

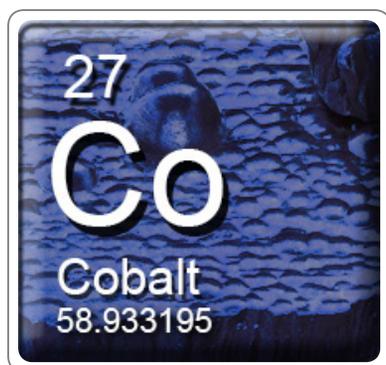


National Toxicology Program  
U.S. Department of Health and Human Services

# Report on Carcinogens

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

April 2016





**National Toxicology Program**

U.S. Department of Health and Human Services

**Report on Carcinogens Monograph on  
Cobalt and Cobalt Compounds  
That Release Cobalt Ions *In Vivo***

April 22, 2016

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

**This Page Intentionally Left Blank**

## 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 NTP, with assistance from other Federal health and regulatory agencies and nongovernmental institutions, prepares the report for the Secretary, Department of HHS. The most recent RoC, the 13th Edition (2014), is available at <http://ntp.niehs.nih.gov/go/roc>.

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

### Monograph contents

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

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

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

### **Process for preparation of the cancer hazard evaluation component**

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

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

### ***Key scientific questions and issues relevant for the cancer evaluation***

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

#### **Questions related to the evaluation of human exposure information**

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

**Questions related to the evaluation of disposition and toxicokinetics**

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

**Questions related to the evaluation of human cancer studies**

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

**Questions related to the evaluation of cancer studies in experimental animals**

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

**Questions related to the evaluation of mechanistic data and other relevant data**

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

### **Approach for obtaining scientific and public input**

To help address the approach to identify a common mode of action involving the cobalt ion for cobalt compounds, additional scientific input was requested early in the review process to define the scope of the review, i.e., what cobalt compound(s) could reasonably be included in this evaluation? Based on input from several scientific experts at a Cobalt Information Group Meeting convened at NIEHS on October 7, 2104, the scope of the evaluation was recommended to include cobalt and cobalt compounds that release cobalt ions *in vivo*. Technical advisors for the review of cobalt are identified on the “CONTRIBUTORS” page.

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

### **Methods for writing the cancer evaluation component of the monograph**

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

The preparation of the RoC monograph for cobalt began with development of a literature search strategy to obtain information relevant to the topics listed above for Sections 1 through 6 using search terms developed in collaboration with a reference librarian (see Protocol). The approximately 7500 citations identified from these searches were uploaded to web-based systematic review software for evaluation by two separate reviewers using inclusion/exclusion criteria, and 484 references were selected for final inclusion in the monograph using these criteria.

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

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

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

### RoC Listing Criteria

#### ***Known To Be Human Carcinogen:***

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

#### ***Reasonably Anticipated To Be Human Carcinogen:***

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

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

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

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

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

## Contributors

### Office of the Report on Carcinogens (ORoC), Division of the National Toxicology Program (NTP)

*Conducted technical review and evaluation and proposed the preliminary listing recommendation*

Ruth Lunn, DrPH  
Director, ORoC

Gloria D. Jahnke, DVM, DABT  
Diane L. Spencer, MS;  
Co-Project Lead

### Integrated Laboratory Systems, Inc. (Support provided through NIEHS Contract Number HHSN273201100004C)

*Conducted technical review and evaluation*

Sanford Garner, PhD  
Principal Investigator  
Stanley Atwood, MS, DABT;  
Co-Project Lead

Andrew Ewens, PhD, DABT  
Alton Peters, MS  
Pamela Schwingl, PhD

*Provided administrative support*

Ella Darden, BS

Tracy Saunders, BS

### Other Members of the RoC Monograph Team (NIEHS/NTP except as noted)

*Provided advice on defining the scope of review (i.e., served on the Information Group) or on issues related to the cancer hazard evaluation*

Mamta Behl, PhD, DABT  
(Information Group Moderator)  
Chad Blystone, PhD, DABT  
Janet Carter, MPH (OSHA/DOL)  
Michelle Hooth, PhD, DABT

Arun Pandiri, PhD, DACVP  
Matthew Stout, PhD, DABT  
Erik Tokar, PhD  
John Wheeler, PhD, DABT  
(ATSDR/CDC)

## Peer Review

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

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

1. To comment on the draft cancer evaluation component for cobalt and certain cobalt compounds, specifically, whether it was technically correct and clearly stated, whether the NTP had objectively presented and assessed the scientific evidence, and whether the scientific evidence was adequate for applying the RoC listing criteria.
2. To comment on the draft profile for cobalt and certain cobalt compounds, specifically, whether the scientific justification presented in the profile supported the NTP's preliminary policy decision on the RoC listing status of the substance.

The Panel was asked to vote on the following questions:

3. Whether the scientific information presented from human cancer studies supported the NTP's preliminary level of evidence conclusion of cobalt and cobalt compounds that release cobalt ions *in vivo*.<sup>1</sup>
4. Whether the scientific information presented from studies in experimental animals supported the NTP's preliminary level of conclusion of cobalt and cobalt compounds that release cobalt ions *in vivo*.<sup>1</sup>
5. Whether NTP's preliminary policy decision for 'cobalt and cobalt compounds that release cobalt ions *in vivo*'<sup>1</sup> in the RoC.

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

---

<sup>1</sup> During the meeting the Panel recommended using the definition of "certain cobalt compounds," i.e., "cobalt compounds that release cobalt ions *in vivo*" in the listing rather than the word "certain."

---

**Peer-Review Panel Members****Melissa A. McDiarmid, MD, MPH,  
DABT (Chair)**

Professor, Medicine and  
Epidemiology & Public Health  
Director, Division of Occupational and  
Environmental Medicine  
University of Maryland School of  
Medicine  
Baltimore, MD

**Robert F. Herrick, DS**

Senior Lecturer on Industrial Hygiene  
Department of Environmental Health  
Harvard School of Public Health  
Boston, MA

**C. William Jameson, PhD**

Consultant  
CWJ Consulting, LLC  
Cape Coral, FL

**Clark Lantz, PhD**

Professor  
Cellular and Molecular Medicine  
The University of Arizona  
Tucson, AZ

**John LaPres, PhD**

Associate Professor  
Department of Biochemistry and Molecular  
Biology  
Michigan State University  
East Lansing, MI

**Marie-Elise Parent, PhD**

Professor, Epidemiology and Biostatistics  
Unit  
INRS-Institut Armand-Frappier  
Université du Québec  
Laval, Quebec  
Canada

**Michael V. Pino, DVM, PhD, DACVP**

Consultant, Veterinary Toxicological  
Pathology and Preclinical Drug  
Development  
Albuquerque, NM

**Lisa De Roo, MPH, PhD**

Department of Global Public Health and  
Primary Care  
University of Bergen  
Norway

**John Pierce Wise, Sr., PhD**

Professor and University Scholar  
Department of Pharmacology and  
Toxicology  
School of Medicine  
University of Louisville  
Louisville, KY

**Anatoly Zhitkovich, PhD**

Professor, Medical Science  
Department of Pathology and Laboratory  
Medicine  
Brown University  
Providence, RI

**This Page Intentionally Left Blank**

## Table of Contents

### Part 1: Draft Cancer Hazard Evaluation

1	Chemical identification and properties .....	1
1.1	Properties of cobalt metal and cobalt compounds, both soluble and poorly soluble .....	1
1.2	Water solubility and bioaccessibility .....	3
1.2.1	Water solubility .....	3
1.2.2	Bioaccessibility .....	3
1.3	Variability of valence .....	4
1.4	Summary .....	5
2	Human Exposure.....	7
2.1	Mining and production .....	7
2.2	Use.....	9
2.3	Recycling of electronic and electrical waste .....	10
2.4	Biomonitoring and environmental monitoring for cobalt .....	11
2.4.1	Evidence of exposure.....	11
2.4.2	Exposure surrogates and analytical methods .....	13
2.5	Characterization of exposure in the workplace .....	14
2.5.1	Cobalt production (metals and salts).....	16
2.5.2	Cemented carbides and bonded diamonds.....	16
2.5.3	Metallurgical-related industries .....	16
2.5.4	Chemicals and pigments .....	17
2.5.5	Electronics, “green” energy, and recycling of electronic and electrical waste .....	18
2.6	Surgical implants.....	18
2.7	Other sources of exposure: Food, consumer and other medical products and tobacco .....	20
2.8	Potential for environmental exposure.....	21
2.8.1	Releases.....	21
2.8.2	Occurrence .....	22
2.8.3	Exposure .....	23
2.9	Summary and synthesis .....	23
3	Disposition and Toxicokinetics.....	25
3.1	Disposition.....	25
3.1.1	Humans .....	25
3.1.2	Experimental animals.....	28
3.2	Toxicokinetics .....	34
3.2.1	Humans .....	34
3.2.2	Experimental animals.....	35
3.3	Synthesis.....	36
4	Human Cancer Studies.....	39
4.1	Selection of the relevant literature.....	39
4.2	Cohort studies and nested case-control studies reporting on lung cancer .....	41
4.2.1	Overview of the methodologies and study characteristics.....	41

4.2.2	Study quality and utility evaluation .....	43
4.2.3	Cancer assessment: Lung .....	45
4.3	Case-control studies .....	57
4.3.1	Overview of the methodologies and study characteristics.....	57
4.3.2	Study quality and utility evaluation .....	58
4.3.3	Cancer assessment: Esophageal cancer .....	59
4.4	Cancer assessment: Other types of cancers .....	65
4.4.1	Other aerodigestive cancers - oral cavity, pharyngeal, and laryngeal cancers.....	65
4.4.2	Other types of cancers.....	66
4.5	NTP listing recommendation.....	66
5	Studies of Cancer in Experimental Animals .....	69
5.1	Carcinogenicity studies .....	69
5.1.1	Overview of the studies .....	69
5.1.2	Study quality assessment .....	72
5.1.3	Assessment of neoplastic findings from carcinogenicity studies .....	75
5.2	Co-carcinogenicity studies .....	107
5.2.1	Overview of the studies .....	107
5.2.2	Overview of the assessment of study quality and utility .....	108
5.2.3	Assessment of findings from co-carcinogenicity studies.....	108
5.3	Synthesis of the findings across studies .....	109
6	Mechanistic and Other Relevant Effects.....	113
6.1	Cobalt particles and cobalt ions.....	113
6.2	Proposed modes of action of cobalt carcinogenicity .....	120
6.2.1	Genotoxicity, inhibition of DNA repair, and related key events .....	122
6.2.2	Oxidative stress and damage.....	126
6.2.3	HIF stabilization and hypoxia mimicry .....	129
6.3	Other biological effects .....	132
6.4	Synthesis.....	132
7	Overall Cancer Evaluation and NTP Listing Recommendation.....	135
7.1	Cobalt and cobalt compounds that release cobalt ion <i>in vivo</i> as a class.....	135
7.1.1	Physicochemical properties and toxicokinetics .....	136
7.1.2	Mechanistic and other relevant data .....	137
7.1.3	Toxicological effects and key events.....	137
7.1.4	Overall synthesis.....	138
7.2	Evidence of carcinogenicity from studies in experimental animals.....	139
7.3	Evidence of carcinogenicity from studies in humans.....	140
8	References.....	141
Appendix A: Literature Search Strategy.....		A-1
A.1	Search strategies.....	A-3
A.2	Updating the literature search .....	A-3
A.3	Review of citations using web-based systematic review software .....	A-3
Appendix B: Chemical Properties and Exposure-Related Information, Clinical Surveys and Studies, and Regulations.....		B-1

B.1	Chemical properties .....	B-1
B.2	Exposure .....	B-2
B.3	Clinical surveys and studies.....	B-19
B.3.1	Studies of lung or laryngeal cancer patients .....	B-19
B.3.2	Other cancers .....	B-20
B.3.3	Synthesis .....	B-21
B.4	Regulations and guidelines .....	B-29
Appendix C: Human Cancer Study Tables .....		C-1
C.1	Methodologies and study characteristics .....	C-1
C.2	Assessment of study quality, sensitivity, and utility of human studies of cobalt.....	C-12
C.2.1	Selection bias and evaluation of methods used to address potential confounding .....	C-13
C.2.2	Evaluation of methods to address confounding.....	C-14
C.2.3	Information bias: exposure assessment and disease endpoints.....	C-19
C.2.4	Information bias - Disease endpoints.....	C-20
C.2.5	Selective reporting and analysis bias in human studies of cobalt.....	C-23
C.2.6	Study sensitivity, quality and utility of cohort and nested case-control studies .....	C-26
Appendix D: Cancer Studies in Experimental Animals .....		D-1
D.1	Study quality .....	D-1
D.1.1	Evaluation methods.....	D-1
D.1.2	Study quality and sensitivity questions and responses .....	D-1
D.2	Overall assessment of study utility .....	D-2
D.3	Study quality assessment and study quality tables: Carcinogenicity studies.....	D-3
Appendix E: Genotoxicity and Cellular Transformation.....		E-1
E.1	<i>In vitro</i> mutagenicity and DNA-damage studies of cobalt compounds in bacteria .....	E-1
E.2	Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes .....	E-6
E.3	<i>In vitro</i> studies of genotoxicity of cobalt compounds in mammalian cells .....	E-10
E.3.1	Rodent cells.....	E-10
E.3.2	Human cells .....	E-11
E.4	Protein binding and DNA repair inhibition by cobalt compounds .....	E-18
E.5	<i>In vivo</i> genotoxicity studies of cobalt compounds in rodents .....	E-18
E.6	Genotoxicity studies of occupational exposure to cobalt .....	E-22
E.7	Cell transformation .....	E-24

## Part 2: Draft cancer hazard profile

Draft Profile .....	P-1
---------------------	-----

### List of Tables

Table 1-1. Physical and chemical properties for cobalt metal and representative cobalt compounds <sup>a,b</sup> .....	2
---	---

Table 2-1. U.S. cobalt compounds production volumes for 2012 exceeding 100,000 pounds per year <sup>a</sup> .....	8
Table 2-2. U.S. imports and exports of cobalt compounds for 2013 (converted from kg by NTP) 8	
Table 2-3. 2012 U.S. consumption and use pattern for cobalt.....	9
Table 2-4. Chemical uses for representative inorganic and organic cobalt compounds.....	10
Table 2-5. Workplace air levels of cobalt.....	14
Table 3-1. Interspecies comparison of lung retention of cobalt oxide (Co <sub>3</sub> O <sub>4</sub> ).....	32
Table 3-2. Elimination half-lives for cobalt administered to experimental animals.....	36
Table 4-1. Cohort and nested case-control studies of exposure to cobalt.....	42
Table 4-2. Bias and quality summary for cohort and nested case-control studies.....	45
Table 4-3. Evidence from cohort and case-control studies on lung cancer and exposure to cobalt.....	47
Table 4-4. Case-control biomarker studies of exposure to cobalt .....	58
Table 4-5. Bias and quality summary for case-control studies.....	59
Table 4-6. Evidence from studies of aerodigestive cancers and exposure to cobalt.....	61
Table 5-1. Overview of cancer studies in experimental animals reviewed .....	71
Table 5-2. Overview of experimental animal carcinogenicity study quality evaluations.....	73
Table 5-3. Lung neoplasms and non-neoplastic lesions in experimental animals exposed to cobalt compounds .....	77
Table 5-4. Injection site neoplasms and non-neoplastic lesions in experimental animals exposed to cobalt compounds.....	89
Table 5-5. Other and distal site neoplasms and relevant non-neoplastic lesions in experimental animals exposed to cobalt compounds .....	99
Table 5-6. Overview of co-carcinogenicity studies in experimental animals reviewed.....	107
Table 5-7. Overall results of carcinogenicity studies in experimental animals sorted by cobalt compound .....	111
Table 6-1. <i>In vitro</i> mechanistic data comparing effects of cobalt nanoparticles, microparticles, and ions.....	114
Table 6-2. Summary assessment of genotoxicity and related effects for cobalt compounds <sup>a</sup> .....	125
Table 7-1. Comparison of chemical and biological properties of cobalt metal and cobalt compounds.....	138
Table 7-2. Carcinogenic effects of cobalt metal and cobalt compounds in experimental animals .....	139
Table B-1. Physical and chemical properties for additional chemical forms of cobalt .....	B-1
Table B-2. Values for urine and blood cobalt levels (means or medians) in the United States and other countries (urine values are plotted in Figure 2-1) .....	B-3
Table B-3. Values for hair and nail cobalt levels in the United States and other countries (hair values are plotted in Figure 2-2) <sup>a</sup> .....	B-16
Table B-4. Findings from studies that measured cobalt in tissues (means or medians) of lung and larynx cancer patients and referents .....	B-22
Table B-5. Findings from studies that measured cobalt in tissues (means or medians) of cancer patients and referents.....	B-25
Table C-1a. Study description and methodologies of cohort studies: Tüchsen <i>et al.</i> (1996) .....	C-2
Table C-1b. Study description and methodologies of cohort studies: Mur <i>et al.</i> (1987).....	C-3
Table C-1c. Study description and methodologies of cohort studies: Moulin <i>et al.</i> (1993).....	C-5
Table C-1d. Study description and methodologies of cohort studies: Moulin <i>et al.</i> (1998).....	C-6

Table C-1e: Study description and methodologies of cohort studies: Wild <i>et al.</i> (2000).....	C-7
Table C-1f: Study description and methodologies of cohort studies: Moulin <i>et al.</i> (2000) .....	C-8
Table C-1g: Study description and methodologies of cohort studies: Grimsrud <i>et al.</i> (2005) ....	C-9
Table C-1h: Study description and methodologies of case-control studies: Rogers <i>et al.</i> (1993).....	C-10
Table C-1i: Study description and methodologies of case-control studies: O'Rorke <i>et al.</i> (2012).....	C-11
Table C-2a. Selection bias and evaluation of methods used to address potential confounding in human studies of cobalt.....	C-16
Table C-2b. Information bias - exposure assessment and disease endpoints in human studies of cobalt .....	C-21
Table C-2c. Selective reporting and analysis bias in human studies of cobalt .....	C-25
Table D-1a. NTP 2014b (rats): Cobalt metal/powder; Inhalation .....	D-4
Table D-1b. NTP 2014b (mice): Cobalt metal/powder; Inhalation .....	D-5
Table D-1c. Hansen 2006: Cobalt metal/powder; Injection .....	D-6
Table D-1d. Jasmin and Riopelle 1976: Cobalt sulfide or cobalt metal; Injection.....	D-8
Table D-1e. Heath and Daniel 1962: Cobalt metal/powder; Injection .....	D-10
Table D-1f. Heath 1956: Cobalt metal/powder; Injection .....	D-12
Table D-1g. NTP 1998 (rats): Cobalt sulfate; Inhalation .....	D-14
Table D-1h. NTP 1998 (mice): Cobalt sulfate; Inhalation .....	D-15
Table D-1i. Shabaan 1977: Cobalt chloride; Injection .....	D-16
Table D-1j. Steinhoff and Mohr 1991: Cobalt oxide; Intratracheal.....	D-18
Table D-1k. Steinhoff and Mohr 1991: Cobalt oxide; Injection (IP) .....	D-20
Table D-1l. Steinhoff and Mohr 1991: Cobalt oxide; Injection (SC).....	D-22
Table D-1m. Gilman and Ruckerbauer 1962 (rats): Cobalt oxide; Injection .....	D-24
Table D-1n. Gilman and Ruckerbauer 1962 (mice): Cobalt oxide; Injection.....	D-26
Table D-1o. Wehner 1977: Cobalt oxide; Inhalation.....	D-28
Table D-2a. Finogenova 1973: Cobalt chloride; Injection .....	D-30
Table D-2b. Kasirsky 1965: Cobalt chloride; Injection.....	D-32
Table D-2c. O'Hara 1971: Cobalt chloride; Injection.....	D-34
Table D-2d. Zeller 1975: Cobalt chloride; Injection .....	D-36
Table D-2e. Orzechowski 1964: Sodium cobaltinitrite; Injection .....	D-38
Table D-2f. Thompson 1965: Sodium cobaltinitrite; Drinking water .....	D-40
Table D-2g. Steinhoff and Mohr 1991: Cobalt oxide; Intratracheal.....	D-41
Table D-2h. Wehner 1977: Cobalt oxide; Inhalation.....	D-43
Table D-3. Overview of experimental animal co-carcinogenicity study quality evaluations...	D-45
Table E-1. <i>In vitro</i> mutagenicity and DNA-damage studies of cobalt compounds in bacteria ...	E-3
Table E-2. Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes .....	E-7
Table E-3. <i>In vitro</i> studies of genotoxic effects of cobalt compounds in mammalian cells .....	E-13
Table E-4. <i>In vitro</i> studies of other (genotoxic-related) effects of cobalt compounds in mammalian cells.....	E-17
Table E-5. Studies of nucleic acid and protein binding and DNA damage/repair inhibition of cobalt compounds.....	E-19
Table E-6. <i>In vivo</i> genotoxicity studies of cobalt compounds in rodents .....	E-21
Table E-7. Genotoxicity studies of occupational exposure to cobalt.....	E-23

Table E-8. <i>In vitro</i> studies of other (genotoxic-related) effects of cobalt compounds in mammalian cells.....	E-24
---	------

### List of Figures

Figure 2-1. Cluster graph of urine cobalt levels from different sources of exposure .....	12
Figure 2-2. Cluster graphs of cobalt levels in hair.....	12
Figure 2-3. Flow of cobalt released from anthropogenic processes .....	22
Figure 3-1. Cobalt disposition.....	25
Figure 3-2. Rate of translocation of cobalt from lung to blood following inhalation of cobalt oxide particles.....	30
Figure 4-1. Forest plot showing lung cancer risk ratios (SIR, SMR, or OR as noted) and 95% CI for epidemiological cohort studies of cobalt exposure.....	57
Figure 6-2. Proposed modes of action of cobalt carcinogenicity.....	121
Figure 6-3. <i>K-ras</i> mutations in lung tumors from cobalt-exposed and non-exposed rodents .....	129
Figure A-1. Literature search strategy and review.....	A-2

---

## **Part 1**

---

# **Draft Cancer Hazard Evaluation**

**Properties and Chemical Identification**

**Human Exposure**

**Disposition (ADME) and Toxicokinetics**

**Human Cancer Studies**

**Studies of Cancer in Experimental Animals**

**Mechanistic Data and Other Relevant Effects**

**Overall Cancer Evaluation and NTP Listing  
Recommendation**

---

**This Page Intentionally Left Blank**

---

# 1 Chemical identification and properties

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

Cobalt (Co) is a naturally occurring transition element with magnetic properties. It is the 33<sup>rd</sup> most abundant element and makes up approximately 0.0025% of the weight of Earth’s crust. Cobalt is a component of more than 70 naturally occurring minerals including arsenides, sulfides, and oxides. The only stable and naturally occurring cobalt isotope is <sup>59</sup>Co. 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, +2 (Co(II)) and +3 (Co(III)).

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

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

## 1.1 Properties of cobalt metal and cobalt compounds, both soluble and poorly soluble

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

Table 1-1. Physical and chemical properties for cobalt metal and representative cobalt compounds<sup>a,b</sup>

Name (+2 valence unless otherwise indicated)	CAS No.	Formula	Molecular weight	Physical form	Density or specific gravity	Solubility (grams per 100 cc cold water)	Particle size, µm (surface area, m <sup>2</sup> /g)	Bioaccessibility <sup>c</sup>
<b><i>Metal</i></b>								
<i>Cobalt</i>	7440-48-4	Co	58.9	Grey hexagonal or cubic metal	8.92	0.00029	7.2 (1.20)	100/100
<i>Cobalt nanoparticles</i>	7440-48-4	Co	58.9	–	–	–	–	–
<b><i>Soluble cobalt compounds</i></b>								
<i>Sulfate heptahydrate</i>	10026-24-1	CoSO <sub>4</sub> •7H <sub>2</sub> O	281.1	Red pink, monoclinic	1.95	60.4	942.0 (3.49)	100/100
<i>Chloride</i>	7646-79-9	CoCl <sub>2</sub>	129.9	Blue hexagonal leaflets	3.36	45	458.0 (0.78)	100/100
<i>Acetate (org.)</i>	71-48-7	Co(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub>	249.1	Red-violet, monoclinic	1.70	34.8	–	98/80
<i>Nitrate</i>	10141-05-6	CoN <sub>2</sub> O <sub>6</sub>	182.9	Red powder or crystals	2.49	67.0	–	96/100
<b><i>Poorly soluble compounds</i></b>								
<i>(II) Oxide</i>	1307-96-6	CoO	74.9	Green-brown cubic	6.45	0.00049	0.692 (4.79)	100/92.4
<i>(II, III) Oxide</i>	1308-06-1	Co <sub>3</sub> O <sub>4</sub>	240.8	Black, cubic	6.07	0.00016	–	2/2 (50% <sup>d</sup> )
<i>2-ethyl-hexanoate (org.)</i>	136-52-7	Co(C <sub>8</sub> H <sub>15</sub> O <sub>2</sub> ) <sub>2</sub>	173.7	Blue liquid (12% Co)	1.01	0.630	0.73 (ND)	100/100
<i>Carbonate (org.)</i>	513-79-1	CoCO <sub>3</sub>	118.9	Red, trigonal	4.13	0.00114	1.834 (103.05)	100/100
<i>Naphthenate (org.)</i>	61789-51-3	Co(C <sub>11</sub> H <sub>7</sub> O <sub>2</sub> ) <sub>2</sub>	401.3	Purple liquid (6% Co)	0.97	0.0293	0.70 (ND)	100/100
<i>Hydroxide</i>	21041-93-0	Co(OH) <sub>2</sub>	93.0	Rose-red, rhomb	3.60	0.00032	–	95/98
<i>Sulfide</i>	1317-42-6	CoS	91.0	Reddish octahedral	5.45	0.00038	–	1/1
<i>Oxalate (org.)</i>	814-89-1	CoC <sub>2</sub> O <sub>4</sub>	147.0	White or reddish	3.02	0.00322	–	37/55
<i>Propionate (org.)</i>	1560-69-6	Co(C <sub>3</sub> H <sub>5</sub> O <sub>2</sub> ) <sub>2</sub>	205.1	Reddish solid	–	7.49	–	91/94
<i>Stearate (org.)</i>	1002-88-6	Co(C <sub>18</sub> H <sub>35</sub> O <sub>2</sub> ) <sub>2</sub>	625.9	Grey solid	–	0.00705	–	14/16

Sources: SciFinder 2015; PubChem Compounds Database 2015; ChemIDplus Database 2015; Cobalt Development Institute (CDI) Report 2006; Hazardous Substances Data Bank (HSDB 2004, 2012); Stopford *et al.* 2003. Personal communication, CDI, July 21, 2015, and October 19, 2015.

org. = organic compound; all others are inorganic.

<sup>a</sup> Cobalt compounds selected for inclusion in the table include those with toxicological data or of commercial importance. All compounds contain Co(II) except where noted. Forms in italics have been tested for carcinogenicity, genetic toxicity, or have mechanistic data; org. = organic compound; all others are inorganic.

<sup>b</sup> Bioaccessibility usually assessed as % solubility in gastric/lysosomal fluids

<sup>c</sup> ( ) = Bioaccessibility assessed by release of cobalt ions into RPMI 1640 culture medium in the presence of canine alveolar macrophages after 2 weeks of culture (Kreyling *et al.* 1990).

## 1.2 Water solubility and bioaccessibility

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

### 1.2.1 Water solubility

Cobalt sulfate, chloride, and nitrate tend to be soluble in water, while oxides (including the mixed oxide,  $\text{Co}_3\text{O}_4$ ), hydroxides, and sulfides tend to be poorly soluble or insoluble (Lison 2015). Organic cobalt compounds can be either soluble (e.g., cobalt(II) acetate) or insoluble (e.g., cobalt carbonate, cobalt(II) oxalate, cobalt propionate, cobalt stearate, cobalt naphthenate, cobalt 2-ethyl-hexanoate) (CDI 2006) (see Table 1-1). The water solubility of cobalt compounds is largely pH dependent, and cobalt is generally more mobile in acidic solutions than in alkaline solutions.

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

### 1.2.2 Bioaccessibility

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

Some compounds that appear to be insoluble in these tests might actually be soluble in biological fluids *in vivo*. This is due to the inability of a simple model with an artificial biological fluid and a cobalt compound not providing for the potential equilibrium between cellular compartments such as the lysosomes with the cytoplasm and ultimately the extracellular fluid. Thus, additional assays that are more physiologically relevant because of the presence of lung cells are potentially more informative. For example, although very low values ( $\leq 2\%$ ) for bioavailability have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the test conditions reported in Table 1-1, other test conditions in the presence of lung cells have indicated that  $\text{Co}_3\text{O}_4$  (cobalt (II,III) oxide) releases cobalt ions. Kreyling *et al.* (1990) reported that cobalt ions dissolved from  $\text{Co}_3\text{O}_4$  particles of different sizes

were ultimately released into the culture medium (RPMI 1640) in the presence of canine alveolar macrophages with up to 50% solubilized from 0.3  $\mu\text{m}$  particles after 2 weeks of culture (larger particles released 2% to 5% under the same conditions). The differences in findings between this study and the 2% solubility in gastric or lysosomal fluid may be due to the interaction of cobalt particles within cells as the soluble fraction of an initial particle mass of  $\text{Co}_3\text{O}_4$  increased with time when the particles were taken up by alveolar macrophages in culture compared with the solubility in culture medium alone. Moreover, Ortega *et al.* (2014), found that intracellular concentrations of solubilized cobalt ions were similar for  $\text{Co}_3\text{O}_4$  and cobalt chloride in human lung cells, suggesting that  $\text{Co}_3\text{O}_4$  would release cobalt ions *in vivo* (see Section 6.1 for details). A similar result was reported for CoO nanoparticles, which increased intracellular cobalt ion concentration in human lung fibroblasts in culture in a concentration-dependent manner (Smith *et al.* 2014).

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

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

### 1.3 Variability of valence

As noted above, cobalt exists primarily as Co(II) and Co(III), and Co(II) is much more stable in aqueous solution (Nilsson *et al.* 1985, Paustenbach *et al.* 2013). Electron-donor ligands (e.g.,  $\text{NH}_3$ ) can stabilize Co(III) in aqueous solution (IARC 1991). In acid solution, Co(II) is the stable form in the absence of electron-donor ligands, and Co(III) ions are so unstable that they quickly reduce to Co(II), oxidizing water and liberating oxygen. In contrast, air or hydrogen peroxide can oxidize Co(II) to the Co(III) complex, which is more stable in alkaline solutions containing ammonium hydroxide or cyanide. This interconversion between Co(II) and Co(III) is important in the use of cobalt compounds as catalysts and paint driers (IARC 1991, Paustenbach *et al.* 2013).

Cobalt is present in its stable +2 valence state in the environment and in most commercially available cobalt compounds, with the exception of the mixed oxide (Co(II,III) or  $\text{Co}_3\text{O}_4$ ) (IARC 1991, Paustenbach *et al.* 2013). Some simple salts of cobalt in its +3 valence state (e.g.,  $\text{Co}_2\text{O}_3$ ) have been used commercially. Cobalt compounds of commercial and toxicological interest

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

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

#### 1.4 Summary

Cobalt metal particles have been found to be 100% bioaccessible (i.e., dissolving to release cobalt ions) in both artificial gastric and lysosomal fluids. The soluble compounds, cobalt(II) sulfate heptahydrate and cobalt(II) chloride, and the poorly soluble compounds, cobalt(II) oxide, cobalt bis(2-ethyl hexanoate), cobalt carbonate, and cobalt naphthenate, also were completely (or almost completely) soluble in the two acidic fluids. The metals and poorly soluble compounds tended to be less bioaccessible in neutral biological fluids, which is consistent with the pH dependence for releasing cobalt ions in solution. Although very low values ( $\leq 2\%$ ) for bioavailability in artificial gastric and lysosomal fluids have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the same test conditions, more informative tests (e.g., using more physiologically relevant conditions) in the presence of lung cells have shown higher bioavailability values for cobalt(II, III) oxide (i.e.,  $\text{Co}_3\text{O}_4$ ) in culture media in the presence of alveolar macrophages. Other studies have reported uptake of  $\text{Co}_3\text{O}_4$  by lung cells, which suggests that compound would release ions *in vivo*.

**This Page Intentionally Left Blank**

## 2 Human Exposure

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

### 2.1 Mining and production

Cobalt is most often found in ores associated with copper or nickel, but may also be a by-product of zinc, lead, and platinum-group metals (Davis 2000, CDI 2006). Cobalt-containing ores often contain arsenic, such as safflorite,  $\text{CoAs}_2$ ; skutterudite,  $\text{CoAs}_3$ ; erythrite,  $\text{Co}_3(\text{AsO}_4)_2 \cdot 8\text{H}_2\text{O}$ ; and glaucodot,  $\text{CoAsS}$  (Davis 2000, ATSDR 2004, CDI 2006). 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 (Rufe 2010, Farquharson 2015, Mining Technology Market and Customer Insight 2015). 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<sup>a</sup>**

CAS Number <sup>b</sup>	Cobalt compound	Quantity (lb) <sup>c</sup>
7440-48-4	Cobalt	23,384,002
21041-93-0	Cobalt hydroxide (Co(OH) <sub>2</sub> )	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 <sub>3</sub> O <sub>4</sub> )	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 tellurate	192,900
61789-51-3	Cobalt naphthenate	100,000–500,000

<sup>a</sup>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<sub>2</sub>) and cobalt chloride (7646-79-9, CoCl<sub>2</sub>) production levels for 2012 were withheld by the manufacturers.

<sup>b</sup>CAS# were identified from multiple sources: ChemIDplus Database 2015; EPA Chemical Data Reporting 2012; PubChem Compounds Database 2015; Ullmann's Encyclopedia of Industrial Chemistry (2012).

<sup>c</sup>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	— <sup>a</sup>
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	— <sup>a</sup>
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	— <sup>a</sup>
Unwrought cobalt alloys	2,132,331	— <sup>a</sup>
Unwrought cobalt excluding alloys, including powders	16,151,599	— <sup>a</sup>

Source: USITC 2014.

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

## 2.2 Use

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

In 2012, the reported U.S. consumption of cobalt was approximately 8,420 metric tons (Shedd 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 <sup>a</sup>	699	8.3
Steels	548	6.5
Miscellaneous and unspecified	63	0.7

Source: Shedd 2014b.

<sup>a</sup>Includes magnetic, nonferrous, and wear-resistant alloys and welding materials.

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

**Metallurgical uses** of cobalt include use in superalloys (IARC 1991, Davis 2000); 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).

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

**Chemical uses** of cobalt compounds include as pigments for glass, ceramics, and enamels, as driers for paints, varnishes, or lacquers, as catalysts, as adhesives and enamel frits (naphthenate, stearate, oxide), as trace mineral additives for animal diets, and in rechargeable batteries (see Section 2.2.4) (IARC 1991, ATSDR 2004, CDI 2006, WHO 2006) (see Table 2-4). Compounds of commercial importance are the oxides, hydroxide, chloride, sulfate, nitrate, phosphate, carbonate, acetate, oxalate, and other carboxylic acid derivatives (IARC 1991). A past use of cobalt (as cobalt sulfate) was as an additive in some beers to increase the stability of the foam (NTP 1998).

**Table 2-4. Chemical uses for representative inorganic and organic cobalt compounds**

Use	Inorganic					Organic			
	Cl <sup>-</sup>	OH <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	O <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	2-EH	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	Pro
Adhesives				X		X			
Animal diets			X	X	X		X	X	
Batteries		X	X						
Catalysts	X	X	X	X			X	X	
Driers		X		X		X	X		X
Pigments	X		X	X	X		X	X	

Sources: CDI 2006, Donaldson and Beyersmann 2012, Richardson and Meshri 2001.

Cl<sup>-</sup> = chloride, OH<sup>-</sup> = hydroxide, NO<sub>3</sub><sup>-</sup> = nitrate, O<sup>2-</sup> = oxide, SO<sub>4</sub><sup>2-</sup> = sulfate, 2-EH = 2-ethyl-hexanoate, C<sub>2</sub>H<sub>3</sub>O<sub>2</sub><sup>-</sup> = acetate, CO<sub>3</sub><sup>2-</sup> = carbonate, Pro = propionate

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

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

### 2.3 Recycling of electronic and electrical waste

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

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

## 2.4 Biomonitoring and environmental monitoring for cobalt

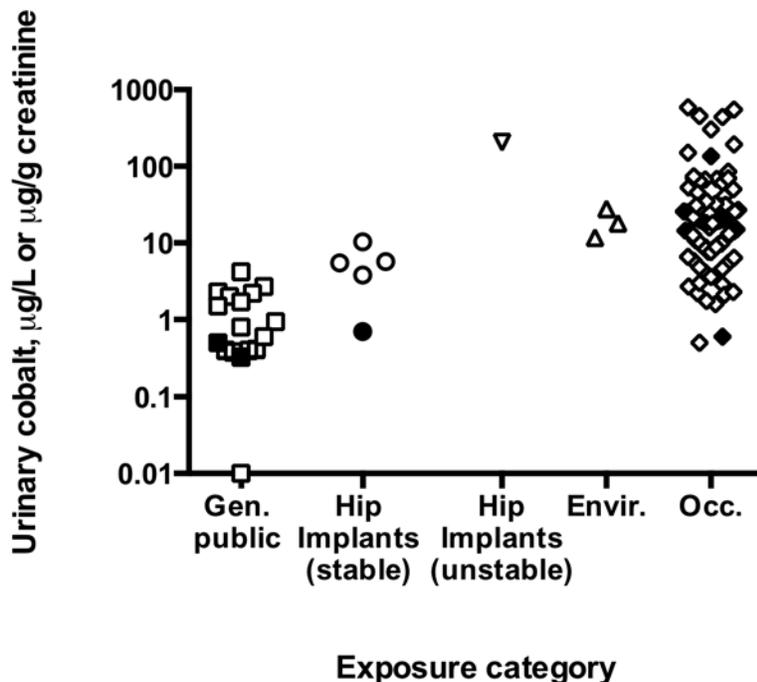
Information on biomonitoring and environmental monitoring for cobalt discussed below includes evidence of exposure (Section 2.4.1) and exposure surrogates and analytical methods (Section 2.4.2).

### 2.4.1 Evidence of exposure

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

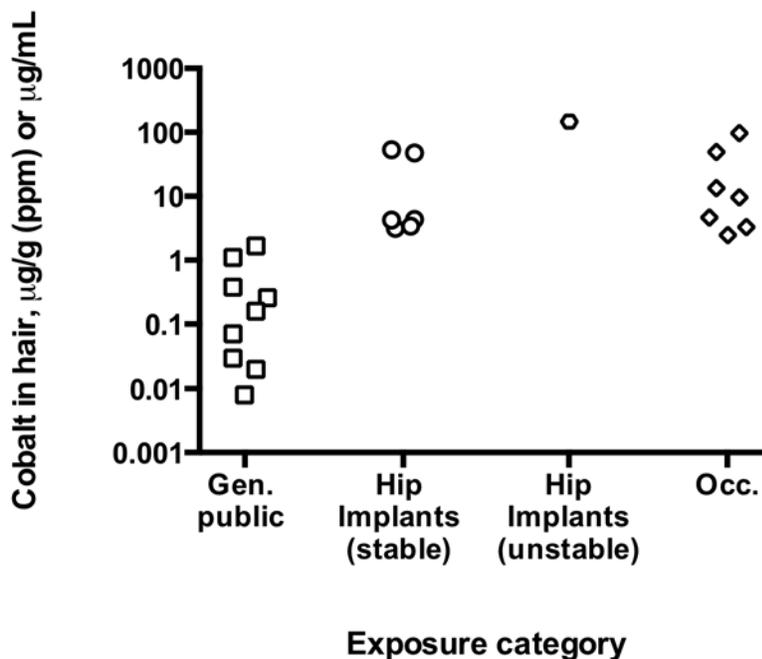
Studies measuring cobalt in the urine of people exposed to cobalt from different sources indicate that the highest levels were generally seen in workers and patients with failed hip implants; with lower levels of exposure in patients with normal implants, people potentially exposed to cobalt from the environment, or in the general public (source of exposure unknown). (See Figure 2-1, which depicts the mean [or median] levels of urinary cobalt in these populations from the studies reported in Appendix B, Table B-2.) The geometric mean urinary cobalt concentration for the U.S. general public for the most recent National Health and Nutrition Examination Survey (NHANES) year (2011 to 2012) for which data are available is 0.326  $\mu\text{g/L}$ ; urinary cobalt measurements in the U.S. general public have remained consistent since 1999, with the geometric mean values ranging from 0.316 to 0.379  $\mu\text{g/L}$  (CDC 2015).

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



**Figure 2-1. Cluster graph of urine cobalt levels from different sources of exposure**

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



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

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

## 2.4.2 Exposure surrogates and analytical methods

### Exposure surrogates

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

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

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

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

### Analytical methods

Analytical methods for cobalt in biological materials include graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), differential pulse cathodic stripping voltammetry (DPCSV), and colorimetric determination (ATSDR 2004). Technical improvements using the Zeeman background correction in GF-AAS have increased specificity and lowered the background (see IUPAC guidelines in Cornelis *et al.* 1995). The colorimetric method generally has limited utility because it has poor sensitivity (Alessio and Dell'Orto 1988). The ICP-AES method is used by NIOSH for exposure to elements in blood and urine (NIOSH 1994a), 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<sub>12</sub>, cannot be determined with these methods (IARC 2006, WHO 2006).

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

## 2.5 Characterization of exposure in the workplace

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

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

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

**Table 2-5. Workplace air levels of cobalt**

Exposure scenario (Country)	Cobalt in workplace air mean (range) in µg/m <sup>3</sup>	Reference(s)
<i>Cobalt production</i>		
Production of cobalt metal and cobalt salts (Belgium)	127.5 (2–7,700)	Swennen <i>et al.</i> 1993
Production of cobalt salts (Russian Federation)	(0.05–50)	Talakin <i>et al.</i> 1991
Nickel refining (Russian Federation)	Up to 4	Thomassen <i>et al.</i> 1999

Exposure scenario (Country)	Cobalt in workplace air mean (range) in $\mu\text{g}/\text{m}^3$	Reference(s)
Production of cobalt metal and cobalt salts (Finland)	< 100	Linna <i>et al.</i> 2003
Conversion of cobalt metal to cobalt oxide (South Africa)	9,900 (highest reported)	Coombs 1996
Nickel refining (Norway)	< 150 <sup>a</sup>	Grimsrud <i>et al.</i> 2005
<b>Metallurgical uses</b>		
Metallurgical (United States)	ND–32,000 <sup>b</sup>	NIOSH 1972, Hervin and Reifschneider 1973, Daniels <i>et al.</i> 1986, Deng <i>et al.</i> 1990, Decker 1991, Deitchman <i>et al.</i> 1994, Kiefer <i>et al.</i> 1994, McCleery <i>et al.</i> 2001, Marsh and Esmen 2007, Beaucham <i>et al.</i> 2014
Production of Stellite, a cobalt-containing alloy (NR)	Several hundred $\mu\text{g}/\text{m}^3$	Simcox <i>et al.</i> 2000
Production of Stellite, a cobalt-containing alloy (NR)	9	Kennedy <i>et al.</i> 1995
Welding with Stellite, a cobalt-containing alloy (NR)	160	Ferri <i>et al.</i> 1994
<b>Cemented carbides (hard metals) and bonded diamonds</b>		
Cemented carbides and bonded diamonds (United States)	ND–1,622.1	Edmonds <i>et al.</i> 1981, McManus 1982, Kerndt <i>et al.</i> 1986, Bryant <i>et al.</i> 1987, Salisbury and Seligman 1987, Tharr and Singal 1987, Burr <i>et al.</i> 1988, Burr and Sinks 1988, Sahakian <i>et al.</i> 2009
Use of cobalt-containing diamond tools (Italy)	690 115 (with improved ventilation)	Ferdenzi <i>et al.</i> 1994
Use of cobalt-containing diamond tools (NR)	(0.1–45)	van den Oever <i>et al.</i> 1990
<b>Chemicals and pigments</b>		
Chemicals (United States)	ND–21	Apol 1976, Rosensteel <i>et al.</i> 1977, Zey 1985, Almaguer 1987, Hall 2003, Burr <i>et al.</i> 2005, Chen <i>et al.</i> 2008, Durgam and Aristeguieta 2010
Painting porcelain plates with cobalt compounds (Denmark)	80 26 (after Danish surveillance program)	Christensen and Poulsen 1994, Christensen 1995, Poulsen <i>et al.</i> 1995
<b>Electronics, “green” energy, and recycling</b>		
Electronics and “green” energy (United States)	ND–1.17	Thoburn and Larsen 1976, Beaucham <i>et al.</i> 2014
Recycling batteries to recover cobalt (NR)	Up to 10	Hengstler <i>et al.</i> 2003

Source: IARC 2006, <http://www2a.cdc.gov/hhe/search.asp>.

NR = Not reported.

<sup>a</sup>Reported as 0.15 mg/m<sup>3</sup>. Among the 3,500 personal samples from the breathing zone taken, cobalt values above 50 mg/m<sup>3</sup> [50,000 µg/m<sup>3</sup>] (3 measurements) were excluded.

<sup>b</sup>OSHA noted that this sample appeared to be tampered with. The next highest value was 21,000 µg/m<sup>3</sup>.

### 2.5.1 Cobalt production (metals and salts)

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

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

### 2.5.2 Metallurgical-related industries

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

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

### 2.5.3 Cemented carbides and bonded diamonds

Exposure to cobalt can occur in hard-metal production, processing, and use and during the maintenance and re-sharpening of hard-metal tools and blades. Air levels of cobalt vary across different stages of the hard-metals manufacturing process, with levels for operations involving

cobalt metal powder often reaching maximum levels between 1,000 and 10,000  $\mu\text{g}/\text{m}^3$  (NTP 2009). Continuous recycling of coolants used during the grinding of hard-metal tools after sintering and during maintenance and re-sharpening has been reported to increase concentrations of dissolved cobalt in the metal-working fluid, which can be a source of exposure to ionic cobalt in aerosols from the coolants (IARC 2006). Wet grinding processes are reported to produce higher cobalt concentrations than dry grinding processes due to coolant mist emissions.

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

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

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

#### 2.5.4 Chemicals and pigments

Cobalt concentrations in workplace air at Danish porcelain factories using cobalt-aluminate spinel or cobalt silicate dyes have been reported to exceed the Danish hygienic standard by 1.3- to 172-fold (Tüchsen *et al.* 1996) (see Section 4). Due to improvements made to workplace conditions in the 1982 to 1992 time period, concentrations of cobalt in workplace air decreased from 1,356  $\text{nmol}/\text{m}^3$  [80  $\mu\text{g}/\text{m}^3$ ] to 454  $\text{nmol}/\text{m}^3$  [26  $\mu\text{g}/\text{m}^3$ ] and worker urinary cobalt decreased from 100-fold to 10-fold above median concentration of controls (IARC 1991, 2006). Several studies have been published reporting urine and blood cobalt levels for pottery or plate painters in Denmark and cloisonne workers in Japan (see Table B-2). The mean urine levels were generally elevated, with levels in the 10s of  $\mu\text{g}/\text{g}$  creatinine for the pottery or plate painters, but <

2 µg/L for the glaze workers in cloisonne production. Mean blood levels did not exceed 3 µg/L for any of the studies identified. No cobalt levels in hair or nails were identified for workers in these industries.

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

### 2.5.5 Electronics, “green” energy, and recycling of electronic and electrical waste

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

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

## 2.6 Surgical implants

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

Total hip implants consist of (1) femoral head attached to a stem that is inserted in the thigh bone (usually made of ceramic or metal) and (2) a socket or cup that is anchored in the pelvis, which can be made of metal, ceramic or polyethylene. Cobalt-chromium-molybdenum (CoCrMo) alloy is the predominant alloy used in metal-containing implants, e.g., metal on metal (MoM) implants (both articulating surfaces are metal), polyethylene on metal or metal on ceramic implants); other metals such as nickel, tungsten, iron, aluminum, and titanium may also be used in implants. A MoM resurfacing hip prosthesis consists of a femoral head capped with a metal covering. MoM hip implants may release a greater number and smaller particles than other types of implants and their use is declining in the United States (Bradberry *et al.* 2014, Devlin *et al.* 2013).

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

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

One in eight total hip implants requires revision within 10 years, and 60% of those are due to wear-related complications (Bradberry *et al.* 2014). Release of metal (wear debris) from implants results from friction between the bearing surfaces and corrosion from non-moving parts, which is caused by body fluids contacting the metal surfaces or by formation of an electrochemical couple between different metal components (Sampson and Hart 2012). The Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom issued a safety alert that proposed a level of 7  $\mu\text{g/L}$  cobalt in blood as an action level for further clinical investigation and action (MHRA 2012) and 10  $\mu\text{g/L}$  in serum was proposed by the Mayo Clinic in the United States (Mayo Clinic 2015). Dunstan *et al.* (2005) also reported blood cobalt levels of 19 and 52  $\mu\text{g/L}$  for two individuals with radiologically loose metal-on-metal hip implants. In rare cases, high levels of cobalt from failed implants may be associated with toxicity. A review of literature published since 1950 identified 18 case reports of hip implant patients with cobalt-associated systematic toxicity (such as cardio-, neuro-, or ocular toxicity) and found that the median cobalt

blood levels were 506 µg/L; range = 353 to 6,521) among 10 patients with failed ceramic implants and 34.5 µg/L (range = 13.6 to 398.6) among 8 patients with MoM implants (Bradberry *et al.* 2014). Removal of a joint replacement device that is associated with high cobalt ion levels generally results in decreased cobalt ion levels as reported by Rodriguez de la Flor (2013) for 11 hip implant patients before revision with mean serum cobalt of 25.8 µg/L, which decreased to 12.1 µg/L after revision surgery (see Table B-2). Only one study (Rodriguez de la Flor *et al.* 2013) was identified that reported mean levels in urine (~205 µg/l) and hair (47.1 µg/g) (see Figure 2-2, and Table B-2) for unstable hip implants and no data were identified for cobalt levels in nails.

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

The general public is exposed to cobalt primarily through consumption of food and to a lesser degree through inhalation of ambient air and ingestion of drinking water; average daily cobalt intake from food has been reported to be 11 µg/day (ATSDR 2004, Lison 2015). Although this amount includes cobalt as part of both vitamin B<sub>12</sub> 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<sub>12</sub>. No estimate for an average dietary intake of cobalt in the United States was identified. Reported values for cobalt content of foods can vary due to differences in environmental cobalt levels, analytical difficulties, and inadequate analytical techniques.

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

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

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<sub>12</sub> absorption.

## **2.8 Potential for environmental exposure**

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

### **2.8.1 Releases**

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

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

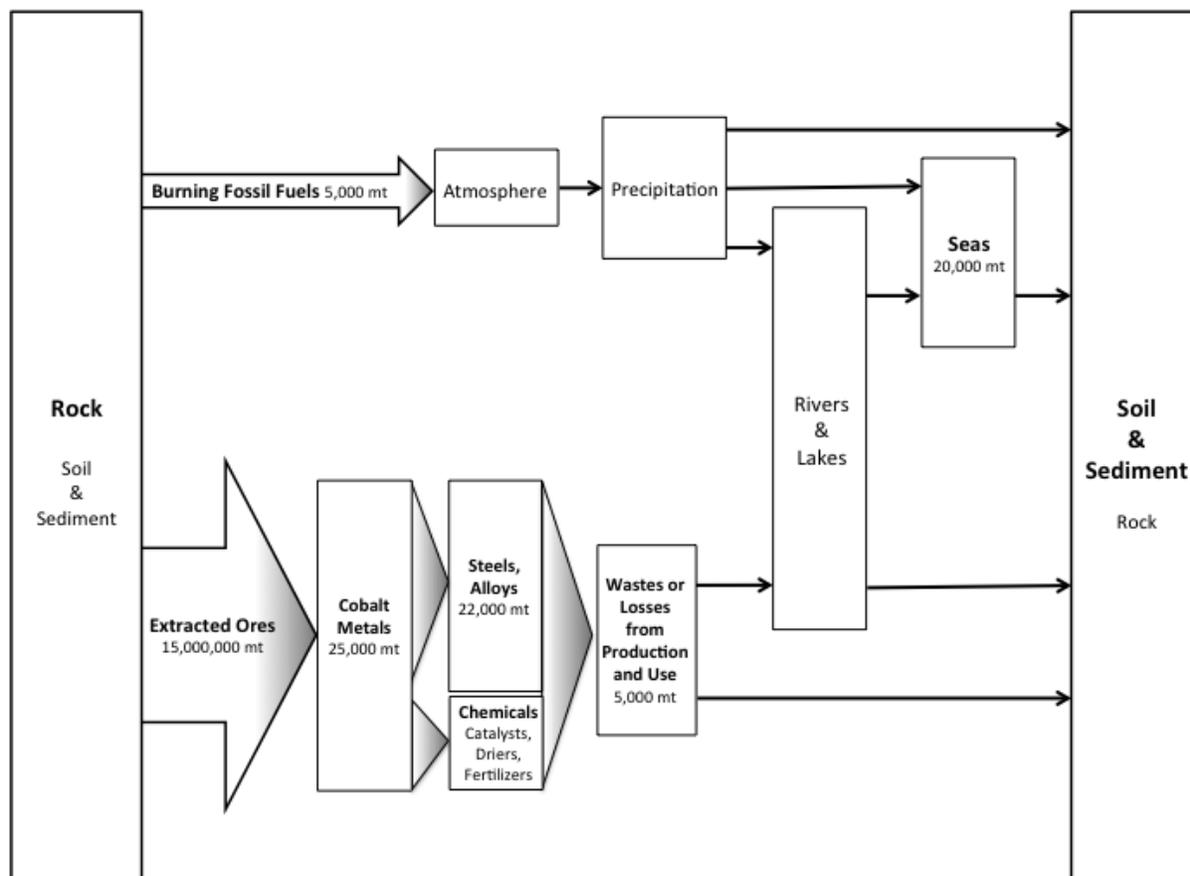


Figure 2-3. Flow of cobalt released from anthropogenic processes

Adapted from Shedd 1993, CDI 2006.

### 2.8.2 Occurrence

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

Median cobalt concentration in U.S. drinking water has been reported to be  $< 2.0 \text{ } \mu\text{g/L}$ ; however, levels as high as  $107 \text{ } \mu\text{g/L}$  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  $4 \text{ } \mu\text{g/L}$  in seawater and from  $0.1$  to  $10 \text{ } \mu\text{g/L}$  in freshwater and groundwater (IARC 2006).

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

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

### 2.8.3 Exposure

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

## 2.9 Summary and synthesis

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

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

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

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

implants and occupational exposures but higher than those reported for exposures from the environment or in the general public.

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

### 3 Disposition and Toxicokinetics

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

#### 3.1 Disposition

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

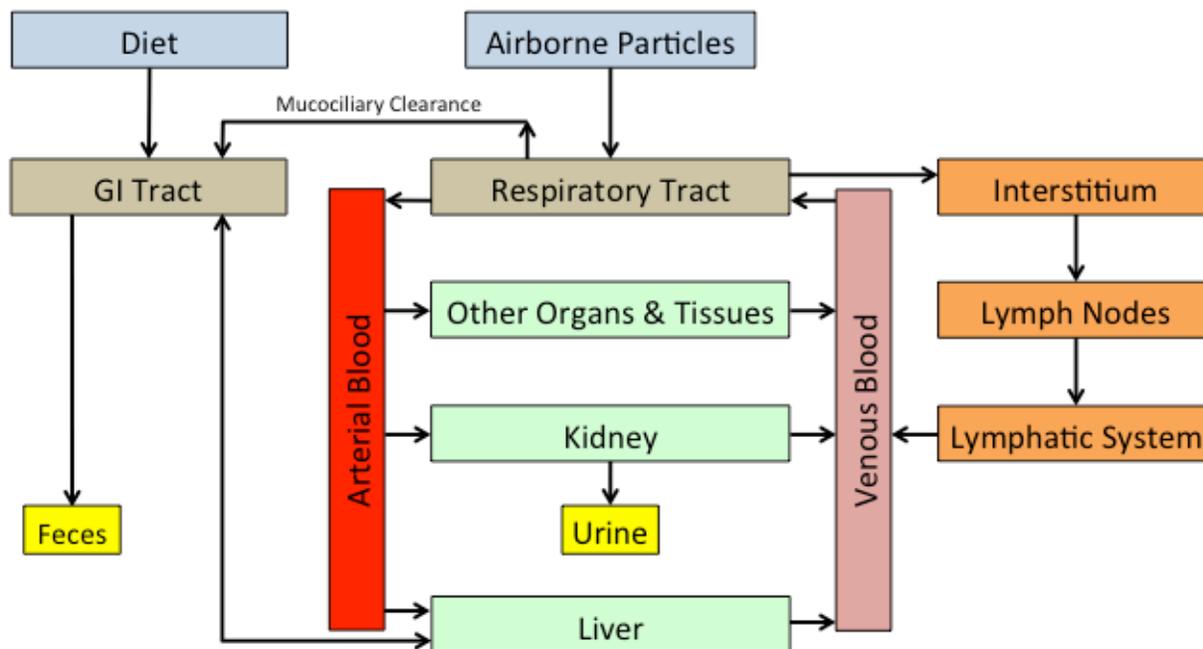


Figure 3-1. Cobalt disposition

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

#### 3.1.1 Humans

Dietary intake of cobalt has been reported as the largest source of exposure for the general population; an average daily intake of cobalt in Canada was reported as 11  $\mu\text{g}/\text{day}$  (ATSDR

2004) (see Section 2.7). Most of the cobalt in the diet is inorganic with a very small fraction from vitamin B<sub>12</sub> (Lison 2015). The normal range of cobalt concentrations (nonoccupational exposure) in the blood and urine are about 0.1 to 0.5 µg/L and < 2 µg/L, respectively (IARC 2006, Paustenbach *et al.* 2013) (see Section 2). About 90% to 95% of cobalt in blood is bound to serum albumin while the concentration of free cobalt is about 5% to 12% of the total cobalt concentration (Simonsen *et al.* 2012, Paustenbach *et al.* 2013). Letourneau *et al.* (1972) showed that a dose of vitamin B<sub>12</sub> 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<sub>12</sub> organometallic complex (WHO 2006, Paustenbach *et al.* 2013).

### Absorption

Cobalt absorption from the gastrointestinal (GI) tract is highly variable, with reported values ranging from < 5% to 97% (Smith *et al.* 1972, IARC 2006, WHO 2006, Paustenbach *et al.* 2013, NTP 2014b, Holstein *et al.* 2015). Unice *et al.* (2012) suggested a central tendency value of 25% for GI absorption of soluble inorganic cobalt while Unice *et al.* (2014) assumed GI absorption of 20% to 45% for aqueous forms and 10% to 25% for solid forms. Cobalt concentrations in whole blood increased 9 to 36 times above normal background concentrations in volunteers who ingested a liquid dietary supplement that contained cobalt chloride for up to 16 days (Tvermoes *et al.* 2013). Soluble cobalt compounds are better absorbed than insoluble forms (Christensen *et al.* 1993, Christensen and Poulsen 1994). For example, men and women volunteers who ingested tablets containing soluble cobalt chloride (CoCl<sub>2</sub>) 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<sub>3</sub>O<sub>4</sub>) (Christensen *et al.* 1993). Controlled studies in human volunteers also indicate that GI uptake is higher in women than in men with adjusted mean whole blood concentrations about two-fold higher in women (Christensen *et al.* 1993, Finley *et al.* 2013). The higher cobalt uptake in women may be due to a higher incidence of iron deficiency since cobalt absorption efficiency is higher in individuals with iron deficiency (31% to 71% compared to 18% to 44% in control subjects) (Valberg *et al.* 1969, Sorbie *et al.* 1971). Meltzer *et al.* (2010) reported that cobalt whole blood concentrations were significantly elevated in women with low serum ferritin concentrations compared to women with higher serum ferritin concentrations and in women with mild to moderate anemia compared to women with only slightly reduced hemoglobin. Low iron status was a prerequisite for high blood concentrations of cobalt; however, not everyone with low iron status had increased blood levels of cobalt. These data suggest that cobalt and iron may share a common gastrointestinal uptake mechanism that may be upregulated with anemia or iron deficiency (Paustenbach *et al.* 2013). Other nutritional factors may affect cobalt absorption due to the formation of complexes with certain organic anions (e.g., amino acids) present in foods.

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

impaction. These particles are readily cleared through mucociliary action and swallowed. Smaller particles escape inertial impaction and deposit in the bronchiolar or alveolar regions via sedimentation and diffusion. Particles deposited in the respiratory tract may dissolve and be absorbed into the blood or undergo phagocytosis or endocytosis by macrophages. In addition, some nanoparticles can translocate rapidly from the lungs to the mediastinal lymph nodes and bloodstream (Luyts *et al.* 2013). Recent *in vitro* studies with human lung cells show that water-insoluble cobalt oxide particles (CoO or Co<sub>3</sub>O<sub>4</sub>) 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 (Papis *et al.* 2009, Ortega *et al.* 2014, Smith *et al.* 2014, Sabbioni *et al.* 2014a). Controlled aerosol studies using human volunteers show that about half of the initial lung burden of inhaled cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) 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.* 2004, Larese Filon *et al.* 2007, Larese Filon *et al.* 2009). The mean permeation flux was 0.0123 µg/cm<sup>2</sup>/hr, the lag time was 1.55 hr, and the permeation coefficient was 0.00037 cm/hr. Median cobalt concentrations in the receiving phase indicated that significantly more (~400 fold) cobalt penetrated damaged skin compared with intact skin (Larese Filon *et al.* 2009). Cobalt was detected in its ionic form in both the donor and the receiving phase. Significant amounts of cobalt also remained within the skin. These experiments showed that skin absorption was closely related to the capacity of synthetic sweat to oxidize metallic cobalt powder to soluble cobalt ions. No significant dermal absorption occurred when cobalt was dispersed in a saline solution (Larese Filon *et al.* 2004).

### **Distribution and excretion**

Numerous studies have shown that cobalt is found in blood, urine, hair, nails, and most other tissues. (See (1) Section 2.3, Figure 2-1, Figure 2-2, or Appendix B, Table B-2 to B-3 for studies of cobalt levels in blood, urine, hair, and nails in specific exposed groups and the general population and (2) Appendix B, Table B-4 for cobalt levels reported in surrogate (hair and nails) or target tissues from cancer patients and referent groups, e.g., patients with other non-cancer diseases or healthy controls reported in several clinical studies.) In humans, inorganic cobalt is distributed to liver, kidney, heart, and spleen with lower concentrations found in bone, hair, lymph, brain, and pancreas (WHO 2006, Paustenbach *et al.* 2013). Cobalt chloride administered intravenously (i.v.) or orally to human volunteers was distributed primarily to the liver (Smith *et al.* 1972, Jansen *et al.* 1996). 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 (Wappelhorst *et al.* 2002, Rudge *et al.* 2009). Most of the cobalt in plasma is bound to leukocytes or plasma proteins with a maximum free fraction of 12%. Free cobalt is also taken up by red blood cells via a membrane transport pathway shared with calcium (Simonsen *et al.* 2011, Simonsen *et al.* 2012). Uptake of cobalt by red blood cells is practically irreversible because the ions bind to hemoglobin and are not extruded by the calcium pump. Thus, it has been speculated that cobalt partitions primarily into tissues with high calcium turnover and accumulates in tissues with slow turnover of cells although cobalt can be detected in most tissues. Although elevated concentrations of cobalt have been reported in the liver and kidney (oral or parental exposure) or lung (inhalation of insoluble particles), cobalt levels in the body do not appear to increase in any specific organ with age (IARC 2006, Paustenbach *et al.* 2013, Lison 2015).

Renal excretion of absorbed cobalt is rapid over the first days following exposure but is followed by a second, slower phase that lasts several weeks (IARC 2006, Simonsen *et al.* 2012). However, a small proportion (~10%) is retained in the tissues with a biological half-life of 2 to 15 years. Controlled experimental studies in humans indicate that 3% to 99% of an orally administered dose of cobalt is excreted in the feces and primarily represents unabsorbed cobalt (WHO 2006). Fecal elimination decreases as cobalt solubility increases. Following i.v. administration of cobalt chloride to 6 volunteers, fecal elimination accounted for about 2% to 12% of the administered dose while about 28% to 56% was eliminated in the urine after 8 days (Smith *et al.* 1972). Valberg *et al.* (1969) reported similar results in subjects administered cobalt by intramuscular injections and followed for 10 days (~6% excreted in feces and 58% in urine). Solubility and particle size affect elimination following inhalation exposure (WHO 2006). Clearance of cobalt particles from the lungs has been reported to follow three-phase kinetics (see Section 3.2.1). Large particles are rapidly cleared from the upper airways via the mucociliary pathway, swallowed, and eliminated in the feces. Urinary excretion of inhaled cobalt particles increases with time. Foster *et al.* (1989) reported that following inhalation of cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) particles, about 17% was cleared mechanically to the gastrointestinal tract and eliminated in the feces within the first week. After 6 months, about 33% of the initial lung burden was eliminated in the urine and about 28% was eliminated in the feces.

### 3.1.2 Experimental animals

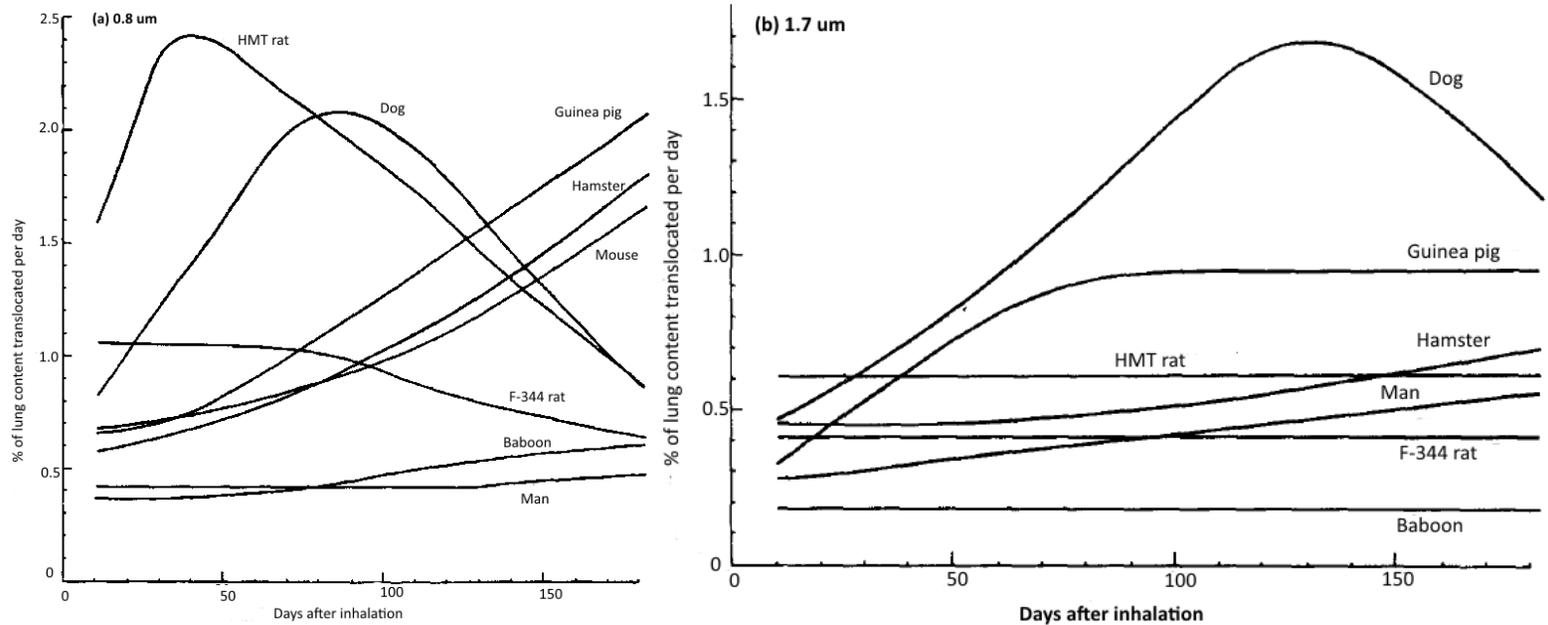
The disposition of cobalt has been investigated in mice, rats, hamsters, guinea pigs, rabbits, dogs, miniature swine, and baboons and show some similarities with human studies. These data are briefly reviewed below. As in humans, cobalt as part of vitamin B<sub>12</sub> is an essential micronutrient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

#### Absorption

Cobalt absorption in experimental animals is highly variable and depends on the chemical form of the compound, age of the animal, species, and nutritional status (Ayala-Fierro *et al.* 1999, WHO 2006, NTP 2014b). In rats, cobalt chloride was absorbed more efficiently from the gastrointestinal tract than insoluble cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) (13% to 34% compared to 1% to 3%)

(NTP 2014b). Gastrointestinal absorption of soluble cobalt compounds was lower in cows (1% to 2%) and guinea pigs (4% to 5%) compared with rats. Cobalt absorption was 3% to 15% greater in young rats and guinea pigs than in adults (Naylor and Harrison 1995). As observed in humans, cobalt absorption was increased in iron-deficient rats (Thomson *et al.* 1971).

Inhalation studies of cobalt metal, cobalt oxides, or soluble cobalt salts in experimental animals show that dissolved cobalt is absorbed rapidly from the lungs while a small percentage is absorbed over several months (IARC 1991, Kyono *et al.* 1992, NTP 1998, IARC 2006, Leggett 2008, NTP 2014b). 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<sub>3</sub>O<sub>4</sub>) translocated to blood per day (i.e., dissolution of particles and absorption into the blood) varied according to particle size, particle surface area, species, and time (Andre *et al.* 1989, Bailey *et al.* 1989, Collier *et al.* 1989, Patrick *et al.* 1989, Kreyling *et al.* 1991a). Initially, translocation of the smaller particles (0.8 μm) ranged from about 0.4%/day in baboons to about 1.4%/day in HMT (inbred strain) rats. Initial translocation rates for the larger particles (1.7 μm) were lower in all species and ranged from about 0.2%/day in baboons to 0.6%/day in HMT rats (Bailey *et al.* 1989). Translocation rates for higher density Co<sub>3</sub>O<sub>4</sub> particles were about a factor of 3 slower than for less dense particles (Bailey *et al.* 1989, Kreyling *et al.* 1991a). Translocation rates reported by Bailey *et al.* (1989) showed a variety of different forms with time, particularly for the smaller particles; this is discussed further in the following section (Figure 3-2). Translocation of cobalt from the lung to the blood also was significantly faster in younger rats compared with older rats (Collier *et al.* 1991).



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

Source: Bailey *et al.* 1989. Used with permission.

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

### Distribution and excretion

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

Distribution of cobalt following inhalation exposure is similar to that observed for other routes with the exception of greater retention in the lung for both soluble and insoluble cobalt (Wehner and Craig 1972, Kerfoot *et al.* 1975, Kreyling *et al.* 1986, Bailey *et al.* 1989, Patrick *et al.* 1989, Bucher *et al.* 1990, Collier *et al.* 1991, Kyono *et al.* 1992, Patrick *et al.* 1994, NTP 2014b). Long-term retention of insoluble cobalt particles and soluble cobalt salts deposited in the lung shows wide interspecies variation and represents a potential continuing source of cobalt ion release (Bailey *et al.* 1989, Kreyling *et al.* 1991a, Patrick *et al.* 1994). In addition, some particles can translocate to the pulmonary interstitium where they are cleared from the lungs through the lymphatic system (Pauluhn 2009). Nanoparticles also may penetrate the alveolar membrane and distribute to extrapulmonary tissues via the circulation (Mo *et al.* 2008). The average size of the long-term retention component in humans is greater than in experimental animals (Bailey *et al.* 1989, Leggett 2008). Retention of insoluble cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) 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<sub>3</sub>O<sub>4</sub>)**

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

Source: Bailey *et al.* 1989.

ND = no data.

<sup>a</sup>Calculated as the fraction of lung content (measured as activity of <sup>57</sup>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<sub>3</sub>O<sub>4</sub> 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<sub>3</sub>O<sub>4</sub> 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  $\text{Co}_3\text{O}_4$  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  $\text{Co}(\text{NO}_3)_2$  in the same species (Bailey *et al.* 1989, Patrick *et al.* 1994). These data suggest that interspecies differences in the time-dependent absorption rates (i.e., translocation of dissolved cobalt from the lung to the blood) for inhaled  $\text{Co}_3\text{O}_4$  particles were not explained by differences in the fraction of dissolved cobalt retained long-term in lung tissue. Kreyling *et al.* (1991b) also found little interspecies variation in pH within alveolar macrophages; therefore, interspecies differences in translocation rates were not explained by differences in phagolysosomal pH. Alternative explanations for these interspecies differences could include a second long-term phase of lung retention as particles or as particle fragments (Patrick *et al.* 1994).

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

Cobalt excretion occurs rapidly with the majority of the administered dose eliminated within hours to a few days after exposure ceases (Gregus and Klaassen 1986, Paustenbach *et al.* 2013). Cobalt is excreted in the urine, feces, and bile with similar excretion patterns reported for all species studied (ATSDR 2004, WHO 2006, NTP 2014b). 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%) (Gregus and Klaassen 1986, Ayala-Fierro *et al.* 1999). Soluble cobalt compounds are cleared from the lungs at a faster rate than less soluble compounds. The rate of urinary excretion correlates with the rate of translocation of cobalt from the lungs to the blood while fecal excretion rates correlate with the rate of mechanical clearance

of cobalt particles from the lung (ATSDR 2004, WHO 2006). Following oral exposure, cobalt is primarily excreted in the feces but the rate decreases as cobalt particle solubility increases (WHO 2006). However, species and sex differences in cobalt excretion rates have been reported. Cobalt urinary excretion rates ( $\mu\text{g}/16 \text{ hr}$ ) 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  $\text{CoCl}_2$  solution to the lungs or inhaled as an aerosol) ranged from 0.002% of the initial body content per day in HMT rats to 0.026% per day in dogs (Patrick *et al.* 1994). Mean daily fecal excretion rates ranged from 0.0009% (dog) to 0.004% (HMT rat).

### 3.2 Toxicokinetics

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

#### 3.2.1 Humans

The kinetics of inhaled cobalt are determined by mechanical (mucociliary) clearance and by translocation to blood and the lymphatic system (Figure 3-1) (ATSDR 2004). Foster *et al.* (1989) calculated average translocation and mechanical clearance rates of inhaled cobalt oxide ( $\text{Co}_3\text{O}_4$ ) 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  $\mu\text{m}$ . Inhalation studies in workers and volunteers exposed to cobalt have shown that the elimination of poorly soluble cobalt metal or cobalt oxides ( $\text{CoO}$  or  $\text{Co}_3\text{O}_4$ ) from the lungs is multiphasic with reported half-lives for the phases of 2 to 44 hours, 10 to 78 days, and years (Newton and Rundo 1971, Apostoli *et al.* 1994, Beleznyay and Osvay 1994, Mosconi *et al.* 1994a, WHO 2006, NTP 2014b). The elimination pattern was independent of the degree of exposure. About 17% of the initial lung burden was eliminated within the first week while about 40% was retained at 6 months after exposure (Foster *et al.* 1989, WHO 2006). These elimination phases likely involve mucociliary clearance of cobalt particles from the tracheobronchial region, macrophage-mediated clearance of cobalt particles from the lungs, and long-term retention and clearance from the lung. The slower clearance with time likely reflects cobalt that is bound to cellular components in the lung (Kreyling *et al.* 1986, Foster *et al.* 1989, ATSDR 2004, WHO 2006). Studies in human volunteers administered cobalt chloride by i.v. injection also showed a multiphasic elimination pattern (Letourneau *et al.* 1972, Smith *et al.* 1972, Jansen *et al.* 1996, Holstein *et al.* 2015). These studies showed that 36% to 44% of the administered dose is cleared with a biological half-life of 6 to 12 hours, 45% to 56% is cleared with a biological half-life of 2 days to 60 days, and 9% to 11% is cleared with a biological half-life of 600 to 800 days (Paustenbach *et al.* 2013). Jansen *et al.* (1996) reported an apparent volume of distribution at steady state of 48 L that likely reflected 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 human studies. Unice *et al.* (2012, 2014) further refined this model by incorporating different gastrointestinal absorption rates, adding compartments to account for albumin-bound cobalt in intravascular and extravascular fluid, and accounting for additional parameters such as total blood volume, red blood cell age, and urinary excretion rates. The model was a reasonably good predictor of cobalt blood and urine concentrations measured in male and female volunteers who ingested a cobalt supplement for 16 days to 3 months (Tvermoes *et al.* 2013, Tvermoes *et al.* 2014, Unice *et al.* 2014).

### 3.2.2 Experimental animals

Lung clearance kinetics of cobalt particles include both mechanical transport and translocation (Bailey *et al.* 1989, Kreyling *et al.* 1991a). Lung clearance of inhaled cobalt metal particles in rats and mice showed a well-defined two-phase elimination profile following 3-month or 2-year studies (NTP 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  $^{60}\text{CoCl}_2$  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**

Reference	Species: exposure route	Compound(s)	Elimination T <sub>1/2</sub>		
			Phase 1	Phase 2	Phase 3
Ayala-Fierro <i>et al.</i> 1999	Male F344 rats: i.v.	CoCl <sub>2</sub>	1.3 hr	4.3 hr	19 hr
Ayala-Fierro <i>et al.</i> 1999	Male F344 rats: oral	CoCl <sub>2</sub>	0.9 <sup>a</sup> hr	4.6 hr	22.9 hr
Menzel <i>et al.</i> 1989	Male SD rats: inhalation	CoCl <sub>2</sub>	1.8 hr	3.7–8.7 <sup>b</sup> hr	–
Kyono <i>et al.</i> 1992	Male SD rats: inhalation	Co metal	52.8 <sup>c</sup> hr 52.8 <sup>d</sup> hr	156 <sup>c</sup> hr 172.8 <sup>d</sup> hr	–
Kreyling <i>et al.</i> 1986	Male beagles: inhalation (endotracheal tube)	Co <sub>3</sub> O <sub>4</sub> Co <sub>3</sub> O <sub>4</sub> + CoO Co(NO <sub>3</sub> ) <sub>2</sub>	0.5 d 1–4 d 0.8 d	6–80 <sup>e</sup> d 20–86 <sup>e</sup> d 27 d	300–380 d 340–440 d 400 d

– = No data.

<sup>a</sup>Absorption half-life.

<sup>b</sup>Calculated from elimination rate constants of 0.188 h<sup>-1</sup> (single exposure) and 0.08 h<sup>-1</sup> (repeat exposure).

<sup>c</sup>Lung.

<sup>d</sup>Blood.

<sup>e</sup>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.

**This Page Intentionally Left Blank**

## 4 Human Cancer Studies

### Introduction

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

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

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

1. Selection of the relevant literature included in the cancer evaluation (Section 4.1 and Cobalt Protocol [NTP 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. Level of evidence conclusion for carcinogenicity (sufficient, limited, or inadequate) of cobalt from human studies (Section 4.5).

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

#### 4.1 Selection of the relevant literature

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

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

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

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

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

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

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

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

## 4.2 Cohort studies and nested case-control studies reporting on lung cancer

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

### 4.2.1 Overview of the methodologies and study characteristics

For each of the reviewed cohort studies, detailed data on study design, methods, and findings were systematically extracted from relevant publications, as described in the study protocol, and into Table 4-1, Tables C-1a-g in [Appendix C](#), and Table 4-2 in Section 4.2.2.

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

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

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

Two publications reported on overlapping populations of hard-metal workers. The first was a historical mortality cohort and nested case-control study of lung cancer among 7,459 workers at 10 hard-metal producing factories in France (Moulin *et al.* 1998) where activities also included powder metallurgy processes. The second was a sub-study of lung cancer among 2,860 workers in the largest hard-metal producing factory in France (the factory was included in the Moulin *et al.* [1998] study, with an additional year of follow-up included) which also produced magnets and stainless steel with cobalt, and cobalt powders by calcination and reduction of cobalt hydroxide (Wild *et al.* 2000). This study also provided complete job histories.

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

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

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

The description of study methods and characteristics of each study is included in 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 <i>et al.</i> 1996	<b>Danish porcelain painters</b> 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 <i>et al.</i> 1987 Moulin <i>et al.</i> 1993 (follow-up)	<b>French electrochemical workers</b> <i>Mur et al. 1987</i> 1950–1980 N = 1,143 males <i>Moulin et al. 1993</i> 1950–1988	Historical mortality cohort study (SMR) and nested case-control analysis (OR) <i>Mur et al. 1987</i> ICD-8: All causes; trachea, bronchus, and lung (162); buccal cavity/pharynx/larynx (140–149, 161) <i>Moulin et al. 1993</i>	Production of cobalt, cobalt salts and oxides. Company records classified workers exclusively employed in one of four work groups including cobalt production workshop <i>Mur et al. 1987</i> Cohort analysis: Only or never employed in cobalt production Nested case-control analysis:

Reference	Population	Design and outcome (cancer sites)	Exposure: Cobalt compounds, assessment, metrics
	N = 1,148 Number of cobalt production workers NR	ICD-8: All causes; bronchus, lung (162); brain (191)	Ever/never employed in cobalt production <i>Moulin et al. 1993</i> Mortality SMR analysis Only vs. never employed in cobalt production
<i>Moulin et al. 1998</i> (multi-plant)	<b>French hard-metal workers</b> <i>Moulin et al. 1998</i>	Nested case-control analysis (OR) and historical mortality cohort study (SMR)	Production of magnets, stainless steel, and cobalt powders “Other” cobalt exposure may have included metallic and ionized cobalt
<i>Wild et al. 2000</i> (sub-study of largest plant)	1945–1991 N = 7,459 men and women; 68 cases and 180 controls <i>Wild et al. 2000</i> 1950–1992 N = 2,860 men and women Number of workers employed in cobalt production only NR	<i>Moulin et al. 1998</i> ICD-8: Lung (162) <i>Wild et al. 2000</i> ICD-8: Lung (162)	Semi-quantitative JEM <i>Moulin et al. 1998</i> Duration, intensity and cumulative exposure <i>Wild et al. 2000</i> Ever exposed
<i>Moulin et al. 2000</i>	<b>French stainless and alloyed steel worker cohort</b> 1968–1991 N = 4,897; 54 cases and 160 controls	Nested case-control analysis (OR) within a historical mortality cohort study ICD-8: Lung (162)	Steel production and casting of stainless steel, nickel, ferro-chromium, and other ferroalloys in which iron, chromium, nickel, and cobalt compounds are used Powder manufacture of metallic powders Semi-quantitative JEM Duration, intensity, and cumulative exposure
<i>Grimsrud et al. 2005</i> methods described in <i>Grimsrud et al. 2002</i> , <i>Grimsrud et al. 2003</i>	<b>Nickel refinery worker cohort</b> 1952–1995 N = 5,389; 213 cases and 524 controls	Nested case-control analysis (OR) within an incidence cohort study; Norwegian Cancer Registry ICD NR: Lung	Cobalt present in raw materials and intermediates in refinery and produced electrolytically in an electrowinning process Breathing zone personal samples for cobalt and nickel JEM Quantitative cumulative exposure

#### 4.2.2 Study quality and utility evaluation

This section provides an overview of the adequacy of the cohort and nested case-control studies to inform the cancer hazard evaluation (see [Appendix C](#) for details on the assessment). This assessment considers factors related to study quality (potential for selection and attrition bias, information bias regarding exposure and outcome, and concern for inadequate analytical methods, selected reporting, and inadequate methods or information to evaluate confounding)

and study sensitivity (e.g., such as adequate numbers of individuals exposed to substantial levels of cobalt). The ratings for each of these factors are provided in Table 4-2 and a detailed description of the rationale for the rating is provided in [Appendix C](#).

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

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

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

**Table 4-2. Bias and quality summary for cohort and nested case-control studies**

Citation	Bias						Quality <sup>a</sup>	Utility <sup>b</sup>
	Selection	Exposure	Outcome	Confounding methods	Adequacy of analysis	Selective reporting	Sensitivity	Integration
<b>Porcelain painters</b> Tüchsen <i>et al.</i> 1996	++	++	+++	++	++	+++	+	++
<b>Electrochemical workers</b> Moulin <i>et al.</i> 1993 with Mur <i>et al.</i> 1987	++	++	++	+	+++	+++	+	+
<b>Hard-metal workers</b> Moulin <i>et al.</i> 1998	++	++/+++	+++	+	+++	+++	++	++
Wild <i>et al.</i> 2000	++	++/+++	+++	+	++	+++	++	++
<b>Stainless and alloyed steel workers</b> Moulin <i>et al.</i> 2000	+++	++	+++	+	+++	+++	++	++
<b>Nickel refinery workers</b> Grimsrud <i>et al.</i> 2005	+++	+++	+++	++	+++	+++	+++	+++

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

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

### 4.2.3 Cancer assessment: Lung

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

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

### Background information

Lung cancer is the third most common cancer in the United States, making up 13.5% of all new cancers. The age-adjusted annual lung cancer rates (including trachea and bronchus) (per 100,000 males or females) in the United States from 2007 to 2011 (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 from the individual studies are discussed below and presented in Table 4-3. The available cohort and nested case-control studies of cobalt and lung cancer include a cohort of Danish female porcelain painters, a cohort of French electrochemical workers, a French multi-centric cohort of hard-metal factory workers, a related cohort of workers from the largest factory in the multi-centric French hard-metal factory cohort, a cohort of French stainless and alloyed steel workers, and a cohort of Norwegian nickel-refinery workers.

Table 4-3. Evidence from cohort and case-control studies on lung cancer and exposure to cobalt

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

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Co-variables controlled	Comments, strengths, and weaknesses	
Mur <i>et al.</i> 1987 Nested case-control France 1950–1980	Electrochemical plant workers Cases: 9; controls: 18 Exposure assessment method: company records	<b>Lung (162)</b>				None	<p><b>Confounding:</b> Cases (deaths from lung cancer) were matched to controls (deaths from cause other than cancer) for year of birth, age at death, and smoking habits; smoking data on only 30% of the cohort; co-exposures to nickel and arsenic were not controlled.</p> <p><b>Strengths:</b> Nested design reduces concern of potential confounding from life style factors</p> <p><b>Limitations:</b> Small numbers with limited information on exposures (only ever/never employment in cobalt production department); also, 46% of the cohort was hired prior to the start of follow-up which could induce a downward bias in the effect estimate due to over-prevalence of healthier workers (left-truncation).</p>
		Ever worked in cobalt production	4	OR [4.0 (0.66–24.4)]			
Moulin <i>et al.</i> 1993 Cohort France Extended follow-up of the Mur <i>et al.</i> 1987 study through 1988	Electrochemical workers Cohort I: N = 1,148; Cohort II: N = 870; number of cobalt workers NR Exposure assessment method: company records	<b>Lung (162)</b>				Age	<p><b>Confounding:</b> No reported control for period effects, duration, or time since first exposure; no consideration of smoking; potential co-exposures to nickel and arsenic from its presence in cobalt ore not controlled.</p> <p><b>Strengths:</b> Cobalt production workers exposed primarily to cobalt compounds.</p> <p><b>Limitations:</b> Small number of exposed cases in overall or sub-cohort; low power to detect an effect; concern about outcome misclassification; potential for selection bias due to left-truncation</p>
		Exclusive employment in cobalt production, Cohort I	3	OR 0.85 (0.18–2.5)			
		Exclusive employment in 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-variables controlled	Comments, strengths, and weaknesses
		Cohort I				
		Ever worked in cobalt production, Cohort II	4	1.18 (0.32–3.03)		
Moulin <i>et al.</i> 1998 Nested case-control France 1968–1991	Workers in all 10 hard-metal factories in France Cases: 61; controls: 180 Exposure assessment method: JEM	<b>Lung (162)</b>				No information on actual exposure level or average exposure duration for the cohort. <b>Confounding:</b> Potential concern for exposure to other lung carcinogens, which were not controlled in the cobalt alone analyses. <b>Strengths:</b> Exposure-response analyses with multiple exposure metrics; JEM validated for atmospheric concentrations of cobalt; incident cohort reducing the potential for left truncation; internal analysis reducing the impact of the reported HWE; and lagged analysis. <b>Limitations:</b> 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)	Age		
	Exposure intensity trend	15	2.05 (0.94–4.45)			
	Exposure duration	15	2.2 (0.99–4.87)			
	Unweighted cumulative exposure	15	1.83 (0.86–3.91)			
	Frequency weighted cumulative exposure trend	15	2.03 (0.94–4.39)			
Wild <i>et al.</i> 2000 Cohort France 1968–1992	Hard-metal workers - Largest plant in France 2,216 men and 644 women Exposure assessment method: JEM	<b>Lung (162)</b>				No information on actual exposure level or average exposure duration for the cohort <b>Confounding:</b> Potential exposure to lung carcinogens which were not controlled in cobalt-only analyses. <b>Strengths:</b> Incident cohort; lagged analysis. <b>Limitations:</b> External analysis only presented; no exposure metrics except for
	Cobalt except in hard metals	15	SMR 1.95 (1.09–3.22)	Age, sex		

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Co-variables controlled	Comments, strengths, and weaknesses	
						ever/never provided.	
Moulin <i>et al.</i> 2000 Nested case-control France 1968–1992	Stainless and alloyed steel workers Cases: 54 (17 cobalt exposed); controls: 162 (67 cobalt exposed) Exposure assessment method: JEM	<b>Lung (162)</b>					No information on actual exposure level or average exposure duration for the cohort <b>Confounding:</b> Potential confounding from exposure to chromium and/or nickel, and iron; controlled for smoking <b>Strengths:</b> Semi-quantitative JEM; exposure metrics including duration and cumulative dose, frequency weighted and unweighted provided; HWE mitigated by use of internal analyses. <b>Limitations:</b> Known carcinogens had non-significant ORs < 1.0, indicating that the study had low sensitivity to detect an effect.
		Exposed, Crude	17	OR 0.64 (0.33–1.25)			
		Exposed, known smoking status, Crude	12	0.62 (0.26–1.46)			
		Exposed, known smoking status, smoking adjusted	12	0.43 (0.16–1.14)			
		Exposed, known smoking status, PAH, silica, and smoking adjusted	12	0.44 (0.17–1.16)			
Grimsrud <i>et al.</i> 2005 Nested case-control Norway 1910–1995	Nickel refinery workers Cases: 213; controls: 525 Exposure assessment method: JEM	<b>Lung</b>				Smoking	<b>Exposure levels</b> ( $\mu\text{g}/\text{m}^3$ ): high (144–3,100); medium (29.7–142); low (0.31–29.5). <b>Confounding:</b> No multivariate estimates for the categorical variable (low, high, medium exposures) were possible due to collinearity with nickel. Continuous rise in OR controlled for smoking and co-exposures. <b>Strengths:</b> Quantitative cobalt levels
		Rise in OR per $\text{mg}/\text{m}^3 \times \text{years}$ , smoking adjusted	NR	OR 1.3 (0.9–1.8)			
		Low (0.31–29.5 $\mu\text{g}/\text{m}^3 \times \text{years}$ )	49	1.5 (0.6–3.8)			

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Co-variables controlled	Comments, strengths, and weaknesses	
		Med (29.7–142 $\mu\text{g}/\text{m}^3 \times$ years)	73	2.4 (1–5.6)			
		High (144–3,100 $\mu\text{g}/\text{m}^3 \times$ years)	82	2.9 (1.2–6.8)			
		<b>Lung</b>					
		Rise in OR per $\text{mg}/\text{m}^3 \times$ years, smoking and co-exposure adjusted	NR	0.7 (0.3–1.4)	Smoking, nickel, sulfuric acid mists, asbestos, arsenic		
		<b>Lung</b>					
		Cobalt electrolysis workshop, 0.03–2.2 yr	23	1.6 (0.8–3)			
		Cobalt electrolysis workshop, 2.3–11.8 yr	44	2.8 (1.5–5)			

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

*Porcelain painters*

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

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

An elevated lung cancer SIR, similar in magnitude to that reported in the exposed group, was also observed in the referents; a comparison of the exposed departments with the reference department gave a relative risk ratio of 1.2 (95% CI = 0.4 to 3.8). The referents were reported to be top glaze decorators employed in a department without cobalt exposure. Data from a previous publication in this factory (Raffn *et al.* 1988) indicated an overlap of cobalt levels in referents and exposed individuals, suggesting that the referents in the Tüchsen *et al.* 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 (Raffn *et al.* 1988, Prescott *et al.* 1992). Based on a calculation of the weighted average of exposed and unexposed respondents from both studies taken over the total sample size of the two studies, and disregarding the specific cobalt compound to which workers were exposed, the smoking rate is calculated to be 52% for exposed and 38% for referent women. The rate of smoking among exposed women is close to that of skilled Danish women taken in 1982 (47%) and 1987 (55%); and the rate of smoking in the referent group is similar to, but lower than, the rate in the general population of Danish women (43% and 42% in these two years). This suggests that there may be a non-smoking cause for the increased rate of lung cancer in the referent population, which might be due either to misclassification of cobalt exposure, or to another unmeasured confounder. It is also possible that cobalt-exposed workers are also exposed to the same unmeasured confounder, although data from the substudy indicates that levels of silica, nickel, and dust were very low based on air monitoring done in 1981 (Raffn *et al.* 1988). The porcelain painters cohort provides inconclusive evidence for a carcinogenic effect of cobalt and lung cancer because of the finding of similarly elevated levels of lung cancer among the referents.

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

### *Electrochemical workers*

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

However, in an extension of the follow-up of the same cohort (Moulin *et al.* 1993) the SMR for lung cancer among French-born workers exclusively employed in cobalt production was 1.16, (95% CI = 0.24 to 3.40), based on 3 observed deaths. (Confidence in the SMR for the entire cohort is lower because of high loss to follow-up and strong healthy worker effect due to 24% foreign-born workers). In addition, Moulin *et al.* 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 reclassified 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 reported matching cases and controls on smoking status; however, no explanation was given regarding the methods of matching given the small percentage of workers with information on smoking status. Moulin *et al.* did not address smoking in the analysis, but reported no excess of mortality from circulatory and respiratory diseases, suggesting that smoking is unlikely to be higher in this cohort than in the local French referent population.

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

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

reducing concerns about confounding from the HWE, however, selection bias due to left-truncation remains a concern.

#### *French hard-metal worker cohorts*

The populations included in the two studies of cobalt exposure and lung cancer among hard-metal workers overlap, and both studies report either statistically significant elevated risks, or borderline statistically significant risks, of lung cancer among those exposed to cobalt without tungsten carbide. Moulin *et al.* (1998) first reported results from the multi-center study of 10 hard-metal factories in France. In the internal nested case-control analysis (Moulin *et al.* 1998), based on 15 exposed cases, a borderline statistically significant increased risk of lung cancer was associated with exposure to “cobalt alone or simultaneously with agents other than tungsten carbide” (levels 2 to 9) compared with little or no exposure (levels 0 or 1) (OR = 2.21, 95% CI = 0.99 to 4.90). Regarding the presence of an exposure-response relationship, Moulin *et al.* 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.* 2000). 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 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.* (2000) 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 exposures were not included in the cobalt model. However, these exposures were also not associated with lung cancer risk in these analyses.

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

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

*Norwegian nickel refinery workers*

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

fully adjusted model for this cobalt variable (including smoking as well as all co-exposures) could not be calculated due to collinearity.

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

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

### **Integration of evidence across studies**

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

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

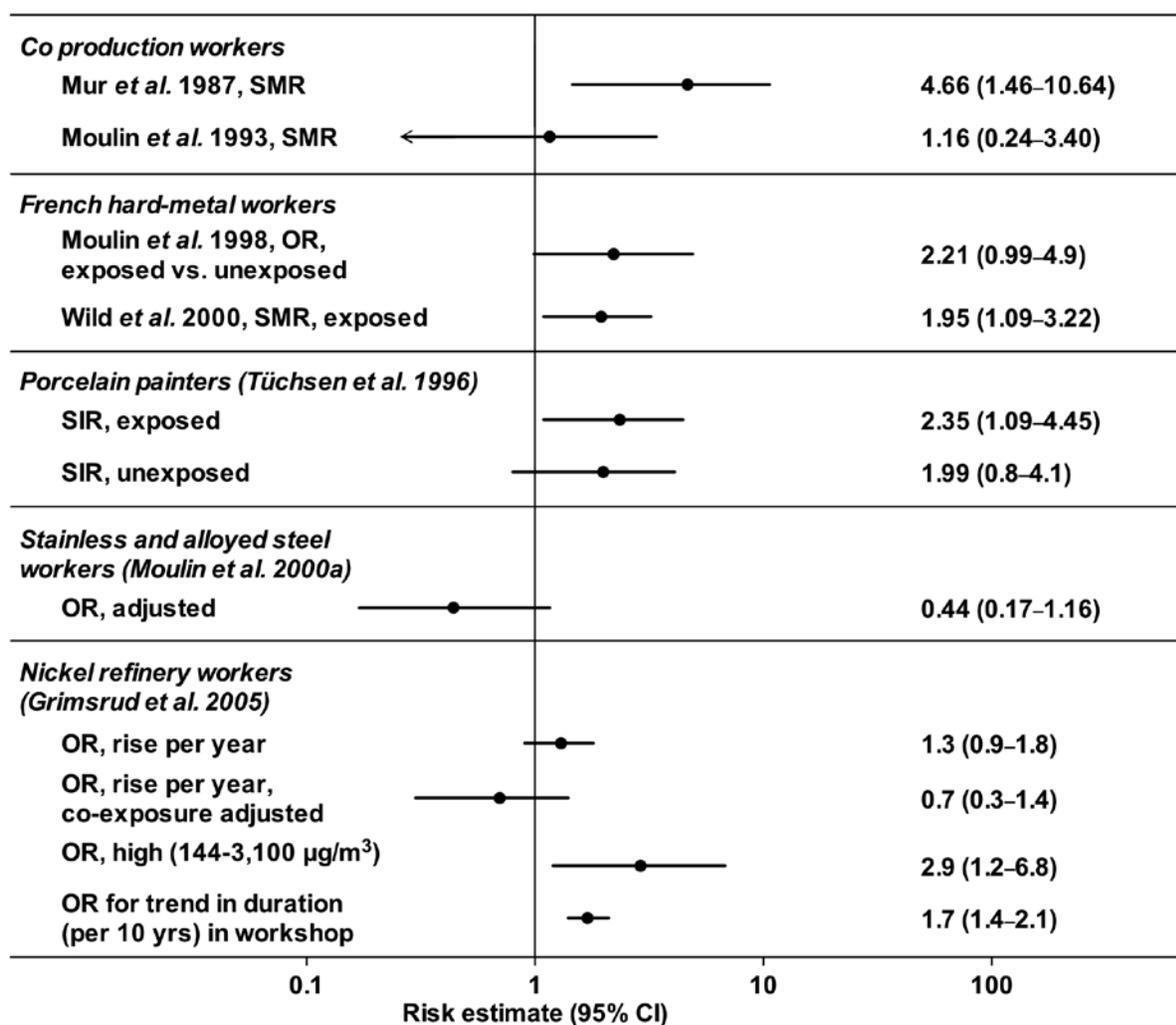


Figure 4-1. Forest plot showing lung cancer risk ratios (SIR, SMR, or OR as noted) and 95% CI for epidemiological cohort studies of cobalt exposure

### 4.3 Case-control studies

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

#### 4.3.1 Overview of the methodologies and study characteristics

The available epidemiological studies that satisfy the criteria for inclusion in the review consist of two population-based case-control studies of metals in biological tissues of cancer cases (lung, esophageal, oral cavity, and laryngeal cancers) and controls published in the literature between 1986 and 2012 (Table 4-4). Both of these studies (Rogers *et al.* 1993, O'Rorke *et al.* 2012) 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 <i>et al.</i> 1993	Population-based case-control biomarker study Western WA state USA 1983–1987 501 cases (153 laryngeal, 73 esophageal, 359 oral cavity cancers)/434 controls	ICD-O Larynx (140.0–141.9) Esophagus (143.0–146.9) Oral cavity (148.0–150.9; 161.0–161.9)	Source and type of compounds unknown Cobalt levels in toenails measured Tertiles (ppm)
Rogers <i>et al.</i> 1993	Population-based case-control biomarker study Ireland FINBAR* study 2002–2004	ICD not reported Esophagus Barrett’s esophagus (metastatic precursor to esophageal cancer)	Source and type of compounds unknown Cobalt levels in toenails measured Tertiles (log transformed - cut points µg/g)

\*FINBAR = Factors Influencing the Barrett’s Adenocarcinoma Relationship.

### 4.3.2 Study quality and utility evaluation

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

Both of the case-control studies of cobalt in toenails have either low/minimal or some concern for most biases except for exposure assessment and sensitivity. Their overall low utility to inform the cancer hazard evaluation, however, is due to the potentially irrelevant window of exposure. Toenail clippings likely reflect an integrated exposure that occurred 12 to 18 months prior to clipping, and toenail samples were collected after cancer diagnosis in these studies. Many factors (including disease) can affect nail growth and metal deposition. The available studies (that evaluated cobalt levels and cancer stage [lung or laryngeal] are conflicting, thus it unclear whether the cancer process can affect cobalt levels in toenails Kuo *et al.* 2006, Benderli Cihan *et al.* 2011, Klatka *et al.* 2011). 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, which indicated no differences in cobalt levels, suggesting that reverse causality may not be a concern.

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

Citation	Bias <sup>a</sup>						Quality <sup>a</sup>	Utility <sup>b</sup>
	Selection	Exposure	Outcome	Confounding methods	Adequacy of analysis	Selective reporting	Sensitivity	Integration
Rogers <i>et al.</i> 1993	+++	+	+++	+++	+++	+++	+	+
O'Rorke <i>et al.</i> 1993	++	+	+++	+++	+++	+++	+	+

<sup>a</sup>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.

<sup>b</sup>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 and, however, have distinct risk factors and trends. esophageal adenocarcinoma, with risk factors being white race, increasing age, body fatness, and male gender, is the predominant histological type among men, while for women, esophageal squamous-cell cancer is more common and rates are still increasing in several European countries. Unlike esophageal squamous-cell carcinoma, alcohol is not a risk factor for either Barrett's esophagus or for esophageal adenocarcinoma (Anderson *et al.* 2009, Kubo *et al.* 2009, Freedman *et al.* 2011); however, smoking is a risk factor for both subtypes and Barrett's esophagus (Cook *et al.* 2010).

Barrett's esophagus is a condition of intestinal metaplasia in which tissue that is similar to the lining of the intestine replaces the tissue lining the esophagus. The prevalence of Barrett's

esophagus is estimated to be between 1.6% and 6.8% (Gilbert *et al.* 2011), although a more precise estimate is not possible as many patients are asymptomatic, and its natural history has been difficult to assess. Barrett's esophagus has an extended latency period prior to progressing to cancer (Butt and Kandel 2014). A recent meta-analysis of studies reports incidence rates for the development of esophageal cancer in nondysplastic Barrett's esophagus of 0.33% per year and 0.19% for short-segment Barrett's esophagus (Desai *et al.* 2012). About 5% of patients with esophageal adenocarcinoma have a pre-cancer diagnosis of Barrett's esophagus (Corley *et al.* 2002); but its presence conveys a 30- to 40-fold increased risk of esophageal carcinoma (Sharma 2004). As incidence of esophageal adenocarcinoma has increased more than six-fold in the last decade, investigations of the risk factors for Barrett's esophagus have been of interest (Jemal *et al.* 2013). Barrett's esophagus incidence increases with age; the prevalence among non-Hispanic whites is 6.1% compared to 1.7% among Hispanics and 1.6% among blacks; and the male/female ratio is about 2:1 (Abrams *et al.* 2008), similar to esophageal cancer.

#### **Evidence from individual studies**

Both of the case-control studies (Rogers *et al.* 1993, O'Rorke *et al.* 2012) 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 <i>et al.</i> 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	<b>Esophagus (143.0–146.9)</b>				Age, sex, smoking (pack-years), alcohol (drink-years), beta-carotene (mg/day), energy intake (kcal/day), ascorbic acid (mg/day)	<b>Exposure levels:</b> Tertiles of cobalt in toenails; highest level = 0.17 ppm  <b>Confounding:</b> Cases and controls were matched on key likely confounders. No information provided about correlation of cobalt with other measured trace metal levels, and nutrients not correlated with cobalt were kept in the model because they resulted in ORs closer to the null. ORs for esophageal cancer were significantly elevated for iron and calcium  <b>Strengths:</b> Population-based study; histologically confirmed cancers; cases and controls from same source population.  <b>Limitations:</b> Not all samples reflect pre-diagnostic window of exposure. No USDA data available on cobalt levels in food as measured by a food frequency questionnaire. Single sample collected even though cobalt in toenails shown to have low reproducibility; window of exposure a concern with long latency cancer.
		< 0.05	92	OR = 1.0			
		0.05–0.17	127	2.4 (0.8–7.2)			
		> 0.17	66	9 (2.7–30)			
		<b>Larynx (140.0–141.9)</b>				Age, sex, smoking (pack-years), energy intake (kcal/day), beta-carotene (mg/day), ascorbic acid (mg/day), alcohol (drink-years)	
		< 0.05	114	OR = 1.0			
		0.05–0.17	168	2 (1–3.8)			
		> 0.17	62	1 (0.4–2.6)			
		<b>Oral cavity cancer (148.0–150.9; 161.0–161.9)</b>				Age, sex, smoking (pack-years), alcohol (drink-years), energy intake (kcal/day), ascorbic acid (mg/day), beta-carotene (mg/day)	
		< 0.05	135	OR = 1.0			
		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 <i>et al.</i> 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	<b>Esophageal cancer</b>				Age, sex, GI reflux, education, <i>H. pylori</i> infection, location, smoking	<b>Exposure levels:</b> Average ( $\mu\text{g/g}$ ) $\pm$ SD: cases = $0.02 \pm 0.06$ ; controls = $0.02 \pm 0.04$ . Range: cases = 0.002–0.60; controls = 0.002–0.47 <b>Confounding:</b> No correlation of cobalt levels with selenium, chromium, zinc, mercury, and cerium reported, nor were other metals included in models. <b>Strengths:</b> Population based; histologically confirmed cancer. <b>Limitations:</b> Differences in sources of cases and controls in N. Ireland and Rep. of Ireland may introduce some selection bias; low participation rate in controls, especially in Rep. of Ireland. Not all samples reflect pre-diagnostic window of exposure. Single sample collected even though cobalt in toenails shown to have low reproducibility; window of exposure a concern with long latency cancer.
		< -5.4824	34	OR = 1.0			
		$\geq$ -5.4824	39	1.06 (0.57–1.98)			
		$\geq$ -4.4705	52	1.54 (0.84–2.85)			
		Trend-test <i>P</i> -value: 0.16					
		<b>Esophageal cancer</b>				Age, sex	
		< -5.4824	34	OR = 1.0			
		$\geq$ -5.4824	39	1.13 (0.64–1.99)			
		$\geq$ -4.4705	52	1.54 (0.9–2.68)			
		Trend-test <i>P</i> -value: 0.11					
		<b>Barrett's Esophagus</b>				Age, sex, GI reflux, <i>H. pylori</i> infection, smoking habits, energy intake, location	
		< -5.4824	55	OR = 1.0			
		$\geq$ -5.4824	54	1.08 (0.55–2.1)			
		$\geq$ -4.4705	64	1.97 (1.01–3.85)			
		Trend-test <i>P</i> -value: 0.05					
		<b>Barrett's Esophagus</b>				Age, sex	
< 5.4824	55	OR = 1.0					
$\geq$ 5.4824	54	0.97 (0.59–1.59)					
$\geq$ 4.4705	64	1.18 (0.72–1.93)					
Trend-test <i>P</i> -value: 0.5							
Mur <i>et al.</i> 1987 Cohort France 1950–1980	Electrochemical workers N = 1,143; number of cobalt production workers NR ~ 25% of	<b>Buccal cavity, pharynx, larynx (140-149, 161)</b>				Age, year of death	Exposure duration: 60% worked greater than 10 years; 75% hired before 1975.
		Employed only in cobalt Production	2	SMR 3.36 (0.29–10.3)			

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Co-variables controlled	Comments, strengths, and weaknesses
	current staff at time of publication  <b>Exposure assessment method:</b> company records					<p><b>Confounding:</b> Likely inadequate control for smoking; however, likely co-exposure to nickel and arsenic with no control for co-exposures.</p> <p><b>Strengths:</b> Cobalt production workers exposed primarily to cobalt compounds.</p> <p><b>Limitations:</b> Small number of exposed cases; high loss to follow-up (20%); potential for selection bias due to left-truncation</p>

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

***Western Washington state study of aerodigestive cancers***

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

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

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

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

***Finbar study – Ireland***

O’Rourke *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 ( $\geq -4.4705$ , log transformed values equivalent to  $\geq 0.011$   $\mu\text{g/g}$ ) (OR = 1.97, 95% CI = 1.01 to 3.85), with a significant ( $P = 0.05$ ) linear test for trend. Both of the estimates were adjusted for age, sex, smoking, location (Northern Ireland or Republic of

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

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

### Integration of the evidence across studies

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

## 4.4 Cancer assessment: Other types of cancers

### 4.4.1 Other aerodigestive cancers - oral cavity, pharyngeal, and laryngeal cancers

The available data to evaluate cobalt in relation to other aerodigestive cancers, specifically cancers of the oral cavity, pharynx, and larynx, consist of the electrochemical workers cohort study (Mur *et al.* 1987), and one population-based case-control biomarker study (Rogers *et al.* 1993). The first publication from the electrochemical workers cohort (Mur *et al.*) 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 ( $\geq 0.17$  ppm) of cobalt concentration in toenails compared to the lowest

level (< 0.05 ppm) of cobalt (OR = 1.9, 95% CI = 1.0 to 3.6). The OR was elevated but not significant for those with medium levels (0.05 to 0.17 ppm) of cobalt concentration compared to those with low levels (OR = 1.5, 95% CI = 0.9 to 2.6). The exposure-response test for trend was not significant (*P*-value not reported). The finding was present in both *in situ*/localized tumors and individuals with regional/distant tumors. In this study, diet was not found to be an explanation for the higher risks, and tobacco and alcohol levels were controlled in the analyses.

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

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

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

#### 4.4.2 Other types of cancers

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

#### 4.5 NTP listing recommendation

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

The relevant data for evaluation of exposure specifically to cobalt are from studies of five major cohorts of workers exposed to cobalt in Denmark (Tüchsen *et al.* 1996), France (Mur *et al.* 1987, Moulin *et al.* 1993, Moulin *et al.* 1998, Wild *et al.* 2000, Moulin *et al.* 2000), Norway (Grimsrud

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

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

**This Page Intentionally Left Blank**

## 5 Studies of Cancer in Experimental Animals

This section reviews and assesses the evidence from carcinogenicity studies in experimental animals exposed to cobalt and cobalt compounds that release cobalt ions *in vivo* (hereinafter referred to as cobalt). Cancer and co-carcinogen studies in experimental animals were identified using methods described in the protocol and literature search strategy document (<http://ntp.niehs.nih.gov/go/730697>). In all, 23 publications (16 carcinogenicity and 9 co-carcinogenicity studies) were identified that met the following inclusion criteria: reported on the presence or absence of neoplastic and related non-neoplastic lesion, had concurrent or historical control group, and had an observational duration of 12 months or greater for rats and mice or were co-carcinogen exposure studies (initiation/promotion and other co-carcinogen studies that isolate the effect of cobalt compound exposures). Some of these publications overlap since some co-carcinogenicity studies had a cobalt exposure alone group and a corresponding control as part of their design. Several studies were excluded from the review because they did not have concurrent controls or controls from a closely related study. These included Hopps *et al.* (1954), Delahant (1955), Gilman (1962), Nowak (1966), and Gunn *et al.* (1967). Studies of cobalt alloys cobalt composites, cobalt compounds containing other metals, and radioactive cobalt in experimental animals were not considered to be informative because of potential confounding by other carcinogens. The cobalt alloys that were tested in experimental animals also contained other metals shown to be carcinogenic in experiments such as nickel and chromium and thus it would not be possible to separate any effects due to cobalt from those due to the other metals .(See 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 level of evidence conclusion for the carcinogenicity of cobalt compounds that release cobalt ions *in vivo* as a class from studies in experimental animals is provided in Section 7, which provides the rationale for evaluating them as a class.

### 5.1 Carcinogenicity studies

#### 5.1.1 Overview of the studies

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

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

**Table 5-1. Overview of cancer studies in experimental animals reviewed**

Strain (sex)	Substance	Route	Exposure period/ study duration	Reference
<b><i>Cobalt metal</i></b>				
Rat F344/NTac (M&F)	Cobalt metal	Inhalation	2 yr/2 yr	NTP 2014b
Mouse B6C3F <sub>1</sub> (M&F)	Cobalt metal	Inhalation	2 yr/2 yr	NTP 2014b
Rat Sprague-Dawley (M)	Cobalt metal [nano] and	IM inj.	Single dose/ 1 yr	Hansen <i>et al.</i> 2006
	Cobalt metal [bulk] <sup>a</sup>	SC inj.		
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
<b><i>Soluble cobalt compounds</i></b>				
Rat F344/N (M&F)	Cobalt sulfate heptahydrate	Inhalation	2 yr/2 yr	NTP 1998
Mouse B6C3F <sub>1</sub> (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 <i>et al.</i> 1977
<b><i>Poorly soluble cobalt compounds</i></b>				
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 <i>et al.</i> 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

<sup>a</sup>Both cobalt compounds tested in the same animal.

### 5.1.2 Study quality assessment

Each of these primary studies was systematically evaluated for its ability to inform the cancer hazard evaluation using a series of signaling questions related to the following study performance elements: population, exposure conditions, outcome assessment, potential confounding, and statistics and reporting (see Protocol for Preparing the RoC Monograph on Cobalt [NTP 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(II) oxide (Wehner *et al.* 1977, Steinhoff and Mohr 1991) and three injection studies of cobalt metal or cobalt sulfide in two publications (Steinhoff and Mohr 1991, Hansen *et al.* 2006) 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, Gilman and Ruckerbauer 1962, Heath and Daniel 1962, Jasmin and Riopelle 1976, Shabaan *et al.* 1977), there were major concerns for several potential biases; thus, these studies were considered to have lower utility. Most of these studies had low sensitivity or incomplete necropsies. Poor reporting of methods and results was also a common problem and in some studies there were concerns about potential confounding. Historical controls from a related study by the same authors were used in lieu of concurrent controls in one study (Heath and Daniel 1962). Overall, the major limitations in the studies with low and moderate utility were primarily (but not exclusively) due to low sensitivity and for these cases there is little concern that these limitations would decrease confidence in a positive finding.

Table 5-2. Overview of experimental animal carcinogenicity study quality evaluations

Study		Quality									Sensitivity			Overall utility	
		Controls	Historical data	Randomization	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 <i>et al.</i> 2006 <sup>a</sup>	Cobalt metal and nano	+++	No	NR	NR	++	+++	+++	++	++	++	++	+	+	Moderate
Jasmin and Riopelle 1976 <sup>a</sup>	Cobalt metal and sulfide	+++	No	NR	++	+	NR	++	++	++	++	++	++	+	Low
Heath and Daniel 1962	Cobalt metal	+	Yes <sup>b</sup>	NR	++	+	NR	++	++	+	++	+	++	+++	Low
Heath 1956	Cobalt metal	++	Yes <sup>b</sup>	NR	++	+	NR	++	++	+	++	+	++	+++	Low
NTP 1998 R	Cobalt sulfate	+++	Yes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	High
NTP 1998 M	Cobalt sulfate	+++	Yes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	High
Shabaan <i>et al.</i> 1977	Cobalt chloride	++	Yes <sup>b</sup>	NR	NR	+	++	+	+	+	++	++	++	+	Low

Study	Quality										Sensitivity			Overall utility	
	Controls	Historical data	Randomization	Purity	Dosing	Treatment-related survival	Pathology	Con-founding	Reporting & analysis	Animal model	Stat power	Duration			
Steinhoff and Mohr 1991- (intratracheal)	Cobalt(II) oxide	+++	No	NR	++	++	++	++	++	++	++	+++	+++	+++	Moderate
Steinhoff and Mohr 1991 - (IP)	Cobalt(II) oxide	+++	No	NR	++	+	NR	++	++	++	++	+++	+	+++	Moderate
Steinhoff and Mohr 1991 - (SC)	Cobalt(II) oxide	+++	No	NR	++	++	NR	++	++	++	++	++	+	+++	Moderate
Gilman and Ruckerbauer 1962 R	Cobalt(II) oxide	+++	No	NR	+	+	++	++	+	+	+	+++	+	++	Low
Gilman and Ruckerbauer 1962 M	Cobalt(II) oxide	+++	No	NR	+	+	++	++	+	++	++	++	++	+++	Low
Wehner <i>et al.</i> 1977	Cobalt(II) oxide		No	NR											

+++ = high quality/little to no concerns, ++ = moderate quality/moderate concerns, + = low quality/high concerns, 0 = inadequate, NR = not reported; M = mice; R = rats.

<sup>a</sup>Includes test results for two forms of cobalt, so considered two studies.

<sup>b</sup>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, intrapleural, and intrarenal). In addition, in some inhalation studies, some tumors were observed in sites distal from the site of administration. Findings for cobalt compounds across organ sites are discussed in Section 5.3.

#### Lung (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(II) oxide (Steinhoff and Mohr 1991)– caused lung neoplasms after exposure by inhalation or intratracheal instillation. Study results for six respiratory exposure studies are reported in Table 5-3 including two studies in mice, three studies in rats, and one study in hamsters. Four of these studies were high-quality, well-designed, and well-conducted studies (NTP 1998, 2014b) and all had either high (NTP 1998, 2014b) or moderate (Wehner *et al.* 1977, Steinhoff and Mohr 1991) utility for evaluating potential cancer hazards.

Four studies found strong evidence that cobalt (both cobalt metal and cobalt sulfate) causes lung tumors in both mice and rats (NTP 1998, 2014b). 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<sup>3</sup>; medium, 2.5 mg/m<sup>3</sup>; high, 5 mg/m<sup>3</sup>) 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 were very high; when adjusted for intercurrent mortality, incidences in the high-dose groups were 81% for male rats, 69% for female rats, 94% for male mice, and 88% for female mice. In addition, dose-related significant increases in multiplicity (animals with more than one lung tumor) of carcinoma were also found for all dose groups in male and female mice and male rats and in the high-dose (5 mg/m<sup>3</sup>) 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 in mid- and high-dose groups in females and high-dose group in males.

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

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

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

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

Table 5-3. Lung neoplasms and non-neoplastic lesions in experimental animals exposed to cobalt compounds

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N*) (%)	Comments		
NTP 2014b Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Multiple alveolar/bronchiolar carcinoma</b>				Survival in exposed groups was similar to controls.  <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals.  <b>Limitations:</b> Decreases in body weight in mid and high dose rats.  <b>Other comments:</b> Historical controls were limited (100 rats).  <b>Significantly increased non-neoplastic lesions:</b> Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels Bronchiolar hyperplasia (pre-neoplastic) - all dose levels	
			0 mg/m <sup>3</sup>	17	0/50 (0%)			
			1.25 mg/m <sup>3</sup>	20	6/50 (12%)*			
			2.5 mg/m <sup>3</sup>	16	14/50 (28%)**			
			5 mg/m <sup>3</sup>	16	30/50 (60%)**			
			<b>Alveolar/bronchiolar carcinoma<sup>a</sup></b>					
			0 mg/m <sup>3</sup>	17	0/50 (0%)			
			1.25 mg/m <sup>3</sup>	20	16/50 (38%***)			
			2.5 mg/m <sup>3</sup>	16	34/50 (77%***)			
			5 mg/m <sup>3</sup>	16	36/50 (81%***)			
			Trend-test <i>P</i> -value: 0.001					
			<b>Multiple alveolar/bronchiolar adenoma</b>					
			0 mg/m <sup>3</sup>	17	1/50 (2%)			
			1.25 mg/m <sup>3</sup>	20	3/50 (6%)			
			2.5 mg/m <sup>3</sup>	16	2/50 (4%)			
			5 mg/m <sup>3</sup>	16	6/50 (12%)			
			<b>Alveolar/bronchiolar adenoma<sup>a</sup></b>					
			0 mg/m <sup>3</sup>	17	2/50 (5%)			
			1.25 mg/m <sup>3</sup>	20	10/50 (24%)*			
			2.5 mg/m <sup>3</sup>	16	10/50 (23%)*			
			5 mg/m <sup>3</sup>	16	14/50 (33%***)			
			Trend-test <i>P</i> -value: 0.011					
<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>a</sup></b>								
0 mg/m <sup>3</sup>	17	2/50 (5%)						
1.25 mg/m <sup>3</sup>	20	25/50 (58%***)						

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>†</sup> ) (%)	Comments
			2.5 mg/m <sup>3</sup>	16	39/50 (85%)***	
			5 mg/m <sup>3</sup>	16	44/50 (94%)***	
			Trend-test <i>P</i> -value: 0.001			
			<b>Cystic keratinizing epithelioma</b>			
			0 mg/m <sup>3</sup>	17	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (2%)	
			2.5 mg/m <sup>3</sup>	16	0/50 (0%)	
			5 mg/m <sup>3</sup>	16	1/50 (2%)	
<b>NTP 2014b</b> Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Multiple alveolar/bronchiolar carcinoma</b>			<b>Survival</b> was significantly decreased in the mid-dose group. <b>Strengths:</b> A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> A significant decrease in survival of female rats and decreases in body weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited (100 rats). <b>Significantly increased non-neoplastic lesions:</b> Alveolar hyperplasia (pre-neoplastic) - all
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	4/50 (8%)	
			2.5 mg/m <sup>3</sup>	24	3/50 (6%)	
			5 mg/m <sup>3</sup>	25	18/50 (36%)**	
			<b>Alveolar/bronchiolar carcinoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	9/50 (21%)***	
			2.5 mg/m <sup>3</sup>	24	17/50 (42%)***	
			5 mg/m <sup>3</sup>	25	30/50 (69%)***	
			Trend-test <i>P</i> -value: 0.001			
			<b>Multiple alveolar/bronchiolar adenoma</b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	1/50 (2%)	
			2.5 mg/m <sup>3</sup>	24	3/50 (6%)	
			5 mg/m <sup>3</sup>	25	4/50 (8%)	
<b>Alveolar/bronchiolar adenoma<sup>a</sup></b>						
0 mg/m <sup>3</sup>	35	2/50 (5%)				

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>†</sup> ) (%)	Comments
			1.25 mg/m <sup>3</sup>	26	7/50 (16%)	dose levels. Bronchiolar hyperplasia (pre-neoplastic) - all dose levels
			2.5 mg/m <sup>3</sup>	24	9/50 (22%)*	
			5 mg/m <sup>3</sup>	25	13/50 (31%)**	
			Trend-test <i>P</i> -value: 0.002			
			<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	2/50 (4%)	
			1.25 mg/m <sup>3</sup>	26	15/50 (35%***)	
			2.5 mg/m <sup>3</sup>	24	20/50 (49%***)	
			5 mg/m <sup>3</sup>	25	38/50 (86%***)	
			Trend-test <i>P</i> -value: 0.001			
			<b>Squamous cell carcinoma</b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	0/50 (0%)	
			2.5 mg/m <sup>3</sup>	24	0/50 (0%)	
			5 mg/m <sup>3</sup>	25	1/50 (2%)	
			<b>Cystic keratinizing epithelioma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	4/50 (10%) <sup>i</sup>	
			2.5 mg/m <sup>3</sup>	24	1/50 (3%) <sup>i</sup>	
			5 mg/m <sup>3</sup>	25	2/50 (5%) <sup>i</sup>	
Trend-test <i>P</i> -value: 0.002						
<b>NTP 2014b</b> Mouse (B6C3F <sub>1</sub> /N) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Multiple alveolar/bronchiolar carcinoma</b>		<b>Survival</b> significantly decreased at 2.5 and 5 mg/m <sup>3</sup> . <b>Strengths:</b> A well-designed study in almost all factors such	
		0 mg/m <sup>3</sup>	39	3/50 (6%)		
		1.25 mg/m <sup>3</sup>	31	18/49 (36%)**		
		2.5 mg/m <sup>3</sup>	29	24/50 (48%)**		
			5 mg/m <sup>3</sup>	25	36/50 (72%)**	

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>†</sup> ) (%)	Comments
			Trend-test <i>P</i> -value: 0.001			as long observation period, sufficient dose levels, adequate number of animals.  <b>Limitations:</b> A significant decrease in survival of male mice and decrease in body weight in high dose mice  <b>Significantly increased non-neoplastic lesions:</b> Alveolar/bronchiolar epithelium hyperplasia (pre-neoplastic) - all dose levels Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels Bronchiolar epithelium hyperplasia (pre-neoplastic) - high dose
			<b>Alveolar/bronchiolar carcinoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	39	11/50 (23%)	
			1.25 mg/m <sup>3</sup>	31	38/49 (79%)***	
			2.5 mg/m <sup>3</sup>	29	42/50 (88%)***	
			5 mg/m <sup>3</sup>	25	46/50 (94%)***	
			Trend-test <i>P</i> -value: 0.001			
			<b>Multiple alveolar/bronchiolar adenoma</b>			
			0 mg/m <sup>3</sup>	39	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	31	1/49 (2%)	
			2.5 mg/m <sup>3</sup>	29	1/50 (2%)	
			5 mg/m <sup>3</sup>	25	0/50 (0%)	
			<b>Alveolar/bronchiolar adenoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	39	7/50 (15%)	
			1.25 mg/m <sup>3</sup>	31	11/49 (25%)	
			2.5 mg/m <sup>3</sup>	29	15/50 (36%)*	
			5 mg/m <sup>3</sup>	25	3/50 (7%)	
			<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	39	16/50 (33%)	
			1.25 mg/m <sup>3</sup>	31	41/49 (85%)***	
2.5 mg/m <sup>3</sup>	29	43/50 (90%)***				
5 mg/m <sup>3</sup>	25	47/50 (96%)***				
Trend-test <i>P</i> -value: 0.001						
<b>NTP 2014b</b> Mouse (B6C3F <sub>1</sub> /N) Female (5–6 wk old)	Cobalt metal 98% pure, mass median	Inhalation (dry particulate) 6 hr/day, 5	<b>Multiple alveolar/bronchiolar carcinoma</b>		<b>Survival</b> in exposed groups was similar to controls.	
		0 mg/m <sup>3</sup>	36	1/49 (10%)		
		1.25 mg/m <sup>3</sup>	36	7/50 (50%)*		

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>†</sup> ) (%)	Comments		
105 wk	aerodynamic diameter 1–3 µm)	day/wk × 105 wk	2.5 mg/m <sup>3</sup>	27	20/50 (76%)**	<p><b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals.</p> <p><b>Limitations:</b> Decrease in body weight in high</p> <p><b>Significantly increased non-neoplastic lesions:</b> 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</p>		
			5 mg/m <sup>3</sup>	26	24/50 (86%)**			
			Trend-test <i>P</i> -value: 0.001					
			<b>Alveolar/bronchiolar carcinoma<sup>a</sup></b>					
			0 mg/m <sup>3</sup>	36	5/49 (11%)			
			1.25 mg/m <sup>3</sup>	36	25/50 (54%***			
			2.5 mg/m <sup>3</sup>	27	38/50 (79%***			
			5 mg/m <sup>3</sup>	26	43/50 (88%***			
			Trend-test <i>P</i> -value: 0.001					
			<b>Multiple alveolar/bronchiolar adenoma</b>					
			0 mg/m <sup>3</sup>	36	0/49 (0%)			
			1.25 mg/m <sup>3</sup>	36	1/50 (2%)			
			2.5 mg/m <sup>3</sup>	27	0/50 (0%)			
			5 mg/m <sup>3</sup>	26	1/50 (2%)			
			<b>Alveolar/bronchiolar adenoma<sup>a</sup></b>					
			0 mg/m <sup>3</sup>	36	3/49 (7%)			
			1.25 mg/m <sup>3</sup>	36	9/50 (20%)			
			2.5 mg/m <sup>3</sup>	27	8/50 (19%)			
			5 mg/m <sup>3</sup>	26	10/50 (25%)*			
			Trend-test <i>P</i> -value: 0.037					
			<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>a</sup></b>					
			0 mg/m <sup>3</sup>	36	8/49 (18%)			
			1.25 mg/m <sup>3</sup>	36	30/50 (64%***			
2.5 mg/m <sup>3</sup>	27	41/50 (85%***						
5 mg/m <sup>3</sup>	26	45/50 (92%***						
Trend-test <i>P</i> -value: 0.001								

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments	
<b>NTP 1998</b> Rat (F344) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Alveolar/bronchiolar carcinoma<sup>b</sup></b>				<b>Survival</b> in exposed groups was similar to controls. <b>Strengths:</b> A well-designed study in all factors <b>Limitations:</b> None. <b>Significantly increased non-neoplastic lesions:</b> Alveolar epithelium metaplasia - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - all dose levels
			0 mg/m <sup>3</sup>	17	0/50 (0%)		
			0.3 mg/m <sup>3</sup>	15	0/50 (0%)		
			1.0 mg/m <sup>3</sup>	21	3/48 (11%)		
			3.0 mg/m <sup>3</sup>	15	1/50 (7%)		
			<b>Alveolar/bronchiolar adenoma<sup>b</sup></b>				
			0 mg/m <sup>3</sup>	17	1/50 (2%)		
			0.3 mg/m <sup>3</sup>	15	4/50 (18%)		
			1.0 mg/m <sup>3</sup>	21	1/48 (2%)		
			3.0 mg/m <sup>3</sup>	15	6/50 (28%)		
			<b>Alveolar/bronchiolar adenoma or carcinoma combined<sup>b</sup></b>				
			0 mg/m <sup>3</sup>	17	1/50 (2%)		
			0.3 mg/m <sup>3</sup>	15	4/50 (18%)		
			1.0 mg/m <sup>3</sup>	21	4/48 (13%)		
3.0 mg/m <sup>3</sup>	15	7/50 (34%)*					
Trend-test <i>P</i> -value: 0.032							
<b>NTP 1998</b> Rat (F344) Female (6 wk old) 2 yr	Cobalt sulfate (99% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Alveolar/bronchiolar carcinoma<sup>b</sup></b>				Survival in exposed groups was similar to controls. <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> None. <b>Significantly increased</b>
			0 mg/m <sup>3</sup>	28	0/50 (0%)		
			0.3 mg/m <sup>3</sup>	25	2/49 (8%)		
			1.0 mg/m <sup>3</sup>	26	6/50 (20%)*		
			3.0 mg/m <sup>3</sup>	30	6/50 (18%)*		
			Trend-test <i>P</i> -value: 0.023				
			<b>Alveolar/bronchiolar adenoma<sup>b</sup></b>				
			0 mg/m <sup>3</sup>	28	0/50 (0%)		
			0.3 mg/m <sup>3</sup>	25	1/49 (3%)		
			1.0 mg/m <sup>3</sup>	26	10/50 (36%)*		

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
			3.0 mg/m <sup>3</sup>	30	9/50 (30%)* <sup>***</sup>	<b>non-neoplastic lesions:</b> Alveolar epithelium metaplasia - all dose levels; Alveolar epithelium hyperplasia (pre-neoplastic) - high dose; Alveolar epithelium hyperplasia, atypical (pre-neoplastic) - high dose
			Trend-test <i>P</i> -value: 0.001			
			<b>Alveolar/bronchiolar adenoma or carcinoma combined<sup>b</sup></b>			
			0 mg/m <sup>3</sup>	28	0/50 (0%)	
			0.3 mg/m <sup>3</sup>	25	3/49 (11%) <sup>c</sup>	
			1.0 mg/m <sup>3</sup>	26	15/50 (51%)* <sup>***c</sup>	
			3.0 mg/m <sup>3</sup>	30	15/50 (46%)* <sup>***c</sup>	
			Trend-test <i>P</i> -value: 0.001			
			<b>Squamous cell carcinoma</b>			
			0 mg/m <sup>3</sup>	28	0/50 (0%)	
			0.3 mg/m <sup>3</sup>	25	0/49 (0%)	
			1.0 mg/m <sup>3</sup>	26	1/50 (2%)	
			3.0 mg/m <sup>3</sup>	30	1/50 (2%)	
			<b>Alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma combined<sup>b</sup></b>			
			0 mg/m <sup>3</sup>	28	0/50 (0%)	
			0.3 mg/m <sup>3</sup>	25	3/49 (11%)	
			1.0 mg/m <sup>3</sup>	26	16/50 (54%)* <sup>***</sup>	
			3.0 mg/m <sup>3</sup>	30	16/50 (49%)* <sup>***</sup>	
Trend-test <i>P</i> -value: 0.001						
<b>NTP 1998</b> Mice (B6C3F <sub>1</sub> ) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure mass median aerodynamic diameter 1–3 μm	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Alveolar/bronchiolar carcinoma<sup>b</sup></b>			<b>Survival</b> in exposed groups was similar to controls.  <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels,
			0 mg/m <sup>3</sup>	22	4/50 (13%)	
			0.3 mg/m <sup>3</sup>	31	5/50 (16%)	
			1.0 mg/m <sup>3</sup>	24	7/50 (25%)	
			3.0 mg/m <sup>3</sup>	20	11/50 (44%)* <sup>d</sup>	
			Trend-test <i>P</i> -value: 0.006			
<b>Alveolar/bronchiolar adenoma<sup>b</sup></b>						

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
			0 mg/m <sup>3</sup>	22	9/50 (30%)	adequate number of animals.. <b>Limitations:</b> None. <b>No significant increase in non-neoplastic lesions.</b>
			0.3 mg/m <sup>3</sup>	31	12/50 (31%)	
			1.0 mg/m <sup>3</sup>	24	13/50 (41%)	
			3.0 mg/m <sup>3</sup>	20	18/50 (55%)* <sup>e</sup>	
			Trend-test <i>P</i> -value: 0.018			
			<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>b</sup></b>			
			0 mg/m <sup>3</sup>	22	11/50 (36%)	
			0.3 mg/m <sup>3</sup>	31	14/50 (37%)	
			1.0 mg/m <sup>3</sup>	24	19/50 (57%)	
			3.0 mg/m <sup>3</sup>	20	28/50 (79%)* <sup>*,f</sup>	
			Trend-test <i>P</i> -value: 0.001			
<b>NTP 1998</b> Mice (B6C3F <sub>1</sub> ) Female (6 wk old) 2 yr	Cobalt sulfate 99% pure mass median aerodynamic diameter 1–3 μm	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Alveolar/bronchiolar carcinoma<sup>b</sup></b>			<b>Survival</b> in exposed groups was similar to controls. <b>Strengths:</b> A well-designed study in all factors and survival was similar to controls. <b>Limitations:</b> None. <b>No significant increase in non-neoplastic lesions.</b>
			0 mg/m <sup>3</sup>	34	1/50 (3%)	
			0.3 mg/m <sup>3</sup>	37	1/50 (3%)	
			1.0 mg/m <sup>3</sup>	32	4/50 (9%)	
			3.0 mg/m <sup>3</sup>	28	9/50 (25%)* <sup>*,g</sup>	
			Trend-test <i>P</i> -value: 0.001			
			<b>Alveolar/bronchiolar adenoma<sup>b</sup></b>			
			0 mg/m <sup>3</sup>	34	3/50 (9%)	
			0.3 mg/m <sup>3</sup>	37	6/50 (15%)	
			1.0 mg/m <sup>3</sup>	32	9/50 (25%)	
			3.0 mg/m <sup>3</sup>	28	10/50 (33%)* <sup>*,h</sup>	
			Trend-test <i>P</i> -value: 0.024			
			<b>Alveolar/bronchiolar carcinoma or adenoma combined<sup>b</sup></b>			
			0 mg/m <sup>3</sup>	34	4/50 (12%)	
0.3 mg/m <sup>3</sup>	37	7/50 (18%)				

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
			1.0 mg/m <sup>3</sup>	32	13/50 (33%)* <sup>i</sup>	
			3.0 mg/m <sup>3</sup>	28	18/50 (50%)* <sup>***i</sup>	
			Trend-test <i>P</i> -value: 0.001			
<b>Steinhoff and Mohr 1991</b> Rat (Sprague-Dawley) Male (10 wk old) life-span	Cobalt(II) oxide "Chemically pure." 80% of particles were 5–40 μm)	Intratracheal instillation (dry particulate) 1 dose/2 wk × 18 doses, then 1 dose/4 weeks × 11 doses (up to 30th dose), then 1 dose/2 weeks × 9 doses (total 39 doses)	<b>Bronchioalveolar carcinoma</b>			<b>Survival</b> in exposed groups was similar as controls.  <b>Strengths:</b> Two dose levels tested in a high number of both sexes of rats for two years, with observations for the lifespan without any significant difference in survival compared to untreated controls.  <b>Limitations:</b> Only the high-dose group received full necropsies. Details of the chemical and animal husbandry were not reported.  <b>Significantly increased non-neoplastic lesions:</b> Bronchioalveolar 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%) <sup>j</sup>	
			<b>Bronchioalveolar adenoma</b>			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	2/50 (4%)	
			<b>Bronchioalveolar adenomas or bronchioalveolar carcinomas combined</b>			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	5/50 (10%)*	
			<b>Benign squamous epithelial tumor</b>			
			0 mg/kg bw	NR	0/50 (0%)	
2 mg/kg bw	NR	1/50 (2%)				
10 mg/kg bw	NR	0/50 (0%)				
<b>Steinhoff and Mohr 1991</b> Rat (Sprague-Dawley) Female (10 wk old) life-span	Cobalt(II) oxide "Chemically pure" 80% of particles were 5–40 μm)	Intratracheal instillation (dry particulate) 1 dose/2 wk × 18 doses, then 1 dose/4 weeks ×	<b>Bronchioalveolar carcinoma</b>			<b>Survival</b> in exposed groups was similar to controls.  <b>Strengths:</b> Two dose levels tested in a high number of both sexes of
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	0/50 (0%)	
			10 mg/kg bw	NR	1/50 (2%)	
			<b>Bronchioalveolar adenoma</b>			

Reference & year, animal, study duration	Substance, purity, size	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>+</sup> ) (%)	Comments
			0 mg/kg bw	NR	0/50 (0%)	rats for two years, with observations for the lifespan without any significant difference in survival compared to untreated controls.  <b>Limitations:</b> Only the high-dose group received full necropsies. Details of the chemical and animal husbandry were not reported.  <b>Significantly increased non-neoplastic lesions:</b> Bronchioalveolar proliferation - both dose levels.
			2 mg/kg bw	NR	1/50 (2%)	
			10 mg/kg bw	NR	0/50 (0%)	
			<b>Bronchioalveolar adenoma or bronchioalveolar carcinoma combined</b>			
			0 mg/kg bw	NR	0/50 (0%)	
			2 mg/kg bw	NR	1/50 (2%)	

\* =  $P$ -value  $\leq 0.05$ ; \*\* =  $P$ -value  $\leq 0.01$ ; \*\*\* =  $P$ -value  $\leq 0.001$ . NR = Not reported, wk = week, yr = year.

<sup>+</sup> = Number of animals necropsied for NTP 2014b and NTP 1998 (each group started with 50 animals per sex in the NTP studies) and is the number of animals at the beginning of the study for all other studies.

<sup>a</sup>Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

<sup>b</sup>Adjusted percent incidence based on Kaplan-Meier estimated incidence at the end of the study after adjustment for intercurrent mortality.

<sup>c</sup>Increased over historical control levels with a mean of 7/650 and range of 0% to 4%.

<sup>d</sup>Increased over historical control levels with a mean of 75/947 and range of 0% to 16%.

<sup>e</sup>Increased over historical control levels with a mean of 141/947 and range of 6% to 36%.

<sup>f</sup>Increased over historical control levels with a mean of 205/947 and range of 10% to 42%.

<sup>g</sup>Increased over historical control levels with a mean of 38/939 and range of 0% to 12%.

<sup>h</sup>Increased over historical control levels with a mean of 61/939 and range of 0% to 14%.

<sup>i</sup>Increased over historical control levels with a mean of 97/939 and range of 0% to 16%.

<sup>j</sup>Includes adenocarcinoma (2) and bronchioalveolar adenocarcinoma (1).

**Injection sites (subcutaneous, intramuscular, intraperitoneal, intrapleural, and intrarenal)**

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

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

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

by poor survival at the beginning of the study (eight rats died within three days) caused by the injections. Sarcomas originating from muscle tissue were only found in studies that injected cobalt metal by intramuscular injection (rhabdomyofibrosarcoma or rhabdomyosarcoma) or intrapleural injection (cardiac or intercostal muscle rhabdomyosarcoma). Relatively high incidences in sarcomas were observed in both studies although the studies had limited sensitivity because only a few animals were tested at only one dose.

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

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

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

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

Table 5-4. Injection site neoplasms and non-neoplastic lesions in experimental animals exposed to cobalt compounds

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N*) (%)	Comments
<b>Hansen <i>et al.</i> 2006</b> Rat (Sprague-Dawley) Male (NR) 12 mo	Cobalt metal [bulk and nano]- Bulk metal particles: 6.5 mm diameter by 1 mm in height; surface area to mass ratio of 4.73; nano-particles: 50–200 nm in size (average 120 nm); surface area to mass ratio of 50,000; PVC (bulk and nano)- bulk PVC, without additives: 10 mm in diameter by 1 mm in height; surface area to mass ratio of 4.2; PVC nano-particles: 60–170 nm in size (average 130 nm); surface area to mass ratio of 50,000.)	Nano (dry particles): IM implant (left side of vertebra) Single dose Bulk (solid metal): SC implant (right side of vertebra) Single dose	<b>Fibroblastic proliferation 6 months</b>			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. <b>Strengths:</b> Tested multiple materials in addition to cobalt and thus able to provide information on whether effects were due to physical state. <b>Limitations:</b> Inert polyvinyl chloride particles were used as a negative control. Only a small number of males were tested at a single dose level. Short duration and unable to fully evaluate effects from cobalt bulk particles.
			0 cm <sup>2</sup>	4	0	
			Nano 2 cm <sup>2</sup>	4	2	
			Bulk 2 cm <sup>2</sup>	4	0	
			<b>Sarcoma 6 months</b>			
			0 cm <sup>2</sup>	4	0	
			Nano 2 cm <sup>2</sup>	4	1	
			Bulk 2 cm <sup>2</sup>	4	0	
			<b>Fibroblastic proliferation 8 months</b>			
			0 cm <sup>2</sup> (12 mo)	6	0/6 (0%)	
			Nano 2 cm <sup>2</sup>	6	1/6 (16.7%)	
			Bulk 2 cm <sup>2</sup>	6	1/6 (16.7%)	
			<b>Sarcoma 8 months</b>			
			0 cm <sup>2</sup> (12 mo)	6	0/6 (0%)	
			Nano 2 cm <sup>2</sup>	6	5/6 (83.3%)[**]	
Bulk 2 cm <sup>2</sup>	6	0/6 (0%)				
<b>Heath 1956</b> Rat (Hooded) Male (2–3 mo old) life span	Cobalt metal "Spectroscopically pure" Particle size: 3.5 × 3.5 μm to 17 × 12 μm)	IM inj. (in fowl serum) Single dose	<b>Rhabdomyofibrosarcoma or sarcoma combined</b>			<b>Survival:</b> 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
						<p><b>Strengths:</b> Observation duration was sufficient and both sexes were tested.</p> <p><b>Limitations:</b> Incomplete reporting of many elements. Limited sensitivity due to only one dose level and few rats tested. Full necropsies were not reported.</p>
<p><b>Heath 1956</b> Rat (Hooded) Female (2–3 mo old) life span</p>	<p>Cobalt metal "Spectroscopically pure" Particle size: 3.5 × 3.5 μm to 17 × 12 μm)</p>	<p>Series I and Series II i.m. inj. (in fowl serum) Single dose</p>	<b>Sarcoma (Rhabdomyofibrosarcoma or fibrosarcoma)</b>			<p><b>Survival:</b> 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.</p> <p><b>Strengths:</b> Observation duration was sufficient and both sexes were tested.</p> <p><b>Limitations:</b> Incomplete reporting of many elements. Limited sensitivity due to only one dose level and few rats tested. Full necropsies were not reported.</p> <p><b>Other comments:</b> Series I used a concurrent</p>
			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 <sup>+</sup> ) (%)	Comments
						control, but Series II used the same controls, which was non-concurrent. 6/7 sarcoma in Series I and 2/5 in Series II were rhabdomyo-fibrosarcoma
<b>Heath and Daniel 1962</b> Rat (Hooded) Female (2–3 mo old) 28 months	Cobalt metal Purity not reported, Particle size: 3.5 × 3.5 μm to 17 × 12 μm)	Intrathoracic inj. (in serum) Single injection	<b>Mixed sarcoma intrathoracic region</b>			<b>Survival</b> was only reported for exposed rats, which was 12/20 on day 3 and 11/20 after 11 months. <b>Strengths:</b> Observation duration was sufficient <b>Limitations:</b> 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. <b>Other comments:</b> 3 of 4 tumors originated in part from cardiac muscle, which are very rare.
			0 mg/dose <sup>a</sup>	NR	0/10 (0%)	
			28 mg/dose	11	4/12 (33%)	
<b>Jasmin and Riopelle 1976</b> Rat (Sprague-Dawley)	Cobalt metal NR	Intrarenal placement (in glycerin)	<b>Kidney neoplasm NOS</b>			<b>Survival</b> was not reported. <b>Strengths:</b> Moderate
			0 mg/rat	NR	0/16 (0%)	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>+</sup> ) (%)	Comments
Female (120–140 g) 12 months		Single dose	10 mg/rat	NR	0/18 (0%)	number of animals. <b>Limitations:</b> 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.
<b>Shabaan et al. 1977</b> Rat (Wistar) Male (4 wk old) 8 and 12 mo	Cobalt chloride NR	SC inj. (in saline) 1 dose/day × 5 days, then 9 days off, then 1 dose/day × 5 days (total 19 days)	<b>Injection site and non-injection fibrosarcoma</b>			Treatment-related decrease in <b>survival</b> ; 16/20 survived at 8 months and 11/20 survived at 12 months. <b>Limitations:</b> Exposure resulted in persistent hyperlipaemia and high mortality. Animals dying before the end of observation period were not examined for tumors. The tumors at injection sites and non-injection sites weren't clearly reported <b>Other comments:</b> 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 <sup>†</sup> ) (%)	Comments
						(Fisher's Exact Test) reported by IARC.
<b>Steinhoff and Mohr 1991</b> Rat (Sprague-Dawley) Male and Female (10 wk old) life span	Cobalt(II) oxide "Chemically pure, 80% of particles were 5–40 µm)	i.p. inj. (in saline) 1 dose/2 mo × 6 mo	<b>Sarcoma</b>			<b>Survival</b> was not reported. <b>Strengths:</b> Both sexes of rats were tested with a long duration of observation. <b>Limitations:</b> 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 <b>Other comments:</b> Results were reported as combined for males and females.
			0 mg/kg	NR	1/20 (5%)	
			200 mg/kg	NR	3/20 (15%)[*]	
			<b>Mesothelioma</b>			
			0 mg/kg	NR	0/20 (0%)	
			200 mg/kg	NR	1/20 (5%)	
			<b>Histiocytoma</b>			
0 mg/kg	NR	1/20 (5%)				
			200 mg/kg	NR	10/20 (50%)[**]	
<b>Steinhoff and Mohr 1991</b> Rat (Sprague-Dawley) Male (10 wk old) life span	Cobalt oxide ("Chemically pure", 80% of particles were 5–40 µm)	s.c. inj. (in saline) 1 inj/day, 5 day/week × 730 days	<b>Histiocytoma or sarcoma combined</b>			<b>Survival</b> was not reported. <b>Strengths:</b> Duration of exposure and observation were sufficient. One dose level was tested, at two intensity levels and two untreated control groups used. <b>Limitations:</b> Limited sensitivity due to few
			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
						animals per group and only males tested. Limited histological examination. Incomplete reporting of many elements.
<b>Gilman and Ruckerbauer 1962</b> Rat (Wistar) Male and female (2–3 mo old) 489 days	Cobalt oxide purity not reported, particle size was < 5 µm	i.m. inj. (in aqueous suspension of penicillin G procaine) Single dose	<b>Sarcoma</b>			<b>Survival</b> was similar to control at 90 days. <b>Strengths:</b> The duration of observation was sufficient and both sexes were tested. <b>Limitations:</b> 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. <b>Other comments:</b> Results were reported as combined for males and females.
			0 mg/rat	10	0/10 (0%)	
			30 mg/rat	10	5/10 (50%)*]	
<b>Gilman and Ruckerbauer 1962</b> Mouse (Swiss) Female (2–3 mo old) 751 days	Cobalt oxide purity not reported, particle size was < 5 µm	i.m. inj. (in aqueous suspension of penicillin G procaine) Single dose	<b>Sarcoma</b>			<b>Survival</b> was similar to control at 90 days. <b>Strengths:</b> The duration of observation and the numbers of animals per group were sufficient.
			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 <sup>+</sup> ) (%)	Comments
						<b>Limitations:</b> Limited sensitivity due to only a single dose was given at one dose level, without a rationale, to females only. Half of the mice were survivors from a preliminary study who received unwashed cobalt, which was known to contain other toxic chemicals. Bedding was periodically dusted with rotenone powder. Incomplete reporting for many elements.
<b>Jasmin and Riopelle 1976</b> Rat (Sprague-Dawley) Female (120–140 g) 12 months	Cobalt sulfide NR	Intrarenal placement (in glycerin) Single dose	<b>Kidney neoplasm NOS</b>			<b>Survival</b> was not reported. <b>Strengths:</b> Moderate number of rats per groups. <b>Limitations:</b> 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  $\leq 0.05$ ; \*\* =  $P$ -value  $\leq 0.01$ ; \*\*\* =  $P$ -value  $\leq 0.001$ .

NR = Not reported, inj. = injection, i.p. = intraperitoneal, i.m. = intramuscular, s.c. = subcutaneous, wk = week, mo = month.

<sup>+</sup> = 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, 10 animals were originally assigned to each group (Hansen 2006) or the number of animals that survived beyond day 4, 10 control and 20 exposed animals were originally assigned (Heath and Daniel 1962).

[ ] = Statistical significance calculated by NTP using Fisher's Exact Test.

<sup>a</sup>Historical control group from earlier study by the same author.

### Other neoplasms including those at distal sites

Several lines of evidence support systemic exposure of rats and mice to cobalt. Cobalt concentrations and burdens increased with increasing exposure concentrations in all studies in all tissues examined; however, tissue burdens normalized by exposure concentration showed increased levels only in the liver (NTP 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 that tested cobalt metal and cobalt sulfate (see Table 5-5) (Wehner *et al.* 1977, NTP 1998, 2014b). In the four NTP studies, cobalt metal and cobalt sulfate heptahydrate were each tested in both mice and rats, but adrenal gland neoplasms developed only in rats. One study reported a single adrenal gland neoplasm in hamsters exposed to cobalt(II) oxide (Wehner *et al.* 1977). There is a high background of adrenal tumors in the male rats in the two NTP studies. Adrenal gland neoplasms can develop because of damage to lungs that causes obstructive sequelae by causing systemic hypoxemia, leading to chronic stimulation of catecholamine release by the adrenal medulla and subsequent neoplastic development (NTP 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 ( $5 \text{ mg/m}^3$ ) and all malignant pheochromocytoma, malignant or benign pheochromocytoma combined, and benign pheochromocytoma in both the mid- ( $2.5 \text{ mg/m}^3$ ) 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 ( $3 \text{ mg/m}^3$ ) group in females and the mid-dose ( $1 \text{ mg/m}^3$ ) group in males. Females had a significant trend of increasing tumor incidence with increasing dose for benign pheochromocytoma and all tumor types combined. Hyperplasia was significantly increased in females and the high-dose, but was significantly decreased in the low-dose ( $0.3 \text{ mg/m}^3$ ) males.

Wehner *et al.* (1977) reported finding a single adrenal gland adenoma in the cortex of hamsters after inhalation of cobalt(II) oxide. Wehner *et al.* only tested one dose level,  $10 \text{ mg/m}^3$ , 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 (NTP 2014b, Behl *et al.* 2015). A non-significant increase in the incidence of kidney tumors was observed in male rats. It is not clear whether the kidney tumors were treatment related. Tumors were not observed in the pancreas, kidney, or hematopoietic system of rats exposed to cobalt sulfate or mice exposed to either form of cobalt. Findings are presented in Table 5-5 and briefly summarized below.

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

The incidence of kidney neoplasms (adenoma or carcinoma combined) was higher (although not significantly so) in the low- and high-dose male rats compared to the concurrent controls and a significant trend was observed. The incidence exceeded the historical controls for all routes of administration, but the historical controls are limited as mentioned above. Four of the five neoplasms were adenomas. In analyses of standard and extended evaluations, a significant trend was observed; two of the seven neoplasms in the high-dose group were carcinomas. Kidney neoplasms are relatively rare, so non-significant increases may be related to cobalt exposure (NTP 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) <sup>a</sup> (%)	Comments
<i>Adrenal gland</i>						
<b>NTP 2014b</b> Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal 98% pure mass median aerodynamic diameter 1–3 μm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Bilateral malignant pheochromocytoma</b>			<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> Decreases in body weight in mid and high dose rats. <b>Other comments:</b> Historical controls were limited (100 rats). <b>No significantly increased non-neoplastic lesions.</b>
			0 mg/m <sup>3</sup>	17	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	20	0/50 (0%)	
			2.5 mg/m <sup>3</sup>	16	0/50 (0%)	
			5 mg/m <sup>3</sup>	16	7/50 (14%)**	
			<b>Malignant pheochromocytoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	17	2/50 (5%)	
			1.25 mg/m <sup>3</sup>	20	2/50 (5%)	
			2.5 mg/m <sup>3</sup>	16	9/50 (21%)*	
			5 mg/m <sup>3</sup>	16	16/50 (39%)**	
			Trend-test <i>P</i> -value: 0.001			
			<b>Benign pheochromocytoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	17	15/50 (36%)	
			1.25 mg/m <sup>3</sup>	20	23/50 (54%)	
			2.5 mg/m <sup>3</sup>	16	37/50 (81%)**	
			5 mg/m <sup>3</sup>	16	34/50 (76%)**	
			Trend-test <i>P</i> -value: 0.001			
			<b>Malignant or benign combined pheochromocytoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	17	17/50 (40%)	
			1.25 mg/m <sup>3</sup>	20	23/50 (54%)	
2.5 mg/m <sup>3</sup>	16	38/50 (83%)**				
5 mg/m <sup>3</sup>	16	41/50 (91%)**				
Trend-test <i>P</i> -value: 0.001						

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
<b>NTP 2014b</b> Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal 98% pure, mass median aerodynamic diameter 1–3 µm	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Bilateral malignant pheochromocytoma</b>			<b>Survival</b> was significantly decreased in the mid-dose group. <b>Strengths:</b> A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> A significant decrease in survival of female rats. Decreases in body weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited, as Fischer 344/NTac rats (100 rats). <b>Significantly increased non-neoplastic lesions:</b> Hyperplasia - low and medium
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	1/50 (2%)	
			2.5 mg/m <sup>3</sup>	24	1/49 (2%)	
			5 mg/m <sup>3</sup>	25	4/50 (8%)*	
			<b>Malignant pheochromocytoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	2/50 (5%)	
			2.5 mg/m <sup>3</sup>	24	3/49 (8%)	
			5 mg/m <sup>3</sup>	25	11/50 (27%)***	
			Trend-test <i>P</i> -value: 0.001			
			<b>Bilateral benign pheochromocytoma</b>			
			0 mg/m <sup>3</sup>	35	2/50 (4%)	
			1.25 mg/m <sup>3</sup>	26	4/50 (8%)	
			2.5 mg/m <sup>3</sup>	24	8/49 (16%)*	
			5 mg/m <sup>3</sup>	25	19/50 (38%)**	
			<b>Benign pheochromocytoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	6/50 (14%)	
			1.25 mg/m <sup>3</sup>	26	12/50 (27%)	
			2.5 mg/m <sup>3</sup>	24	22/49 (52%)***	
			5 mg/m <sup>3</sup>	25	36/50 (81%)***	
			Trend-test <i>P</i> -value: 0.001			
			<b>Malignant or benign combined pheochromocytoma<sup>a</sup></b>			
0 mg/m <sup>3</sup>	35	6/50 (14%)				
1.25 mg/m <sup>3</sup>	26	13/50 (29%)				
2.5 mg/m <sup>3</sup>	24	23/49 (55%)***				
5 mg/m <sup>3</sup>	25	40/50 (89%)***				
Trend-test <i>P</i> -value: 0.001						

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments		
<b>NTP 1998</b> Rat (F344) Male (6 wk old) 2 yr	Cobalt sulfate 99% pure	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Benign pheochromocytoma<sup>b</sup></b>				<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors and survival was similar to controls such as long observation period, sufficient dose levels, adequate number of animals.. <b>Limitations:</b> None. <b>No significantly increased non-neoplastic lesions.</b>	
			0 mg/m <sup>3</sup>	17	14/50 (51%)			
			0.3 mg/m <sup>3</sup>	15	19/50 (70%)			
			1.0 mg/m <sup>3</sup>	21	23/48 (72%)			
			3.0 mg/m <sup>3</sup>	15	20/50 (71%)			
			<b>Malignant, benign, or complex pheochromocytoma combined<sup>b</sup></b>					
			0 mg/m <sup>3</sup>	17	15/50 (52%)			
			0.3 mg/m <sup>3</sup>	15	19/50 (70%)			
			1.0 mg/m <sup>3</sup>	21	25/48 (74%)* <sup>c</sup>			
			3.0 mg/m <sup>3</sup>	15	20/50 (71%)			
<b>NTP 1998</b> Rat (F344) Female (6 wk old) 2 yr	Cobalt sulfate 99% pure	Inhalation (dry particulate) 6 hr/day, 5 days/wk × 105 wk	<b>Benign pheochromocytoma<sup>b</sup></b>				<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors such as such as long observation period, sufficient dose levels, adequate number of animals.. <b>Limitations:</b> None <b>Significantly increased non-neoplastic lesions:</b> Adrenal gland: hyperplasia - high dose.	
			0 mg/m <sup>3</sup>	28	2/48 (5%)			
			0.3 mg/m <sup>3</sup>	25	1/49 (3%)			
			1.0 mg/m <sup>3</sup>	26	3/50 (9%)			
			3.0 mg/m <sup>3</sup>	30	8/48 (26%)*			
			Trend-test <i>P</i> -value: 0.004					
			<b>Malignant, benign, or complex combined<sup>b</sup></b>					
			0 mg/m <sup>3</sup>	28	2/48 (4%)			
			0.3 mg/m <sup>3</sup>	25	1/49 (2%)			
			1.0 mg/m <sup>3</sup>	26	4/50 (8%)			
			3.0 mg/m <sup>3</sup>	30	10/48 (21%)* <sup>d</sup>			
			Trend-test <i>P</i> -value: 0.001					

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
<b>Wehner <i>et al.</i> 1977</b> Hamster (Syrian Golden, random bred ENG:ELA) Male (2 mo old) Lifespan	Cobalt(II) oxide Purity not reported Median diameter of particles 0.14 µm, Median mass diameter 0.45 µm Geometric standard deviation 1.9 µm	Inhalation (dry particulate) 7 hr/day, 5 days/wk × lifespan	<b>Adenoma (cortex)</b>			<b>Survival</b> in exposed group is similar to control <b>Strengths:</b> Duration of exposure and observation were sufficient. <b>Limitations:</b> Incomplete reporting. Low sensitivity because of relatively poor survival of both exposed and controls, only a single dose level was tested with no justification for choosing that level. <b>Other comments:</b> 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%)	
<b>Pancreas</b>						
<b>NTP 2014b</b> Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Carcinoma<sup>a</sup></b>			<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels,
			0 mg/m <sup>3</sup>	17	2/50 (5%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (3%)	
			2.5 mg/m <sup>3</sup>	16	5/48 (13%) <sup>c</sup>	
			5 mg/m <sup>3</sup>	16	6/49 (15%) <sup>c</sup>	
			Trend-test <i>P</i> -value: 0.021			

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>a</sup> ) (%)	Comments
			<b>Adenoma<sup>a</sup></b>			adequate number of animals. <b>Limitations:</b> Decreases in body weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited (100 rats). <b>No significantly increased non-neoplastic lesions.</b>
			0 mg/m <sup>3</sup>	17	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (3%)	
			2.5 mg/m <sup>3</sup>	16	6/48 (15%)*	
			5 mg/m <sup>3</sup>	16	3/49 (8%)	
			<b>Carcinoma or adenoma combined<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	17	2/50 (5%)	
			1.25 mg/m <sup>3</sup>	20	2/50 (5%)	
			2.5 mg/m <sup>3</sup>	16	10/48 (25%)* <sup>c</sup>	
			5 mg/m <sup>3</sup>	16	9/49 (23%)* <sup>c</sup>	
			Trend-test <i>P</i> -value: 0.002			
<b>NTP 2014b</b> Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 μm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Carcinoma<sup>a</sup></b>			<b>Survival</b> was significantly decreased in the mid-dose group. <b>Strengths:</b> A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> A significant decrease in survival of female rats. Decreases in body weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited (100 rats). <b>No significantly</b>
			0 mg/m <sup>3</sup>	35	1/50 (2%)	
			1.25 mg/m <sup>3</sup>	26	0/50 (0%)	
			2.5 mg/m <sup>3</sup>	24	0/50 (0%)	
			5 mg/m <sup>3</sup>	25	3/50 (7%) <sup>f</sup>	
			<b>Adenoma</b>			
			0 mg/m <sup>3</sup>	35	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	26	0/50 (0%)	
			2.5 mg/m <sup>3</sup>	24	0/50 (0%)	
			5 mg/m <sup>3</sup>	25	1/50 (2%)	
			<b>Carcinoma or adenoma combined<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	35	1/50 (2%)	
			1.25 mg/m <sup>3</sup>	26	0/50 (0%)	
			2.5 mg/m <sup>3</sup>	24	0/50 (0%)	
5 mg/m <sup>3</sup>	25	3/50 (7%) <sup>f</sup>				

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>+</sup> ) (%)	Comments
						<b>increased non-neoplastic lesions</b>
<i>Hematopoietic system</i>						
<b>NTP 2014b</b> Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Mononuclear cell leukemia<sup>a</sup></b>			<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> None. <b>Other comments:</b> Historical controls were limited (100 rats). <b>No significantly increased non-neoplastic lesions.</b>
			0 mg/m <sup>3</sup>	17	21/50 (49%)	
			1.25 mg/m <sup>3</sup>	20	25/50 (58%)	
			2.5 mg/m <sup>3</sup>	16	22/50 (50%)	
			5 mg/m <sup>3</sup>	16	22/50 (48%)	
<b>NTP 2014b</b> Rat (F344/NTac) Female (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Mononuclear cell leukemia<sup>a</sup></b>			<b>Survival</b> was significantly decreased in the mid-dose group. <b>Strengths:</b> A well-designed study in almost all factors such as long observation period, sufficient dose levels, adequate number of animals. <b>Limitations:</b> A significant decrease in survival of female rats. Decreases in body
			0 mg/m <sup>3</sup>	35	16/50 (36%)	
			1.25 mg/m <sup>3</sup>	26	29/50 (62%)* <sup>g</sup>	
			2.5 mg/m <sup>3</sup>	24	28/50 (61%)* <sup>g</sup>	
			5 mg/m <sup>3</sup>	25	27/50 (59%)* <sup>g</sup>	

Reference & year, animal, study duration	Substance & purity	Dosing regimen	Dose levels	# Animals at sacrifice	Tumor incidence (n/N <sup>+</sup> ) (%)	Comments
						weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited (100 rats). <b>No significantly increased non-neoplastic lesions</b>
<b>Kidney</b>						
<b>NTP 2014b</b> Rat (F344/NTac) Male (5–6 wk old) 105 wk	Cobalt metal (98% pure, mass median aerodynamic diameter 1–3 µm)	Inhalation (dry particulate) 6 hr/day, 5 day/wk × 105 wk	<b>Tubule adenoma<sup>a</sup></b>			<b>Survival</b> was similar to controls. <b>Strengths:</b> A well-designed study in all factors. <b>Limitations:</b> Decreases in body weight in mid- and high-dose rats. <b>Other comments:</b> Historical controls were limited (100 rats.) <b>No significantly increased non-neoplastic lesions</b>
			0 mg/m <sup>3</sup>	17	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (3%) <sup>h</sup>	
			2.5 mg/m <sup>3</sup>	16	0/50 (0%)	
			5 mg/m <sup>3</sup>	16	3/50 (8%) <sup>h</sup>	
			<b>Tubule carcinoma or adenoma<sup>a</sup></b>			
			0 mg/m <sup>3</sup>	17	0/50 (0%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (3%) <sup>h</sup>	
			2.5 mg/m <sup>3</sup>	16	0/50 (0%)	
			5 mg/m <sup>3</sup>	16	4/50 (10%) <sup>h</sup>	
			Trend-test <i>P</i> -value: 0.018			
			<b>Tubule carcinoma or adenoma<sup>ai</sup></b>			
			0 mg/m <sup>3</sup>	17	3/50 (8%)	
			1.25 mg/m <sup>3</sup>	20	1/50 (3%)	
			2.5 mg/m <sup>3</sup>	16	1/50 (2%)	
			5 mg/m <sup>3</sup>	16	7/50 (17%)	
Trend-test <i>P</i> -value: 0.023						

\**P*-value < 0.05; \*\**P*-value < 0.01; \*\*\**P*-value < 0.01.

<sup>+</sup> = Number of animals necropsied for NTP 2014b and NTP 1998 (each group started with 50 animals per sex in the NTP studies) and is the number of animals at the beginning of the study for all other studies.

NR = Not reported, M = male, F = female, hr = hour, wk = week, mo = month, yr = year.

<sup>a</sup>Adjusted percent incidence based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

<sup>b</sup>Adjusted percent incidence based on Kaplan-Meier estimated incidence at the end of the study after adjustment for intercurrent mortality.

<sup>c</sup>Increased over historical control levels with a mean of 176/623 and range of 8% to 50%.

<sup>d</sup>Increased over historical control levels with a mean of 39/608 and range of 2% to 14%.

<sup>e</sup>Increased over historical control levels with a mean of 2/100 and range of 0% to 4%.

<sup>f</sup>Increased over historical control levels with a mean of 1/100 and range of 0% to 2%.

<sup>g</sup>Increased over historical control levels with a mean of 35/100 and range of 32% to 38%.

<sup>h</sup>Increased over historical control levels with a mean of 1/100 and range of 0% to 2%.

<sup>i</sup>Analyzed by standard and extended evaluation. In a standard evaluation a single section of each kidney is examined histologically, while in an extended evaluation, three to four additional sections, taken at 1 mm intervals are examined histologically.

## 5.2 Co-carcinogenicity studies

### 5.2.1 Overview of the studies

Nine co-carcinogen studies were identified that tested soluble compounds, including four studies using cobalt chloride (Kasirsky *et al.* 1965, O'Hara *et al.* 1971, Finogenova 1973, Zeller 1975) and three studies using sodium cobaltinitrite (Orzechowski *et al.* 1964, Thompson *et al.* 1965, O'Hara *et al.* 1971); and a poorly soluble compound, cobalt(II) oxide, in two studies (Wehner *et al.* 1977, Steinhoff and Mohr 1991) (see Table 5-6). Most co-carcinogen studies were conducted in mice, though two studies were conducted in rats (Zeller 1975, Steinhoff and Mohr 1991) 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 CBAx57B <sub>1</sub> (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 <i>et al.</i> 1971
Mouse CF-1 (M&F)	Cobalt chloride	IP inj.	methylcholanthrene dermal	10 wk/ 10wk	Kasirsky <i>et al.</i> 1965
Mouse CF-1 (M&F)	Sodium cobaltinitrite	IP inj.	methylcholanthrene dermal	5 wk/ 17 wk	O'Hara <i>et al.</i> 1971
Mouse CF-1 (M&F)	Sodium cobaltinitrite	Drinking water	methylcholanthrene dermal	11 wk/1 1wk	Thompson <i>et al.</i> 1965
Mouse CF-1 (M&F)	Sodium cobaltinitrite	IP inj.	methylcholanthrene dermal	72 days/ 75 days	Orzechowski <i>et al.</i> 1964
Rat Sprague-Dawley (F)	Cobalt(II) oxide	Intratracheal instill.	benzo[ <i>a</i> ]pyrene intratracheal instill.	47 wk/ lifespan	Steinhoff and Mohr 1991
Hamster Syrian Golden (M)	Cobalt(II) oxide	Inhalation	cigarette smoke inhalation	Lifespan/ lifespan	Wehner <i>et al.</i> 1977

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

### 5.2.2 Overview of the assessment of study quality and utility

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

### 5.2.3 Assessment of findings from co-carcinogenicity studies

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

#### Skin

Four co-carcinogenicity studies of cobalt and methylcholanthrene were reviewed (Orzechowski *et al.* 1964, Kasirsky *et al.* 1965, Thompson *et al.* 1965, O'Hara *et al.* 1971, Finogenova 1973). 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 (Orzechowski *et al.* 1964, Kasirsky *et al.* 1965, Thompson *et al.* 1965). In the fourth study, no differences were seen in the onset or latency of neoplasm development for either skin "cancer NOS" or papilloma from the addition of cobalt chloride (Finogenova 1973). The authors didn't report any tumor incidences.

#### Lung

Two co-carcinogenicity studies used either inhalation or intratracheal instillation as the route of exposure for both the cobalt compound and the known carcinogen (Wehner *et al.* 1977, Steinhoff and Mohr 1991). Steinhoff and Mohr administered benzo[*a*]pyrene and cobalt(II) oxide to female rats by intratracheal instillation. The addition of cobalt(II) oxide increased the incidence of squamous-cell carcinoma of the lung (Steinhoff and Mohr 1991). An adenocarcinoma was also reported in the group exposed to both compounds, but not in the group exposed to just benzo[*a*]pyrene. However, the incidence of adenocarcinoma was not significantly increased by cobalt(II) oxide. Wehner exposed male hamsters to cigarette smoke and cobalt(II) oxide by

inhalation (Wehner *et al.* 1977). No significant change in tumor incidence from the addition of cobalt(II) oxide was reported, but the locations of the neoplasms were not clearly reported.

### **Liver and nose**

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

### **Locations of unspecified neoplastic or non-neoplastic lesions**

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

## **5.3 Synthesis of the findings across studies**

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

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

Most of the neoplasms induced by cobalt compounds occur at the site of administration. Lung tumors are only seen in inhalation or intratracheal instillation studies and tissue sarcoma

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

The most widely studied form of cobalt was cobalt metal. Lung tumors were observed in rats and mice in both sexes after inhalation exposure (NTP 1998, 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 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(II) oxide) caused both lung neoplasms (after intratracheal instillation) in male rats and sarcoma and histiocytoma in several studies of male and/or female rats after injection by various methods (s.c., i.m., i.p.) (Gilman and Ruckerbauer 1962, Steinhoff and Mohr 1991). Inhalation exposure to cobalt(II) oxide did not increase the incidences of lung tumors in Syrian golden hamsters, but the hamster is a less sensitive model for evaluating lung carcinogenicity (Steinhoff and Mohr 1991, McInnes *et al.* 2013) 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
<b><i>Cobalt metal</i></b>					
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 <sub>1</sub> /N (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F	NTP 2014b
Cobalt metal [Nano] <sup>a</sup>	Rat Sprague-Dawley (M)	i.m. inj.	Single dose/1 yr	Injection site Sarcoma, M	Hansen <i>et al.</i> 2006
Cobalt metal [Bulk] <sup>a</sup>	Rat Sprague-Dawley (M)	s.c. inj.	Single dose/1 yr	No increased incidence in tumors Fibroblastic proliferation (non-neoplasia)	Hansen <i>et al.</i> 2006
Cobalt metal	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/1 yr	No increased incidence in tumors	Jasmin and Riopelle 1976
Cobalt metal	Rat Hooded (F)	Intrathoracic	Single dose/2.3 yr	Injection-site sarcoma [including rhabdomyosarcoma of cardiac and intercostal muscle, mixed	Heath and Daniel 1962
Cobalt metal	Rat Hooded (M&F)	i.m. inj.	Single dose/lifespan	Injection-site sarcoma [rhabdomyofibrosarcoma, M&F; sarcoma M; fibrosarcoma F	Heath 1956
<b><i>Soluble cobalt compounds</i></b>					
Cobalt sulfate heptahydrate	Rat F344/N (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma ,M&F Adrenal	NTP 1998

Substance	Strain (sex)	Route	Exposure period/ study duration	Results	Reference
				Benign or malignant pheochromocytoma, F	
Cobalt sulfate heptahydrate	Mouse B6C3F <sub>1</sub> (M&F)	Inhalation	2 yr/2 yr	Lung Alveolar/bronchiolar adenoma and carcinoma, M&F	NTP 1998
Cobalt chloride	Rat Wistar (M)	s.c. inj.	8–12 mo/8–12 mo	Injection site Fibrosarcoma, M  Non-injection site Fibrosarcoma. M	Shabaan <i>et al.</i> 1977
<b>Poorly soluble cobalt compounds</b>					
Cobalt oxide	Rat Sprague-Dawley (M&F)	Intratracheal instill.	1.5 yr/lifespan	Lung Alveolar/bronchiolar carcinoma, benign squamous epithelial neoplasm, or alveolar/bronchiolar adenoma combined M	Steinhoff and Mohr 1991
Cobalt oxide	Rat Sprague-Dawley (M&F)	i.p. inj.	6 mo/lifespan	Injection site Histiocytoma and sarcoma, M&F	Steinhoff and Mohr 1991
Cobalt oxide	Rat Sprague-Dawley (M)	s.c. inj.	730 day/lifespan	Injection site Histiocytoma and sarcoma, M	Steinhoff and Mohr 1991
Cobalt oxide	Rat Wistar (M&F)	i.m. inj.	Single dose/1.3 yr	Injection site Sarcoma, M&F	Gilman and Ruckerbauer 1962
Cobalt oxide	Mouse Swiss (F)	i.m. inj.	Single dose/2 yr	No increased incidence in tumors	Gilman and Ruckerbauer 1962
Cobalt oxide	Hamster Syrian Golden (M)	Inhalation	Lifespan/lifespan	No increased incidence in tumors	Wehner <i>et al.</i> 1977
Cobalt sulfide	Rat Sprague-Dawley (F)	Intrarenal inj.	Single dose/1 yr	No increased incidence in tumors	Jasmin and Riopelle 1976

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

<sup>a</sup>Cobalt bulk and nanoparticle tested in the same animal in the Hansen *et al.* study

## 6 Mechanistic and Other Relevant Effects

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

### 6.1 Cobalt particles and cobalt ions

Studies with toxic metals in general, and cobalt specifically, show that solubility and particle size can play an important role in metal-induced toxicity, genotoxicity, and carcinogenicity (Smith *et al.* 2014). The main cobalt compounds studied for toxicological effects (including both micro- and nanoparticles) are metallic cobalt (Co(0)), cobalt(II) oxide (CoO), cobalt(II,III) oxide (Co<sub>3</sub>O<sub>4</sub>), and various cobalt(II) salts (e.g., cobalt sulfate, cobalt chloride) (Beyersmann and Hartwig 2008, Ortega *et al.* 2014, Smith *et al.* 2014, Sabbioni *et al.* 2014a, Lison 2015). Many cobalt(II) salts are readily soluble in water and biological fluids (see Section 1).

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

**Table 6-1. *In vitro* mechanistic data comparing effects of cobalt nanoparticles, microparticles, and ions**

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

Reference	Cobalt form (size, nm) and cell types	Cytotoxicity	Genotoxicity <sup>a</sup>	ROS	Cellular uptake
Horie <i>et al.</i> 2012	CoO NP (> 10) CoCl <sub>2</sub>  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 <i>et al.</i> 2009	Co <sub>3</sub> O <sub>4</sub> NP (45) CoCl <sub>2</sub>  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 <i>et al.</i> 2007	Co <sub>3</sub> O <sub>4</sub> NP (20-75) Co <sub>3</sub> O <sub>4</sub> /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 <i>et al.</i> 2015	CoNP (2-60) CoCl <sub>2</sub>  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 <sub>2</sub> 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 <sub>2</sub> did not significantly increase ROS.	No data

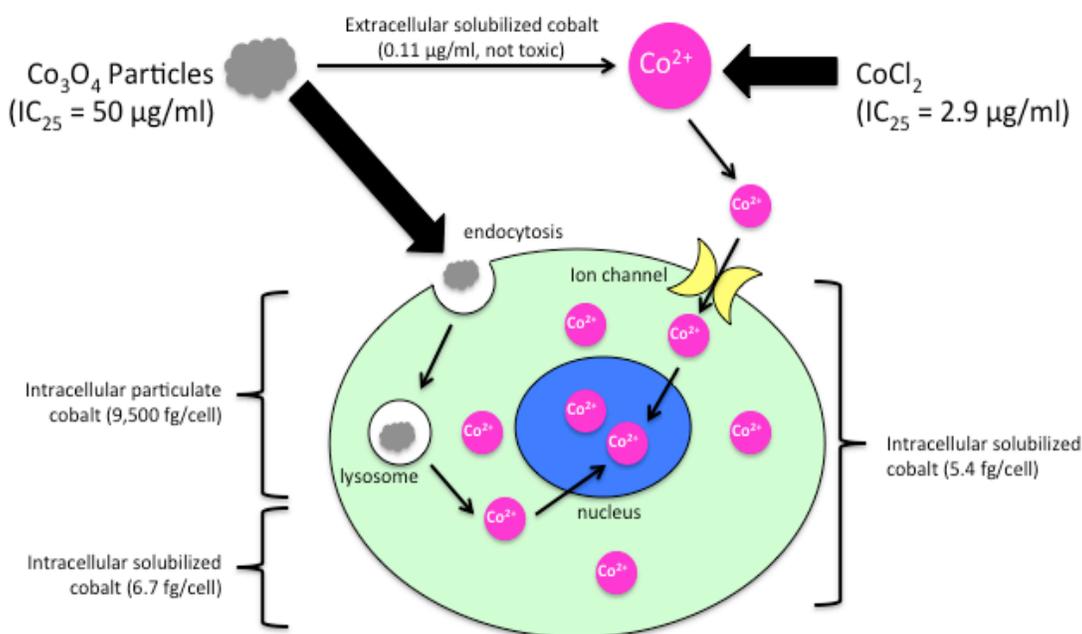
Reference	Cobalt form (size, nm) and cell types	Cytotoxicity	Genotoxicity <sup>a</sup>	ROS	Cellular uptake
Annangi <i>et al.</i> 2014	CoNP (30.7 ± 20.2)  Ogg1 <sup>+/+</sup> and Ogg1 <sup>-/-</sup> mouse embryo fibroblasts (MEF)	NPs induced dose-dependent cytotoxicity in wild-type and knockout MEF cells (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 <i>et al.</i> 2011	Co NP (10–50) CoCl <sub>2</sub>  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 <i>et al.</i> 2009	Co NP (20–500) CoCl <sub>2</sub>  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 <sub>2</sub> induced DNA damage only.	No data	No data
Kwon <i>et al.</i> 2009	Co NP (30) CoSO <sub>4</sub>  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 types	Cytotoxicity	Genotoxicity <sup>a</sup>	ROS	Cellular uptake
Colognato <i>et al.</i> 2008	Co NP (100–500) CoCl <sub>2</sub>  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 <sup>-5</sup> M but similar toxicity at > 2 x 10 <sup>-5</sup> 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 <i>et al.</i> 2007	Co NP (28) CoCl <sub>2</sub>  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).

<sup>a</sup>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  $\text{Co}_3\text{O}_4$  particles, human lung cells exposed to the  $\text{IC}_{25}$  (inhibitory concentration at which the ATP content was reduced by 25% compared to non-exposed cells) of cobalt chloride (2.9  $\mu\text{g}/\text{mL}$ ) or  $\text{Co}_3\text{O}_4$  (50  $\mu\text{g}/\text{mL}$ ) had similar intracellular concentrations of solubilized cobalt (6.5 fg/cell for  $\text{Co}_3\text{O}_4$  compared to 5.4 fg/cell for cobalt chloride) (Figure 6-1). Smith *et al.* (2014) also reported that at intracellular cobalt concentrations less than 1,000  $\mu\text{M}$ , the cytotoxic effects of cobalt chloride and  $\text{CoO}$  to human lung fibroblasts were similar while cobalt chloride was more cytotoxic than  $\text{CoO}$  at intracellular concentrations greater than 1,000  $\mu\text{M}$ . 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 (Simonsen *et al.* 2012, Paustenbach *et al.* 2013, Ortega *et al.* 2014, Smith *et al.* 2014).



**Figure 6-1. Cellular uptake of cobalt particles and ions**

Source: Ortega *et al.* 2014

Because of similar physical/chemical properties, cobalt ions compete with essential divalent metal ions (e.g., calcium, copper, zinc, iron, manganese, and magnesium) for absorption, specific receptor activation, and ion channel transport (Paustenbach *et al.* 2013). For example, cobalt absorption is increased in humans and animals with iron deficiency suggesting that these metals share a common uptake mechanism (Thomson *et al.* 1971). Further, cobalt ions have the same

size and charge as zinc ions; therefore, both ions bind to the same types of ligands (e.g., oxygen, nitrogen, and sulfur groups of biomolecules) (Beyersmann and Hartwig 2008). The bioavailability of cobalt ions *in vivo* is limited because of extensive binding (90% to 95%) to serum proteins (e.g., albumin,  $\alpha_2$ -macroglobulin) (Simonsen *et al.* 2012, Paustenbach *et al.* 2013). Thus, the concentration of free, ionized cobalt in serum is about 5% to 12% of the total cobalt concentration (Simonsen *et al.* 2012). However, Heath *et al.* (1969) demonstrated that myoblasts exposed to cobalt-bound protein complexes (primarily globulin and albumin), but not to cobalt chloride, developed cytological alterations (e.g., enlarged hyperchromatic nucleoli, chromocenters, and nuclei) in actively growing cultures that were similar to those seen in pre-malignant myoblasts *in vivo*. In contrast, myoblasts exposed to cobalt chloride were either killed or showed no cytological abnormalities when exposed to sublethal concentrations.

Differences in toxicity reported for cobalt particles and ions may be partially explained by differences in cellular uptake mechanisms (see Figure 6-1). Cobalt ions first saturate binding sites in the extracellular milieu and on cell surfaces and, after saturation, are actively transported inside the cell via metal ion transport systems such as calcium channels or divalent metal ion transporters (Garrick *et al.* 2003, Simonsen *et al.* 2012, Smith *et al.* 2014, Sabbioni *et al.* 2014a). 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<sub>3</sub>O<sub>4</sub>) particles show that these particles readily enter cells through endocytosis via a clathrin-mediated pathway (called a Trojan-horse type mechanism) and are partially solubilized in the low pH environment within the lysosomes (Limbach *et al.* 2007, Papis *et al.* 2009, Ortega *et al.* 2014, Smith *et al.* 2014). Although the intracellular solubilized cobalt content was small compared to the intracellular particulate content, the data suggest that the solubilized fraction was responsible for the overall toxicity to human lung cells (Ortega *et al.* 2014, Smith *et al.* 2014).

Endocytosis of Co<sub>3</sub>O<sub>4</sub> particles was a more efficient uptake pathway compared to the specific transport or ionic pumps involved with uptake of cobalt ions (Ortega *et al.* 2014). These studies also demonstrated that concentrations of extracellular solubilized cobalt were too low to induce cytotoxicity and that particle-to-cell contact was necessary to generate high intracellular cobalt levels. Further, cobalt particles taken up by lung cells can lead to long-term intracellular release of toxic metal ions. Similarly, cobalt metal nanoparticles are internalized by phagocytosis and endocytosis and spread rapidly to the cytoplasm, cellular organelles, and nucleus where they release cobalt ions (Ponti *et al.* 2009, Sabbioni *et al.* 2014a). 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.* (2014a) also reported that the intracellular distribution of cobalt in Balb/3T3 cells was different following exposure to cobalt nanoparticles compared to cobalt ions. Cells exposed to cobalt nanoparticles had a higher nuclear fraction and a lower cytoplasmic fraction than cells exposed to cobalt ions. The amount of cobalt bound to DNA was significantly greater in cells

exposed to cobalt microparticles than nanoparticles but was the lowest in cells exposed to cobalt ions (tested concentrations were 10 and 100  $\mu\text{M}$  for 4 hours). Intracellular distribution studies in primary rhabdomyosarcoma induced by intramuscular injection of metallic cobalt also reported that most of the total cellular content of cobalt was associated with the nuclear fraction and was bound by components of the nucleoplasm, chromatin, and nucleoli (Heath and Webb 1967, Webb *et al.* 1972).

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

## 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 (Beyersmann and Hartwig 2008, Koedrith and Seo 2011, Angelé-Martínez *et al.* 2014). These include (1) oxidative stress, (2) DNA repair modulation, and (3) disturbances of signal transduction pathways that affect cell growth and differentiation. Modes of action most likely involved in cobalt-induced carcinogenesis are consistent with these general mechanisms and include: (1) genotoxicity and inhibition of DNA repair, (2) induction of reactive oxygen species (ROS) and oxidative damage, and (3) induction of hypoxia-like responses by activating hypoxia-inducible factors (HIFs) (see Figure 6-2) (Lison *et al.* 2001, De Boeck *et al.* 2003a, Simonsen *et al.* 2011, Magaye *et al.* 2012, Simonsen *et al.* 2012, Green *et al.* 2013, Smith *et al.* 2014).

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

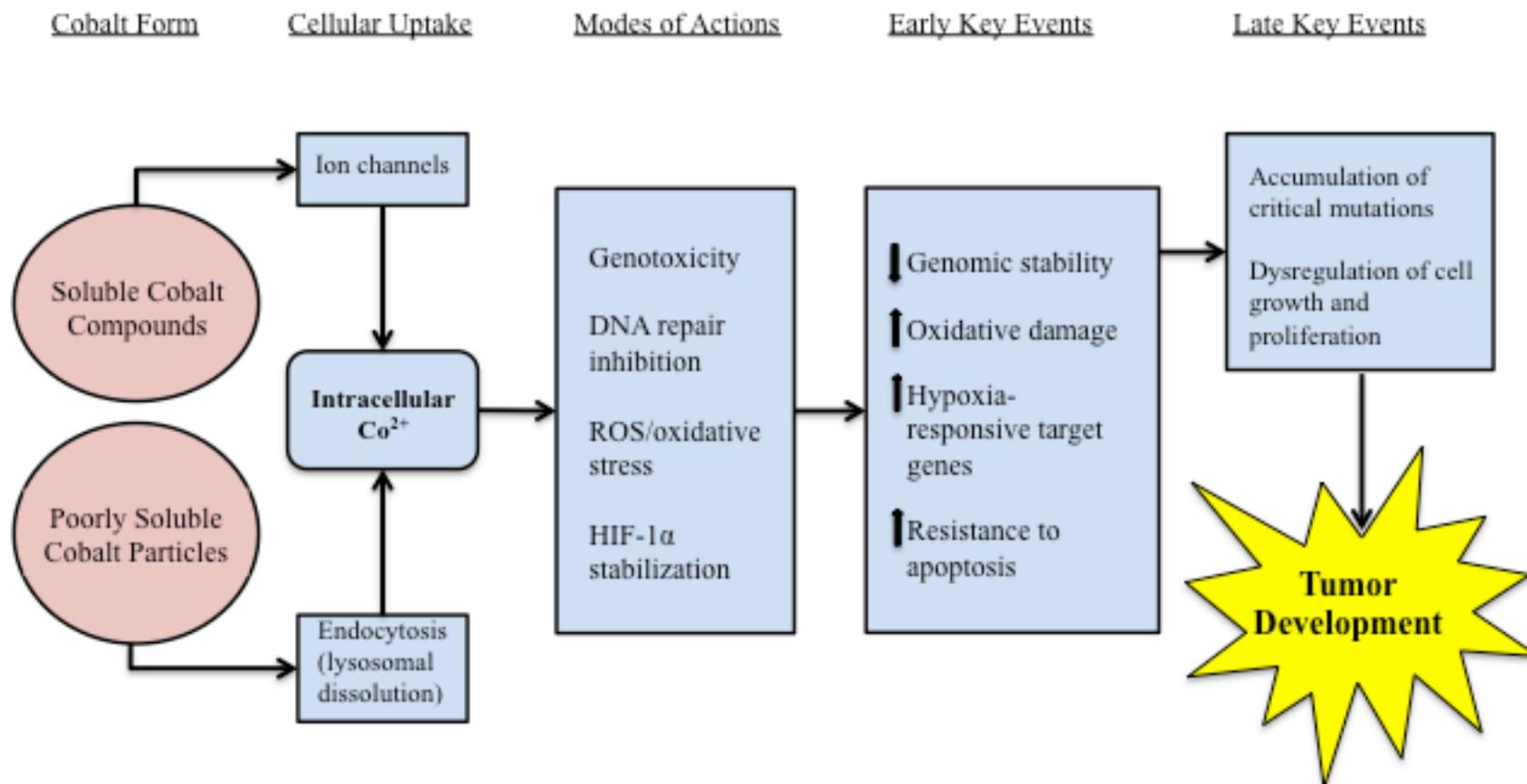


Figure 6-2. Proposed modes of action of cobalt carcinogenicity.

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

### 6.2.1 Genotoxicity, inhibition of DNA repair, and related key events

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

#### Overview of genotoxicity findings

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

Some recent *in vitro* studies are consistent with the earlier data and show that cobalt ions and particles induce genotoxic effects in human and animal cells, but the studies also compared effects and relative potency of cobalt ions and particles (Table 6-1) (Colognato *et al.* 2008, Ponti *et al.* 2009, Patel *et al.* 2012, Alarifi *et al.* 2013, Smith *et al.* 2014). Smith *et al.* (2014) compared the effect of CoO particles with cobalt chloride and reported similar genotoxic effects (primarily chromatid lesions); however, particle-to-cell contact was required to induce genotoxicity from CoO. Soluble cobalt also induced cell-cycle arrest at a much lower intracellular cobalt concentration than CoO. Alarifi *et al.* (2013) compared Co<sub>3</sub>O<sub>4</sub> nanoparticles and cobalt chloride and reported that both forms caused DNA damage in human HepG2 cells but the nanoparticles were more potent. Two studies investigated the genetic effects of metallic cobalt nanoparticles in Balb/3T3 mouse fibroblast (Patel *et al.* 2012) and human leukocytes (Colognato *et al.* 2008). Cobalt nanoparticles induced DNA strand breaks, micronuclei, and cell transformation in mouse fibroblasts and DNA damage in human leukocytes. Cobalt ions had no effect in human leukocytes but induced DNA damage in mouse fibroblasts. Ponti *et al.* (2009) reported that cobalt chloride induced double-strand breaks in human lung epithelial cells and that the effects were increased with co-exposure to nickel chloride.

Potential molecular mechanisms for cobalt-induced genotoxicity (primarily clastogenic effects) include (1) a direct effect of cobalt(II) ions to induce oxidative damage to DNA through a Fenton-like mechanism (see Section 6.2.2), and (2) an indirect effect of cobalt(II) ions to inhibit

repair of DNA damage caused by endogenous events or induced by other agents (IARC 2006, Lison 2015). These mechanisms are discussed below.

### **Inhibition of DNA repair**

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

### **Key events**

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

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

Cobalt metal particles, water-soluble cobalt compounds (cobalt chloride, cobalt sulfate, cobalt acetate), and water-insoluble cobalt compounds (cobalt sulfides) induced cell transformation in different types of rodent cells (C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), or BALB/3T3 cells) (Casto *et al.* 1979, Abbracchio *et al.* 1982, Costa *et al.* 1982, Kerckaert *et al.* 1996, Doran *et al.* 1998, Ponti *et al.* 2009, Annangi *et al.* 2014, Sighinolfi *et al.* 2014, Sabbioni *et al.* 2014b). A few studies of either cobalt chloride Ponti *et al.* 2009, Sabbioni *et al.* 2014b) or cobalt metal (Doran *et al.* 1998) were negative, which could be due to differences in experimental conditions such as the types of cells, because positive findings were found for the same cobalt forms using other experimental conditions. Sabbioni *et al.* (2014b) reported that Type III foci in Balb/3TC cells induced by cobalt nanoparticles and microparticles were inhibited by ascorbic acid and suggested that the response was dependent on ROS production and lipid peroxidation. Another study found that mouse fibroblast cells without a DNA base-pair excision gene (8-oxoguanine glycosylate, *Ogg1*<sup>-/-</sup>) were more sensitive to cobalt nanoparticle-induced cellular transformation (after 12 weeks of exposure to sub-toxic doses) compared to wild type cells (*Ogg1*<sup>+/+</sup>) (Annangi *et al.* 2014). Ogg is involved in the repair of 8-oxoguanine and thus, this study supports the role of oxidative DNA damage in cobalt carcinogenicity. Oxidative stress is discussed in more detail in the following section.

Table 6-2. Summary assessment of genotoxicity and related effects for cobalt compounds<sup>a</sup>

Endpoint (Test system)	Cobalt chloride		Cobalt sulfate		Cobalt nitrate		Cobalt(II) oxide		Cobalt(II,III) oxide		Cobalt acetate		Cobalt metal		Cobalt sulfide		Cobalt nanoparticles	
	<i>In vitro</i> <sup>b</sup>	<i>In vivo</i>	<i>In vitro</i> <sup>b</sup>	<i>In vitro</i> <sup>c</sup>	<i>In vivo</i>	<i>In vitro</i> <sup>b</sup>	<i>In vivo</i>	<i>In vitro</i> <sup>b</sup>	<i>In vivo</i>	<i>In vitro</i> <sup>c</sup>	<i>In vitro</i> <sup>c</sup>	<i>In vitro</i> <sup>c</sup>	<i>In vitro</i> <sup>c</sup>					
<b><i>Mutation</i></b>																		
Mutation (prokaryotes)	(-)		(-)											(-)				
Mutation (eukaryotes)	±				+											±		-
<b><i>Chromosomal damage/cytogenetic effects</i></b>																		
Chromosomal aberrations	+	+	+	-	±													
Micronucleus induction	±	+												+		-		
Recombination	+				+													+
Gene conversion	(+)																	
Aneuploidy	+	+	+															
Sister chromatid exchange	+																	
<b><i>DNA damage and repair</i></b>																		
DNA damage/ strand breaks or bases	+		+	+				+			+		+			+		+
DNA repair inhibition	+										+		+					
<b><i>Binding/cross-links</i></b>																		
DNA-protein crosslinks	+		+										+					+
DNA-protein binding inhibition	+				+													

Sources: IARC 2006 review and additional primary references as described in tables and text.

Positive +, mostly positive evidence (+), mixed results ±, mostly negative evidence (-), and negative -.

<sup>a</sup>No column is included for *in vivo* results if none were identified.

<sup>b</sup>Results shown are for -S9; test +S9 was negative.

<sup>c</sup>Results shown are for -S9; not tested with the addition of metabolic activation (S9).

### 6.2.2 Oxidative stress and damage

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) induce oxidative and nitritative 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* (Kasprzak 2002, Valko *et al.* 2005, Valko *et al.* 2006, Beyersmann and Hartwig 2008, Jomova and Valko 2011, Koedrith and Seo 2011). 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- $\alpha$ ) (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 (Petit *et al.* 2005, Valko *et al.* 2005, Jomova and Valko 2011, Romero *et al.* 2014).

#### Mechanisms of ROS

Direct interactions between cobalt metal or ions and oxygen or lipids can generate ROS. High concentrations (10 mg/mL) of aqueous suspensions of Co(0) metal particles can react with dissolved oxygen to generate hydrogen peroxide and hydroxyl radicals in the presence of superoxide dismutase (SOD) as illustrated below (reactions 1-3) (Leonard *et al.* 1998, Jomova and Valko 2011, Lee *et al.* 2012). 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) (Shi *et al.* 1993, Mao *et al.* 1996, Leonard *et al.* 1998). 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 (Vaca *et al.* 1988, Shi *et al.* 1993, Barrera 2012). 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.



### Evidence for cobalt-induced oxidative stress

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

Both cobalt ions and cobalt metal can catalyze the formation of ROS *in vivo* and *in vitro* (Moorhouse *et al.* 1985, Kadiiska *et al.* 1989, Kawanishi *et al.* 1989, Lewis *et al.* 1991, Hanna *et al.* 1992, Lewis *et al.* 1992, Kawanishi *et al.* 1994, Zou *et al.* 2001, Dick *et al.* 2003, Pourahmad *et al.* 2003, Kotake-Nara and Saida 2007, Limbach *et al.* 2007, Peters *et al.* 2007, Papis *et al.* 2009, Qiao *et al.* 2009, Patel *et al.* 2012, Alarifi *et al.* 2013, Annangi *et al.* 2014, Scharf *et al.* 2014, Chattopadhyay *et al.* 2015). 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 (Zou *et al.* 2001, Pulido and Parrish 2003). 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  $\text{Co}_3\text{O}_4$ , and Lewis *et al.* (1991, 1992) reported evidence of oxidative stress in hamster lung following exposure to cobalt ions *in vivo* and *in vitro*. Evidence of oxidative stress included decreased levels of reduced glutathione, increased levels of oxidized glutathione, and increased activity of the pentose phosphate pathway.

Simultaneous incubation with hydrogen peroxide potentiated cobalt-induced increases in levels of oxidized glutathione and pentose phosphate pathway activity. Although the data suggested that oxidation of glutathione occurred as an early event in cobalt-induced lung toxicity, the data did not indicate that glutathione oxidation related directly to the observed toxicity. Thus, oxidative effects that occur at sites other than the glutathione system might mediate cobalt toxicity.

### **Oxidative stress and DNA damage**

As mentioned above, one of the likely mechanisms for cobalt particles and ions to induce genetic damage is through ROS and oxidative stress. Several types of DNA damage are associated with ROS including single- and double-strand breaks, base modifications, deoxyribose modification, and DNA cross-linking (Klaunig *et al.* 2010). If not repaired prior to DNA replication, DNA damage can lead to cell death, mutations, replication errors, and genomic instability. Kasprzak *et al.* (1994) reported oxidative damage to DNA in the liver, kidney, and lung of rats injected with cobalt ions.

Two studies, using different cobalt forms, evaluated *K-ras* mutations (Figure 6-3) in cobalt-induced lung neoplasms of B6C3F<sub>1</sub> mice (cobalt metal and cobalt sulfate heptahydrate) or F344/NTac rats (cobalt metal only). Rodents were exposed by inhalation (NTP 1998, 2014a, Hong *et al.* 2015). Both studies found a higher frequency of G to T transversions in codon 12 of the *K-ras* gene in cobalt-induced neoplasms compared to spontaneous lung neoplasms from historical control or other laboratory control rodents. In contrast, the predominant type of *K-ras* mutation observed in spontaneous lung tumors from historical control mice was G to A transitions. No *K-ras* mutations were observed in spontaneous lung tumors in the concurrent or historical control rats. *K-ras* G to T transversion mutations are associated with the production of 8-hydroxydeoxyguanosine, an oxidative DNA lesion that is formed when ROS reacts with deoxyguanosine (Klaunig *et al.* 2010, Itsara *et al.* 2014). This is also consistent with the mutation pattern observed in bacteria (i.e., results were correlated with ability of the tester strain to detect mutational events at G:C base pairs) (Hong *et al.* 2015) G to T transversions are also the most common type of mutation observed in human lung tumors in the *p53* gene (Harty *et al.* 1996). These studies suggest that oxidative DNA damage may play a role in cobalt-mediated lung tumorigenicity.

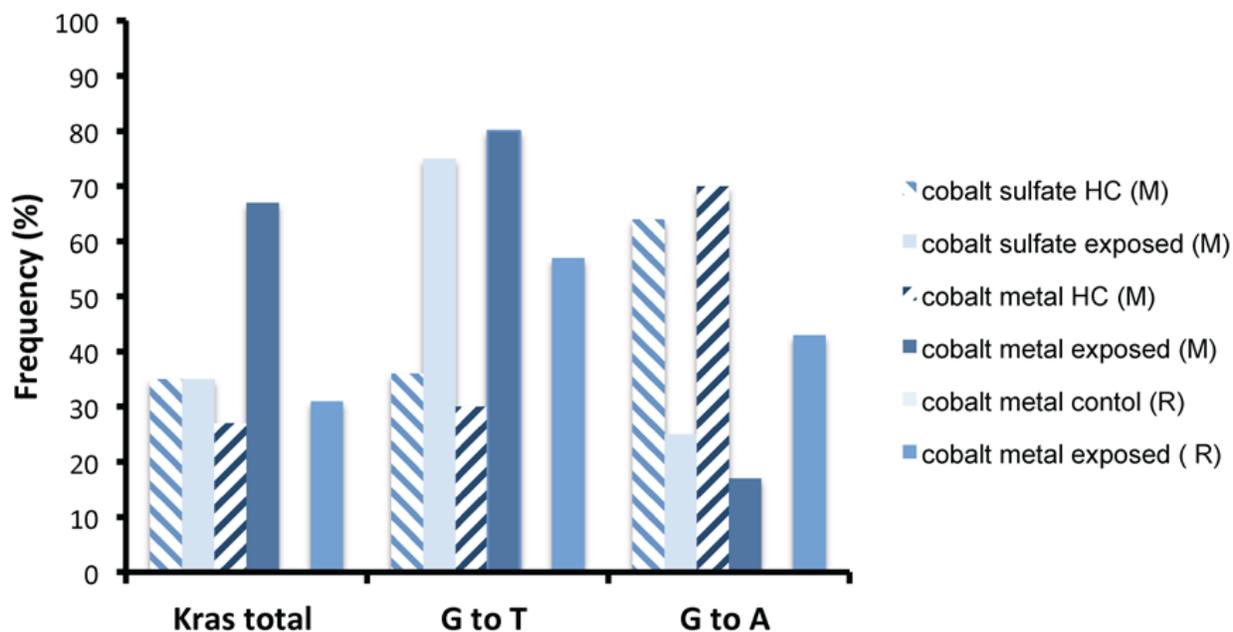


Figure 6-3. K-ras mutations in lung tumors from cobalt-exposed and non-exposed rodents

Sources: NTP 1998, 2014a, Hong *et al.* 2015.

HC = historical control, M = mouse, R = rat.

Frequency of K-ras mutations from lung tumors in mice or rats exposed to cobalt metal or cobalt sulfate and spontaneous tumors. Total K-ras is the incidences of any K-ras mutation detected in all samples and includes mutations in codon 12, 13, and 61. G to T and G to A is the frequency of these specific mutations occurring in codon 12 only. i.e., the total number of K-ras mutations in codon 12 is the denominator.

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

### 6.2.3 HIF stabilization and hypoxia mimicry

Oxygen homeostasis in mammals is tightly regulated in order to provide sufficient oxygen levels to body tissues and cells while minimizing production of ROS (Bracken *et al.* 2003). HIFs are heterodimers composed of a labile  $\alpha$  subunit and a stable  $\beta$  subunit and are the primary transcriptional regulators that mediate the cellular response to hypoxia (Salnikow *et al.* 2004, Forooghian *et al.* 2007, Galanis *et al.* 2008, Befani *et al.* 2013, Zhang *et al.* 2014, Davidson *et al.* 2015). The  $\alpha$  subunit is post-translationally regulated by oxygen and is rarely detectable at normal oxygen tension, while the  $\beta$  subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed. There are three known isoforms of the  $\alpha$  subunit (HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$ ) in humans and mammals; however, the HIF-1 $\alpha$  subunit

is the most widely studied (Zhang *et al.* 2014, Wolff *et al.* 2013, Forooghian *et al.* 2007, Bracken *et al.* 2003). HIF-1 $\alpha$  and HIF-2 $\alpha$  share significant structural homology and function and are expressed in multiple tissues and cell types in response to hypoxia (Befani *et al.* 2013). Less is known about HIF-3 $\alpha$ ; however, it is a transcriptional target of HIF-1 $\alpha$  (Tanaka *et al.* 2009). Most studies with cobalt investigated its effects on HIF-1 $\alpha$ ; however, Befani *et al.* (2013) reported that cobalt stimulated HIF-1-dependent gene expression in two human liver cancer cell lines but inhibited HIF-2-dependent gene expression. Therefore, the following discussion is limited to HIF-1 $\alpha$ .

### **Cobalt-induced HIF stabilization**

HIF-1 overexpression and enhanced transcriptional activity are linked to cancer initiation and progression. There is strong experimental support that cobalt is a potent inducer of HIF-1 $\alpha$  activation. Cobalt metal particles, cobalt chloride, and cobalt sulfate heptahydrate promote a hypoxia-like state *in vivo* and *in vitro*, even with normal molecular oxygen pressure, by stabilizing HIF-1 $\alpha$  (Maxwell and Salnikow 2004, Beyersmann and Hartwig 2008, Galanis *et al.* 2009, Qiao *et al.* 2009, Xia *et al.* 2009, Saini *et al.* 2010a, Saini *et al.* 2010b, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Nyga *et al.* 2015). 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 (Wang and Semenza 1995, Ardyanto *et al.* 2008, Fu *et al.* 2009). Cobalt chloride-induced hypoxia also increased the invasiveness of one primary breast cancer cell line (Fu *et al.* 2009). Human A549 lung adenocarcinoma cells exposed to cobalt chloride overexpressed Cap43, a hypoxia-regulated gene (Salnikow *et al.* 2000). Increased expression of Cap43 was reported in tumors and serum of lung cancer patients compared to adjacent normal tissues and may be predictive of tumor angiogenesis and poor prognosis (Azuma *et al.* 2012, Wang *et al.* 2012). Permenter *et al.* (2013) investigated gene expression and intracellular protein abundance in two rat liver cell lines exposed to cobalt chloride. Many genes, proteins, and pathways were modulated, which were mainly due to induction of a hypoxia-like response and oxidative stress. These data were consistent with gene expression profiling in hypoxia signaling in human hepatocellular carcinoma (Hep3B) cells exposed to cobalt chloride (Vengellur *et al.* 2005). Cobalt nanoparticles and ions also induced a time-dependent increase in HIF-target genes and expression of proinflammatory cytokines in the U937 human monocytic cell line (Nyga *et al.* 2015).

Under normal oxygen conditions, the iron-containing oxygen-sensing enzymes (prolyl hydroxylases) and an asparagine hydroxylase that hydroxylate specific proline or asparagine residues in the HIF-1 $\alpha$  subunits (Maxwell and Salnikow 2004). Hydroxylated HIF-1 $\alpha$  binds to a multiprotein complex that contains the von Hippel-Lindau (VHL) tumor suppressor. VHL acts as part of an ubiquitin ligase complex resulting in rapid ubiquitination and proteolysis of HIF-1 $\alpha$ . Under hypoxic conditions, HIF-1 $\alpha$  subunits are not hydroxylated, and consequently the protein is stabilized and translocates to the nucleus where it binds with a HIF-1 $\beta$  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-1 $\alpha$  such as prolyl

hydroxylases and VHL (Davidson *et al.* 2015). Possible mechanisms by which cobalt ions activate HIF-1 include replacing iron in the regulatory prolyl hydroxylases or depleting intracellular ascorbate (a cofactor for prolyl hydroxylase activity), thus, deactivating these enzymes (Maxwell and Salnikow 2004, Salnikow *et al.* 2004, Qiao *et al.* 2009, Davidson *et al.* 2015). Kang *et al.* (2006) reported that metal-induced (cobalt or nickel) HIF-1 $\alpha$  stabilization was reversed in human lung carcinoma A549 cells when the cells were treated simultaneously with iron and metal ions. Oxidative stress does not appear to be a primary mechanism of cobalt-induced HIF activation. Salnikow *et al.* (2000) found that while cobalt and nickel produced oxidative stress in A549 cells, activation of HIF-1-dependent genes was independent of ROS formation. Although the mitochondria are a main target of cobalt toxicity and generate ROS that trigger hypoxia-induced transcription, cobalt activates hypoxia-induced transcription via a mitochondria-independent mechanism (Chandel *et al.* 1998, Karovic *et al.* 2007). In a study with rat hepatocytes, lysosomes were shown to be the source of ROS formation with redox transition metals (including cobalt), while the mitochondria were the source of ROS formation for non-redox or poor redox cycling transition metals (Pourahmad *et al.* 2003). Nyga *et al.* (2015) also reported evidence that HIF-1 $\alpha$  stabilization in human macrophages treated with cobalt metal nanoparticles or cobalt ions occurred via an ROS-independent pathway.

### Hypoxia mimicry

HIF-1 $\alpha$  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 (Wang and Semenza 1995, Beyersmann and Hartwig 2008, Greim *et al.* 2009, Saini *et al.* 2010a, Saini *et al.* 2010b, Simonsen *et al.* 2012, Gao *et al.* 2013). The evidence suggests that HIF-1 $\alpha$  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 (Lee *et al.* 2001, Maxwell and Salnikow 2004, Hammond and Giaccia 2005, Ardyanto *et al.* 2008, Greim *et al.* 2009).

HIF-1 $\alpha$  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 (Maxwell and Salnikow 2004, Paul *et al.* 2004, Galanis *et al.* 2008, Galanis *et al.* 2009, Cheng *et al.* 2013). Greim *et al.* (2009) also identified hypoxia and HIF activation as a relevant mechanism for pheochromocytoma in rats. Further evidence for a role of HIF-1 in cancer is as follows: (1) enhanced glycolytic and angiogenic activities are hallmarks of many tumors and are consequences of HIF-1 activation, (2) immunolabelling for HIF-1 $\alpha$  subunits confirms there is a common activation in solid tumors, (3) genetic studies comparing tumor growth with and without HIF-1 have generally shown that tumors without specific HIF subunits have decreased vascularization and growth, (4) a number of pathways implicated in cancer progression increase activation of the HIF-1 pathway in normoxia and hypoxia, and (5) as described above, the VHL tumor suppressor protein is required to regulate HIF-1 (Maxwell and Salnikow 2004). VHL loss of function results in constitutive HIF activation and an increased risk of developing cancer and is evident in people with VHL disease. VHL

disease is a hereditary cancer syndrome that is caused by inactivation of the VHL protein (Ben-Skowronek and Kozaczuk 2015). This disease is characterized by the development of multiple vascular tumors including pheochromocytomas, pancreatic islet cell tumors, renal cell tumors, retinal and central nervous system hemangioblastomas, and others. Loss of the VHL protein results in elevated levels of HIF and leads to increased production of VEGF, platelet-derived growth factor (PDGF), transforming growth factor  $\alpha$  (TGF- $\alpha$ ) and other hypoxia-responsive transcripts that promote cell growth and angiogenesis. HIF also contributes to overproduction of tyrosine hydroxylase and catecholamines in pheochromocytomas.

### 6.3 Other biological effects

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

Potential mechanisms for these endpoints might be some of the modes of action discussed in Section 6.2 as well as other cobalt-related biological responses. Oxidative damage and inflammatory events are characteristics of fibrosing alveolitis (hard-metal lung disease) and lung cancer and there is some evidence that lung fibrosis is a risk factor for lung cancer (IARC 2006). Lung injury may be due in part to cobalt-induced apoptosis which is primarily mediated via loss of mitochondrial membrane potential and release of cytochrome c and apoptosis-inducing factor (AIF) (Araya *et al.* 2002, Pulido and Parrish 2003, Karovic *et al.* 2007, Battaglia *et al.* 2009). Cobalt also competes with essential divalent metal ions (e.g., calcium, copper, zinc, iron, manganese, and magnesium) for absorption, specific receptor activation, and ion channel transport (Paustenbach *et al.* 2013) and interferes with a number of iron-containing proteins such as the iron regulatory protein 1 (IRP1)/iron response element (IRE) system and various iron-sulfur cluster proteins that are important for maintaining iron homeostasis, energy production, metabolism, gene expression, DNA/RNA processing and repair, and defense against oxidative stress (Lee *et al.* 2006, Sheftel *et al.* 2010, Davidson *et al.* 2015). Thus, disruption of iron homeostasis could potentially lead to numerous adverse health effects, including cancer.

### 6.4 Synthesis

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

activation of HIF-1 $\alpha$  likely contribute to cobalt-induced neoplastic development and progression. All of these mechanisms are relevant to humans.

**This Page Intentionally Left Blank**

## 7 Overall Cancer Evaluation and NTP Listing Recommendation

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

### *NTP listing recommendation*

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

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

### 7.1 Cobalt and cobalt compounds that release cobalt ion *in vivo* as a class

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

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

- Physicochemical properties and toxicokinetics (Section 7.1.1)

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

### 7.1.1 Physicochemical properties and toxicokinetics

Physicochemical properties and toxicokinetic data for cobalt metal and various cobalt compounds were presented in Sections 1 and 3. Solubility, particle size, bioavailability, and cellular uptake and retention affect toxicity. These data show the following general rank order for aqueous solubility: cobalt(II) salts > cobalt metal > cobalt oxides. Bioaccessibility, defined as the availability of a metal for absorption when dissolved in artificial body fluids, is often used as an *in vitro* surrogate for bioavailability testing (Stopford *et al.* 2003). Bioaccessibility measurements showed the same general rank order as aqueous solubility at near neutral pH but, in acidic solutions associated with lysosomes (pH 4.5) or gastric fluid (pH 1.5), bioaccessibility was 100% or near 100% for cobalt metal and several cobalt compounds tested including water-soluble and several poorly soluble compounds indicating that they release cobalt ions in solution (see Table 1-1). Although very low values ( $\leq 2\%$ ) for bioavailability in artificial gastric and lysosomal fluids have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the same test conditions, other, more informative (e.g., physiologically relevant) test conditions in the presence of lung cells (Section 1.2.2) have found higher bioavailability values for cobalt(II,III) oxide (i.e.,  $\text{Co}_3\text{O}_4$ ) in culture media with alveolar macrophages and other studies have reported its uptake by lung cells, which suggests that  $\text{Co}_3\text{O}_4$  would release ions *in vivo*.

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

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

the body. Elimination of cobalt particles and ions is multiphasic with fast, intermediate, and slow phases; however, soluble compounds are cleared faster with a smaller fraction of the dose retained long term.

### 7.1.2 Mechanistic and other relevant data

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

### 7.1.3 Toxicological effects and key events

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

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

when complexed with endogenous chelators such as glutathione or anserine. In addition, mutations in lung tumors induced by cobalt sulfate or cobalt metal included G to T transversions that are characteristic of oxidative damage.

#### 7.1.4 Overall synthesis

Several biological endpoints were identified from physicochemical, toxicological, and mechanistic data for cobalt metal, cobalt chloride, cobalt sulfate, and cobalt oxides (CoO and Co<sub>3</sub>O<sub>4</sub>). These cobalt forms were the most studied and included both soluble and poorly soluble forms (see Table 7-1 for synthesis of available information). Data for two cobalt oxides, CoO and Co<sub>3</sub>O<sub>4</sub>, were combined because both are poorly water-soluble, but are bioavailable (under more informative, physiologically relevant, test conditions in the presence of lung cells; see Sections 1.2.2 and 7.1.1) since they enter cells by endocytosis and release Co ions in the lysosomes, and induced similar biological effects (e.g., genotoxicity and cytotoxicity). Although data was not available for all endpoints for each oxide, overall the mechanistic data support the inclusion of both oxides in the class of cobalt compounds that release ions *in vivo*.

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

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

Endpoint	Soluble cobalt salts	Cobalt metal	Poorly soluble cobalt compounds
	CoCl <sub>2</sub> and/or CoSO <sub>4</sub>	Particles	CoO and/or Co <sub>3</sub> O <sub>4</sub>
Bioaccessibility			
<i>Lysosomal fluid</i>	+	+	+ <sup>a</sup>
<i>Gastric fluid</i>	+	+	+ <sup>b</sup>
Cellular uptake	+	+	+ <sup>c</sup>
Cytotoxicity	+	+	+ <sup>c</sup>
ROS	+ <sup>d</sup>	+	+ <sup>e</sup>
HIF-1 $\alpha$ stabilization	+	+	+ <sup>f</sup>
DNA repair inhibition	+ <sup>d</sup>	+	ND
Genotoxicity <i>in vitro</i>	+	+	+ <sup>g</sup>
Genotoxicity <i>in vivo</i>	+ <sup>d</sup>	-	ND
Animal carcinogenicity	+ <sup>h</sup>	+	+ <sup>i</sup>

ND = No data, + = positive, - = negative.

<sup>a</sup>CoO = 92.4% (Stopford *et al.* 1993); Co<sub>3</sub>O<sub>4</sub> = 50% by release of cobalt ions into RPMI 1640 culture medium in the presence of canine alveolar macrophages after 2 weeks of culture (Kreyling *et al.* 1990).

<sup>b</sup>CoO = 100% (Stopford *et al.* 1993); Co<sub>3</sub>O<sub>4</sub> = ND.

<sup>c</sup>CoO = + (Smith *et al.* 2014); Co<sub>3</sub>O<sub>4</sub> = + (Ortega *et al.* 2014).

<sup>d</sup>CoCl<sub>2</sub> = +; CoSO<sub>4</sub> = ND.

<sup>e</sup>CoO = + (Chattopadhyay *et al.* 2015); Co<sub>3</sub>O<sub>4</sub> = + (Alarifi *et al.* 2013).

<sup>f</sup>CoO = ND; Co<sub>3</sub>O<sub>4</sub> = + (Ortega *et al.* 2014).

<sup>g</sup>CoO =  $\pm$  for chromosomal aberrations (Horie *et al.* 2012); Co<sub>3</sub>O<sub>4</sub> = + for DNA damage (Kain *et al.* 2012, Alarifi *et al.* 2013).

<sup>h</sup>CoCl<sub>2</sub> = + by local injection; CoSO<sub>4</sub> = + by inhalation.

<sup>i</sup>CoO = + by intratracheal, intraperitoneal, and subcutaneous injection; Co<sub>3</sub>O<sub>4</sub> = ND.

## 7.2 Evidence of carcinogenicity from studies in experimental animals

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

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

**Table 7-2. Carcinogenic effects of cobalt metal and cobalt compounds in experimental animals**

Animal neoplasms	Soluble cobalt salts		Cobalt metal	Poorly soluble cobalt compounds
	CoCl <sub>2</sub>	CoSO <sub>4</sub>	particles	CoO
Lung	ND	+	+	+
Adrenal gland	ND	+	+	ND
Pancreatic islet cell	ND	–	+	ND
Mononuclear cell leukemia	ND	–	+	ND
Kidney	ND	–	±	ND
Injection site sarcomas	+	ND	+	+

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

### 7.3 Evidence of carcinogenicity from studies in humans

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

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

## 8 References

1. Abbracchio MP, Heck JD, Costa M. 1982. The phagocytosis and transforming activity of crystalline metal sulfide particles are related to their negative surface charge. *Carcinogenesis* 3(2): 175-180. (Supported by NIEHS, the U.S. EPA and the Department of Energy. Authors affiliated with University of Texas, TX. )
2. Abrams JA, Fields S, Lightdale CJ, Neugut AI. 2008. Racial and ethnic disparities in the prevalence of Barrett's esophagus among patients who undergo upper endoscopy. *Clin Gastroenterol Hepatol* 6(1): 30-34. (Supported by NCI. Authors affiliated with Columbia University Medical Center, NY.)
3. Adachi S, Takemoto K, Ohshima S, Shimizu Y, Takahama M. 1991. Metal concentrations in lung tissue of subjects suffering from lung cancer. *Int Arch Occup Environ Health* 63(3): 193-197. (Support not reported. Authors affiliated with Saitama Medical School, Japan.)
4. Adami G, Smarrelli D, Martinelli B, Acquavita A, Reisenhofer E. 2003. Cobalt blood levels after total hip replacement (THR): a new follow-up study in Trieste (Italy). *Ann Chim* 93(1-2): 1-10. (Supported by the University of Trieste. Authors affiliated with University of Trieste, Italy; "Maggiore" Hospital, Italy; LBM, Italy.)
5. Adámik M, Bažantová P, Navrátilová L, Polášková A, Pečinka P, Holaňová L, Tichý V, Brázdová M. 2015. Impact of cadmium, cobalt and nickel on sequence-specific DNA binding of p63 and p73 in vitro and in cells. *Biochem Biophys Res Commun* 456(1): 29-34. (Supported by the IGA VFU Brno, the Czech Science Foundation and IET. Authors affiliated with Academy of Science of the Czech Republic, Czech Republic; University of Veterinary and Pharmaceutical Sciences, Czech Republic; University of Ostrava, Czech Republic.)
6. Afridi HI, Kazi TG, Kazi NG, Jamali MK, Arain MB, Sirajuddin, Kandhro GA, Shah AQ, Baig JA. 2009. Evaluation of arsenic, cobalt, copper and manganese in biological Samples of Steel mill workers by electrothermal atomic absorption Spectrometry. *Toxicol Ind Health* 25(1): 59-69. (Supported by the Higher Education Commission, Islamabad, Pakistan. Authors affiliated with University of Sindh, Pakistan.)
7. Alarifi S, Ali D, Y AO, Ahamed M, Siddiqui MA, Al-Khedhairi AA. 2013. Oxidative stress contributes to cobalt oxide nanoparticles-induced cytotoxicity and DNA damage in human hepatocarcinoma cells. *Int J Nanomedicine* 8: 189-199. (Supported by the King Saud University. Authors affiliated with King Saud University, Saudi Arabia.)
8. Alessio L, Dell'Orto A. 1988. Biological monitoring of cobalt. In *Biological Monitoring of Toxic Metals*. T.W. C, Friberg L, Nordberg GF, Sager PR, eds. New York, NY: Springer US. pp. 407-417. (Support not reported. Authors affiliated with University of Brescia, Italy.)
9. Alexander CS. 1969. Cobalt and the heart. *Ann Intern Med* 70(2): 411-413. (Support not reported. Author affiliated with University of Minnesota, MN.)

10. Alexandersson R, Lidums V. 1979. [Studies on the effects of exposure to cobalt. IV. Cobalt concentrations in blood and urine as exposure indicators]. *Arbete Halsa* 8: 2-23. (as cited in IARC 2006)
11. Alexandersson R. 1988. Blood and urinary concentrations as estimators of cobalt exposure. *Arch Environ Health* 43(4): 299-303. (Support not reported. Authors affiliated with Sundsvall's Hospital, Sweden.)
12. Alimonti A, Bocca B, Lamazza A, Forte G, Rahimi S, Mattei D, Fiori E, Iacomino M, Schillaci A, De Masi E, Pino A. 2008. A study on metals content in patients with colorectal polyps. *J Toxicol Environ Health A* 71(5): 342-347. (Supported by the LILT, Lega Italiana per la Lotta contro i Tumori (Italian League against Cancer). Authors affiliated with Italian National Institute of Health, Italy; Hospital 'Umberto I', Italy; Hospital 'San Carlo di Nancy', Italy.)
13. Alipázaga MV, Moreno RG, Linares E, Medeiros MH, Coichev N. 2008. DNA damage by sulfite autoxidation catalyzed by cobalt complexes. *Dalton Trans*(41): 5636-5644. (Supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) (Brazilian Agencies))
14. Allen MJ, Myer BJ, Millett PJ, Rushton N. 1997. The effects of particulate cobalt, chromium and cobalt-chromium alloy on human osteoblast-like cells in vitro. *J Bone Joint Surg Br* 79(3): 475-482. (Support not reported. Authors affiliated with Addenbrooke's Hospital, UK.)
15. Almaguer D. 1987. *Health Hazard Evaluation Report. American Cyanamid, Michigan City, IN*. HETA 86-251-1842. National Institute for Occupational Safety and Health. 12 pp.
16. Amacher DE, Paillet SC. 1980. Induction of trifluorothymidine-resistant mutants by metal ions in L5178Y/TK<sup>±</sup> cells. *Mutat Res* 78(3): 279-288. (as cited in IARC 2006)
17. Anard D, Kirsch-Volders M, Elhajouji A, Belpaeme K, Lison D. 1997. In vitro genotoxic effects of hard metal particles assessed by alkaline single cell gel and elution assays. *Carcinogenesis* 18(1): 177-184. (Supported by the Federal Services of the Scientific, Technical and Cultural Affairs of the Prime Minister's Service (Belgium). Authors affiliated with Universite Catholique de Louvain, Belgium; Vrije Univesiteit Brussel, Belgium.)
18. Andersen O. 1983. Effects of coal combustion products and metal compounds on sister chromatid exchange (SCE) in a macrophagelike cell line. *Environ Health Perspect* 47: 239-253. (as cited in IARC 2006)
19. Andersen I, Høgetveit AC. 1984. Analysis of cobalt in plasma by electrothermal atomic absorption spectrometry. *Fresenius Z Anal Chem* 318: 41-44. (as cited in IARC 1991)
20. Anderson LA, Cantwell MM, Watson RG, Johnston BT, Murphy SJ, Ferguson HR, McGuigan J, Comber H, Reynolds JV, Murray LJ. 2009. The association between alcohol and reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma.

- Gastroenterology* 136(3): 799-805. (Supported by an Ireland-Northern Ireland Co-operation Research Project Grant sponsored by the Northern Ireland Research & Development Office, by the Health Research Board, Ireland, and by the Ulster Cancer Foundation. Authors affiliated with Queen's University, Ireland; Royal Group of Hospitals, Ireland; Daisy Hill Hospital, Ireland; National Cancer Registry, Ireland; St. James's Hospital, Ireland.)
21. Andre S, Metivier H, Masse R. 1989. An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles - Part III: Lung clearance of inhaled cobalt oxide particles in baboons. *J Aerosol Sci* 20(2): 205-217. (Support not reported. Authors affiliated with Institut de Protection et de Surete Nucleaire, France.)
  22. Angelé-Martínez C, Goodman C, Brumaghim J. 2014. Metal-mediated DNA damage and cell death: Mechanisms, detection methods, and cellular consequences. *Metallomics* 6(8): 1358-1381. (Supported by the National Science Foundation. Authors affiliated with Clemson University, SC.)
  23. Angerer J. 1989. Cobalt. In *Biologische Arbeitsstoff- Toleranzwerte (BAT-Werte), Arbeitsmedizinisch-toxikologische Begründungen [Biological Occupational Tolerance Value, Occupational Medical-toxicological Basis]*. Henschler D, Lehnert G, eds. Weinheim: VCH-Verlag. pp. 1-13. (as cited in IARC 1991)
  24. Angerer J, Heinrich R, Szadkowski D, Lehnert G. 1985. Occupational Exposure to Cobalt Powder and Salts - Biological Monitoring and Health Effects. In *Proceedings of an International Conference on Heavy Metals in the Environment, Athens, September 1985*. vol. 2. Luxembourg: Commission of the European Community. p. 11 - 13. (Support and affiliations not reported.)
  25. Annangi B, Bach J, Vales G, Rubio L, Marcos R, Hernández A. 2014. Long-term exposures to low doses of cobalt nanoparticles induce cell transformation enhanced by oxidative damage. *Nanotoxicology*(Early Online). (Supported by Universitat Autònoma de Barcelona, UAB PIF, the "Generalitat de Catalunya," and the Spanish Ministry of Education and Science. Authors affiliated with Universitat Autònoma de Barcelona, Spain; ISCIII, Spain.)
  26. Apol AG. 1976. *Health Hazard Evaluation Determination: Rycraft, Inc., Corvallis, Oregon*. HETA 76-38-326. Cincinnati, OH: National Institute for Occupational Health and Safety. 9 pp.
  27. Apostoli P, Porru S, Alessio L. 1994. Urinary cobalt excretion in short time occupational exposure to cobalt powders. *Sci Total Environ* 150(1-3): 129-132. (Support not reported. Authors affiliated with University of Brescia, Italy.)
  28. Applebaum KM, Malloy EJ, Eisen EA. 2011. Left truncation, susceptibility, and bias in occupational cohort studies. *Epidemiology* 22(4): 599-606. (Supported by the Centers for Disease Control, National Institute of Occupational Safety and Health. Authors affiliated with Boston University School of Public Health, MA; American University, Washington, DC; University of California, CA.)

29. Arai F, Yamamura Y, Yoshida M, Kishimoto T. 1994. Blood and urinary levels of metals (Pb, Cr, Cd, Mn, Sb, Co and Cu) in cloisonne workers. *Ind Health* 32(2): 67-78. (Support not reported. Authors affiliated with St. Marianna University School of Medicine, Japan.)
30. Araya J, Maruyama M, Inoue A, Fujita T, Kawahara J, Sassa K, Hayashi R, Kawagishi Y, Yamashita N, Sugiyama E, Kobayashi M. 2002. Inhibition of proteasome activity is involved in cobalt-induced apoptosis of human alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 283(4): L849-858. (Support not reported. Authors affiliated with Toyama Medical and Pharmaceutical University, Japan.)
31. Ardyanto TD, Osaki M, Nagahama Y, Yamaga K, Maeta N, Tamura T, Ito H. 2008. Down-regulation of cobalt-induced HIF-1alpha expression correlates with cell proliferation and apoptosis in human gastric carcinoma cells. *Oncol Rep* 19(2): 339-343. (Support not reported. Authors affiliated with Tottori University, Japan; Sebelas Maret University, Indonesia.)
32. Arlauskas A, Baker RS, Bonin AM, Tandon RK, Crisp PT, Ellis J. 1985. Mutagenicity of metal ions in bacteria. *Environ Res* 36(2): 379-388. (as cited in IARC 2006)
33. Arslan M, Demir H, Arslan H, Gokalp AS, Demir C. 2011. Trace elements, heavy metals and other biochemical parameters in malignant glioma patients. *Asian Pac J Cancer Prev* 12(2): 447-451. (Support not reported. Authors affiliated with Yuzuncu Yil University, Turkey.)
34. Asante KA, Agusa T, Biney CA, Agyekum WA, Bello M, Otsuka M, Itai T, Takahashi S, Tanabe S. 2012. Multi-trace element levels and arsenic speciation in urine of e-waste recycling workers from Agbogbloshie, Accra in Ghana. *Sci Total Environ* 424: 63-73. (Supported by the Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan and Steel Foundation for Environmental Protection Technology, Japan. Authors affiliated with Ehime University, Japan; CSIR Water Research Institute, Ghana; Volta Basin Authority, Burkina Faso; Ehime Prefectural Institute of Public Health and Environmental Science, Japan.)
35. Ashley K, Shulman SA, Brisson MJ, Howe AM. 2012. Interlaboratory evaluation of trace element determination in workplace air filter samples by inductively coupled plasma mass spectrometry. *J Environ Monit* 14(2): 360-367. (Supported by CDC and NIOSH. Authors affiliated with NIOSH, OH; Savannah River Nuclear Solutions, SC; Health and Safety Laboratory, UK.)
36. Asmuss M, Mullenders LH, Eker A, Hartwig A. 2000. Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. *Carcinogenesis* 21(11): 2097-2104. (Supported by the Deutsche Forschungsgemeinschaft. Authors affiliated with University of Karlsruhe, Germany; Leiden University, Netherlands; Erasmus University Rotterdam, Netherlands.)
37. ATSDR. 2004. *Toxicological Profile for Cobalt*. Atlanta, GA: Agency for Toxic Substances and Disease Registry. pp. 207-E203.  
<http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=373&tid=64>.

38. Auffan M, Rose J, Wiesner MR, Bottero JY. 2009. Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro. *Environ Pollut* 157(4): 1127-1133. (Supported by the French National Program ACIFNS (ANR) ‘‘ECCO’’ supported by INSU (French National Institute for Earth Sciences and Astronomy) and the US National Science Foundation. Authors affiliated with Duke University, NC; CNRS/Aix-Marseille Université, France; Europôle de l’Arbois, France.)
39. Ayala-Fierro F, Firriolo JM, Carter DE. 1999. Disposition, toxicity, and intestinal absorption of cobaltous chloride in male Fischer 344 rats. *J Toxicol Environ Health A* 56(8): 571-591. (Supported by the Robert S. Flinn Foundation. Authors affiliated with University of Arizona, AZ; Smith Kline Beecham, NJ.)
40. Azuma K, Kawahara A, Hattori S, Taira T, Tsurutani J, Watari K, Shibata T, Murakami Y, Takamori S, Ono M, Izumi H, Kage M, Yanagawa T, Nakagawa K, Hoshino T, Kuwano M. 2012. NDRG1/Cap43/Drg-1 may predict tumor angiogenesis and poor outcome in patients with lung cancer. *J Thorac Oncol* 7(5): 779-789. (Support not reported. Authors affiliated with Kurume University, Japan; Kinki University, Japan; Kyushu University, Japan; University of Occupational and Environmental Health, Japan; Graduate School of Pharmaceutical Sciences, Japan.)
41. Bailey MR, Kreyling WG, Andre S, Batchelor A, Collier CG, Drosselmeyer E, Ferron GA, Foster P, Haider B, Hodgson A, Masse R, Metivier H, Morgan A, Müller H-L, Patrick G, Pearman I, Pickering S, Ramsden D, Stirling C, Talbot RJ. 1989. An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles - Part I: Objectives and summary of results. *J Aerosol Sci* 20(2): 169-188. (Supported by the Commission of the European Communities. Authors affiliated with National Radiological Protection Board, UK; Institut für Strahlenschutz, UK; Institut de Protection et de Sûreté Nucleaire, France; Medical Research Council Radiobiology Unit, UK; Institut für Genetik und für Toxikologie von Spaltstoffen, Germany; Atomic Energy Establishment Winfrith, UK; Harwell Laboratory, UK; European Institute for Transuranium Elements, Germany.)
42. Bal W, Sokołowska M, Kurowska E, Faller P. 2013. Binding of transition metal ions to albumin: Sites, affinities and rates. *Biochimica Et Biophysica Acta-General Subjects* 1830(12): 5444-5455. (Support not reported. Authors affiliated with Polish Academy of Sciences, Poland; CNRS, France; Université de Toulouse, France.)
43. Baldwin EL, Byl JA, Osheroff N. 2004. Cobalt enhances DNA cleavage mediated by human topoisomerase II alpha in vitro and in cultured cells. *Biochemistry* 43(3): 728-735. (Supported by NIH. Authors affiliated with Vanderbilt University School of Medicine, TN.)
44. Barrera G. 2012. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol* 2012: 137289. (Support not reported. Author affiliated with University of Turin, Italy.)
45. Basu N, Abare M, Buchanan S, Cryderman D, Nam DH, Sirkin S, Schmitt S, Hu H. 2010. A combined ecological and epidemiologic investigation of metal exposures amongst Indigenous peoples near the Marlin Mine in Western Guatemala. *Sci Total Environ* 409(1):

- 70-77. (Supported by the Due Process of Law Foundation, the University of Michigan School of Public Health, Physicians for Human Rights and the Independent International Panel. Authors affiliated with University of Michigan, MI; University of Illinois at Chicago, IL; Physicians for Human Rights, MA.)
46. Battaglia V, Compagnone A, Bandino A, Bragadin M, Rossi CA, Zanetti F, Colombatto S, Grillo MA, Toninello A. 2009. Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsic apoptosis in hepatocyte primary cultures. *Int J Biochem Cell Biol* 41(3): 586-594. (Support not reported. Authors affiliated with Università degli Studi di Padova, Italy; Università di Torino, Italy; Università Ca' Foscari, Italy.)
  47. Bax M. 1981. Lead and impaired abilities. *Develop Med Child Neurol* 23: 565-566. (Support and author affiliations not reported.)
  48. Beaucham CC, Kawamoto MM, Brueck SE. 2014. *Evaluation of Exposure to Metals at an Electronics Scrap Recycling Facility*. HETA 2013-0130-3226. Cincinnati, OH: National Institute for Occupational Safety and Health. 44 pp.
  49. Befani C, Mylonis I, Gkotinakou IM, Georgoulas P, Hu CJ, Simos G, Liakos P. 2013. Cobalt stimulates HIF-1-dependent but inhibits HIF-2-dependent gene expression in liver cancer cells. *Int J Biochem Cell Biol* 45(11): 2359-2368. (Support not reported. Authors affiliated with University of Thessaly, Greece; University of Colorado, CO.)
  50. Behl M, Stout MD, Herbert RA, Dill JA, Baker GL, Hayden BK, Roycroft JR, Bucher JR, Hooth MJ. 2015. Comparative toxicity and carcinogenicity of soluble and insoluble cobalt compounds. *Toxicology* 333: 195-205. (Support not reported. Authors affiliated with NIEHS, NC; Battelle Memorial Institute, OH.)
  51. Belezny E, Osvay M. 1994. Long-term clearance of accidentally inhaled <sup>60</sup>Co aerosols in humans. *Health Phys* 66(4): 392-399. (Support not reported. Authors affiliated with Hungarian Academy of Sciences, Hungary.)
  52. Ben-Skowronek I, Kozaczuk S. 2015. Von Hippel-Lindau Syndrome. *Horm Res Paediatr* 84(3): 145-152. (Support not reported. Authors affiliated with Medical University of Lublin, Poland.)
  53. Bencko V, Wagner V, Wagnerová M, Zavázal V. 1986. Human exposure to nickel and cobalt: biological monitoring and immunobiochemical response. *Environ Res* 40(2): 399-410. (Support not reported. Authors affiliated with Charles University, Czechoslovakia; Regional Hygiene Station of the Central-Bohemian Region, Czechoslovakia; Regional Institute of National Health, Czechoslovakia.)
  54. Benderli Cihan Y, Öztürk Yildirim S. 2011. A discriminant analysis of trace elements in scalp hair of healthy controls and stage-IIIb non-small cell lung cancer (NSCLC) patients. *Biol Trace Elem Res* 144(1-3): 272-294. (Support not reported. Authors affiliated with Kayseri Education and Research Hospital, Turkey; Erciyes University Faculty of Sciences, Turkey.)

55. Benderli Cihan Y, Sözen S, Öztürk Yildirim S. 2011. Trace elements and heavy metals in hair of stage III breast cancer patients. *Biol Trace Elem Res* 144(1-3): 360-379. (Support not reported. Authors affiliated with Kayseri Education and Research Hospital, Turkey; Erciyes University Faculty of Sciences, Turkey.)
56. Benigni R, Bossa C, Tcheremenskaia O, Battistelli CL, Giuliani A. 2015. The Syrian hamster embryo cells transformation assay identifies efficiently nongenotoxic carcinogens, and can contribute to alternative, integrated testing. *Mutat Res*(In press). (Support not reported. Authors affiliated with Istituto Superiore di Sanità, Italy.)
57. Bergomi M, Vinceti M, Nacci G, Pietrini V, Brätter P, Alber D, Ferrari A, Vescovi L, Guidetti D, Sola P, Malagù S, Aramini C, Vivoli G. 2002. Environmental exposure to trace elements and risk of amyotrophic lateral sclerosis: a population-based case-control study. *Environ Res* 89(2): 116-123. (Supported by the Ministry of the University and of Scientific and Technological Research, by the National Research Council, and by the Pietro Manodori Foundation of Reggio Emilia. Authors affiliated with Università di Modena e Reggio Emilia, Italy; Università of Parma, Italy; Hahn-Meitner Institut, Germany; Ospedale Santa Maria Nuova, Italy; Ospedale Bufalini-Marconi, Italy; Ospedale per gli Infermi, Italy.)
58. Bernstein J, Derman P. 2014. Dramatic increase in total knee replacement utilization rates cannot be fully explained by a disproportionate increase among younger patients. *Orthopedics* 37(7): e656-659. (Support not reported. Authors affiliated with University of Pennsylvania, PA; Hospital for Special Surgery. NY.)
59. Beyersmann D, Hartwig A. 1992. The genetic toxicology of cobalt. *Toxicol Appl Pharmacol* 115(1): 137-145. (Support not reported. Authors affiliated with University of Bremen, Germany.)
60. Beyersmann D, Hartwig A. 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch Toxicol* 82(8): 493-512. (Support not reported. Authors affiliated with University of Bremen, Germany; Technical University of Berlin, Germany.)
61. Bibi M, Hashmi MZ, Malik RN. 2015. The level and distribution of heavy metals and changes in oxidative stress indices in humans from Lahore district, Pakistan. *Hum Exp Toxicol*. (No funding received. Authors affiliated with Quaid-i-Azam University, Pakistan; Zhejiang University, China.)
62. Bourg WJ, Nation JR, Clark DE. 1985. The effects of chronic cobalt exposure on passive-avoidance performance in the adult rat. *Bull Psychon Soc* 23(6): 527-530. (Supported by the University Undergraduate Fellows Program at Texas A&M University. Authors affiliated with Texas A&M University, TX; College Station, TX.)
63. Bracken CP, Whitelaw ML, Peet DJ. 2003. The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. *Cell Mol Life Sci* 60(7): 1376-1393. (Supported by the Cancer Council of South Australia, National Heart Foundation, and National Health and Medical Research Council of Australia. Authors affiliated with University of Adelaide, Australia.)

64. Bradberry SM, Wilkinson JM, Ferner RE. 2014. Systemic toxicity related to metal hip prostheses. *Clin Toxicol (Phila)* 52(8): 837-847. (Support not reported. Authors affiliated with West Midlands Poisons Unit, UK; University of Sheffield, UK; West Midlands Centre for Adverse Drug Reactions, UK.)
65. Bresson C, Darolles C, Carmona A, Gautier C, Sage N, Roudeau S, Ortega R, Ansoborlo E, Malard V. 2013. Cobalt chloride speciation, mechanisms of cytotoxicity on human pulmonary cells, and synergistic toxicity with zinc. *Metallomics* 5(2): 133-143. (Supported by the transversal toxicology program from the CEA DSV (France) and Electricité De France (EDF) company. Authors affiliated with Laboratoire de developpement Analytique Nucleaire, France; Lab Biochim System Perturb, France; Univ. Bordeaux, France; CNRS, France; Laboratoire d'Analyse en Soutien aux Exploitants, France; CEA, France.)
66. Broberg K, Engstrom K, Shegufta A. 2015. Gene-environment interactions for metals. In *Handbook on the Toxicology of Metals*. 4th ed., Vol. I: General Considerations. Nordberg GF, Fowler BA, Nordberg M, eds. Waltham, MA: Elsevier. pp. 239-264. (Support and author affiliations not reported.)
67. Brock T, Stopford W. 2003. Bioaccessibility of metals in human health risk assessment: evaluating risk from exposure to cobalt compounds. *J Environ Monit* 5(4): 71N-76N. (Support not reported. Authors affiliated with Duke University, NC.)
68. Bryant C, Rondinelli R, Singal M. 1987. *Health Hazard Evaluation Report: GTE/Valenite Corporation, Westminster, South Carolina*. HETA 85-064-1844. Cincinnati, OH: National Institute for Occupational Health and Safety. 26 pp.
69. Bucher JR, Elwell MR, Thompson MB, Chou BJ, Renne R, Ragan HA. 1990. Inhalation toxicity studies of cobalt sulfate in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol* 15(2): 357-372. (Support not reported. Authors affiliated with NIEHS, NC; Pacific Northwest Laboratories, WA.)
70. Burr G, Sinks T. 1988. *Health Hazard Evaluation Report: General Electric Carbology Systems, Detroit, Michigan*. HETA 85-295-1907. Cincinnati, OH: National Institute for Occupational Health and Safety. 46 pp.
71. Burr G, Singal M, Hartle R, Rondinelli R. 1988. *Health Hazard Evaluation Report: General Electric Company, Evendale, Ohio*. HETA 85-423-1904. Cincinnati, OH: National Institute for Occupational Health and Safety. 25 pp.
72. Burr G, Habes D, Driscoll R, Krake A. 2005. *Health Hazard Evaluation Report: Freudenberg-NOK High Quality Plastics Division, Findlay, Ohio*. HETA 2003-0351-2972. Cincinnati, OH: National Institute for Occupational Health and Safety. 14 pp.
73. Butt J, Kandel G. 2014. Barrett esophagus: when to endoscope. *Clin Endosc* 47(1): 40-46. (Support not reported. Authors affiliated with St. Michael's Hospital, Canada.)
74. Cadex Eletronics Inc. 2015. *BU-205: Types of Lithium Ion*. Updated on 8/17/15. [http://batteryuniversity.com/learn/article/types\\_of\\_lithium\\_ion](http://batteryuniversity.com/learn/article/types_of_lithium_ion). Accessed on 10/14/15.

75. Caicedo M, Jacobs JJ, Reddy A, Hallab NJ. 2007. Analysis of metal ion-induced DNA damage, apoptosis, and necrosis in human (Jurkat) T-cells demonstrates Ni<sup>2+</sup> and V<sup>3+</sup> are more toxic than other metals: Al<sup>3+</sup>, Be<sup>2+</sup>, Co<sup>2+</sup>, Cr<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Mo<sup>5+</sup>, Nb<sup>5+</sup>, Zr<sup>2+</sup>. *J Biomed Mater Res Part A*: 905-913. (Support not reported. Authors affiliated with Rush University Medical Center, IL.)
76. Campbell CA, Peet M, Ward NI. 1988. Vanadium and other trace elements in patients taking lithium. *Biol Psychiatry* 24(7): 775-781. (Support not reported. Authors affiliated with David Rice Hospital, UK; Northern General Hospital, UK; University of Surrey, UK; Fulbourn Hospital, UK.)
77. Cancer Research UK. 2014. *Oesophageal cancer risk factors*. Cancer Research UK. Updated on 5/21/14. <http://www.cancerresearchuk.org/cancer-info/cancerstats/types/oesophagus/riskfactors/oesophageal-cancer-risk-factors - Overview>. Accessed on 3/30/15.
78. Carneiro MF, Grotto D, Batista BL, Rhoden CR, Barbosa F, Jr. 2011. Background values for essential and toxic elements in children's nails and correlation with hair levels. *Biol Trace Elem Res* 144(1-3): 339-350. (Supported by the Secretaria de Saúde do Estado do Rio Grande do Sul, the Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNP, and the Fundação de Amparo à Pesquisa do Estado de São Paulo. Authors affiliated with Universidade de São Paulo, Brazil; Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil.)
79. Casto BC, Meyers J, DiPaolo JA. 1979. Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. *Cancer Res* 39(1): 193-198. (as cited in IARC 2006)
80. Cavallo D, Ciervo A, Fresegna AM, Maiello R, Tassone P, Buresti G, Casciardi S, Iavicoli S, Ursini CL. 2015. Investigation on cobalt-oxide nanoparticles cyto-genotoxicity and inflammatory response in two types of respiratory cells. *J Appl Toxicol*. (Supported by the Italian Ministry of Health. Authors affiliated with Italian Workers' Compensation Authority, Italy.)
81. CDC. 2013. *Biomonitoring Summary: cobalt*. Centers for Disease Control and Prevention. [http://www.cdc.gov/biomonitoring/Cobalt\\_BiomonitoringSummary.html](http://www.cdc.gov/biomonitoring/Cobalt_BiomonitoringSummary.html). Accessed on 1/31/15.
82. CDC. 2015. *Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2015*. Atlanta, GA: Centers for Disease Control and Prevention. 1095 pp.
83. CDI. 2006. *Cobalt Facts*. Cobalt Development Institute. <http://thecdi.com/cobaltfacts.php>. Accessed on 2/12/15.
84. Cereda C, Redaelli ML, Canesi M, Carniti A, Bianchi S. 1994. Widia tool grinding: the importance of primary prevention measures in reducing occupational exposure to cobalt. *Sci Total Environ* 150(1-3): 249-251. (Support not reported. Authors affiliated with Health and Safety Unit, Italy.)

85. Chadwick JK, Wilson HK, White MA. 1997. An investigation of occupational metal exposure in thermal spraying processes. *Sci Total Environ* 199(1-2): 115-124. (Support not reported. Authors affiliated with Health and Safety Laboratory, UK; Commission of the European Communities Joint Research Centre, Italy.)
86. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. 1998. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A* 95(20): 11715-11720. (Supported by the National Heart, Lung, and Blood Institute. Authors affiliated with University of Chicago, IL.)
87. Chattopadhyay S, Dash SK, Tripathy S, Das B, Mandal D, Pramanik P, Roy S. 2015. Toxicity of cobalt oxide nanoparticles to normal cells; an in vitro and in vivo study. *Chem Biol Interact* 226: 58-71. (Support not reported. Authors affiliated with Vidyasagar University, India; Indian Institute of Technology, India.)
88. ChemIDplus. 2015. *ChemIDplus Lite*. National Library of Medicine. <http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp> and search by CAS number. Accessed on 3/24/15.
89. Chen L, Ramsey J, Brueck S. 2008. *Evaluation of Exposures at a Pottery Shop. Health Hazard Evaluation Report: FUNKe Fired Arts (formerly known as Annie's Mud Pie Shop), Cincinnati, Ohio*. HETA 2007-0127-3068. Cincinnati, OH: National Institute for Occupational Health and Safety. 40 pp.
90. Cheng Y, Chen G, Hong L, Zhou L, Hu M, Li B, Huang J, Xia L, Li C. 2013. How does hypoxia inducible factor-1alpha participate in enhancing the glycolysis activity in cervical cancer? *Ann Diagn Pathol* 17(3): 305-311. (Supported by China's Post-doctoral Science Fund and the Natural Science Fund of Hubei Province. Authors affiliated with Renmin Hospital of Wuhan University, China; Hospital of Xiantao City, China; Third Affiliated Hospital of Guangzhou Medical College, China.)
91. Cheuk W, Chan AC, Chan JK, Lau GT, Chan VN, Yiu HH. 2005. Metallic implant-associated lymphoma: a distinct subgroup of large B-cell lymphoma related to pyothorax-associated lymphoma? *Am J Surg Pathol* 29(6): 832-836. (Support not reported. Authors affiliated with Queen Elizabeth Hospital, China.)
92. Cheyns K, Banza Lubaba Nkulu C, Ngombe LK, Asosa JN, Haufroid V, De Putter T, Nawrot T, Kimpanga CM, Numbi OL, Ilunga BK, Nemery B, Smolders E. 2014. Pathways of human exposure to cobalt in Katanga, a mining area of the D.R. Congo. *Sci Total Environ* 490: 313-321. (Support not reported. Authors affiliated with Veterinary and Agrochemical Research Centre, Belgium; Université de Lubumbashi, People's Republic of Congo; Université de Kamina, People's Republic of Congo; Université Catholique de Louvain, Belgium; Royal Museum for Central Africa, Belgium; Department of Public Health and Primary Care, Belgium; Hasselt University, Belgium; KU Leuven, Belgium.)
93. Christensen JM. 1995. Human exposure to toxic metals: factors influencing interpretation of biomonitoring results. *Sci Total Environ* 166: 89-135. (Support not reported. Authors affiliated with National Institute of Occupational Health, Denmark.)

94. Christensen JM, Mikkelsen S. 1986. Cobalt concentration in whole blood and urine from pottery plate painters exposed to cobalt paint. In *Proceedings of an International Conference, Heavy Metals in the Environment, Athens, September 1985*. Lakkas TD, ed. Luxembourg: Commission of the European Communities. pp. 86-88. (as cited in IARC 1991)
95. Christensen JM, Poulsen OM. 1994. A 1982-1992 surveillance programme on Danish pottery painters. Biological levels and health effects following exposure to soluble or insoluble cobalt compounds in cobalt blue dyes. *Sci Total Environ* 150(1-3): 95-104. (Support not reported. Authors affiliated with Danish National Institute of Occupational Health, Denmark.)
96. Christensen JM, Poulsen OM, Thomsen M. 1993. A short-term cross-over study on oral administration of soluble and insoluble cobalt compounds: sex differences in biological levels. *Int Arch Occup Environ Health* 65(4): 233-240. (Supported by Royal Copenhagen A/S and the Danish Working Environment Fund. Authors affiliated with National Institute of Occupational Health, Denmark; Danish Emergency Management Agency, Denmark.)
97. Clyne N, Lins L-E, Pehrsson SK, Lundberg A, Werner J. 1988. Distribution of cobalt in myocardium, skeletal muscle and serum in exposed and unexposed rats. *Trace Elem Med* 5(2): 52-54. (Supported by the Magnus Bergvall's and Tore Nilsson's foundations. Authors affiliated with Karolinska Hospital, Sweden; Swedish Environmental Research Institute, Sweden.)
98. Coleman RF, Herrington J, Scales JT. 1973. Concentration of wear products in hair, blood, and urine after total hip replacement. *Br Med J* 1(5852): 527-529. (as cited in Schaffer *et al* 1999)
99. Collecchi P, Esposito M, Brera S, Mora E, Mazzucotelli A, Oddone M. 1986. The distribution of arsenic and cobalt in patients with laryngeal carcinoma. *J Appl Toxicol* 6(4): 287-289. (Support not reported. Authors affiliated with Istituto Nazionale per la Ricerca sul Cancro, Italy; Clinica Otorinolaringoiatrica 'B dell' Universita, Italy; Dipartimento di Chimica Generale dell' Universita di Pavia, Italy.)
100. Collier CG, Bailey MR, Hodgson A. 1989. An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles - Part V: Lung clearance of inhaled cobalt oxide particles in hamster, rats and guinea pigs. *J Aerosol Sci* 20(2): 233-247. (Supported by the CEC. Authors affiliated with National Radiological Protection Board (NRPB), UK.)
101. Collier CG, Hodgson A, Gray A, Moody JC, Ball A. 1991. The lung clearance kinetics of <sup>57</sup>Co<sub>3</sub>O<sub>4</sub> in rats of various ages. *J Aerosol Sci* 22(4): 537-549. (Supported by the CEC. Authors affiliated with National Radiological Protection Board (NRPB), UK.)
102. Colognato R, Bonelli A, Ponti J, Farina M, Bergamaschi E, Sabbioni E, Migliore L. 2008. Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro. *Mutagenesis* 23(5): 377-382. (Supported by the Italian Ministry of University and Scientific Research. Authors affiliated with University of Pisa, Italy; European Commission, Italy; University of Parma, Italy.)

103. Cook MB, Kamangar F, Whiteman DC, Freedman ND, Gammon MD, Bernstein L, Brown LM, Risch HA, Ye W, Sharp L, Pandeya N, Webb PM, Wu AH, Ward MH, Giffen C, Casson AG, Abnet CC, Murray LJ, Corley DA, Nyren O, Vaughan TL, Chow WH. 2010. Cigarette smoking and adenocarcinomas of the esophagus and esophagogastric junction: a pooled analysis from the international BEACON consortium. *J Natl Cancer Inst* 102(17): 1344-1353. (Supported by NIH, the Chief Scientist Office (Scotland), the Locally Organised Research Scheme, the Special Trustees of the Nottingham University Hospitals, the Medical Research Council, the California Tobacco-Related Research Program, NCI, the Nova Scotia Health Research Foundation, the Northern Ireland Research and Development Office, the Health Research Board, Ireland, the Queensland Cancer Fund, and the National Health and Medical Research Council of Australia. Authors affiliated with NIH, MD; Morgan State University, MD; Queensland Institute of Medical Research, Australia; University of North Carolina School of Public Health, NC; Beckman Research Institute and City of Hope Comprehensive Cancer Center, CA; RTI International, MD; Yale University School of Medicine, CT; Karolinska Institutet, Sweden; National Cancer Registry, Ireland; USC Norris Comprehensive Cancer Center, CA; Information Management Services, MD; University of Saskatchewan, Canada; Queen's University, Ireland; Kaiser Permanente, CA; Fred Hutchinson Cancer Research Center, WA.)
104. Coombs M. 1996. Biological monitoring of cobalt oxide workers. *Int Arch Occup Environ Health* 68(6): 511-512. (Support not reported. Authors affiliated with Sentrachem Ltd., South Africa.)
105. Corley DA, Levin TR, Habel LA, Weiss NS, Buffler PA. 2002. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. *Gastroenterology* 122(3): 633-640. (Supported by the American Digestive Health Foundation/TAP Outcomes Research Award, and the National Institutes of Health Mentored Clinical Scientist Development Award. Authors affiliated with University of California - Berkely and San Francisco, CA; Northern California Kaiser Division of Research, CA; University of Washington, WA.)
106. Cornelis R, Heinzow B, Herber RFM, Molin Christensen J, Paulsen OM, Sabbioni E, Templeton DM, Thomassen Y, Vahter M, Vesterberg O. 1995. Sample collection guidelines for trace elements in blood and urine. *Pure Appl Chem* 67(8/9): 1575-1608. (Support not reported. Authors affiliated with University of Gent, Belgium; Institute for Environmental Toxicology of Schleswig-Holstein, Germany; University of Amsterdam, Netherlands; National Institute for Occupational Health, Denmark, Norway and Sweden; EC Research Centre, Italy; University of Toronto, Canada; Karolinska Institute of Environmental Medicine, Sweden.)
107. Costa M, Heck JD, Robison SH. 1982. Selective phagocytosis of crystalline metal sulfide particles and DNA strand breaks as a mechanism for the induction of cellular transformation. *Cancer Res* 42(7): 2757-2763. (Supported by the Office of Research and Development, Environmental Protection Agency, NIEHS and the the United States Department of Energy. Authors affiliated with University of Texas, TX. )
108. Coyle YM, Hynan LS, Euhus DM, Minhajuddin AT. 2005. An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. *Breast Cancer Res Treat* 92(2): 107-114. (Supported by the Natalie Ornish Fund, the Clay Weed

- Memorial Trust Fund, and CREW Dallas. Authors affiliated with University of Texas Southwestern Medical Center, TX.)
109. Coyle YM, Minahjuddin AT, Hynan LS, Minna JD. 2006. An ecological study of the association of metal air pollutants with lung cancer incidence in Texas. *Journal of Thoracic Oncology* 1(7): 654-661. (Supported by the SPORE in Lung Cancer and the Clay Weed Memorial Trust Fund. Authors affiliated with University of Texas Southwestern Medical Center, TX.)
  110. Daniels WJ, Arnold SJ, Orris P. 1986. *Health Hazard Evaluation Report: Ladish Company, Cudahy, Wisconsin*. HETA 84-102-1653. Cincinnati, OH: National Institute for Occupational Health and Safety. 21 pp.
  111. Davidson T, Ke Q, Costa M. 2015. Selected molecular mechanisms of metal toxicity and carcinogenicity. In *Handbook on the Toxicology of Metals*. 4th ed., Vol. I: General Considerations. Nordberg GF, Fowler BA, Nordberg M, eds. Waltham, MA: Elsevier. pp. 173-196.
  112. Davies AP, Sood A, Lewis AC, Newson R, Learmonth ID, Case CP. 2005. Metal-specific differences in levels of DNA damage caused by synovial fluid recovered at revision arthroplasty. *J Bone Joint Surg Br* 87(10): 1439-1444. (Support not reported. Authors affiliated with Southmead Hospital, UK; King's College Hospital, UK.)
  113. Davis JR, ed. 2000. *Nickel, Cobalt, and Their Alloys*. Materials Park, OH: ASM International. pp. 345-406.
  114. Dawber R, Baran R. 1987. Nail growth. *Cutis* 39(2): 99-103. (Support not reported. Authors affiliated with Slade Hospital, UK; General Hospital Cannes, France.)
  115. De Boeck M, Lison D, Kirsch-Volders M. 1998. Evaluation of the *in vitro* direct and indirect genotoxic effects of cobalt compounds using the alkaline comet assay. Influence of interdonor and interexperimental variability. *Carcinogenesis* 19(11): 2021-2029. (Supported by the Belgian Federal Offices for Scientific, Technical and Cultural Affairs (OSTC) of the Prime Minister's Services. Authors affiliated with Vrije Universiteit Brussel, Belgium; Université Catholique de Louvain, Belgium.)
  116. De Boeck M, Lardau S, Buchet JP, Kirsch-Volders M, Lison D. 2000. Absence of significant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust: a cross-sectional study. *Environ Mol Mutagen* 36(2): 151-160. (Supported by the Belgian Federal Offices for Scientific, Technical and Cultural Affairs and Cobalt Development Institute. Authors affiliated with Vrije Universiteit Brussel, Belgium; Université catholique de Louvain, Belgium.)
  117. De Boeck M, Kirsch-Volders M, Lison D. 2003a. Cobalt and antimony: genotoxicity and carcinogenicity. *Mutat Res* 533(1-2): 135-152. (Support not reported. Authors affiliated with Vrije Universiteit Brussel, Belgium; Université Catholique de Louvain, Belgium.)
  118. De Boeck M, Lombaert N, De Backer S, Finsky R, Lison D, Kirsch-Volders M. 2003b. *In vitro* genotoxic effects of different combinations of cobalt and metallic carbide particles.

- Mutagenesis* 18(2): 177-186. (Supported by the the Belgian Federal Offices for Scientific, Technical and Cultural Affairs of the Prime Minister's Service. Authors affiliated with Vrije Universiteit Brussel, Belgium; Université Catholique de Louvain, Belgium.)
119. De Olivera JV, Boufleur LA, Dos Santos CE, Dias JF, Squeff CH, Silva GR, Ianistcki M, Benvegnu VC, Da Silva J. 2012. Occupational genotoxicity among copper smelters. *Toxicol Ind Health* 28(9): 789-795. (Support not received. Authors affiliated with Universidade Luterana do Brasil, Brazil; Universidade Federal do Rio Grande do Sul, Brazil.)
120. De Palma G, Goldoni M, Catalani S, Carbognani P, Poli D, Mozzoni P, Acampa O, Internullo E, Rusca M, Apostoli P. 2008. Metallic elements in pulmonary biopsies from lung cancer and control subjects. *Acta Biomed* 79 Suppl 1: 43-51. (Support not reported. Authors affiliated with University of Brescia, Italy; University of Parma, Italy; University Hospital of Parma, Italy.)
121. Decker JA. 1991. *Health Hazard Evaluation Report. Rockwell International, Newark, OH.* HETA 90-368-2137. National Institute for Occupational Safety and Health. 23 pp.
122. Deitchman S, Kelly J, Miller A, D. L. 1994. *Health Hazard Evaluation Report. Tinker Air Force Base, Oklahoma City, OK.* HETA 91-0259-2420. National Institute for Occupational Safety and Health. 22 pp.
123. Delahant AB. 1955. An experimental study of the effects of rare metals on animal lungs. *AMA Arch Ind Health* 12(2): 116-120. (Support not reported. Author affiliated with Saranac Laboratory, NY.)
124. Della Torre F, Cassani M, Segale M, Scarpazza G, Pietra R, Sabbioni E. 1990. Trace metal lung diseases: a new fatal case of hard metal pneumoconiosis. *Respiration* 57(4): 248-253. (Support not reported. Authors affiliated with INRCA Casatenovo, Italy; Commission of the European Communities, Italy.)
125. Demir C, Demir H, Esen R, Sehitogullari A, Atmaca M, Alay M. 2011. Altered serum levels of elements in acute leukemia cases in Turkey. *Asian Pac J Cancer Prev* 12(12): 3471-3474. (Support not reported. Authors affiliated with Yuzuncu Yil University, Turkey; Van Training and Research Hospital, Turkey.)
126. Deng X, Tüysüz H. 2014. Cobalt-oxide-based materials as water oxidation catalyst: Recent progress and challenges. *ACS Catal* 4: 3701-3714. (Support not reported. Authors affiliated with Max-Planck Institut für Kohlenforschung, Germany.)
127. Deng J-F, Elliot L, Sinks T, Smith D. 1990. *Health Hazard Evaluation Report. Hitachi Magnetics Corporation, Edmore, MI.* HETA 88-192-1998. National Institute for Occupational Safety and Health. 47 pp.
128. Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, Howden CW. 2012. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 61(7): 970-976. (Support not reported. Authors affiliated with William Beaumont Hospital, MI; Northwestern University Feinberg School of Medicine, IL;

Oakland University, MI. Dr. Howard is a consultant for Takeda, Otsuka, Boehringer-Ingelheim, Novartis, Eisai, and XenoPort; and he has received speaking fees from Takeda, Otsuka, and GlaxoSmithKline)

129. Devlin JJ, Pomerleau AC, Brent J, Morgan BW, Deitchman S, Schwartz M. 2013. Clinical features, testing, and management of patients with suspected prosthetic hip-associated cobalt toxicity: a systematic review of cases. *J Med Toxicol* 9(4): 405-415. (Support not reported. Authors affiliated with Georgia Poison Center, GA; Emory University School of Medicine, GA; Centers for Disease Control and Prevention, GA; University of Colorado School of Medicine, CO; DePuy Companies.)
130. Dick CA, Brown DM, Donaldson K, Stone V. 2003. The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. *Inhal Toxicol* 15(1): 39-52. (Support not reported. Authors affiliated with Napier University, UK; Scottish Biomedical, UK.)
131. Dodion P, Putz P, Amiri-Lamraski MH, Efira A, de Martelaere E, Heimann R. 1983. Immunoblastic lymphoma at the site of an infected vitallium bone plate. *Histopathology* 6: 807-813. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium.)
132. Donaldson JD, Beyersmann D. 2012. Cobalt and Cobalt Compounds. In *Ullman's Encyclopedia of Industrial Chemistry*. vol, 9. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA. pp. 429-465.
133. Dongarrà G, Lombardo M, Tamburo E, Varrica D, Cibella F, Cuttitta G. 2011. Concentration and reference interval of trace elements in human hair from students living in Palermo, Sicily (Italy). *Environ Toxicol Pharmacol* 32(1): 27-34. (Supported by Miur and CNR - IBIM: Institute of Biomedicine and Molecular Immunology "A. Monroy". Authors affiliated with Università di Palermo, Italy; Consiglio Nazionale delle Ricerche, Italy.)
134. Doran A, Law FC, Allen MJ, Rushton N. 1998. Neoplastic transformation of cells by soluble but not particulate forms of metals used in orthopaedic implants. *Biomaterials* 19(7-9): 751-759. (as cited in IARC 2006)
135. Dunstan E, Sanghrajka AP, Tilley S, Unwin P, Blunn G, Cannon SR, Briggs TW. 2005. Metal ion levels after metal-on-metal proximal femoral replacements: a 30-year follow-up. *J Bone Joint Surg Br* 87(5): 628-631. (Support not reported. Authors affiliated with Royal National Orthopaedic Hospital, UK.)
136. Durgam S, Aristeguieta C. 2010. *Evaluation of Potential Exposures at an Electrolytic Manganese Dioxide Processing Plant. Health Hazard Evaluation Report: Erachem Comilog, Inc., New Johnsonville, Tennessee*. HETA 2007-0331-3100. Cincinnati, OH: National Institute for Occupational Safety and Health. 44 pp.
137. ECHA. 2009. *Practical Guide 6: How to Report Read-Across and Categories*. ECHA-10-B-11-EN. Helsinki, Finland: European Chemicals Agency. 34 pp.

138. Edel J, Pozzi G, Sabbioni E, Pietra R, Devos S. 1994. Metabolic and toxicological studies on cobalt. *Sci Total Environ* 150(1-3): 233-244. (Support not reported. Authors affiliated with European Commission, Italy.)
139. Edmonds L, McQuilkin S, Orris P, Daniels W. 1981. *Health Hazard Evaluation Report: Federal Mogul, Metal Removal Tooling Division, Chicago, Illinois*. HETA 80-058-874. Cincinnati, OH: National Institute for Occupational Health and Safety. 13 pp.
140. Egilsson V, Evans IH, Wilkie D. 1979. Toxic and mutagenic effects of carcinogens on the mitochondria of *Saccharomyces cerevisiae*. *Mol Gen Genet* 174(1): 39-46. (as cited in IARC 2006)
141. Elenge MM, Aubry JC, Jacob L, De Brouwer C. 2011. Heavy metal in hair samples of 109 non-industrial (miners) population in Katanga. *Sante* 21(1): 41-46. (No funds received. Authors affiliated with Universit\_e Libre de Bruxelles, Belgique.)
142. EPA. 2012. *EPA Chemical Data Reporting*. U.S. Environmental Protection Agency. [http://java.epa.gov/oppt\\_chemical\\_search/](http://java.epa.gov/oppt_chemical_search/) and search by CAS number. Accessed on 3/24/15.
143. Erturk FA, Ay H, Nardemir G, Agar G. 2013. Molecular determination of genotoxic effects of cobalt and nickel on maize (*Zea mays* L.) by RAPD and protein analyses. *Toxicol Ind Health* 29(7): 662-671. (Support not reported. Authors affiliated with Atatürk University, Turkey.)
144. Evarts E. 2013. *What happens to electric car batteries when the car is retired?* Consumer Reports. <http://www.consumerreports.org/cro/news/2013/10/what-happens-to-electric-car-batteries-when-the-car-is-retired/index.htm>. Accessed on 10/14/15.
145. Fairhall LT, Keenan RG, Brinton HP. 1949. Cobalt and the dust environment of the cemented tungsten carbide industry. *Public Health Rep* 64(15): 485-490. (Support not reported. Authors affiliated with Public Health Service.)
146. Farah SB. 1983. The in vivo effect of cobalt chloride on chromosomes. *Rev Brasil Genet* 6: 433-442. (as cited in IARC 2006)
147. Farquharson JP. 2015. *Formation Metals Inc. - President's Letter to Shareholders*. Formation Metals, Inc. Updated on 2/23/15. <http://www.formationmetals.com/s/CobaltNews.asp?ReportID=697830>. Accessed on 3/6/15.
148. Ferdenzi P, Giaroli C, Mori P, Pedroni C, Piccinini R, Ricci R, Sala O, Veronesi C, Mineo F. 1994. Cobalt powdersintering industry (stone cutting diamond wheels): a study of environmental-biological monitoring, workplace improvement and health surveillance. *Sci Total Environ* 150(1-3): 245-248. (Support not reported. Authors affiliated with SMPIL USL 9, Italy; USL 12 Scandiano, Italy; Industrial Physician, Italy; PMP USL 9, Italy.)
149. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. 2013. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 49(6): 1374-1403. (Supported by the ERA-NET project)

- EUROCOURSE funded within the Seventh Framework Programme of the European Commission. Authors affiliated with IARC, France; CPO – Centre for Epidemiology and Prevention in Oncology in Piedmont, Italy; Erasmus MC, Netherlands; Comprehensive Cancer Centre South (IKZ), Netherlands; National Cancer Registry, Ireland.)
150. Ferri F, Candela S, Bedogni L, Piccinini R, Sala O. 1994. Exposure to cobalt in the welding process with stellite. *Sci Total Environ* 150(1-3): 145-147. (Support not reported. Authors affiliated with Servizio di Medicina Preventiva ed Igiene del Lavoro, Italy; Presidio Multizonale di Prevenzione, Italy.)
  151. Figgitt M, Newson R, Leslie IJ, Fisher J, Ingham E, Case CP. 2010. The genotoxicity of physiological concentrations of chromium (Cr(III) and Cr(VI)) and cobalt (Co(II)): an in vitro study. *Mutat Res* 688(1-2): 53-61. (Supported by the Arthritis and Rheumatism Council. Authors affiliated with Southmead Hospital, UK; Imperial College London, UK; University of Leeds, UK.)
  152. Finley BL, Unice KM, Kerger BD, Otani JM, Paustenbach DJ, Galbraith DA, Tvermoes BE. 2013. 31-day study of cobalt(II) chloride ingestion in humans: pharmacokinetics and clinical effects. *J Toxicol Environ Health A* 76(21): 1210-1224. (Supported by DePuy Orthopedics, Inc. Authors affiliated with Cardno ChemRisk, CA, PA and CO.)
  153. Finogenova MA. 1973. [The influence of cobalt on induced cutaneous carcinogenesis]. *Biull Eksp Biol Med* 74(2): 73-75. (Support not reported. Authors affiliated with Nutrition Institute of the USSR Academy of Medicine, Russia.)
  154. Fleckman P. 1985. Anatomy and physiology of the nail. *Dermatol Clin* 3(3): 373-381. (Support not reported. Author affiliated with University of Washington, WA.)
  155. Fleckman P. 1997. Basic science of the nail unit. In *Nails: Therapy, Diagnosis, Surgery*. Scher RK, Daniel CR, eds. Philadelphia, PA: Saunders. pp. 99-103.
  156. Formation Metals Inc. 2015. *Cobalt - A Better Future*. Vancouver, B.C., Canada: Formation Metals, Inc. 47 pp.
  157. Forooghian F, Razavi R, Timms L. 2007. Hypoxia-inducible factor expression in human RPE cells. *Br J Ophthalmol* 91(10): 1406-1410. (Supported by the Canadian Institutes of Health Research. Authors affiliated with University of Toronto, Canada; Centre for Applied Genomics, Canada; Hospital for Sick Children, Canada.)
  158. Foster PP, Pearman I, Ramsden D. 1989. An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles - Part II: Lung clearance of inhaled cobalt oxide particles in man. *J Aerosol Sci* 20(2): 189-204. (Supported by the UKAEA Core Programme on Radiological Protection Research and the CEC. Authors affiliated with Atomic Energy Establishment, UK.)
  159. Frank E. 2012. *Metal-on-Metal Hip Systems*. U.S. Food and Drug Administration. <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/OrthopaedicandRehabilitationDevicesPanel/UCM310217.pdf> target=.

160. Freedman ND, Murray LJ, Kamangar F, Abnet CC, Cook MB, Nyren O, Ye W, Wu AH, Bernstein L, Brown LM, Ward MH, Pandeya N, Green AC, Casson AG, Giffen C, Risch HA, Gammon MD, Chow WH, Vaughan TL, Corley DA, Whiteman DC. 2011. Alcohol intake and risk of oesophageal adenocarcinoma: a pooled analysis from the BEACON Consortium. *Gut* 60(8): 1029-1037. (Supported by NIH, NCI, the Nova Scotia Health Research Foundation, the Northern Ireland Research & Development Office, the Health Research Board, Ireland, the California Tobacco Related Research Program, the Queensland Cancer Fund, and the National Health and Medical Research Council (NHMRC) of Australia. Authors affiliated with NIH, MD; Queen's University, Ireland; Morgan State University, MD; Karolinska Institutet, Sweden; University of Southern California/Norris Comprehensive Cancer Center, CA; Beckman Research Institute, CA; RTI International, MD; Queensland Institute of Medical Research, Australia; University of Saskatchewan, Canada; Information Management Services, MD; Yale University School of Medicine, CT; University of North Carolina School of Public Health, NC; Fred Hutchinson Cancer Research Center, WA; Kaiser Permanente, CA.)
161. Fu OY, Hou MF, Yang SF, Huang SC, Lee WY. 2009. Cobalt chloride-induced hypoxia modulates the invasive potential and matrix metalloproteinases of primary and metastatic breast cancer cells. *Anticancer Res* 29(8): 3131-3138. (Supported by the National Science Council and the Chi Mei Medical Center. Authors affiliated with Kaohsiung Medical University, Taiwan; Chi Mei Medical Center, Taiwan; Taipei Medical University, Taiwan; Southern Taiwan University, Taiwan.)
162. Fukunaga M, Kurachi Y, Mizuguchi Y. 1982. Action of some metal ions on yeast chromosomes. *Chem Pharm Bull (Tokyo)* 30(8): 3017-3019. (as cited in IARC 2006)
163. Gaines L. 2014. The future of automotive lithium-ion battery recycling: Charting a sustainable course. *Sustainable Mat Technol* 1-2: 2-7. (Supported by the U.S. Department of Energy's Office of Vehicle Technologies. Author affiliated with Argonne National Laboratory, IL.)
164. Galán-Cobo A, Sánchez-Silva R, Serna A, Abreu-Rodríguez I, Muñoz-Cabello AM, Echevarría M. 2013. Cellular overexpression of Aquaporins slows down the natural HIF-2 $\alpha$  degradation during prolonged hypoxia. *Gene* 522(1): 18-26. (Supported by the "Instituto de Salud Carlos III," "La Junta de Andalucía", Consejería de Salud, Consejería de Innovación Ciencia y Empresa, "La Junta de Andalucía, Consejería de Innovación Ciencia y Empresa", Spain and the Spanish Ministry of Science and Innovation. Authors affiliated with Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain; Centro de Investigación Biomédica en Red sobre Enfermedades Respiratorias (CIBERES), Spain.)
165. Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. 2008. Reactive oxygen species and HIF-1 signalling in cancer. *Cancer Lett* 266(1): 12-20. (Supported by a Marie Curie International Reintegration Grant within the 6th European Community Framework Program and the Hellenic Ministry of Education. Authors affiliated with Democritus University of Thrace, Greece.)
166. Galanis A, Karapetsas A, Sandaltzopoulos R. 2009. Metal-induced carcinogenesis, oxidative stress and hypoxia signalling. *Mutation Research - Genetic Toxicology and*

- Environmental Mutagenesis* 674(1-2): 31-35. (Support not reported. Authors affiliated with Democritus University of Thrace, Greece.)
167. Gao S, Zhou J, Zhao Y, Toselli P, Li W. 2013. Hypoxia-response element (HRE)-directed transcriptional regulation of the rat lysyl oxidase gene in response to cobalt and cadmium. *Toxicol Sci* 132(2): 379-389. (Supported by NIH. Authors affiliated with Boston University School of Medicine, MA.)
168. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, Willett WC, Hunter DJ. 1993. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. *Cancer Epidemiol Biomarkers Prev* 2(5): 493-497. (Supported by NIH and NIEHS. Authors affiliated with Harvard School of Public Health, MA; Harvard Medical School and Brigham and Women's Hospital, MA.)
169. Garrick MD, Dolan KG, Horbinski C, Ghio AJ, Higgins D, Porubcin M, Moore EG, Hainsworth LN, Umbreit JN, Conrad ME, Feng L, Lis A, Roth JA, Singleton S, Garrick LM. 2003. DMT1: a mammalian transporter for multiple metals. *Biometals* 16(1): 41-54. (Supported by the NIH, USDA, NSF and EPA. Authors affiliated with SUNY at Buffalo, NY; Environmental Protection Agency, NC; University of South Alabama, AL.)
170. Gerhardsson L, Nordberg GF. 1993. Lung cancer in smelter workers--interactions of metals as indicated by tissue levels. *Scand J Work Environ Health* 19 Suppl 1: 90-94. (Support not reported. Authors affiliated with University of Umeå, Sweden; University Hospital, Sweden.)
171. Gerhardsson L, Wester PO, Nordberg GF, Brune D. 1984. Chromium, cobalt and lanthanum in lung, liver and kidney tissue from deceased smelter workers. *Sci Total Environ* 37(2-3): 233-246. (Supported by the Swedish Work Environment Fund. Authors affiliated with Umeå University, Sweden; Scandinavian Institute of Dental Materials, Norway.)
172. Gerhardsson L, Brune D, Nordberg IG, Wester PO. 1985. Protective effect of selenium on lung cancer in smelter workers. *Br J Ind Med* 42(9): 617-626. (Supported by the Swedish Work Environment Fund. Authors affiliated with University of Umeå, Sweden; University Hospital, Sweden; Scandanavian Institute of Dental Materials, Norway.)
173. Gerhardsson L, Brune D, Lundstrom NG, Nordberg G, Wester PO. 1993. Biological specimen bank for smelter workers. *Sci Total Environ* 140: 157-173. (Supported by the Swedish Work Environment Fund. Authors affiliated with University of Umeå, Sweden; Lund University Hospital, Sweden; D.B. C., Dag Brune Consulting, Norway; University Hospital of Umeå, Sweden.)
174. Gilbert EW, Luna RA, Harrison VL, Hunter JG. 2011. Barrett's esophagus: a review of the literature. *J Gastrointest Surg* 15(5): 708-718. (Support not reported. Authors affiliated with Oregon Health Sciences University, OR.)
175. Gilman JP. 1962. Metal carcinogenesis. II. A study on the carcinogenic activity of cobalt, copper, iron, and nickel compounds. *Cancer Res* 22: 158-162. (Supported by the National Cancer Institute of Canada. Author affiliated with Ontario Veterinary College, Canada.)

176. Gilman JP, Ruckerbauer GM. 1962. Metal carcinogenesis. I. Observations on the carcinogenicity of a refinery dust, cobalt oxide, and colloidal thorium dioxide. *Cancer Res* 22: 152-157. (Supported by the National Cancer Institute of Canada. Authors affiliated with Ontario Veterinary College, Canada.)
177. González-Muñoz MJ, Sánchez-Muniz FJ, Ródenas S, Sevillano MI, Larrea Marín MT, Bastida S. 2010. Differences in metal and metalloid content in the hair of normo- and hypertensive postmenopausal women. *Hypertens Res* 33(3): 219-224. (Supported by projects PR248/01-10161 and AGL Consolider- Ingenio 2010 project reference CSD2007-00016. Authors affiliated with Universidad de Alcalá, Spain; Universidad Complutense, Spain; Universidad Complutense de Madrid, Spain; CSIC, Spain.)
178. Gori C, Zucconi L. 1957. [L'azione citologica indotta da un gruppo di composti inorganici su *Allium cepa*]. *Caryologia* 10: 29-45. (as cited in IARC 2006)
179. Grant K, Goldizen FC, Sly PD, Brune MN, Neira M, van den Berg M, Norman RE. 2013. Health consequences of exposure to e-waste: a systematic review. *Lancet Glob Health* 1(6): e350-361. (Supported by the Children's Health and Environment Program, Queensland Children's Medical Research Institute, The University of Queensland. Authors affiliated with Queensland Children's Medical Research Institute, Australia; University of Queensland, Australia; World Health Organization, Switzerland; Utrecht University, Netherlands.)
180. Green SE, Luczak MW, Morse JL, DeLoughery Z, Zhitkovich A. 2013. Uptake, p53 pathway activation, and cytotoxic responses for Co(II) and Ni(II) in human lung cells: implications for carcinogenicity. *Toxicol Sci* 136(2): 467-477. (Supported by NIEHS. Authors affiliated with Brown University, RI.)
181. Gregus Z, Klaassen CD. 1986. Disposition of metals in rats: a comparative study of fecal, urinary, and biliary excretion and tissue distribution of eighteen metals. *Toxicol Appl Pharmacol* 85(1): 24-38. (Supported by USPHS. Authors affiliated with University of Kansas, KS.)
182. Greim H, Hartwig A, Reuter U, Richter-Reichhelm HB, Thielmann HW. 2009. Chemically induced pheochromocytomas in rats: Mechanisms and relevance for human risk assessment. *Critical Reviews in Toxicology* 39(8): 695-718. (Supported by the Deutsche Forschungsgemeinschaft. Authors affiliated with Technical University of Munich, Germany; Technical University of Berlin, Germany; Bundesinstitut für Risikobewertung, Germany; German Cancer Research Center, Germany.)
183. Grimsrud TK, Berge SR, Haldorsen T, Andersen A. 2002. Exposure to different forms of nickel and risk of lung cancer. *Am J Epidemiol* 156(12): 1123-1132. (Supported by the Norwegian Cancer Society, the Confederation of Norwegian Business and Industry Working Environment Fund, Falconbridge Nikkelverk A/S, and the Cancer Registry of Norway. Authors affiliated with Cancer Registry of Norway, Norway; Falconbridge Nikkelverk A/S, Norway.)
184. Grimsrud TK, Berge SR, Martinsen JI, Andersen A. 2003. Lung cancer incidence among Norwegian nickel-refinery workers 1953-2000. *J Environ Monit* 5(2): 190-197. (Supported

- by the Confederation of Norwegian Business and Industry Working Environment Fund. Authors affiliated with Cancer Registry of Norway, Norway; Falconbridge Nikkelverk A/S, Norway.)
185. Grimsrud TK, Berge SR, Haldorsen T, Andersen A. 2005. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel? *Epidemiology* 16(2): 146-154. (Supported by the Norwegian Cancer Society, the Confederation of Norwegian Business and Industry (CNBI) Working Environment Fund, Falconbridge Nikkelverk A/S, and the Cancer Registry of Norway. Authors affiliated with Cancer Registry of Norway, Norway; Falconbridge Nikkelverk A/S, Norway.)
  186. Gunn SA, Gould TC, Anderson WA. 1967. Specific response of mesenchymal tissue to cancerigenesis by cadmium. *Arch Pathol* 83(6): 493-499. (Supported by NCI. Authors affiliated with University of Miami School of Medicine, FL.)
  187. Guskov A, Eshaghi S. 2012. The mechanisms of Mg<sup>2+</sup> and Co<sup>2+</sup> transport by the CorA family of divalent cation transporters. *Curr Top Membr* 69: 393-414. (Supported by the National Research Foundation and Biomedical Research Council of Singapore. Authors affiliated with Nanyang Technological University, Singapore.)
  188. Guyton KZ, Kyle AD, Aubrecht J, Cogliano VJ, Eastmond DA, Jackson M, Keshava N, Sandy MS, Sonawane B, Zhang L, Waters MD, Smith MT. 2009. Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat Res* 681(2-3): 230-240. (Supported by the U.S. EPA, ILS, Inc, Pfizer, Inc., and NIEHS. Authors affiliated with U.S. EPA, Washington, D.C.; University of California - Berkeley, CA; Pfizer Global Research and Development, CT; IARC, France; University of California - Riverside, CA; Integrated Laboratory Systems, Inc., NC; California Environmental Protection Agency, CA.)
  189. Hall RM. 2003. *Health Hazard Evaluation Report: Sunset Strip Furniture Stripping, Huntington Beach, California*. HETA 2001- 0537-2897. Cincinnati, OH: National Institute for Occupational Health and Safety. 17 pp.
  190. Hamilton-Koch W, Snyder RD, Lavelle JM. 1986. Metal-induced DNA damage and repair in human diploid fibroblasts and Chinese hamster ovary cells. *Chem Biol Interact* 59(1): 17-28. (as cited in IARC 2006)
  191. Hammond EM, Giaccia AJ. 2005. The role of p53 in hypoxia-induced apoptosis. *Biochemical and Biophysical Research Communications* 331(3): 718-725. (Supported by NIH. Authors affiliated with Stanford University, CA.)
  192. Hanna PM, Kadiiska MB, Mason RP. 1992. Oxygen-derived free radical and active oxygen complex formation from cobalt(II) chelates in vitro. *Chem Res Toxicol* 5(1): 109-115. (Support not reported. Authors affiliated with NIEHS, NC; Bulgarian Academy of Sciences, Bulgaria.)
  193. Hansen T, Clermont G, Alves A, Eloy R, Brochhausen C, Boutrand JP, Gatti AM, Kirkpatrick CJ. 2006. Biological tolerance of different materials in bulk and nanoparticulate form in a rat model: sarcoma development by nanoparticles. *J R Soc*

- Interface* 3(11): 767-775. (Supported by the European Commission. Authors affiliated with Johannes Gutenberg-University of Mainz, Germany; Biomatech S.A., France; Università degli studi di Modena e Reggio Emilia, Italy.)
194. Hartung M, Schaller K-H, Brand E. 1982. On the question of the pathogenic importance of cobalt for hard metal fibrosis of the lung. *Int Arch Occup Environ Health* 50: 53-57. (Support not reported. Authors affiliated with Universität Erlangen-Nürnberg, Germany; Chirurgische Klinik im Klinikum Nürnberg, Germany.)
195. Hartung M. 1986. *Lungenfibrosen bei Hartmetallschleifern—Bedeutung der Cobalteinwirkung [Lung Fibrosis in Hard-metal Grinding—Significance of Cobalt Activity]* (Publication Series of Main Associations of Industrial Societies), Bonn: Köllen-Druck & Verlag. (as cited in IARC 1991)
196. Hartwig A. 1998. Carcinogenicity of metal compounds: possible role of DNA repair inhibition. *Toxicol Lett* 102-103: 235-239. (Supported by the Deutsche Forschungsgemeinschaft. Author affiliated with University of Karlsruhe, Germany.)
197. Hartwig A, Kasten U, Boakye-Dankwa K, Schlepegrell R, Beyersmann D. 1990. Uptake and genotoxicity of micromolar concentrations of cobalt chloride in mammalian cells. *Toxicol Environ Chem* 28: 205-215. (as cited in IARC 2006)
198. Hartwig A, Snyder RD, Schlepegrell R, Beyersmann D. 1991. Modulation by Co(II) of UV-induced DNA repair, mutagenesis and sister-chromatid exchanges in mammalian cells. *Mutat Res* 248(1): 177-185. (Supported by the Bundesminister für Forschung und Technologie, Bonn. Authors affiliated with University of Bremen, Germany; Merrell Dow Research Institute, OH.)
199. Hartwig A, Asmuss M, Ehleben I, Herzer U, Kostelac D, Pelzer A, Schwerdtle T, Burkle A. 2002. Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. *Environ Health Perspect* 110 Suppl 5: 797-799. (Supported by the Deutsche Forschungsgemeinschaft, the European Union and Programm Lebensgrundlage Umwelt und ihre Sicherung (BW-PLUS). Authors affiliated with University of Karlsruhe, Germany; University of Newcastle, UK; Institut für Strahlenhygiene, Germany.)
200. Harty LC, Guinee DG, Travis WD, Bennett WP, Jett J, Colby TV, Tazelaar H, Trastek V, Pairolero P, Liotta LA, Harris CC, Caporaso NE. 1996. p53 mutations and occupational exposures in a surgical series of lung cancers. *Cancer Epidemiology Biomarkers & Prevention* 5(12): 997-1003. (Support not reported. Authors affiliated with NIH, MD; Armed Forces Institute of Pathology. Washington. D. C.; Mayo Clinic, MN.)
201. He K. 2011. Trace elements in nails as biomarkers in clinical research. *Eur J Clin Invest* 41(1): 98-102. (Supported by NIH. Author affiliated with University of North Carolina, NC.)
202. Heath JC. 1956. The production of malignant tumours by cobalt in the rat. *Br J Cancer* 10(4): 668-673. (Supported by the British Empire Cancer Campaign. Author affiliated with Strangeways Research Laboratory, UK.)

- 
203. Heath JC, Daniel MR. 1962. The production of malignant tumours by cobalt in the rat: intrathoracic tumours. *Br J Cancer* 16(3): 473-478. (Supported by the British Empire Cancer Campaign. Author affiliated with Strangeways Research Laboratory, UK.)
204. Heath JC, Webb M. 1967. Content and intracellular distribution of the inducing metal in the primary rhabdomyosarcomata induced in the rat by cobalt, nickel and cadmium. *Br J Cancer* 21(4): 768-779. (Supported by the British Empire Cancer Campaign and the Medical Research Council. Authors affiliated with Strangeways Research Laboratory, UK.)
205. Heath JC, Webb M, Caffrey M. 1969. The interaction of carcinogenic metals with tissues and body fluids. Cobalt and horse serum. *Br J Cancer* 23(1): 153-166. (Supported by the British Empire Cancer Campaign and the Medical Research Council. Authors affiliated with Strangeways Research Laboratory, UK.)
206. Henderson RG, Verougstraete V, Anderson K, Arbildua JJ, Brock TO, Brouwers T, Cappellini D, Delbeke K, Herting G, Hixon G, Odnevall Wallinder I, Rodriguez PH, Van Assche F, Wilrich P, Oller AR. 2014. Inter-laboratory validation of bioaccessibility testing for metals. *Regul Toxicol Pharmacol* 70(1): 170-181. (Supported by the Nickel Producers Environmental Research Association, Inc., the Cobalt Development Institute, the International Zinc Association, the European Copper Institute, Eurometaux and the Food Safety and Environmental Stewardship Program at OSU. Authors affiliated with ToxStrategies, Inc., NC; Eurometaux, Belgium; Oregon State University, OR; Adolfo Ibañez University, Chile; Duke University, NC; ECTX bvba, Belgium; Kirby Memorial Health Center, PA; European Copper Institute, Belgium; KTH Royal Institute of Technology, Sweden; International Zinc Association, Belgium; Freie Universität Berlin, Germany; Nickel Producers Environmental Research Association, Inc., NC.)
207. Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte W, Jung D, Mayer-Popken O, Fuchs J, Gebhard S, Bienfait HG, Schlink K, Dietrich C, Faust D, Epe B, Oesch F. 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis* 24(1): 63-73. (Supported by the BMBF and the DFG. Authors affiliated with University of Mainz, Germany; Ministry of Labour Inspection, Germany.)
208. Hennig FF, Raithel HJ, Schaller KH, Dohler JR. 1992. Nickel-, chrom- and cobalt-concentrations in human tissue and body fluids of hip prosthesis patients. *J Trace Elem Electrolytes Health Dis* 6(4): 239-243. (as cited in Schaffer *et al* 1999)
209. Hervin RL, Reifschneider R. 1973. *Health Hazard Evaluation Determination: Steel Tool and Engineering Company, Taylor, Michigan*. HETA 72-42-76. Cincinnati, OH: National Institute for Occupational Health and Safety. 14 pp.
210. Hill AB. 1965. The environment and disease: Association or causation? *Proc R Soc Med* 58: 295-300. (Support not reported. Author affiliated with University of London, UK.)
211. Hillwalker WE, Anderson KA. 2014. Bioaccessibility of metals in alloys: evaluation of three surrogate biofluids. *Environ Pollut* 185: 52-58. (Supported by the OSU Food Safety

- and Environmental Stewardship Program. Authors affiliated with Oregon State University, OR.)
212. Hollins JG, McCullough RS. 1971. Radiation dosimetry of internal contamination by inorganic compounds of cobalt: an analysis of cobalt metabolism in rats. *Health Phys* 21(2): 233-246. (Support not reported. Authors affiliated with National Research Council of Canada, Canada.)
213. Holstein H, Ranebo Y, Raaf CL. 2015. Human metabolism of orally administered radioactive cobalt chloride. *J Environ Radioact* 143: 152-158. (Supported by the Swedish Radiation Safety Authority. Authors affiliated with Skåne University Hospital, Sweden; Barsebäck Kraft AB, Sweden; Lund University, Sweden.)
214. Hong H-H, Hoenerhoff MJ, Ton T-V, Herbert RA, Kissling GE, Hooth MJ, Behl M, Witt KL, Smith-Roe SL, Sills RC, Pandiri AR. 2015. Kras, egfr, and tp53 mutations in B6C3F1/N mouse and F344/NTAC rat alveolar/bronchiolar carcinomas resulting from chronic inhalation exposure to cobalt metal. *Toxicol Pathol* 43(6): 872-882. (Supported by the Divisions of the National Toxicology Program and the Intramural Research Program at the NIEHS, NIH. Authors affiliated with NIEHS, NC; Experimental Pathology Laboratories, Inc., NC.)
215. Hopps HC, Stanley AJ, Shideler AM. 1954. Polycythemia induced by cobalt. III. Histologic studies with evaluation of toxicity of cobaltous chloride. *Am J Clin Pathol* 24(12): 1374-1380. (Support not reported. Authors affiliated with University of Oklahoma School of Medicine, OK.)
216. Horev-Azaria L, Kirkpatrick CJ, Korenstein R, Marche PN, Maimon O, Ponti J, Romano R, Rossi F, Golla-Schindler U, Sommer D, Uboldi C, Unger RE, Villiers C. 2011. Predictive toxicology of cobalt nanoparticles and ions: comparative in vitro study of different cellular models using methods of knowledge discovery from data. *Toxicol Sci* 122(2): 489-501. (Supported by the European Commission. Authors affiliated with Tel Aviv University, Israel; University Medical Center of the Johannes Gutenberg University Mainz, Germany; University Grenoble, France; INSERM, France; European Commission, Italy; Universitaet Muenster, Germany.)
217. Horie M, Fujita K, Kato H, Endoh S, Nishio K, Komaba LK, Nakamura A, Miyauchi A, Kinugasa S, Hagihara Y, Niki E, Yoshida Y, Iwahashi H. 2012. Association of the physical and chemical properties and the cytotoxicity of metal oxide nanoparticles: metal ion release, adsorption ability and specific surface area. *Metallomics* 4(4): 350-360. (Supported by the New Energy and Industrial Technology Development Organization of Japan. Authors affiliated with University of Occupational and Environmental Health, Japan; National Institute of Advanced Industrial Science and Technology, Japan; Research Institute of Science for Safety and Sustainability, Japan; National Metrology Institute of Japan, Japan; Research Institute for Environmental Management Technology, Japan; Gifu University, Japan.)

218. HPD. 2014. *Household Products Database*. National Library of Medicine. Updated on 8/14. <http://householdproducts.nlm.nih.gov/advancedsearch.htm> and select "Ingredient" and search CAS No. Accessed on 10/10/14.
219. HSDB. 2004. *Hazardous Substances Database. Cobalt Bis(2-Ethylhexanoate)*. National Library of Medicine. Updated on 3/5/04. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> and search on CAS number or compound name. Accessed on 11/12/15.
220. HSDB. 2012. *Hazardous Substances Database. Cobaltous Chloride*. National Library of Medicine. Updated on 3/23/12. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> and search on CAS number or compound name. Accessed on 11/12/15.
221. Hughes AW, Sherlock DA, Hamblen DL, Reid R. 1987. Sarcoma at the site of a single hip screw. A case report. *J Bone Joint Surg Br* 69(3): 470-472. (Support not reported. Authors affiliated with General Hospital, UK; Western Infirmary, UK.)
222. Hunter DJ, Morris JS, Chute CG, Kushner E, Colditz GA, Stampfer MJ, Speizer FE, Willett WC. 1990. Predictors of selenium concentration in human toenails. *Am J Epidemiol* 132(1): 114-122. (Supported by NIH. Authors affiliated with Harvard Medical School, MA; Brigham and Women's Hospital, MA; University of Missouri, MO.)
223. IARC. 1991. Cobalt and cobalt compounds. In *Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 52. Lyon, France: International Agency for Research on Cancer. pp. 363-434.
224. IARC. 2006. Metallic cobalt particles (with or without tungsten carbide). In *Cobalt in Hard Metals*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 86. Lyon, France: International Agency for Research on Cancer. pp. 39-155.
225. Ichikawa Y, Kusaka Y, Goto S. 1985. Biological monitoring of cobalt exposure, based on cobalt concentrations in blood and urine. *Int Arch Occup Environ Health* 55(4): 269-276. (as cited in IARC 2006 and IARC 1991)
226. Inaba J, Suzuki-Yasumoto M. 1979. A kinetic study of radionuclide absorption through damaged and undamaged skin of the guinea pig. *Health Phys* 37(4): 592-595. (Support not reported. Authors affiliated with National Institute of Radiological Sciences, Japan.)
227. Inoue T, Ohta Y, Sadaie Y, Kada T. 1981. Effect of cobaltous chloride on spontaneous mutation induction in a *Bacillus subtilis* mutator strain. *Mutat Res* 91(1): 41-45. (as cited in IARC 2006)
228. IPPC. 2014. *Best Available Techniques (BAT) Reference Document for the Non-Ferrous Metal Industries*. MR/GC/EIPPCB/NFM\_Draft\_3. Seville, Spain: European Commission, Integrated Pollution Prevention and Control. 184 pp.
229. Itsara LS, Kennedy SR, Fox EJ, Yu S, Hewitt JJ, Sanchez-Contreras M, Cardozo-Pelaez F, Pallanck LJ. 2014. Oxidative stress is not a major contributor to somatic mitochondrial DNA mutations. *PLoS Genet* 10(2): e1003974. (Supported by a National Institute of

- Neurological Disorders and Stroke fellowship, the National Institute of General Medicine, the Muscular Dystrophy Association, the Nathan Shock Center of Excellence in Basic Biology of Aging, the National Cancer Institute and the National Institute on Aging. Authors affiliated with University of Washington, WA; Mayo Clinic, FL; University of Montana, MT.)
230. Jansen HM, Knollema S, van der Duin LV, Willemsen AT, Wiersma A, Franssen EJ, Russel FG, Korf J, Paans AM. 1996. Pharmacokinetics and dosimetry of cobalt-55 and cobalt-57. *J Nucl Med* 37(12): 2082-2086. (Supported by the Dutch Technology Foundation. Authors affiliated with University of Groningen, Netherlands; University of Nijmegen, Netherlands.)
231. Jantzen C, Jorgensen HL, Duus BR, Sporning SL, Lauritzen JB. 2013. Chromium and cobalt ion concentrations in blood and serum following various types of metal-on-metal hip arthroplasties: a literature overview. *Acta Orthop* 84(3): 229-236. (Support not reported. Authors affiliated with University of Copenhagen, Denmark.)
232. Jasmin G, Riopelle JL. 1976. Renal carcinomas and erythrocytosis in rats following intrarenal injection of nickel subsulfide. *Lab Invest* 35(1): 71-78. (Supported by the Medical Research Council of Canada. Authors affiliated with Universite de Montreal, Canada.)
233. Jemal A, Simard EP, Xu J, Ma J, Anderson RN. 2013. Selected cancers with increasing mortality rates by educational attainment in 26 states in the United States, 1993-2007. *Cancer Causes Control* 24(3): 559-565. (Supported by the Intramural Research Program of the American Cancer Society. Authors affiliated with American Cancer Society, GA; Centers for Disease Control and Prevention, MD.)
234. Jiang H, Liu F, Yang H, Li Y. 2012. Effects of cobalt nanoparticles on human T cells in vitro. *Biol Trace Elem Res* 146(1): 23-29. (Supported by the National Natural Science Foundation of China. Authors affiliated with First Affiliated Hospital of Soochow University, China; Affiliated Hospital of Nantong University, China; First People's Hospital of Taizhou City, China.)
235. Jomova K, Valko M. 2011. Advances in metal-induced oxidative stress and human disease. *Toxicology* 283(2-3): 65-87. (Supported by the Scientific Grant Agency of the Slovak Republic and the Slovak Research and Development Agency of the Slovak Republic. Authors affiliated with Constantine The Philosopher University, Slovakia; Slovak Technical University, Slovakia.)
236. Julander A, Lundgren L, Skare L, Grandner M, Palm B, Vahter M, Liden C. 2014. Formal recycling of e-waste leads to increased exposure to toxic metals: an occupational exposure study from Sweden. *Environ Int* 73: 243-251. (Supported by the Swedish Research Council for Health, Working Life and Welfare and the Karolinska Institutet. Authors affiliated with Karolinska Institutet, Sweden; Stockholm University, Sweden.)
237. Kada T, Kanematsu N. 1978. Reduction of N -methyl-N'-nitrosoguanidine-induced mutations by cobalt chloride in *Escherichia coli*. *Proc Jpn Acad* 54B: 234-237. (as cited in IARC 2006)

- 
238. Kadiiska MB, Maples KR, Mason RP. 1989. A comparison of cobalt(II) and iron(II) hydroxyl and superoxide free radical formation. *Arch Biochem Biophys* 275(1): 98-111. (Support not reported. Authors affiliated with NIEHS, NC.)
239. Kain J, Karlsson HL, Moller L. 2012. DNA damage induced by micro- and nanoparticles--interaction with FPG influences the detection of DNA oxidation in the comet assay. *Mutagenesis* 27(4): 491-500. (Supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, the Swedish Research Council, AF's Foundation for Research and Development. Authors affiliated with Karolinska Institutet, Sweden.)
240. Kanabrocki EL, Kanabrocki JA, Greco J, Kaplan E, Oester YT, Brar SS, Gustafson PS, Nelson DM, Moore CE. 1979. Instrumental analysis of trace elements in thumbnails of human subjects. *Sci Total Environ* 13(2): 131-140. (Supported by WHO. Authors affiliated with Veterans Administration Hospital, IL; Argonne National Laboratory, IL; Loyola University, IL.)
241. Kanematsu N, Hara M, Kada T. 1980. Rec assay and mutagenicity studies on metal compounds. *Mutat Res* 77(2): 109-116. (as cited in IARC 2006)
242. Kang GS, Li Q, Chen H, Costa M. 2006. Effect of metal ions on HIF-1alpha and Fe homeostasis in human A549 cells. *Mutat Res* 610(1-2): 48-55. (Supported by the National Institute of Environmental Health Sciences and the National Cancer Institute. Authors affiliated with NYU School of Medicine, NY.)
243. Kaniass GD, Kouri E, Arvaniti H, Karaiosifidi H, Kouneli S. 1994. Trace element content in breasts with fibrocystic disease. *Biol Trace Elem Res* 43-45: 363-370. (Support not reported. Authors affiliated with N.C.S.R. DEMOKRITOS, Greece; "Helena Venizelou," Greece. )
244. Karovic O, Tonazzini I, Rebola N, Edstrom E, Lovdahl C, Fredholm BB, Dare E. 2007. Toxic effects of cobalt in primary cultures of mouse astrocytes. Similarities with hypoxia and role of HIF-1alpha. *Biochem Pharmacol* 73(5): 694-708. (Supported by the Karolinska Institutet, the Swedish Science Research Council, European Commission, the Swedish Brain Foundation, the T. Nilson Foundation, the Swedish Society of Medicine, the A. Wiberg Foundation and the General Maternity Hospital foundation. Authors affiliated with Karolinska Institutet, Sweden.)
245. Kasirsky G, Gautieri RF, Mann DE, Jr. 1965. Effect of cobaltous chloride on the minimal carcinogenic dose of methylcholanthrene in albino mice. *J Pharm Sci* 54: 491-493. (Support and author affiliations not reported.)
246. Kasperek K, Kiem J, Iyengar GV, Feinendegen LE. 1981. Concentration differences between serum and plasma of the elements cobalt, iron, mercury, rubidium, selenium and zinc determined by neutron activation analysis. *Sci Total Environ* 17: 133-143. (as cited in IARC 1991)

247. Kasprzak KS. 2002. Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis. *Free Radical Biology and Medicine* 32(10): 958-967. (Support not reported. Authors affiliated with NCI, MD.)
248. Kasprzak KS, Zastawny TH, North SL, Riggs CW, Diwan BA, Rice JM, Dizdaroglu M. 1994. Oxidative DNA base damage in renal, hepatic, and pulmonary chromatin of rats after intraperitoneal injection of cobalt(II) acetate. *Chem Res Toxicol* 7(3): 329-335. (Support not reported. Authors affiliated with NCI, MD; Data Management Services, Inc., MD; NIST, MD; Rydygier Medical School, Poland; Program hources, Inc./DynCorp, MD.)
249. Kasten U, Mullenders LH, Hartwig A. 1997. Cobalt(II) inhibits the incision and the polymerization step of nucleotide excision repair in human fibroblasts. *Mutat Res* 383(1): 81-89. (Supported by the Kooperationsbereich Universität/Arbeiterkammer Bremen and by the Deutsche Forschungsgemeinschaft. Authors affiliated with University of Bremen, Germany; Leiden University, Netherlands.)
250. Kawamoto MM, Echt A, Reh CM. 1999. *Health Hazard Evaluation Report: Eagle-Picher Industries, Joplin, Missouri*. HETA 96-0016-2777. Cincinnati, OH: National Institute for Occupational Health and Safety. 36 pp.
251. Kawanishi S, Inoue S, Yamamoto K. 1989. Hydroxyl radical and singlet oxygen production and DNA damage induced by carcinogenic metal compounds and hydrogen peroxide. *Biol Trace Elem Res* 21: 367-372. (Support not reported. Authors affiliated with Kyoto University, Japan.)
252. Kawanishi S, Inoue S, Yamamoto K. 1994. Active oxygen species in DNA damage induced by carcinogenic metal compounds. *Environ Health Perspect* 102 Suppl 3: 17-20. (Support not reported. Authors affiliated with Kyoto University, Japan.)
253. Keegan GM, Learmonth ID, Case CP. 2008. A systematic comparison of the actual, potential, and theoretical health effects of cobalt and chromium exposures from industry and surgical implants. *Crit Rev Toxicol* 38(8): 645-674. (Support not reported. Authors affiliated with University of Bristol, UK; Southmead Hospital, UK.)
254. Kennedy SM, Chan-Yeung M, Marion S, Lea J, Teschke K. 1995. Maintenance of stellite and tungsten carbide saw tips: respiratory health and exposure-response evaluations. *Occup Environ Med* 52(3): 185-191. (Support not reported. Authors affiliated with University of British Columbia, Canada.)
255. Kerckaert GA, LeBoeuf RA, Isfort RJ. 1996. Use of the Syrian hamster embryo cell transformation assay for determining the carcinogenic potential of heavy metal compounds. *Fundam Appl Toxicol* 34(1): 67-72. (Support not reported. Authors affiliated with Procter & Gamble Company, OH.)
256. Kerfoot EJ, Fredrick WG, Domeier E. 1975. Cobalt metal inhalation studies on miniature swine. *Am Ind Hyg Assoc J* 36(1): 17-25. (Supported by the Health Services and Mental Health Administration, Department of Health, Education and Welfare. Authors affiliated with Wayne State University, MI.)

- 
257. Kerndt PR, Rondinelli R, Burr G. 1986. *Health Hazard Evaluation Report: United Technologies Diesel Systems, Springfield, Massachusetts*. HETA 85-254-1722. Cincinnati, OH: National Institute for Occupational Health and Safety. 27 pp.
258. Kharab P, Singh I. 1985. Genotoxic effects of potassium dichromate, sodium arsenite, cobalt chloride and lead nitrate in diploid yeast. *Mutat Res* 155(3): 117-120. (as cited in IARC 2006)
259. Kharab P, Singh I. 1987. Induction of respiratory deficiency in yeast by salts of chromium, arsenic, cobalt and lead. *Indian J Exp Biol* 25(2): 141-142. (as cited in IARC 2006)
260. Kibblewhite MG, Van Rensburg SJ, Laker MC, Rose EF. 1984. Evidence for an intimate geochemical factor in the etiology of esophageal cancer. *Environ Res* 33(2): 370-378. (Supported by the South African National Cancer Association. Authors affiliated with University of Fort Hare, South Africa; National Research Institute for Nutritional Diseases, South Africa.)
261. Kiefer M, Trout D, Decker J, Salisbury S. 1994. *Health Hazard Evaluation Report. General Electric Aircraft Engines, Madisonville, KY*. HETA 94-0151-2475. National Institute for Occupational Safety and Health. 29 pp.
262. Kitahara J, Yamanaka K, Kato K, Lee YW, Klein CB, Costa M. 1996. Mutagenicity of cobalt and reactive oxygen producers. *Mutat Res* 370(3-4): 133-140. (Supported by NIH. Authors affiliated with New York University Medical Center, NY; Nihon University College of Pharmacy, Japan.)
263. Klatka J, Remer M, Dobrowolski R, Pietruszewska W, Trojanowska A, Siwiec H, Charytanowicz M. 2011. The content of cadmium, cobalt and nickel in laryngeal carcinoma. *Arch Med Sci* 7(3): 517-522. (Support not reported. Authors affiliated with Medical University, Poland; District Hospital, Poland; Maria Curie Sklodowska University, Poland; The John Paul II Catholic University, Poland.)
264. Klaunig JE, Kamendulis LM, Hocevar BA. 2010. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol* 38(1): 96-109. (Support not reported. Authors affiliated with Indiana University School of Medicine, IN.)
265. Klein CB, Su L, Rossman TG, Snow ET. 1994. Transgenic gpt+ V79 cell lines differ in their mutagenic response to clastogens. *Mutat Res* 304(2): 217-228. (Supported by NIH and NIEHS. Authors affiliated with New York University Medical Center, NY.)
266. Koedrith P, Seo YR. 2011. Advances in carcinogenic metal toxicity and potential molecular markers. *Int J Mol Sci* 12(12): 9576-9595. (Supported by the Ministry of Environment, Korea, the Korea Research Foundation, the National Research Foundation of Korea and the Ministry of Education, Science and Technology, Korea. Authors affiliated with Dongguk University, Korea.)
267. Kopera E, Schwerdtle T, Hartwig A, Bal W. 2004. Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. *Chem Res Toxicol* 17(11): 1452-1458. (Supported by the

- Polish State Committee for Scientific Research and the Deutsche Forschungsgemeinschaft. Authors affiliated with Polish Academy of Sciences, Poland; Technical University of Berlin, Germany.)
268. Kotake-Nara E, Saida K. 2007. Characterization of CoCl<sub>2</sub>-induced reactive oxygen species (ROS): Inductions of neurite outgrowth and endothelin-2/vasoactive intestinal contractor in PC12 cells by CoCl<sub>2</sub> are ROS dependent, but those by MnCl<sub>2</sub> are not. *Neurosci Lett* 422(3): 223-227. (Supported by a New Energy and Industrial Technology Development Organization (NEDO) fellowship. Authors affiliated with National Institute of Advanced Industrial Science and Technology, Japan; New Energy and Industrial Technology Development Organization (NEDO), Japan.)
269. Kraus T, Schramel P, Schaller KH, Zöbelein P, Weber A, Angerer J. 2001. Exposure assessment in the hard metal manufacturing industry with special regard to tungsten and its compounds. *Occup Environ Med* 58(10): 631-634. (Support not reported. Authors affiliated with University of Erlangen-Nürnberg, Germany; GSF Neuherberg, Germany.)
270. KRC. 2015. *Which Type of Implant is Right For You?* KneeReplacementCosts.com. <http://www.kneereplacementcosts.com/implant-types.html>. Accessed on 4/4/16.
271. Kreyling WG, Ferron GA, Haider B. 1986. Metabolic fate of inhaled Co aerosols in beagle dogs. *Health Phys* 51(6): 773-795. (Support not reported. Authors affiliated with Institut fuer Stahlschutz, Germany.)
272. Kreyling WG, Godleski JJ, Kariya ST, Rose RM, Brain JD. 1990. *In vitro* dissolution of uniform cobalt oxide particles by human and canine alveolar macrophages. *Am J Respir Cell Mol Biol* 2(5): 413-422. (Supported by the U.S. Public Health Service, by the US-FRG cooperative program in pulmonary research and the GSF F+E Vorhaben 71142. Authors affiliated with Gesellschaft für Strahlen- und Umweltforschung mbH München (GSF), Germany; Harvard School of Public Health, MA; New England Deaconess Hospital, MA)
273. Kreyling WG, Andre S, Collier CG, Ferron GA, Metivier H, Schumann G. 1991a. Interspecies comparison of lung clearance after inhalation of monodisperse, solid cobalt oxide aerosol particles. *J Aerosol Sci* 22(4): 509-535. (Supported by CEC. Authors affiliated with Gesellschaft für Strahlen- und Umweltforschung mbH München (GSF), Germany; Commissariat à l'Energie Atomique (CEA), France; National Radiological Protection Board (NRPB), UK.)
274. Kreyling WG, Nyberg K, Nolibé D, Collier CG, Camner P, Heilman P, Lirsac PN, Lundborg M, Matejkova E. 1991b. Interspecies comparison of phagolysosomal pH in alveolar macrophages. *Inhal Toxicol* 3: 91-100. (Supported by the European Late Effects Project Group, the CEC, and the Karolinska Institute. Authors affiliated with Gesellschaft für Strahlen- und Umweltforschung mbH München, Germany; Karolinska Institutet, Sweden; Commissariat à l'Energie Atomique, France; National Radiological Protection Board, UK.)
275. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. 2009. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. *Gastroenterology* 136(3): 806-815. (Supported by NIH and Kaiser Permanente/

- Permanente Medical Group Community Benefits. Authors affiliated with Kaiser Permanente, CA; University of California - Berkeley and San Francisco, CA.)
276. Kuo CY, Wong RH, Lin JY, Lai JC, Lee H. 2006. Accumulation of chromium and nickel metals in lung tumors from lung cancer patients in Taiwan. *J Toxicol Environ Health A* 69(14): 1337-1344. (Support not reported. Authors affiliated with Chung Shan Medical University, China.)
277. Kusaka Y. 1996. Cobalt and nickel induced hard metal asthma. *Toxicol Metals*: 461-468. (Support and author affiliations not reported.)
278. Kusaka Y, Yokoyama K, Sera Y, Yamamoto S, Sone S, Kyono H, Shirakawa T, Goto S. 1986. Respiratory diseases in hard metal workers: an occupational hygiene study in a factory. *Br J Ind Med* 43(7): 474-485. (Support not reported. Authors affiliated with Osaka University, Japan; National Kinlci Chuo Hospital for Chest Diseases, Japan; Osaka City, Department of Radiology, Japan.)
279. Kusama T, Itoh S, Yoshizawa Y. 1986. Absorption of radionuclides through wounded skin. *Health Phys* 51(1): 138-141. (Support not reported. Authors affiliated with University of Tokyo, Japan.)
280. Kwon YM, Xia Z, Glyn-Jones S, Beard D, Gill HS, Murray DW. 2009. Dose-dependent cytotoxicity of clinically relevant cobalt nanoparticles and ions on macrophages in vitro. *Biomed Mater* 4(2): 025018. (Support not reported. Authors affiliated with University of Oxford, UK.)
281. Kyono H, Kusaka Y, Homma K, Kubota H, Endo-Ichikawa Y. 1992. Reversible lung lesions in rats due to short-term exposure to ultrafine cobalt particles. *Ind Health* 30(3-4): 103-118. (Support not reported. Authors affiliated with National Institute of Industrial Health, Japan; Fukui Medical School, Japan; Kansai Medical University, Japan.)
282. Lacy SA, Merritt K, Brown SA, Puryear A. 1996. Distribution of nickel and cobalt following dermal and systemic administration with in vitro and in vivo studies. *J Biomed Mater Res* 32(2): 279-283. (Support not reported. Authors affiliated with Case Western Reserve University, OH.)
283. Laker M. 1982. On determining trace element levels in man: the uses of blood and hair. *Lancet* 2(8292): 260-262. (Support not reported. Authors affiliated with Royal Hospital for Sick Children, UK.)
284. Lantin AC, Mallants A, Vermeulen J, Speybroeck N, Hoet P, Lison D. 2011. Absence of adverse effect on thyroid function and red blood cells in a population of workers exposed to cobalt compounds. *Toxicol Lett* 201(1): 42-46. (Supported by the Cobalt Development Institute. Authors affiliated with Umicore, Belgium; Université catholique de Louvain, Belgium; St. Dymphna Hospital, Belgium.)
285. Larese Filon F, Maina G, Adami G, Venier M, Coceani N, Bussani R, Massiccio M, Barbieri P, Spinelli P. 2004. *In vitro* percutaneous absorption of cobalt. *Int Arch Occup*

- Environ Health* 77(2): 85-89. (Supported by the European Community. Authors affiliated with Università di Trieste, Italy; Università degli Studi di Torino, Italy; Eurand, Italy.)
286. Larese Filon F, Gianpietro A, Venier M, Maina G, Renzi N. 2007. *In vitro* percutaneous absorption of metal compounds. *Toxicol Lett* 170(1): 49-56. (Supported by the European Community. Authors affiliated with Scienze di Medicina Pubblica, Italy; University of Trieste, Italy.)
287. Larese Filon F, D'Agostin F, Crosera M, Adami G, Bovenzi M, Maina G. 2009. *In vitro* absorption of metal powders through intact and damaged human skin. *Toxicol In Vitro* 23(4): 574-579. (Supported by the Italian Minister of University and Research. Authors affiliated with Università di Trieste, Italy.)
288. Lark RM, Ander EL, Cave MR, Knights KV, Glennon MM, Scanlon RP. 2013. Mapping trace element deficiency by cokriging from regional geochemical soil data: A case study on cobalt for grazing sheep in Ireland. *Geoderma* 226-227: 64-78. (Supported by the European Union's INTERREG IVA cross-border Programme managed by the Special EU Programmes Body, the Tellus Border Project TB: 'Application of the Tellus Border soil chemistry data to the agricultural sector in Ireland,' and NERC. Authors affiliated with British Geological Survey, UK; Geological Survey of Ireland, Ireland.)
289. Lee SG, Lee H, Rho HM. 2001. Transcriptional repression of the human p53 gene by cobalt chloride mimicking hypoxia. *FEBS Lett* 507(3): 259-263. (Supported by Research Grants from Molecular Medicine Research Program of the Ministry of Science and Technology (MOST) and Research Fellowships from the Ministry of Education and Human Resources Development. Authors affiliated with Seoul National University, South Korea.)
290. Lee AL, Burgoon LD, Lamb L, Dere E, Zacharewski TR, Hogenesch JB, Lapres JJ. 2006. Identification and characterization of genes susceptible to transcriptional cross-talk between the hypoxia and dioxin signaling cascades. *Chem Res Toxicol* 19(10): 1284-1293. (Supported by HH. Authors affiliated with Michigan State University, MI; Genomics Institute of the Novartis Research Foundation, CA.)
291. Lee JC, Son YO, Pratheeshkumar P, Shi X. 2012. Oxidative stress and metal carcinogenesis. *Free Radical Biology and Medicine* 53(4): 742-757. (Supported by NIH. Authors affiliated with University of Kentucky, KY; Chonbuk National University, South Korea.)
292. Leggett RW. 2008. The biokinetics of inorganic cobalt in the human body. *Sci Total Environ* 389(2-3): 259-269. (Supported by the Office of Radiation and Indoor Air, U. S. Environmental Protection Agency. Author affiliated with Oak Ridge National Laboratory, TN.)
293. Leitao AC, Soares RA, Cardoso JS, Guillobel HC, Caldas LR. 1993. Inhibition and induction of SOS responses in *Escherichia coli* by cobaltous chloride. *Mutat Res* 286(2): 173-180. (as cited in IARC 2006)

- 
294. Leonard S, Gannett PM, Rojanasakul Y, Schwegler-Berry D, Castranova V, Vallyathan V, Shi X. 1998. Cobalt-mediated generation of reactive oxygen species and its possible mechanism. *J Inorg Biochem* 70(3-4): 239-244. (Support not reported. Authors affiliated with NIOSH; West Virginia University, WV.)
295. Leonard SS, Harris GK, Shi X. 2004. Metal-induced oxidative stress and signal transduction. *Free Radic Biol Med* 37(12): 1921-1942. (Support not reported. Authors affiliated with NIOSH, WV.)
296. Letourneau EG, Jack GC, McCullough RS, Hollins JG. 1972. The metabolism of cobalt by the normal human male: whole body retention and radiation dosimetry. *Health Phys* 22(5): 451-459. (Support not reported. Authors affiliated with Department of National Health and Welfare, Canada; National Research Council of Canada, Canada.)
297. Lewis SA, O'Haver TC, Harnly JM. 1985. Determination of metals at the microgram-per-liter level in blood serum by simultaneous multielement atomic absorption spectrometry with graphite furnace atomization. *Anal Chem* 57(1): 2-5. (as cited in IARC 1991)
298. Lewis CP, Demedts M, Nemery B. 1991. Indices of oxidative stress in hamster lung following exposure to cobalt(II) ions: in vivo and in vitro studies. *Am J Respir Cell Mol Biol* 5(2): 163-169. (Supported by the Katholieke Universiteit Leuven and the Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO), Brussels. Authors affiliated with Katholieke Universiteit Leuven, Belgium.)
299. Lewis CP, Demedts M, Nemery B. 1992. The role of thiol oxidation in Cobalt(II)-induced toxicity in hamster lung. *Biochem Pharmacol* 43(3): 519-525. (Supported by the European Science Foundation, the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek and by the Onderzoeksfonds of the Katholieke Universiteit Leuven. Authors affiliated with Katholieke Universiteit Leuven, Belgium.)
300. Lhotka C, Szekeres T, Steffan I, Zhuber K, Zweymuller K. 2003. Four-year study of cobalt and chromium blood levels in patients managed with two different metal-on-metal total hip replacements. *J Orthop Res* 21(2): 189-195. (Supported by the "Gesellschaft zur Forderung der Hiiftchirurgie" in Vienna/Austria. Authors affiliated with Municipality of Vienna Gersthof Orthopaedic Hospital, Austria; Vienna University Hospital, Austria; University of Vienna, Austria; Holy Cross Sisters of Mercy Hospital, Austria.)
301. Li Q, Ke Q, Costa M. 2009. Alterations of histone modifications by cobalt compounds. *Carcinogenesis* 30(7): 1243-1251. (Supported by the Swiss Federal Office of Public Health. Authors affiliated with Institute for Chemical and Bioengineering, Switzerland; Swiss Federal Laboratories for Materials Testing and Research, Switzerland.)
302. Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, Stark WJ. 2007. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. *Environ Sci Technol* 41(11): 4158-4163. (Supported by the Swiss Federal Office of Public Health. Authors affiliated with ETH Zurich, Switzerland; Swiss Federal Laboratories for Materials Testing and Research, Switzerland.)
-

303. Linna A, Oksa P, Palmroos P, Roto P, Laippala P, Uitti J. 2003. Respiratory health of cobalt production workers. *Am J Ind Med* 44(2): 124-132. (Supported by the Finnish Work Environment Fund. Authors affiliated with Outokumpu Zinc Oy, Finland; Tampere University Hospital, Finland.)
304. Linnainmaa M, Kiilunen M. 1997. Urinary cobalt as a measure of exposure in the wet sharpening of hard metal and stellite blades. *Int Arch Occup Environ Health* 69(3): 193-200. (Supported by the Finnish Work Environment Fund. Authors affiliated with Finnish Institute of Occupational Health, Finland.)
305. Lippi G, Franchini M, Guidi GC. 2005. Cobalt chloride administration in athletes: a new perspective in blood doping? *Br J Sports Med* 39(11): 872-873. (Support not reported. Authors affiliated with Università di Verona, Italy; Azienda Ospedaliera di Verona, Italy.)
306. Lison D, De Boeck M, Verougstraete V, Kirsch-Volders M. 2001. Update on the genotoxicity and carcinogenicity of cobalt compounds. *Occup Environ Med* 58(10): 619-625. (Support not reported. Authors affiliated with Universite Catholique de Louvain, Belgium; Vrije Universiteit Brussel, Belgium.)
307. Lison D. 2015. Cobalt. In *Handbook on the Toxicology of Metals*. 4th ed., Vol. II: Specific Metals. Nordberg GF, Fowler BA, Nordberg M, eds. Waltham, MA: Elsevier. pp. 743-763.
308. Liu FC, Qin J, Wu HS, Wu YL, Zhu YL. 2011. Co and Cr accumulation in hair after metal-on-metal hip resurfacing arthroplasty. *ANZ J Surg* 81(6): 436-439. (No funding received. Authors affiliated with Second Military Medical University, China.)
309. Lloyd DR, Carmichael PL, Phillips DH. 1998. Comparison of the formation of 8-hydroxy-2'-deoxyguanosine and single- and double-strand breaks in DNA mediated by fenton reactions. *Chem Res Toxicol* 11(5): 420-427. (Support not reported. Authors affiliated with Haddow Laboratories, UK; Imperial College School of Medicine at St. Mary's, Norfolk Place, UK.)
310. Lucas DR, Miller PR, Mott MP, Kronick JL, Unni KK. 2001. Arthroplasty-associated malignant fibrous histiocytoma: two case reports. *Histopathology* 39(6): 620-628. (Support not reported. Authors affiliated with Karmanos Cancer Institute, MI; Mayo Clinic and Mayo Foundation, NY.)
311. Luyts K, Napierska D, Nemery B, Hoet PH. 2013. How physico-chemical characteristics of nanoparticles cause their toxicity: complex and unresolved interrelations. *Environ Sci Process Impacts* 15(1): 23-38. (Supported by the ENPRA (Risk assessment of engineered nano particles), Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO), KU Leuven and an agentschap voor Innovatie door Wetenschap en Technologie. Authors affiliated with K.U. Leuven, Belgium.)
312. Magaye R, Zhao J, Bowman L, Ding M. 2012. Genotoxicity and carcinogenicity of cobalt-, nickel- and copper-based nanoparticles. *Exp Ther Med* 4(4): 551-561. (Supported by the Ningbo Scientific Project, the Scientific Innovation Team Project of Ningbo, the Foundations of Innovative Research Team of Educational Commission of Zhejiang

- Province, Innovative Research Team of Ningbo and K.C. Wong Magna Fund in Ningbo University. Authors affiliated with Ningbo University, China; NIOSH, WV.)
313. Malcolm S. 1984. Malignant soft tissue tumour at the site of a total hip replacement. *J Bone Joint Surg* 66-B: 629. (as cited in Mallick et al 2009)
314. Mao Y, Liu KJ, Jiang JJ, Shi X. 1996. Generation of reactive oxygen species by Co(II) from H<sub>2</sub>O<sub>2</sub> in the presence of chelators in relation to DNA damage and 2'-deoxyguanosine hydroxylation. *J Toxicol Environ Health* 47(1): 61-75. (Support not reported. Authors affiliated with NCI, MD; Dartmouth Medical School, NH.)
315. Marsh GM, Esmen NA. 2007. *Highlights of the NIOSH Health Hazard Evaluation: Copperhill Smelter Worker Study*. HETA 2001-0088-3048. Cincinnati, OH: National Institute for Occupational Health and Safety. 4 pp.
316. Mates JM, Segura JA, Alonso FJ, Marquez J. 2010. Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. *Free Radic Biol Med* 49(9): 1328-1341. (Supported by the Ministerio de Ciencia y Tecnología of Spain and Junta de Andalucía, Proyectos de Investigación de Excelencia, Convocatoria 2006, Spain. Authors affiliated with Universidad de Málaga, Spain.)
317. Maxwell P, Salnikow K. 2004. HIF-1: an oxygen and metal responsive transcription factor. *Cancer Biol Ther* 3(1): 29-35. (Support not reported. Authors affiliated with Imperial College, UK; NCI, MD.)
318. Mayo Clinic. 2015. *Test ID: COS. Cobalt, serum*. Mayo Medical Laboratories. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/80084>. Accessed on 4/10/15.
319. McCleery RE, Blade LM, Burt SE. 2001. *Health Hazard Evaluation Report: Special Metals Corporation, Princeton Powder Division, Princeton, Kentucky*. HETA 97-0141-2819. Cincinnati, OH: National Institute for Occupational Health and Safety. 41 pp.
320. McDonald I. 1981. Malignant lymphoma associated with internal fixation of a fractured tibia. *Cancer* 48(4): 1009-1011. (Support not reported. Author affiliated with Christchurch Hospital, NZ.)
321. McInnes EF, Ernst H, Germann PG. 2013. Spontaneous neoplastic lesions in control Syrian hamsters in 6-, 12-, and 24-month short-term and carcinogenicity studies. *Toxicol Pathol* 41(1): 86-97. (No funding received. Authors affiliated with Healthscope, Australia; Fraunhofer Institute for Toxicology and Experimental Medicine, Germany; Nycomed GmbH, Germany.)
322. McKinley JM, Ofterdinger U, Young M, Barsby A, Gavin A. 2013. Investigating local relationships between trace elements in soils and cancer data. *Spatial Stat* 5: 25-41. (Supported by the British Geological Survey (BGS) University Funding Initiative, the Public Health Agency for N. Ireland, the Department for Enterprise, Trade and Investment (DETINI) and The Rural Development Programme through the Northern Ireland

- Programme for Building Sustainable Prosperity. Authors affiliated with Queen's University Belfast, UK; Geological Survey Northern Ireland, UK; N. Ireland Cancer Registry, UK.)
323. McLean JR, McWilliams RS, Kaplan JG, Birnboim HC. 1982. Rapid detection of DNA strand breaks in human peripheral blood cells and animal organs following treatment with physical and chemical agents. In *Progress in Mutation Research*. vol. 3. Bora KC, Douglas GR, Nestmann ER, eds. Amsterdam: Elsevier Biomedical Press. pp. 137-141. (as cited in IARC 2006)
324. McManus KP. 1982. *Health Hazard Evaluation Report: N.P.C. Systems, Inc., Milford, New Hampshire*. HETA 81-212-1169. Cincinnati, OH: National Institute for Occupational Health and Safety. 13 pp.
325. Meecham HM, Humphrey P. 1991. Industrial exposure to cobalt causing optic atrophy and nerve deafness: a case report. *J Neurol Neurosurg Psychiatry* 54(4): 374-375. (Support not reported. Authors affiliated with Walton Hospital, UK.)
326. Meltzer HM, Brantsaeter AL, Borch-Johnsen B, Ellingsen DG, Alexander J, Thomassen Y, Stigum H, Ydersbond TA. 2010. Low iron stores are related to higher blood concentrations of manganese, cobalt and cadmium in non-smoking, Norwegian women in the HUNT 2 study. *Environ Res* 110(5): 497-504. (Supported by the Norwegian Research Council. The Nord-Trøndelag Health Study (The HUNT Study) is a collaboration between HUNT Research Centre, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Verdal, Norwegian Institute of Public Health, and Nord-Trøndelag County Council. Authors affiliated with Norwegian Institute of Public Health, Norway; University of Oslo, Norway; National Institute of Occupational Health, Norway; Norwegian Institute of Public Health, Norway; Statistics Norway, Norway.)
327. Menzel DB, Wolpert RL, Francovitch RJ, Shoaf CR, Boger III JR, Tayyeb MI. 1989. Respiratory tract burdens of cobalt from inhalation of soluble aerosols: simulation by a two-compartment model. *Inhal Toxicol* 1: 49-69. (Supported by NIH and the International Life Sciences Risk Research Institute. Authors affiliated with Duke University Medical Center, NC.)
328. Méplan C, Richard MJ, Hainaut P. 2000. Metalloregulation of the tumor suppressor protein p53: zinc mediates the renaturation of p53 after exposure to metal chelators in vitro and in intact cells. *Oncogene* 19(46): 5227-5236. (Supported by the Fondation Volvic pour la recherche sur les oligoéléments (1998) and by an IARC Special Training Award. Authors affiliated with IARC, France; Centre Hospitalier et Universitaire Albert Michalon, France.)
329. MHRA. 2012. *Medical Device Alert: All Metal-on-Metal (MoM) Hip Replacements*. Ref: MDA/2012/036. Medicines and Healthcare Products Regulatory Agency. 7 pp. <https://assets.digital.cabinet-office.gov.uk/media/5485abf6ed915d4c10000273/con155767.pdf>
330. Mikkelsen S, Raffn E, Altman D, Groth S, Christensen JM. 1984. [Helbred og Kobolt]. In *Tvaersnitsundersøgelse af Platte-malere [Health and Cobalt. A Cross-sectional Study of Plate Painters]*. Copenhagen: Arbejdsmitjøfondet. (as cited in IARC 1991)

331. Miller AC, Mog S, McKinney L, Luo L, Allen J, Xu J, Page N. 2001. Neoplastic transformation of human osteoblast cells to the tumorigenic phenotype by heavy metal-tungsten alloy particles: induction of genotoxic effects. *Carcinogenesis* 22(1): 115-125. (Supported by the Armed Forces Radiobiology Research Institute. Authors affiliated with Armed Forces Radiobiology Research Institute, MD; NIH, MD.)
332. Min WK, Kim SY, Oh CW, Kim SJ, Park TI, Koo KH. 2008. Malignant fibrous histiocytoma arising in the area of total hip replacement. *Joint Bone Spine* 75(3): 319-321. (Support not reported. Authors affiliated with Kyungpook National University, South Korea; Goodmoring Hospital, South Korea; Seoul National University, South Korea.)
333. Mining Technology Market and Customer Insight. 2015. *Idaho Cobalt Mine, United States of America*. Mining Technology Market & Customer Insight. <http://www.mining-technology.com/projects/idaho-cobalt-mine/>. Accessed on 3/6/15.
334. Miyaki M, Akamatsu N, Ono T, Koyama H. 1979. Mutagenicity of metal cations in cultured cells from Chinese hamster. *Mutat Res* 68(3): 259-263. (as cited in IARC 2006)
335. Mo Y, Zhu X, Hu X, Tollerud DJ, Zhang Q. 2008. Cytokine and NO release from peripheral blood neutrophils after exposure to metal nanoparticles: In vitro and ex vivo studies. *Nanotoxicology* 2(2): 79-87. (Supported by the American Lung Association and the University of Louisville. Authors affiliated with University of Louisville, KY; Zhejiang University, China; Albert Einstein College of Medicine, NY.)
336. Mochizuki H, Kada T. 1982. Antimutagenic action of cobaltous chloride on Trp-P-1-induced mutations in *Salmonella typhimurium* TA98 and TA1538. *Mutat Res* 95(2-3): 145-157. (as cited in IARC 2006)
337. Mohmand J, Eqani SA, Fasola M, Alamdar A, Mustafa I, Ali N, Liu L, Peng S, Shen H. 2015. Human exposure to toxic metals via contaminated dust: Bio-accumulation trends and their potential risk estimation. *Chemosphere* 132: 142-151. (Supported by the Higher Education Commission and the the IUE, CAS, China. Authors affiliated with COMSATS Institute of Information & Technology, Pakistan; Università, Italy; Chinese Academy of Sciences, China; University of Sargodha, Pakistan; King Abdulaziz University, Saudi Arabia.)
338. Moorhouse CP, Halliwell B, Grootveld M, Gutteridge JM. 1985. Cobalt(II) ion as a promoter of hydroxyl radical and possible 'crypto-hydroxyl' radical formation under physiological conditions. Differential effects of hydroxyl radical scavengers. *Biochim Biophys Acta* 843(3): 261-268. (Supported by the Wellcome Trust and the Arthritis and Rheumatism Council. Authors affiliated with University of London King's College, UK; National Institute for Biological Standards and Control, UK.)
339. Moreno ME, Acosta-Saavedra LC, Meza-Figueroa D, Vera E, Cebrian ME, Ostrosky-Wegman P, Calderon-Aranda ES. 2010. Biomonitoring of metal in children living in a mine tailings zone in Southern Mexico: A pilot study. *Int J Hyg Environ Health* 213(4): 252-258. (Supported by the Mexican Council for Science and Technology. Authors affiliated with Cinvestav, Mexico; Universidad Autonoma de Guerrero, Mexico; Universidad de Sonora, Mexico; Universidad Nacional Autonoma de Mexico, Mexico.)

340. Mosconi G, Bacis M, Vitali MT, Leghissa P, Sabbioni E. 1994a. Cobalt excretion in urine: results of a study on workers producing diamond grinding tools and on a control group. *Sci Total Environ* 150(1-3): 133-139. (Support not reported. Authors affiliated with Ospedali Riuniti of Bergamo, Italy; Commission of the European Communities, Italy.)
341. Mosconi G, Bacis M, Leghissa P, Maccarana G, Arsuffi E, Imbrogno P, Airoidi L, Caironi M, Ravasio G, Parigi PC, Polini S, Luzzana G. 1994b. Occupational exposure to metallic cobalt in the Province of Bergamo. Results of a 1991 survey. *Sci Total Environ* 150(1-3): 121-128. (Support not reported. Authors affiliated with Ospedali Riuniti of Bergamo, Italy; Local Sanitary Units 26 - 32, Italy.)
342. Moulin JJ, Wild P, Mur JM, Fournier-Betz M, Mercier-Gallay M. 1993. A mortality study of cobalt production workers: an extension of the follow-up. *Am J Ind Med* 23(2): 281-288. (Support not reported. Authors affiliated with INRS, France.)
343. Moulin JJ, Wild P, Romazini S, Lasfargues G, Peltier A, Bozec C, Deguerry P, Pellet F, Perdrix A. 1998. Lung cancer risk in hard-metal workers. *Am J Epidemiol* 148(3): 241-248. (Support not reported. Authors affiliated with INRS, France; Institut Universitaire de Medecine du Travail et d'Ergonomie, France; Institut Universitaire de Medecine du Travail du Val de Loire, France; Eramet, France; Sandvik Hard Materials, France.)
344. Moulin JJ, Clavel T, Roy D, Dananché B, Marquis N, Févotte J, Fontana JM. 2000. Risk of lung cancer in workers producing stainless steel and metallic alloys. *Int Arch Occup Environ Health* 73(3): 171-180. (Support not reported. Authors affiliated with INRS, France; Paris La Défense, France; Institut Universitaire de Médecine du Travail de Lyon, France; LECES, France.)
345. Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. 1987. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. *Am J Ind Med* 11(1): 75-81. (Support not reported. Authors affiliated with INRS, France.)
346. Naylor GP, Harrison JD. 1995. Gastrointestinal iron and cobalt absorption and iron status in young rats and guinea pigs. *Hum Exp Toxicol* 14(12): 949-954. (Support not reported. Authors affiliated with National Radiological Protection Board, UK.)
347. Neale G. 1990. B12 binding proteins. *Gut* 31(1): 59-63. (Support not reported. Author affiliated with Addenbrookes Hospital, UK.)
348. Nemery B, Casier P, Roosels D, Lahaye D, Demedts M. 1992. Survey of cobalt exposure and respiratory health in diamond polishers. *Am Rev Respir Dis* 145(3): 610-616. (Supported by the Fonds voor Geneeskundig Wetenschappelijk Onderzoek. Authors affiliated with Katholieke Universiteit te Leuven, Belgium; Fund for Occupational Diseases, Belgium.)
349. Newton D, Rundo J. 1971. The long-term retention of inhaled cobalt-60. *Health Phys* 21(3): 377-384. (Support not reported. Authors affiliated with Atomic Energy Research Establishment, UK; Argonne National Laboratory, IL.)

350. Nielsen NH, Kristiansen J, Borg L, Christensen JM, Poulsen LK, Menne T. 2000. Repeated exposures to cobalt or chromate on the hands of patients with hand eczema and contact allergy to that metal. *Contact Dermatitis* 43(4): 212-215. (Supported by the European Commission. Authors affiliated with University of Copenhagen, Denmark; National Institute of Occupational Health, Denmark; National University Hospital, Denmark.)
351. Nilsson K, Jensen BS, Carlsen L. 1985. The migration chemistry of cobalt. *Eur Appl Res Rept - Nucl Sci Technol* 7(1): 23-86. (Performed in the frame of the R&D Programme "Management and disposal of radioactive waste." Authors affiliated with Risø National Laboratory, Denmark.)
352. NIOSH. 1972. *Health Hazard Evaluation Report: Crane Company, St. Louis, Missouri*. HETA 71-11-2. Cincinnati, OH: National Institute for Occupational Health and Safety. 6 pp.
353. NIOSH. 1987. *Health Hazard Evaluation Report: GTE/Valenite Corporation, Westminster, South Carolina*. HETA 85-064-1844. U.S. Department of Health and Human Services, National Institute for Occupational Health and Safety.
354. NIOSH. 1990. *National Occupational Hazard Survey (1981-1983)*. National Institute for Occupational Safety and Health. Last updated 7/1/90.  
<http://www.cdc.gov/noes/noes4/73470sco.html>,  
<http://www.cdc.gov/noes/noes4/x8305sco.html>.
355. NIOSH. 1994a. Elements in Blood or Tissue: Method 8005. In *NIOSH Manual of Analytical Methods*. 4th edition. National Institute for Occupational Health and Safety. 6 pp.
356. NIOSH. 1994b. Cobalt and compounds, as Co: Method 7027. In *NIOSH Manual of Analytical Methods*. 4th edition. National Institute for Occupational Health and Safety. 3 pp.
357. NIOSH. 2003. Elements on wipes: Method 9102. In *NIOSH Manual of Analytical Methods*. 4th edition. National Institute for Occupational Health and Safety. 5 pp.
358. Nishioka H. 1975. Mutagenic activities of metal compounds in bacteria. *Mutat Res* 31(3): 185-189. (as cited in IARC 2006)
359. NIST. 2005. *Why Are There No Volume Li-ion Battery Manufacturers in the United States: Chapter II - Rationale for Li-ion Case* National Institute of Standards and Technology. Updated on 1/3/2007. <http://www.atp.nist.gov/eao/wp05-01/chapt2.htm>. Accessed on 5/22/15.
360. Novick N. 2013. *Understanding Implants in Knee and Hip Replacement*. Hospital for Special Surgery. [https://www.hss.edu/conditions\\_understanding-implants-in-knee-and-hip-replacement.asp](https://www.hss.edu/conditions_understanding-implants-in-knee-and-hip-replacement.asp). Accessed on 4/4/16.
361. Nowak HF. 1966. Neoplasia in mouse skeletal muscles under the influence of polyester resin activator. *Arch Immunol Ther Exp (Warsz)* 14(6): 774-778. (Support not reported. Author affiliated with Department of Pathologic Anatomy, School of Medicine, Bialystok.)

362. NTP. 1998. *Toxicology and Carcinogenesis Studies of Cobalt Sulfate Heptahydrate (CAS No. 10026-24-1) in F344/N Rats and B6C3F1/N Mice (Inhalation Studies)*. Technical Report Series No. 471. NIH Publication No. 98-3961. Research Triangle Park, NC: National Toxicology Program. 268 pp.
363. NTP. 2002. *Report on Carcinogens Background Document for Cobalt Sulfate*. Research Triangle Park, NC: National Toxicology Program. 71 pp.
364. NTP. 2009. *Report on Carcinogens Background Document for Cobalt-Tungsten Carbide: Powders and Hard metals*. Research Triangle Park, NC: National Toxicology Program. 180 pp.
365. NTP. 2014a. Cobalt-tungsten carbide: Powders and hard metals. In *Report on Carcinogens*. 13th Edition. Research Triangle Park, NC: National Toxicology Program.  
<http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.
366. NTP. 2014b. *Toxicology Studies of Cobalt Metal (CAS No. 7440-48-4) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTac Rats and B6C3F1/N Mice (Inhalation Studies)*. Technical Report Series No. 581. NIH Publication No. 14-5932. Research Triangle Park, NC: National Toxicology Program. 308 pp.
367. NTP. 2014c. *Cobalt and Certain Cobalt Compounds Protocol*. Research Triangle Park, NC: National Toxicology Program. 50 pp.  
[http://ntp.niehs.nih.gov/ntp/roc/protocols/cobalt\\_508.pdf](http://ntp.niehs.nih.gov/ntp/roc/protocols/cobalt_508.pdf).
368. NTP. 2014d. Cobalt sulfate. In *Report on Carcinogens*. 13th Edition. Research Triangle Park, NC: National Toxicology Program.  
<http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.
369. Nyga A, Hart A, Tetley TD. 2015. Importance of the HIF pathway in cobalt nanoparticle-induced cytotoxicity and inflammation in human macrophages. *Nanotoxicology*: 1-13. (Supported by the Technology Strategy Board. Authors affiliated with Imperial College London, UK; University College London, UK.)
370. O'Hara GP, Mann DE, Jr., Gautieri RF. 1971. Effect of cobalt chloride and sodium cobaltinitrite on the growth of established epithelial tumors induced by methylcholanthrene. *J Pharm Sci* 60(3): 473-474. (Support not reported. Authors affiliated with American Foundation for Pharmaceutical Education.)
371. O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, Group FS. 2012. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *Int J Cancer* 131(8): 1882-1891. (Supported by the Centre for Health Improvement, Queen's University Belfast, Northern Ireland, the Northern Ireland Research & Development Office, the Health Research Board, Ireland, the Ulster Cancer Foundation and the Charitable Fund of the Royal Groups of Hospitals, Belfast. Authors affiliated with Queens University Belfast, UK; NCI, MD; University of Missouri-Columbia, MO.)

372. OECD. 2014. *Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party On Chemicals, Pesticides and Biotechnology. Guidance on Grouping of Chemicals, Second Edition*. OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 194, ENV/JM/MONO(2014)4. Paris, France: Organisation for Economic Co-operation and Development. 141 pp.
373. Ogawa HI, Sakata K, Inouye T, Jyosui S, Niyitani Y, Kakimoto K, Morishita M, Tsuruta S, Kato Y. 1986. Combined mutagenicity of cobalt(II) salt and heteroaromatic compounds in *Salmonella typhimurium*. *Mutat Res* 172(2): 97-104. (as cited in IARC 2006)
374. Ogawa HI, Shibahara T, Iwata H, Okada T, Tsuruta S, Kakimoto K, Sakata K, Kato Y, Ryo H, Itoh T, *et al.* 1994. Genotoxic activities in vivo of cobaltous chloride and other metal chlorides as assayed in the *Drosophila* wing spot test. *Mutat Res* 320(1-2): 133-140. (as cited in IARC 2006)
375. Ogawa HI, Ohyama Y, Ohsumi Y, Kakimoto K, Kato Y, Shirai Y, Nunoshiba T, Yamamoto K. 1999. Cobaltous chloride-induced mutagenesis in the supF tRNA gene of *Escherichia coli*. *Mutagenesis* 14(2): 249-253. (as cited in IARC 2006)
376. Ohno I. 2010. Electroless Deposition of Alloys. In *Modern Electroplating*. 5th edition. Schlesinger M, Paunovic M, eds.: John Wiley & Sons. pp. 499-506.
377. Ortega R, Bresson C, Darolles C, Gautier C, Roudeau S, Perrin L, Janin M, Floriani M, Aloin V, Carmona A, Malard V. 2014. Low-solubility particles and a Trojan-horse type mechanism of toxicity: the case of cobalt oxide on human lung cells. *Part Fibre Toxicol* 11: 14. (Supported by the CEA DSV (France) and Electricité De France (EDF) company. Authors affiliated with University of Bordeaux, France; CEA, DEN, DPC, SEARS, Laboratoire de développement Analytique Nucléaire, France; CEA, DSV, IBEB, Lab Biochim System Perturb, France; CEA, DEN, DPC, SEARS, Laboratoire d'Analyse en Soutien aux Exploitants, France; IRSN, France; University of Nîmes, France.)
378. Orzechowski RF, Gautieri RF, Mann DE, Jr. 1964. Effect of sodium cobaltinitrite on the minimal carcinogenic dose-50 (Mcd-50) of methylcholanthrene in albino mice. *J Pharm Sci* 53: 388-391. (Supported by NCI, NIH. Authors affiliated with Temple University, PA.)
379. Ostapczuk P, Valenta P, Stoeppler M, Nürnberg HW. 1983. Voltammetric determination of nickel and cobalt in body fluids and other biological materials. In *Chemical Toxicology and Clinical Chemistry of Metals*. Brown SS, Savory J, eds. London: Academic Press. pp. 61-64. (as cited in IARC 1991)
380. Pagano DA, Zeiger E. 1992. Conditions for detecting the mutagenicity of divalent metals in *Salmonella typhimurium*. *Environ Mol Mutagen* 19(2): 139-146. (as cited in IARC 2006)
381. Palecek E, Brazdova M, Cernocka H, Vlk D, Brazda V, Vojtesek B. 1999. Effect of transition metals on binding of p53 protein to supercoiled DNA and to consensus sequence in DNA fragments. *Oncogene* 18(24): 3617-3625. (Supported by the Grant Agency of the Czech Republic, Volkswagen Stiftung, the Grant Agency of the Academy of Sciences CR and IGA MH CR. Authors affiliated with Academy of Sciences of the Czech Republic, Czech Republic; Masaryk Memorial Cancer Institute, Czech Republic.)

382. Palit S, Sharma A, Talukder G. 1991a. Cytotoxic effects of cobalt chloride on mouse bone marrow cells in vivo. *Cytobios* 68(273): 85-89. (Supported by the University Grants Commission and Council of Scientific and Industrial Research. Authors affiliated with University of Calcutta, India; Vivekananda Institute of Medical Sciences, India.)
383. Palit S, Ghosh AK, Sharma A, Talukder G. 1991b. Modification of the clastogenic effects of cobalt by calcium in bone marrow cells of mice in vivo. *Cytologia* 56: 373-377. (As cited in WHO 2006)
384. Palit S, Sharma A, Talukder G. 1991c. Chromosomal aberrations induced by cobaltous chloride in mice in vivo. *Biol Trace Elem Res* 29(2): 139-145. (as cited in WHO 2006)
385. Palit S, Sharma A, Talukder G. 1991d. Protection by chlorophyllin against induction of chromosomal aberrations by cobalt in bone marrow cells of mice in vivo. *Fitoterapia* 62(5): 425-428. (as cited in WHO 2006)
386. Papis E, Rossi F, Raspanti M, Dalle-Donne I, Colombo G, Milzani A, Bernardini G, Gornati R. 2009. Engineered cobalt oxide nanoparticles readily enter cells. *Toxicol Lett* 189(3): 253-259. (Support not reported. Authors affiliated with Insubria University, Italy; University of Milan, Italy; Centro di Ricerca Interuniversitario Politecnico di Milano e Università dell'Insubria "The Protein Factory", Italy.)
387. Pasha Q, Malik SA, Iqbal J, Shah MH. 2007. Characterization and distribution of the selected metals in the scalp hair of cancer patients in comparison with normal donors. *Biological Trace Element Research* 118(3): 207-216. (Supported by the Higher Education Commission, Government of Pakistan. Authors affiliated with Quaid-i-Azam University, Pakistan; POF Hospital, Pakistan.)
388. Patel E, Lynch C, Ruff V, Reynolds M. 2012. Co-exposure to nickel and cobalt chloride enhances cytotoxicity and oxidative stress in human lung epithelial cells. *Toxicol Appl Pharmacol* 258(3): 367-375. (Support not reported. Authors affiliated with Washington College, MD; U.S. Army Research Institute of Environmental Medicine, MA.)
389. Paton GR, Allison AC. 1972. Chromosome damage in human cell cultures induced by metal salts. *Mutat Res* 16(3): 332-336. (as cited in IARC 2006)
390. Patrick G, Batchelor AL, Stirling C. 1989. An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles - Part VI: Lung clearance of inhaled cobalt oxide particles in SPF Fischer rats. *J Aerosol Sci* 20(2): 249-255. (Support not reported. Authors affiliated with MRC Radiobiology Unit, UK.)
391. Patrick G, Stirling C, Kreyling WG, Poncy JL, Duserre C, Collier CG, Godleski J, Brain JD. 1994. Interspecies comparison of the clearance of ionic cobalt from the lungs. *Inhal Toxicol* 6: 225-240. (Supported by the Commission of the European Communities. Authors affiliated with Medical Research Council Radiobiology Unit, UK; GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Germany; Commissariat à l'Energie Atomique, France; National Radiological Protection Board, UK; Harvard School of Public Health, MA.)

- 
392. Paul SAM, Simons JW, Mabeesh NJ. 2004. HIF at the crossroads between ischemia and carcinogenesis. *Journal of Cellular Physiology* 200(1): 20-30. (Supported by the Avon Foundation, NIH, CaP CURE Foundation and the M.K. Humanitarian Association and Parisian. Authors affiliated with Ludwig-Maximilians-University, Germany; Emory University School of Medicine, GA; Tel Aviv Sourasky Medical Center, Israel.)
393. Pauluhn J. 2009. Comparative pulmonary response to inhaled nanostructures: Considerations on test design and endpoints. *Inhalation Toxicology* 21(Suppl. 1): 40-54. (Supported by the German Federal Ministry of Education and Research. Author affiliated with Bayer HealthCare AG, Germany.)
394. Paustenbach DJ, Tvermoes BE, Unice KM, Finley BL, Kerger BD. 2013. A review of the health hazards posed by cobalt. *Crit Rev Toxicol* 43(4): 316-362. (Supported by DePuy Orthopaedics, Inc. Authors affiliated with ChemRisk, LLC, CA, CO and PA.)
395. Pellet F, Perdrix A, Vincent M, Mallion J-M. 1984. [Biological levels of urinary cobalt]. *Arch Mal Prof* 45: 81-85. (as cited in IARC 2006 and IARC 1991)
396. Permenter MG, Dennis WE, Sutto TE, Jackson DA, Lewis JA, Stallings JD. 2013. Exposure to cobalt causes transcriptomic and proteomic changes in two rat liver derived cell lines. *PLoS One* 8(12): e83751. (Supported by the U.S. Army Medical Research and Materiel Command, Military Operational Medicine Research Program. Authors affiliated with Excet, Inc., MD; US Army Center for Environmental Health Research, MD; Naval Research Laboratory, Washington, D.C.)
397. Petering HG, Yeager DW, Witherup SO. 1973. Trace metal content of hair. II. Cadmium and lead of human hair in relation to age and sex. *Arch Environ Health* 27(5): 327-330. (Supported by NIH. Authors affiliated with University of Cincinnati, OH.)
398. Peters K, Unger RE, Gatti AM, Sabbioni E, Tsaryk R, Kirkpatrick CJ. 2007. Metallic nanoparticles exhibit paradoxical effects on oxidative stress and pro-inflammatory response in endothelial cells in vitro. *Int J Immunopathol Pharmacol* 20(4): 679-689. (Supported by the Deutsche Forschungsgemeinschaft and the European Commission. Authors affiliated with Johannes Gutenberg University, Germany; University of Modena and Reggio Emilia, Italy; European Commission, Italy.)
399. Petit A, Mwale F, Tkaczyk C, Antoniou J, Zukor DJ, Huk OL. 2005. Induction of protein oxidation by cobalt and chromium ions in human U937 macrophages. *Biomaterials* 26(21): 4416-4422. (Supported by the Orthopaedic Research Fund of the Sir Mortimer B. Davis-Jewish General Hospital. Authors affiliated with McGill University, Canada.)
400. Polyzois I, Nikolopoulos D, Michos I, Patsouris E, Theocharis S. 2012. Local and systemic toxicity of nanoscale debris particles in total hip arthroplasty. *J Appl Toxicol* 32(4): 255-269. (Support not reported. Authors affiliated with National and Kapodistrian University of Athens, Greece; Asclepion Voulas General Hospital, Greece.)
401. Ponti J, Sabbioni E, Munaro B, Broggi F, Marmorato P, Franchini F, Colognato R, Rossi F. 2009. Genotoxicity and morphological transformation induced by cobalt nanoparticles and cobalt chloride: an in vitro study in Balb/3T3 mouse fibroblasts. *Mutagenesis* 24(5): 439-

445. (Supported by the European Commission, Joint Research Centre, Nanobiosciences Unit, project: 'NanoBioTechnology for Health'. Authors affiliated with European Commission, Italy.)
402. Posma FD, Dijkstra SK. 1985. Serum and urinary cobalt levels as indicators of cobalt exposure in hard metal workers. In *Proceedings of an International Conference, Heavy Metals in the Environment, Athens, September 1985*. Lekkas TD, ed. Luxembourg: Commission of the European Communities. pp. 89-91. (as cited in IARC 2006 and IARC 1991)
403. Poulsen OM, Olsen E, Christensen JM, Vinzent P, Petersen OH. 1995. Geltape method for measurement of work related surface contamination with cobalt containing dust: Correlation between surface contamination and airborne exposure. *Occup Environ Med* 52: 827-833. (as cited in IARC 2006)
404. Pourahmad J, O'Brien PJ, Jokar F, Daraei B. 2003. Carcinogenic metal induced sites of reactive oxygen species formation in hepatocytes. *Toxicol In Vitro* 17(5-6): 803-810. (Support not reported. Authors affiliated with Shaheed Beheshti University of Medical Sciences, Iran; University of Toronto, Canada.)
405. Prazmo W, Balbin E, Baranowska H, Ejchart A, Putrament A. 1975. Manganese mutagenesis in yeast. II. Conditions of induction and characteristics of mitochondrial respiratory deficient *Saccharomyces cerevisiae* mutants induced with manganese and cobalt. *Genet Res* 26(1): 21-29. (as cited in IARC 2006)
406. Prescott E, Netterstrom B, Faber J, Hegedus L, Suadican P, Christensen JM. 1992. Effect of occupational exposure to cobalt blue dyes on the thyroid volume and function of female plate painters. *Scand J Work Environ Health* 18(2): 101-104. (Supported by SID and Royal Copenhagen. Authors affiliated with University Hospital of Copenhagen, Denmark; Herlev University Hospi, Denmark; Frederiksberg Hospital, Denmark; Danish National Institute of Occupational Health, Denmark.)
407. PubChem. 2015. *PubChem Compound*. National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/pccompound> and search by Compound Identification Number. Accessed on 3/24/15.
408. Pulido MD, Parrish AR. 2003. Metal-induced apoptosis: mechanisms. *Mutat Res* 533(1-2): 227-241. (Support not reported. Authors affiliated with Texas A&M University System Health Science Center, TX.)
409. Putrament A, Baranowska H, Ejchart A, Jachymczyk W. 1977. Manganese mutagenesis in yeast. VI. Mn<sup>2+</sup> uptake, mitDNA replication and ER induction: comparison with other divalent cations. *Mol Gen Genet* 151(1): 69-76. (as cited in IARC 2006)
410. Qayyum MA, Shah MH. 2014. Comparative Assessment of Selected Metals in the Scalp Hair and Nails of Lung Cancer Patients and Controls. *Biological Trace Element Research* 158(3): 305-322. (Supported by the Higher Education Commission, Government of Pakistan. Authors affiliated with Quaid-i-Azam University, Pakistan.)

411. Qiao Y, Ma L. 2013. Quantification of metal ion induced DNA damage with single cell array based assay. *Analyst* 138(19): 5713-5718. (Supported by Bankhead-Copley Cancer Research Program of Florida Department of Health. Authors affiliated with University of Central Florida, FL.)
412. Qiao H, Li L, Qu ZC, May JM. 2009. Cobalt-induced oxidant stress in cultured endothelial cells: prevention by ascorbate in relation to HIF-1alpha. *Biofactors* 35(3): 306-313. (Supported by NIH and the Vanderbilt Diabetes Research and Training Center. Authors affiliated with Vanderbilt University School of Medicine, TN.)
413. Raffn E, Mikkelsen S, Altman DG, Christensen JM, Groth S. 1988. Health effects due to occupational exposure to cobalt blue dye among plate painters in a porcelain factory in Denmark. *Scand J Work Environ Health* 14(6): 378-384. (Supported by the Danish Work Environment Fund. Authors affiliated with Danish Labour Inspectorate, Denmark; Rigshospitale, Denmark; Panum Institute, Denmark; Clinical Research Centre UK; Danish National Institute of Occupational Health, Denmark; Finsen Institute, Denmark.)
414. Rasgele PG, Kekecoglu M, Muranli FDG. 2013. Induction of micronuclei in mice bone marrow cells by cobalt and copper chlorides. *Arch Environ Prot* 39(1): 75-82. (Support not reported. Authors affiliated with Duzce University, Turkey; Trakya University, Turkey.)
415. Reddy SB, Charles MJ, Kumar MR, Reddy BS, Anjaneyulu C, Raju GJN, Sundareswar B, Vijayan V. 2002. Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. *Nucl Instr Methods Phys Res B* 196: 333-339. (Supported by Andhra University. Authors affiliated with Andhra University, India; Andhra Medical College, India; Institute of Physics, India.)
416. Reinardy HC, Syrett JR, Jeffree RA, Henry TB, Jha AN. 2013. Cobalt-induced genotoxicity in male zebrafish (*Danio rerio*), with implications for reproduction and expression of DNA repair genes. *Aquat Toxicol* 126: 224-230. (Supported by the Government of the Principality of Monaco, the International Atomic Energy Agency and Plymouth University Marine Institute. Authors affiliated with University of Plymouth, UK; University of Technology, Australia; University of Tennessee, TN.)
417. Resende de Souza Nazareth H. 1976. [Efeito do cloreto de cobalto em não-disjunção]. *Cie Cult* 28: 1472-1475. (as cited in IARC 2006)
418. Retriev Technologies. 2015. *Lithium Ion*. <http://www.retrievtech.com/recycling/lithium-ion>. Accessed on 10/14/15.
419. Richardson HW, Meshri DT. 2001. Cobalt compounds. In *Kirk-Othmer Encyclopedia of Chemical Technology*. vol. 7. John Wiley & Sons, Inc. pp. 229-249.
420. Richter PA, Bishop EE, Wang J, Swahn MH. 2009. Tobacco smoke exposure and levels of urinary metals in the U.S. youth and adult population: the National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Int J Environ Res Public Health* 6(7): 1930-1946. (Support not reported. Authors affiliated with CDC, GA; RTI International, GA; Georgia State University, GA.)

421. Robison SH, Cantoni O, Costa M. 1982. Strand breakage and decreased molecular weight of DNA induced by specific metal compounds. *Carcinogenesis* 3(6): 657-662. (as cited in IARC 2006)
422. Rodríguez de la Flor M, Hernández-Vaquero D, Fernández-Carreira JM. 2013. Metal presence in hair after metal-on-metal resurfacing arthroplasty. *J Orthop Res* 31(12): 2025-2031. (Supported by the Spanish Society of Orthopedic Surgery and Traumatology. Authors affiliated with Hospital San Agustín, Spain; Julian Clavería, Spain.)
423. Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. 1993. A case-control study of element levels and cancer of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev* 2(4): 305-312. (Supported by NCI. Authors affiliated with SUNY Health Science Center, NY; Fred Hutchinson Cancer Research Center, WA; University of Washington, WA.)
424. Romero A, Ramos E, De Los Ríos C, Egea J, Del Pino J, Reiter RJ. 2014. A review of metal-catalyzed molecular damage: Protection by melatonin. *Journal of Pineal Research* 56(4): 343-370. (Supported by the Miguel Servet Program. Authors affiliated with Universidad Complutense de Madrid, Spain; Universidad Autonoma de Madrid, Spain; Hospital Universitario de la Princesa, Spain; University of Texas Health Science, Center at San Antonio, TX.)
425. Rosensteel RE, Meyer CR, Flesch JP. 1977. *Health Hazard Evaluation Determination: Reinell Boats, Inc., Poplar Bluff, Missouri*. HETA 75-150-378. Cincinnati, OH: National Institute for Occupational Health and Safety. 55 pp.
426. Rossman TG, Molina M, Meyer LW. 1984. The genetic toxicology of metal compounds: I. Induction of lambda prophage in E coli WP2s(lambda). *Environ Mutagen* 6(1): 59-69. (as cited in IARC 2006)
427. Rudge CV, Röllin HB, Nogueira CM, Thomassen Y, Rudge MC, Odland JØ. 2009. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. *J Environ Monit* 11(7): 1322-1330. (Supported by the Brazilian Federal Agency for Graduate Studies. Authors affiliated with São Paulo State University, Brazil; Medical Research Council, South Africa; University of Pretoria, South Africa; University of the Witwatersrand, South Africa; National Institute for Occupational Health, South Africa; National Institute for Occupational Health, Norway; University of Tromsø, Norway; University of Aarhus, Denmark.)
428. Rufe PF. 2010. *Testimony for United States Senate Committee on Energy and Natural Resources, September 30, 2010*. Salmon, ID: Formation Capital Corporation, U.S. 164 pp.
429. Saat NZM, Chow SY, Ghazali AR, Hamid ZA, Lubis SH, Mohamed N, Ishak I, Othman H. 2013. Study of heavy metal levels in nails and hairs among vegetable farmers in Malaysia. *Res J Appl Sci* 8(9): 449-455. (Supported by the Ministry of Higher Education Malaysia and the Universiti Kebangsaan Malaysia. Authors affiliated with Jalan Raja Muda Abdul Aziz, Malaysia.)

430. Sabbioni E, Minoia C, Pietra R, Mosconi G, Forni A, Scansetti G. 1994a. Metal determinations in biological specimens of diseased and non-diseased hard metal workers. *Sci Total Environ* 150(1-3): 41-54. (Support not reported. Authors affiliated with European Commission, Italy; Fondazione Clinica del Lavoro, Italy; Ospedali Riuniti di Bergamo, Italy; University of Milan, Italy; University of Turin, Italy.)
431. Sabbioni E, Mosconi G, Minoia C, Seghizzi P. 1994b. The European Congress on Cobalt and Hard Metal Disease. Conclusions, highlights and need of future studies. *Sci Total Environ* 150(1-3): 263-270. (Supported by the Associazione Italiana Ricerca sul Cancro (AIRC). Authors affiliated with Veneto Nanotech SCpA, Italy; European Commission, Italy; Phi-Science srl Chemical and Safety Consulting, Italy; Ext-ECEurope, Italy; "Università G. d'Annunzio" Foundation, Italy; University of Insubria, Italy; Polytechnic University of Milan, Italy; G. d'Annunzio University, Italy.)
432. Sabbioni E, Fortaner S, Farina M, Del Torchio R, Petrarca C, Bernardini G, Mariani-Costantini R, Perconti S, Di Giampaolo L, Gornati R, Di Gioacchino M. 2014a. Interaction with culture medium components, cellular uptake and intracellular distribution of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts. *Nanotoxicology* 8(1): 88-99. (Supported by the Unit of "Immunotoxicology and Allergy" of Ce.S.I., ECVAM Unit, the "Fondi Ateneo per la Ricerca" from the University of Insubria and the "Associazione Italiana Ricerca sul Cancro." Authors affiliated with European Center for the Sustainable Impact of Nanotechnologies, Italy; European Commission, Italy; Phi-Science srl Chemical and Safety Consulting, Italy; Ext-EC-Europe, Italy; Aging Research Center, Italy; University of Insubria, Italy; Polytechnic University of Milan, Italy; d'Annunzio University, Italy.)
433. Sabbioni E, Fortaner S, Farina M, Del Torchio R, Olivato I, Petrarca C, Bernardini G, Mariani-Costantini R, Perconti S, Di Giampaolo L, Gornati R, Di Gioacchino M. 2014b. Cytotoxicity and morphological transforming potential of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts: an in vitro model. *Nanotoxicology* 8(4): 455-464. (Supported by the Associazione Italiana Ricerca sul Cancro. Authors affiliated with European Center for the Sustainable Impact of Nanotechnologies, Italy; European Commission, Italy; Phi-Science srl Chemical and Safety Consulting, Italy; Ext-ECEurope, Italy; "Università G. d'Annunzio" Foundation, Italy; University of Insubria, Italy; Polytechnic University of Milan, Italy; G. d'Annunzio University, Italy.)
434. Sahakian N, Stefaniak A, Day G, Kanwal R. 2009. *Report on Respiratory Symptoms and Disease among Cemented Tungsten Carbide Workers. Health Hazard Evaluation Report: Metalworking Products, Huntsville, Gurley, and Grant, Alabama.* HETA 2003-0257-3088. Cincinnati, OH: National Institute for Occupational Safety and Health. 316 pp.
435. Saini Y, Greenwood KK, Merrill C, Kim KY, Patial S, Parameswaran N, Harkema JR, LaPres JJ. 2010a. Acute cobalt-induced lung injury and the role of hypoxia-inducible factor 1 alpha in modulating inflammation. *Toxicological Sciences* 116(2): 673-681. (Supported by NIH. Authors affiliated with Michigan State University, MI.)
436. Saini Y, Kim KY, Lewandowski R, Bramble LA, Harkema JR, Lapres JJ. 2010b. Role of hypoxia-inducible factor 1{alpha} in modulating cobalt-induced lung inflammation. *Am J*

- Physiol Lung Cell Mol Physiol* 298(2): L139-147. (Supported by NIEHS. Authors affiliated with Michigan State University, MI.)
437. Salisbury S, Seligman PJ. 1987. *Health Hazard Evaluation Report: Wheel Trueing Tool Company, Columbia, South Carolina*. HETA 84-288-1847. Cincinnati, OH: National Institute for Occupational Health and Safety. 38 pp.
438. Salnikow K, Su W, Blagosklonny MV, Costa M. 2000. Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species-independent mechanism. *Cancer Res* 60(13): 3375-3378. (Supported by NIH. Authors affiliated with New York University School of Medicine, NY; NIH, MD.)
439. Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, Kasprzak KS. 2004. Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. *J Biol Chem* 279(39): 40337-40344. (Supported by NIEHS. Authors affiliated with NIH, MD; University of Texas Southwestern Medical Center, TX; Brown University, RI.)
440. Sampson B, Hart A. 2012. Clinical usefulness of blood metal measurements to assess the failure of metal-on-metal hip implants. *Ann Clin Biochem* 49(Pt 2): 118-131. (Supported by the The London Implant Retrieval Centre (LIRC), which is funded by the British Orthopaedic Association (BOA). Authors affiliated with Imperial College Healthcare NHS Trust, UK; Imperial College, UK.)
441. Sarmiento-González A, Marchante-Gayón JM, Tejerina-Lobo JM, Paz-Jiménez J, Sanz-Medel A. 2008. High-resolution ICP-MS determination of Ti, V, Cr, Co, Ni, and Mo in human blood and urine of patients implanted with a hip or knee prosthesis. *Anal Bioanal Chem* 391(7): 2583-2589. (Supported by Acuña y Fombona S.A, FICYT and the Ministry of Science and Technology of Spain. Authors affiliated with University of Oviedo, Spain.)
442. Savarino L, Cadossi M, Chiarello E, Baldini N, Giannini S. 2013. Do ion levels in metal-on-metal hip resurfacing differ from those in metal-on-metal tha at long-term followup. *Clin Orthop Relat Res* 471: 2964-2971. (Supported by the Italian Ministry of the Health, Financial Support for Scientific Research. Authors affiliated with Rizzoli Orthopaedic Institute, Italy; Bologna University, Italy.)
443. Savarino L, Cadossi M, Chiarello E, Fotia C, Greco M, Baldini N, Giannini S. 2014. How do metal ion levels change over time in hip resurfacing patients? A cohort study. *Sci World J* 2014: 291925. (Authors affiliated with Rizzoli Orthopaedic Institute, Italy; Bologna University, Italy.)
444. Scansetti G, Lamon S, Talarico S, Botta GC, Spinelli P, Sulotto F, Fantoni F. 1985. Urinary cobalt as a measure of exposure in the hard metal industry. *Int Arch Occup Environ Health* 57(1): 19-26. (as cited in IARC 2006 and IARC 1991)
445. Scansetti G, Botta GC, Spinelli P, Reviglione L, Ponzetti C. 1994. Absorption and excretion of cobalt in the hard metal industry. *Sci Total Environ* 150(1-3): 141-144. (Support not reported. Authors affiliated with Università di Torino, Italy; Servizio di Igiene Pubblica, Italy.)

- 
446. Scansetti G, Maina G, Botta GC, Bambace P, Spinelli P. 1998. Exposure to cobalt and nickel in the hard-metal production industry. *Int Arch Occup Environ Health* 71(1): 60-63. (Support not reported. Authors affiliated with Università degli Studi di Torino, Italy.)
447. Schaffer AW, Pilger A, Engelhardt C, Zweymueller K, Ruediger HW. 1999. Increased blood cobalt and chromium after total hip replacement. *J Toxicol Clin Toxicol* 37(7): 839-844. (Support not reported. Authors affiliated with University of Vienna, Austria.)
448. Scharf B, Clement CC, Zolla V, Perino G, Yan B, Elci SG, Purdue E, Goldring S, Macaluso F, Cobelli N, Vachet RW, Santambrogio L. 2014. Molecular analysis of chromium and cobalt-related toxicity. *Sci Rep* 4: 5729. (Supported by the University of Tuscia. Authors affiliated with Albert Einstein College of Medicine, NY; Hospital for Special Surgery, NY.)
449. Schumacher-Wittkopf E, Angerer J. 1981. [A practical method for the determination of cobalt in urine (author's transl)]. *Int Arch Occup Environ Health* 49(1): 77-81. (as cited in IARC 1991)
450. SciFinder. 2015. *SciFinder*. <http://www.cas.org/products/scifinder>. Accessed on 4/30/15.
451. SEER. 2015a. *SEER Cancer Statistics Factsheets: Lung and Bronchus Cancer*. National Cancer Institute. <http://seer.cancer.gov/statfacts/html/lungb.html>. Accessed on 3/30/15.
452. SEER. 2015b. *SEER Cancer Statistics Factsheets: Esophageal Cancer*. National Cancer Institute. <http://seer.cancer.gov/statfacts/html/esoph.html>. Accessed on 3/30/15.
453. SEER. 2015c. *SEER Cancer Stat Fact Sheets*. National Cancer Institute. <http://seer.cancer.gov/statfacts/>. Accessed on 3/30/15.
454. Shabaan AA, Marks V, Lancaster MC, Dufeu GN. 1977. Fibrosarcomas induced by cobalt chloride (CoCl<sub>2</sub>) in rats. *Lab Anim* 11(1): 43-46. (Support not reported. Authors affiliated with University of Surrey, UK; MRC Laboratory Animals Centre, UK.)
455. Shacklette HT, Boerngen JG. 1984. *Element concentrations in soils and other surficial materials of the conterminous United States*. U.S. Geological Survey Professional Paper 1270. Washington, D.C.
456. Sharma P. 2004. Review article: prevalence of Barrett's oesophagus and metaplasia at the gastro-oesophageal junction. *Aliment Pharmacol Ther* 20 Suppl 5: 48-54; discussion 61-42. (Support not reported. Author affiliated with University of Kansas School of Medicine, MO.)
457. Shedd KB. 1993. *The Materials Flow of Cobalt in the United States*. IC 9350. United States Department of the Interior. 31 pp.
458. Shedd KB. 2014a. *U.S. Geological Survey, Mineral Commodity Summaries, February 2014: Cobalt*. <http://minerals.usgs.gov/minerals/pubs/commodity/cobalt/>. Accessed on 2/12/15.

459. Shedd KB. 2014b. *USGS 2012 Minerals Yearbook: Cobalt [Advance Release]*. <http://minerals.usgs.gov/minerals/pubs/commodity/cobalt/index.html> - myb. Accessed on 2/12/15.
460. Sheftel A, Stehling O, Lill R. 2010. Iron-sulfur proteins in health and disease. *Trends Endocrinol Metab* 21(5): 302-314. (Supported by the Deutsche Forschungsgemeinschaft, Rhön Klinikum AG, von Behring-Röntgen Stiftung, Max-Planck Gesellschaft, Alexander-von-Humboldt Stiftung, Fonds de la Recherche en Santé Québec, Canadian Institutes of Health Research, and Fonds der chemischen Industrie. Authors affiliated with Philipps-Universität Marburg, Germany.)
461. Shi X, Dalal NS, Kasprzak KS. 1993. Generation of free radicals from model lipid hydroperoxides and H<sub>2</sub>O<sub>2</sub> by Co(II) in the presence of cysteinyl and histidyl chelators. *Chem Res Toxicol* 6(3): 277-283. (Support not reported. Authors affiliated with NCI, MD; West Virginia University, WV.)
462. Shukla SJ, Huang R, Simmons SO, Tice RR, Witt KL, Vanleer D, Ramabhadran R, Austin CP, Xia M. 2012. Profiling environmental chemicals for activity in the antioxidant response element signaling pathway using a high throughput screening approach. *Environ Health Perspect* 120(8): 1150-1156. (Supported by NIH and the U.S. EPA. Authors affiliated with NIH, MD and NC; U.S. EPA, NC; )
463. Sidaginamale RP, Joyce TJ, Lord JK, Jefferson R, Blain PG, Nargol AVF, Langton DJ. 2013. Blood metal ion testing is an effective screening tool to identify poorly performing metal-on-metal bearing surfaces. *Bone Joint Res* 2: 84-95. (Supported by the British Orthopaedic Association/Joint Action. Authors affiliated with University Hospital of North Tees, UK; Newcastle University, UK.)
464. Sighinolfi GL, Artoni E, Gatti AM, Corsi L. 2014. Carcinogenic potential of metal nanoparticles in BALB/3T3 cell transformation assay. *Environ Toxicol*. (Support not reported. Authors affiliated with University of Modena and Reggio Emilia, Italy; Institute for Advanced Sciences Convergence & Int'l Clean Water Institute, VA.)
465. Simcox NJ, Stebbins A, Guffey S, Atallah R, Hibbard R, Camp J. 2000. Hard metal exposures. Part 2: Prospective exposure assessment. *Appl Occup Environ Hyg* 15(4): 342-353. (Support not reported. Authors affiliated with University of Washington, WA.)
466. Simonsen LO, Brown AM, Harbak H, Kristensen BI, Bennekou P. 2011. Cobalt uptake and binding in human red blood cells. *Blood Cells Mol Dis* 46(4): 266-276. (Support not reported. Authors affiliated with University of Copenhagen, Denmark; University of Nottingham, UK.)
467. Simonsen LO, Harbak H, Bennekou P. 2012. Cobalt metabolism and toxicology--a brief update. *Sci Total Environ* 432: 210-215. (Support not reported. Authors affiliated with University of Copenhagen, Denmark.)
468. Singh I. 1983. Induction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisiae*. *Mutat Res* 117(1-2): 149-152. (as cited in IARC 2006)

- 
469. Slotnick MJ, Nriagu JO. 2006. Validity of human nails as a biomarker of arsenic and selenium exposure: A review. *Environ Res* 102(1): 125-139. (Supported by NCI and the U.S. EPA. Authors affiliated with University of Michigan School of Public Health, MI.)
470. Smith IC, Carson BL. 1981. Section C. Geochemistry and Occurrence. Section D. Environmental Transport. In *Trace Metals in the Environment*. vol. 6 - Cobalt. Smith IC, Carson BL, eds. Ann Arbor, MI: Ann Arbor Science Publishers. pp. 359-367. (as cited in Paustenbach *et al.* 2013)
471. Smith ML, Seo YR. 2002. p53 regulation of DNA excision repair pathways. *Mutagenesis* 17(2): 149-156. (Support not reported. Authors affiliated with Indiana University School of Medicine, IN; Walther Cancer Institute, IN.)
472. Smith T, Edmonds CJ, Barnaby CF. 1972. Absorption and retention of cobalt in man by whole-body counting. *Health Phys* 22(4): 359-367. (Support not reported. Authors affiliated with University College Hospital Medical School, UK.)
473. Smith LJ, Holmes AL, Kandpal SK, Mason MD, Zheng T, Wise JP, Sr. 2014. The cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells. *Toxicol Appl Pharmacol* 278(3): 259-265. (Supported by ARO and the Maine Center for Toxicology and Environmental Health. Authors affiliated with University of Southern Maine, ME; University of Maine, ME; Yale School of Public Health, CT.)
474. Snyder RD, Davis GF, Lachmann PJ. 1989. Inhibition by metals of X-ray and ultraviolet-induced DNA repair in human cells. *Biol Trace Elem Res* 21: 389-398. (as cited in IARC 2006)
475. Sorbie J, Olatunbosun D, Corbett WE, Valberg LS. 1971. Cobalt excretion test for the assessment of body iron stores. *Can Med Assoc J* 104(9): 777-782. (Support not reported. Authors affiliated with Queen's University, Canada; Kingston General Hospital, Canada.)
476. Sprince NL, Chamberlin RI, Hales CA, Weber AL, Kazemi H. 1984. Respiratory disease in tungsten carbide production workers. *Chest* 86(4): 549-557. (Support not reported. Authors affiliated with Harvard Medical School, MA; Massachusetts Institute of Technology, MA; Massachusetts Eye and Ear Infirmary, MA.)
477. Steinhoff D, Mohr U. 1991. On the question of a carcinogenic action of cobalt-containing compounds. *Exp Pathol* 41(4): 169-174. (Support not reported. Authors affiliated with Bayer AG, Germany.)
478. Stopford W, Turner J, Cappellini D, Brock T. 2003. Bioaccessibility testing of cobalt compounds. *J Environ Monit* 5(4): 675-680. (Supported by the Cobalt Development Institute. Authors affiliated with Duke University Medical Center, NC; Angeline Kirby Memorial Health Center, PA.)
479. Suardi R, Belotti L, Ferrari MT, Leghissa P, Caironi M, Maggi L, Alborghetti F, Storto T, Silva T, Piazzolla S. 1994. Health survey of workers occupationally exposed to cobalt. *Sci Total Environ* 150(1-3): 197-200. (Support not reported. Authors affiliated with Local

- Sanitary Unit 29, Italy; Ospedali Riuniti of Bergamo, Italy; Local Sanitary Unit 28, Italy; Local Sanitary Unit 30, Italy.)
480. Sunderman FW, Jr., Hopfer SM, Swift T, Rezuke WN, Ziebka L, Highman P, Edwards B, Folcik M, Gossling HR. 1989. Cobalt, chromium, and nickel concentrations in body fluids of patients with porous-coated knee or hip prostheses. *J Orthop Res* 7(3): 307-315. (Supported by Howmedica Inc. and the Northeast Utilities Company. Authors affiliated with University of Connecticut School of Medicine, CT.)
481. Suzuki T, Yamamoto R. 1982. Organic mercury levels in human hair with and without storage for eleven years. *Bull Environ Contam Toxicol* 28(2): 186-188. (Supported by the Japanese Ministry of Education and Culture. Authors affiliated with University of Tokyo, Japan; Tohoku University School of Medicine, Japan.)
482. Suzuki Y, Shimizu H, Nagae Y, Fukumoto M, Okonogi H, Kadokura M. 1993. Micronucleus test and erythropoiesis: effect of cobalt on the induction of micronuclei by mutagens. *Environ Mol Mutagen* 22(2): 101-106. (Support not reported. Authors affiliated with Jikei University School of Medicine, Japan; CIBA-GEIGY, Japan; Takarazuka, Japan.)
483. Swennen B, Buchet JP, Stănescu D, Lison D, Lauwerys R. 1993. Epidemiological survey of workers exposed to cobalt oxides, cobalt salts, and cobalt metal. *Br J Ind Med* 50(9): 835-842. (Support not reported. Authors affiliated with Union Minière, Belgium; Catholic University of Louvain, Belgium.)
484. Talakin YN, Ivanova LA, Kostetskaya NI, Komissarov VN, Belyaeva IV. 1991. [Hygienic characteristics of working conditions and health state of workers engaged in the production of cobalt salts]. *Gig Tr Prof Zbl* 1(10-11). (as cited in IARC 2006)
485. Tanaka T, Wiesener M, Bernhardt W, Eckardt KU, Warnecke C. 2009. The human HIF (hypoxia-inducible factor)-3alpha gene is a HIF-1 target gene and may modulate hypoxic gene induction. *Biochem J* 424(1): 143-151. (Supported by the Alexander von Humboldt-Foundation, the Roche Foundation for Anemia Research, the ELAN-Fonds of the University of Erlangen-Nuremberg, Germany and the German Research Foundation. Authors affiliated with University Clinic Erlangen, Germany; University of Erlangen-Nuremberg, Germany.)
486. Tayton KJ. 1980. Ewing's sarcoma at the site of a metal plate. *Cancer* 45(2): 413-415. (Support not reported. Author affiliated with Cardiff Royal Infirmary, UK.)
487. Tharr D, Singal M. 1987. *Health Hazard Evaluation Report: Eccles Saw and Tool Company, Cincinnati, Ohio*. HETA 85-415-1806. Cincinnati, OH: National Institute for Occupational Health and Safety. 21 pp.
488. Thoburn T, Larsen LB. 1976. *Health Hazard Evaluation Determination: Marathon Battery Company, Waco, Texas*. HETA 74-16-272. Cincinnati, OH: National Institute for Occupational Health and Safety. 40 pp.

489. Thomas RG, Furchner JE, London JE, Drake GA, Wilson JS, Richmond CR. 1976. Comparative metabolism of radionuclides in mammals--X. Retention of tracer-level cobalt in the mouse, rat, monkey and dog. *Health Phys* 31(4): 323-333. (Supported by the U.S. Energy Research and Development Administration. Authors affiliated with Holifield National Laboratory, TN; Los Alamos Scientific Laboratory, NM.)
490. Thomassen Y, Nieboer E, Ellingsen D, Hetland S, Norseth T, Odland JØ, Romanova N, Chernova S, Tchachtchine VP. 1999. Characterisation of workers' exposure in a Russian nickel refinery. *J Environ Monit* 1(1): 15-22. (Supported by the Nickel Producers Environmental Research Association, the Kola Research Laboratory for Occupational Health, McMaster University, the National Institute of Occupational Health of Norway, and the University of Tromsø. Authors affiliated with National Institute of Occupational Health, Norway; McMaster University, Canada; University of Tromsø, Norway; St. Petersburg State Technical University, Russia; Kola Research Laboratory for Occupational Health, Russia.)
491. Thompson RS, Gautieri RF, Mann DE, Jr. 1965. Effect of chronic oral administration of sodium cobaltinitrite and sodium nitrite on the minimal carcinogenic dose of methylcholanthrene in albino mice. *J Pharm Sci* 54(4): 595-598. (Supported by the Damon Runyon Foundation. Authors affiliated with Temple University, PA.)
492. Thomson AB, Valberg LS, Sinclair DG. 1971. Competitive nature of the intestinal transport mechanism for cobalt and iron in the rat. *J Clin Invest* 50(11): 2384-2394. (Supported by the Medical Research Council. Authors affiliated with Queens University, Canada.)
493. TRI. 2014a. *TRI Explorer Chemical Report. On-site Disposal to Class I Underground Injection Wells, RCRA Subtitle C Landfills, and Other Landfills*. U.S. Environmental Protection Agency. <http://www.epa.gov/triexplorer>. Last accessed on 10/13/14.
494. TRI. 2014b. *TRI Explorer Chemical Report. TRI On-site and Off-site Reported Disposed of or Otherwise Released (in pounds), Trend Report for Facilities in All industries, for Cobalt Chemical, U.S.* U.S. Environmental Protection Agency. <http://www.epa.gov/triexplorer>. Last accessed on 10/14/14.
495. TRI. 2014c. *TRI Explorer Chemical Report. TRI On-site and Off-site Reported Disposed of or Otherwise Released (in pounds), Trend Report for Facilities in All industries, for Cobalt Compounds Chemical, U.S.* U.S. Environmental Protection Agency. <http://www.epa.gov/triexplorer>. Last accessed on 10/13/14.
496. TRI. 2014d. *TRI EZ Search in Envirofacts. Toxic Chemical Releases to the Environment (in pounds), for Cobalt and Cobalt Compounds, U.S.* U.S. Environmental Protection Agency. <http://www.epa.gov/enviro/facts/tri/ez.html>. Last accessed on 10/29/14.
497. Tso WW, Fung WP. 1981. Mutagenicity of metallic cations. *Toxicol Lett* 8(4-5): 195-200. (as cited in IARC 2006)
498. Tüchsen F, Jensen MV, Villadsen E, Lynge E. 1996. Incidence of lung cancer among cobalt-exposed women. *Scand J Work Environ Health* 22(6): 444-450. (Supported by the

- Danish Working Environment Fund and the Danish Health Fund. Authors affiliated with National Institute of Occupational Health, Denmark; Danish Cancer Society, Denmark.)
499. Tvermoes BE, Finley BL, Unice KM, Otani JM, Paustenbach DJ, Galbraith DA. 2013. Cobalt whole blood concentrations in healthy adult male volunteers following two-weeks of ingesting a cobalt supplement. *Food Chem Toxicol* 53: 432-439. (Supported by DePuy Orthopaedics, Inc. Authors affiliated with ChemRisk, LLC, CO, CA and PA.)
500. Tvermoes BE, Unice KM, Paustenbach DJ, Finley BL, Otani JM, Galbraith DA. 2014. Effects and blood concentrations of cobalt after ingestion of 1 mg/d by human volunteers for 90 d. *Am J Clin Nutr* 99(3): 632-646. (Supported by DePuy Orthopaedics Inc. Authors affiliated with Cardno ChemRisk LLC, CA, CO and PA.)
501. Underwood EJ. 1977. Cobalt. In *Trace Elements in Human and Animal Nutrition*. 4th ed. New York: Academic Press. p. 132-158.
502. Unice KM, Monnot AD, Gaffney SH, Tvermoes BE, Thuett KA, Paustenbach DJ, Finley BL. 2012. Inorganic cobalt supplementation: prediction of cobalt levels in whole blood and urine using a biokinetic model. *Food Chem Toxicol* 50(7): 2456-2461. (Supported by DePuy Orthopaedics. Authors affiliated with ChemRisk, LLC, CA, PA and CO.)
503. Unice KM, Kerger BD, Paustenbach DJ, Finley BL, Tvermoes BE. 2014. Refined biokinetic model for humans exposed to cobalt dietary supplements and other sources of systemic cobalt exposure. *Chem Biol Interact* 216: 53-74. (Supported by DePuy Orthopaedics, Inc. Authors affiliated with Cardno ChemRisk LLC, PA, CA, NY and CO.)
504. USITC. 2014. *USITC Interactive Tariff and Trade Dataweb*. United States International Trade Commission. [http://dataweb.usitc.gov/scripts/user\\_set.asp](http://dataweb.usitc.gov/scripts/user_set.asp) and search on HTS no. 2836999050, 2836991000, 2833291000, 2833299100, 8105200000, 8105209000, 8105203000, 8105206000, 2836991000, 2833291000, 8105209000, 8105203000, 8105900000, 2915293000, 2827396000, 2605000000, 2822000000, 8105300000. Accessed on 10/14.
505. USPTO (1958). Reducing the tendency of beer towards gushing and increasing its foam stability. United States Patent Office. Richard Stanley Wrey Thorne H, Denmark, assignor to Alfred Jorgenson's gaeringsfsiologiske Laboratorium, Copenhagen, Denmark, a firm. Patent No. 2,865,755: 3 pp.
506. Vaca CE, Wilhelm J, Harms-Ringdahl M. 1988. Interaction of lipid peroxidation products with DNA. A review. *Mutat Res* 195(2): 137-149. (Supported by The Swedish Institute, Stockholm, and by the International Seminar in Physics, University of Uppsala. Authors affiliated with University of Stockholm, Sweden; Charles University, Czechoslovakia.)
507. Valberg LS, Ludwig J, Olatunbosun D. 1969. Alteration in cobalt absorption in patients with disorders of iron metabolism. *Gastroenterology* 56(2): 241-251. (Supported by the Medical Research Council of Canada. Authors affiliated with Kingston General Hospital, Canada; Queen's University, Canada.)

- 
508. Vales G, Demir E, Kaya B, Creus A, Marcos R. 2013. Genotoxicity of cobalt nanoparticles and ions in *Drosophila*. *Nanotoxicology* 7(4): 462-468. (Supported by the Universitat Autònoma de Barcelona, the Akdeniz University and the Council of Higher Education, the Generalitat de Catalunya and the the Management Unit of Research Projects of Akdeniz University. Authors affiliated with Universitat Autònoma de Barcelona, Spain; Akdeniz University, Turkey; CIBER Epidemiología y Salud Pública, Spain.)
509. Valko M, Morris H, Cronin MT. 2005. Metals, toxicity and oxidative stress. *Curr Med Chem* 12(10): 1161-1208. (Supported by the Leverhulme Trust, VEGA and APVT. Authors affiliated with Slovak Technical University, Slovakia; Liverpool John Moores University, UK.)
510. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160(1): 1-40. (Supported by VEGA and APVT. Authors affiliated with Slovak Technical University, Slovakia; University of Reading, UK.)
511. van den Oever R, Roosels D, Douwen M, Vanderkeel J, Lahaye D. 1990. Exposure of diamond polishers to cobalt. *Ann Occup Hyg* 34(6): 609-614. (Support not reported. Authors affiliated with National Confederation of Christian Sickness Funds, Belgium; Fund of Occupational Diseases, Belgium; Metallurgie Hoboken-Overpelt Sint Theresiastraat, Belgium.)
512. van der List JJ, van Horn JR, Slooff TJ, ten Cate LN. 1988. Malignant epithelioid hemangioendothelioma at the site of a hip prosthesis. *Acta Orthop Scand* 59(3): 328-330. (Support not reported. Authors affiliated with University of Nijmegen, Netherlands.)
513. Van Goethem F, Lison D, Kirsch-Volders M. 1997. Comparative evaluation of the in vitro micronucleus test and the alkaline single cell gel electrophoresis assay for the detection of DNA damaging agents: genotoxic effects of cobalt powder, tungsten carbide and cobalt-tungsten carbide. *Mutat Res* 392(1-2): 31-43. (Supported by the Belgian Federal Office for Scientific, Technical and Cultural affairs. Authors affiliated with Free University Brussels, Belgium; Catholic University of Louvain, Belgium.)
514. Vendittoli PA, Roy A, Mottard S, Girard J, Lusignan D, Lavigne M. 2010. Metal ion release from bearing wear and corrosion with 28 mm and large-diameter metal-on-metal bearing articulations: a follow-up study. *J Bone Joint Surg Br* 92(1): 12-19. (Supported by Zimmer, Warsaw, Indiana. Authors affiliated with University of Montreal, Canada; Salem University Hospital, France.)
515. Vengellur A, Phillips JM, Hogenesch JB, LaPres JJ. 2005. Gene expression profiling of hypoxia signaling in human hepatocellular carcinoma cells. *Physiol Genomics* 22(3): 308-318. (Support not reported. Authors affiliated with Michigan State University, MI; Genomics Institute of the Novartis Research Foundation, CA.)
516. Versieck J, Hoste J, Barbier F, Steyaert H, De Rudder J, Michels H. 1978. Determination of chromium and cobalt in human serum by neutron activation analysis. *Clin Chem* 24(2): 303-308. (as cited in IARC 1991)

517. Visuri T, Pukkala E, Paavolainen P, Pulkkinen P, Riska EB. 1996. Cancer risk after metal on metal and polyethylene on metal total hip arthroplasty. *Clin Orthop Relat Res*(329 Suppl): S280-289. (Support not reported. Authors affiliated with Central Military Hospital, Finland; Finish Cancer Registry, Finland; Orthopedic Hospital of the Invalid Foundation, Finland; University of Helsinki, Finland; Dextra Medical Cente, Finland.)
518. Visuri T, Pulkkinen P, Paavolainen P. 2006. Malignant tumors at the site of total hip prosthesis. Analytic review of 46 cases. *J Arthroplasty* 21(3): 311-323. (No funds received for this report. Authors affiliated with Central Military Hospital, Finland; University of Helsinki, Finland; Hospital of the Invalid Foundation, Finland.)
519. Visuri T, Borg H, Pulkkinen P, Paavolainen P, Pukkala E. 2010. A retrospective comparative study of mortality and causes of death among patients with metal-on-metal and metal-on-polyethylene total hip prostheses in primary osteoarthritis after a long-term follow-up. *BMC Musculoskelet Disord* 11: 78. (Support not reported. Authors affiliated with Research Institute of Military Medicine, Finland; Central University Hospital, Finland; University of Helsinki, Finland; Orton Orthopaedic Hospital, Finland; Finnish Cancer Registry, Finland; University of Tampere, Finland.)
520. Voroshilin SI, Plotko EG, Fink TV, Nikiforova V. 1978. [Cytogenetic effect of inorganic wolfram, zinc, cadmium and cobalt compounds on human and animal somatic cells]. *Tsitol Genet* 12(3): 241-243. (as cited in IARC 2006)
521. Wan R, Mo Y, Feng L, Chien S, Tollerud DJ, Zhang Q. 2012. DNA damage caused by metal nanoparticles: involvement of oxidative stress and activation of ATM. *Chem Res Toxicol* 25(7): 1402-1411. (Supported by the Health Effects Institute, the American Lung Association, the American Heart Association, the Basic Award of Clinical & Translational Sciences Pilot Grant Program from UofL, an Intramural Research Incentive Grants from UofL and NIESH. Authors affiliated with University of Louisville, KY.)
522. Wang GL, Semenza GL. 1995. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270(3): 1230-1237. (Supported by the Council for Tobacco Research, the Lucille P. Markey Charitable Trust, and the NIDDK, National Institutes of Health. Authors affiliated with Johns Hopkins University School of Medicine, MD.)
523. Wang G, Hazra TK, Mitra S, Lee HM, Englander EW. 2000. Mitochondrial DNA damage and a hypoxic response are induced by CoCl<sub>2</sub> in rat neuronal PC12 cells. *Nucleic Acids Res* 28(10): 2135-2140. (Supported by the Shriners Hospitals for Children. Authors affiliated with University of Texas Medical Branch, TX.)
524. Wang T, Fu J, Wang Y, Liao C, Tao Y, Jiang G. 2009. Use of scalp hair as indicator of human exposure to heavy metals in an electronic waste recycling area. *Environ Pollut* 157(8-9): 2445-2451. (Supported by National Science Foundation of China and the National Basic Research Program of China. Authors affiliated with Chinese Academy of Sciences, China; Shangyu Environmental Protection Bureau, China.)
525. Wang D, Tian X, Jiang Y. 2012. NDRG1/Cap43 overexpression in tumor tissues and serum from lung cancer patients. *J Cancer Res Clin Oncol* 138: 1813-1820. (Support not reported. Authors affiliated with China Medical University, China.)

- 
526. Wappelhorst O, Kuhn I, Heidenreich H, Markert B. 2002. Transfer of selected elements from food into human milk. *Nutrition* 18(4): 316-322. (Supported by Bundesamt für Strahlenschutz, Neuherberg. Authors affiliated with International Graduate School of Zittau, Germany.)
527. Webb M, Heath JC, Hopkins T. 1972. Intranuclear distribution of the inducing metal in primary rhabdomyosarcomata induced in the rat by nickel, cobalt and cadmium. *Br J Cancer* 26(4): 274-278. (Supported by the Medical Research Council and the Cancer Research Campaign. Authors affiliated with Strangeways Research Laboratory, UK.)
528. Wedrychowski A, Schmidt WN, Hnilica LS. 1986. DNA-protein crosslinking by heavy metals in Novikoff hepatoma. *Arch Biochem Biophys* 251(2): 397-402. (Supported by NIH. Authors affiliated with Vanderbilt University, TN.)
529. Wehner AP, Craig DK. 1972. Toxicology of inhaled NiO and CoO in Syrian golden hamsters. *Am Ind Hyg Assoc J* 33(3): 146-155. (Supported by NCI. Authors affiliated with Battelle Pacific Northwest Laboratories, WA.)
530. Wehner AP, Busch RH, Olson RJ, Craig DK. 1977. Chronic inhalation of cobalt oxide and cigarette smoke by hamsters. *Am Ind Hyg Assoc J* 38(7): 338-346. (Supported by NCI. Authors affiliated with Battelle Pacific Northwest Laboratories, WA.)
531. White MA, Dyne D. 1994. Biological monitoring of occupational cobalt exposure in the United Kingdom. *Sci Total Environ* 150(1-3): 209-213. (Support not reported. Authors affiliated with Health and Safety Executive, UK. )
532. WHO. 2006. *Cobalt and Inorganic Cobalt Compounds*. Concise International Chemical Assessment Document 69. Geneva, Switzerland: World Health Organization. 93 pp.
533. Wild P, Perdrix A, Romazini S, Moulin JJ, Pellet F. 2000. Lung cancer mortality in a site producing hard metals. *Occup Environ Med* 57(8): 568-573. (Support not reported. Authors affiliated with INRS, France; Institut Universitaire de Médecine du Travail et de l'Environnement de Grenoble, France.)
534. Witkiewicz-Kucharczyk A, Bal W. 2006. Damage of zinc fingers in DNA repair proteins, a novel molecular mechanism in carcinogenesis. *Toxicol Lett* 162(1): 29-42. (Supported by the Polish Ministry of Science. Authors affiliated with Polish Academy of Science, Poland; National Research Institute, Poland.)
535. Witzleb WC, Ziegler J, Krummenauer F, Neumeister V, Guenther KP. 2006. Exposure to chromium, cobalt and molybdenum from metal-on-metal total hip replacement and hip resurfacing arthroplasty. *Acta Orthop* 77(5): 697-705. (Support not reported. Authors affiliated with Dresden University of Technology, Germany.)
536. Wolff M, Jelkmann W, Dunst J, Depping R. 2013. The Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT/HIF-1beta) is influenced by hypoxia and hypoxia-mimetics. *Cell Physiol Biochem* 32(4): 849-858. (Support not reported. Authors affiliated with Universität zu Lübeck, Germany; Klinik für Strahlentherapie, Germany.)

537. Wong PK. 1988. Mutagenicity of heavy metals. *Bull Environ Contam Toxicol* 40(4): 597-603. (as cited in IARC 2006)
538. Wragg J, Cave M, Basta N, Brandon E, Casteel S, Denys S, Gron C, Oomen A, Reimer K, Tack K, Van de Wiele T. 2011. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. *Sci Total Environ* 409(19): 4016-4030. (Support not reported. Authors affiliated with British Geological Survey, UK; Ohio State University, OH; National Institute for Public Health and the Environment, Netherlands; University of Missouri, MO; INERIS, France; DHI Water Environment Health, Denmark; Royal Military College of Canada, Canada; University of Ghent, Belgium.)
539. Xia M, Huang R, Sun Y, Semenza GL, Aldred SF, Witt KL, Inglese J, Tice RR, Austin CP. 2009. Identification of chemical compounds that induce HIF-1alpha activity. *Toxicol Sci* 112(1): 153-163. (Supported by NIEHS and NIH. Authors affiliated with National Institutes of Health, MD; University of Michigan, MI; Johns Hopkins University School of Medicine, MD; SwitchGear Genomics, CA; National Institute of Environmental Health Sciences, NC.)
540. Yamada K. 2013. Cobalt: its role in health and disease. *Met Ions Life Sci* 13: 295-320. (Support not reported. Author affiliated with Uniformed Services University of the Health Sciences, MD.)
541. Yesilada E. 2001. Genotoxicity testing of some metals in the Drosophila wing somatic mutation and recombination test. *Bull Environ Contam Toxicol* 66(4): 464-469. (as cited in IARC 2006)
542. Yildiz M, Cigerci IH, Konuk M, Fidan AF, Terzi H. 2009. Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. *Chemosphere* 75(7): 934-938. (Support not reported. Authors affiliated with Afyon Kocatepe University, Turkey.)
543. Yin DZ. 1990. [Comparison of serum trace element spectrum of liver cancer patients and healthy adults]. *Zhonghua Zhong Liu Za Zhi* 12(3): 200-202. (Support unknown due to foreign language. Authors affiliated with Academia Sinica, China.)
544. Yokoiyama A, Kada T, Kuroda Y. 1990. Antimutagenic action of cobaltous chloride on radiation-induced mutations in cultured Chinese hamster cells. *Mutat Res* 245(2): 99-105. (as cited in IARC 2006)
545. Zeh A, Planert M, Siegert G, Lattke P, Held P, Hein W. 2007. Release of cobalt and chromium ions into the serum following implantation of the metal-on-metal maverick-type artificial lumbar disc (Medtronic Sofamor Danek). *Spine* 32(3): 348-352. (No funds were received in support of this work. Authors affiliated with Martin-Luther-University of Halle/Wittenberg, Germany; Dresden University Hospital's Institute for Clinical Chemistry and Laboratory Medicine, Germany.)
546. Zeh A, Becker C, Planert M, Lattke P, Wohlrab D. 2009. Time-dependent release of cobalt and chromium ions into the serum following implantation of the metal-on-metal

- Maverick™ type artificial lumbar disc (Medtronic Sofamor Danek). *Arch Orthop Trauma Surg* 129: 741-746. (Support not reported. Authors affiliated with Martin-Luther-University of Halle-Wittenberg, Germany; Carl Gustav Carus University of Dresden, Germany.)
547. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ Mol Mutagen* 19 Suppl 21: 2-141. (as cited in IARC 2006)
548. Zeller WJ. 1975. [Investigation of the influence of copper and cobalt ions on the carcinogenesis by diethyl-nitrosamine (DNA) in Wistar rats (author's transl)]. *Arch Geschwulstforsch* 45(7): 634-636. (Support not reported. Author affiliated with German Center for Cancer Research, Germany.)
549. Zey JN. 1985. *Health Hazard Evaluation Report: Wyoming High School, Wyoming, Ohio*. HETA 85-085-1615. Cincinnati, OH: National Institute for Occupational Health and Safety. 23 pp.
550. Zhang Q, Kusaka Y, Donaldson K. 2000. Comparative pulmonary responses caused by exposure to standard cobalt and ultrafine cobalt. *J Occup Health* 42: 179-184. (Supported by the Ministry of Education, Science and Culture, Japan. Authors affiliated with Fukui Medical University, Japan; Napier University, UK.)
551. Zhang L, Lv J, Sun S. 2012a. Elements in lung tissues of patients from a high lung cancer incidence area of China. *Biol Trace Elem Res* 148(1): 7-10. (Supported by the State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco Environmental Sciences, Chinese Academy of Sciences. Authors affiliated with National Environmental Monitoring Center, China; Supreme People's Procuratorate, China; PLA General Hospital, China.)
552. Zhang H, Ji Z, Xia T, Meng H, Low-Kam C, Liu R, Pokhrel S, Lin S, Wang X, Liao YP, Wang M, Li L, Rallo R, Damoiseaux R, Telesca D, Madler L, Cohen Y, Zink JI, Nel AE. 2012b. Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. *ACS Nano* 6(5): 4349-4368. (Supported by the US Public Health Service, National Science Foundation, and the EPA. Authors affiliated with University of California, CA; University of Bremen, Germany; Universitat Rovira i Virgili, Spain.)
553. Zhang P, Yao Q, Lu L, Li Y, Chen PJ, Duan C. 2014. Hypoxia-inducible factor 3 is an oxygen-dependent transcription activator and regulates a distinct transcriptional response to hypoxia. *Cell Rep* 6(6): 1110-1121. (Supported by NSF and the Natural Scientific Foundation of China. Authors affiliated with University of Michigan, MI; Ocean University of China, China.)
554. Zhu S, Zhang Y, Gao Y, Wang XJ, Chen T, Yang Y, Shi R, Jin P, Tian Y, Shen XM. 2011. [Correlation between level of metallic elements in urine and childhood acute leukemia]. *Zhonghua Yu Fang Yi Xue Za Zhi* 45(2): 146-149. (Support unknown due to foreign language. Authors affiliated with Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, China.)

555. Zou W, Yan M, Xu W, Huo H, Sun L, Zheng Z, Liu X. 2001. Cobalt chloride induces PC12 cells apoptosis through reactive oxygen species and accompanied by AP-1 activation. *J Neurosci Res* 64(6): 646-653. (Supported by the Chinese Academy of Sciences. Authors affiliated with Chinese Academy of Sciences, China.)

**This Page Intentionally Left Blank**

## 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).

## 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 <sup>2</sup> :	centimeters squared
cm <sup>3</sup> :	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:	<i>Federal Register</i>
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 <sub>ow</sub> :	logarithm of octanol/water partition coefficient
M:	male
m <sup>3</sup> :	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
$\mu\text{g}$ :	microgram

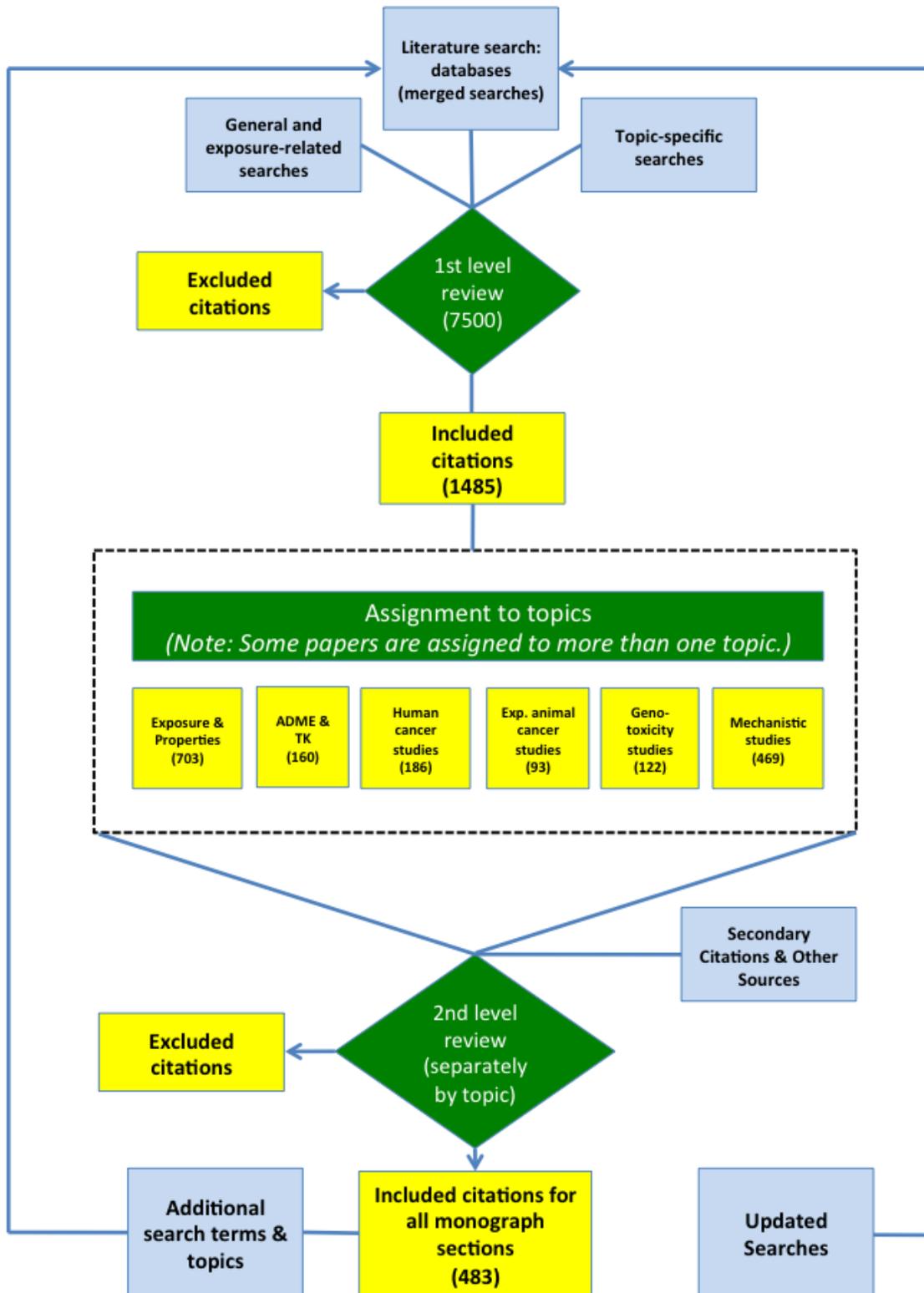
## Appendix A: Literature Search Strategy

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

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

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

Figure A-1. Literature search strategy and review



### A.1 Search strategies

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

1. General data search: This search covers a broad range of general data sources for information relevant to many or all of the wide range of monograph topics pertaining to cobalt.
2. Exposure-related data search: This search covers a broad range of potential sources for exposure-related information and physical-chemical properties.
3. Database searches in PubMed, Scopus, and Web of Science: The majority of the primary literature used to draft the cobalt monograph was identified from searches of these three extensive databases available through the NIEHS Library. Searches for cobalt were combined with the search terms for each of the monograph topics listed above to create the specific literature searches.
4. Searches for human cancer studies are somewhat unique because they involve the identification of search terms for exposure scenarios that might result in exposure of people to cobalt. For cobalt, these exposure-related search terms were based on uses of cobalt identified from the EPA's TRI database and the Chemical Data Report rule website.
5. QUOSA library of occupational case-control studies search of the QUOSA-based library of more than 6,000 occupational case-control studies, approximately 95% of which are currently available as searchable full-text pdfs, was conducted using the "cobalt."
6. Secondary sources: Citations identified from authoritative reviews or from primary references located by literature search, together with publications citing key papers identified using the Web of Science, "Cited Reference Search," were also added.

### A.2 Updating the literature search

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

### A.3 Review of citations using web-based systematic review software

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

**This Page Intentionally Left Blank**

## Appendix B: Chemical Properties and Exposure-Related Information, Clinical Surveys and Studies, and Regulations

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

### B.1 Chemical properties

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

**Table B-1. Physical and chemical properties for additional chemical forms of cobalt**

Name	CAS No.	Formula	Molecular weight	Physical form	Solubility (grams per 100 cc cold water)	Bioaccessibility (% solubility in gastric/lysosomal fluids)
Hydroxide oxide	12016-80-7	CoOOH	91.9	Solid	0.00007	21/42
Trihydroxide	1307-86-4	Co(OH) <sub>3</sub>	110.0	Pasty liquid	0.00013	65/80
Lithium dioxide	12190-79-3	CoO <sub>2</sub> Li	97.9	Solid	0.00003	26/4
Isononanoate	84255-52-7	Co(C <sub>9</sub> H <sub>17</sub> O <sub>2</sub> ) <sub>2</sub>	373.4	Liquid	0.705	91/89
Neodecanoate	27253-31-2	Co(C <sub>10</sub> H <sub>19</sub> O <sub>2</sub> ) <sub>2</sub>	401.5	Liquid	0.0772	83/72
Acetyl acetate	14024-48-7	Co(C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> ) <sub>2</sub>	257.2	Solid	0.516	100/96
Borate propionate	91782-61-5	BO <sub>3</sub> (CoC <sub>3</sub> H <sub>5</sub> O <sub>2</sub> ) <sub>3</sub>	454.8	Solid	3.33	89/88
Borate 2-ethylhexanoate	91782-60-4	BO <sub>3</sub> (CoC <sub>8</sub> H <sub>15</sub> O <sub>2</sub> ) <sub>3</sub>	665.3	Solid	0.994	88/92

Name	CAS No.	Formula	Molecular weight	Physical form	Solubility (grams per 100 cc cold water)	Bioaccessibility (% solubility in gastric/lysosomal fluids)
Borate neodecanoate	68457-13-6	$\text{BO}_3(\text{CoC}_{10}\text{H}_{19}\text{O}_2)_3$	749.5	Pasty liquid	0.190	100/87
Tallate	61789-52-4	-	-	Pasty liquid		62/48
Resinate	68956-82-1	-	-	Solid	0.00335	9/14

## B.2 Exposure

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

**Table B-2. Values for urine and blood cobalt levels (means or medians) in the United States and other countries (urine values are plotted in Figure 2-1; values plotted for the general public or unexposed controls are shown below in *bold italic* while those for other exposures are in bold)<sup>a</sup>**

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt (µg/L) <sup>a</sup>	Serum plasma, or blood cobalt (µg/L) <sup>a</sup>
<b>General Public</b>				
Bibi <i>et al.</i> 2015 <i>Pakistan</i>	Control group that never drank arsenic-contaminated water from 48 total subjects, including children (10–15 yr) and 2 groups of adults (25–35 yr; 40–50 yr)	Control (N not reported)	<i>Mean (SE) Units NR<sup>h</sup></i> <b>0.07 ± 0.01</b>	<i>Blood Mean (SE) Units NR</i> 0.08 ± 0.03
Bradberry <i>et al.</i> 2014 <i>United Kingdom</i>	Normal ranges for UK			<i>Blood or serum</i> < 0.6
NHANES (2011–2012) <i>United States</i>		(2,504)	<b>0.326<sup>b,c</sup> GM</b>	
Dunstan <i>et al.</i> 2005 <i>England</i>	Controls for patients receiving a hip implant	Controls (4)	Mean (95% CI) <b>0.6 (0.40–0.74)</b>	<i>Blood Mean (95% CI)</i> 0.69 (0.43–0.89)
De Boeck <i>et al.</i> 2000 Belgium, Norway, Finland (cobalt refineries), Sweden, England (hard-metal plants)	Matched controls from 2 cobalt refineries and two hard-metal producing plants	Controls (27)	GM ± SD <b>1.7 ± 1.6</b>	
Nemery <i>et al.</i> 1992 <i>Belgium</i>	Workers in workshops in the diamond polishing industry (sawing diamonds or drawing jewelry) not thought to be exposed to cobalt (controls for occupational study)	(48)	<b>2.3 ± 1.8</b>	

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
Angerer <i>et al.</i> 1989 (as reported in IARC 1991) Germany	NR	(NR)	<b>0.01</b> (NR)	<i>Blood (range)</i> 0.2–1.3
Alexandersson 1988 <i>Sweden</i>	Workers not exposed to cobalt (controls for occupation study)	(25)	<b>[0.4</b> (0.1–2.2)]	<i>Blood</i> [0.5 (0.1–1.2)]
Christensen and Mikkelsen 1986 <i>Not specified</i>	Porcelain workers without exposure to cobalt (controls for occupational study)	(46)	$\mu\text{g/g creatinine}^{\text{e,f}}$ <b>[0.8</b> (0.04–10.7)]	<i>Blood</i> 0.24 (0.05–0.6)
Hartung 1986 (as reported in IARC 1991) <i>Not specified</i>		(NR)		<i>Serum</i> 0.1 (NR)
Ichikawa <i>et al.</i> 1985 <i>Not specified</i> Japan	Office workers (controls for occupational study)	(20)	<b>2.0</b> $\pm$ 1.0	<i>Blood</i> 1.9 $\pm$ 1.1
Lewis <i>et al.</i> 1985 (as reported in IARC 1991) <i>Not specified</i>				<i>Serum</i> 0.28 (NR)
Scansetti <i>et al.</i> 1985 <i>Italy</i>	“White collar” workers (control for occupational study)	(NR)	<b>0.41</b> $\pm$ 0.22	
Andersen and Høgetveit 1984 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)		<i>Plasma</i> 0.15 $\pm$ 0.07

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
Mikkelsen <i>et al.</i> 1984 (as cited in IARC 1991) <i>Not specified</i>		(NR)	<b>0.94</b> (0.05–13.8)	
Ostapczuk <i>et al.</i> 1983 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)		<i>Blood</i> 0.09 ± 0.02
Kasperek <i>et al.</i> 1981 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)		<i>Plasma</i> 0.195 ± 0.015
Schumacher-Wittkopf and Angerer 1981 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)	<b>0.38</b> (0.1–0.75)	
Alexandersson and Swensson 1979 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)		<i>Blood</i> 0.5 ± 0.1
Versieck <i>et al.</i> 1978 (as reported in IARC 1991) <i>Not specified</i>	NR	(NR)		<i>Serum</i> 0.108 ± 0.06

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

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

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
Hennig <i>et al.</i> 1992 (as cited in Schaffer <i>et al.</i> 1999) <i>Not specified</i>	Metal-on-metal total hip replacement patients	(10)	<b>3.8<sup>d</sup></b> (2.1–286.2)	
Sunderman <i>et al.</i> 1989 <i>United States</i>	Metal-on-polyethylene hip (N =21) Pre and post implantation NB: The pre-operative group included patients receiving either Ti-Al-V or Co-Cr prostheses and knee implants	Controls (42) Patients (Time sampled) 6–120 wk PO (NR)	$\mu\text{g/g creatinine}^{\text{c}}$ <b>0.5</b> $\pm$ 0.4 (SE) <b>0.7</b> $\pm$ 0.3	<i>Serum</i> 0.05 $\pm$ 0.01 (SE) 0.13 $\pm$ 0.05
<b>Medical implants (unstable)</b>				
Bradberry <i>et al.</i> 2014 <i>Not reported; authors from UK</i>	Failed hip replacement patients (18) with systemic toxicity (e.g., neuro-ocular toxicity, cardiotoxicity, thyroid toxicity)	Total with levels (17) Metal-on metal (8) Ceramic (9)		<i>Median peak</i> 398 (14–6521) 34.5 (13.6–399) 506 (353–6521)
Rodriguez de la Flor <i>et al.</i> 2013 <i>Spain</i>	Hip implants requiring revision surgery	Before revision (11) After revision (11)	<b>205.6</b> $\pm$ 310.6 44.3 $\pm$ 94.9	<i>Serum</i> 25.8 $\pm$ 40.6 13.1 $\pm$ 29.3
Dunstan <i>et al.</i> 2005 <i>England</i>	Patients receiving a metal on metal hip implant because of bone cancer; 10 survivors with samples, 5 of which retained their original implants	Radiologically loose hip implants (2)	Mean (95% CI) 205 (140, 270)	Mean (95% CI) 35.5 (19, 52)
<b>Environmental exposure</b>				
Cheyens <i>et al.</i> 2014 <i>D.R. Congo</i>	Adults and children (< 14 yrs) living in urban and rural communities close to metal mining and/or refining plants or villages near a lake	<i>Controls</i> Children (24) Adults (57)	$\mu\text{g/g creatinine}^{\text{c}}$ Mean <sup>b</sup> (25%–75% CI) 4.2 (2.6–7.2) 2.7 (1.3–5.3)	

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
	receiving effluents from metal refining plants	<i>Lake</i> Children (13) Adults (47)	14.0 (7.5–21.0) 9.4 (4.0–19.8)	
		<i>Polluted communities</i> Children (32) Adults (79)	<b>27.9</b> (13.4–62.7) <b>11.7</b> (7.1–22.0)	
Moreno <i>et al.</i> 2010 <i>Mexico</i>	Urine cobalt levels in children in the Taxco mining area of Southern Mexico  Reference values were reported in the literature for children from other countries	Reference Children (35)	<b>2.2</b> (NR) <b>18<sup>d</sup></b> (3–47)	
<b>Occupational exposure</b>				
<b>Cobalt production: Refining</b>				
Lantin <i>et al.</i> 2011 <i>Belgium</i>	Cobalt refinery workers	Cobalt production (249)	$\mu\text{g/g creatinine}$ <sup>f</sup> <b>3.90<sup>e</sup></b> (0.3–204)	<i>Blood</i> 1.0 (< 0.5–32.0)
De Boeck <i>et al.</i> 2000 Belgium, Norway, Finland (cobalt refineries), Sweden, England (hard metal plants)	Workers exposed to cobalt dust from 2 refineries and hard metal dust from two hard-metal producing plants, location not specified	Co refinery workers (24) Hard metal workers (29)	GM $\pm$ SD <b>21.5 <math>\pm</math> 2.1</b> <b>19.9 <math>\pm</math> 2.4</b>	
Thomassen <i>et al.</i> 1999 <i>Russia</i>	Nickel refinery workers	Roasting (25) Anode casting [old] (20) Anode casting [new] (11) Electrorefining [old] (23) Electrorefining [new] (10) Rinsing [old] (18) Rinsing [new] (12))	<b>5.8 <math>\pm</math> 5.7</b> <b>8.4 <math>\pm</math> 9.0</b> <b>14 <math>\pm</math> 37</b> <b>2.9 <math>\pm</math> 4.8</b> <b>2.7 <math>\pm</math> 2.4</b> <b>8.7 <math>\pm</math> 11</b> <b>16 <math>\pm</math> 19</b>	

**Cobalt production: Cobalt salts or oxide**

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
Coombs 1996 South Africa	Cobalt oxide workers, pre- and post-environmental and exposure controls (plant converts cobalt metal to cobalt oxide)	Pre-controls (43 samples across 8 job titles) Post-controls (91 samples across 12 job titles)	<b>454.6</b> $\mu\text{g/g}$ creatinine <sup>c</sup> <b>68.5</b> <sup>i</sup>	
Swennen <i>et al.</i> 1993 Belgium	Production of cobalt powder, oxides, and salts	Day and shift Mon; pre-shift (82) Mon; post-shift (82) Fri; pre-shift (82) Fri; post-shift (82)	$\mu\text{g/g}$ creatinine <sup>b,f</sup> <b>21.1</b> (0.3–488) <b>52.9</b> (2.7–2245) <b>40.6</b> (0.9–1288) <b>69.8</b> (1.6–2038)	<i>Blood</i> 9.7 (2.0–120) 11.0 (2.0–120) 11.2 (2.0–110) 12.7 (2.0–120)
Angerer <i>et al.</i> 1985 Germany	Foundry workers using cobalt as a powder (4 groups) or salt (3 groups)	All 7 groups (40)	Range of means <b>18.9–438.4</b>	<i>Blood</i> 4.9–47.9
<b>Metallurgical</b>				
Beaucham <i>et al.</i> 2015 (State NR), United States	Metallurgical workers at an orthopedic implant manufacturing company	(21)	<b>0.6</b> <sup>d</sup> (0.3–2.0)	
Deng <i>et al.</i> 1990 Michigan, United States	Metallurgical Site visit; 261 urine samples from 39 workers; up to 7 specimens /person per day	Monday Pre-shift (33) Post-shift (32) Tuesday Pre-shift (34) Post-shift (34) Wednesday Pre-shift (36) Post-shift (36) Thursday Pre-shift (35) Post-shift (0)	$\mu\text{g Co/mg creatinine}$ <sup>e</sup> <b>21.3</b> $\pm$ 28.6 <b>44.3</b> $\pm$ 65.7 <b>26.4</b> $\pm$ 32.1 <b>50.4</b> $\pm$ 71.7 <b>36.3</b> $\pm$ 56.7 <b>50.2</b> $\pm$ 78.0 <b>40.8</b> $\pm$ 58.6 ND	
<b>Cemented carbides (hard meals) and bonded diamonds (diamond abrasives)</b>				

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt (µg/L) <sup>a</sup>	Serum plasma, or blood cobalt (µg/L) <sup>a</sup>
Sahakian <i>et al.</i> 2009 <i>Alabama, United States</i>	Cemented carbides and bonded diamonds Site visit at three cemented tungsten manufacturing facilities; urine collected post shift near end of work week	All plants (84) Areas with air levels > recommended exposure limits Reclamation (7) Powder mixing (5) Milling (5) Spray drying (2) Pressing (10) Shaping (4)	µg Co/g creatinine GM <sup>b</sup> (95% CI) <b>9.6</b> (7.1–12.8) <b>25.2</b> (8.7–73.2) <b>14.5</b> (2.5–84.9) <b>134.7</b> (96–189) <b>20.0</b> (0.9–438) <b>30.3</b> (14.9–61.8) <b>25.7</b> (5.0–133.5)	<i>Blood</i> GM <sup>b</sup> (95% CI) 2.0 (1.5–2.5) 4.0 (1.2–13.2) 3.7 (0.6–23.6) 15.6 (11.2–22) 3.2 (1.8–5.8) 3.7 (2.6–5.2) 3.3 (2.2–5.1)
Kraus <i>et al.</i> 2001 <i>Germany</i>	Hard-metals production workers (87) in different workshops	Forming (23) Pressing (30) Heavy alloy production (3) Powder processing (14) WC production (4) Sintering (6) Grinding (5) Maintenance (2)	µg/g creatinine <sup>e</sup> <b>13.5</b> (0.8–106.4) <b>5.5</b> (0.4–35.9) <b>1.6</b> (1.1–2.2) <b>28.5</b> (0.8–227.8) <b>2.1</b> (0.3–5.7) <b>4.1</b> (0.3–9.6) <b>2.2</b> (0.2–6.0) <b>3.0</b> (1.3–4.7)	
Linnainmaa and Kiilunen 1997 <i>Finland</i>	Hard metal workers; manufacturing or sharpening blades at 16 workplaces	(131)	<b>14.2</b> (0.5–160)	
Ferdenzi <i>et al.</i> 1994 <i>Italy</i>	Production of diamond cutting wheels workers (20)	Pre- & post-workplace modifications (Friday end of shift) 1988 (NR) 1991 (15)	<b>550</b> <b>85</b>	
Mosconi <i>et al.</i> 1994b	Diamond abrasive production and hard metal production	People exposed (314; numbers not reported for		

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
<i>Province of Bergamo, Italy</i>		subgroups) Diamond abrasive production Mould-filling Sintering Grinding Mechanical-working Hard-metals exposures Grinding Tool production Hard metal alloy filling Other	<b>587</b> (39–2100) <b>193</b> (102–390) <b>151</b> (34–520) <b>67.2</b> (14–165) <b>31.5</b> (0.8–730) <b>19.4</b> (0.8–100) <b>4.8</b> (0.8–18) 2.85 (0.8–72)	
Sabbioni <i>et al.</i> 1994a <i>Italy</i>	Hard metal workers (251) in four locations; three were hard metal mfg/tool production; Pavia produced diamond wheels	Bergamo (88) Milan (24 urine, 20 blood) Pavia- powder mixing (23) Turin (28)	<b>303.6</b> $\pm$ 837.5 <b>13.9</b> $\pm$ 9.7 <b>61.1</b> $\pm$ 58.2 <b>32.5</b> $\pm$ 35.7	<i>Blood</i> 45.6 $\pm$ 66.9 5.06 $\pm$ 4.37 NR NR
Suardi <i>et al.</i> 1994 <i>Italy</i>	Diamond abrasive production and grinding activities workers (159)	Producer (6) Grinder (87) Hard-metal form grinder (9) Others (76) Total (178)	$\mu\text{g/g creatinine}$ <sup>f</sup> <b>50.17</b> $\pm$ 24.30 <b>10.89</b> $\pm$ 15.23 <b>7.67</b> $\pm$ 5.94 4.55 $\pm$ 7.31 9.17 $\pm$ 14.79	
Nemery <i>et al.</i> 1992 <i>Belgium</i>	Diamond polishers using cobalt-containing disks workers in 10 workshop (194); 5 with low Co exposure and 5 with high exposure	Exposure category Low (73) High (86)	<b>9.0</b> $\pm$ 7.2 <b>25.2</b> $\pm$ 23.8	
Burr and Sinks 1988 <i>Michigan, United</i>	Cemented carbides and bonded diamonds Site visit; 149 urine samples	Monday Pre-shift (19)	Creatinine adjusted <b>10.8</b> $\pm$ 7.0	

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt (µg/L) <sup>a</sup>	Serum plasma, or blood cobalt (µg/L) <sup>a</sup>	
<i>States</i>	from 24 tool manufacturing workers (up to 7 specimens/person per day)	Post-shift (20) Tuesday	<b>19.0</b> ± 14.8		
		Pre-shift (21) Post-shift (23)	<b>18.3</b> ± 20.3 <b>25.1</b> ± 21.4		
		Wednesday			
		Pre-shift (20) Post-shift (23)	<b>15.0</b> ± 15.4 <b>27.0</b> ± 21.9		
		Thursday			
		Pre-shift (21) Post-shift (0)	<b>22.1</b> ± 2 6.2 ND		
NIOSH 1987 <i>South Carolina, United States</i>		Cemented carbides and bonded diamonds Site visit for grinding of tungsten carbide tools (post-sintering process) ()	Pre-shift (10)	µg/g creatinine <sup>e</sup> <b>[10.5]</b> (4.7–19.0)	
			Post-shift (10)	<b>[18.1]</b> (8.4–27.7)	
	Change from pre-to post – shift (10)		<b>[7.6]</b> (1.4–15.1)		
Kusaka 1986, 1996 <i>Asia</i>	Hard metal asthma patients (8)	(4)	(1–29)	<i>Blood</i> (2.8–4.2)	
Posma and Dijkstra 1985 <i>The Netherlands</i>	Hard metal production workers	6 subgroups (27)	µg/g creatinine <sup>e</sup>	<i>Blood</i>	
		Sawing (3)	<b>64.3</b> (45–102)	18.3 (9.6–32)	
		Pressing/mixing (4)	<b>45.1</b> (31–56)	11.5 (10.4–12.9)	
		Grinding (10)	<b>25.5</b> (5.8–39)	8.6 (4.0–14.6)	
		Sintering (3)	<b>6.4</b> (2.5–11.1)	2.0 (< 0.3–4.4)	
Pellet <i>et al.</i> 1984 (as reported in IARC 1991) <i>Not specified</i>	Hard metal production workers	Co powder production (6)	<b>35.1</b>		
		Pre-sintered WC (15)	<b>9.6</b>		
		Hard metal use (7)	<b>11.7</b>		
<b>Chemicals and pigments: Pottery or plate painting or cloisonné</b>					
Christensen and Poulsen 1994; Raffin <i>et al.</i> 1988;	Adult female pottery painters at two factories	10 yr surveillance date	µg/g creatinine <sup>e,f</sup>	<i>Blood</i> <sup>g</sup>	
		1982 (46)	<b>[69.5]</b>		
		1984 (49)	<b>[21.7]</b>		

Reference Location	Study population	Exposure group (# people or samples)	Urine cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>	Serum plasma, or blood cobalt ( $\mu\text{g/L}$ ) <sup>a</sup>
Christensen and Mikkelsen 1986 <i>Denmark</i>		1989 (145)	[12.7]	
		1991 (107)	[9.8]	
		Type of Co exposure		
		soluble cobalt (46)		2.16 ± 3.72
		slightly soluble cobalt (15)		0.63 ± 0.27
Arai <i>et al.</i> 1994 <i>Japan</i>	Cloisonne workers	Exposure timing/1982 (46)		
		After 6 wk vacation	[4.8 (< 0.1–26.2)]	[0.5 ± 0.3]
		4 wk after returning to work	[77.0 (2.2–848.0)]	[2.2 ± 3.7]
<b>Other or unspecified</b>				
Afridi <i>et al.</i> 2009 <i>Pakistan</i>	Steel mill workers	Non-exposed (75)	1.5 ± 0.4	1.2 ± 0.3
		Quality control (35)	2.3 ± 0.4	2.4 ± 0.6
		Production (56)	3.6 ± 0.6	3.9 ± 0.8
Scansetti <i>et al.</i> 1998 <i>Italy</i>	Not specified	Monday (6)	13.2 ± 9.8	
		Thursday (6)	30.9 ± 21.9	
Chadwick <i>et al.</i> 1997 <i>United Kingdom</i>	Thermal spraying workers (34) at 6 worksites in the industrial processes		$\mu\text{g/g creatinine}^{\text{d,e,f}}$	
		Grit blasting (5)	6.6 (0.16–11.0)	
		Plasma spraying (89)	0.5 (0.16–139)	
Meecham and Humphrey 1991 <i>Not reported; authors from UK</i>	Unspecified occupational exposure to Co	Detonation gun spraying (27)	8.9 (0.73–34.1)	
		(1)	NA	234

Co= cobalt NF = no studies found, NR = not reported in IARC or primary reference; PO = post-operation; WC = tungsten carbide.

<sup>a</sup>Mean ± standard deviation or range () unless stated otherwise. Studies are arranged within groups by most recent to oldest by publication date.

<sup>b</sup>Geometric mean.

<sup>c</sup>Reported urinary cobalt concentration is the geometric mean for the most recent (2011-2012) National Health and Nutrition Examination (NHANES) survey year for which data are available. Urinary cobalt data ranged from 0.316 to 0.379  $\mu\text{g/L}$  for 1999 to 2012 (CDC 2015).

<sup>d</sup>Median.

<sup>e</sup>It is generally accepted that 1 L of urine contains 1 g creatinine.

<sup>f</sup>Value reported by authors as  $\mu\text{g}/\text{mmol}$  creatinine or  $\mu\text{mol}/\text{mmol}$  creatinine; converted to  $\mu\text{g}/\text{g}$  creatinine using the following conversion factor: 1 mol creatinine = 113.1 g creatinine; nmol/L blood converted to  $\mu\text{g}/\text{L}$  using the MW of cobalt = 58.9.

<sup>g</sup>Values were reported in units of  $\mu\text{g}/\text{mg}$  creatinine; however,  $\mu\text{g}/\text{mg}$  creatinine appears to be a typographical error. All other HHEs reported urine values in either  $\mu\text{g}/\text{L}$  or  $\mu\text{g}/\text{g}$  creatinine.

<sup>h</sup>Not graphed because units not reported by authors.

<sup>i</sup>Values reported are weighted means across the 8 or 12 job titles.

**Table B-3. Values for hair and nail cobalt levels in the United States and other countries (hair values are plotted in Figure 2-2; values plotted for the general public or unexposed controls are shown below in *bold italic* while those for other exposures are in bold)<sup>a</sup>**

Reference Location	Study Population	Exposure group, number of people or samples (N)	Hair concentration, $\mu\text{g/g}^b$	Nails concentration, $\mu\text{g/g}^b$
<b>General Population</b>				
Carneiro <i>et al.</i> 2011 Brazil	Healthy male and female urban students 12–18 years of age from 9 public schools in Porto Alegre	(126)	<b>0.008</b> $\pm$ 0.007	0.08 $\pm$ 0.1
Dongarrá <i>et al.</i> 2011 Sicily	Students 11–13 years of age	Overall (136) Males (38) Females (98)	0.19 $\pm$ 0.33 <b>0.26</b> $\pm$ 0.51 <b>0.16</b> $\pm$ 0.22	
Elenge <i>et al.</i> 2011 Province of Katanga, Congo	General, non-industrialized population (medical students with no occupational history of exposure to metals) in the copper-belt.	109	Mean (5 to 95 percentile) <b>1.67</b> (0.8–2.02)	
González-Muñoz <i>et al.</i> 2010 Spain	Normotensive and hypertensive postmenopausal women	Normotensive (12) Hypertensive (14)	Median <b>0.02</b> (0.01–0.03) <b>0.03</b> (0.02–0.3)	
Bergomi <i>et al.</i> 2002 Emilia-Romagna region, No. Italy	Randomly sampled controls for an ALS study enrolled in the Italian National Health Service	Controls (40)		Median (25–75 percentile) 0.018 (0.009–0.041)
Campbell <i>et al.</i> 1988 UK	Healthy controls (hospital staff, volunteers) not on medication	Controls (160)	$\mu\text{g/mL}$ <b>0.07</b> $\pm$ 0.02	

Reference Location	Study Population	Exposure group, number of people or samples (N)	Hair concentration, $\mu\text{g/g}^b$	Nails concentration, $\mu\text{g/g}^b$
Kanabrocki <i>et al.</i> 1979 Unknown – U.S. institutions	General population - details NR	Females (9) Males (11)		0.07 (0.02–0.15) 0.04 (0.01–0.15)
<b>Environmental</b>				
Bibi <i>et al.</i> 2015 Lahore district, Punjab province, Pakistan	Persons of various ages from 3 sites near industrial areas used for agricultural purposes ranked as high, medium, and low exposure and controls living far from arsenic-contaminated regions	Risk area Low risk (12) Medium risk (12) High risk (12) Control (12)		$0.59 \pm 0.02^c$ $0.53 \pm 0.04$ $0.32 \pm 0.07$ $0.22 \pm 0.07$ $P = 0.00$
<b>Occupational</b>				
Afridi <i>et al.</i> 2009 Pakistan	Steel mill workers and non-exposed males 25– 55 years of age	Production (56) Quality control (35) Control (75)	$4.67 \pm 0.8$ $2.48 \pm 0.5$ $1.1 \pm 0.2$	
Sabbioni <i>et al.</i> 1994a Bergamo, Milan, Pavia, and Turin, Italy	Hard metal workers (male and female) from four plants; three were hard metal manufacturing/tool production; hair and nail data not available from the diamond workers	Bergamo; hair (90); toenails (92) Milan; hair (22); toenails (23) Turin; hair (28); toenails (NR)	$49.09 \pm 114.2$ $9.6 \pm 10.7$ $13.4 \pm 25.3$	$53.8 \pm 107.2$ $18.9 \pm 27.3$
Bencko <i>et al.</i> 1986 Czech Republic	Nickel (Ni) and Co production workers, and age-matched healthy workers unexposed to Co	Exposed to Co (30) Exposed to Ni (33) Unexposed (27)	$96.8 \pm 59.7$ $3.3 \pm 2.0$ $0.38 \pm 0.27$	

Reference Location	Study Population	Exposure group, number of people or samples (N)	Hair concentration, $\mu\text{g/g}^b$	Nails concentration, $\mu\text{g/g}^b$
<b>Hip Implants</b>				
Rodriguez de la Flor <i>et al.</i> 2013 Spain	Patients with metal-on-metal resurfacing arthroplasty before and after revision surgery	Before revision surgery (i.e., unstable) (11)	<b>147.4</b> $\pm$ 233.3	
		After revision surgery (11)	47.11 $\pm$ 74.20 <i>P</i> = 0.249	
Liu <i>et al.</i> 2011 China	Patients with metal-on-metal hip resurfacing arthroplasty  Compared with patients with metal-on-polyethylene hip arthroplasty	Metal-on-metal (22)		
		Preop	<b>4.4</b> $\pm$ 2.13	
		6 mos post-op	<b>53.3</b> $\pm$ 11.84	
		12 mos post-op	<b>47.4</b> $\pm$ 10.0 <i>P</i> = 0.0000	
		Polyethylene bearings (22)		
		Preop	<b>3.2</b> $\pm$ 2.42	
	6 mo post-op	<b>3.4</b> $\pm$ 1.69		
	12 mo post-op	<b>4.2</b> $\pm$ 2.46 <i>P</i> = 0.3371		

NR = not reported.

<sup>a</sup>Reported values are means  $\pm$  standard deviations or ranges () except where noted (e.g., medians or percentiles). Studies are arranged from most recent to oldest by publication date.

<sup>b</sup>Other units noted in table.

<sup>c</sup>No units were reported by the authors in this paper, but Bibi *et al.* (2015b) reported results for arsenic for the same population as  $\mu\text{g/g}$  hair and nails.

### B.3 Clinical surveys and studies

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

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

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

#### B.3.1 Studies of lung or laryngeal cancer patients

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

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

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

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

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

### B.3.2 Other cancers

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

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

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

of leukemia found non-significantly higher levels of cobalt compared to healthy subjects, one measuring cobalt in serum in acute leukemia patients (Demir *et al.* 2011) and the other measuring cobalt in urine of childhood leukemia patients (Zhu *et al.* 2011). In the sixth study, cobalt concentrations were similar from brain cancer patients and “healthy humans” (Arslan *et al.* 2011).

### B.3.3 Synthesis

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

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

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

**Table B-4. Findings from studies that measured cobalt in tissues (means or medians) of lung and larynx cancer patients and referents**

Reference	Population	Cancer tissue	Number of subjects	Category	Co levels (µg/g dry tissue)	Exposure methods	Comments
Adachi <i>et al.</i> 1991	Japanese clinical survey	Lung cancer	224	Lung cancer	0.33 ± 1.49	Dried and digested; atomic absorption spectrophotometer (AAS)	NS
	Autopsies from deaths (men and women) due to lung cancer and other cancers from the same medical center	Lung tissue	1,715	Other cases	0.27 ± 0.41		
Benderli Cihan and Öztürk Yildirim 2011	Turkish hospital-based case-control study	Lung cancer	67	NSCLC	[0.0031 ± 0.011]*	3 g; inductively coupled plasma mass spectrometry (ICP-MS)	<i>P</i> < 0.05
	Male non-small cell lung cancer (NSCLC; stage IIIB) and controls from the same geographical region using similar inclusion/criteria; similar age and ethnic background; all smokers	Hair	74	Controls	[0.0004 ± 0.0005]		
De Palma <i>et al.</i> 2008	Italian clinical study NSCLC and controls (men and women) undergoing pulmonary resection (lung metastasis from other cancers and lung disease) from the same hospital; smoking higher in cases	Lung cancer	45	NSCLC (non-tumor tissue)	0.07 (0.05–0.11)	Dried and digested; ICP-MS; standard	NS No differences in cobalt levels in non-tumor tissue in occupationally exposed (to metals) vs. non-exposed subjects and in smokers vs. non-smokers
		Lung biopsies	45	NSCLC (tumor)	0.05 (0.01–0.10)		
			8	Controls	0.04 (0.02–0.18)		
Gerhardsson <i>et al.</i> 1993, Gerhardsson <i>et al.</i> 1985,	Swedish male smelter workers and rural and urban referents (deaths)	Lung and other cancers Lung tissue	7	Workers/lung Cancer	[0.015]	Freeze dried; irradiated; neutron activation analysis (NAA)	Mean exposure duration 31.2 ± 8.4 yr Metal concentrations

Reference	Population	Cancer tissue	Number of subjects	Category	Co levels (µg/g dry tissue)	Exposure methods	Comments
			24	Workers/all cancer	[0.016]**		
			29	Workers/cardio-vascular	[0.016]***		
			12	Workers/other causes	[0.016]*		
			65	All workers	[0.015]***		
			14	Rural referents	[0.007]		
Kuo <i>et al.</i> 2006	Taiwanese hospital based case-control study; 1994–1998  Cases (82% men) had primary lung cancer presenting at a veterans hospital. Controls (81% men) had lung disease presenting at the veterans hospital and 2 other teaching hospitals.	Lung cancer Lung tissue	57	Lung cancer cases	0.18 ± 0.03*	Dried and digested; AAS; standard references	Cases were older and smoked more than controls  Cobalt levels were similar in non-smokers and smokers
			40	Controls (lung disease)	0.25 ± 0.06		
		25	Adenocarcinoma	0.11 ± 0.01			
		35	Squamous-cell carcinoma	0.23 ± 0.04			
		39	Stage I/II	0.20 ± 0.04			
		21	Stage I/III	0.15 ± 0.02			
Qayyum and Shah 2014	Pakistan case-referent study: lung patients and controls  Newly diagnosed patients from medical center and matched volunteer controls from same localities	Lung cancer Scalp hair	56	Cases	10.77 ± 1.599*	Hair (3g); nails (1g) dried; flame atomic absorption spectrophotometry (FAAS); 3 subsamples/sample; standard references	Cases were more likely to be male and smoked more than controls  Cobalt levels and variables- stage (nails & hair): decreasing 1 to 3 Inconsistent patterns between nails and hair for other
			54	Controls	6.787 ± 0.873		
		Lung cancer Nails	56	Cases	51.36 ± 10.47*		
			54	Controls	45.38 ± 7.491		

Reference	Population	Cancer tissue	Number of subjects	Category	Co levels (µg/g dry tissue)	Exposure methods	Comments
							variables such as sex, location, smoking
Zhang <i>et al.</i> 2012a	Chinese case-series (clinical) Malignant and normal tissue from lung cancer patients in regions with high lung cancer incidence	Lung cancer Lung tissue and tumor	30 30	Malignant tumor/lung cancer Normal tissue/lung cancer	[0.00012 ± 0.00005] [0.00025 ± 0.00016]	Dried, powder and digested; ICP-MS; standard references, spiked entire process; blanks to test for contamination	Number of subjects and whether the tumor and non-tumor tissue is from same subject not clear <i>P</i> = 0.051
Collecchi <i>et al.</i> 1986	Italian clinical study Males without known exposure to arsenic and cobalt with laryngeal carcinoma and "normal males"	Larynx cancer Larynx tissue Plasma	15 15 15 11	Malignant tissue/laryngeal cancer Non-malignant tissue/laryngeal cancer Laryngeal cancer Controls	0.069 ± 0.007** 0.040 ± 0.007 18.27 ± 2.10*** ng/mL 0.73 ± 0.10	Radioactive NAA; standard references	Population undefined; spiked samples
Klatka <i>et al.</i> 2011	Polish clinical survey Male laryngeal cancer patients: tumor and normal tissue from the same patient	Larynx cancer Larynx tissue	43 43 29 14 19 24	Laryngeal carcinoma Non-tumor tissue Stage 3 tumor Stage 4 tumor Rural regions Urban regions	0.031 ± 0.0375 0.017 ± 0.013 0.025 ± 0.034* 0.044 ± 0.043 0.046 ± 0.050* 0.019 ± 0.017	Digested; plasma optical emission spectrometry (ICP-OES); separate tissue dried for calibration validated with reference material	

Table B-5. Findings from studies that measured cobalt in tissues (means or medians) of cancer patients and referents

Reference	Population	Cancer tissue	Number of subjects	Category	Co levels (µg/g dry tissue)	Exposure methods	Comments
Pasha <i>et al.</i> 2007	Pakistan clinical survey; 2001 to 2003	All cancers	111	Cancer patients	24.6 ± 1.5*	Flame atomic absorption spectrophotometry (FAAS); 3 samples/person; reference	Normal donors matched for age group. Types of cancer not reported
	Men and women cancer patients from two hospitals (15 to 93 yr) and normal donors from same region	Hair	113	Normal donors	6.10 ± 0.36		
Gerhardsson <i>et al.</i> 1993, Gerhardsson <i>et al.</i> 1985, Gerhardsson <i>et al.</i> 1984	Swedish retired copper smelter workers and 8 rural referents Tissue from deceased subjects who died of cancer and other causes	All cancers	10	Workers/cancer	[0.012]	Freeze dried; irradiated; neutron activation analysis (NAA)	47 workers were retired for 0 to 10 years and 18 workers were retired for 11 to 23 years; mean retirement = 7.2 ± 5.9 yr
		Liver	8	Workers/cardio-vascular	[0.011]		
			2	Workers/other causes	[0.015]		
			20	All workers	[0.011]		
			8	Rural referents	[0.016]		
		All cancers	10	Workers/cancer	[0.003]		
		Kidney	8	Workers/cardio-vascular	[0.003]		
			3	Workers/other causes	[0.006]		
			21	All workers	[0.003]		
			8	Rural referents	[0.001]		
Kantias <i>et al.</i> 1994	Greek clinical survey Women (23) undergoing biopsy because of	Breast	17	Fibrocystic disease	0.051 ± 0.045	Samples and standards irradiated; radioactive count	Differences in cobalt levels between disease groups not
		breast tissue	6	Fibroadenoma	0.10 ± 0.17		

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

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

**This Page Intentionally Left Blank**

## B.4 Regulations and guidelines

### 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  $\geq 0.5$  mg cobalt as a cobalt salt per dosage unit and which recommend administration rates of  $\geq 0.5$  mg per dose and  $\geq 2$  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<sup>3</sup> 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<sup>3</sup> for cobalt and inorganic compounds; = 0.1 mg/m<sup>3</sup> for cobalt carbonyl and cobalt hydrocarbonyl.

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

**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<sup>3</sup>; industrial air = 0.0014 µg/m<sup>3</sup>; tap water = 6 µg/L.

**National Institute for Occupational Safety and Health (NIOSH)**

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

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

**This Page Intentionally Left Blank**

## Appendix C: Human Cancer Study Tables

This appendix contains background information related to the cancer assessment on cobalt and certain cobalt compounds in humans including detailed (1) data information on study design, methods, and findings for human cancer studies (Tables C-1 to C-2) and (2) detailed information on the quality assessment of the individual studies (Tables C-3 to C-5).

### C.1 Methodologies and study characteristics

The data from the 7 cohort studies, which include four nested case-control studies (Table C-1), and 2 case-control studies on esophageal and head and neck cancers (Table C-2), were systematically extracted from relevant publications and are summarized in the tables below. Some of the studies were conducted on overlapping populations. The cohort studies are organized by occupational group and listed in chronological order (earliest studies first) similar to Table 4-1.

**Table C-1a. Study description and methodologies of cohort studies: Tüchsen *et al.* (1996)**

Field	Description
<b>Reference</b>	<i>Tüchsen et al. (1996)</i> Tüchsen F, Jensen MV, Villadsen E, Lynge E. Incidence of lung cancer among cobalt-exposed women. <i>Scand J Work Environ Health.</i> 1996 Dec;22(6):444-50. PubMed PMID: 9000312.
<b>Study-design type</b>	Cohort
<b>Location and enrollment dates</b>	Copenhagen, Denmark; Jan 1, 1943 (Factory 1) or Jan 1, 1962 (Factory 2) - Dec 31, 1992
<b>Population description</b>	Danish women porcelain plate workers.
<b>Eligibility criteria</b>	All women employed at any time in two underglaze porcelain plate departments (Factory 1 and Factory 2); and all female top glaze decorators in a department without cobalt exposure (Factory 1).
<b>Cohort details</b>	Population size: 1394 total; 874 cobalt-exposed workers, 520 unexposed workers. Loss-to-follow-up: 13 (0.92%) Referent Group: External (SIR); also calculated SIR for unexposed workers.
<b>Outcome data source</b>	Followed for death and emigration using data in the Central Population Register and the municipal population registers. Cancer cases identified by linkage to Danish Cancer Register (ICD-7).
<b>Exposure assessment</b>	Company records
<b>Exposure assessment notes</b>	Exposure to cobalt-aluminate spinel and/or cobalt silicate at 2 factories. Detailed information on work history; exposure monitoring data was reported for air and urine from the 1980s which was not used in the exposure assessment; calendar period was adjusted for in analysis.
<b>Exposure-level</b>	Employment in factories/departments with or without cobalt
<b>Co-exposures</b>	Nickel, silica
<b>Analysis methods and control for confounding</b>	Analytical methods: Personnel files for permanently ill persons may have been removed in earlier years, potentially resulting in an underestimate of incidence. Covariates: Age Confounder consideration: Calculation of expected number of cancer cases took five year age groups and calendar periods in consideration. No HWE, No control for other variables; unclear if calendar period was controlled

Table C-1b. Study description and methodologies of cohort studies: Mur *et al.* (1987)

Field	Description			
Reference	<i>Mur et al. (1987)</i> Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. <i>Am J Ind Med.</i> 1987;11(1):75-81. PubMed PMID: 3812499.			
Study-design type	Cohort and nested case control study			
Location and enrollment dates	France; 1950-1980			
Population description	<b>Male electrochemical workers</b> including cobalt production workers			
Eligibility criteria	N = 1,143. All men employed for at least one year at a cobalt production plant producing cobalt, cobalt salt and oxides, and sodium between 1950 and 1980; hired between 1900 and 1979.			
Cohort details	<i>Population size:</i> N = 1,143; number of cobalt production workers not reported but ~ 25% of current staff at publication <i>Loss-to-follow-up:</i> 17.9% for cobalt production workers; 75% hired before 1975. <i>Referent Group:</i> Internal and external comparing cohort mortality to male mortality in France			
Case-control description and eligibility criteria		<i>Population size</i>	<i>Response rates</i>	<i>Source</i>
	Cases	9	NR	All lung cancer cases from cohort
	Controls	18	NR	Two controls/case were matched on year of birth and age at death and "smoking habits" (undefined); controls were selected from among those dying of conditions other than cancer.
Outcome data source	Vital status ascertained by registry offices in the birth places of Frenchmen, and at embassies and consulates for foreign-born. Cause of death (ICD-8) ascertained by physicians and medical records. 80% of causes of death determined and classified.			
Exposure assessment	Company records			
Exposure assessment notes	Job histories grouped according to employment in general service, maintenance, sodium or cobalt production. Only included those with exclusive employment in any of these departments. No Co levels reported, nor were prior measurements available.			
Exposure-level	60% worked greater than 10 years; 75% hired before 1975			
Co-exposures	Arsenic, nickel			
Analysis methods and control for confounding	<i>Analytical methods:</i> <i>Covariates:</i> age, year of death SMR all cause mortality = 0.77 ( $P < 0.01$ ); no methods to control HWE; all cause mortality for cobalt production = SMR 1.29 (0.86–1.87). <i>Analytical method:</i> matched case-control study <i>Covariates:</i> None			

Field	Description
	<i>Confounder consideration:</i> Cases (deaths from lung cancer) were matched to controls (deaths from cause other than cancer) for year of birth, age at death, and smoking habits

**Table C-1c. Study description and methodologies of cohort studies: Moulin *et al.* (1993)**

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

Table C-1d. Study description and methodologies of cohort studies: Moulin *et al.* (1998)

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

Table C-1e. Study description and methodologies of cohort studies: Wild *et al.* (2000)

Field	Description
Reference	Wild <i>et al.</i> (2000) Wild P, Perdrix A, Romazini S, Moulin JJ, Pellet F. Lung cancer mortality in a site producing hard metals. <i>Occup Environ Med.</i> 2000 Aug;57(8):568-73. PubMed PMID: 10896965.
Study-design type	Cohort
Location and enrollment dates	France; January 1968–December 1992
Population description	Hard metal workers in the largest such factory in France (included in the Moulin <i>et al.</i> 1998 paper).
Eligibility criteria	Subjects who had worked at least 3 months between January 1, 1950 and June 30, 1992, and were still alive by January 1, 1968. 80% of cohort were hired prior to 1970. Mean follow-up 18.6 years
Cohort details	<i>Population size:</i> 2,216 men and 644 women <i>Loss-to-follow-up:</i> 20.2%; Foreign-born workers terminated before 1968 censored and considered lost to follow-up <i>Referent Group:</i> External analysis using "local death rates" as comparison.
Outcome data source	Vital status ascertained by registry offices of birthplaces and computer database of all deaths in France starting in 1978. Cause of death obtained by matching the file of dead subjects with the national file of causes of death from 1968, coded to ICD-8 of disease before 1978, and to ICD-9 for disease after 1978; 96% of causes could be retrieved.
Exposure assessment	JEM
Exposure assessment notes	Semi-quantitative (JEM) exposure assessment based on administrative records and interviews with colleagues; 320 job periods assigned estimates of exposure to cobalt and tungsten carbide - Intensity score from 0 (no exposure) to 9 (highest exposure level); frequency score of <10%, 10-50%, and >50% of work time. Ever or only employment in the "powder production workshop" was also used as an indicator of potential exposure to cobalt.
Exposure-level	NR
Co-exposures	PAHs, certain chromium compounds, certain nickel compounds, silica, cobalt-tungsten carbide, asbestos, arsenic compounds, cadmium compounds, nitrosamines, benzene
Analysis methods and control for confounding	<i>Analytical methods:</i> <i>Covariates:</i> Age, unclear if these are crude estimates <i>Confounder consideration:</i> conducted separate smoking analyses

Table C-1f. Study description and methodologies of cohort studies: Moulin *et al.* (2000)

Field	Description			
Reference	<i>Moulin et al. (2000)</i> Moulin JJ, Clavel T, Roy D, Dananche B, Marquis N, Fevotte J, Fontana JM. 2000. Risk of lung cancer in workers producing stainless steel and metallic alloys. <i>Int Arch Occup Environ Health</i> 73(3): 171-180. PMID 10787132			
Study-design type	Nested Case-Control			
Location and enrollment dates	France; January 1, 1968 - December 31, 1992			
Population description	<b>Male and female workers in a French factory producing stainless and alloyed steel.</b>			
Case-control description and eligibility criteria		<i>Population size</i>	<i>Response rates</i>	<i>Source</i>
	Cases	54 (17 Co-exposed)	NR	All workers who died from lung cancer determined thru death certificate and medical record matching process.
	Controls	162 (67 Co-exposed)	NR	3 controls / case sampled from those under follow-up at the date of death, had completed 1 year of employment, and known to be alive on this date, same gender, and DOB within 6 months of deceased case.
Exposure assessment	JEM			
Exposure assessment notes	Semi-quantitative JEM had 5 levels of exposure no exposure, occasional, and low, medium, and high exposure. Frequency was coded as 10% to 100% of working time; low, medium, and high probability of accuracy of intensity and frequency codes was included. Increasing exposure levels, duration of exposure, and cumulative dose (frequency weighted and unweighted)			
Exposure-level	NR			
Co-exposures	Iron, acid mists, PAHs, asbestos, silica, chromium and/or nickel			
Analysis methods and control for confounding	<i>Analytical methods:</i> Analyses were lagged. <i>Covariates:</i> PAHs, age, gender, silica, smoking ever/never <i>Confounder consideration:</i> Co correlated in a reported matrix with Chromium and/or Nickel, and Iron, but neither of these were included in the multivariate analysis			

Table C-1g. Study description and methodologies of cohort studies: Grimsrud *et al.* (2005)

Field	Description			
Reference	<i>Grimsrud et al. (2005)</i> Grimsrud TK, Berge SR, Haldorsen T, Andersen A. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel? <i>Epidemiology</i> . 2005 Mar;16(2):146-54. PubMed PMID: 15703528.			
Study-design type	Nested Case-Control			
Location and enrollment dates	Norway; 1910–1995			
Population description	Norwegian nickel refinery workers			
Case-control description and eligibility criteria		<i>Population size</i>	<i>Response rates</i>	<i>Source</i>
	Cases	213	NR	lung cancers diagnosed from 1952-1995 and in the Cancer Registry of Norway during this time.
	Controls	525	NR	3 controls / case randomly drawn among cohort members at risk at the time of dx (incidence density sampling), free of lung CA, and born within 24 months of the case's DOB, and matched by gender. Controls drawn in a 1:1 ratio for cases diagnosed before 1970.
Exposure assessment	JEM			
Exposure assessment notes	A semi-quantitative JEM was developed for various species of nickel based on 5900 personal measurements; this was supplemented with 3500 personal samples from the breathing zone for cobalt.			
Exposure-level	In $\mu\text{g}/\text{m}^3$ : High (144–3100); Medium (29.7–142); Low (0.31–29.5)			
Co-exposures	Nickel, arsenic, asbestos, sulfuric acid mists			
Analysis methods and control for confounding	<i>Analytical methods:</i> <i>Covariates:</i> smoking <i>Confounder consideration:</i> No multivariate estimates were possible due to collinearity with nickel.			

Table C-1h. Study description and methodologies of case-control studies: Rogers *et al.* (1993)

Field	Description			
Reference	Rogers <i>et al.</i> (1993) Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. A case-control study of element levels and cancer of the upper aerodigestive tract. <i>Cancer Epidemiol Biomarkers Prev.</i> 1993 Jul-Aug;2(4):305-12. PubMed PMID: 8348053.			
Study-design type	Case-Control			
Location and enrollment dates	Western WA state, USA; 9/1/83 - 2/28/87			
Population description	<b>Population based randomly selected controls and cases from SEER</b>			
Case-control description and eligibility criteria		<i>Population size</i>	<i>Response rates</i>	<i>Source</i>
	Cases	N = 507; N = 153 laryngeal, N = 73 esophageal, N = 359 oral cavity cancers	52.8% providing toenail samples	Laryngeal, esophageal or oral cavity cancers of epithelial origin identified from local SEER registry with positive histological findings; some cases confirmed by cytology and followed with attending physician.
	Controls	N = 434	66.4% providing toenail samples	Controls from same area as cases selected by random digit dialing and frequency matched by sex and age in 5 year intervals of cases.
Exposure assessment	Personal monitoring			
Exposure assessment notes	Dietary sources of trace elements of cobalt, iron, calcium, zinc, chromium explored; toenails collected and cleaned; personnel blinded to case status; formation of nail matrix takes 8-24 mos; median time from dx to interview was 6.5 mos, so samples likely to represent prediagnostic levels; qx data on occupation collected but not reported.			
Exposure-level	Tertiles of cobalt in toenails; highest level 0.17 ppm			
Co-exposures	Iron, calcium, zinc, chromium			
Analysis methods and control for confounding	<p><i>Analytical methods:</i> "Exposed cases" in this table refers to both cases and controls combined; exposed cases alone NR.</p> <p><i>Covariates:</i> age, alcohol (drink years), ascorbic acid mg/day, beta-carotene, mg/day, energy intake, kcal/day, sex, smoking (pack-years). Correlations between cobalt and other measured metals not provided; and models for cobalt did not include other metal levels.</p> <p><i>Confounder consideration:</i> 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.</p>			

Table C-1i. Study description and methodologies of case-control studies: O'Rorke *et al.* (2012)

Field	Description			
Reference	O'Rorke <i>et al.</i> (2012) O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, FINBAR Study Group. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. <i>Int J Cancer</i> . 2012 Oct 15;131(8):1882-91. PubMed PMID: 22262413.			
Study-design type	Case-control study			
Location and enrollment dates	All Ireland (Republic and Northern); 3/2002 - 12/2004			
Population description	<b>Population based cases and controls</b>			
Case-control description and eligibility criteria		<i>Population size</i>	<i>Response rates</i>	<i>Source</i>
	Cases	N = 137 esophageal cancer; N = 182 Barrett's esophagus	Esophageal CA = 38.6%; Barrett's esophagus = 66.9	No. Ireland: Esophageal cases ( $\leq 85$ yrs) identified from electronic path records from all path labs. Rep of Ireland: cases identified from the main referral hospitals diagnosing and treating esophageal CA. Pathology review and histologically confirmation; excluding in situ CA. Barrett's esophagus - pts with $\geq 3$ cm of Barrett's mucosa at endoscopy or biopsy showed specialized intestinal metaplasia. Pts with dysplasia on histology excluded.
	Controls	221	35.5	Adults (35 to 84) without history of esophageal or other gastrointestinal cancer or known diagnosis of Barrett's esophagus; frequency matched by sex and age (5 yrs). Selected at random from general practitioner (GP) list (No. Ireland) and from 4 GP practices (2 rural and 2 urban) in Dublin and Cork areas that reflected the distribution of the Rep. Ireland cases
Exposure assessment	Personal monitoring			
Exposure assessment notes	Cobalt level in toenails; tertile cutpoints of log(e) transformed Co based on control distribution. Questionnaire for demographics, lifestyle habits, diet, manual/non-manual occupation, and medical history; anthropometric measurements; personnel blinded as to case status.			
Exposure-level	Average ( $\mu\text{g/g}$ ) $\pm$ SD: cases – $0.02 \pm 0.06$ ; controls – $0.02 \pm 0.04$ . Range: cases 0.002 – 0.60; controls – 0.002 – 0.47			
Co-exposures	Selenium, iron, chromium, zinc measured			
Analysis methods and control for confounding	<p><i>Analytical methods:</i></p> <p><i>Covariates:</i> GI reflux, H. pylori infection, age, education, energy intake, location, sex, smoking, smoking habits</p> <p><i>Confounder consideration:</i> 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.</p>			

## C.2 Assessment of study quality, sensitivity, and utility of human studies of cobalt

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

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

- *Low/minimal concerns*: Information from study designs and methodologies indicate that they are close to the ideal study characteristics and that the potential for bias is unlikely or minimal, recognizing general limitations of observational studies. [+++ high quality]
- *Some concerns*: Study designs or methodologies are less than ideal, indicating possible bias. [++ medium quality]
- *Major concerns*: Study designs or methodologies suggest that the potential for a specific type of bias is likely albeit depending on the direction and distortion of the potential bias, the study may have some limited utility. [+ low quality]
- *Critical concern*: Distortion of bias would make study findings unreliable for cancer hazard identification. [0 rating]
- *No information*: The information in the study is inadequate to evaluate the level of concern for the domain.

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

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

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

The overall study judgment is not meant to be an algorithm that sums up the ratings across domains. The quality of the exposure assessment and potential for exposure misclassification and

potential confounding was given considerable weight in ranking the studies. In addition, studies with high probability of systematic (i.e., differential) biases were rated low.

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

Selection bias and evaluation of methods used to address potential confounding (Table C-2a)

Information bias: exposure and outcome misclassification (Table C-2b)

Selective reporting and analysis bias (Table C-2c)

Study sensitivity, quality and utility of cohort and nested case-control studies (Table 3-2-4)

Study sensitivity, quality and utility of case-control studies (Table 3-2-5)

### **C.2.1 Selection bias and evaluation of methods used to address potential confounding**

#### ***Cohort and nested case-control studies***

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

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

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

In the electrochemical workers cohort (Moulin *et al.* 1993) 46% of cohort members had been hired prior to the start of follow-up, with the likely effect of inducing a downward bias in the effect estimate. Such left-censoring can result in a cohort with healthier prevalent hires who have remained working from earlier periods of exposure. The Moulin *et al.* (2000) study indirectly addressed the healthy worker survival effect (HWSE) indicating that cases and controls in the

nested study were matched on age and were reported to have similar distributions for the date of hire, suggesting there was little concern for HWSE.

### **Case-control studies**

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

In the FINBAR study, there is some concern that selection bias may be operating in the selection of cases and controls from the Republic of Ireland in this study (O'Rorke *et al.* 2012). Cases were identified from the "main" hospitals involved in the diagnosis and treatment of esophageal cancer including the national referral center for esophageal cancer. In contrast, Republic of Ireland controls were selected at random from two urban and two rural general practices, purportedly reflecting the urban/rural distribution of esophageal cancer cases in the Republic. However, smoking rates among the controls suggest that participating controls may not be fully representative of the case population or of those who did not submit toenail samples. Current smoking was higher in the cases, as expected, but the level of smoking in the controls was lower than that of the general population (16% of controls returning toenail samples, and 17.7% in all controls; 23.6% of males 55 years and over). Among those not returning toenail samples, the proportion of current smokers was higher (27%).

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

### **C.2.2 Evaluation of methods to address confounding**

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

### **Cohort and nested case-control studies**

Most of the cohort and nested case-control studies conducted age-, sex-, and calendar year- or period-standardized comparisons in external analyses (SMR or SIR); and in some cases restricted internal analyses to men (when there were small numbers of women workers) in internal analyses. All studies provided information about or directly controlled for smoking; however, the quality of these data ranged widely. Some conducted sub-studies of smoking habits of some proportion of the workers (ranging from <30% to 70%) (Tüchsen *et al.* 1996, Moulin *et al.* 1993, Mur *et al.* 1987); others categorized workers as "ever-never" smokers (Moulin *et al.* 2000), and

others were able to incorporate detailed information on former and current smokers and their level of smoking (Grimsrud *et al.* 2005). While smoking is a strong risk factor for lung cancer, there was no evidence of smoking being strongly associated with cobalt exposure in any of the studies.

Regarding co-exposures, studies among the hard-metal workers (Wild *et al.* 2000, Moulin *et al.* 1998), stainless and alloyed steel workers (Moulin *et al.* 2000) and nickel refinery workers (Grimsrud *et al.* 2005) assessed co-exposures to several known IARC carcinogens; however, in none was information on co-exposures either sufficiently reported, or controlled. Based on communications with the author (Dr. Wild) it is unlikely that co-exposures were controlled in the hard-metal studies. Furthermore, exposure to cobalt (not in the presence of tungsten carbide) ranged from exposure to pure cobalt in cobalt powder production workshops to mixed exposures, with potential exposure to lung carcinogens in the other production workshops. In the stainless and alloyed steel workers (Moulin *et al.* 2000) no control was indicated for metals most closely correlated with cobalt. In the Grimsrud *et al.* study, data were available to evaluate the role of cobalt on lung cancer controlling for a number of other carcinogens. Their focus was to understand the confounding effect of co-exposures with nickel (correlation,  $r = 0.63$ ); however, they were not able to separate the effects of cobalt from nickel as all nickel workers were exposed to cobalt. The electrochemical worker study (Moulin *et al.* 1993, Mur *et al.* 1987) and the porcelain painters (Tüchsen *et al.* 1996) did not collect information on co-exposures, although an earlier paper on this cohort provided information about low levels of nickel ( $<10\%$  of Danish occupational limit of  $0.1 \text{ mg/m}^3$ ), silica (no detectable concentrations), and dust (average of  $7.6 \text{ mg/m}^3$ ) measured in 1981 prior to changes in practices that reduced air levels of cobalt somewhat. The electrochemical workers cohort may have been exposed to arsenic, which is added during the production process, and nickel and arsenic are contained in cobalt ore, but no measurements of these were taken.

### **Case-control studies**

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

**Table C-2a. Selection bias and evaluation of methods used to address potential confounding in human studies of cobalt**

Study	Selection bias	Methods used to address potential confounding
Tüchsen <i>et al.</i> 1996	<i>Rating:</i> ++; Direction ↓ <i>Rationale:</i> No HWE was evident; only external analyses comparing cancer incidence in the Danish population. Differential selection out of the cohort could have occurred as the authors mentioned that records of ill persons may have been removed potentially resulting in an underestimate of the true incidence of cancer.	<i>Rating:</i> ++; ↑ <i>Rationale:</i> No internal or statistical analysis controlling for smoking or potential carcinogens; however, minimal exposure to occupational co-exposures indicated from measurements in 1981 and smoking data on subset of the population
Mur <i>et al.</i> 1987 Moulin <i>et al.</i> 1993	<i>Rating:</i> ++ (Mur for case-control analysis); ++ (Moulin 1993; French nationals analysis); ↓ <i>Rationale:</i> HWE present based on significant decreases in all-cause mortality rates (Mur <i>et al.</i> 1987) in the entire cohort, but not for workers exposed only to cobalt. High loss to follow-up in both studies due to foreign-born workers, with no information provided to assess if the loss was related to exposure. Moulin <i>et al.</i> 1993 provided a restricted analysis for French nationals in addition to a whole-cohort analysis, to mitigate a potential bias. However, 46% of the cohort was hired prior to the start of follow-up (left-truncation), which could induce a downward bias in the effect estimate.	<i>Rating:</i> +; ↑ <i>Rationale:</i> Mur <i>et al.</i> reported smoking data from admin records available from 30% of the cohort (Mur <i>et al.</i> 1987), although cases and controls were reported as matched on smoking status with no report on methods of matching. Moulin <i>et al.</i> 1993 did not adjust for smoking. Potential exposure to nickel and arsenic from cobalt ore and/or processing methods, however, no analysis conducted controlling for potential carcinogens. Internal analysis (nested case-control) available in the Mur <i>et al.</i> 1987 study helps minimize potential confounding.
Moulin <i>et al.</i> 1998	<i>Rating:</i> ++ for nested case-control analysis; ↓ <i>Rationale:</i> High loss to follow-up largely due to foreign-born workers who were right censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that loss was related to exposure. Concerns with statistically significant decreases in all-cause mortality rates (HWE) mitigated by nested case-control analysis. Left-truncation not an issue in this incidence cohort.	<i>Rating:</i> +; ↑ <i>Rationale:</i> Ever vs. never smoking data collected, and ever/never data on co-exposures in the JEM. Unlikely if models for cobalt alone included smoking and co-exposures (based on communication with author). Little information provided on cobalt co-exposures. Internal analyses may help reduce potential confounding from life style factors. “Other cobalt exposures” were a mix of cobalt exposures from pure cobalt alone to other cobalt (not tungsten carbide) production activities with exposure to other carcinogens and not well defined.

Study	Selection bias	Methods used to address potential confounding
Wild <i>et al.</i> 2000	<p><i>Rating:</i> ++; ↓</p> <p><i>Rationale:</i> High loss to follow-up largely due to foreign-born workers who were censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that the loss was related to exposure. No case-control analysis of cobalt alone provided. Left-truncation not an issue in this incidence cohort.</p>	<p><i>Rating:</i> +; ↑</p> <p><i>Rationale:</i> Smoking data abstracted from factory health records and for earlier smoking from former colleagues. Co-exposures to several IARC carcinogens likely (no correlations provided) and assessed (ever/never) from JEM exposure. Unlikely that smoking and co-exposures were included in estimate for cobalt alone exposure. See Moulin <i>et al.</i> 1998 also.</p>
Moulin <i>et al.</i> 2000	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> HWE effect based on significant decreases in all-cause mortality rates in external analysis; cross-sectional cohort, internal analyses may have mitigated potential HWE bias. Nested case-control study of living controls matched on age at selection with the case; similar as to their distribution of year of hire, suggesting that it is unlikely that the HWSE was operating.</p>	<p><i>Rating:</i> ++; ↑</p> <p><i>Rationale:</i> Statistical analysis controlled for smoking (ever-never) for 71% of subjects and some co-exposures (levels assigned from JEM). However, the models did not control for co-exposure to other metals (nickel/chromium and iron), which were correlated with exposure to cobalt, although not to lung cancer in these data.</p>
Grimsrud <i>et al.</i> 2005	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> No information about whether HWE was present in the original cohort, but only cases who were identified from 1970 onwards were included; controls were matched to cases by gender and age and at risk at the time of the case diagnosis, and were similar with respect to year of first employment, reducing the concern with HWSE. However, some concern remains given that sick workers hired at the same dates as healthy workers could move from higher to lower exposure groups. No analysis was conducted to account for this possibility. Participation rates were 94% for both cases and controls.</p>	<p><i>Rating:</i> ++; ↑</p> <p>Statistical analyses controlled for smoking (5 categories) and exposure to lung carcinogens (arsenic, asbestos, sulfuric acid mists and nickel). Due to the high correlation between cobalt and nickel exposure, categorical levels of cobalt variable could not be retained in the fully adjusted model. Only smoking adjusted exposure response estimates were available.</p>

Study	Selection bias	Methods used to address potential confounding
Rogers <i>et al.</i> 1993	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Ascertained all cases of aerodigestive cancer from the Western Washington State SEER cancer registry and used random digit dialing to identify controls in the same defined area as controls.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol and tobacco use among cases and controls. Differences in education, not occupation reported. Food frequency questionnaire based on usual dietary habits 10 years prior to the interview. Other metals measured included zinc, chromium, iron and calcium; however no correlations were shown with cobalt, nor were these metals included in models.</p>
O'Rorke <i>et al.</i> 2012	<p><i>Rating:</i> ++</p> <p><i>Rationale:</i> Methods for case and control selection vary by location. Northern Ireland cases identified systematically from electronic pathology records across the country; cases in the Republic were identified from "main" hospitals involved in the diagnosis and treatment of esophageal cancer. Potential for cases not referred to the major referral centers to be excluded. Very low participation rates and lower smoking rates in controls suggests the potential for selection bias is present due to lifestyle factors related to esophageal cancer.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol, manual labor, and tobacco use among cases and controls. Dietary intake based on food frequency questionnaire assessing diet and alcohol use 5 years previously. Other metals measured included selenium, chromium, zinc, mercury, and cerium; however no correlation with cobalt was indicated, nor were these other metal included in the models for cobalt.</p>

### C.2.3 Information bias: exposure assessment and disease endpoints

#### ***Cohort and nested case-control studies***

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

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

The most concern about exposure misclassification existed primarily for studies in which cobalt exposure was simply defined as employment in particular workshops (Moulin *et al.* 1993, Mur *et al.* 1987), or in factory departments (Tüchsen *et al.* 1996). Previously published data from the porcelain painters (Raffn *et al.* 1988) indicated an overlap of cobalt levels in referents and exposed individuals, suggesting that the referents in the Tüchsen *et al.* paper may not have been “unexposed.” In addition, exposure assessment in these studies did not differentiate workers according to exposure level. Potential misclassification of exposure would arise from lack of information on job tasks, use, and exposure conditions.

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

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

### **Case-control studies**

Both population-based studies were conducted to determine the relationship of low levels of metals derived primarily from dietary sources to esophageal cancer, Barrett's esophagus, and other aero-digestive cancers. However, a major concern about information bias in exposure assessment exists for both studies because the window of exposure implied by measuring metals in nails for cancer outcomes may not be appropriate. Nail samples were collected a median of 6.5 months after diagnosis in the Rogers *et al.* study, at or near the time of study enrollment in both studies. O'Rorke *et al.* (2012) reported that 76.7% of esophageal cancer cases had habitually clipped their toenails prior to admission to hospital. Toenail clippings likely reflect an integrated exposure that occurred 12 to 18 months prior to clipping (Fleckman 1997). Given that Barrett's esophagus, esophageal and aerodigestive cancers have long latency periods, clippings that reflect at most an exposure window 12 months prior to diagnosis may or may not reflect ongoing exposure related to the development of cancer. In addition, several known factors can influence nail growth and possibly affect the time window of exposure represented by the sample. For example, nail growth is faster during pregnancy, and in warmer climates (Fleckman 1997). Certain medical conditions have been shown to increase or decrease the rate of nail growth, and age and sex affect nail growth with faster nail growth in men, and declining rates with increasing age (Dawber and Baran 1987; Fleckman, 1985). Trace element deposition in nails may be influenced by several factors including those that are correlated with cancer (e.g., immobilization, decreased circulation, malnutrition, weight loss, age, gender, changes in diet and smoking and alcohol consumption) (Slotnick and Nriagu 2006, Hunter *et al.* 1990). Furthermore, reproducibility of cobalt specifically, in toenails from multiple samples over time has been reported to have intermediate to high within-person variability suggesting that sampling at any one point in time may not reflect long-term exposure (Garland *et al.* 1993). Another concern is whether the cancer process itself could alter deposition of cobalt in the nails, resulting in reverse causality. Two studies of cobalt levels by cancer stage in patients with lung cancer (Benderli-Cihan *et al.* 2011; Kuo *et al.* 2006) and one with laryngeal cancer (Klatka *et al.* 2011) show contradictory findings, with no difference, increasing, and decreasing cobalt levels with increasing stage of disease. Rogers stratified cases by stage at diagnosis (*in situ*, localized versus regional, and distant), and by the time from diagnosis to interview (which was either < 7 months or > 7 months); there were no significant differences by stage or by time from diagnosis to interview, suggesting that reverse causality was not operating in this study.

#### **C.2.4 Information bias - Disease endpoints**

##### **Cohort and nested case-control studies**

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

The remaining cohorts, with the exception of the first analysis of the electrochemical workers (Mur *et al.* 1987) were based on mortality data obtained primarily from death certificates (Moulin *et al.* 2000, Wild *et al.* 2000, Moulin *et al.* 1998, Moulin *et al.* 1993). For lung cancer

results and cancers with similarly low survival rates (lung and esophageal cancer 5-year survival rates, 16.8% and 17.8%, respectively), mortality data adequately reflect incidence.

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

### Case-control studies

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

**Table C-2b. Information bias - exposure assessment and disease endpoints in human studies of cobalt**

Study	Exposure misclassification	Outcome misclassification
Tüchsen <i>et al.</i> 1996	<p>Rating: ++, Direction ↓</p> <p>Rationale: Exposure designated by employment in a department considered to include exposure to cobalt or not; calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level or duration</p>	<p>Rating: +++</p> <p>Rationale; Incident cases of lung cancer obtained through linkage with the Danish Cancer Registry</p>
Mur <i>et al.</i> 1987 Moulin <i>et al.</i> 1993	<p>Rating: ++, ↓</p> <p>Rationale: Exposure was assigned based on job location in various workshops including cobalt production, calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level or duration.</p>	<p>Rating: ++</p> <p>Some evidence that lung cancer cases may have been missed. Cause of death ascertained by physician interviews and medical records (Mur <i>et al.</i>), and only by death certificates in the re-analysis (Moulin <i>et al.</i>), resulting in one of four cases being dropped. Mortality may have missed cases certain cases with better survival (e.g., laryngeal, oral cavity and pharyngeal cancers), which were first reported by Mur <i>et al.</i>, but not by Moulin <i>et al.</i></p>

Study	Exposure misclassification	Outcome misclassification
Moulin <i>et al.</i> 1998	<p><i>Rating:</i> ++ to +++, ↓</p> <p><i>Rationale:</i> JEM based on expert judgment and limited data from records. Intensity scores (0-9), and frequency of work time exposed (&lt;10%, 10-50%, &gt;50%). JEM exposure scores for cobalt validated but did not include cobalt powder production areas.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Mortality data are adequate for lung cancer, which has a low survival rate.</p>
Wild <i>et al.</i> 2000	<p><i>Rating:</i> ++ to +++, ↓</p> <p><i>Rationale:</i> Similar JEM as Moulin 1998. As the focus of the study was on hard metals, exposures within other workshops (i.e., cobalt powder production) were assessed in less detail or were not precise as those for cobalt-tungsten carbide.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Mortality data are adequate for lung cancer, which has a low survival rate.</p>
Moulin <i>et al.</i> 2000	<p><i>Rating:</i> ++, ↓</p> <p><i>Rationale:</i> JEM for specific job periods based on expert's subjective quantification which was based on interviews with former and current workers in each workplace and on measurements in other French factories and published results from the literature. No airborne exposure level measurements used to validate these judgments and little information on past exposures. Categories of exposure were based on frequency, intensity, duration, and probability.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Mortality data, which is adequate for lung cancer that has a low survival rate</p>
Grimsrud <i>et al.</i> 2005	<p><i>Rating:</i> +++, ↓</p> <p><i>Rationale:</i> Quantitative JEM developed for nickel analysis using time- and department-specific exposure estimates plus a cobalt surrogate intensity measurement based on estimated levels of time- and department-specific periods from 3,500 personal samples of cobalt air concentrations from the breathing zone. Low level of concern for non-differential exposure misclassification as monitoring did not begin until 1973 and personnel files carried some degree of uncertainty concerning the earlier decades.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Incident cases of lung cancer obtained through linkage with the Norwegian Cancer Registry.</p>
Rogers <i>et al.</i> 1993	<p><i>Rating:</i> +; direction not known</p> <p><i>Rationale:</i> Unlike other elements investigated, cobalt levels in food measured by a food frequency questionnaire were not available from USDA, Window of exposure implied by a single measurement of cobalt in nails (12-18 months exposure) for cancer outcomes</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Cases identified through the local SEER cancer registry; diagnosis based on a positive histological finding or a positive cytology with follow-up to the attending physician to confirm the diagnosis. Potential controls with a history of any cancer were excluded.</p>

Study	Exposure misclassification	Outcome misclassification
O'Rorke <i>et al.</i> 2012	<p>may not be valid for induction of esophageal cancer. Single sample of toenails shown to have low to intermediate reproducibility for cobalt (Garland <i>et al.</i> 1993).</p> <p><i>Rating:</i> +; direction not known</p> <p><i>Rationale:</i> Window of exposure implied by a single measurement of cobalt in nails (12-18 months exposure) for cancer outcomes may not be valid for cancer induction of esophageal cancer or Barrett's esophagus; single sample of toenails shown to have low to intermediate reproducibility for cobalt (Garland <i>et al.</i> 1993).</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Esophageal adenocarcinoma cases had a histologic confirmation of adenocarcinoma within the esophagus, and excluded <i>in situ</i> cancers. Available clinical and histologic records (surgical and radiological reports) were reviewed by 3 authors and a pathologist to confirm location of the tumor in the esophagus. Potential controls had no history of esophageal or any gastrointestinal cancer or Barrett's esophagus.</p>

### C.2.5 Selective reporting and analysis bias in human studies of cobalt

#### **Cohort and nested case-control studies of cobalt**

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

The analysis of the nickel refinery workers (Grimsrud *et al.* 2005) is the strongest in terms of its methods, assumptions, and statistical analysis, using categorical and continuous variables were reported and methods of model fitting described. The nested case-control analyses of stainless-steel workers (Moulin *et al.* 2000) and hard-metal workers (Moulin *et al.* 1998) were also considered adequate and used appropriate models to evaluate exposure-response relationships.

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

#### **Case-control studies**

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

Concerns with analysis bias were low/minimal in both of these studies, although O'Rorke *et al.* (2012) provided somewhat more detail about the assumptions and methods of their analyses. The O'Rorke *et al.* study, after log transforming the toenail element concentrations, used a backwards

elimination approach using multivariate logistic regression investigating the association between tertiles of toenail trace element concentrations and the risk of esophageal cancer. Rogers *et al.* (1993) reported a categorical analysis using unconditional logistic regression to calculate ORs as estimates of the relative risk for each cancer, adjusting for the effects of potentially confounding factors. The primary table in the Rogers *et al.* study combined cases and controls, so it was not possible to ascertain the distribution of cases and controls across tertiles of cobalt.

**Table C-2c. Selective reporting and analysis bias in human studies of cobalt**

<b>Study</b>	<b>Selective reporting</b>	<b>Analysis</b>
Tüchsen <i>et al.</i> 1996	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: ++</i> <i>Rationale:</i> SMR external analysis only conducted; no internal analyses conducted to account for potential confounding, or induction period. No exposure-response analyses.
Mur <i>et al.</i> 1987 Moulin <i>et al.</i> 1993	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: +++</i> <i>Rationale:</i> Conducted cobalt-specific external and internal analyses, and did not report exposure-response analyses or particular methods for analysis of the data (Mur); Moulin restricted analyses to French-born workers exposed to cobalt exclusively, to minimize bias from selection or confounding. Unclear whether adjustments for censoring or recent exposures (lagging) were considered to address the large number of electrochemical workers who worked fewer than 10 years, which would address potential bias from inclusion of short-term workers with potentially lower exposures.
Moulin <i>et al.</i> 1998	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: +++</i> <i>Rationale:</i> Conducted internal cobalt analysis with trend analyses for exposure to cobalt alone by levels of intensity, duration, and cumulative (weighted or unweighted) doses using a fit of the ranks of the recoded variables as a test for trend.
Wild <i>et al.</i> 2000	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: ++</i> <i>Rationale:</i> Conducted external SMR analysis only for cobalt alone with little documentation of what the models included
Moulin <i>et al.</i> 2000a	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: +++</i> <i>Rationale:</i> Methods, assumptions, and statistical analysis are described in detail. Analyses using categorical and continuous variables were reported and methods of model fitting described
Grimsrud <i>et al.</i> 2005	<i>Rating: +++</i> <i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	<i>Rating: +++</i> <i>Rationale:</i> Methods, assumptions, and statistical analysis are described in detail. Analyses using categorical and continuous variables were reported and methods of model

Study	Selective reporting	Analysis
		fitting described; and an indicator variable was included to denote first employment prior to 1930 when exposure assessments were not as reliable
Rogers <i>et al.</i> 1993	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Categorical analyses using unconditional logistic regression to calculate ORs as estimates of the relative risk of each cancer, adjusting for the effects of potentially confounding factors.</p>
O'Rorke <i>et al.</i> 2012	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.</p>	<p><i>Rating:</i> +++</p> <p><i>Rationale:</i> Backwards elimination approach using multivariate logistic regression investigating the association between tertiles of toenail trace element concentrations (log transformed) and the risk of esophageal cancer</p>

### C.2.6 Study sensitivity, quality and utility of cohort and nested case-control studies

#### **Cohort and nested case-control studies**

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

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

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

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

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

### **Case-control studies**

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

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

**This Page Intentionally Left Blank**

## Appendix D: Cancer Studies in Experimental Animals

### D.1 Study quality

#### D.1.1 Evaluation methods

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

#### D.1.2 Study quality and sensitivity questions and responses

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

##### Population (selection of study animals)

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

##### Quality of the exposure

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

**Quality of the endpoint assessment**

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

**Potential for confounding**

- Are there concerns about the potential for confounding?

**Analysis and reporting**

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

**Sensitivity**

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

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

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

**D.2 Overall assessment of study utility**

An overall assessment of study utility is based on consideration of both the potential for bias (i.e., limitations) and consideration of study sensitivity, and the studies are broadly grouped into the four categories below. Studies having critical concerns for important issues will generally be considered to be inadequate to inform the evaluation. It should also be noted that some concerns about a study element (such as inadequate observation and/or exposure period and statistical power) would decrease the sensitivity of a study to detect an effect; however, if despite these

limitations positive findings were described, these studies would inform a cancer assessment. Some studies, such as co-carcinogen studies, have less utility for determining whether a substance is a cancer hazard but may provide utility regarding mechanism of action or other issues and thus utility would be rated based on the purpose of the study.

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

### **D.3 Study quality assessment and study quality tables: Carcinogenicity studies**

The following tables contain the study quality assessment (rating and rationale for the rating) for each study. The studies are organized by study type, and then by metal type or compound, followed by route of exposure. For summary tables of study quality across all studies, see Tables 5-2 (carcinogenicity) and D-3 (co-carcinogenicity).

Table D-1a. NTP 2014b (rats): Cobalt metal/powder; Inhalation

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	Yes Historical controls were reported.
<b>Randomization</b>	+++ Random allocation was done.
<b>Exposure</b>	
<b>Chemical purity</b>	+++ The cobalt was 98% pure and tested for stability.
<b>Dosing regimen</b>	+++ Dose levels were based on the 3-month studies, and three dose levels were tested.
<b>Survival</b>	++ A significant decrease in survival of females and significant decrease in body weight of both sexes was reported.
<b>Pathology</b>	+++ Full necropsies were performed and a quality assurance program was in place to verify the histopathological evaluations.
<b>Confounding</b>	+++ Little to no sources of confounding. Animal husbandry and disease surveillance were well conducted and chemical purity was tested by a third party.
<b>Reporting and analysis</b>	+++ Full details were reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic rats were used.
<b>Statistical power</b>	+++ Large number of animals used.
<b>Study duration</b>	+++ Near lifespan duration of 2 years was used. Although survival rates were low in both control and exposed groups at the end of the study, survival did not decline after ~ 80 weeks and there was $\geq 90\%$ probability of survival at 75 weeks. Thus, there is minimal concern that the low survival rates at the end of the study limited the sensitivity to observed treatment-related cancer effects.

**Overall utility: High**

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

Table D-1b. NTP 2014b (mice): Cobalt metal/powder; Inhalation

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

**Overall utility: High**

A well-designed study in all factors, but with a significant decrease in survival of male mice.

Table D-1c. Hansen 2006: Cobalt metal/powder; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Untreated controls were not included but PVC particles, which are assumed to be inert, were used.
<b>Historical data</b>	No No historical controls were available.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information on the chemical purity was reported, only information on the particle size of the bulk metal and the surface to mass ratios of the bulk metal and nanoparticles.
<b>Dosing regimen</b>	++ Only a single dose and single dose level was given, but the dose level was not reported. Particles were reported to cover a specific area of tissue; continuous exposure to cobalt nanoparticles.
<b>Survival</b>	+++ Survival was lower than PVC controls after 8 months, but it was due to moribund tumor growth.
<b>Pathology</b>	+++ Complete necropsies were performed.
<b>Confounding</b>	++ Details of animal husbandry were not reported and neither was chemical purity; however, it was stated that animals were looked after in accordance with European standard requirements and that animals were observed daily for clinical abnormalities. The negative controls underwent the same procedures of implanting particles.
<b>Reporting and analysis</b>	++ Neither the chemical purity, dose level, age of rats, nor statistical analysis were reported.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only male rats were tested, so sex differences can't be determined.
<b>Statistical power</b>	+ Few animals per group were tested. Two time points were used to sacrifice animals, which reduced the effective number of animals at each sacrifice and for each reported incidence, thus lowering the statistical power of each individual time point.

Study utility domain Question	Rating Rationale
Study duration	+ Treated animals were sacrificed at 6 and 8 months; animal sacrificed at 8 rather than 12 months because of tumor growth from exposure to cobalt nanoparticles. Controls were sacrificed at 6 and 12 months. Duration may not be adequate for evaluating cobalt metal.

**Overall utility: Moderate**

The longest duration of observation was 8 months (due to tumors induced by cobalt nanoparticles) and two forms of cobalt metal were tested in the same individual rat, bulk metal particles and nanoparticles. Duration may not be adequate for evaluating cobalt metal. Complete necropsies were performed. Inert polyvinyl chloride particles were used as a negative control. Only a small number of males were tested with a single dose level, though dose was never fully reported. Poor reporting of chemical purity and animal husbandry.

Table D-1d. Jasmin and Riopelle 1976: Cobalt sulfide or cobalt metal; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent vehicle controls were used.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ The chemical purity was reported as "reagent grade".
<b>Dosing regimen</b>	+ Only a single dose was given at one dose level without a reported basis for that level. Dose was lower than that used in other injection studies.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	++ The level of necropsy was not full: in addition to the kidney, study looked for metastases in the abdomen and thorax, but not the entire body.
<b>Confounding</b>	++ No information about animal husbandry, including disease surveillance, was reported and chemical purity was only reported as "reagent grade."
<b>Reporting and analysis</b>	++ Details were not reported for animal husbandry or disease surveillance and chemical purity reported was limited as it was reported as "reagent grade". Survival information was not reported and number of animals at sacrifice is unclear.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only non-transgenic female rats were tested.
<b>Statistical power</b>	++ A moderate number of animals per group were used and survival was not reported.
<b>Study duration</b>	+ 12 months.

**Overall utility: Low**

A moderate number of rats per group was used; however, sensitivity was limited by short observation period, use of only a single dose level, which was lower than that used in other studies and testing in only females. No information on animal husbandry, including disease surveillance; chemical purity or stability, and number of animals at sacrifice were poorly

reported. Full necropsies were not performed, though the abdominal and thoracic cavities were examined.

**Table D-1e. Heath and Daniel 1962: Cobalt metal/powder; Injection**

<b>Study utility domain</b>	<b>Rating</b>
<b>Question</b>	<b>Rationale</b>
<b>Selection of animals</b>	
<b>Controls</b>	+ There was no concurrent control, but there was a historical control from Heath 1954, which was cited in the paper as the reason for not having a concurrent control.
<b>Historical data</b>	Yes (limited) Controls from an earlier studies were used in place of concurrent controls.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ Only stated as "spectroscopically pure."
<b>Dosing regimen</b>	+ Only one dose was given at one dose level and the number of injections was not reported but assumed to be single injection. Rationale for dose not provided.
<b>Survival</b>	NR Unable to determine if there were treatment-related survival effects since survival of controls from the 1956 study was not reported. Survival in exposed group was low, with 8 out of 20 rats dying only days after the injection; however, survival was good after initial deaths; deaths may have been due to technical difficulties with the intrapleural injections.
<b>Pathology</b>	++ Only looked at the injection site; full necropsies were not reported.
<b>Confounding</b>	++ No information regarding animal husbandry conditions and disease surveillance were reported, but chemical purity was reported as "spectroscopically" pure.
<b>Reporting and analysis</b>	+ No animal husbandry or necropsy methods were reported and chemical purity was reported only as "spectroscopically pure."
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only non-transgenic female rats were tested.
<b>Statistical power</b>	+ Small numbers of animals. Survival was low due to deaths caused within the first 3 days from difficulties with the intrapleural injection.
<b>Study duration</b>	+++ 28 months.

**Overall utility: Low**

The duration of observation was over two years long, but exposure was only a single dose. There was no concurrent control, but there was a historical control. No statistics were done, a low number of animals was used, and necropsies were not done; only skin tumors were histologically examined. No information was reported about chemical purity or stability, or animal husbandry.

Table D-1f. Heath 1956: Cobalt metal/powder; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	++ Untreated controls were used in the first series of experiments, but not in the second series; however, untreated controls from the first series could be used as historical or non-concurrent controls for the second series of studies. The survival of untreated controls was not reported.
<b>Historical data</b>	Yes (limited) The untreated controls from the first series of studies was used as a historical control for the second series of studies.
<b>Randomization</b>	NR No information about randomization was reported.
<b>Exposure</b>	
<b>Chemical purity</b>	++ Purity was reported as "spectroscopically pure."
<b>Dosing regimen</b>	+ Only one dose level was used with no explanation as to why that level was chosen. The duration of treatment was not reported, but was assumed to be a single dose.
<b>Survival</b>	NR No data reported for untreated controls; two of 10 males, 4 of 10 females (Series I) and 0/10 females (Series II) without tumors died before sacrificed.
<b>Pathology</b>	++ No methods reported, but it was stated that no other tumors besides local tumors were found, though a metastasis to the lymph nodes were found, suggesting necropsies were performed, but the extent of the necropsies is not known.
<b>Confounding</b>	++ No information regarding animal husbandry conditions and disease surveillance were reported, but chemical purity was reported as "spectroscopically" pure.
<b>Reporting and analysis</b>	+ No reporting of animal husbandry, disease surveillance, randomization, treatment duration, necropsy methods, or survival for untreated controls.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Both male and female rats were used in the first series of studies, but only females were tested in the second series.
<b>Statistical power</b>	+ Small numbers of animals were tested.
<b>Study duration</b>	+++ Duration of observation was near the animals' expected lifespan.

**Overall utility: Low**

Observation duration was sufficient and both sexes were tested. However, there was no reporting of animal husbandry, necropsy methods, or chemical stability. Only one dose level was tested, and a small number of male and female rats was used. Full necropsies were not reported to have been performed.

Table D-1g. NTP 1998 (rats): Cobalt sulfate; Inhalation

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	Yes Historical controls were reported.
<b>Randomization</b>	+++ Random allocation was done.
<b>Exposure</b>	
<b>Chemical purity</b>	+++ The cobalt was 99% pure and tested for stability.
<b>Dosing regimen</b>	+++ Dose levels were based on 16-day and 13-week studies, and three dose levels were tested.
<b>Survival</b>	+++ Survival was high and not affected by cobalt exposure.
<b>Pathology</b>	+++ Full necropsies were performed and a quality assurance program was in place to verify the histopathological evaluations.
<b>Confounding</b>	+++ Little to no sources of confounding. Animal husbandry and disease surveillance were well conducted and chemical purity was tested by a third party.
<b>Reporting and analysis</b>	+++ Full details were reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic rats were used.
<b>Statistical power</b>	+++ Large number of animals used.
<b>Study duration</b>	+++ Near lifespan duration of 2 years was used.

**Overall utility: High**

A well-designed study in all factors and survival was similar to controls.

Table D-1h. NTP 1998 (mice): Cobalt sulfate; Inhalation

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	Yes Historical controls were reported.
<b>Randomization</b>	+++ Random allocation was done.
<b>Exposure</b>	
<b>Chemical purity</b>	+++ The cobalt was 99% pure and tested for stability.
<b>Dosing regimen</b>	+++ Dose levels were based on 16-day and 13-week studies, and three dose levels were tested.
<b>Survival</b>	+++ Survival was high and not affected by cobalt exposure.
<b>Pathology</b>	+++ Full necropsies were performed and a quality assurance program is in place to verify the histopathological evaluations.
<b>Confounding</b>	+++ Little to no sources of confounding. Animal husbandry and disease surveillance were well-conducted and chemical purity was tested by a third party.
<b>Reporting and analysis</b>	+++ Full details were reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic mice were tested.
<b>Statistical power</b>	+++ Large number of animals used.
<b>Study duration</b>	+++ Near lifespan duration of 2 years was used.

**Overall utility: High**

A well-designed study in all factors and survival was similar to controls.

Table D-1i. Shabaan 1977: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	++ Untreated controls were not reported for the 8-month study, but were for the 12-month study and could be used for the 8-month study as a non-concurrent control.
<b>Historical data</b>	Yes (limited) There were untreated controls that were sacrificed at the 12-month time point that could serve as non-concurrent or historical controls for the 8-month study. Fewer neoplasms would be expected at 8 months than at 12 months and no neoplasms were found at 12. months, so using 12month untreated controls seems justified.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	+ Only one dose tested and no basis given for choosing that level. Animals were treated 19 days. Treated animals developed persistent hyperlipaemia
<b>Survival</b>	++ Mortality of exposed rats was high compared to controls; 11/20 survived at 12 months and 16/20 survived at 8 months
<b>Pathology</b>	+ Only survivors were necropsied; those that died before 8 months or 12 months were not examined.
<b>Confounding</b>	
	+ No information about chemical purity or disease surveillance was reported and animal husbandry was poorly reported.
<b>Reporting and analysis</b>	
	+ Untreated controls were not clearly reported, neither was animal husbandry. Chemical purity and the rationale for the single dose level used was not reported. Statistics were not calculated. The route of exposure was reported as s.c. injection, but the tumors developed in sites outside of the reported injection sites (central abdominal wall) and the authors didn't differentiate which sites were injection sites and which were non-injection sites.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only male non-transgenic rats were tested.
<b>Statistical power</b>	++ A reasonable number of animals tested, but there was a significant decrease in survival of the exposed rats.

Study utility domain	Rating
Question	Rationale
<b>Study duration</b>	+ 8 months (experiment 1) or 12 months (experiment 2). The 8-month study would not normally meet inclusion criteria, but since tumors were induced it was included.

**Overall utility: Low**

Exposure was only for 19 days and animals that did not survive to the end of the experiments were not necropsied and there was a significant decrease in survival of exposed rats, so the studies may underestimate the true results. Only a single injection was given to male rats; females were not tested. The tumors and injection sites were not clearly reported and tumor sites were not designated as injection site or non-injection sites

**Table D-1j. Steinhoff and Mohr 1991: Cobalt oxide; Intratracheal**

<b>Study utility domain</b>	<b>Rating</b>
<b>Question</b>	<b>Rationale</b>
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	No No historical controls were available.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Dose levels were randomly verified by gravimetric measurements taken several times during the study.
<b>Dosing regimen</b>	++ Two dose levels were given every 2 or 4 weeks for close to 2 years (39 doses).
<b>Survival</b>	++ Data are not provided, but it was stated in the results that there were "no appreciable differences."
<b>Pathology</b>	++ Only the high-dose group was fully necropsied. Histological examinations were done on organs with gross lesions suspected of having tumors and all respiratory tracts in the low-dose group or controls.
<b>Confounding</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
<b>Reporting and analysis</b>	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic rats were used.
<b>Statistical power</b>	+++ Large number of animals were used; survival was reported as similar to controls.
<b>Study duration</b>	+++ Observation duration was for lifespan.

**Overall utility: Moderate**

Two dose levels were tested in a high number of both sexes of rats for two years, with treatment and observations for the lifespan without any significant difference in survival compared to untreated controls. However, only the high-dose group received full necropsies. Details of the chemical and animal husbandry were not reported.

Table D-1k. Steinhoff and Mohr 1991: Cobalt oxide; Injection (IP)

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	No No historical controls were available.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure".
<b>Dosing regimen</b>	+ One dose level was given every 2 months for 6 months with no explanation as to what the dose level was based on.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	++ All organs and tissues suspected of having tumors and all tumors in the injection region were evaluated by histological examination.
<b>Confounding</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
<b>Reporting and analysis</b>	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic rats were used.
<b>Statistical power</b>	+ There were only 10 animals per sex per group tested and survival was not reported.
<b>Study duration</b>	+++ Observation duration was for lifespan.

**Overall utility: Moderate**

Both sexes of rats were tested with a long duration of observation. However, reporting of animal husbandry, including disease surveillance and chemical purity was poor and survival was not reported. There was a small number of animals per group, only one dose level was tested,

exposure was for less than one year and histological examination was only done on organs with gross tumors, all of which would limit the sensitivity to detect an effect.

Table D-11. Steinhoff and Mohr 1991: Cobalt oxide; Injection (SC)

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls were used.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
<b>Dosing regimen</b>	++ Only a single dose level was tested, but it was given at two intensities, either weekly or daily (1/5 the level) for 2 years. There was no reported basis for that dose level.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	++ All organs and tissues suspected of having tumors and all tumors in the injection region were evaluated by histological examination.
<b>Confounding</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
<b>Reporting and analysis</b>	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only male rats were tested.
<b>Statistical power</b>	+ There were only 10 animals per group and survival was not reported.
<b>Study duration</b>	+++ Observation duration was for lifespan.

**Overall utility: Moderate**

Duration of exposure and observation were sufficient; one dose level was tested, but it was tested at two intensity levels. However, few animals per group were used and only included males; histological exam was only done on organs with gross tumors, which would limit the sensitivity

to detect an effect. Reporting of animal husbandry, including disease surveillance, and chemical purity were poor and no survival data was provided.

**Table D-1m. Gilman and Ruckerbauer 1962 (rats): Cobalt oxide; Injection**

<b>Study utility domain Question</b>	<b>Rating Rationale</b>
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls (vehicle – aqueous suspension of penicillin G procaine) were used.
<b>Historical data</b>	No No historical controls were available..
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was "washed" to remove water soluble impurities and was < 5 µm particle size. Cobalt was administered in an aqueous suspension of penicillin G procaine.
<b>Dosing regimen</b>	+ Only a single dose given at one dose level, but preliminary tests using unwashed unwashed particles, which contained an unknown water-soluble toxin that had killed many mice. Rats tolerated dose.
<b>Survival</b>	++ No survival-related effects at 90 days, only time period reported.
<b>Pathology</b>	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
<b>Confounding</b>	+ Animals and bedding periodically dusted with rotenone powder; not clear if the same rats were used from the preliminary experiment using unwashed cobalt.
<b>Reporting and analysis</b>	+ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis. Tumor incidences were reported as both sexes combined.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic rats were used.
<b>Statistical power</b>	+ Only 10 animals were tested and they were reported as both sexes combined.
<b>Study duration</b>	++ 1.3 years.

**Overall utility: Low**

The duration of observation was sufficient and both sexes were tested. However, only a single dose was given at one dose level with results reported as both sexes combined; few animals per group were tested. Reporting was poor and lacked information on chemical purity or stability, animal husbandry, and necropsy methods. Animal bedding was periodically dusted with rotenone powder and half of the exposed group had been administered unwashed cobalt, which was known to contain additional chemicals.

Table D-1n. Gilman and Ruckerbauer 1962 (mice): Cobalt oxide; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent controls (vehicle – aqueous suspension of penicillin G procaine) were used.
<b>Historical data</b>	No No historical controls were available..
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was "washed" to remove water soluble impurities and was < 5 µm particle size. Cobalt was administered in an aqueous suspension in penicillin G procaine.
<b>Dosing regimen</b>	+ Only a single dose given at one dose level, but preliminary tests using unwashed particles, which contained an unknown water-soluble toxin that had killed many animals, was reported. Half the animals died in this study between 2 <sup>nd</sup> and 6 <sup>th</sup> day.
<b>Survival</b>	++ No treatment-related survival effects at 90 days, only time period reported.
<b>Pathology</b>	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
<b>Confounding</b>	+ Animals and bedding periodically dusted with rotenone powder. Half of the animals were given washed particles and the other half were survivors of animals given unwashed particles in a preliminary experiment, which contained an unknown water-soluble toxin that had killed many animals.
<b>Reporting and analysis</b>	++ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only non-transgenic female mice were tested.
<b>Statistical power</b>	++ A large number of animals were tested; however, survival was only reported for 90 days.
<b>Study duration</b>	+++ 2 years.

**Overall utility: Low**

The duration of observation and the numbers of animals per group were sufficient. Survival was only reported for 90 days. However, only a single dose was given (without rationale for level), to females only; half of them received unwashed cobalt, which was known to contain other chemicals. Reporting was poor and lacked chemical purity or stability, animal husbandry, and necropsy methods, though metastasis was reported suggesting necropsies were performed. Bedding was periodically dusted with rotenone powder.

Table D-1o. Wehner 1977: Cobalt oxide; Inhalation

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Concurrent untreated controls (sham-smoked) were used.
<b>Historical data</b>	No No historical controls were available.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ There was no information on chemical purity or stability of the cobalt.
<b>Dosing regimen</b>	++ Single dose level with no justification for choosing that level, but administered for life.
<b>Survival</b>	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls although survival was low in both groups.
<b>Pathology</b>	+++ Detailed necropsies were performed.
<b>Confounding</b>	++ Animal conditions were partly reported, but chemical purity and disease surveillance were not reported.
<b>Reporting and analysis</b>	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as "carcinoma" or "polyp" without saying what tissue they originated from is meaningless.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only male hamsters were tested. Hamsters are less sensitive for evaluating lung tumors.
<b>Statistical power</b>	+ A large number of animals was used; however, statistical power was reduced by poor survival (fewer than 10 animals were alive at 18 months) in both cobalt-exposed and the corresponding control animals (IARC 1999).
<b>Study duration</b>	+++ Duration of treatment and observation was for the animals' lifespans.

**Overall utility: Moderate**

Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported.

Only a single dose level was tested (with no justification for choosing that dose level) in a large number of male hamsters. There was relatively poor survival in both exposed and control groups.

## Study quality tables: Co-carcinogen studies

Table D-2a. Finogenova 1973: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	++ Two dose levels (10-fold apart from each other) of cobalt were tested and were given twice a week for only 8 weeks, but this is a co-carcinogen study.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	++ Only local skin tumors were reported and described as to histologic grade. Full necropsies were not conducted. Because it's a co-carcinogen study and tumors from the known carcinogen are what is of interest and the types of induced tumors are already known or expected, lack of a full necropsy is less critical than for a carcinogenicity study.
<b>Confounding</b>	NR No information about chemical purity or animal husbandry, including disease surveillance, was reported.
<b>Reporting and analysis</b>	+ Very poor reporting. There is no chemical purity, stability, or homogeneity reported and no information on animal husbandry disease surveillance, duration of observation, extent of necropsy, survival, or tumor incidence (only tumor latency was reported).
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only females of non-transgenic mice were tested.
<b>Statistical power</b>	+++ Large number of animals used, but survival was not reported.
<b>Study duration</b>	++ The duration of observation was unreported, but was at least 24 weeks, however, this is a co-carcinogen study.

---

Study utility domain Question	Rating Rationale
Assay utility	+ Co-Carcinogen study

---

**Overall utility: Low**

All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. A large number of female animals per group were given two dose levels. However, there was very poor reporting. Incidence was not reported, only tumor latency and onset were reported. Survival, chemical purity or stability, animal husbandry, duration of observation, and extent of necropsy were not reported.

Table D-2b. Kasirsky 1965: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	++ A single dose level of the known carcinogen and four dose levels of cobalt chloride were given for about 10 weeks, though the exact duration of exposure was not clearly reported.
<b>Survival</b>	+ Survival was only reported for three dose levels of cobalt chloride in the first trial, but not in any groups of the second trial or the carcinogen alone control of the first trial. The high cobalt chloride dose level of the first trial reported conflicting survival between Table 1 and the summary at the end of the paper.
<b>Pathology</b>	+ No necropsies were performed, just measurement of tumor size by external examination and histological exam of excised tumors.
<b>Confounding</b>	++ Very few details were reported about animal husbandry and no information was reported about chemical purity.
<b>Reporting and analysis</b>	++ Results were not reported per sex and the duration was not clearly reported. Survival for all groups, chemical purity, and animal husbandry were not reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic mice were tested.
<b>Statistical power</b>	++ Sufficient number of animals of each sex, but results were not reported as per sex. Survival was not clearly reported.
<b>Study duration</b>	++ It was not clearly reported, but is thought to have been at least 72 days, though this is a co-carcinogen study.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Neither duration, survival, chemical purity, stability or animal husbandry were clearly reported. Results were not reported per sex. A full necropsy was not conducted, so this study is only relevant to skin tumors induced by the carcinogen.

Table D-2c. O'Hara 1971: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ There was no untreated group and no cobalt alone group, but it's similar to a co-carcinogen study and did have a known carcinogen alone group.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	Inadequate Cobalt was administered after tumors had already started to develop; this is more like a tumor treatment study. Only two very similar dose levels were tested: 50 and 60 mg/kg.
<b>Survival</b>	+++ The high dose level caused an increase in mortality, however low survival was caused by tumors. Statistical analysis was not done on survival.
<b>Pathology</b>	++ No full necropsies were reported; only local skin tumors were reported.
<b>Confounding</b>	
	++ Very few details reported about animal husbandry and no information reported about chemical characteristics.
<b>Reporting and analysis</b>	++ Chemical purity or stability or disease surveillance methods were not reported and animal husbandry conditions were poorly reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic mice were tested.
<b>Statistical power</b>	+++ The high-dose level group had a decrease in survival, but statistics were not calculated for survival and the low survival was caused by tumors.
<b>Study duration</b>	++ 17 weeks, but this is a co-carcinogen study and neoplasms were induced before cobalt exposure started.
<b>Assay utility</b>	Inadequate Co-carcinogen study

**Overall utility: Inadequate**

This study has little utility for evaluation because the cobalt was not administered until after tumors had developed. No necropsies were performed; it only looked at local tumors. Only two, closely spaced dose levels were tested. A good number of mice per group was used. Chemical purity or stability and animal husbandry or disease surveillance were not reported. All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity.

Table D-2d. Zeller 1975: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ A cobalt chloride alone group was said to have been tested, but no results of that group were reported. The reported control was the known carcinogen alone, which is appropriate for a co-carcinogen study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	+ Only one dose level of cobalt, given for 43 weeks, was tested with no rationale for choosing that dose level.
<b>Survival</b>	+++ There was no significant effect on survival from cobalt.
<b>Pathology</b>	++ Pathology procedures not described but histopathological evaluations were done on the respiratory tract and the liver.
<b>Confounding</b>	
	NR No information on animal husbandry conditions, disease surveillance, or chemical purity were reported.
<b>Reporting and analysis</b>	+ Poor reporting of chemical purity or stability, animal husbandry, or necropsy methods. Tumor incidences were reported as both sexes combined, making it impossible to examine sex differences.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Both sexes of non-transgenic rats were tested; however, tumor incidences were reported as both sexes combined.
<b>Statistical power</b>	+ Only 12 animals per group per sex were tested, but tumor incidences were reported as both sexes combined.
<b>Study duration</b>	+++ Duration of treatment was 43 weeks and duration of observation was for the animals' lifespan.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. The study had a long duration of observation. However, poor reporting of chemical purity/stability, animal husbandry, necropsy methods, or necropsy with histological descriptions only of the respiratory tract and liver. Only one dose level was tested, without a reported rationale, on a small number of males and females, with tumor incidences reported as both sexes combined.

Table D-2e. Orzechowski 1964: Sodium cobaltinitrite; Injection

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	+++ Six dose levels were given over 72 days and maximum dose level was based on toxicity in preliminary studies.
<b>Survival</b>	+++ Survival was high and similar to known carcinogen only controls.
<b>Pathology</b>	+ Necropsies were not done, only histological examination of tumors were done.
<b>Confounding</b>	++ Very few details reported about animal husbandry and no information was reported about chemical purity.
<b>Reporting and analysis</b>	+ No chemical purity or animal husbandry were reported. Tumor incidences were reported as both sexes combined.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Both sexes of non-transgenic mice were used, though tumor incidence were only reported as both sexes combined.
<b>Statistical power</b>	+++ Large number of animals per group and experiments were conducted in triplicate and survival was high and similar to controls.
<b>Study duration</b>	++ A very short duration of 75 days was used, but it's a co-carcinogenicity study and tumors were induced.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Tumor incidences were reported as both

sexes combined. No information on chemical purity or stability or animal husbandry were reported. Six dose levels were tested, which were based on preliminary studies and a high number of animals per group was used, with experiments conducted in triplicate. Necropsies were not performed; histological examination was conducted only on tumors.

Table D-2f. Thompson 1965: Sodium cobaltinitrite; Drinking water

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	NR No information about chemical purity, stability, or homogeneity was provided.
<b>Dosing regimen</b>	+++ Three dose levels were tested. The duration of treatment was not reported, but assumed to be about 11 weeks, but this is a co-carcinogen study.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	+ No full necropsies were performed, just histology of the tumors and hematology measurements.
<b>Confounding</b>	++ Very little information about animal husbandry and no information about chemical purity or disease surveillance.
<b>Reporting and analysis</b>	++ Nothing was reported for chemical purity or stability, disease surveillance, survival, or duration of treatment. Dosing regimen and duration of observation were not clearly reported.
<b>Sensitivity</b>	
<b>Animal model</b>	+++ Both sexes of non-transgenic mice were tested.
<b>Statistical power</b>	+++ Large number of animals per group, but survival was not reported.
<b>Study duration</b>	++ Not clearly reported, assumed to be about 11 weeks, but this is a co-carcinogen study.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Both sexes of mice were tested with

three dose levels. Nothing was reported for chemical purity or stability, survival, duration of treatment, and animal husbandry and the dosing regimen were not clearly reported. Full necropsies were not done; histology was only performed on tumors.

**Table D-2g. Steinhoff and Mohr 1991: Cobalt oxide; Intratracheal**

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ No untreated control was used, but a benzo[a]pyrene-only control was included, which is consistent with a co-carcinogenicity study.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
<b>Dosing regimen</b>	+ Only a single dose level was used and given for 47 weeks for cobalt and 13 wk for benzo[a]pyrene.
<b>Survival</b>	NR No survival information was reported.
<b>Pathology</b>	++ Only organs with gross lesions and the respiratory tract were examined histologically.
<b>Confounding</b>	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
<b>Reporting and analysis</b>	++ Methods were poorly reported and lacked details about the chemical and animal husbandry.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only female rats were tested.
<b>Statistical power</b>	+++ A large number of animals were used and survival was similar to controls.
<b>Study duration</b>	++ Treatment with cobalt was for 47 weeks, benzo[a]pyrene was given for 13 wk, while observation was lifespan.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. One dose level was tested on a high number of females for almost a year, with observations for their lifespan. However, only partial necropsies were performed. Details of the chemical, animal husbandry, and survival were not reported.

Table D-2h. Wehner 1977: Cobalt oxide; Inhalation

Study utility domain Question	Rating Rationale
<b>Selection of animals</b>	
<b>Controls</b>	+++ Controls were cigarette smoke alone.
<b>Historical data</b>	No No historical controls were used.
<b>Randomization</b>	NR No information about randomization was provided.
<b>Exposure</b>	
<b>Chemical purity</b>	+ There was no information on chemical purity or stability of the cobalt and the composition of the cigarettes is complex and variable. However, information on the type of research cigarette (Kentucky IRI research cigarettes) was provided.
<b>Dosing regimen</b>	++ Single dose level with no justification for choosing that level, but administered for life.
<b>Survival</b>	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls.
<b>Pathology</b>	+++ Detailed necropsies were performed.
<b>Confounding</b>	+ Chemical purity and disease surveillance were not reported. The uncertainty of the composition of the cigarettes may contribute to confounding.
<b>Reporting and analysis</b>	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as "carcinoma" or "polyp" without saying from which tissue they originated is meaningless.
<b>Sensitivity</b>	
<b>Animal model</b>	++ Only male hamsters were tested.
<b>Statistical power</b>	+++ A large number of animals were used and survival was similar to controls.
<b>Study duration</b>	+++ Duration of treatment and observation was for the animals' lifespan.
<b>Assay utility</b>	+ Co-carcinogen study

**Overall utility: Low**

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported. Only a single dose level was tested (with no rationale for choosing that dose level).

Table D-3. Overview of experimental animal co-carcinogenicity study quality evaluations

Study	Metal	Controls	Historical data	Randomization	Purity	Dosing	Survival	Pathology	Confounding	Reporting & Analysis	Animal Model	Stats	Duration	Assay Utility	Overall utility
Finogenova 1973	Cobalt chloride	+++	No	NR	NR	++	NR	++	NR	+	++	+++	++	+	Low
Kasirsky <i>et al.</i> 1965	Cobalt chloride	+++	No	NR	NR	++	+	+	++	++	+++	++	++	+	Low
O'Hara <i>et al.</i> 1971	Cobalt chloride Sodium cobaltinitrite	+++	No	NR	NR	0	+++	++	++	++	+++	+++	++	0	Inadequate
Zeller 1975	Cobalt chloride	+++	No	NR	NR	+	+++	++	NR	+	++	+	+++	+	Low
Orzechowski <i>et al.</i> 1964	Sodium cobaltinitrite	+++	No	NR	NR	+++	+++	+	++	+	++	+++	++	+	Low
Thompson <i>et al.</i> 1965	Sodium cobaltinitrite	+++	No	NR	NR	+++	NR	+	++	++	+++	+++	++	+	Low
Steinhoff and Mohr 1991	Cobalt oxide	+++	No	NR	++	+	NR	++	++	++	++	+++	++	+	Low
Wehner <i>et al.</i> 1977	Cobalt oxide	+++	No	NR	+	++	+++	+++	+	+	++	+++	+++	+	Low

+++ = high quality/little to no concerns, ++ = moderate quality/moderate concerns, + = low quality/high concerns, 0 = inadequate, NR = not reported.

**This Page Intentionally Left Blank**

## Appendix E: Genotoxicity and Cellular Transformation

This section describes the assessment of studies evaluating genetic and related effects from exposure to cobalt compounds, and provides the background for the discussion of genotoxicity as a possible mode of action for cobalt-induced carcinogenicity (see Section 6). The genotoxicity data summarized in Section 6.2.1 and Table 6-2 are described more fully below.

Cobalt metal and several cobalt compounds have been tested in short-term assays to evaluate mutagenicity, DNA damage, and other potential genotoxic effects. These compounds include several forms of cobalt: (1) the water-soluble salts cobalt chloride (and its hexahydrate), cobalt sulfate (and its heptahydrate), and cobalt nitrate (and its hexahydrate); (2) a water-insoluble compound, cobalt oxide; (3) an organic water-soluble compound, cobalt acetate; (4) cobalt metal and (5) the cobalt particles: cobalt sulfide(s) and cobalt nanoparticles. Most of the genotoxicity studies identified reported on tests using cobalt chloride (or its hexahydrate). The specific cobalt compound form (i.e., hydrate) tested is indicated when provided by the study authors. The oxidation state of the cobalt salts, oxide and acetate compounds in this section is +2 (cobalt(II)), unless indicated otherwise.

A discussion of the genotoxic effects of certain cobalt compounds reported for *in vitro* and *in vivo* assays are presented below and a compilation of studies is provided in tabular form for each section. An overall summary call for genetic and related effects is provided for the compounds by endpoint in Table 6-2 in Section 6.2.1; the calls are based on the integration of the evidence from an authoritative review (namely IARC 2006) and several primary studies published since the IARC review.

### E.1 *In vitro* mutagenicity and DNA-damage studies of cobalt compounds in bacteria

Cobalt metal and two water-soluble cobalt salts (cobalt chloride and its hexahydrate, cobalt sulfate heptahydrate) were tested for mutagenicity in prokaryotic (bacterial) systems (see Table E-1 for study details and sources not provided in text). Results for mutagenicity are mostly negative for cobalt compounds in bacterial tester strains; however, there were some positive results in a few studies that detect mutations involving GC base pairs, although the evidence was not entirely consistent.

Cobalt chloride was reported to be mutagenic in only some of the studies in three *Salmonella* strains (TA97, TA98, and TA1537) (Pagano and Zeiger 1992, Wong 1988) but not in other strains (TA100, TA102, TA1535, TA1538, TA2637). It was also mutagenic in one of several studies in *Escherichia coli* (Ogawa *et al.* 1999) but not *Bacillus subtilis*. Cobalt sulfate heptahydrate was positive in TA100 but not in TA98 and TA1537. A growth inhibition assay for cobalt chloride in *B. subtilis* had mixed results in two studies; however, the test that showed growth inhibition (positive results) was conducted using preincubation, which is a more sensitive assay than the standard plate incorporation assay. Cobalt metal was positive in the *Salmonella* strains in which it was tested (TA98 and TA100) but not in *E. coli*.

Positive results in a particular set of *Salmonella typhimurium* and *E. coli* bacterial tester strains can suggest very specific types of mutations. Tester strains TA100, TA102 and TA1535 are generally considered indicators of base-pair substitution. The frameshift mutation detected by *S.*

*typhimurium* strain TA98 is a disruption of a dinucleotide run of (CG)<sub>4</sub> residues, while TA100 detects base-pair mutations in a codon for proline (GGG sequence) in the *histidine G46* gene. Reverse mutations at the *trpE* ochre (TAA) codon can be identified by positive results in the *E. coli* WP2 *uvrA*/pKM101 strain. The results for these strains in the studies identified in this review are not strong; however, sequencing of the *supF* tRNA mutational reporter gene in bacteria exposed to cobalt chloride showed that both frameshift and base-pair substitution occurred at G:C base pairs (Ogawa *et al.* 1999).

Overall, the results for cobalt-induced bacterial mutagenicity are considered mostly negative without the addition of S9 and are completely negative in all assays with S9. The negative results for the assays that included the addition of S9 (and which were positive without S9) may be due to the presence of radical-scavenging enzymes in the mixture, which could eliminate a mutagenic effect; alternatively, proteins in the S9 mixture could bind the cobalt ions, rendering them ineffective as a mutagen.

Some studies reported anti-mutagenic effects. Potent anti-mutagenic effects were observed for cobalt chloride on reverse mutations induced by 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) in *Salmonella* strains TA98 and TA1538. Cobalt chloride hexahydrate showed an anti-mutagenic effect (inhibition of spontaneous mutation) when tested in *B. subtilis* (Inoue *et al.* 1981).

Table E-1. *In vitro* mutagenicity and DNA-damage studies of cobalt compounds in bacteria

Compound	Reference	LED/HID	Results		Comments and conclusions
			-S9	+S9	
<b><i>Reverse mutation/ Salmonella typhimurium/ TA100</i></b>					
Cobalt chloride	Ogawa <i>et al.</i> 1986*	NR	-		Negative -S9
Cobalt chloride hexahydrate	Tso and Fung 1981*	23,800 µg/mL	-		Negative -S9 in two studies
	Arlauskas <i>et al.</i> 1985*	NR	-		
Cobalt sulfate heptahydrate	Zeiger <i>et al.</i> 1992, NTP1998	100 µg/plate	+	(+)	Positive -S9; weak positive +S9
Cobalt metal	NTP 2014b	500 µg/plate -S9	(+)	-	Weak positive -S9; negative +S9
		7,500 µg/plate +S9			
<b><i>Reverse mutation/ Salmonella typhimurium/ TA102</i></b>					
Cobalt chloride	Wong 1988*	40 µg/mL [approx. 100 µg/plate]	-	-	Negative ±S9
<b><i>Reverse mutation/ Salmonella typhimurium/ TA1535</i></b>					
Cobalt chloride	Arlauskas <i>et al.</i> 1985*	NR	-		Negative -S9 in 2 of 2 studies; negative +S9
	Wong 1988*	40 µg/mL	-	-	
Cobalt sulfate heptahydrate	Zeiger <i>et al.</i> 1992, NTP 1998	10,000 µg/plate	-	-	Negative ±S9
<b><i>Reverse mutation/ Salmonella typhimurium/ TA97</i></b>					
Cobalt chloride	Pagano and Zeiger 1992*	13 µg/mL [approx. 32 µg/plate]	+		Positive -S9; Preincubation assay (generally more sensitive than standard plate incorporation assay)
<b><i>Reverse mutation/ Salmonella typhimurium/ TA98</i></b>					
Cobalt chloride	Arlauskas <i>et al.</i> 1985*	NR	-		Positive -S9 in 1 of 3 studies; negative +S9
	Ogawa <i>et al.</i> 1986*	NR	-		
	Wong 1988*	40 µg/mL [approx. 100 µg/plate]	+	-	
Cobalt chloride hexahydrate	Mochizuki and Kada 1982*	20 µg/mL	-		Anti-mutagenic effect on Trp-P-1-induced reverse mutations; same effect in TA1538, so independent of plasmid pKM101 (which is in TA98 but not TA1538).
Cobalt sulfate heptahydrate	Zeiger <i>et al.</i> 1992,	10,000 µg/plate	-	-	Negative ±S9

Compound	Reference	LED/HID	Results		Comments and conclusions
			-S9	+S9	
	NTP 1998				
Cobalt metal	NTP 2014b	100 µg/plate -S9 7,500 µg/plate +S9	+	-	Positive -S9 (weak effect, not well-correlated with dose); negative +S9
<b>Reverse mutation/ Salmonella typhimurium/ TA1537</b>					
Cobalt chloride	Arlauskas <i>et al.</i> 1985* Ogawa <i>et al.</i> 1986* Wong 1988*	NR 130,000 µg/plate 40 µg/mL [approx. 100 µg/plate]	- - +	-	Positive -S9 in 1 of 3 studies; negative +S9 [note: recommended maximum dose for assay is generally 5,000 to 6,000 µg/plate, depending on toxicity]
<b>Reverse mutation/ Salmonella typhimurium/ TA1538</b>					
Cobalt chloride	Arlauskas <i>et al.</i> 1985*	NR	-		Negative -S9
Cobalt chloride hexahydrate	Mochizuki and Kada 1982*	20 µg/mL	-		Anti-mutagenic effect on Trp-P-1-induced reverse mutations; same effect in both strains, independent of plasmid pKM101, which is in TA98 but not TA1538
<b>Reverse mutation/ Salmonella typhimurium/ TA2637</b>					
Cobalt chloride	Ogawa <i>et al.</i> 1986*	130,000 µg/plate	-		Negative -S9; strain detects bulky DNA adduct formation
<b>Mutation/ Escherichia coli strain WP2 uvrA/pKM101</b>					
Cobalt chloride hexahydrate	Arlauskas <i>et al.</i> 1985*	NR	-		Negative -S9
Cobalt chloride hexahydrate	Kada and Kanematsu 1978* Leitao <i>et al.</i> 1993*	20 µg/mL 50 µg/mL	- -		Negative -S9 Induced anti-mutagenic effect (inhibition of mutagenesis induced by N-methyl-N'-nitrosoguanidine) in two studies
Cobalt metal	NTP 2014b	450 µg/plate	-	-	Negative ±S9
<b>Mutation/ Escherichia coli strain SY1032/pKY241 supF tRNA locus</b>					
Cobalt chloride	Ogawa <i>et al.</i> 1999*	2.6 µg/mL	+		Positive -S9
<b>Prophage induction/ Escherichia coli</b>					
Cobalt chloride	Rossmann <i>et al.</i> 1984*	415 µg/mL [approx. 1037 µg/plate]	-		Negative -S9
<b>Reverse mutation/ Bacillus subtilis strain NIG 1125</b>					
Cobalt chloride hexahydrate	Inoue <i>et al.</i> 1981*	30 µg/mL	-		Anti-mutagenic effect (inhibition of spontaneous mutation)

Compound	Reference	LED/HID	Results		Comments and conclusions
			-S9	+S9	
<b><i>Growth inhibition/ Bacillus subtilis rec strain strains H17</i></b>					
Cobalt chloride	Nishioka 1975*	325 µg/plate	-		Positive -S9 in 1 of 2 studies; positive study used 'cold preincubation' procedure
	Kanematsu <i>et al.</i> 1980*	325 µg/plate	+		

\*As cited by IARC 2006.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weak positive, - = negative.

## E.2 Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes

Cobalt water-soluble compounds (cobalt chloride, cobalt nitrate, and cobalt sulfate) and nanoparticles were tested for mutations, DNA damage and chromosomal damage in numerous studies in non-mammalian eukaryotes. Mostly positive effects were observed in yeast, plants, insects, nematodes, and zebrafish, for genotoxic activity of a variety of cobalt compounds for the evaluated endpoints. These include mutation (cobalt chloride and cobalt nitrate hexahydrate), gene conversion (cobalt chloride), DNA damage (cobalt chloride, cobalt nitrate hexahydrate, and cobalt sulfate), chromosomal aberration (cobalt chloride and cobalt sulfate) and aneuploidy (cobalt sulfate). Recombination was reported as treatment-related in *Drosophila* studies on cobalt chloride, cobalt nitrate hexahydrate, and cobalt nanoparticles. None of these studies reported using the addition of a metabolic activation mixture (S9). The results of the genotoxicity studies of cobalt compounds tested in non-mammalian eukaryotes are described below and are summarized in Table E-2.

In fungi, cobalt chloride treatment resulted in induction of gene mutation and conversion in several assays. In the yeast *Saccharomyces cerevisiae*, cobalt chloride was at least weakly mutagenic in five of eight studies identified (Kharab and Singh 1987, 1985, Egilsson *et al.* 1979, Putrament *et al.* 1977, Prazmo *et al.* 1975), depending on the type of mutation. Respiratory deficiency mutations are consistently positive, while others, such as for the *ilv* gene, were negative; the significance of this difference is not clear. Gene conversion was observed as at least weakly positive at the *trp* locus in the yeast *S. cerevisiae* D7 in all three of the studies reported (Kharab and Singh 1985, Singh 1983, Fukunaga *et al.* 1982).

Studies on tissues from two plants reported DNA damage due to exposure to cobalt chloride for *Allium cepa* bulbs (Yildiz *et al.* 2009) and cobalt nitrate hexahydrate treatment of *Zea mays* seedlings (Erturk *et al.* 2013). Chromosomal aberrations were reported after cobalt chloride exposure in the Yildiz *et al.* study and also in an earlier *A. cepa* study with cobalt sulfate (Gori and Zucconi 1957), which also reported the induction of aneuploidy.

Cobalt soluble salts (cobalt chloride and cobalt nitrate hexahydrate) caused somatic mutation and/or recombination in *Drosophila melanogaster* fruit flies strain *mwh/flr3* (Vales *et al.* 2013, Yesilada 2001, Ogawa *et al.* 1994). In the study of cobalt nanoparticles, Vales *et al.* (2013) used the somatic mutation wing spot assay with strain *mwh/TM3* to distinguish somatic mutations from recombination that suggested that the genotoxic effect for the *mwh/flr3* is not due to mutation (see study details in Table E-2).

Fish have been used to assess genotoxic effects of cobalt compounds. Direct DNA damage was reported in sperm from exposed male zebrafish (*Danio rerio*) for both cobalt chloride and cobalt sulfate, showing a dose-dependent increase for both compounds.

Table E-2. Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes

Compound	Reference	LED/HID	Results (-S9) <sup>a</sup>	Comments and conclusions	
<b>FUNGI (Yeast)</b>					
<b>Mutation/ <i>Saccharomyces cerevisiae</i></b>					
Cobalt chloride	Prazmo <i>et al.</i> 1975*	'Petite' mutation	260 µg/mL	+	Some positive results for mutation in yeast, especially respiratory deficiency type
	Egilsson <i>et al.</i> 1979*	SBTD-2B, respiratory deficiency	640 µg/mL	(+)	
	Putrament <i>et al.</i> 1977*	Respiratory deficiency	520 µg/mL	+	
	Putrament <i>et al.</i> 1977*	Strain 197/2d	520 µg/mL	-	
		Erythromycin-resistant mutation/ <i>ilv</i> mutation DL7:			
	Fukunaga <i>et al.</i> 1982*	1,300 µg/mL	-		
	Singh 1983*	13,000 µg/ml	-		
	Kharab and Singh 1985*	3,000 µg/mL	(+)		
	Kharab and Singh 1987*	'Petite' mutation/DL7 respiratory deficiency: 750 µg/mL	+		
<b>Gene conversion (<i>trp</i>)/<i>Saccharomyces cerevisiae</i> D7</b>					
Cobalt chloride	Fukunaga <i>et al.</i> 1982*	1,300 µg/mL	+	Positive for 3 of 3 studies	
	Singh 1983*	13,000 µg/mL	(+)		
	Kharab and Singh 1985*	1,500 µg/mL	(+)		
<b>PLANTS (Onion or corn)</b>					
<b>DNA damage/ <i>Allium cepa</i> or <i>Zea mays</i></b>					
Cobalt chloride	Yildiz <i>et al.</i> 2009	5.5 ppm	+	Positive in comet assay in <i>Allium cepa</i> bulbs	
Cobalt nitrate hexahydrate	Erturk <i>et al.</i> 2013	5 mM	+	Genomic template instability increases with cobalt exposure levels in <i>Zea mays</i> seedlings	
<b>Chromosomal aberrations/ <i>Allium cepa</i></b>					
Cobalt chloride	Yildiz <i>et al.</i> 2009	5.5 ppm	+	Positive in anaphase-telophase chromosome aberration assay in <i>Allium cepa</i> bulbs	
Cobalt sulfate	Gori and Zucconi 1957*	3 µg/mL	+	Positive results	

Compound	Reference	LED/HID	Results (-S9) <sup>a</sup>	Comments and conclusions		
<i>Aneuploidy/ Allium cepa</i>						
Cobalt sulfate	Gori and Zucconi 1957*	15 µg/mL	+	Positive results/ dosed 5 d, then water 3d		
<i>INSECTS (Drosophila melanogaster; fruit fly)</i>						
<i>Mutation or mitotic recombination/ wing spot test</i>						
Cobalt chloride	Ogawa <i>et al.</i> 1994*	<i>mwh/flr</i>	260 µg/mL	+	Positive results for single and total mutant spots at high doses (10 mM) of ionic cobalt indicate CoCl <sub>2</sub> is more genotoxic than nanoparticles in this assay (see below).	
	Ogawa <i>et al.</i> 1994*	<i>mwh/TM3</i>	1040 µg/mL	-		
	Vales <i>et al.</i> 2013	<i>mwh/flr</i> <sup>3</sup> wings				
		small spots	10 mM	+		
		large spots	10 mM	i		
		twin spots	10 mM	i		
total	10 mM	+				
Cobalt nitrate hexahydrate	Yesilada 2001*	Strain <i>mwh/flr</i> <sup>3</sup> wings			Positive effects; additional details were not provided in review paper	
		Mutations, chromosomal deletion, nondisjunction				
		291 µg/mL	+			
		Mitotic recombination				
		2,910 µg/mL	+			
Cobalt nanoparticles	Vales <i>et al.</i> 2013	<i>mwh/flr</i> <sup>3</sup> wings			Dose-dependent induction of small, but not large, spots indicates slow progression of nanoparticles to reach the wing imaginal disks. Single mutant spots result from both somatic mutation and somatic recombination; twin spots only result from somatic mutation.	
		small spots	1 mM	+		
		large spots	10 mM	-		
		twin spots	10 mM	i		
		total	5 mM	+		
		<i>mwh/TM3</i> wings				Results negative for this assay, suggesting that effect for experiment above is due to somatic recombination and <u>not</u> mutation.
		small spots	1 mM	i		
		large spots	10 mM	-		
		total	10 mM	i		
<i>FISH (Danio rerio; zebrafish)</i>						
<i>DNA damage</i>						

Compound	Reference	LED/HID	Results (-S9) <sup>a</sup>	Comments and conclusions
Cobalt chloride	Reinardy <i>et al.</i> 2013	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water
Cobalt sulfate	Reinardy <i>et al.</i> 2013	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water

\*As cited by IARC 2006.

<sup>a</sup>No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, - = negative, i = inconclusive.

### E.3 *In vitro* studies of genotoxicity of cobalt compounds in mammalian cells

Cobalt compounds have been evaluated for genotoxic effects in mammalian cells *in vitro* in rodent (mouse, hamster, and rat) and human cells. In general, no major species differences (with one possible exception) were observed, albeit not all types of effects were tested in cells from all species. Although most studies tested cobalt chloride, a greater number of different cobalt compounds were tested compared to the other experimental systems including another soluble cobalt salt (cobalt sulfate), an organic water-soluble cobalt compound and insoluble cobalt forms including cobalt metal, cobalt nanoparticles, and cobalt sulfide particles.

Overall, there is strong evidence that all types of cobalt compounds are clastogenic and induced DNA damage in both human and animal cells and most of the compounds (except cobalt metal) caused cellular transformation in animal cell lines (see Section E.7). There is also some evidence that a soluble cobalt compound (cobalt chloride, which was the only compound tested for most of the endpoints) induces sister chromatid exchange and aneuploidy. However, mixed results were reported for mutagenicity in animal cells for a variety of cobalt compounds and chromosomal aberrations in human cells. Cobalt nanoparticles caused micronuclei in both rodent and human cells; however, findings for other compounds differ by species with positive findings for cobalt chloride and cobalt metal in human cells and negative findings for cobalt chloride in rodent cells.

All of the described studies in mammalian cells were performed without the addition of exogenous S9 metabolic activation mixture. Results for the *in vitro* studies in mammalian cells are discussed below and genotoxic and related effects are summarized in Tables E-3 and E-4.

#### E.3.1 Rodent cells

Rodent cells were tested *in vitro* for genotoxicity (mutagenicity, DNA strand breaks, sister chromatid exchange, and micronuclei) and related effects (i.e., cellular transformation and apoptosis) with soluble cobalt salts (cobalt chloride) and some relatively insoluble forms or particles (cobalt oxide, cobalt sulfides and particles, cobalt metal and nanoparticles) (see Table E-3).

There is strong evidence that different types of cobalt compounds (both soluble and relatively insoluble forms) cause DNA damage. Positive results were reported for cobalt chloride, cobalt metal, and cobalt nanoparticles in BALB/3T3 cells (Ponti *et al.* 2009, Anard *et al.* 1997); cobalt chloride and cobalt sulfides in Chinese hamster ovary (CHO) cells (Hamilton-Koch *et al.* 1986, Robison *et al.* 1982), as well as in rat neuronal PC12 cell mitochondria (Wang *et al.* 2000). The only negative study reported using a different type of assay (nucleoid sedimentation) in CHO cells to test cobalt chloride (Hamilton-Koch *et al.* 1986).

Cobalt assays for mutagenicity generally gave negative results; conflicting results may be due in part to the type of loci evaluated in the various assays. Cobalt chloride and cobalt sulfide caused mutations in studies using V79 Chinese hamster fibroblast cells *Hprt* locus (Hartwig *et al.* 1990, Miyaki *et al.* 1979) and for the transgenic G12 *Gpt*, but not for the normal fibroblast *Gpt* locus (Kitahara *et al.* 1996). Both assays testing cobalt chloride hexahydrate were negative, but they tested different gene loci, one at the *Tk* locus of mouse lymphoma L5178Y cells (Amacher and Paillet 1980) and the 8AG locus of V79 cells (Yokoiyama *et al.* 1990). Thus, the disparity of results may be due to the specific locus tested in these assays; the *Hprt* locus was positive in the

two studies where it was evaluated for cobalt chloride, while the other assays looked at different loci. Mixed results were also reported for cobalt sulfide tested in a Chinese hamster transgenic cell line; the *Gpt* locus for G10 was negative while the G12 strain tested positive. These cell lines have different *Gpt* locus insertion sites and differ in their response to clastogens. When compared with G10, the G12 strain has a lower spontaneous mutant frequency (30 compared with 100 per million cells) and is highly sensitive to insoluble metal (nickel) compounds, with mutant induction of 20 to 30 fold for G12, compared with only 2 to 3 times the number of spontaneous mutants induced for G10 (Klein *et al.* 1994).

In cytogenetic assays, cobalt chloride induced sister chromatid exchange (SCE) in mouse macrophage-like cells (Andersen 1983). SCE involves double-strand DNA breaks and is induced by agents that form DNA adducts or interfere with DNA replication and/or repair. Cobalt nanoparticles (Ponti *et al.* 2009), but not cobalt chloride (Ponti *et al.* 2009, Suzuki *et al.* 1993), caused micronucleus induction.

### E.3.2 Human cells

Human cells were tested *in vitro* for genotoxicity (DNA strand breaks, sister chromatid exchange, micronuclei, chromosomal aberrations, and aneuploidy) with soluble cobalt salts (cobalt acetate, cobalt chloride, cobalt nitrate, and some relatively insoluble forms or particles (cobalt oxide, cobalt metal, and nanoparticles) (see Table E-3).

There is strong evidence that different types of cobalt compounds caused DNA damage after exposure *in vitro* in human cells, similar to that of the rodent cells described previously. DNA damage, such as strand breaks, was reported after cobalt chloride treatment in assays in several human cell lines including diploid fibroblasts, mononuclear leukocytes, HepG2 cells, H460 lung epithelial cells, and T-cells (Alarifi *et al.* 2013, Patel *et al.* 2012, Caicedo *et al.* 2007, Davies *et al.* 2005, De Boeck *et al.* 1998, Hartwig *et al.* 1990, Hamilton-Koch *et al.* 1986, McLean *et al.* 1982). Negative results were reported in studies that used different techniques like nucleoid sedimentation (Hamilton-Koch *et al.* 1986) or different cell types like peripheral blood leukocytes (Colognato *et al.* 2008). Interestingly, T-cells did not show DNA damage in the comet assay for cobalt chloride but did for cobalt nanoparticles in the same study (Jiang *et al.* 2012). Treatment with cobalt metal also gave very strong positive results for lymphocytes, mononuclear leukocytes, and normal fetal fibroblasts (Qiao and Ma 2013, De Boeck *et al.* 2003b, De Boeck *et al.* 1998, Anard *et al.* 1997, Van Goethem *et al.* 1997). Cobalt nanoparticles and cobalt oxide nanoparticles gave positive results in all identified studies for lymphocytes, HepG2 cells, A549 lung epithelial cells, and bronchial BEAS-2B bronchial cells (Cavallo *et al.* 2015, Alarifi *et al.* 2013, Jiang *et al.* 2012, Kain *et al.* 2012, Wan *et al.* 2012, Colognato *et al.* 2008).

Evidence that cobalt compounds cause chromosomal damage comes primarily from studies using human lymphocytes or lung fibroblast cells. Both soluble (cobalt chloride) and insoluble (cobalt metal and cobalt nanoparticles) cobalt forms induced micronucleus formation (Colognato *et al.* 2008, De Boeck *et al.* 2003b, Miller *et al.* 2001, Van Goethem *et al.* 1997). Chromosomal aberrations were evaluated after exposure to various forms of cobalt, with mixed results possibly related to cell type or exposure level and not compound solubility. Cobalt chloride hexahydrate and cobalt oxide were positive for aberrations in lung fibroblast cells (Smith *et al.* 2014, Figgitt *et al.* 2010); however, exposure to cobalt oxide, cobalt acetate tetrahydrate, and cobalt nitrate did

not induce chromosomal aberrations in lymphocytes, diploid fibroblasts or mononuclear leukocytes (Voroshilin *et al.* 1978, Paton and Allison 1972). These results appear to be related to cell type but not compound solubility, although intracellular soluble cobalt has been shown to be more cytotoxic than particulate cobalt in human lung fibroblasts at levels above 1 mM. For example, the relative survival for 1.7 mM cobalt chloride treated cells was 29% but was 55% survival for the same concentration of cobalt oxide (Smith *et al.* 2014). Regarding the negative results in the study by Paton and Allison, the top dose of 0.015 µg/mL cobalt nitrate to treat fibroblasts may have been too low to see an effect in the assay.

Cobalt chloride induced sister chromatid exchange in lymphocytes (Andersen 1983) as well as aneuploidy in lymphocytes and primary fibroblasts (Figgitt *et al.* 2010, Resende de Souza Nazareth 1976).

Table E-3. *In vitro* studies of genotoxic effects of cobalt compounds in mammalian cells

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
<b>RODENT CELLS</b>				
<b><i>Mutation/ V79 Chinese hamster lung fibroblasts (V79) or Mouse lymphoma (MOLY), hamster transgenic cell line or BALB/3T3 mouse cells</i></b>				
Cobalt chloride	Miyaki <i>et al.</i> 1979*	26 µg/mL	(+)	V79 – mixed results <i>Hprt</i> locus
	Hartwig <i>et al.</i> 1990*	13 µg/mL	+	<i>Hprt</i> locus
	Kitahara <i>et al.</i> 1996*	13 µg/mL	–	<i>Gpt</i> locus
	Kitahara <i>et al.</i> 1996*	6.5 µg/mL	+	Transgenic G12, <i>Gpt</i> locus
Cobalt chloride hexahydrate	Yokoiyama <i>et al.</i> 1990*	2 µg/mL	–	V79 – 8AG locus, negative results
	Amacher and Paillet 1980*	57.11 µg/mL	–	MOLY L5178Y cells, <i>Tk</i> locus, negative results
Cobalt sulfide (CoS <sub>2</sub> and CO <sub>3</sub> S <sub>4</sub> ) particles	Kitahara <i>et al.</i> 1996*	1 µg/mL	–	Chinese hamster transgenic cell lines (derived from V79) G10, <i>Gpt</i> locus, negative results
		0.5 µg/mL	+	G12, <i>Gpt</i> locus, positive results
<b><i>DNA damage/strand breaks/ alkaline elution, sucrose gradient, or Q-PCR/3T3 mouse cells, Chinese Hamster Ovary (CHO) cells, BALB/3T3 cells/neuronal cell mitochondria</i></b>				
Cobalt chloride	Hamilton-Koch <i>et al.</i> 1986*	260 µg/mL (ASG)	+	CHO cells – positive using alkaline sucrose gradient but not nucleoid sedimentation in the same study
	Hamilton-Koch <i>et al.</i> 1986*	1,300 µg/mL (NS)	–	
	Ponti <i>et al.</i> 2009	1 µM	+	Positive in BALB/3T3 cells – 2 hr. exposure to sub-toxic dose
	Wang <i>et al.</i> 2000	100 µM	+	Positive in rat neuronal PC12 cell mitochondria
Cobalt (metal)	Anard <i>et al.</i> 1997*	1 µg/mL	+	BALB/3T3 mouse cells – positive for alkaline elution; used purified DNA
Cobalt sulfides (CoS <sub>2</sub> and CO <sub>3</sub> S <sub>4</sub> ) particles	Robison <i>et al.</i> 1982*	10 µg/mL	+	CHO cells – Positive using sucrose gradient
Cobalt metal nanoparticles	Ponti <i>et al.</i> 2009	1 µM	+	Positive in BALB/3T3 cells – 2 hr. exposure to sub-toxic dose
<b><i>Micronucleus formation/ mouse cells</i></b>				
Cobalt chloride	Ponti <i>et al.</i> 2009	10 µM	–	Negative for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
Cobalt chloride hexahydrate	Suzuki <i>et al.</i> 1993*	50 µg/mL	–	Negative for MN induction in BALB/c mouse bone marrow

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
Cobalt metal nanoparticles	Ponti <i>et al.</i> 2009	1 µM	+	Positive for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
<i>Sister chromatid exchange/ mouse macrophage-like cells P388D<sub>1</sub></i>				
Cobalt chloride	Andersen 1983*	13 µg/mL	+	Positive for SCE induction
<b>HUMAN CELLS</b>				
<i>DNA damage - strand breaks/ several cell types/ alkaline elution, alkali-labile sites, or comet assay</i>				
Cobalt chloride	McLean <i>et al.</i> 1982*	6.5 µg/mL	+	Mostly positive results White blood cells – fluorescence analysis of DNA unwinding Diploid fibroblasts
	Hamilton-Koch <i>et al.</i> 1986*	650 µg/mL	+	Alkaline sucrose gradient
	Hamilton-Koch <i>et al.</i> 1986*	1,300 µg/mL	+	Nick translation
	Hamilton-Koch <i>et al.</i> 1986*	1,300 µg/mL	–	Nucleoid sedimentation
	Hartwig <i>et al.</i> 1990*	65 µg/mL	+	Nucleoid sedimentation
	De Boeck <i>et al.</i> 1998*	0.3 µg/mL	+	Mononuclear leukocytes – comet assay
	Alarifi <i>et al.</i> 2013	10 µg/mL	+	HepG2 hepatocarcinoma cells (24 hr) – comet assay
	Colognato <i>et al.</i> 2008	100 µM	–	Negative in peripheral blood leukocytes – comet assay
	Patel <i>et al.</i> 2012	150 µM	+	Damage in H460 lung epithelial cells – comet assay
	Caicedo <i>et al.</i> 2007	5 mM	+	Damage in CD4+ T-cells obtained from lymphoma Jurkat cell line
	Davies <i>et al.</i> 2005	0.84 µM	+	Damage for artificial spiked fluids – comet assay
	Jiang <i>et al.</i> 2012	30 µM	–	Negative for DNA damage on T-cells – comet assay
Cobalt chloride hexahydrate	Anard <i>et al.</i> 1997*	25 µg/mL	–	Negative for lymphocytes using alkaline elution
Cobalt metal	Anard <i>et al.</i> 1997*	3.0 µg/mL	+	Positive for lymphocytes using alkaline elution
	Anard <i>et al.</i> 1997*	4.5 µg/mL	+	Positive in several studies, for mononuclear leukocytes for DNA single-strand breaks and alkali-labile sites, and alkaline comet assay
	Van Goethem <i>et al.</i> 1997*	0.6 µg/mL	+	
	De Boeck <i>et al.</i> 1998*	0.3 µg/mL	+	
	De Boeck <i>et al.</i> 2003b*	0.6 µg/mL	+	
	Qiao and Ma 2013	5µM	+	Positive for normal fetal fibroblast cells in single cell array assay

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions	
Cobalt nanoparticles	Cognato <i>et al.</i> 2008	50 µM	+	Positive in peripheral blood leukocytes – comet assay	
	Wan <i>et al.</i> 2012	5µg/ml	+	Positive in exposed A549 lung epithelial cells – comet assay	
	Jiang <i>et al.</i> 2012	3µM	+	Positive for DNA damage in T-cells– comet assay	
Cobalt oxide (Co <sub>3</sub> O <sub>4</sub> ) nanoparticles	Kain <i>et al.</i> 2012	20 µg/mL	+	Positive in A549 lung cell line (DNA breaks)	
	Kain <i>et al.</i> 2012	20 µg/mL	+	Positive in BEAS-2B lung cell (DNA breaks and oxidative damage)	
	Alarifi <i>et al.</i> 2013	5 µg/mL	+	Positive in HepG2 hepatocarcinoma cells (24 hr) – comet assay	
	Cavallo <i>et al.</i> 2015	A549			Positive in alveolar A549 and bronchial BEAS-2B cells, for both direct and oxidative damage – comet assay
		20 µg/mL		+	
		BEAS-2B		+	
	40 µg/mL (direct)		+		
	µg/mL (oxidative)		+		
<b><i>Micronucleus formation/ binucleates, cytochalasin-B assay/ lymphocytes or osteoblast-like cell line</i></b>					
Cobalt chloride	Cognato <i>et al.</i> 2008	40 µM	+	Positive in peripheral blood leukocytes, with clear trend for increase, high variability in response of donors	
Cobalt metal	Van Goethem <i>et al.</i> 1997*	0.6 µg/mL	+	Positive for micronuclei induction in three studies with different cell types	
	Miller <i>et al.</i> 2001*	0.75 µg/ml	+		
	De Boeck <i>et al.</i> 2003b*	3 µg/mL	+		
Cobalt nanoparticles	Cognato <i>et al.</i> 2008	40 µM	+	Increase in peripheral blood leukocytes, high variability among donors, less effective than cobalt chloride in same study	
<b><i>Chromosomal aberrations/ lung fibroblast cells or lymphocytes</i></b>					
Cobalt chloride hexahydrate	Fairhall <i>et al.</i> 1949	1.3 ppb	+	Induced significant increase of total aberrations in primary fibroblasts In WTHBF-lung fibroblast cells – soluble cobalt induces more cytotoxicity and cell cycle arrest than particulate (cobalt oxide, see below) but both produced similar levels of genotoxicity; chromosomal damage significant (p<0.05).	
	Smith <i>et al.</i> 2014	50 µM	+		
Cobalt nitrate	Paton and Allison 1972*	0.015 µg/mL	-	Negative in diploid fibroblasts WI38 (derived from embryonic lung tissue) and MRC-5 (derived from fetal lung tissue) toxic dose Negative in mononuclear leukocytes (toxic dose)	
		0.15 µg/mL	-		
Cobalt oxide (CoO)	Smith <i>et al.</i> 2014	0.5 µg/mL	+	Lung fibroblast cells – significant chromosome damage at <i>P</i> < 0.05	

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
Cobalt acetate tetrahydrate	Voroshilin <i>et al.</i> 1978*	0.6 µg/mL	-	Negative in lymphocytes
<i>Sister chromatid exchange (SCE)/ lymphocytes</i>				
Cobalt chloride	Andersen 1983*	1.3 µg/mL	+	Positive for SCE in lymphocytes
<i>Aneuploidy/ lymphocytes</i>				
Cobalt chloride	Resende de Souza Nazareth 1976*	3.7 µg/mL	+	Positive for aneuploidy in lymphocytes
Cobalt chloride hexahydrate	Figgitt <i>et al.</i> 2010	25 ppb	+	Induced significant increase in aneuploidy in primary fibroblasts

\*As cited by IARC 2006.

<sup>a</sup>No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weakly positive, - = negative.

Table E-4. *In vitro* studies of other (genotoxic-related) effects of cobalt compounds in mammalian cells

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
<i>Cell transformation/ C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), or BALB/3T3 cells</i>				
Cobalt chloride	Doran <i>et al.</i> 1998*	5 µg/mL	+	Positive in C3H10T1/2 mouse fibroblast cells
	Ponti <i>et al.</i> 2009	70 µM	-	Negative in BALB/3T3 cells (72 hr exposure)
Cobalt sulfate monohydrate	Kerckaert <i>et al.</i> 1996*	0.125 µg/mL	+	Positive in SHE cells
Cobalt acetate	Casto <i>et al.</i> 1979*	0.2 mM (approx. 35.4 µg/mL)	+	Positive for cell transformation enhancement by simian adenovirus SA7/SHE cells
Cobalt metal	Doran <i>et al.</i> 1998*	500 µg/mL	-	Negative in C3H10T1/2 mouse fibroblast cells, even at high exposure
Cobalt metal nanoparticles	Ponti <i>et al.</i> 2009	7 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Sighinolfi <i>et al.</i> 2014	10 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Annangi <i>et al.</i> 2014	0.05 µg/ml	+	Cell transformation in mouse embryo fibroblasts after 12 wk exposure to sub-toxic dose; <i>Ogg1</i> <sup>+/+</sup> and <i>Ogg1</i> <sup>-/-</sup> with knockout cells more sensitive
Cobalt sulfide (CoS, amorphous)	Abbracchio <i>et al.</i> 1982* and Costa <i>et al.</i> 1982*	10 µg/mL	(+)	Positive in Syrian hamster embryo cells
Cobalt sulfide (CoS <sub>2</sub> , crystalline)	Abbracchio <i>et al.</i> 1982* and Costa <i>et al.</i> 1982*	1 µg/mL	+	Positive in Syrian hamster embryo cells

\*As cited by IARC 2006.

<sup>a</sup>No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose; + = positive; (+) = weakly positive; - = negative.

#### **E.4 Protein binding and DNA repair inhibition by cobalt compounds**

Protein binding and DNA repair inhibition due to exposure to several cobalt compounds (including cobalt chloride, cobalt sulfate, cobalt nitrate, cobalt acetate, cobalt metal, and cobalt nanoparticles) have been evaluated; the available studies are summarized in Table E-5 and discussed in Section 6.3 on potential mechanisms of carcinogenesis. Protein binding is important in the consideration of genotoxicity assay results for cobalt compounds because cobalt binding *in vivo*, e.g., to serum proteins, could render it less effective than when tested *in vitro* for the same endpoint.

#### **E.5 *In vivo* genotoxicity studies of cobalt compounds in rodents**

Several studies tested different type of cobalt compounds, including a water-soluble salt (cobalt chloride), cobalt acetate, and cobalt metal for genotoxic effects *in vivo*. The available data suggests that cobalt compounds are clastogenic and can induce DNA and chromosomal damage, i.e., micronucleus formation, chromosomal aberrations, and aneuploidy, as shown by results reported for *in vitro* assays. The results of the *in vivo* studies are discussed below and summarized in Table E-6.

DNA damage was observed after i.p. exposure to cobalt acetate in the Fischer rat, with strongest results observed for cells from the kidney, liver, then lung (Kasprzak *et al.* 1994). In two studies, cobalt chloride exposure *in vivo* induced micronucleus formation in mouse bone marrow after i.p. injection (Rasgele *et al.* 2013, Suzuki *et al.* 1993) but results were negative in a third study of cobalt metal in murine peripheral blood lymphocytes after inhalation exposure (NTP 2014b). Route of exposure and tissue type varied between these studies; one or both of these factors may be the cause of the disparate results. Dose-dependent increases in chromosomal breaks and aberrations were reported in Swiss mouse bone marrow after oral exposure to a single dose of cobalt chloride in the test animals ((Palit *et al.* 1991d, Palit *et al.* 1991c, Palit *et al.* 1991b, Palit *et al.* 1991a), as cited in (WHO 2006)). Aneuploidy was observed in hamster bone marrow and testes after i.p. injection of either cobalt chloride or cobalt chloride hexahydrate (Farah 1983).

Table E-5. Studies of nucleic acid and protein binding and DNA damage/repair inhibition of cobalt compounds

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
<b>Binding/ crosslinks</b>				
Cobalt chloride	Wedrychowski <i>et al.</i> 1986*	130 µg/mL	+	Positive for DNA-protein crosslinks/ in rat Novikoff ascites hepatoma cells
	Palecek <i>et al.</i> 1999*	>13 µg/mL 78 µg/mL	+ (full) +	Inhibition of p53 protein-DNA binding for consensus sequence and supercoiled DNA
Cobalt chloride hexahydrate	Sabbioni <i>et al.</i> 2014a	10µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) much lower (0.0019 ng/10 <sup>6</sup> cells) than for microparticles or nanoparticles (see below)
Cobalt sulfate	Lloyd <i>et al.</i> 1998	20 µM	+	Salmon sperm DNA generative cross-links, induced single (not double) strand DNA damage
Cobalt metal ion	Bal <i>et al.</i> 2013	NTS 110 µM A 90 µM B 11 µM	+	Cobalt binds to human serum albumin at three sites: N-terminal site (NTS), A and B; they had different affinities –site B was the strongest
Cobalt nanoparticles	Sabbioni <i>et al.</i> 2014a	10 µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) Binding 1.7 ng/10 <sup>6</sup> cells
Cobalt microparticles	Sabbioni <i>et al.</i> 2014a	10 µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) Binding 9.2 ng/10 <sup>6</sup> cells
Cobalt ions (see comments)	Alipázaga <i>et al.</i> 2008	1.0 mM	+	Cobalt binds to O <sub>2</sub> in presence of glycylglycylhistidine, directly forming adducts; cobalt was prepared from cobalt carbonate reaction with perchloric acid
<b>Inhibition of DNA repair</b>				
Cobalt chloride hexahydrate	Hartwig <i>et al.</i> 1991 and Kasten <i>et al.</i> 1997*	12 µg/mL (incision and polymerization step)	+	Positive for Inhibition of nucleotide excision repair of UV-induced DNA damage, alkaline unwinding/ repair VH16 fibroblasts; data shown for Kasten <i>et al.</i> (same research group, subsequent publication)
		48 µg/mL (ligation step)	-	
Cobalt chloride hexahydrate	Kasten <i>et al.</i> 1997*	86 µg/mL (incision step)	+	Inhibition of UV-induced cyclobutane pyrimidine dimers, alkaline unwinding + T4 endonuclease V/VH16 fibroblasts

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
Cobalt (metal)	De Boeck <i>et al.</i> 1998*	1.2 µg/mL (with 5.5 µg/mL MMS post-treatment)	+	Positive for DNA repair inhibition, alkaline Comet assay/ human mononuclear leukocytes
		1.2 µg/mL (with 5.5 µg/mL MMS co-exposure)	+	
Cobalt acetate	Snyder <i>et al.</i> 1989*	100 µg/mL	+	Inhibition of repair of UV-induced pyrimidine dimers, nucleoid sedimentation in HeLa S-3 cells
<b><i>Inhibition/inactivation of protein</i></b>				
Cobalt chloride	Asmuss <i>et al.</i> 2000*	6.5 µg/mL (XPA)	+	Positive for inhibition of xeroderma pigmentosum group A (XPA) protein (with Zn finger domain) binding to UV-irradiated oligonucleotide [XPA is a zinc finger protein involved in nucleotide excision repair, but no effect on bacterial Fpg protein (Zn finger domain)]
		130 µg/mL (Fpg)	-	
Cobalt nitrate hexahydrate	Kopera <i>et al.</i> 2004	10 µM	+	Reported substitution for zinc in the zinc finger derived from the DNA repair protein XPA

\*As cited by IARC 2006.

<sup>a</sup>No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, - = negative.

Table E-6. *In vivo* genotoxicity studies of cobalt compounds in rodents

Compound	Species/sex/#	Reference	Exposure	Results	Comments and conclusions
<b>DNA damage</b>					
Cobalt acetate	F344/CR rat male and female/12 per group	Kasprzak <i>et al.</i> 1994	i.p. one dose 50 or 100 µmol 2 or 10 d	Kidney + Liver + Lung +	Damage in kidney > liver > lung cells; retention of cobalt in kidney and liver, less in lung
<b>Micronucleus formation/ peripheral blood lymphocytes</b>					
Cobalt metal	B6C3F1/N mouse/male and female	NTP 2014a	Inhalation 3 months 10 mg/m <sup>3</sup>	–	Negative for micronuclei induction in peripheral blood lymphocytes
<b>Micronucleus formation/ bone marrow</b>					
Cobalt chloride	Swiss albino mouse/male/5 per group	Rasgele <i>et al.</i> 2013	i.p. 24 hr 22.5 mg/kg 48 hr 11.2 mg/kg	+ +	Significant increase in micronucleated polychromatic erythrocytes; no toxicity observed; distilled water control
Cobalt chloride hexahydrate	BALB/c AnNCrj mouse/male	Suzuki <i>et al.</i> 1993*	i.p. 50 mg/kg bw	+; also enhanced formation with other mutagens	Micronuclei enhanced, compared with other mutagens used (20 mg/kg DMH, 50 mg/kg benzo(a)pyrene, and 200 mg/kg 2-naphthylamine)
<b>Chromosomal breaks and chromosomal aberrations (bone marrow)</b>					
Cobalt chloride	Swiss mouse/male	Palit <i>et al.</i> 1991d, Palit <i>et al.</i> 1991c, Palit <i>et al.</i> 1991b, Palit <i>et al.</i> 1991a**	Oral Single dose 4.96 to 19.8 mg/kg	+	Dose response increase in chromosomal damage
<b>Aneuploidy, pseudodiploidy, and hyperploidy/ bone marrow and testes (meiosis I)</b>					
Cobalt chloride	Hamster	Farah 1983*	i.p. 400 mg/kg bw total dose	+(bone marrow and testes)	Exposure is total dose over 9 days
Cobalt chloride hexahydrate	Hamster	Farah 1983*	i.p. 400 mg/kg bw total dose	+(bone marrow and testes)	Exposure is total dose over 9 days

\*As cited by (IARC 2006), \*\* as cited by WHO (CICAD) (2006).

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, – = negative.

## E.6 Genotoxicity studies of occupational exposure to cobalt

The available database for evaluating occupational exposure to cobalt and genetic effects is inadequate because of the paucity of studies, exposure to other genotoxic agents, or small numbers of exposed workers. Two studies reported by IARC are not reviewed because they were not specific for cobalt exposure and a more recent study of occupational exposure was identified; however, a study of Brazilian copper smelter workers is also not reviewed because blood cobalt levels were similar among unexposed controls (De Olivera *et al.* 2012). Two studies are reviewed below, only one of which is of cobalt workers (De Boeck *et al.* 2000); however, the second study is briefly reviewed (Hengstler *et al.* 2003) because it conducted multivariate analyses (see Table E-7).

De Boeck *et al.* (2000) measured 8-hydroxydeoxyguanosine, DNA damage (comet assay), and micronuclei in lymphocytes from workers at cobalt refinery facilities in Belgium, Norway, and Finland, cobalt hard workers, and unexposed controls from the same plants. All genetic markers were similar between the cobalt-exposed workers and non-exposed workers. Limitations of the study were small numbers of workers and the measurement of damage at least 40 hours after exposure.

In the second study, DNA strand breaks were measured in 78 German workers producing or recycling cadmium or cadmium products. In analysis considering only exposure to single metals, DNA single-strand breaks correlated better with cobalt concentrations (measured in air and urine) than cadmium concentrations (air and blood). Logistic analysis evaluating all variables as well as interactions between metals found that the increases in DNA strand breaks were explained by cobalt (air), cadmium (air), cadmium (blood), and interaction between lead and cobalt in the air (Hengstler *et al.* 2003).

Table E-7. Genotoxicity studies of occupational exposure to cobalt

Endpoint	Population	Reference	Exposure assessment	Results	Comments and conclusions
DNA damage Cobalt in air and urine	Workers in 10 facilities producing or recycling cadmium or its products in Germany  (N = 78; 62 men and 16 women)	Hengstler <i>et al.</i> 2003	Cobalt meas in air (range): 0-10 $\mu\text{g}/\text{m}^3$  Cobalt in urine – to air  Cobalt in urine – to air (normalized to creatinine)	+  R = 0.453 P = 0.000  R = 0.504 P = 0.000	Co-exposures to cadmium and lead contribute to DNA strand breaks in logistic regression models.  DNA single strand breaks correlated with cobalt conc in air (P < 0.001, R = 0.401).
DNA damage 8-Hydroxy-deoxyguanosine (8-OHdG)  Micronuclei	Refinery workers (three facilities – in Belgium, Norway and Finland) exposed to cobalt (analysis = 24); workers exposed to hard metal plants in one plant (analysis = 29) and unexposed workers from the four facilities (analysis = 27)  Workers and exposed workers from one plant were excluded from the analysis because of older population and higher 8-OHdG	De Boeck <i>et al.</i> 2000	Cobalt in urine ( $\mu\text{g}$ cobalt per gram creatinine)  Level (SD; range): Controls: 1.7 (1.6; 0.6 - 5.5) Exposed: 21.5 (2.1 (5.0-82.5)	–  (all three endpoints)	Negative for DNA damage measured in lymphocytes – comet assay; cobalt levels were measured in urine  Exposure equivalent to 20 $\mu\text{g}/\text{m}^3$ of cobalt in air  Exposure assessment from samples on Friday, and genetic damage assessed from samples the following Monday

R = correlation coefficient.

## E.7 Cell transformation

Cobalt compounds caused cellular transformation, which may be related to genotoxicity but is not a genotoxic effect *per se* (see Table E-8). The Syrian hamster embryo (SHE) transformation assay identifies non-genotoxic carcinogens with 80% to 90% accuracy and detection for genotoxic carcinogens is even higher (Benigni *et al.* 2015). Cell-transformation assays in SHE and other cell lines were positive for three soluble cobalt compounds (cobalt chloride, cobalt sulfate monohydrate, and cobalt acetate) (Ponti *et al.* 2009, Doran *et al.* 1998, Kerckaert *et al.* 1996, Casto *et al.* 1979), cobalt nanoparticles (Annangi *et al.* 2014, Sighinolfi *et al.* 2014, Ponti *et al.* 2009, Sabbioni *et al.* 2014b), cobalt microparticles (Sabbioni *et al.* 2014b), and cobalt sulfide (Abbracchio *et al.* 1982, Costa *et al.* 1982), but negative for cobalt metal (Doran *et al.* 1998).

**Table E-8. *In vitro* studies of other (genotoxic-related) effects of cobalt compounds in mammalian cells**

Compound	Reference	Concentration (LED or HID)	Results (-S9) <sup>a</sup>	Comments and conclusions
<i>Cell transformation/ C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), BALB/3T3 cells</i>				
Cobalt chloride	Doran <i>et al.</i> 1998*	5 µg/mL	+	Positive in C3H10T1/2 mouse fibroblast cells
	Ponti <i>et al.</i> 2009	70 µM	-	Negative in BALB/3T3 cells (72 hr exposure)
Cobalt sulfate monohydrate	Kerckaert <i>et al.</i> 1996*	0.125 µg/mL	+	Positive in SHE cells
Cobalt acetate	Casto <i>et al.</i> 1979*	0.2 mM (approx. 35.4 µg/mL)	+	Positive for cell transformation enhancement by simian adenovirus SA7/ SHE cells
Cobalt metal	Doran <i>et al.</i> 1998*	500 µg/mL	-	Negative in C3H10T1/2 mouse fibroblast cells, even at high exposure
Cobalt nanoparticles	Ponti <i>et al.</i> 2009	7 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Sabbioni <i>et al.</i> 2014b	5 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Sighinolfi <i>et al.</i> 2014	10 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	Annangi <i>et al.</i> 2014	0.05 µg/ml	+	Cell transformation, mouse embryo fibroblasts, 12 wk exposure to sub-toxic dose; <i>Ogg1</i> <sup>+/+</sup> and <i>Ogg1</i> <sup>-/-</sup> with knockout cells more sensitive
Cobalt microparticles	Sabbioni <i>et al.</i> 2014b	1 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
Cobalt sulfide (CoS, amorphous)	Abbracchio <i>et al.</i> 1982* and Costa <i>et al.</i> 1982*	10 µg/mL	(+)	Positive in Syrian hamster embryo cells
Cobalt sulfide (CoS <sub>2</sub> , crystalline)	Abbracchio <i>et al.</i> 1982* and Costa <i>et al.</i> 1982*	1 µg/mL	+	Positive in Syrian hamster embryo cells

\*As cited by IARC 2006.

<sup>a</sup>No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weakly positive, - = negative.

**Part 2**

**RoC Substance Profile**

**This Page Intentionally Left Blank**

---

## Cobalt and Cobalt Compounds That Release Cobalt Ions *In Vivo*

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

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

Reasonably anticipated to be human carcinogens<sup>1</sup>

### 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 cobalt compounds that release cobalt ions *in vivo* supersedes the previous listing of cobalt sulfate in the Report on Carcinogens and applies to the class cobalt and cobalt compounds that release cobalt ions *in vivo* as defined below.

### Carcinogenicity

Cobalt and cobalt compounds that release cobalt ions *in vivo* 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. Mechanistic data indicate that the release of cobalt ions *in vivo* is a key event for cobalt-induced carcinogenicity. The available data show that cobalt metal and cobalt compounds that release cobalt ions *in vivo* (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, IARC 2006, NTP 2014).

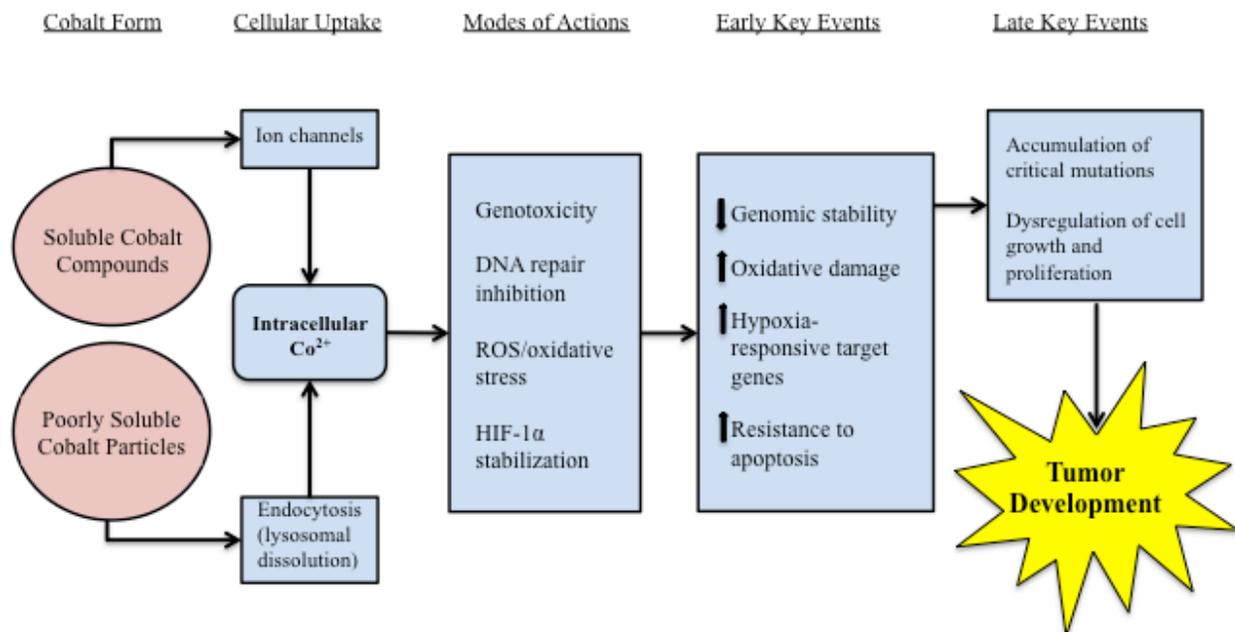
Both water-soluble cobalt compounds, which release ions in extracellular fluids, and poorly water-soluble cobalt particles, which release cobalt ions intracellularly in lysosomes, are included in this grouping. Evidence for cellular uptake of particles of cobalt metal and poorly water-soluble cobalt compounds and subsequent intracellular release of cobalt ions includes increased intracellular cobalt ion concentrations and cytotoxicity *in vitro* (Sabbioni *et al.* 1994, Peters *et al.* 2007, Ortega *et al.* 2014, Smith *et al.* 2014) as well as solubility in biological fluids *in vitro* (e.g., gastric and lysosomal fluids), as discussed under “Properties” below. Vitamin B<sub>12</sub>, which is an essential cobalt-containing nutrient, does not meet the criteria for this listing because it does not release cobalt ions as it 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.

---

<sup>1</sup>NTP listing recommendation proposed for the RoC.



### Mechanistic events in cobalt carcinogenicity

Although the mechanism(s) of action for cobalt-induced carcinogenic effects are not completely understood, several key events have been identified that are related to biologically plausible 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 1 $\alpha$  (HIF-1 $\alpha$ ).

Cobalt is clastogenic 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. Two potential mechanisms for genotoxicity include: (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 DNA damage have been shown in *in vivo* studies in rat kidney, liver, and lung (Kasprzak *et al.* 1994). 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, IARC 2006, NTP 2014). In addition to generating DNA damage, ROS induced by cobalt also activate stress-response genes and redox-sensitive transcription factors (e.g., NF- $\kappa$ B, AP1, p53, Nrf2) (Valko *et al.* 2005, Valko *et al.* 2006, Beyersmann and Hartwig 2008, Shukla *et al.* 2012, Davidson *et al.* 2015, PubChem 2015). ROS has been proposed to initiate tumor development by mutagenesis and/or promotion of tumor growth by dysregulation of cell growth and proliferation.

Finally, a well-established biological effect of cobalt is to mimic hypoxia by stabilizing HIF-1 $\alpha$  (Maxwell and Salnikow 2004, Greim *et al.* 2009, Saini *et al.* 2010a, Saini *et al.* 2010b, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Nyga *et al.* 2015). HIF-1 $\alpha$  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-1 $\alpha$  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, Galanis *et al.* 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, 2014b, 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, Smith *et al.* 2014, Sabbioni *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); which could be caused by direct or indirect mechanisms.

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 for 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). In the study of nanoparticles by Hansen *et al.* 2006, no tumors were observed after implantation of substances (e.g., titanium dioxide and silicon dioxide) with the same physical characteristics (i.e., surface to volume ratio) as cobalt, which suggests that the tumors were due to carcinogenic properties of cobalt and not just to a reaction to any physical implant.

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 cobalt compounds that release cobalt ions *in vivo*. 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 joint implants) were not considered to be informative, because the extent of cobalt exposure is unknown and 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 addition, the studies had limited sensitivity to detect a true risk because of small number of cases, crude exposure assessment, or concern about healthy worker related effects.

In the case-control studies, cobalt exposure was assessed in a single sample of toenail clippings taken at or several months after diagnosis of esophageal cancer. Measurements of cobalt in toenails reflect an integrated exposure that occurred 12-18 months prior to clipping, raising the question whether levels found in toenails close to, and in many cases after cancer diagnosis, reflect the relevant period of exposure for long latency cancer.

### **Properties**

Cobalt and cobalt compounds that release cobalt ions *in vivo* as a class are related largely by their chemical properties, specifically bioavailability.

#### **Bioavailability**

The carcinogenic and toxic effects of cobalt and cobalt compounds begin with the release of cobalt ions *in vivo*. The bioavailability of a metal species can be predicted by its solubility in biological fluids, such as synthetic equivalents of gastric and intestinal fluids (for ingestion exposure); alveolar, interstitial, and lysosomal fluids (for inhalation exposure) and by studies in

cultured cells. Results from studies in biological fluids are shown in the table below, along with other chemical and physical properties of cobalt metal and these cobalt compounds. These 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, personal communication from CDI on July 21, 2015, and October 19, 2015), suggesting that they will release ions *in vivo*. Although very low values ( $\leq 2\%$ ) for bioavailability have been reported for the sulfide and mixed (II,III) oxide and intermediate values (14% to 55%) for stearate and oxalate under the same test conditions, other, more informative tests with more physiologically relevant test conditions (e.g., longer term [2 weeks] studies with 0.3  $\mu\text{m}$  particles in culture medium in the presence of alveolar macrophages), have reported a 50% solubility value for  $\text{Co}_3\text{O}_4$  (e.g., cobalt(II, III)). In addition,, Ortega *et al.* (2014), found that intracellular concentrations of solubilized cobalt ions were similar for  $\text{Co}_3\text{O}_4$  and cobalt chloride in human lung cells, suggesting that  $\text{Co}_3\text{O}_4$  would release cobalt ions *in vivo*. 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 all affect the solubility of cobalt in biological fluids.

## Physical and chemical properties for cobalt metal and some cobalt compounds

Form <sup>a</sup>	CAS No. <sup>b</sup>	Formula	Molec. weight	Physical form	Density or specific gravity	Water solubility (g/100 cc) <sup>c</sup>	Bioaccessibility (% solubility in gastric/lysosomal fluids) <sup>d</sup>
<i>Cobalt metal</i>	7440-48-4	Co <sup>e</sup>	58.9 <sup>e</sup>	grey hexagonal or cubic metal <sup>e</sup>	8.92 <sup>e</sup>	0.00029 <sup>i</sup>	100/100
<b>Water-soluble compounds</b>							
<i>Sulfate heptahydrate</i>	10026-24-1	CoSO <sub>4</sub> •7H <sub>2</sub> O <sup>g</sup>	281.1 <sup>g</sup>	red pink, monoclinic <sup>g</sup>	1.95 <sup>g</sup>	60.4 <sup>g</sup>	100/100
<b>Chloride</b>	7646-79-9	CoCl <sub>2</sub> <sup>h</sup>	129.8 <sup>h</sup>	blue hexagonal leaflets <sup>h</sup>	3.36 <sup>h</sup>	45 <sup>h</sup>	100/100
<i>Acetate (org.)</i>	71-48-7	Co(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> <sup>g</sup>	249.1 <sup>g</sup>	red-violet, monoclinic <sup>g</sup>	1.70 <sup>g</sup>	34.8 <sup>i</sup>	98/80 <sup>i</sup>
<i>Nitrate</i>	10141-05-6	CoN <sub>2</sub> O <sub>6</sub> <sup>e</sup>	182.9 <sup>e</sup>	red powder or crystals <sup>e</sup>	2.49 <sup>e</sup>	67.0 <sup>i</sup>	96/100 <sup>i</sup>
<b>Poorly water-soluble compounds</b>							
<i>(II) Oxide</i>	1307-96-6	CoO <sup>g</sup>	74.9 <sup>g</sup>	green-brown cubic <sup>g</sup>	6.45 <sup>g</sup>	0.00049 <sup>i</sup>	100/92.4
<i>(II, III) Oxide</i>	1308-06-1	Co <sub>3</sub> O <sub>4</sub> <sup>b</sup>	240.8 <sup>g</sup>	black, cubic <sup>g</sup>	6.07 <sup>g</sup>	0.00016 <sup>i</sup>	2/2 <sup>i</sup> (50 <sup>o</sup> %)
<i>2-Ethylhexanoate (org.)</i>	136-52-7	Co(C <sub>8</sub> H <sub>15</sub> O <sub>2</sub> ) <sub>2</sub> <sup>g</sup>	173.7 <sup>h</sup>	blue liquid (12% Co) <sup>g</sup>	1.01 <sup>g</sup>	0.630 <sup>i</sup>	100/100
<i>Carbonate (org.)</i>	513-79-1	CoCO <sub>3</sub> <sup>g</sup>	118.9 <sup>g</sup>	red, trigonal <sup>g</sup>	4.13 <sup>g</sup>	0.00114 <sup>i</sup>	100/100
<i>Naphthenate (org.)</i>	61789-51-3	Co(C <sub>11</sub> H <sub>7</sub> O <sub>2</sub> ) <sub>2</sub> <sup>e</sup>	401.3 <sup>e</sup>	purple liquid (6% Co) <sup>g</sup>	0.97 <sup>g</sup>	0.0293 <sup>i</sup>	100/100
<i>Hydroxide</i>	21041-93-0	Co(OH) <sub>2</sub> <sup>b</sup>	93.0 <sup>g</sup>	rose-red, rhomb <sup>g</sup>	3.60 <sup>g</sup>	0.00032 <sup>g</sup>	95/98 <sup>i</sup>
<i>Sulfide</i>	1317-42-6	CoS <sup>b</sup>	91.0 <sup>g</sup>	reddish octahedral <sup>g</sup>	5.45 <sup>g</sup>	0.00038 <sup>g</sup>	1/1 <sup>i</sup>
<i>Oxalate (org.)</i>	814-89-1	CoC <sub>2</sub> O <sub>4</sub> <sup>g</sup>	147.0 <sup>g</sup>	white or reddish <sup>g</sup>	3.02 <sup>g</sup>	0.00322 <sup>i</sup>	37/55 <sup>i</sup>
<i>Propionate (org.)</i>	1560-69-6	Co(C <sub>3</sub> H <sub>5</sub> O <sub>2</sub> ) <sub>2</sub> <sup>e</sup>	205.1 <sup>e</sup>	reddish solid <sup>i</sup>	–	7.49 <sup>i</sup>	91/94 <sup>i</sup>
<i>Stearate (org.)</i>	1002-88-6	Co(C <sub>18</sub> H <sub>35</sub> O <sub>2</sub> ) <sub>2</sub> <sup>e</sup>	625.9 <sup>e</sup>	grey solid <sup>i</sup>	–	0.00705 <sup>i</sup>	14/16 <sup>i</sup>

<sup>a</sup> Cobalt compounds selected for inclusion in the table include those with toxicological data or of commercial importance. All compounds contain Co(II) except where noted. Forms in italics have been tested for carcinogenicity, genetic toxicity, or have mechanistic data; org. = organic compound; all others are inorganic.

<sup>b</sup>SciFinder 2015.

<sup>c</sup>Solubility data were converted to g/100 cc as necessary.

<sup>d</sup>Stopford *et al.* 2003, <sup>e</sup>PubChem 2015, <sup>f</sup>ChemIDplus 2015, <sup>g</sup>CDI 2006, <sup>h</sup>HSBD 2004, 2012, <sup>i</sup>Personal communication, CDI, July 21, 2015, October 19, 2015.

<sup>j</sup>Kreyling *et al.* 1990.

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<sub>3</sub>O<sub>4</sub>), 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  $^{59}\text{Co}$  (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 ( $\text{Co}_3\text{O}_4$ ) (IARC 1991, Paustenbach *et al.* 2013, Lison 2015) and some simple salts of Co(III) (e.g.,  $\text{Co}_2\text{O}_3$ ). 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; recycling of electronic and electrical waste can result in releases of cobalt to the environment (though more of a global than U.S. concern). 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 <sup>a</sup>	699	8.3
Steels	548	6.5
Miscellaneous and unspecified	63	0.7

Source: Shedd 2014b.

<sup>a</sup>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.

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

– = no data found.

<sup>a</sup>No specific Schedule B code (i.e., 10-digit classification numbers administered and used by the U.S. Commerce Department to collect and publish statistics on physical goods exported from the United States to another country) identified.

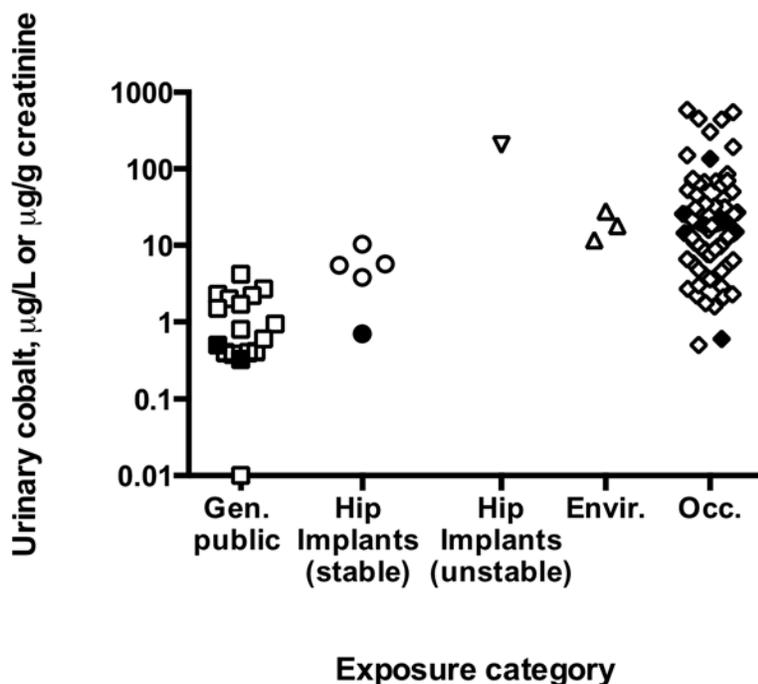
<sup>b</sup>Cobalt chloride production data for 2012 were withheld by the manufacturer.

<sup>c</sup>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

Gen. public = general public exposure, Envir. = environmental exposure, Occ. = occupational exposure. Filled symbols = U.S. data; open symbols = non-U.S. data. Each graph point represents a different study.

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 2014). 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 for the following industries: (1) production of cobalt metal or salts, (2) metallurgical-related industries, (3) cemented carbides and bonded diamonds, (4) chemicals and pigments, and (5) electronics, “green” energy, and recycling. Occupational exposure has been documented by measurements of cobalt in ambient workplace air (see the table below) and in blood, urine (see the figure above), 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 ( $> 1,000 \mu\text{g}/\text{m}^3$  in some instances) (NTP 2009) and for production of cobalt salts or metallurgical-related industries ( $> 10,000 \mu\text{g}/\text{m}^3$  in some instances) (IARC 2006). The highest cobalt levels in urine, blood, hair, and nails were also associated with exposure to cobalt powders.

Industry	Cobalt in workplace air (range, in $\mu\text{g}/\text{m}^3$ )
Production of cobalt metal or salts	2–50,000
Metallurgical-related industries <sup>a</sup>	ND–21,000 <sup>b</sup>
Cemented carbides and bonded diamonds <sup>a</sup>	ND–1,622
Chemicals and pigments <sup>a</sup>	ND–80
Electronics, “green” energy, and recycling <sup>a</sup>	ND–10

Source: IARC 2006, <http://www2a.cdc.gov/hhe/search.asp>.

ND = Not detected.

<sup>a</sup>Range for cobalt in workplace air includes U.S. data from NIOSH Hazard Evaluation and Technical Assistance (HETA) surveys.

<sup>b</sup>One higher value was reported; however, OSHA noted that sample appeared to be tampered with.

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, patients receiving orthopedic joint replacements may be exposed to cobalt. Most, but not all, hip, knee, and shoulder replacements have at least one articular bearing surface composed of cobalt-chromium molybdenum alloy. If the bearing surface(s) or modular taper junction(s) of the total joint replacement are composed of CoCrMo alloy, cobalt ions may be released into the body throughout the lifetime of the device (Sampson and Hart 2012, Devlin *et al.* 2013). Urine levels identified from studies of hip implants reported as stable or that did not specifically address stability ranged from  $\sim 0.7$  to  $12 \mu\text{g}/\text{L}$ . 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  $7 \mu\text{g}/\text{L}$  in the United Kingdom (MHRA 2012) and at  $10 \mu\text{g}/\text{L}$  in the United States by the Mayo Clinic (2015). Dunstan *et al.* (2005) also reported blood cobalt levels of 19 and  $52 \mu\text{g}/\text{L}$  for two individuals with unstable metal-on-metal implants (radiologically loose). In rare cases, high levels of cobalt from failed implants may be associated with toxicity.

### **Environmental exposure**

The TRI reported that in 2013, on- and off-site industrial releases of cobalt and cobalt compounds totaled 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  $0.4 \text{ ng/m}^3$  (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  $2.0 \text{ }\mu\text{g/L}$ ; however, levels as high as  $107 \text{ }\mu\text{g/L}$  have been reported (ATSDR 2004). Cobalt concentrations have been reported to range from 0.01 to  $4 \text{ }\mu\text{g/L}$  in seawater and from 0.1 to  $10 \text{ }\mu\text{g/L}$  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).

Data for individuals exposed to cobalt from the environment are limited, but 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).

### **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; average daily cobalt intake from food has been reported to be  $11 \text{ }\mu\text{g/day}$  (ATSDR 2004, Lison 2015). Although this amount includes cobalt as part of both vitamin B<sub>12</sub> 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<sub>12</sub>. 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  $2.3 \text{ }\mu\text{g/g}$  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  $\geq 0.5$  mg cobalt as a cobalt salt per dosage unit and which recommend administration rates of  $\geq 0.5$  mg per dose and  $\geq 2$  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<sup>3</sup> 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<sup>3</sup> for cobalt and inorganic compounds; = 0.1 mg/m<sup>3</sup> for cobalt carbonyl and cobalt hydrocarbonyl.

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

### **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<sup>3</sup>; industrial air = 0.0014 µg/m<sup>3</sup>; tap water = 6 µg/L.

### **National Institute for Occupational Safety and Health (NIOSH)**

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

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

## References

ATSDR. 2004. *Toxicological Profile for Cobalt*. Atlanta, GA: Agency for Toxic Substances and Disease Registry. pp. 207-E203. <http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=373&tid=64>.

Behl M, Stout MD, Herbert RA, Dill JA, Baker GL, Hayden BK, Roycroft JR, Bucher JR, Hooth MJ. 2015. Comparative toxicity and carcinogenicity of soluble and insoluble cobalt compounds. *Toxicology* 333: 195-205.

Beyersmann D, Hartwig A. 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch Toxicol* 82(8): 493-512.

Brock T, Stopford W. 2003. Bioaccessibility of metals in human health risk assessment: evaluating risk from exposure to cobalt compounds. *J Environ Monit* 5(4): 71N-76N.

CDC. 2013. *Biomonitoring Summary: cobalt*. Centers for Disease Control and Prevention. [http://www.cdc.gov/biomonitoring/Cobalt\\_BiomonitoringSummary.html](http://www.cdc.gov/biomonitoring/Cobalt_BiomonitoringSummary.html). Accessed on 1/31/15.

CDC. 2014. *Fourth National Report on Human Exposure to Environmental Chemicals*. Atlanta, GA: Centers for Disease Control and Prevention. 514 pp.

CDI. 2006. *Cobalt Facts*. Cobalt Development Institute. <http://thecdi.com/cobaltfacts.php>. Accessed on 2/12/15.

ChemIDplus. 2015. *ChemIDplus Lite*. National Library of Medicine. <http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp> and search by CAS number. Accessed on 3/24/15.

Cheng Y, Chen G, Hong L, Zhou L, Hu M, Li B, Huang J, Xia L, Li C. 2013. How does hypoxia inducible factor-1 $\alpha$  participate in enhancing the glycolysis activity in cervical cancer? *Ann Diagn Pathol* 17(3): 305-311.

Cheyns K, Banza Lubaba Nkulu C, Ngombe LK, Asosa JN, Haufroid V, De Putter T, Nawrot T, Kimpanga CM, Numbi OL, Ilunga BK, Nemery B, Smolders E. 2014. Pathways of human exposure to cobalt in Katanga, a mining area of the D.R. Congo. *Sci Total Environ* 490: 313-321.

Davidson T, Ke Q, Costa M. 2015. Selected molecular mechanisms of metal toxicity and carcinogenicity. In *Handbook on the Toxicology of Metals*. 4th ed., Vol. I: General Considerations. Nordberg GF, Fowler BA, Nordberg M, eds. Waltham, MA: Elsevier. pp. 173-196.

Davis JR, ed. 2000. *Nickel, Cobalt, and Their Alloys*. Materials Park, OH: ASM International. pp. 345-406.

Devlin JJ, Pomerleau AC, Brent J, Morgan BW, Deitchman S, Schwartz M. 2013. Clinical features, testing, and management of patients with suspected prosthetic hip-associated cobalt toxicity: a systematic review of cases. *J Med Toxicol* 9(4): 405-415.

Dunstan E, Sanghrajka AP, Tilley S, Unwin P, Blunn G, Cannon SR, Briggs TW. 2005. Metal ion levels after metal-on-metal proximal femoral replacements: a 30-year follow-up. *J Bone Joint Surg Br* 87(5): 628-631.

Farquharson JP. 2015. *Formation Metals Inc. - President's Letter to Shareholders*. Formation Metals, Inc. Updated on 2/23/15. <http://www.formationmetals.com/s/CobaltNews.asp?ReportID=697830>. Accessed on 3/6/15.

Galán-Cobo A, Sánchez-Silva R, Serna A, Abreu-Rodríguez I, Muñoz-Cabello AM, Echevarría M. 2013. Cellular overexpression of Aquaporins slows down the natural HIF-2 $\alpha$  degradation during prolonged hypoxia. *Gene* 522(1): 18-26.

Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. 2008. Reactive oxygen species and HIF-1 signalling in cancer. *Cancer Lett* 266(1): 12-20.

Galanis A, Karapetsas A, Sandaltzopoulos R. 2009. Metal-induced carcinogenesis, oxidative stress and hypoxia signalling. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 674(1-2): 31-35.

Gao S, Zhou J, Zhao Y, Toselli P, Li W. 2013. Hypoxia-response element (HRE)-directed transcriptional regulation of the rat lysyl oxidase gene in response to cobalt and cadmium. *Toxicol Sci* 132(2): 379-389.

Gilman JP, Ruckerbauer GM. 1962. Metal carcinogenesis. I. Observations on the carcinogenicity of a refinery dust, cobalt oxide, and colloidal thorium dioxide. *Cancer Res* 22: 152-157.

- Greim H, Hartwig A, Reuter U, Richter-Reichhelm HB, Thielmann HW. 2009. Chemically induced pheochromocytomas in rats: Mechanisms and relevance for human risk assessment. *Critical Reviews in Toxicology* 39(8): 695-718.
- Grimsrud TK, Berge SR, Haldorsen T, Andersen A. 2005. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel? *Epidemiology* 16(2): 146-154.
- Hansen T, Clermont G, Alves A, Eloy R, Brochhausen C, Boutrand JP, Gatti AM, Kirkpatrick CJ. 2006. Biological tolerance of different materials in bulk and nanoparticulate form in a rat model: sarcoma development by nanoparticles. *J R Soc Interface* 3(11): 767-775.
- Heath JC. 1956. The production of malignant tumours by cobalt in the rat. *Br J Cancer* 10(4): 668-673.
- Heath JC, Daniel MR. 1962. The production of malignant tumours by cobalt in the rat: intrathoracic tumours. *Br J Cancer* 16(3): 473-478.
- HPD. 2014. *Household Products Database*. National Library of Medicine. Updated on 8/14. <http://householdproducts.nlm.nih.gov/advancedsearch.htm> and select "Ingredient" and search CAS No. Accessed on 10/10/14.
- HSDB. 2004. *Hazardous Substances Database. Cobalt Bis(2-Ethylhexanoate)*. National Library of Medicine. Updated on 3/5/04. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> and search on CAS number or compound name. Accessed on 11/12/15.
- HSDB. 2012. *Hazardous Substances Database. Cobaltous Chloride*. National Library of Medicine. Updated on 3/23/12. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> and search on CAS number or compound name. Accessed on 11/12/15.
- IARC. 1991. Cobalt and cobalt compounds. In *Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 52. Lyon, France: International Agency for Research on Cancer. pp. 363-434.
- IARC. 2006. Metallic cobalt particles (with or without tungsten carbide). In *Cobalt in Hard Metals*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 86. Lyon, France: International Agency for Research on Cancer. pp. 39-155.
- Jasmin G, Riopelle JL. 1976. Renal carcinomas and erythrocytosis in rats following intrarenal injection of nickel subsulfide. *Lab Invest* 35(1): 71-78.
- Kasprzak KS, Zastawny TH, North SL, Riggs CW, Diwan BA, Rice JM, Dizdaroglu M. 1994. Oxidative DNA base damage in renal, hepatic, and pulmonary chromatin of rats after intraperitoneal injection of cobalt(II) acetate. *Chem Res Toxicol* 7(3): 329-335.
- Kreyling WG, Godleski JJ, Kariya ST, Rose RM, Brain JD. 1990. In vitro dissolution of uniform cobalt oxide particles by human and canine alveolar macrophages. *Am J Respir Cell Mol Biol* 2(5): 413-422.
- Lison D. 2015. Cobalt. In *Handbook on the Toxicology of Metals*. 4th ed., Vol. II: Specific Metals. Nordberg GF, Fowler BA, Nordberg M, eds. Waltham, MA: Elsevier. pp. 743-763.

- Maverick T. 2015. *Cobalt Shortage Put Brakes on Electric Car*. Wall Street Daily. <http://www.wallstreetdaily.com/2015/01/13/cobalt-electric-car-battery/>. Accessed on 5/18/15.
- Maxwell P, Salnikow K. 2004. HIF-1: an oxygen and metal responsive transcription factor. *Cancer Biol Ther* 3(1): 29-35.
- Mayo Clinic. 2015. *Test ID: COS. Cobalt, serum*. Mayo Medical Laboratories. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/80084>. Accessed on 4/10/15.
- MHRA. 2012. *Medical Device Alert: All Metal-on-Metal (MoM) Hip Replacements*. Ref: MDA/2012/036. Medicines and Healthcare Products Regulatory Agency. 7 pp. <https://assets.digital.cabinet-office.gov.uk/media/5485abf6ed915d4c10000273/con155767.pdf>
- Moulin JJ, Wild P, Mur JM, Fournier-Betz M, Mercier-Gallay M. 1993. A mortality study of cobalt production workers: an extension of the follow-up. *Am J Ind Med* 23(2): 281-288.
- Moulin JJ, Wild P, Romazini S, Lasfargues G, Peltier A, Bozec C, Deguerry P, Pellet F, Perdrix A. 1998. Lung cancer risk in hard-metal workers. *Am J Epidemiol* 148(3): 241-248.
- Moulin JJ, Clavel T, Roy D, Dananché B, Marquis N, Févotte J, Fontana JM. 2000. Risk of lung cancer in workers producing stainless steel and metallic alloys. *Int Arch Occup Environ Health* 73(3): 171-180.
- Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. 1987. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. *Am J Ind Med* 11(1): 75-81.
- Neale G. 1990. B12 binding proteins. *Gut* 31(1): 59-63.
- Nilsson K, Jensen BS, Carlsen L. 1985. The migration chemistry of cobalt. *Eur Appl Res Rept - Nucl Sci Technol* 7(1): 23-86.
- NIOSH. 1990. *National Occupational Hazard Survey (1981-1983)*. National Institute for Occupational Safety and Health. Last updated 7/1/90. <http://www.cdc.gov/noes/noes4/73470sco.html>, <http://www.cdc.gov/noes/noes4/x8305sco.html>.
- NTP. 1998. *Toxicology and Carcinogenesis Studies of Cobalt Sulfate Heptahydrate (CAS No. 10026-24-1) in F344/N Rats and B6C3F1/N Mice (Inhalation Studies)*. Technical Report Series No. 471. NIH Publication No. 98-3961. Research Triangle Park, NC: National Toxicology Program. 268 pp.
- NTP. 2009. *Report on Carcinogens Background Document for Cobalt-Tungsten Carbide: Powders and Hard Metals*. Research Triangle Park, NC: National Toxicology Program. 180 pp.
- NTP. 2014. *Toxicology Studies of Cobalt Metal (CAS No. 7440-48-4) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTac Rats and B6C3F1/N Mice (Inhalation Studies)*. Technical Report Series No. 581. NIH Publication No. 14-5932. Research Triangle Park, NC: National Toxicology Program. 308 pp.
- Nyga A, Hart A, Tetley TD. 2015. Importance of the HIF pathway in cobalt nanoparticle-induced cytotoxicity and inflammation in human macrophages. *Nanotoxicology*: 1-13.

O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, Group FS. 2012. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *Int J Cancer* 131(8): 1882-1891.

Ortega R, Bresson C, Darolles C, Gautier C, Roudeau S, Perrin L, Janin M, Floriani M, Aloin V, Carmona A, Malard V. 2014. Low-solubility particles and a Trojan-horse type mechanism of toxicity: the case of cobalt oxide on human lung cells. *Part Fibre Toxicol* 11: 14.

Paul SAM, Simons JW, Mabweesh NJ. 2004. HIF at the crossroads between ischemia and carcinogenesis. *Journal of Cellular Physiology* 200(1): 20-30.

Paustenbach DJ, Tvermoes BE, Unice KM, Finley BL, Kerger BD. 2013. A review of the health hazards posed by cobalt. *Crit Rev Toxicol* 43(4): 316-362.

Peters K, Unger RE, Gatti AM, Sabbioni E, Tsaryk R, Kirkpatrick CJ. 2007. Metallic nanoparticles exhibit paradoxical effects on oxidative stress and pro-inflammatory response in endothelial cells in vitro. *Int J Immunopathol Pharmacol* 20(4): 679-689.

PubChem. 2015. *PubChem Compound*. National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/pccompound> and search by Compound Identification Number. Accessed on 3/24/15.

Richter PA, Bishop EE, Wang J, Swahn MH. 2009. Tobacco smoke exposure and levels of urinary metals in the U.S. youth and adult population: the National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Int J Environ Res Public Health* 6(7): 1930-1946.

Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. 1993. A case-control study of element levels and cancer of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev* 2(4): 305-312.

Sabbioni E, Minoia C, Pietra R, Mosconi G, Forni A, Scansetti G. 1994. Metal determinations in biological specimens of diseased and non-diseased hard metal workers. *Sci Total Environ* 150(1-3): 41-54.

Sabbioni E, Fortaner S, Farina M, Del Torchio R, Petrarca C, Bernardini G, Mariani-Costantini R, Perconti S, Di Giampaolo L, Gornati R, Di Gioacchino M. 2014. Interaction with culture medium components, cellular uptake and intracellular distribution of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts. *Nanotoxicology* 8(1): 88-99.

Saini Y, Greenwood KK, Merrill C, Kim KY, Patial S, Parameswaran N, Harkema JR, LaPres JJ. 2010a. Acute cobalt-induced lung injury and the role of hypoxia-inducible factor 1 alpha in modulating inflammation. *Toxicological Sciences* 116(2): 673-681.

Saini Y, Kim KY, Lewandowski R, Bramble LA, Harkema JR, Lapres JJ. 2010b. Role of hypoxia-inducible factor 1 {alpha} in modulating cobalt-induced lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 298(2): L139-147.

Sampson B, Hart A. 2012. Clinical usefulness of blood metal measurements to assess the failure of metal-on-metal hip implants. *Ann Clin Biochem* 49(Pt 2): 118-131.

SciFinder. 2015. *SciFinder*. <http://www.cas.org/products/scifinder>. Accessed on 4/30/15.

Shabaan AA, Marks V, Lancaster MC, Dufeu GN. 1977. Fibrosarcomas induced by cobalt chloride (CoCl<sub>2</sub>) in rats. *Lab Anim* 11(1): 43-46.

- Shedd KB. 1993. *The Materials Flow of Cobalt in the United States*. IC 9350. United States Department of the Interior. 31 pp.
- Shedd KB. 2014a. *U.S. Geological Survey, Mineral Commodity Summaries, February 2014: Cobalt*. <http://minerals.usgs.gov/minerals/pubs/commodity/cobalt/>. Accessed on 2/12/15.
- Shedd KB. 2014b. *USGS 2012 Minerals Yearbook: Cobalt [Advance Release]*. <http://minerals.usgs.gov/minerals/pubs/commodity/cobalt/index.html - myb>. Accessed on 2/12/15.
- Shukla SJ, Huang R, Simmons SO, Tice RR, Witt KL, Vanleer D, Ramabhadran R, Austin CP, Xia M. 2012. Profiling environmental chemicals for activity in the antioxidant response element signaling pathway using a high throughput screening approach. *Environ Health Perspect* 120(8): 1150-1156.
- Smith LJ, Holmes AL, Kandpal SK, Mason MD, Zheng T, Wise JP, Sr. 2014. The cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells. *Toxicol Appl Pharmacol* 278(3): 259-265.
- Steinhoff D, Mohr U. 1991. On the question of a carcinogenic action of cobalt-containing compounds. *Exp Pathol* 41(4): 169-174.
- Stopford W, Turner J, Cappellini D, Brock T. 2003. Bioaccessibility testing of cobalt compounds. *J Environ Monit* 5(4): 675-680.
- TRI. 2014a. *TRI Explorer Chemical Report. TRI On-site and Off-site Reported Disposed of or Otherwise Released (in pounds), Trend Report for Facilities in All industries, for Cobalt Chemical, U.S.* U.S. Environmental Protection Agency. <http://www.epa.gov/triexplorer>. Last accessed on 10/14/14.
- TRI. 2014b. *TRI EZ Search in Envirofacts. Toxic Chemical Releases to the Environment (in pounds), for Cobalt and Cobalt Compounds, U.S.* U.S. Environmental Protection Agency. <http://www.epa.gov/enviro/facts/tri/ez.html>. Last accessed on 10/29/14.
- Tüchsen F, Jensen MV, Villadsen E, Lynge E. 1996. Incidence of lung cancer among cobalt-exposed women. *Scand J Work Environ Health* 22(6): 444-450.
- Valko M, Morris H, Cronin MT. 2005. Metals, toxicity and oxidative stress. *Curr Med Chem* 12(10): 1161-1208.
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160(1): 1-40.
- Wehner AP, Busch RH, Olson RJ, Craig DK. 1977. Chronic inhalation of cobalt oxide and cigarette smoke by hamsters. *Am Ind Hyg Assoc J* 38(7): 338-346.
- WHO. 2006. *Cobalt and Inorganic Cobalt Compounds*. Concise International Chemical Assessment Document 69. Geneva, Switzerland: World Health Organization. 93 pp.
- Wild P, Perdrix A, Romazini S, Moulin JJ, Pellet F. 2000. Lung cancer mortality in a site producing hard metals. *Occup Environ Med* 57(8): 568-573.

**This Page Intentionally Left Blank**



Division of the National Toxicology Program  
National Institute of Environmental Health Sciences  
National Institutes of Health  
P.O. Box 12233, MD K2-14  
Research Triangle Park, NC 27709

NIH# 16-5987  
ISSN# 2331-267X