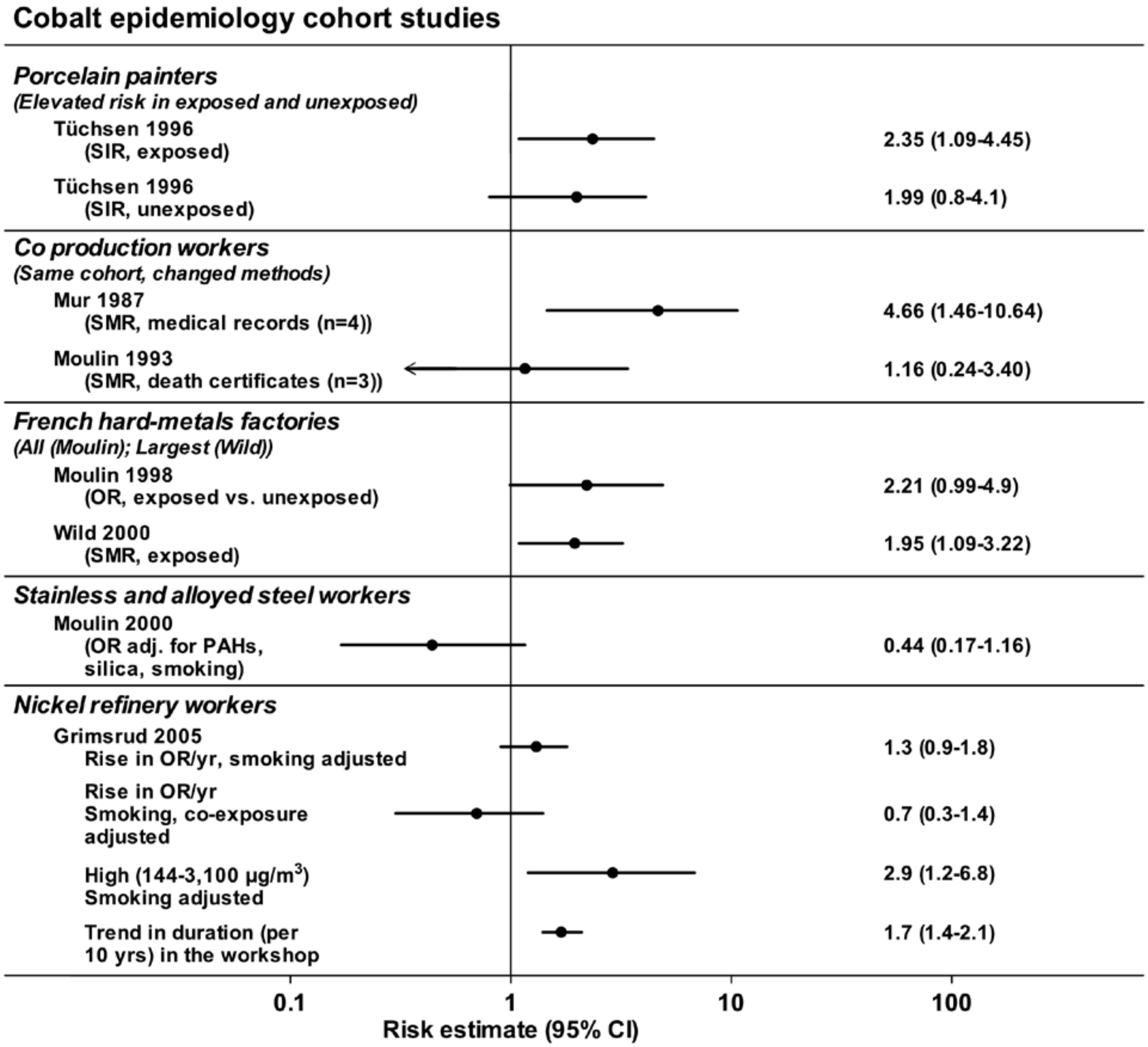
Although the design of this study was of high quality, due to the collinearity with exposure to nickel, this study cannot separate out the effects of cobalt and nickel on lung cancer and thus the findings from the study are unclear.

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.

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 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. 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, controlled 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 risk ratios (SIR, SMR or OR as noted) and 95% CI for epidemiological 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 epidemiologic 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, Tables C-1h,i in Appendix C, and Table 4-6 in Section 4.3.2.

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)
(O'Rorke *et al.* 2012)	Population-based case-control biomarker study Ireland FINBAR* study 2002–2004 137 cases/221 controls	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 and potential for reverse causation. 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.* conducted stratified analyses on tumor stage and time of diagnosis that may be of help to evaluate the potential for reverse causality.

Table 4-5. Bias and quality summary for case-control studies

	Bias[a]						Quality[a]	Utility[b]
Citation	Selection	Exposure	Outcome	Confounding methods	Adequacy of analysis	Selective reporting	Sensitivity	Integration
Rogers *et al.* (1993)	+++	+	+++	+++	+++	+++	+	+
O'Rorke *et al.* (1993)	++	+	+++	+++	+++	+++	+	+

[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 (SEER 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 UK 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 (EAC) and esophageal squamous-cell cancer (ESCC), however, have distinct risk factors and trends. EAC, with risk factors being white race, increasing age, body fatness, and male gender, is the predominant histological type among men, while for women, ESCC 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 (Freedman *et al.* 2011, Anderson *et al.* 2009, 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 of 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.* limited their analysis to esophageal adenocarcinoma, while no histologic information was provided by Rogers *et al*., thus it is likely that the Rogers *et al*. 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)**				Tertiles of cobalt in toenails; highest level 0.17 ppm **Confounding:** 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 **Strengths:** Population-based study; histologically confirmed cancers; cases and controls from same source population; matching on key likely confounders **Limitations:** Toenail levels pre-diagnostic, but reverse causation can't be ruled out. No information provided about correlation of cobalt with other trace elements.
		< 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 (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)		

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
(O'Rorke *et al.* 2012) Case-Control All Ireland (Republic and Northern) 3/2002–12/2004	All Ireland population based study of 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	**Esophageal cancer**				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 **Confounding:** 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. **Strengths:** Population based; histologically confirmed cancer; data on broad range of co-exposures and co-variates collected. **Limitations:** Differences in sources of cases and controls in No Ireland and Rep of Ireland may introduce some selection bias; low participation rate in controls, especially in Rep of Ireland; no correlation information of cobalt with other trace elements provided.
		< -5.4824	34	OR = 1.0	Age, sex, GI reflux, education, H. pylori infection, location, smoking	
		≥ -5.4824	39	1.06 (0.57–1.98)		
		≥ -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) Cohort France 1950–1980	Electrochemical workers N=1143; number of cobalt production workers NR ~ 25% of current staff at time of	**Buccal cavity, pharynx, larynx (140-149, 161)**				60% worked greater than 10 years; 75% hired before 1975 **Confounding:** SMR all cause mortality = 0.77 (*P* < 0.01); no methods to control HWE; all cause mortality for cobalt
		Cobalt Production	2	SMR 3.36 (0.29–10.29)	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
	publication **Exposure assessment method:** company records					production = SMR 1.29 (0.86–1.87). **Strengths:** Cobalt production workers exposed primarily to cobalt compounds; estimates of cancer risk among those exclusively employed in cobalt production. **Limitations:** Small number of exposed cases; high loss to follow-up (20%); smoking history only known on 30% of cases; co-exposure to nickel and arsenic; significant HWE in this cross-sectional cohort; no adjustments for HWE or HWSE.

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 risks factors for esophageal cancer can reasonably be ruled out, except for the metals, as none have previously been associated with esophageal cancer. 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 may 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*. 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. Reverse causality due to trace element deposition in nails influenced by factors associated with cancer (e.g., weight loss, age, gender, changes in diet and smoking and alcohol consumption) (Slotnick and Nriagu 2006, Hunter *et al.* 1990) may be a possibility. However, the authors stated (data not shown) that elevated risks of esophageal cancer were found in individuals with *in situ*/localized tumors as well as those with regional/distant tumors, and no significant differences were found in stratified analysis by the time from diagnosis to tumor (< 7 months or ≥ 7 month), suggesting that reverse causality may not be a concern.

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), energy intake, gastro-esophageal reflux, and *H. pylori* infection. O’Rorke *et al.* reported no information regarding the correlation of 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.* 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. In addition, reverse causality is possible due to trace element deposition in nails being influenced by factors associated with cancer that could create a bias away from the null, and explain the elevated cobalt levels found in this study. Barrett's esophagus, like esophageal cancer, has a prolonged latency period; however, it is unknown whether the disease progression of Barrett's esophagus could influence deposition of trace metals in nails. Similar to the Rogers *et al.* 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 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*.) provided an SMR for buccal cavity, pharyngeal, and laryngeal cancers for those working in cobalt production. Rogers *et al*. 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) (SEER 2015c); 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 cancers

The available data to evaluate cobalt in relation to other 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 (Tüchsen *et al.* 1996, Moulin *et al.* 1993) (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 Preliminary listing recommendation

There is inadequate evidence from studies in humans to evaluate the association between exposure to cobalt and cancer. While almost all the cohort studies reported approximately a doubling of the risk of lung cancer from exposure to various cobalt compounds, cobalt exposure was likely correlated with exposure to other known lung carcinogens, which complicates the interpretation of the results. Increased risks of esophageal cancer were found in two population-based case-control studies; however, cobalt exposure was assessed in toenail samples at or after cancer diagnosis. Thus, it is unclear whether cobalt levels in the toenails reflected exposure to cobalt preceding cancer or resulted from changes due to tumor formation.

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5 Studies of Cancer in Experimental Animals

This section reviews and assesses the evidence from carcinogenicity studies in experimental animals exposed to cobalt and certain cobalt compounds. 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 studies (16 carcinogenicity and 9 co-carcinogenicity studies) were identified that met the inclusion criteria. 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. The criteria to evaluate exposure specific to cobalt and/or cobalt compounds require studies that either had observational durations > 12 months 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) and that report on the presence or absence of neoplastic and related non-neoplastic lesions. Several studies were excluded from the review because they did not have concurrent controls. These included Hopps *et al.* (1954), Delahant (1955), Gilman (1962), Nowak (1966), and Gunn *et al.* (1967). Studies of cobalt alloys and radioactive cobalt in experimental animals were not considered to be informative because of potential confounding by other carcinogens. (See IARC 2006 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 preliminary level of evidence conclusion for the carcinogenicity of cobalt and certain cobalt compounds 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 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).

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 2014b)
Mouse B6C3F$_1$ (M&F)	Cobalt metal	Inhalation	2 yr/2 yr	(NTP 2014b)
Rat Sprague-Dawley (M)	Cobalt metal [nano] and Cobalt metal [bulk]	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.

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 2014c]). An overview of the quality evaluations for the carcinogenicity studies is shown in Table 5-3 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 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 (Heath 1956, Heath and Daniel 1962, Gilman and Ruckerbauer 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	Randomi-zation	Purity	Dosing	Treatment-related survival	Pathology	Con-founding	Reporting & analysis	Animal model	Stat power	Duration	
(NTP 2014b) -R	**Cobalt metal**	+++	**Yes**	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	**High**
(NTP 2014b) -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**

Study		Quality									Sensitivity			Overall utility
		Controls	Historical data	Randomi-zation	Purity	Dosing	Treatment-related survival	Pathology	Con-founding	Reporting & analysis	Animal model	Stat power	Duration	
(Steinhoff and Mohr 1991)-(intratracheal)	**Cobalt oxide**	+++	**No**	**NR**	++	++	++	++	++	++	+++	+++	+++	**Moderate**
(Steinhoff and Mohr 1991) - (IP)	**Cobalt oxide**	+++	**No**	**NR**	++	+	**NR**	++	++	++	+++	+	+++	**Moderate**
(Steinhoff and Mohr 1991) - (SC)	**Cobalt oxide**	+++	**No**	**NR**	++	++	**NR**	++	++	++	++	+	+++	**Moderate**
(Gilman and Ruckerbauer 1962) – R	**Cobalt oxide**	+++	**No**	**NR**	+	+	++	++	+	+	+++	+	++	**Low**
(Gilman and Ruckerbauer 1962) – M	**Cobalt oxide**	+++	**No**	**NR**	+	+	++	++	+	++	++	++	+++	**Low**
(Wehner *et al.* 1977)	**Cobalt 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 Tables 5-3 to 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, intrarenal, and intrapleural). In addition, in some inhalation studies, some tumors were observed in sites distal from the route of administration. Findings for cobalt compounds across organ sites are discussed in Section 5.3.

Lung (Table 5-3)

Different types of cobalt compounds – cobalt metal (NTP 2014b), a soluble cobalt salt, cobalt sulfate heptahydrate (NTP 1998), and a poorly soluble cobalt compound, cobalt 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 2014b, 1998) and all had either high (NTP 2014b, 1998) 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 2014b, 1998). 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 2014b). 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 was 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 2014b). 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 at 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 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, only those with gross lesions 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 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	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
NTP 2014b Rat (F344/NTac) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation 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. **Limitations:** Decreases in body weight in mid and high dose rats, **Other comments:** Historical controls were limited, as Fischer 344/NTac rats have only been used in two NTP carcinogenicity studies and so it is based on only 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]			
			0 mg/m³	17	2/50 (5%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
			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 2014b Rat (F344/NTac) Female 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation 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. **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, as Fischer 344/NTac rats have only been used in two NTP carcinogenicity studies and so it is based on only 100 rats. Significantly increased
			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%)	
			2.5 mg/m³	24	3/50 (6%)	
			5 mg/m³	25	4/50 (8%)	
			Alveolar/bronchiolar adenoma[a]			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N^+) (%)	Comments, strengths, and limitations
			0 mg/m³	35	2/50 (5%)	non-neoplastic lesions: Alveolar hyperplasia (pre-neoplastic) - all dose levels. Bronchiolar hyperplasia (pre-neoplastic) - all dose levels
			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]	
			Trend-test *P*-value: 0.002			
NTP 2014b Mouse ($B6C3F_1$/N) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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
			0 mg/m³	39	3/50 (6%)	
			1.25 mg/m³	31	18/49 (36%)**	
			2.5 mg/m³	29	24/50 (48%)**	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
			5 mg/m³	25	36/50 (72%)**	almost all factors. **Limitations:** A significant decrease in survival of male mice and decrease in body weight in high dose mice. **Other comments:** 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
			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]			
			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 2014b Mouse ($B6C3F_1$/N)	Cobalt metal (98% pure, mass	Inhalation 6 hr/day, 5 day/wk ×	**Multiple alveolar/bronchiolar carcinoma**			Survival in exposed groups was similar to
			0 mg/m³	36	1/49 (10%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
Female 105 wk	median aerodynamic diameter 1-3 µm)	105 wk	1.25 mg/m³	36	7/50 (50%)*	controls. **Strengths:** A well-designed study in all factors. **Limitations:** Decrease in body weight in high dose mice. **Other comments:** 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) – mid- and high-dose levels
			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%)	
			1.25 mg/m³	36	9/50 (20%)	
			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%)***	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
			Trend-test *P*-value: 0.001			
NTP 1998 Rat (F344) Male 2 yr	Cobalt sulfate (99% pure)	Inhalation 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. **Other comments:** 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%)	
			3.0 mg/m³	15	7/50 (34%)*	
			Trend-test *P*-value: 0.032			
NTP 1998 Rat (F344) Female 2 yr	Cobalt sulfate (99% pure)	Inhalation 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. **Other comments:** Significantly increased non-neoplastic lesions: Alveolar epithelium
			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%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N^+) (%)	Comments, strengths, and limitations
			1.0 mg/m³	26	10/50 (36%)***	metaplasia - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - high dose; Alveolar epithelium hyperplasia, atypical (pre-neoplastic) - high dose
			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%)	
			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 2 yr	Cobalt sulfate (99% pure)	Inhalation 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
			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]	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N+) (%)	Comments, strengths, and limitations
			Trend-test *P*-value: 0.006			was similar to controls. **Limitations:** None.
			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]	
			Trend-test *P*-value: 0.001			
NTP 1998 Mice ($B6C3F_1$) Female 2 yr	Cobalt sulfate (99% pure)	Inhalation 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.
			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			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
			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 life-span	Cobalt oxide ("Chemically pure." 80% of particles were 5–40 μm)	Intratracheal instillation 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)	**Bronchio/alveolar carcinoma**			Survival and body weight were the same as controls. **Strengths:** Two dose levels were 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 husbandry were not reported. **Other comments:** Significantly increased non-neoplastic lesions: Bronchio/alveolar proliferation - both dose levels.
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	3/50 (6%)[1]	
			Bronchio/alveolar adenoma			
			0 mg/kg bw	NR	0/50 (0%)	
			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%)	

1

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
Steinhoff and Mohr 1991 Rat (Sprague-Dawley) Female life-span	Cobalt oxide ("Chemically pure", 80% of particles were 5–40 µm)	Intratracheal instillation 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)	**Bronchio/alveolar carcinoma**			Survival in exposed groups was similar to controls. **Strengths:** Two dose levels were 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 husbandry were not reported. **Other comments:** Significantly increased non-neoplastic lesions: Bronchio/alveolar proliferation - both dose levels.
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	1/50 (2%)	
			Bronchio/alveolar 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%)	
			Bronchio/alveolar adenoma or bronchio/alveolar carcinoma combined			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	1/50 (2%)	
			10 mg/kg bw	NR	1/50 (2%)	

* = *P*-value ≤ 0.05; ** = *P*-value ≤ 0.01; *** = *P*-value ≤ 0.001. NR = Not reported.
[+] = Number of animals necropsied for NTP 2014b and NTP 1998 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 oxide) by injection increased injection-site tumors in several studies in rats (Hansen *et al.* 2006, Steinhoff and Mohr 1991, Shabaan *et al.* 1977, Gilman and Ruckerbauer 1962, Heath and Daniel 1962, Heath 1956). 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 (Steinhoff and Mohr 1991, Hansen *et al.* 2006) or low utility (Heath *et al.* 1956, Heath and Daniel 1962, Gilman and Ruckerbauer 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 4.4).

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 PVC and sacrificed at six and twelve months. Local sarcomas developed around the site of the nanoparticles in one of four rats at the 6-month sacrifice and in 5 of 6 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 cause 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 Ni/Co 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 and Daniel 1962, Heath 1956) 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 (8 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 and Daniel 1962, Heath 1956), suggesting that the dose might have been to 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 oxide was injected (i.p., s.c., i.m.) into rats in three studies (Steinhoff and Mohr 1991, Gilman and Ruckerbauer 1962) 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, strengths, and limitations
Hansen 2006 Rat (Sprague-Dawley) Male 12 mo	Cobalt metal [nano and bulk] (Bulk metal particles were 50-200 nm in size, with an average of 120 nm. The surface area to mass ratio was 4.75 and for the nano-particles it was 50,000, but the size of the nano-particles were not reported.)	Nano: IM implant (left side of vertebra) Single dose Bulk: 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 cm²	4	0	
			Nano 2 cm²	4	2	
			Bulk 2 cm²	4	0	
			Sarcoma 6 months			
			0 cm²	4	0	
			Nano 2 cm²	4	1	
			Bulk 2 cm²	4	0	
			Fibroblastic proliferation 8 months			
			0 cm² (12 mo)	6	0/6 (0%)	
			Nano 2 cm²	6	1/6 (16.7%)	
			Bulk 2 cm²	6	1/6 (16.7%)	
			Sarcoma 8 months			
			0 cm² (12 mo)	6	0/6 (0%)	
			Nano 2 cm²	6	5/6 (83.3%)[**]	
			Bulk 2 cm²	6	0/6 (0%)	
Heath 1956 Rat (Hooded) Male life span	Cobalt metal ("Spectroscopically pure," Particle size: 3.5 × 3.5 µm to 17 × 12 µm)	IM inj. in fowl serum Single dose	**Rhabdomyofibrosarcoma or sarcoma combined**			No data was given on the survival of untreated controls. 2/10 treated males without tumors died before final sacrificed.
			0 mg/rat	NR	0/10 (0%)	
			28 mg/rat	8	4/10 (40%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
						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 life span	Cobalt metal ("Spectroscopically pure," Particle size: 3.5 × 3.5 µm to 17 × 12 µm)	Series I and Series II IM inj. in fowl serum Single dose	**Sarcoma (Rhabdomyofibrosarcoma or fibrosarcoma)**			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
			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%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N^+) (%)	Comments, strengths, and limitations
						controls, which was non-concurrent. 6/7 sarcoma in Series I and 2/5 in Series II were rhabdomyo-fibrosarcoma
Heath and Daniel 1962 Rat (Hooded) Female 28 months	Cobalt metal (Purity not reported, Particle size: 3.5x3.5 µm to 17x12 µm)	Intrathoracic inj. (in serum) Single injection	**Mixed sarcoma intrathoracic region**			Survival was only reported for exposed rats, which was 12/20 on day 3 and 11/20 after 11 months. **Strengths:** Observation duration was sufficient and both sexes were tested. **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.
			0 mg/dose[a]	NR	0/10 (0%)	
			28 mg/dose	11	4/12 (33%)	
Jasmin and Riopelle 1976 Rat (Sprague-Dawley)	Cobalt metal NR	Intrarenal placement Single dose	**Kidney neoplasm NOS**			Survival was not reported. **Strengths:** Moderate
			0 mg/rat	NR	0/16 (0%)	
			10 mg/rat	NR	0/18 (0%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N^+) (%)	Comments, strengths, and limitations
Female 12 months						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.
Shabaan 1977 Rat (Wistar) Male 8 and 12 mo	Cobalt chloride NR	SC inj. 1 dose/day × 5 days, then 9 days off, then 1 dose/day × 5 days (total 19 days)	**Injection site and non-injection fibrosarcoma**			Treatment-related decrease in survival; 16/20 survived at 8 months and 11/20 survived at 12 months. **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 months controls used as comparison group. Statistical testing
			0 mg/kg bw 12 mo	19	0/19 (0%)	
			40 mg/kg bw 8 mo	16	6/16 (30%)[**]	
			40 mg/kg bw 12 mo	11	8/11 (40%)[***]	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N+) (%)	Comments, strengths, and limitations
						(Fisher's Exact Test) reported by IARC.
Steinhoff and Mohr 1991 Rat (Sprague-Dawley) Male and Female life span	Cobalt oxide ("Chemically pure," 80% of particles were 5–40 µm)	IP inj. 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 life span	Cobalt oxide ("Chemically pure", 80% of particles were 5–40 µm)	SC inj. 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 intensity levels and two untreated control groups used. **Limitations:** Limited sensitivity due to few animals per group and
			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%)[*]	
			10 mg/kg/wk	NR	4/10 (40%)[*]	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
						only males tested. Limited histological examination. Incomplete reporting of many elements.
Gilman and Ruckerbauer 1962 Rat (Wistar) Male and female 489 days	Cobalt oxide (purity not reported, particle size was < 5 µm)	IM inj. 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 Mouse (Swiss) Female 751 days	Cobalt oxide (purity not reported, particle size was < 5 µm)	IM inj. Single dose	**Sarcoma**			Survival was similar to control at 90 days. **Strengths:** The duration of observation and the numbers of animals per group were sufficient. **Limitations:** Limited sensitivity due to only a
			0 mg/mouse	48	0/51 (0%)	
			20 mg/mouse	46	0/50 (0%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N+) (%)	Comments, strengths, and limitations
						single dose was given at one dose level, without a rationale, to females only. Half of the rats 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 12 months	Cobalt sulfide NR	Intrarenal placement 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.
+ = Number of animals at the beginning of the study, except for Hansen 2006 and Heath and Daniel 1962, which used the number of animals that were examined at the time of sacrifice (Hansen 2006) or the number of animals that survived beyond day 4 (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 and distal site neoplasms and non-neoplastic lesions

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 2014b; 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 testing cobalt metal and cobalt sulfate (see Table 5-5) (NTP 2014b, 1998, 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 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 from damage to lungs that cause obstructive sequela by causing systemic hypoxemia, leading to chronic stimulation of catecholamine release by the adrenal medulla and subsequent neoplastic development (NTP 2014b). 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 2014b). 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 at the low-dose (0.3 mg/m^3) males.

Wehner reported finding a single adrenal gland adenoma in the cortex of hamsters after inhalation of cobalt oxide. (Wehner *et al.* 1977). Wehner 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 2014b). 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 carcinomas observed in female rats exceeded the historical controls for all routes of administration and thus may 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. The onset of leukemia in females was shorter in cobalt-exposed groups, but no statistical calculations were done to tell if the differences were significant. 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 2014b). 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, strengths, and limitations
Adrenal gland						
NTP 2014b Rat (F344/NTac) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation 6 hr/day, 5 day/wk × 105 wk	**Bilateral malignant pheochromocytoma**			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, as Fischer 344/NTac rats have only been used in two carcinogenicity studies and so it is based on only 100 rats.
			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%)***	
			Trend-test *P*-value: 0.001			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N+) (%)	Comments, strengths, and limitations
NTP 2014b Rat (F344/NTac) Female 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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. **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 have only been used in two carcinogenicity studies and so it is based on only 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]			
			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%)***	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
			Trend-test *P*-value: 0.001			
NTP 1998 Rat (F344) Male 2 yr	Cobalt sulfate (99% pure)	Inhalation 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.. **Limitations:** None.
			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 2 yr	Cobalt sulfate (99% pure)	Inhalation 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. **Limitations:** None. **Other comments:** Significantly increased non-neoplastic lesions: Adrenal gland: Hyperplasia - high dose
			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%)	
			1.0 mg/m³	26	4/50 (8%)	
			3.0 mg/m³	30	10/48 (21%)*[d]	
			Trend-test *P*-value: 0.001			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N+) (%)	Comments, strengths, and limitations
Wehner 1977 Hamster (Syrian Golden, random bred ENG:ELA) Male Lifespan	Cobalt oxide (Purity not reported, the median diameter of particles was 0.14 µm, with a median mass diameter of 0.45 µm and a geometric standard deviation of 1.9 µm)	Inhalation 7 hr/day, 5 days/wk × lifespan	**Adenoma (cortex)**			Survival in exposed group is similar to control but is poor in both groups. **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 dose 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%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
Pancreas						
NTP 2014b Rat (F344/NTac) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation 6 hr/day, 5 day/wk × 105 wk	**Carcinoma[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, as Fischer 344/NTac rats have only been used in two carcinogenicity studies and so it is based on only 100 rats.
			0 mg/m³	17	2/50 (5%)	
			1.25 mg/m³	20	1/50 (3%)	
			2.5 mg/m³	16	5/48 (13%)[e]	
			5 mg/m³	16	6/49 (15%)[e]	
			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			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
NTP 2014b Rat (F344/NTac) Female 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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. **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 have only been used in two NTP carcinogenicity studies and so it is based on only 100 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]			
			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]	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N^+) (%)	Comments, strengths, and limitations
Hematopoietic system						
NTP 2014b Rat (F344/NTac) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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. **Limitations:** None. **Other comments:** Historical controls were limited, as Fischer 344/NTac rats have only been used in two carcinogenicity studies and so it is based on only 100 rats.
			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 2014b Rat (F344/NTac) Female 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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 all factors. **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 have only been used in two NTP carcinogenicity studies and so it is based on only 100 rats.
			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]	
			5 mg/m³	25	27/50 (59%)*[g]	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# animals at sacrifice	Tumor incidence (n/N[+]) (%)	Comments, strengths, and limitations
Kidney						
NTP 2014b Rat (F344/NTac) Male 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation 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, as Fischer 344/NTac rats have only been used in two NTP carcinogenicity studies and so it is based on only 100 rats.
			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]			
			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 2014b and NTP 1998 and is the number of animals at the beginning of the study for all other studies.
NR = Not reported.
[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.

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 (Zeller 1975, Finogenova 1973, O'Hara *et al.* 1971, Kasirsky *et al.* 1965) and three studies using sodium cobaltinitrite (O'Hara *et al.* 1971, Thompson *et al.* 1965, Orzechowski *et al.* 1964); and a poorly soluble compound, cobalt 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/ 8wk	(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/ 10wk	(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	11wk/1 1wk	(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 to be used to evaluate the carcinogenicity of cobalt, because they 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 oxide), while three studies showed no effect from cobalt (cobalt chloride and cobalt oxide) and three studies reported a decrease (cobalt chloride and sodium cobaltinitrite) in neoplastic incidence with the additional exposure to cobalt compounds.

Skin

Four co-carcinogenicity studies of cobalt and methylcholanthrene were reviewed (Finogenova 1973, O'Hara *et al.* 1971, Kasirsky *et al.* 1965, Thompson *et al.* 1965, Orzechowski *et al.* 1964). 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, Thompson *et al.* 1965, Orzechowski *et al.* 1964). 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 administered benzo[*a*]pyrene and cobalt oxide to female rats by intratracheal instillation. The addition of cobalt 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 oxide. Wehner exposed male hamsters to cigarette smoke and cobalt oxide by inhalation

(Wehner *et al.* 1977). No significant change in tumor incidence from the addition of cobalt 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.

Unspecified neoplasm or non-neoplastic lesion locations

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 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, in two studies, no tumors were observed after implantation or injection of other materials (titanium dioxide, silicon dioxide, or thorium dioxide), whereas tumors were observed after implantation of cobalt metal (Hansen *et al*. 2006) or injection of a cobalt compound, cobalt oxide (Gilman and Ruckerbauer 1962). Hansen *et al*. found that the materials (titanium dioxide and silicon dioxide) that did not induce neoplasms had the same physical characteristics (i.e., surface to volume ratio) as those that did (cobalt and nickel), which suggests that the tumors were due to carcinogenic properties of cobalt and not just to a reaction to any physical implant. 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 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, 2014b), 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 *et al.* 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 2014b). 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 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.) (Steinhoff and Mohr 1991, Gilman and Ruckerbauer 1962). Inhalation exposure to cobalt 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 2014b)
Cobalt metal	Mouse $B6C3F_1$/N (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma M&F	(NTP 2014b)
Cobalt metal [Nano]	Rat Sprague-Dawley (M)	IM inj.	Single dose/1 yr	Injection site Sarcoma M	(Hansen *et al.* 2006)
Cobalt metal [Bulk]	Rat Sprague-Dawley (M)	SC inj.	Single dose/1 yr	Negative Fibroblastic proliferation (non-neoplasia)	(Hansen *et al.* 2006)
Cobalt metal	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/ 1 yr	Negative	(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)	IM inj.	Single dose/lifespan	Injection-site sarcoma [rhabdomyofibrosarcoma M&F; sarcoma M; fibrosarcoma F	(Heath 1956)
Soluble cobalt compounds					
Cobalt	Rat F344/N	Inhalation	2 yr/2 yr	Lung	(NTP 1998)

Substance	Strain (sex)	Route	Exposure period/ study duration	Results	Reference
sulfate heptahydrate	(M&F)			Alveolar/bronchiolar adenoma and carcinoma M&F Adrenal Benign or malignant pheochromocytoma F	
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)	SC 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)	IP inj.	6 mo/lifespan	Injection site Histiocytoma and sarcoma M&F	(Steinhoff and Mohr 1991)
Cobalt oxide	Rat Sprague-Dawley (M)	SC inj.	730 day/lifespan	Injection site Histiocytoma and sarcoma M	(Steinhoff and Mohr 1991)
Cobalt oxide	Rat Wistar (M&F)	IM inj.	Single dose/1.3 yr	Injection site Sarcoma M&F	(Gilman and Ruckerbauer 1962)
Cobalt oxide	Mouse Swiss (F)	IM inj.	Single dose/2 yr	Negative	(Gilman and Ruckerbauer 1962)
Cobalt oxide	Hamster Syrian Golden (M)	Inhalation	Lifespan/lifespan	Negative	(Wehner *et al.* 1977)
Cobalt sulfide	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/1 yr	Negative	(Jasmin and Riopelle 1976)

6 Mechanistic and Other Relevant Effects

This section discusses the relative role of cobalt ions and particles in cobalt toxicity (Section 6.1), several proposed modes of actions for cobalt carcinogenicity (Section 6.2), cobalt levels in neoplastic and non-neoplastic tissue from cancer patients (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. The genetic and related effects of cobalt and cobalt compounds are reviewed in Appendix E.

6.1 Cobalt particles and cobalt ions

Studies with toxic metals in general 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) (Lison 2015, Ortega *et al.* 2014, Sabbioni *et al.* 2014, Smith *et al.* 2014, Beyersmann and Hartwig 2008). Many cobalt(II) salts are readily soluble in water and biological fluids (see Section 1).

Cobalt particles and ions induce similar biological effects *in vivo* and *in vitro* (e.g., cytotoxicity, genotoxicity, apoptosis, and at high concentrations, necrosis with an inflammatory response) (Smith *et al.* 2014, Simonsen *et al.* 2012). In particular, the effects of chronic exposure to cobalt and cobalt compounds on the respiratory system in humans and experimental animals are well documented (IARC 2006, ATSDR 2004, IARC 1991). Effects include respiratory irritation, diminished pulmonary function, asthma, and interstitial fibrosis. Respiratory effects have been observed in workers employed in cobalt refineries, hard metal workers, diamond polishers, and ceramic dish painters. Several *in vitro* studies that specifically compared the cellular uptake and/or molecular and cellular effects (e.g., cytotoxicity, genetic toxicity, ROS production) of cobalt ions and particles (i.e., cobalt metal nanoparticles or cobalt 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 (Simonsen *et al.* 2012, Mo *et al.* 2008, Peters *et al.* 2007, Zhang *et al.* 2000). In addition, relatively soluble cobalt particles (e.g., cobalt metal) are generally more cytotoxic and genotoxic than cobalt ions (Sabbioni *et al.* 2014, Ponti *et al.* 2009, Peters *et al.* 2007) and cobalt ions are generally more cytotoxic than cobalt particles with low solubility (e.g., cobalt oxide) (Table 6-1) (Ortega *et al.* 2014, Smith *et al.* 2014, Papis *et al.* 2009). 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 type	Cytotoxicity	Genotoxicity[a]	ROS	Cellular uptake
(Sabbioni *et al.* 2014)	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.	No data	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 type	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

Reference	Cobalt form (size, nm) and cell type	Cytotoxicity	Genotoxicity[a]	ROS	Cellular uptake
(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.
(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.

Reference	Cobalt form (size, nm) and cell type	Cytotoxicity	Genotoxicity[a]	ROS	Cellular uptake
(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 x 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.
(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). 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, Smith *et al.* 2014, Paustenbach *et al.* 2013, Simonsen *et al.* 2012).

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) and formation of insoluble complexes in the presence of physiological concentrations of phosphates (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. 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 (Sabbioni *et al.* 2014, Smith *et al.* 2014, Simonsen *et al.* 2012, Garrick *et al.* 2003). 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 (Ortega *et al.* 2014, Smith *et al.* 2014, Papis *et al.* 2009, Limbach *et al.* 2007). Although the intracellular solubilized cobalt content was small compared to the intracellular particulate content, the solubilized fraction was shown to be 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. 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 rapidly spread to the cytosol, cellular organelles, and nucleus where they release cobalt ions (Sabbioni *et al.* 2014, Ponti *et al.* 2009). 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.* (2014) 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 cytosolic 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 (Webb *et al.* 1972, Heath and Webb 1967).

The *in vivo* toxicity and carcinogenicity of soluble cobalt sulfate heptahydrate and cobalt metal particles from the NTP (2014b, 1998) bioassays were recently compared (Behl *et al.* 2015). Contrary to expectations, the data indicated that cobalt metal was more toxic and carcinogenic than the cobalt salt based on the incidence and spectrum of lung neoplasms and extent of systemic lesions. However, 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 (Sabbioni *et al.* 2014, Smith *et al.* 2014, Peters *et al.* 2007).

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, Koedrith and Seo 2011, Beyersmann and Hartwig 2008). 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 stress, and (3) induction of hypoxia-like responses by activating hypoxia-inducible factor 1 (HIF-1) (see Figure 6-1) (Smith *et al.* 2014, Green *et al.* 2013, Magaye *et al.* 2012, Simonsen *et al.* 2012, Simonsen *et al.* 2011, De Boeck *et al.* 2003a, Lison *et al.* 2001). In addition, these modes of action also affect cell signaling pathways and gene expression that likely contribute to neoplastic development and progression (Davidson *et al.* 2015, Nyga *et al.* 2015, Verstraelen *et al.* 2014, Permenter *et al.* 2013, Malard *et al.* 2007). Experimental evidence for these modes of action is briefly reviewed below.

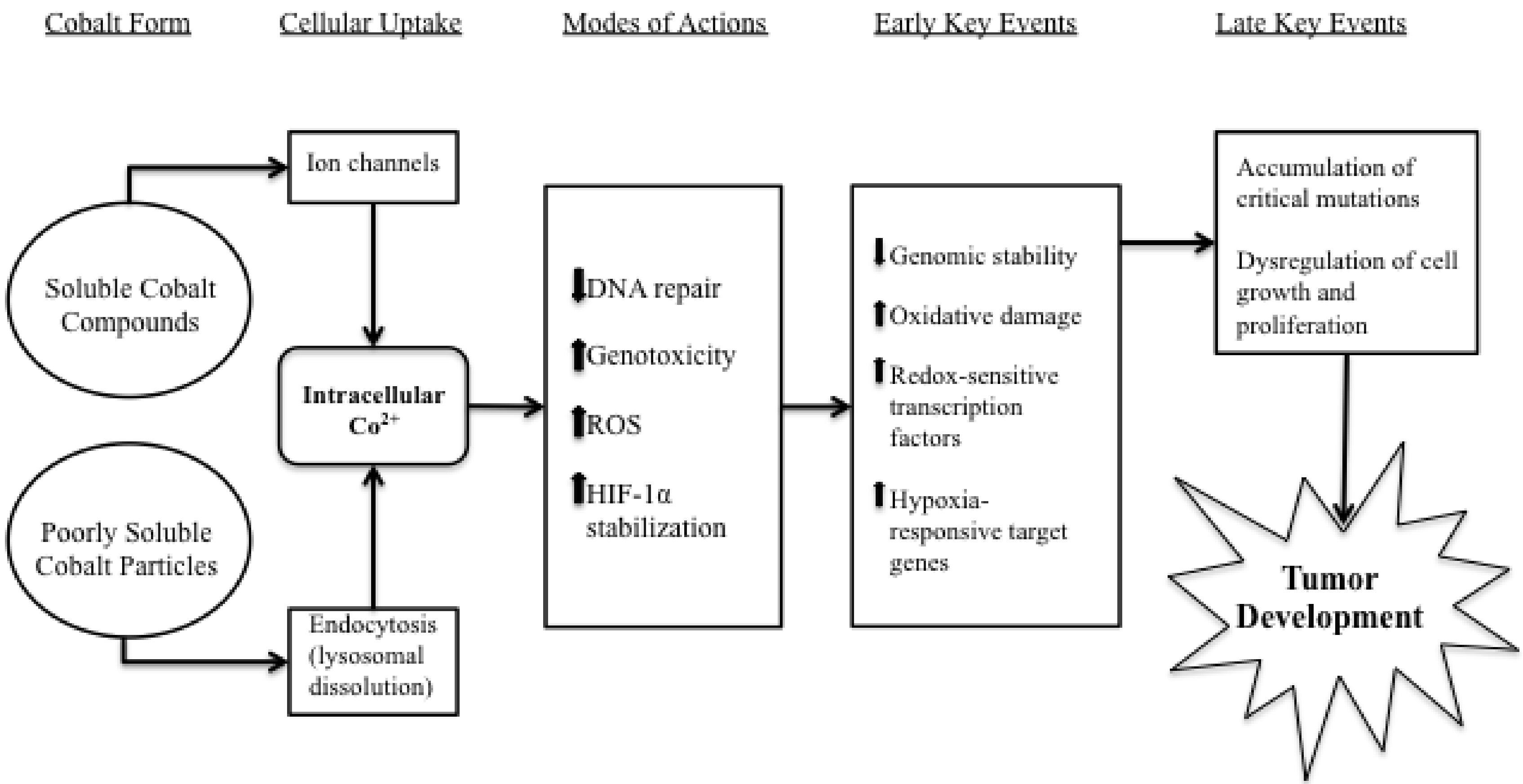

Figure 6-1. Proposed modes-of-action of cobalt carcinogenicity.

(adapted from Beyersmann and Hartwig 2008, De Boeck *et al.* 2003a)

6.2.1 Genotoxicity, inhibition of DNA repair, and related effects

This section addresses genotoxicity and related biological adverse effects (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).

The genotoxicity and related effects for cobalt metal and soluble and insoluble cobalt compounds are reviewed in Appendix E and summarized here (see Table 6-2). Increases in gene mutations, DNA strand breaks, sister chromatid exchange, micronuclei, aneuploidy, chromosomal aberrations, DNA-protein crosslinks, inhibition of DNA repair, and cell transformation were reported in mammalian cells *in vitro* following exposure to cobalt, but cobalt compounds were mostly non-mutagenic in bacterial assays. 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. Dose-dependent responses were reported in some of these studies, supporting the evidence for a genotoxic effect *in vivo*.

More 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 they also compare effects and relative potency of cobalt ions and particles (Table 6-1) (Smith *et al.* 2014, Alarifi *et al.* 2013, Patel *et al.* 2012, Ponti *et al.* 2009, Colognato *et al.* 2008). 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 fibroblast 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.

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 2008, IARC 2006, Beyersmann and Hartwig 1992). 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 (Koedrith and Seo 2011, Beyersmann and Hartwig 2008). 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 (Beyersmann and Hartwig 2008, Witkiewicz-Kucharczyk and Bal 2006, Baldwin *et al.* 2004, Kopera *et al.* 2004, Asmuss *et al.* 2000, Hartwig 1998, Kasten *et al.* 1997, Hartwig *et al.* 1991). 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). The co-mutagenic effects of cobalt observed *in vitro* are consistent with one study by Steinhoff and Mohr (1991) that reported co-carcinogenic effects of cobalt oxide and benzo[*a*]pyrene for squamous-cell carcinoma of the lung described in Section 5.2.3.

Genotoxicity assays with cobalt salts and cobalt metal demonstrate a mutagenic potential and at least two molecular mechanisms seem to apply: (1) a direct effect of cobalt(II) ions to induce oxidative damage to DNA through a Fenton-like mechanism, and (2) an indirect effect of cobalt(II) ions through inhibition of repair of DNA damage caused by endogenous events or induced by other agents (Lison 2015, IARC 2006).

Table 6-2. Summary assessment of genotoxicity and related effects for cobalt compounds

	Cobalt chloride		Cobalt sulfate		Cobalt nitrate		Cobalt oxide		Cobalt acetate		Cobalt metal		Cobalt sulfide		Cobalt nanoparticles	
Endpoint (Test system)	***In vitro*** [a]	***In vivo***	***In vitro*** [a]	***In vivo***	***In vitro*** [b]	***In vivo***	***In vitro*** [b]	***In vivo***	***In vitro*** [b]	***In vivo***	***In vitro*** [a]	***In vivo***	***In vitro*** [b]	***In vivo***	***In vitro*** [b]	***In vivo***
Mutation																
Mutation (prokaryotes)	(–)[1]		(–)[1]								(–)[1]					
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	+				+											
Other endpoints																
Transformation	±		+						+		–		+		+	
Apoptosis	+										+					

Sources: IARC (2006) review and additional primary references as described in tables and text.
Positive +, mostly positive evidence (+), mixed results ±, mostly negative evidence (–), and negative –.

[a]Results shown are for –S9; test +S9 was negative.
[b]Results shown are for –S9; not tested with the addition of metabolic activation (S9).

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6.2.2 Oxidative stress

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* (Jomova and Valko 2011, Koedrith and Seo 2011, Beyersmann and Hartwig 2008, Valko *et al.* 2006, Valko *et al.* 2005, Kasprzak 2002). Oxidative stress has been demonstrated to be one of the principle injury mechanisms through which metal and metal oxide nanoparticles induce adverse health effects (Zhang *et al.* 2012b). 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 (Romero *et al.* 2014, Jomova and Valko 2011, Petit *et al.* 2005, Valko *et al.* 2005). Petit *et al.* (2005) reported that cobalt ions induced a time- and dose-dependent protein oxidation in human U937 macrophages that was inhibited by glutathione. In addition to generating DNA damage, ROS also activate redox-sensitive transcription factors (e.g., NF-κB, AP1, p53) (Beyersmann and Hartwig 2008, Valko *et al.* 2006, Valko *et al.* 2005). These 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.

Both cobalt ions and cobalt metal can catalyze the formation of ROS *in vivo* and *in vitro* (Chattopadhyay *et al.* 2015, Annangi *et al.* 2014, Scharf *et al.* 2014, Alarifi *et al.* 2013, Patel *et al.* 2012, Papis *et al.* 2009, Qiao *et al.* 2009, Kotake-Nara and Saida 2007, Limbach *et al.* 2007, Peters *et al.* 2007, Dick *et al.* 2003, Pourahmad *et al.* 2003, Zou *et al.* 2001, Kawanishi *et al.* 1994, Hanna *et al.* 1992, Lewis *et al.* 1992, 1991, Kadiiska *et al.* 1989, Kawanishi *et al.* 1989, Moorhouse *et al.* 1985). 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.* (1992, 1991) 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 may mediate cobalt toxicity. Kasprzak *et al.* (1994) also 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 6-2) 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 2014a, 1998). 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 adducts that result from oxidative damage (Itsara *et al.* 2014, Klaunig *et al.* 2010) and are consistent with the mutation pattern in bacteria (i.e., positive in strains detecting mutational events at G:C base pairs). G to T 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 suggest that oxidative damage may play a role in cobalt-mediated lung tumorigenicity.

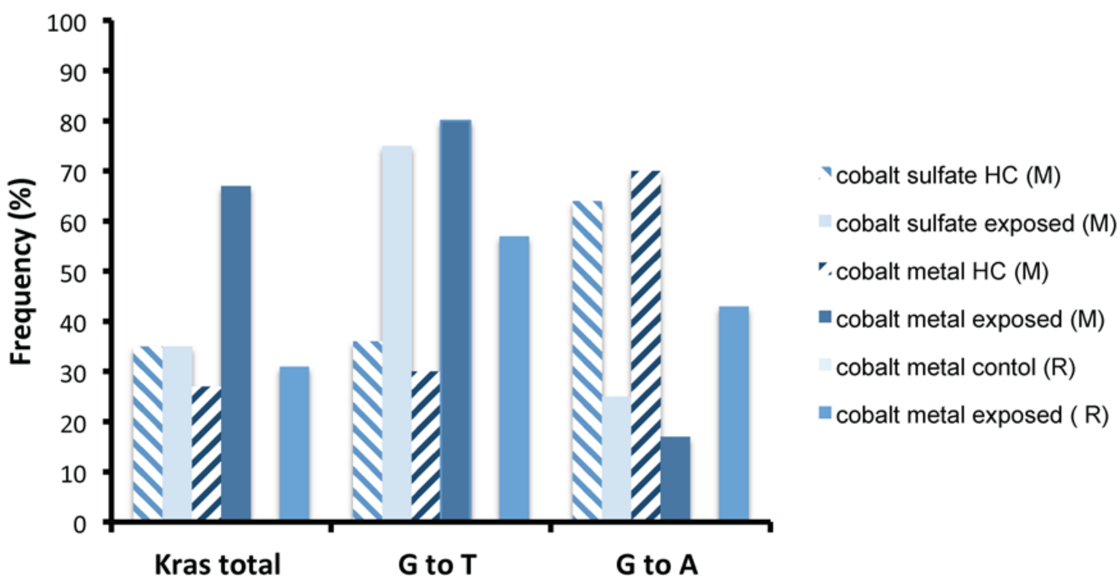

Figure 6-2. K-*ras* mutations in lung tumors from cobalt-exposed and non-exposed rodents

Source: (Hong *et al.* 2015, NTP 2014a, 1998).
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.

Direct interactions between cobalt metal or ions and oxygen or lipids can generate ROC. 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 (reactions 1-3) (Lee *et al.* 2012, Jomova and Valko 2011, 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 (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.

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

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

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

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

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 (Paustenbach *et al.* 2013, Beyersmann and Hartwig 2008). 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 Hypoxia mimicry and HIF-1 stabilization

HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits and is the key mediator of hypoxia response (Davidson *et al.* 2015, Galanis *et al.* 2008, Salnikow *et al.* 2004). The β subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT) is constitutively expressed while HIF-1α is oxygen sensitive. HIF-1 overexpression and enhanced transcriptional activity are linked to cancer initiation and progression. There is strong experimental support that

HIF-1 activation is involved in cobalt-induced carcinogenesis. 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 (Nyga *et al.* 2015, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Saini *et al.* 2010b, Saini *et al.* 2010a, Galanis *et al.* 2009, Qiao *et al.* 2009, Xia *et al* 2009, Beyersmann and Hartwig 2008, Maxwell and Salnikow 2004). 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 (Fu *et al.* 2009, Ardyanto *et al.* 2008, Wang and Semenza 1995). Cobalt chloride-induced hypoxia also increased the invasiveness of one primary breast cancer cell line (Fu *et al.* 2009). Under normal oxygen conditions, the iron-containing oxygen sensing enzymes (oxygenases) hydroxylate specific proline and asparagine residues in the HIF-1α subunits (Maxwell and Salnikow 2004). Hydroxylated HIF-1α binds to a multiprotein complex that contains the VHL tumor suppressor. VHL acts as part of an 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 oxygenases or depleting intracellular ascorbate (a cofactor for prolyl hydroxylase activity), thus, deactivating these enzymes (Davidson *et al.* 2015, Qiao *et al.* 2009, Maxwell and Salnikow 2004, Salnikow *et al.* 2004). Oxidative stress has also been investigated as a possible mechanism of cobalt-induced HIF activation; however, Salnikow *et al.* (2000) showed that activation of HIF-1-dependent genes was independent from ROS formation. 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.

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 (Gao *et al.* 2013, Simonsen *et al.* 2012, Saini *et al.* 2010b, Saini *et al.* 2010a, Greim *et al.* 2009, Beyersmann and Hartwig 2008, 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 (Greim *et al.* 2009, Ardyanto *et al.* 2008, Hammond and Giaccia 2005, Maxwell and Salnikow 2004, Lee *et al.* 2001). 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 pheochromocytomas 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.

6.2.4 Cell signaling and gene expression modulation

Cell signaling pathways control the expression of numerous genes that play important roles in carcinogenesis and many of these pathways are known targets of metal toxicity (Broberg *et al.* 2015, Davidson *et al.* 2015). Signaling pathways, receptors and transcription factors affected by cobalt include MAPKs, HIF-1, p53, AP-1, VEGF, P13K/Akt, and NFκB (Davidson *et al.* 2015, Lee *et al.* 2012, Mates *et al.* 2010, Leonard *et al.* 2004). Cobalt-mediated effects in humans and animal cell lines, which may be direct by interaction with proteins, or indirect through formation of ROS, are briefly reviewed here.

Human A549 lung adenocarcinoma cells exposed to cobalt chloride overexpressed the *N*-myc downstream regulated gene 1 (NDRG1/Cap43) (Salnikow *et al.* 2000). Increased expression of NDRG1/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).

Malard *et al.* (2007) also reported that A549 cells exposed to cobalt chloride differentially expressed 85 genes including potential cobalt carriers, tumor suppressors, transcription factors, and genes linked to stress response. Most of these had never been described as related to cobalt stress and only 7 of 85 genes matched HIF-1 target genes. In another study, cobalt oxide nanoparticles at non-cytotoxic concentrations induced mainly downregulation of gene transcription in A549 and bronchial BEAS-2B cells (Verstraelen *et al.* 2014). BEAS-2B cells were more sensitive than A549 cells having higher numbers of differentially expressed transcripts at a 10-fold lower concentration. Two distinct clusters of upregulated genes were observed in both cell lines that were associated with metabolic processes. Between 1% and 14% of the differentially expressed transcripts encoded markers involved in immune processes. 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).

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 human hepatocellular carcinoma (Hep3B) cells exposed to cobalt chloride (Vengellur *et al.* 2005).

6.3 Cobalt tissue levels from patients with lung and other cancers

Several publications were identified that measured trace metals (such as heavy metals and essential metals) in tissue (such as tumor 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) (see Appendix B). For most studies, the source of the exposure was unknown. Overall, several studies found statistically significant 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 (Qayyum and Shah 2014, Benderli Cihan and Öztürk Yildirim 2011), 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. 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 and there were several limitations such as co-exposure to other metals and limited methods to select cases and controls, and the source of the exposure is unknown.

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 principle 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 DNA, proteins, and lipids. 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 Preliminary 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 a preliminary listing recommendation.

Preliminary listing recommendation

"Cobalt and certain cobalt compounds" are reasonably anticipated to be human carcinogens based on sufficient evidence from studies in experimental animals and supporting mechanistic data. "Certain" refers to those cobalt compounds – including soluble and poorly water-soluble cobalt compounds and particles – that can release cobalt ions *in vivo*, which mechanistic data indicate are key for cobalt-induced carcinogenicity.

Because mechanistic data are key for the evaluation of cobalt and certain cobalt compounds, that topic is discussed first (Section 7.1), followed by a discussion of the rationale for grouping cobalt and certain cobalt compounds as a class (Section 7.2). The scientific data supporting the conclusion of sufficient evidence of cobalt and certain cobalt compounds from studies in experimental animals is discussed in Section 7.3, and the conclusions from the cancer studies in human studies is briefly summarized in Section 7.4.

7.1 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 genotoxicity and inhibition of DNA repair, ROS and oxidative damage, and 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 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.

7.2 Cobalt and certain cobalt compounds 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 (OECD

2014, ECHA 2009). 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 certain cobalt compounds 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 certain cobalt compounds was warranted. These endpoints are compared in Table 7-1 for several cobalt compounds in Section 7.2.3 and discussed below.

In addition to the mechanistic data, other data relevant for chemical grouping include the following:

- Physicochemical properties and toxicokinetics (Section 7.2.1);
- Toxicological effects related to a common functional group (i.e., the cobalt ion) (Section 7.2.2); and
- Overall synthesis (Section 7.2.3).

7.2.1 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 all cobalt compounds tested including water-soluble and poorly soluble compounds indicating that they release cobalt ions in solution.

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, 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.

7.2.2 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.

7.2.3 Overall synthesis

Several biological endpoints were identified from physicochemical, toxicological, and mechanistic data for cobalt metal, cobalt chloride, cobalt sulfate, and cobalt oxide (CoO and Co3O4). These cobalt forms were the most studied and included both soluble and insoluble forms. The data are synthesized and compared on a semi-quantitative scale in Table 7-1. Symbols (i.e., −, +, ++, +++) and colors are used to designate the relative effect of the various cobalt forms (see explanation below). 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 to an analogous cobalt compound within the table.

Table 7-1 also includes particle size (i.e., < 100 nm or > 100 nm) for cobalt metal and cobalt oxides to compare relative effects of nanoparticles with larger particles. Although many of the *in vitro* studies included direct comparisons of endpoints for cobalt ions and cobalt particles, few studies provided a direct comparison of particles of different sizes. In those cases, determinations for the particle sizes were made relative to cobalt ions. There also were a few cases where the available studies were inconsistent. In those cases, it was not possible to determine the relative effects of the particles or compounds; therefore, the effects were considered equivalent. However, cell designations in Table 7-1 should only be compared within a given row or, in some cases, within a column (i.e., bioaccessibility data or animal neoplasms) because the relative scales vary by endpoint. For example, the cell designation for lung tumors induced by cobalt sulfate indicates a less potent response when compared to lung tumors induced by cobalt metal but should not be compared to the cell designation for adrenal tumors induced by cobalt metal.

Table 7-1. Comparison of chemical and biological properties of cobalt metal and cobalt compounds.

	Soluble compounds		Cobalt metal		Poorly soluble compounds Cobalt oxide[a]	
Endpoint	**$CoCl_2$**	**$CoSO_4$**	**> 100 nm**	**< 100 nm**	**>100 nm**	**<100 nm**
Bioacessibility[b]						
Alveolar	+++	+++	++	ND	+	ND
Interstitial/serum	+++	+++	++	++	+	+
Lysosome	+++	+++	+++	+++	+++	+++
Gastric	+++	+++	+++	ND	+++	ND
Cellular uptake	+	ND	+++	+++	+++	+++
Cytotoxicity	++	++	+++	+++	+	++
ROS	+	ND	++	++	ND	++
HIF-1α stabilization	+++	+++	ND	+++	ND	ND
DNA repair inhibition	+	ND	+	ND	ND	ND
Genotoxicity *in vitro*	+	+	+	+	+	++
Genotoxicity *in vivo*	+	ND	–	ND	ND	ND
Animal neoplasms						
Lung	ND	++	+++	ND	+	ND
Adrenal gland	ND	+	++	ND	ND	ND
Pancreatic islet	ND	–	+	ND	ND	ND
Kidney	ND	–	–	ND	ND	ND
Mononuclear cell leukemia	ND	–	+	ND	ND	ND
Injection site[c]	+	ND	+++	+++	+++	ND

ND = no data, – = no effect, + = positive effect, ++ = greater positive effect, +++ = greatest positive effect. (Note: the relative scales vary by endpoint.)

[a] Includes CoO and Co_3O_4.

[b] Dissolution in artificial extraction fluids or culture medium after 48 to 72 hours.

[c] Includes subcutaneous, intramuscular, intraperitoneal, and intrathoracic injection or implantation studies.

7.3 Evidence of carcinogenicity from studies in experimental animals

There is sufficient evidence for the carcinogenicity of cobalt and certain cobalt compounds (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. 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 oxide). A comparison of the inhalation studies conducted by NTP of cobalt metal and cobalt sulfate suggest 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.

7.4 Evidence of carcinogenicity from studies in humans

There is inadequate evidence from studies in humans to evaluate the association between exposure to cobalt and cancer. While almost all the cohort studies reported approximately a doubling of the risk of lung cancer from exposure to various cobalt compounds, cobalt exposure was likely correlated with exposure to other known lung carcinogens, which complicates the interpretation of the results. Increased risks of esophageal cancer were found in two population-based case-control studies; however, cobalt exposure was assessed in toenail samples at or after cancer diagnosis. Thus, it is unclear whether cobalt levels in the toenails reflected exposure to cobalt preceding cancer or resulted from changes due to tumor formation.

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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).

Part 2

Draft Profile

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Cobalt and Certain Cobalt Compounds

CAS No. 7440-48-4 (Cobalt metal)

No separate CAS No. assigned for cobalt compounds as a class

Reasonably anticipated to be human carcinogens

Introduction

The compound cobalt sulfate was first listed in the Eleventh Report on Carcinogens in 2004 as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. The listing of cobalt and certain cobalt compounds supersedes the previous listing of cobalt sulfate in the Report on Carcinogens and applies to cobalt and certain cobalt compounds as defined below.

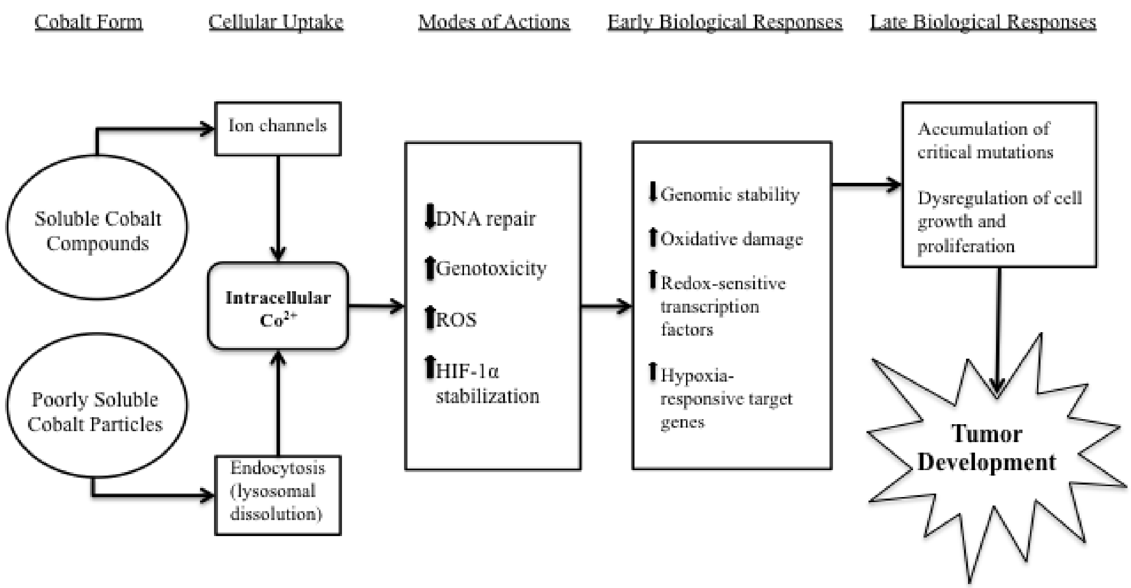
Carcinogenicity

Cobalt and certain cobalt compounds are *reasonably anticipated to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data from studies on mechanisms of carcinogenesis. "Certain cobalt compounds" are defined as compounds that release cobalt ions *in vivo*, which mechanistic data indicate is a key event for cobalt-induced carcinogenicity. The available data show that cobalt metal and certain cobalt compounds (regardless of their solubility in water) act via similar modes of action and induce similar cytotoxic, genotoxic, and carcinogenic effects, and that the cobalt ion is largely responsible for the toxicity and carcinogenicity (NTP 1998, 2014, IARC 2006).

Both water-soluble cobalt compounds, which release ions in extracellular fluids, and poorly water-soluble cobalt particles, which release cobalt ions intracellularly in lysosomes, are included in this grouping. Cobalt metal and all of the cobalt water-soluble and poorly water-soluble cobalt compounds that have been evaluated have been found to be soluble in biological fluids (e.g., gastric and lysosomal fluids), as discussed under "Properties" below, suggesting that they will release cobalt ions *in vivo* (Hillwalker and Anderson 2014, Brock and Stopford 2003, Stopford *et al.* 2003). Vitamin B_{12}, which is an essential cobalt-containing nutrient, does not meet the criteria for "certain cobalt compounds," because it does not release cobalt ions and passes through the body intact while bound to specific carrier proteins (Neale 1990).

Mechanisms of Carcinogenesis and Other Relevant Data

The key events related to toxicity and carcinogenicity 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 (as shown in the diagram below). The first step in the carcinogenicity or toxicity process is the release of cobalt ions *in vivo*. Water-soluble cobalt compounds release ions into extracellular fluids, and poorly water-soluble cobalt particles release cobalt ions intracellularly in lysosomes.



Mechanistic events in cobalt carcinogenicity

Several key events have been identified that are related to biologically plausibile modes of actions and are applicable to all cobalt forms that release cobalt ions *in vivo.* These events include inhibition of DNA repair, genotoxicity, generation of reactive oxygen species (ROS) and oxidative damage, and stabilization of hypoxia-inducible factor 1α (HIF-1α).

Cobalt is mutagenic in mammalian cells and induces DNA strand breaks and chromosome damage *in vitro*. Only a few *in vivo* genotoxicity studies were available, but the results were generally consistent with those of *in vitro* studies. Although the mechanisms of cobalt-induced genetic damage are not completely understood, the literature suggests two possible mechanisms: (1) a direct effect of cobalt(II) ions to induce oxidative damage to DNA, and/or (2) an indirect effect through inhibition of DNA repair (Smith *et al.* 2014, Lison 2015).

Cobalt is also a redox-active transition metal, and *in vitro* studies have shown 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 have been shown in *in vivo* studies in rat kidney, liver, and lung. Also, a higher frequency of G to T transversion mutations in the K-*ras* oncogene (a common mutation associated with oxidative DNA damage) were found in cobalt induced lung tumors in mice and rats compared to spontaneous lung tumors (NTP 1998, 2014, IARC 2006). In addition to directly inducing DNA damage, ROS also activate a number of redox-sensitive transcription factors (e.g., nuclear factor κB, activator protein 1, and tumor protein p53) that have been linked to carcinogenesis because of their role in regulating inflammation, cell proliferation, differentiation, angiogenesis, and apoptosis (Valko *et al.* 2005, 2006, Beyersmann and Hartwig 2008). Thus, ROS may initiate tumor development by mutagenesis and/or promote tumor growth by dysregulation of cell growth and proliferation.

Finally, a well-established biological effect of cobalt is to mimic hypoxia by stabilizing HIF-1α (Maxwell and Salnikow 2004, Greim *et al.* 2009, Saini *et al.* 2010a,b, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Nyga *et al.* 2015). 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. HIF-1α overexpression has been linked to cancer initiation and progression and is a common characteristic of many human cancers (Paul *et al.* 2004, Galanis *et al.* 2008, 2009, Cheng *et al.* 2013).

Although most of the toxicological effects of cobalt are attributed to the cobalt ion, direct toxic effects of cobalt particles also contribute, as evidenced by the greater toxicity of cobalt metal than of cobalt sulfate in National Toxicology Program (NTP) rodent bioassays (NTP 1998 2014, Behl *et al.* 2015). Differences in the relative toxicity reported for cobalt particles and ions may be partially explained by differences in cellular uptake mechanisms, a synergistic effect between the particles and metal on ROS production, and differences in intracellular cobalt accumulation and distribution (Peters *et al.* 2007, Sabbioni *et al.* 2014, Smith *et al.* 2014).

Cancer Studies in Experimental Animals

Exposure of experimental animals to cobalt metal or cobalt compounds caused tumors in two rodent species, at several different tissue sites, and by several different routes of exposure. This conclusion is based on studies in rats and mice exposed to cobalt metal (five studies), water-soluble cobalt compounds (two studies with cobalt sulfate and one study with cobalt chloride), and poorly water-soluble cobalt compounds (four studies with cobalt oxide). Studies of cobalt alloys and radioactive cobalt in experimental animals were not considered to be informative because of potential confounding by other carcinogens.

Inhalation exposure of rats and mice to cobalt metal (NTP 2014) or cobalt sulfate (NTP 1998) or intratracheal instillation of cobalt oxide in rats (Steinhoff and Mohr 1991) caused lung tumors (alveolar/bronchiolar adenoma and carcinoma). In addition, inhalation exposure of rats to cobalt metal caused squamous-cell tumors of the lung (primarily cystic keratinizing epithelioma) in females and possibly in males.

In inhalation studies of cobalt metal in rats, tumors were also induced at sites distant from the lung, including tumors of the pancreas (islet-cell adenoma or carcinoma combined) in males and of the hematopoietic system (mononuclear-cell leukemia) in females, indicating a systemic effect (NTP 2014). Increased incidence of neoplasms in the kidney (adenoma or carcinoma combined) in male rats and pancreas (carcinoma) in female rats may have been related to cobalt metal inhalation (NTP 2014). Exposure to cobalt metal or cobalt sulfate induced adrenal gland tumors (benign and malignant pheochromocytoma). The inhalation-exposure studies conducted with cobalt metal and cobalt sulfate suggested that cobalt metal was more carcinogenic than cobalt sulfate at a similar cobalt concentration, based on the incidence and spectrum of lung tumors and the extent of systemic lesions (Behl *et al.* 2015). This finding is consistent with the greater cytotoxicity of and ROS induction for cobalt metal in the *in vitro* mechanistic studies described above.

In rats, local injection of cobalt at various anatomic locations caused tumors at the injection sites. Although these studies were less robust than the inhalation studies and sarcomas are common in injection studies in rats on a variety of compounds, the consistency of the tumor types and findings across different cobalt forms provide supporting evidence of carcinogenicity of cobalt. Intraperitoneal or intramuscular injection of the poorly water-soluble compound cobalt oxide caused histiocytoma and/or sarcoma at the injection site (Gilman and Ruckerbauer 1962, Steinhoff and Mohr 1991), and subcutaneous injection of the water-soluble compound cobalt chloride caused fibrosarcoma (Shabaan *et al.* 1977). Intramuscular or intrathoracic injection of cobalt metal (Heath 1956, Heath and Daniel 1962) or nanoparticles (Hansen *et al.* 2006) caused sarcoma (primarily rhabdomyofibrosarcoma, rhabdomyosarcoma, or fibrosarcoma

A few studies in rodents (Gilman and Ruckerbauer 1962, Jasmin and Riopelle 1976, Wehner *et al.* 1977) found no tumors at certain tissue sites following exposure to the same forms of cobalt that caused tumors in other studies; however, these studies generally lacked sensitivity to detect an effect, because of the use of a less sensitive animal model, shorter study duration, or lower exposure levels.

Cancer Studies in Humans

The data available from studies in humans are inadequate to evaluate the relationship between human cancer and exposure specifically to cobalt and certain cobalt compounds. The data relevant to the evaluation were from studies of five independent cohorts of workers, primarily evaluating lung cancer, and two population-based case-control studies of esophageal and other cancers of the aerodigestive tract, one in Ireland (O'Rorke *et al.* 2012) and the other in the state of Washington (Rogers *et al.* 1993). The cohorts included (1) porcelain painters in Denmark (Tüchsen *et al.* 1996), (2) cobalt production workers in an electrochemical plant in France reported in two publications (Mur *et al.* 1987, Moulin *et al.* 1993), (3) two overlapping cohorts of cobalt–tungsten carbide hard-metals workers in France (Moulin *et al.* 1998, Wild *et al.* 2000), (4) stainless- and alloyed-steel workers in France (Moulin *et al.* 2000), and (5) nickel refinery workers in Norway (Grimsrud *et al.* 2005). Studies of cobalt alloys in humans (primarily metal-on-metal implants) were not considered to be informative, because they were not specific to cobalt exposure.

Although increased risks of lung cancer were found in most of the cohort studies, and increases in esophageal cancer were suggested in the two case-control studies, 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 study limitations. In the cohort studies, hard-metal (Moulin *et al.* 1998, Wild *et al.* 2000) and nickel refinery workers (Grimsrud *et al.* 2005) were also exposed to known lung carcinogens; excess risks were also found among the “unexposed” referent pottery workers; and the excess risk found in an earlier cohort study of cobalt production workers (Mur *et al.* 1987) was no longer present in a later update of the cohort (Moulin *et al.* 1993). In the case-control studies, cobalt exposure was assessed in toenail samples taken at or after diagnosis of esophageal cancer. It therefore is unclear whether cobalt levels in the toenails reflected a relevant period of exposure, or whether cobalt exposure preceded cancer or resulted from changes due to tumor formation.

Properties

Cobalt and certain cobalt compounds as a class are related largely by their chemical properties, specifically bioavailability.

Bioavailability

Because the carcinogenic and toxic effects of cobalt and certain cobalt compounds begin with the release of cobalt ions *in vivo*, the bioavailability of cobalt ions is critical for consideration of carcinogenicity. The bioavailability of a metal species is determined by its solubility in biological fluids. Bioaccessibility studies (testing solubility in synthetic biological fluids) have demonstrated that cobalt metal and both water-soluble and poorly water-soluble cobalt compounds can dissolve and release cobalt ions in some biological fluids (Brock and Stopford 2003, Stopford *et al.* 2003), suggesting that they will release ions *in vivo*. *In vitro* bioaccessibility studies using synthetic equivalents of gastric fluid (for ingestion exposure) and lysosomal fluids (for inhalation exposure) (Brock and Stopford 2003, Stopford *et al.* 2003, Hillwalker and Anderson 2014) confirmed the complete (or mostly complete) solubility of cobalt metal, representative water-soluble cobalt compounds (cobalt sulfate heptahydrate and chloride), and both organic and inorganic poorly water-soluble cobalt compounds (cobalt oxide, 2-ethyl-hexanoate, carbonate, and naphthenate) in both of these low-pH biological fluids. The bioaccessibility results are shown in the table below, along with other chemical and physical properties of cobalt metal and these cobalt compounds. Results with other biological fluids, such as serum and intestinal, alveolar, and interstitial fluids, indicate that species of cobalt compound, particle size and surface area, and the pH of the surrogate fluid can affect the solubility of cobalt in biological fluids.

Physical and chemical properties for cobalt metal and cobalt compounds that have been tested for bioaccessibility

Form[a]	CAS No.[b]	Formula	Molec. weight	Physical form	Density or specific gravity	Water solubility (g/100 cc)[c]	Bioaccessibility (% solubility in gastric/ lysosomal fluids)[d]
Cobalt metal	7440-48-4	Co[e]	58.9[e]	grey hexagonal or cubic metal[e]	8.92[e]	0.000875[f]	100/100
Water-soluble compounds							
Sulfate heptahydrate	10026-24-1	$CoSO_4 \cdot 7H_2O$[g]	281.1[g]	red pink, monoclinic[g]	1.95[g]	60.4[g]	100/100
Chloride	7646-79-9	$CoCl_2$[h]	129.8[h]	blue hexagonal leaflets[h]	3.36[h]	45[h]	100/100
Poorly water-soluble compounds							
Oxide	1307-96-6	CoO[g]	74.9[g]	green-brown cubic[g]	6.45[g]	i[g]	100/92.4
2-Ethylhexanoate (org.)	136-52-7	$Co(C_8H_{15}O_2)_2$[g]	173.7[h]	blue liquid (12% Co)[g]	1.01[g]	28.8 (20°C)[k]	100/100
Carbonate (org.)	513-79-1	$CoCO_3$[g]	118.9[g]	red, trigonal[g]	4.13[g]	i[g]	100/100
Naphthenate (org.)	61789-51-3	$Co(C_{11}H_7O_2)_2$[e]	401.3[e]	purple liquid (6% Co)[g]	0.97[g]	34.3 (20°C)[j]	100/100

[a]All compounds contain Co(II). Forms in italics have been tested for carcinogenicity, genetic toxicity, or have mechanistic data; org. = organic compound; all others are inorganic.
[b]SciFinder (2015)
[c]Solubility in cold water unless otherwise indicated; i = insoluble;
[d]Stopford *et al*. 2003, [e]PubChem 2015, [f]ChemIDplus 2015, [g]CDI 2006, [h]HSBD 2015, [i]ATSDR 2004, [j]MorningStar 2005a, [k]MorningStar 2005b.

The solubility of cobalt compounds in water is largely pH dependent, and cobalt is generally more mobile in acidic solutions than in alkaline solutions (IARC 1991, Paustenbach *et al.* 2013). Sulfates, nitrates, and chlorides of cobalt tend to be soluble in water, whereas oxides (including the mixed oxide, Co_3O_4), hydroxides, and sulfides tend to be poorly soluble or insoluble in water (Lison 2015). Organic cobalt compounds can be either soluble, as with cobalt(II) acetate, or insoluble, as with cobalt(II) carbonate and cobalt(II) oxalate (CDI 2006). In addition to low pH, solubilization of some poorly water-soluble compounds in biological fluids may be enhanced in the presence of binding proteins (IARC 2006).

Chemical characteristics

Cobalt (Co) is a naturally occurring transition element with magnetic properties. It is the 33rd most abundant element, making 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 (ATSDR 2004, WHO 2006). Metallic cobalt, Co(0), exists in two allotropic forms, hexagonal and cubic, which are stable at room temperature (IARC 1991, ATSDR 2004, WHO 2006). Cobalt predominantly occurs in two oxidation states, Co(II) and Co(III). Co(II) is much more stable than Co(III) in aqueous solution (Nilsson *et al.* 1985, Paustenbach *et al.* 2013) and is present in the environment and in most commercially available cobalt compounds (e.g., cobalt chloride, sulfide, and sulfate). Co(III) is also present in some commercially available cobalt compounds, including the mixed oxide (Co_3O_4) (IARC 1991, Paustenbach *et al.* 2013, Lison 2015) and some simple salts of Co(III) (e.g., Co_2O_3). Important salts of carboxylic acids include formate, acetate, citrate, naphthenate, linoleate, oleate, oxalate, resinate, stearate, succinate, sulfamate, and 2-ethylhexanoate.

Use

Cobalt and cobalt compounds are used in numerous commercial, industrial, and military applications. On a global basis, the largest use of cobalt is in rechargeable battery electrodes. In 2012, the reported U.S. consumption of cobalt and cobalt compounds was approximately 8,420 metric tons, the majority used for superalloys (Shedd 2014b). Major uses for metallic cobalt include production of superalloys, cemented carbides, and bonded diamonds. Cobalt nanoparticles are used in medical applications (e.g., sensors, magnetic resonance imaging contrast enhancement, drug delivery), and cobalt nanofibers and nanowires are used in industrial applications. Cobalt compounds are used as pigments for glass, ceramics, and enamels (oxides, sulfate, and nitrate), as driers for paints, varnishes, or lacquers (hydroxide, oxides, propionate, acetate, tallate, naphthenate, and 2-ethylhexanoate), as catalysts (hydroxide, oxides, carbonate, nitrate, acetate, oxalate, and sulfide), as adhesives and enamel frits (naphthenate, stearate, and oxides), and as trace mineral additives in animal diets (carbonate, sulfate, nitrate, oxides, and acetate). U.S. consumption of cobalt and cobalt compounds in 2012 is summarized in the following table.

End use	Metric tons of cobalt content	Percent of total consumption
Superalloys	4,040	48.0
Chemicals and ceramics	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 2014b.
[a]Includes magnetic, nonferrous, and wear-resistant alloys and welding materials.

The fastest-growing use for cobalt in recent years has been in high-capacity, rechargeable batteries, including nickel-cadmium, nickel-metal hydride, and lithium-ion batteries for electric vehicles and portable electronic devices such as smart phones and laptops (Maverick 2015). Many other uses for cobalt exist, including in integrated circuit contacts and semiconductor production. An emerging area of use is as a key element in several forms of "green" energy technology applications, including gas-to-liquids and coal-to-liquids processes, oil desulfurization, clean coal, solar panels, wind and gas turbines, and fuel cells, and in cobalt-based catalysts for sunlight-driven water-splitting to convert solar energy into electrical and chemical energy.

Production

Cobalt metal is produced as a by-product from ores associated with copper, nickel, zinc, lead, and platinum-group metals and is most often chemically combined in its ores with sulfur and arsenic (Davis 2000, CDI 2006). The largest cobalt reserves are in the Congo (Kinshasa), Australia, Cuba, Zambia, Canada, Russia, and New Caledonia, with very limited production in the United States in recent years (Shedd 2014a). Except for a negligible amount of by-product cobalt produced from mining and refining of platinum-group metal ores, the United States did not refine cobalt in 2012 (Shedd 2014b). Cobalt has not been mined in the United States in over 30 years (ATSDR 2004); however, a primary cobalt mine, mill, and refinery were being established in Idaho in 2015 (Farquharson 2015). In 2012, 2,160 metric tons of cobalt was recycled from scrap. No cobalt has been sold from the National Defense Stockpile since 2009.

Metallic cobalt and several cobalt compounds are high-production-volume (HPV) chemicals, based on their annual production or importation into the United States in quantities of at least 1 million pounds. Recent volumes of U.S. production, imports, and exports of cobalt metal and HPV cobalt compounds are listed in the following table.

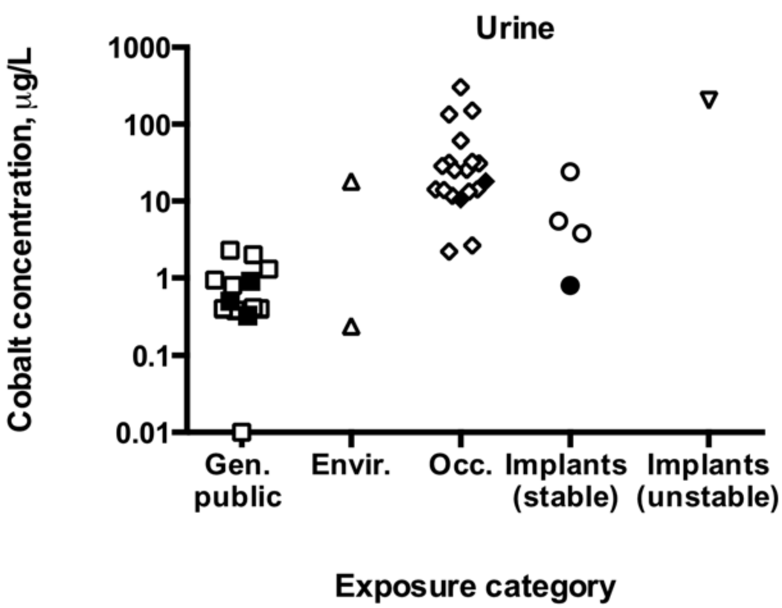
	Quantity (lb)		
Cobalt category	**Production (2012)**	**Imports (2013)**	**Exports (2013)**
Metal (excluding alloys)	23,384,002	16,151,599	–[a]
Compounds:			
Acetates	1 million to < 10 million	342,918	520,996
Carbonates	1,038,821	1,193,856	–[a]
Chlorides	–[b]	215,661	14,304
2-Ethylhexanoate	4,294,523	–	–
Hydroxide	4,709,137	–	–
Oxides	1 million to < 10 million	5,300,984[c]	902,467[c]
Propionate	1 million to < 10 million	–	–
Sulfate	1 million to < 10 million	1,319,004	–[a]

– = no data found.
[a]No specific U.S. Census Bureau Schedule B export code was identified.
[b]Cobalt chloride production data for 2012 were withheld by the manufacturer.
[c]The reported value is for cobalt hydroxide and oxides combined.

Exposure

A significant number of people living in the United States are exposed to cobalt, based on several lines of evidence, including biological monitoring data demonstrating exposure in occupationally and non-occupationally exposed populations. Data from the U.S. Environmental Protection Agency's Toxics Release Inventory (TRI) indicate that production- and use-related releases of cobalt compounds have occurred at numerous industrial facilities in the United States.

In biomonitoring studies that measured cobalt in the urine of people exposed to cobalt from various sources, the highest levels generally were due to occupational exposures and failed hip implants; lower levels were due to exposure from normal implants or the environment. Low levels were also observed in the general population (with unknown sources of exposure). The following graph shows the mean or median levels of urinary cobalt for the general public and for groups with known exposures. Data are reported for both U.S. and non-U.S. exposures; occupational and medical implant exposures outside the United States can be informative because of the similar production methods and implant compositions worldwide.



Urine levels of cobalt for various exposed groups

Filled symbols = U.S. data; open symbols = non-U.S. data.

Urinary cobalt measurements in the U.S. general public have remained consistent since 1999, with geometric mean values between 0.316 and 0.379 µg/L, according to the National Health and Nutrition Examination Survey (NHANES) (CDC 2015). Urinary cobalt is considered a good indicator of absorbed cobalt (IARC 2006, WHO 2006), especially from recent exposures (ATSDR 2004). Levels of cobalt in blood (including whole blood, plasma, and serum) show a pattern similar to that for urinary cobalt levels.

Occupational exposure

The primary route of occupational exposure to cobalt is via inhalation of dust, fumes, or mists or gaseous cobalt carbonyl. Dermal contact with cemented carbide (i.e., hard-metal) powders and cobalt salts can result in systemic uptake. Occupational exposure to cobalt occurs during refining of cobalt; production and use of cobalt alloys; hard-metal production, processing, and use; maintenance and re-sharpening of hard-metal tools and blades; manufacture and use of cobalt-containing diamond tools; and use of cobalt-containing pigments and driers. Workers regenerating spent catalysts may also be exposed to cobalt sulfides. Occupational exposure has been documented by measurements of cobalt in ambient workplace air and in blood, urine, nails, and hair, and lung tissue from workers or deceased workers (IARC 1991, ATSDR 2004, IARC 2006, CDC 2013). The highest levels of cobalt in workplace air are generally for hard-metal manufacture involving cobalt metal powders (1,000 to 10,000 μg/m^3) (NTP 2009) and for production of cobalt acetate, chloride, nitrate, oxide, and sulfate (IARC 2006).

The National Institute of Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that approximately 386,500 workers were potentially exposed to cobalt and cobalt compounds (NIOSH 1990).

Surgical implants

As mentioned above, cobalt implants are a major source of exposure to cobalt in patients receiving orthopedic joint replacements, especially hip implants, which contain cobalt in cobalt-chromium-molybdenum alloys (Sampson and Hart 2012, Devlin *et al.* 2013). Implants may fail because of excessive wear or corrosion by body fluids, increasing the levels of cobalt released from the implants (Sampson and Hart 2012). A recommended level of blood cobalt for further clinical investigation and action has been set at 7 μg/L in the United Kingdom (MHRA 2012) and at 10 μg/L in the United States by the Mayo Clinic (2015).

Environmental exposure

Evidence of the potential for environmental exposure to cobalt comes from biomonitoring studies that found elevated levels of cobalt in people who lived near mining operations in Guatemala (Basu *et al.* 2010) and Mexico (Moreno *et al.* 2010). The TRI reported that in 2013, on- and off-site industrial releases of cobalt and cobalt compounds totalled approximately 5.5 million pounds from 723 facilities in the United States (TRI 2014a). Calculations based on media-specific release data from TRI indicate that releases to land accounted for 82% of total releases in 2013. Worldwide, approximately 75,000 metric tons of cobalt enters environment annually (Shedd 1993, CDI 2006) with similar amounts coming from natural sources (40,000 metric tons) and anthropogenic sources (35,000 metric tons) (Shedd 1993, CDI 2006, TRI 2014b).

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 and compounds) reported from outside the United States. The median cobalt concentration in U.S. drinking water has been reported to be less than 2.0 µg/L; however, levels as high as 107 µg/L have been reported (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 fresh water and groundwater (IARC 2006). Studies have reported cobalt soil concentrations ranging from 0.1 to 50 ppm. However, soils near ore deposits, phosphate rock, or ore-smelting facilities or soils contaminated by airport or highway traffic or near other source areas may contain higher concentrations (IARC 2006).

Other sources of exposure to the general public

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; daily cobalt intake from food has been reported to range from 5 to 50 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}. In the 1960s, some breweries added cobalt salts to beer to stabilize the foam (resulting in exposures of 0.04 to 0.14 mg cobalt/kg body weight), but cobalt is no longer added to beer (ATSDR 2004). Higher cobalt intake may result from consumption of over-the-counter or prescription mineral preparations containing cobalt compounds.

Other potential sources of exposure include consumer products and tobacco smoking. Cobalt is present in only a few consumer products, including cleaners, detergents, soaps, car waxes, and a nickel metal hydride battery (5% to 10% cobalt) (ATSDR 2004, HPD 2014). Various brands of tobacco have been reported to contain cobalt at concentrations ranging from less than 0.3 to 2.3 μg/g dry weight, and 0.5% of the cobalt content is transferred to mainstream smoke (WHO 2006). However, urinary cobalt levels (unadjusted for creatinine) for cigarette-smoke-exposed and unexposed NHANES participants for survey years 1999 to 2004 did not differ significantly (Richter *et al.* 2009).

Regulations

Coast Guard, Department of Homeland Security

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

Department of Transportation (DOT)

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

Environmental Protection Agency (EPA)

Clean Air Act

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

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.

Comprehensive Environmental Response, Compensation, and Liability Act

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

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.

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.

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.

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).

Guidelines

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) (end of shift at end of workweek) = 15 µg/L for cobalt in urine.

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.

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 µg/m^3; industrial air = 0.0014 µg/m^3; tap water = 6 µg/L.

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).

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