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

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

Appendices

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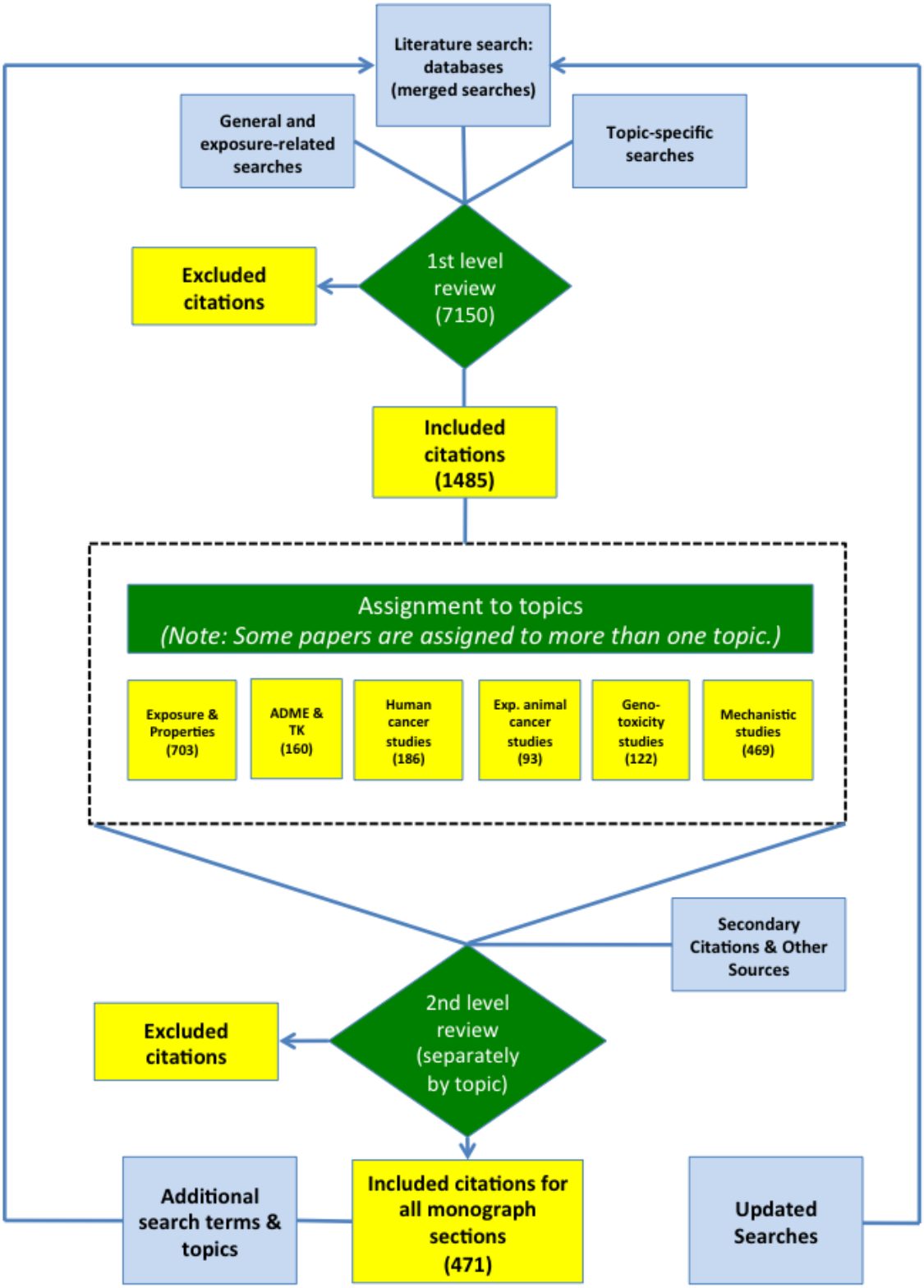
Appendix A: Literature Search Strategy

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/37898.

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.

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

This appendix reports exposure information for cobalt levels in urine and blood (Section B.1, 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 Exposure

The values for urine cobalt listed below are illustrated in Figure 2-1 in Section 2. Values identified as measurements made on people living in the United States are listed first in each section of 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.

Table B-1. 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)

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
General Public (not occupationally exposed)				
United States				
NHANES		2,504	0.326[b, c]	
(Sunderman *et al.* 1989b)	Controls for hip implants	42	0.5 µg/g creatinine[f]	
	Pre-op values for hip implant patients	24	0.9 µg/g creatinine[f]	
Non-United States				
(Adami *et al.* 2003) Italy	Metal-on-metal total hip replacement controls	15		0.3
(Bradberry *et al.* 2014) Not reported; authors from UK.	Failed hip replacements Normal ranges for UK			< 0.6 (in blood or serum)
(Lhotka *et al.* 2003) Not reported; hip manufacturers are European.	Hip replacement controls	31	NA	0.7

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
(Sidaginamale *et al.* 2013) UK	Metal on metal hip arthroplasty controls	3,042		0.5[e]
(Witzleb *et al.* 2006) Not reported; replacements were from UK and Switzerland.	Controls	130		0.25
(Zeh *et al.* 2007) Not reported; prostheses from Germany and procedure carried out in Germany.	Metal-on-metal artificial lumbar disc Controls (5) Serum cobalt			0.62
(Zeh *et al.* 2009) Not reported; prostheses from Germany and procedure carried out in Germany.	Metal-on-metal artificial lumbar disc controls	5		0.72 (serum)
Unclear				
(Alexandersson 1988)[d] Sweden			0.4	0.5 (blood)
(Alexandersson and Swensson 1979)[b]			NA	0.5 (blood)
(Alexandersson and Lidums 1979)[d] Sweden			0.4	0.5 (blood)
(Andersen and Høgetveit 1984)[b]			NA	0.15 (plasma)

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
(Angerer 1989)[d] Germany			0.01	0.2–1.3 (blood)
(Christensen and Mikkelsen 1986)[d]			0.8 µg/g creatinine[f, g]	0.24 (blood)
(Collecchi *et al.* 1986)[b]			NA	0.73 (plasma)
(Hartung 1986)[b]			NA	0.1
(Hartung *et al.* 1982)[d]			1.3	NA
(Ichikawa *et al.* 1985)[d] Japan			2.0	1.9 (blood)
(Kasperek *et al.* 1981)[b]			NA	0.195 (plasma)
(Lewis *et al.* 1985)[b]			NA	0.28 (serum)
(Mikkelsen *et al.* 1984)[d]			0.94	
Nemery *et al.* 1992 Belgium		48	2.3	
(Ostapczuk *et al.* 1983)			NA	0.09 (blood)
(Sarmiento-Gonzalez *et al.* 2008) Not reported; hip and knee joints made in US.	Hip or knee prostheses controls	9		0.565
(Scansetti *et al.* 1985)[d] Italy			0.41	NA
(Schumacher-Wittkopf and Angerer 1981)[d]			0.38	NA
(Versieck *et al.* 1978)[b]			NA	0.108 (serum)

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
Environmental exposure				
Non-United States				
(Basu *et al.* 2010) Central America	Marlin Mine (gold and silver) in Guatemala	23	0.24[e]	0.4
(Moreno *et al.* 2010) Mexico	Urine cobalt levels in children in the Taxco mining area of Southern Mexico	35	18[e]	
Occupational exposure				
United States				
(NIOSH 1987b)	Post-sintering	10 10	Preshift: 10.5 µg/g creatinine[f] Postshift: 18.12 µg/g creatinine[f]	
Non-United States				
(Alexandersson and Lidums 1979) The Netherlands	Not specified		134	10.5 (blood)
(Angerer *et al.* 1985) Germany	40 foundry workers		NA	26
Arai *et al.* (1994) Japan	Cloisonne glaze workers	49	1.75	1.5
(Cereda *et al.* 1994) Italy	Post-sintering	6 8	28.5[b] 2.66[b]	
Chadwick *et al.* (1997) United Kingdom	Thermal spraying processes	 5 89 27	(µg/g creatinine[f]) Grit blasting- 6.6 Plasma spraying- 5.1 Detonation gun spraying- 8.9	NA
Christensen and Poulsen (1994)	Pottery painting	8	14.5 µg/g creatinine[f]	

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
Denmark				
Coombs 1996 South Africa	Cobalt oxide workers	91	10.7 µg/g creatinine[f]	NA
(Della Torre *et al.* 1990) Italy	Post-sintering	6	14.17	4.0 (blood)
Ferdenzi *et al.* (1994) Italy	Production of diamond cutting wheels	15	1988 samples 550 1991 samples (after workplace modifications) 85	NA
Hengstler *et al.* (2003) Germany	Workers exposed to cadmium and cobalt	91	20 µg/g creatinine[f]	NA
(Kraus *et al.* 2001) Germany	Hard-metals production	 23 30 3 14 4 6 5 2	(µg/g creatinine[f]) Forming- 13.5 Pressing- 5.5 Heavy alloy production- 1.6 µg/g Powder processing- 28.5 Tungsten carbide production- 2.1 Sintering- 4.1 Grinding- 2.2 Maintenance- 3.0	
(Kusaka *et al.* 1986) Asia	(Pre-sintering)	22	NR	2.8 & 42 (blood)
Kusaka 1996 Asia	(Post-sintering)		NR	3.2 & 4
(Lantin *et al.* 2011) Belgium	249 foundry workers		NA	1
(Linnainmaa and Kiilunen 1997)	Post-sintering	131	14.2	

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
Finland				
Nemery *et al.* 1992 Belgium	Diamond polishers using cobalt-containing disks	86	25.2	NA
(Pellet *et al.* 1984)	Pre-sintering		11.7	
(Posma and Dijstelberger 1985) The Netherlands	Post-sintering	10	25.5 µg/g creatinine[f]	
(Raffn *et al.* 1988) Denmark	46 plate painters		NA	2.1
(Sabbioni *et al.* 1994a) Italy	Pre-sintering	23	61.1	NR
(Sabbioni *et al.* 1994a) Italy	Post-sintering	88	303.6	45.6 (blood)
(Sabbioni *et al.* 1994a) Italy	Not specified	24, 20 (blood) 28	Milan: 13.9 Turin: 32.5	5.06
(Scansetti *et al.* 1998) Italy	Not specified	6 6	13.23 30.87	
Suardi *et al.* (1994) Italy	Diamond abrasive production	 6 87 9 76	(µg/g creatinine[f]) Diamond abrasive producers- 50.17 Grinders- 10.89 Hard-metal form grinders- 7.67 Others- 4.55	NA
Swennen *et al.* 1993) Belgium	Production of cobalt powder, oxides, and salts	82	47.6 µg/g creatinine[f]	10.8

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
Thomassen *et al.* (1999) Russia	Nickel refinery workers	346	7.8	
White and Dyne (1994) United Kingdom	Several industries using cobalt	~400 workers total, but breakdown by task not reported	(µg/g creatinine[f]) Manufacture of cobalt powders, salts, and pigments- 48.4 hard-metal manufacture- 9.9 Hard-metal finishing- 8.8 Other metal working- < 1.6	
Unclear				
(Meecham and Humphrey 1991) Not reported; authors from UK.	1 adult occupationally exposed to Co		NA	234
(Mosconi *et al.* 1994b)	Post-sintering	NR	31.5 & 151	
Medical implants (stable)				
United States				
(Sunderman *et al.* 1989b)	Hip implants after implantation	28	0.8 µg/g creatinine[f]	
Non-United States				
(Adami *et al.* 2003) Italy	Metal-on-metal total hip replacement	15		4.1
(Lhotka *et al.* 2003) Not reported; hip manufacturers are European.	(Hip replacement)		NA	*Implant #1*
		24 (immediate PO)		3.23
		27 (3-6 mo)		10.88
		27 (12-15 mo)		23.34
		28 (35-38 mo)		36.55
		25 (42-48 mo)		16.95
				Implant #2
		24 (immediate PO)		8.13
		25 (3-6 mo)		14.83

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
		27 (12-15 mo) 26 (35-38 mo) 26 (42-48 mo)		23.34 171.35 27.66
(Savarino *et al.* 2014) Not reported; authors from Italy.	Hip resurfacing	14 (2 yr) 19 (5 yr) 22 (9 yr)		1.17 1.13 0.90
(Sidaginamale *et al.* 2013) UK	Metal on metal hip arthroplasty			*Implant #1 (416)* (467) 2.99 μg/L (median) (range 0.20–228) *Implant #2 (165)* 2.29 μg/L (median) (range 0.65–195 Implant #3 *2.63 μg/L (median)* (range 0.37–204)
(Witzleb *et al.* 2006) Not reported; replacements were from UK and Switzerland.	NA	56 (3 mo) 23 (24mo) 23 (24 mo) 3 (24 mo)		*Implant #1* 2.17 4.28 *Implant #2, bilateral* 3.18 Implant #2, unilateral 1.70
(Zeh *et al.* 2007) Not reported; prostheses from Germany and procedure carried out in Germany.	Metal-on-metal artificial lumbar disc implants	10		4.97 (serum)
(Zeh *et al.* 2009) Not reported; prostheses from	Metal-on-metal artificial lumbar disc implants	10 (Follow-up #1) 10 (Follow-up #2)		4.75 1.89

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
Germany and procedure carried out in Germany.				
Unclear				
(Coleman *et al.* 1973) (as cited in Schaffer *et al.* 1999)	Metal-on-metal total hip replacement		24.0	
(Hennig *et al.* 1992) (as cited in Schaffer *et al.* 1999)	Metal-on-metal total hip replacement		3.8[e]	
(Sarmiento-Gonzalez *et al.* 2008) Not reported; hip and knee joints made in US.	Hip or knee prostheses	11 (< 5 yrs) 10 (> 5 yrs) 12 (< 5 yrs) 10 (> 5 yrs) 11 (<5 yrs) 11 (> 5 yrs)		Implant #1 0.312 0.297 Implant #2 0.333 0.281 Implant #3 0.224 0.497
(Schaffer *et al.* 1999) Not reported; authors from Austria and prostheses from Austria	Metal-on-metal total hip replacement		5.5[e]	
Medical implants (unstable)				
United States				
NF				
Non-United States				
(Bradberry *et al.* 2014) Not reported; authors from UK.	Failed hip replacements	8 10		*Metal-on-metal* 34.5[e] *Ceramic* 506[e]
Unclear				
(Dunstan *et al.* 2005)	Radiologically loose hip	2	205	35.5

Reference	Sample population	Number of samples (N)	Urine cobalt conc.[a]	Serum plasma, or blood cobalt conc.[a]
England	implants			

Source: (Finley *et al.* 2012).
[a]Units are µg/L unless stated otherwise.
[b]Geometric mean.
[c]Reported urinary cobalt concentration is the geometric mean for the most recent (2011-2012) National Health and Nutrition Examination (NHANES) survey year for which data are available. Urinary cobalt data ranged from 0.316 to 0.379 µg/L for 1999 to 2012 (CDC 2015).
[d]As cited in (IARC 1991).
[e]Median.
[f]It is generally accepted that 1 L of urine contains 1 g creatinine.
[g]Value reported as 0.09 µg/mmol creatinine; converted to µg/g creatinine using the following conversion factor: 1 mol creatinine = 113.1 g creatinine.

Table B-2. Values for hair and nail cobalt levels (means or medians) in the United States and other countries (hair values are plotted in Figure 2-2.)

Author and Date	Location	Sample Population	Hair conc.	Nails conc.
General Population UNITED STATES				
(Kanabrocki *et al.* 1979)	unknown - US institutions	General population - details NR		Females 0.07 (0.02–0.15) Males 0.04 (0.01–0.15) $P < 0.05$
General Population Non-UNITED STATES				
(Elenge *et al.* 2011)	Katanga. Sante; Congo	General, non-industrialized population (medical students with no occupational hx with metals) of the copper-belt, Province of Katanga.	**Mean** µg/g 1.67 5th %ile = 0.80; 95%ile = 2.02	
(Carneiro *et al.* 2011)	Brazil	Healthy male and female urban students ages 12-18 years of age	**Mean (SD)** 0.008 (0.007) µg/g	**Mean (SD)** 0.08 (0.1) µg/g
Dongarrá *et al.* 2011	Sicily	Students 11-13 years of age	**Mean (SD)** 0.19 (0.33) µg/g Males 0.26 (0.51) Females 0.16 (0.22)	
(González-Muñoz et al. 2010)	Spain	Normotensive post menopausal women	**Median** (min, max) µg/g 0.017 (0.013, 0.026)	
(Bergomi *et al.* 2002)	Emilia-Romagna region, No. Italy	Randomly sampled controls for an ALS study enrolled in the Italian NHS.		25th %ile 0.009 50th %ile 0.015 75th %ile 0.031
(Campbell *et al.* 1988)	UK	Healthy controls (hosp staff, volunteers) on no medication	**Mean** (SD) µg/ml Controls 0.0670 (0.0232) P = NS	
Environmental Non UNITED STATES				
(Bibi *et al.* 2015)	Lahore district,	Persons of various ages from 3 sites near		**Mean** By age

Author and Date	Location	Sample Population	Hair conc.	Nails conc.
	Punjab province, Pakistan	industrial areas used for agricultural purposes ranked as high, medium, and low exposure Compared with people living far from Arsenic contaminated regions of Lahore who never drank Arsenic contaminated water		10–15 yr 0.47 25–35 yr 0.38 40–50 yr 0.47 *P* = 0.55 By risk area Low risk 0.59 Medium risk 0.53 High risk 0.32 Control 0.22 *P* = 0.00
(Mohmand *et al.* 2015)	Punjab, Pakistan	Sampled in rural, urban, and industrial areas of the city	**Mean** (SD) ppm Rural 0.3 (0.2) Urban 0.2 (0.1) Industrial 0.2 (0.1)	**Mean** (SD) ppm Rural 1.7 (2.2) Urban 0.2 (0.2) Industrial 0.2 (0.1)
Occupational **Non UNITED STATES**				
(Saat *et al.* 2013)	Malaysia	Vegetable farmers	ppm 0.01211 +/- 0.00158	ppm 0.01491 +/- 0.00164
(Afridi *et al.* 2009)	Pakistan	Steel mill workers and Non-exposed males 25–55 years of age	**Mean** (SD), **Range** µg/g Production 4.67 (0.8) (3.57–5.29) QC 2.48 (0.5) (1.89–3.09) Control 1.1 (0.2) (0.88–1.36)	
(Sabbioni *et al.* 1994a)	Italy	Hard metal M and F workers from four plants and seven individuals, 15–65 years of age, with up to 22 yrs duration of employment; N = 251, with 23 diseased workers); Diseased subjects had asthma and/or a fibrosis; And Non-diseased workers	**Mean** (SD), **Range** µg/g Diseased workers 49.088 (114.194), 0.110–910 MD = 16.475 **Non-Diseased**	**Mean (SD), range** µg/g Diseased workers 53.792 (107.175), 0.109–580 MD = 15.250 **Non-Diseased**

Author and Date	Location	Sample Population	Hair conc.	Nails conc.
			workers 9.607 (10.664), 1.050–29.90 MD = 4.8	**workers** 18.9 (27.33), 2-109 MD = 6.6
(Bencko *et al.* 1986)	Czech Republic.	Nickel and cobalt production workers And age-matched healthy workers unexposed to Co	**Mean** µg/g Exposed 96.8 Unexposed 0.38	
Hip Implants (Stable) **Non UNITED STATES**				
(Rodriguez de la Flor *et al.* 2013)	Spain	Patients with metal-on-metal resurfacing arthroplasty before and after revision surgery	**Mean (range)** µg/g **Mean (µg/g), range** Before revision surgery 147.4 (3.7–618.8) After revision surgery 47.11 (0.3–205.8) p=0.249	
Liu et al. 2011	China	Patients with metal-on metal hip resurfacing arthroplasty Compared with Pts with metal-on-polyethylene hip arthroplasty	**Mean** (µg/g) (SD) **Metal-on-metal** Preop 4.3532 (2.1346) 6 mos post-op 53.2882 (11.8431) 12 mos post-op 47.3995 (10.0417) **Polyethylene bearings** Preop 3.1460 (2.4172) 6 mos post-op 3.3920 (1.6864) 12 mos post-op 4.2170 (2.4552)	
(Coleman *et al.* 1973)	UK	Patients with total hip replacement compared to those without hip implant	**Mean (range) ppm** Cases 0.42 (0.06–2.3) Controls 0.22 (0.07–0.49)	

B.2 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 Appendix B 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.2.1 discusses the studies of patients with cancers of the lung and larynx, which have been identified as cancer sites of interest, and Section B.2.2 discusses studies of patients with cancer or tumors at other tissue sites (breast, brain, colon, leukemia, and thyroid).

B.2.1 Studies of lung or laryngeal cancer patients

Appendix Table B-3 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.2.2 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. In addition to these studies, the occupational study of copper smelter workers discussed above for lung cancer (Gerhardsson *et al.* 1993, Gerhardsson *et al.* 1985, Gerhardsson *et al.* 1984), measured cobalt in liver and kidney tissues. In contrast to the findings for lung tissues, cobalt concentrations in liver and kidney tissue were similar among deceased workers as the rural referents (Gerhardsson *et al.* 1984).

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

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

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

B.2.3 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 (Kanias *et al.* 1994) or levels were higher in the referent group (lung disease) compared to lung cancer patients (Kuo *et al.* 2006). In a series of studies (Gerhardsson *et al.* 1993, Gerhardsson *et al.* 1985, Gerhardsson *et al.* 1984), cobalt levels were higher in lung tissues (but not liver or kidney) from cancer cases from deceased cobalt-exposed workers compared to the same type of tissue from the rural referent group who died from other causes.

Studies comparing cobalt levels in tumor and non-tumor tissue (from the same or different subjects) or by cancer stage were conflicting and were limited by only one or two studies available for each type of cancer. Higher levels of cobalt were found in tumors of the larynx (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-3. Findings from studies that measured cobalt in tissues (means or medians) of lung cancer patients and referents

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

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	Correlation of cobalt with scandium in fibroadenoma and with zinc with combined fibroadenoma & fibrocystic disease
(Benderli Cihan *et al.* 2011)	Turkish clinical study Breast cancer from one hospital and volunteers or employees at the hospital (same age)	breast (stage III) hair	52 52	cancer patients healthy humans	0.664 ± 0.566* 0.269 ± 0.390	3 g; ICP-MS	Cobalt was correlated with several other heavy metals in cancer patients
(Arslan *et al.* 2011)	Turkey clinical survey Patient with malignant glial tumors operated from one clinical center and healthy humans.	brain serum	22 22	Cancer patients healthy humans	0.04 ± 0.03 (µg/dL) 0.03 ± 0.03 (µg/dL)	frozen; atomic absorption spectrophotometer (AAS)	No information on healthy humans NS
(Alimonti *et al.* 2008)	Italian clinical survey Male and female patients with colorectal polyps and control group from same hospital	colorectal polyps colorectal tissue	17 17 15	tumor/polyps Normal tissue/polyps normal tissue/controls	[0.019 ± 0.016]* [0.04 ± 0.02] [0.03 ± 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
(Demir *et al.* 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 *et al.* 2011)	Chinese case-control; 2007–2008 Newly diagnosed male and female cases (71) of childhood leukemia (15 yr or less) at a hospital and sex- and age-matched controls	urine childhood leukemia	71 113	childhood leukemia cases controls	0. 98 (0.57–2. 28) ng/mg creatinine 0.77 (0.44–1. 44) ng/mg	inductively coupled plasma mass spectrometry (ICP-MS)	NS
(Yin 1990)	Chinese clinical survey Male and female liver cancer cases (930) and age-matched healthy adults selected from the same hospital	liver cancer serum	30 30	liver cancer cases healthy adults	0.0085 ± 0.0017 ppm* 0.0035 ± 0.0012 ppm	ICP-AES	Cobalt levels correlated with other metals
(Reddy *et al.* 2002)	Indian thyroid samples Normal thyroid, adenoma, carcinoma samples from four subjects from pathology dept.	thyroid cancer thyroid tissue	NR NR NR	carcinoma adenoma normal thyroid	17.9 ± 2 11.6 ± 1.2 11.3 ± 1.2	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
(Adachi *et al.* 1991)	Japanese clinical survey	lung cancer	224	lung cancer	0.33 ± 1.49	dried and digested; atomic absorption	NS

Reference	Population	Cancer tissue	Number of subjects	Category	Co levels (µg/g dry tissue)	Exposure methods	Comments
			1,715	others cases	0.27 ± 0.41		
(Benderli Cihan and Öztürk Yildirim 2011)	Turkish hospital-based case-control study Male non-small cell lung cancer (NSCLC; stage IIIB) and controls from the same geographical region using similar inclusion/criteria; similar age and ethnic background; all smokers	lung cancer hair	67 74	NSCLC controls	[0.0031 ± 0.011]* [0.0004 ± 0.0005]	3 g; inductively coupled plasma mass spectrometry (ICP-MS)	$P < 0.05$
(De Palma *et al.* 2008)	Italian clinical study NSCLC and controls (men and women) undergoing pulmonary resection (lung metastasis from other cancers and lung disease) from the same hospital; smoking higher in cases	lung cancer lung biopsies	45 45 8	NSCLC (non-tumor tissue) NSCLC (tumor) controls	0.07 (0.05–0.11) 0.05 (0.01–0.10) 0.04 (0.02–0.18)	dried and digested; ICP-MS; standard	NS No differences in cobalt levels in non-tumor tissue in occupationally exposed (to metals) vs. non-exposed subjects and in smokers vs. non-smokers
(Gerhardsson *et al.* 1993,	Swedish male smelter workers and rural and	lung and other cancers	7	workers/lung cancer	[0.015]	freeze dried; irradiated; neutron	Mean exposure duration 31.2 ± 8.4

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/cardiovascular	[0.016]***		
			12	workers/other causes	[0.016]*		
			65	all workers	[0.015]***		
			14	rural referents	[0.007]		
(Kuo *et al.* 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 variables such as
			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
							sex, location, smoking
(Zhang *et al.* 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 *P* = 0.051
(Collecchi *et al.* 1986)	Italian clinical study Males without known exposure to arsenic and cobalt with laryngeal carcinoma and "normal males"	larynx cancer larynx tissue 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 *et al.* 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	

B.3 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 ≥ 0.5 mg cobalt as a cobalt salt per dosage unit and which recommend administration rates of ≥ 0.5 mg per dose and ≥ 2 mg per 24-hour period.

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

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

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

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

Occupational Safety and Health Administration (OSHA)

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

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

Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

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

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

Consumer Product Safety Commission (CPSC)

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

Environmental Protection Agency (EPA)

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

National Institute for Occupational Safety and Health (NIOSH)

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

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

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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 (Table 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 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
Reference	*Tüchsen et al. (1996)* Tüchsen F, Jensen MV, Villadsen E, Lynge E. Incidence of lung cancer among cobalt-exposed women. Scand J Work Environ Health. 1996 Dec;22(6):444-50. PubMed PMID: 9000312.
Study-design type	Cohort
Location and enrollment dates	Copenhagen, Denmark; Jan 1, 1943 (Factory 1) or Jan 1, 1962 (Factory 2) - Dec 31, 1992
Population description	Danish women porcelain plate workers.
Eligibility criteria	All women employed at any time in two underglaze porcelain plate departments (Factory 1 and Factory 2); and all female top glaze decorators in a department without cobalt exposure (Factory 1).
Cohort details	Population size: 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.
Outcome data source	Followed for death and emigration using data in the Central Population Register and the municipal population registers. Cancer cases identified by linkage to Danish Cancer Register (ICD-7).
Exposure assessment	Company records
Exposure assessment notes	Exposure to cobalt-aluminate spinel and/or cobalt silicate at 2 factories. Detailed information on work history; exposure monitoring data was reported for air and urine from the 1980s which was not used in the exposure assessment; calendar period was adjusted for in analysis.
Exposure-level	Employment in factories/departments with or without cobalt
Co-exposures	Nickel, silica
Analysis methods and control for confounding	Analytical methods: Personnel files for permanently ill persons may have been removed in earlier years, potentially resulting in an underestimate of incidence. Covariates: Age Confounder consideration: Calculation of expected number of cancer cases took 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	*Mur et al. (1987)* Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. Am J Ind Med. 1987;11(1):75-81. PubMed PMID: 3812499.			
Study-design type	Cohort and nested case control study			
Location and enrollment dates	France; 1950-1980			
Population description	**Male electrochemical workers** including cobalt production workers			
Eligibility criteria	N = 1,143. All men employed for at least one year at a cobalt production plant producing cobalt, cobalt salt and oxides, and sodium between 1950 and 1980; hired between 1900 and 1979.			
Cohort details	*Population size:* N = 1,143; number of cobalt production workers not reported but ~ 25% of current staff at publication *Loss-to-follow-up:* 17.9% for cobalt production workers; 75% hired before 1975. *Referent Group:* Internal and external comparing cohort mortality to male mortality in France			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	9	NR	All lung cancer cases from cohort
	Controls	18	NR	Two controls/case were matched on year of birth and age at death and "smoking habits" (undefined); controls were selected from among those dying of conditions other than cancer.
Outcome data source	Vital status ascertained by registry offices in the birth places of Frenchmen, and at embassies and consulates for foreign-born. Cause of death (ICD-8) ascertained by physicians and medical records. 80% of causes of death determined and classified.			
Exposure assessment	Company records			
Exposure assessment notes	Job histories grouped according to employment in general service, maintenance, sodium or cobalt production. Only included those with exclusive employment in any of these departments. No Co levels reported, nor were prior measurements available.			
Exposure-level	60% worked greater than 10 years; 75% hired before 1975			
Co-exposures	Arsenic, nickel			
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* age, year of death SMR all cause mortality = 0.77 ($P < 0.01$); no methods to control HWE; all cause mortality for cobalt production = SMR 1.29 (0.86–1.87). *Analytical method case control study* *Covariates:* None			

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

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

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

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

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

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

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

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

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

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

Field	Description			
Reference	*Grimsrud et al. (2005)* Grimsrud TK, Berge SR, Haldorsen T, Andersen A. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel?. Epidemiology. 2005 Mar;16(2):146-54. PubMed PMID: 15703528.			
Study-design type	Nested Case-Control			
Location and enrollment dates	Norway; 1910–1995			
Population description	**Norwegian nickel refinery workers**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	213	NR	lung cancers diagnosed from 1952-1995 and in the Cancer Registry of Norway during this time.
	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 $\mu g/m^3$: 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	*Analytical methods:* *Covariates:* smoking *Confounder consideration:* 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 et al. (1993)* Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. A case-control study of element levels and cancer of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev. 1993 Jul-Aug;2(4):305-12. PubMed PMID: 8348053.			
Study-design type	Case-Control			
Location and enrollment dates	Western WA state, USA; 9/1/83 - 2/28/87			
Population description	**Population based randomly selected controls and cases from SEER**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	N = 507; N = 153 laryngeal, N = 73 esophageal, N = 359 oral cavity cancers	52.8% providing toenail samples	Laryngeal, esophageal or oral cavity cancers of epithelial origin identified from local SEER registry with positive histological findings; some cases confirmed by cytology and followed with attending physician.
	Controls	N = 434	66.4% providing toenail samples	Controls from same area as cases selected by random digit dialing and frequency matched by sex and age in 5 year intervals of cases.
Exposure assessment	personal monitoring			
Exposure assessment notes	Dietary sources of trace elements of cobalt, iron, calcium, zinc, chromium explored; toenails collected and cleaned; personnel blinded to case status; formation of nail matrix takes 8-24 mos; median time from dx to interview was 6.5 mos, so samples likely to represent prediagnostic levels; qx data on occupation collected but not reported.			
Exposure-level	Tertiles of Cobalt in toenails; highest level 0.17 ppm			
Co-exposures	Iron, calcium, zinc, chromium			
Analysis methods and control for confounding	*Analytical methods:* "Exposed cases" in this table refers to both cases and controls combined; exposed cases alone NR. *Covariates:* age, alcohol (drink years), ascorbic acid mg/day, beta-carotene, mg/day, energy intake, kcal/day, sex, smoking (pack-years) *Confounder consideration:* Nutrients in the model did not greatly confound the relationship between exposure and disease, but inclusion resulted in ORs closer to the null. ORs for Esophageal cancer significantly elevated for iron and calcium			

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

Field	Description			
Reference	*O'Rorke et al. (2012)* O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, FINBAR Study Group. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. Int J Cancer. 2012 Oct 15;131(8):1882-91. PubMed PMID: 22262413.			
Study-design type	Case-control study			
Location and enrollment dates	All Ireland (Republic and Northern); 3/2002 - 12/2004			
Population description	**Population based cases and controls**			
Case-control description and eligibility criteria		*Population size*	*Response rates*	*Source*
	Cases	N = 137 esophageal cancer; N = 182 Barrett's esophagus	Esophageal CA = 38.6%; Barrett's esophagus = 66.9	No. Ireland: Esophageal cases (≤ 85 yrs) identified from electronic path records from all path labs. Rep of Ireland: cases identified from the main referral hospitals diagnosing and treating esophageal CA. Pathology review and histologically confirmation; excluding in situ CA. Barrett's esophagus - pts with >= 3 cm of Barrett's mucosa at endoscopy or biopsy showed specialized intestinal metaplasia. Pts with dysplasia on histology excluded.
	Controls	221	35.5	Adults (35 to 84) without history of esophageal or other gastrointestinal cancer or known diagnosis of Barrett's esophagus; frequency matched by sex and age (5 yrs). Selected at random from general practitioner (GP) list (No. Ireland) and from 4 GP practices (2 rural and 2 urban) in Dublin and Cork areas that reflected the distribution of the Rep. Ireland cases
Exposure assessment	Personal monitoring			
Exposure assessment notes	Cobalt level in toenails; tertile cutpoints of log(e) transformed Co based on control distribution. Questionnaire for demographics, lifestyle habits, diet, manual/non-manual occupation, and medical history; anthropometric measurements; personnel blinded as to case status.			
Exposure-level	Average (μg/g) ± SD: cases – 0.02 ± 0.06; controls – 0.02 ± 0.04. Range: cases 0.002 – 0.60; controls – 0.002 – 0.47			
Co-exposures	Selenium, iron, chromium, zinc			
Analysis methods and control for confounding	*Analytical methods:* *Covariates:* GI reflux, H. pylori infection, age, education, energy intake, location, sex, smoking, smoking habits *Confounder consideration:* Unadjusted model almost identical results to the age and sex adjusted model., other metals measured included selenium, chromium, zinc, mercury, cerium. No correlation with cobalt reported. Not in models.			

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

This Appendix provides (1) an assessment of the study quality, sensitivity, and utility of the human studies to inform the cancer hazard evaluation, and (2) study quality and utility summaries for cohort studies and for case-control studies. Each primary study was systematically evaluated for its utility to inform the cancer hazard identification using core, signaling and follow-up questions outlined in the protocol (NTP 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.* 2000a, 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, these workers were either right-censored 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). No information to assess or methods to correct for potential right censoring were employed in these studies. Moulin *et al.* (2000a) addressed the possibility of a healthy worker survival effect (HWSE directly), and lagged the exposure in these analyses and matched on employment in the nested case-control study. The possibility of an HWSE also existed for the electrochemical workers (Moulin *et al.* 1993) as this study included prevalent workers. The existence of an HWSE is likely to bias the effect estimate downward.

Evidence of a healthy worker effect (HWE) based on external analyses showing statistically significant decreases in all-cause mortality rates was present in the Moulin *et al.* (1993) (foreign-born only), Moulin *et al.* (1998), and Moulin *et al.* (2000a) studies. In each study, internal analyses were conducted that have the effect of minimizing the effect the HWE, although no adjustment in any analysis was made for time since hire (in the case of Moulin *et al.* [1993], an internal analysis was conducted in the earlier study (Mur *et al.* [1987]).

In the electrochemical workers cohort (Moulin *et al.* 1993) and the Tüchsen et al. (1996) porcelain painters, the start of the follow-up and start of exposure did not coincide, indicating

that potentially healthier prevalent hires may have been selected into the cohort which could induce a downward bias in the effect estimate.

Case-control studies

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

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

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

C.2.2 Evaluation of methods to address confounding

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

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 substudies 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.* 2000a), 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 mg/m^3), silica (no detectable concentrations), and dust (average of 7.6 mg/m^3) measured in 1981 prior to changes in practices that reduced air levels of cobalt somewhat. The electrochemical workers cohort may have been exposed to arsenic, which is added during the production process, and nickel is 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, although the authors reported no matrix of correlations among the multiple metals analyzed in these studies. 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 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 *et al.* 1996)	*Rating*: **++;** Direction ↓ *Rationale:* No HWE was evident; only external analyses comparing cancer incidence in the Danish population. The start of the follow-up and start of exposure did not coincide, indicating that potentially healthier prevalent hires may have been selected into the cohort which could induce a downward bias in the exposure estimate. Inadequate information to determine if women who worked prior to the start of follow-up were included in the cohort. It's possible that records of some permanently ill workers were not included in newer registers, resulting in an underestimate of the true incidence of cancer.	*Rating;* **++;** ↑ *Rationale*: Smoking data available from auxiliary sub-studies only; minimal exposure to occupational co-exposures indicated from measurements in 1981. No internal or statistical analysis controlling for smoking or potential carcinogens.
(Mur *et al.* 1987) (Moulin *et al.* 1993)	*Rating:* ++ (Mur for case-control analysis); ++ (Moulin 1993; French nationals analysis); ↓ *Rationale:* HWE effect based on significant decreases in all-cause mortality rates (Mur *et al. 1987*); internal analysis reported would minimize bias. High loss to follow-up in both studies due to foreign-born workers, with no information provided to assess if the loss was related to exposure. Moulin *et al.* 1993 provided a restricted analysis for French nationals in addition to a whole-cohort analysis, to mitigate a potential bias. HWSE is a possibility given the mix of prevalent and new hires in the cohort; however, no exposure-response estimates were provided.	*Rating;* +; ↑ *Rationale*: Mur et al. reported smoking data from admin records available from 30% of the cohort (Mur *et al.* 1987), although cases and controls were reported as matched on smoking status with no report on methods of matching. Moulin *et al.* 1993 did not adjust for smoking. No major occupational co-exposure identified. Internal analysis (nested case-control) available in the Mur *et al.* 1987 study to help minimize potential confounding. No analysis controlling for potential carcinogens.

Study	Selection bias	Methods used to address potential confounding
(Moulin *et al.* 1998)	*Rating: ++ for nested case-control analysis;* ↓ *Rationale:* High loss to follow-up largely due to foreign-born workers who were right censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that loss was related to exposure. Concerns with statistically significant decreases in all-cause mortality rates (HWE) mitigated by nested case-control analysis. Left-truncation not an issue in this incidence cohort.	*Rating;* +; ↑ Rationale: Ever vs. never smoking data collected, and ever/never data on co-exposures in the JEM. Unlikely if models for cobalt alone included smoking and co-exposures (based on communication with author). Little information provided on cobalt co-exposures. Internal analyses may help reduce potential confounding from 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.
(Wild *et al.* 2000)	*Rating:* ++; ↓ *Rationale:* High loss to follow-up largely due to foreign-born workers who were censored at the last date of employment; no g-methods or additional information provided to assess/mitigate potential HWSE. No indication that the loss was related to exposure. No case-control analysis of cobalt alone provided. Left-truncation not an issue in this incidence cohort.	*Rating;* +; ↑ *Rationale*; Smoking data abstracted from factory health records and for earlier smoking from former colleagues. Co-exposures to several IARC carcinogens likely (no correlations provided) and assessed (ever/never) from JEM exposure. Unlikely that smoking and co-exposures were included in estimate for cobalt alone exposure. See Moulin et al. 1998 also.
(Moulin *et al.* 2000a)	*Rating:* +++ *Rationale:* HWE effect based on significant decreases in all-cause mortality rates in external analysis; cross-sectional cohort, internal analyses may have mitigated potential HWE bias. Nested case-control study of living controls matched on age at selection with the case; similar as to their distribution of year of hire, suggesting that it is unlikely that the HWSE was operating.	*Rating:* ++; ↑ *Rationale:* Statistical analysis controlled for smoking (ever-never) for 71% of subjects and some co-exposures (levels assigned from JEM). However, the models did not control for co-exposure to other metals (nickel/chromium and iron), which were correlated with exposure to cobalt, although not to lung cancer in these data.

Study	Selection bias	Methods used to address potential confounding
(Grimsrud *et al.* 2005)	*Rating:* +++ *Rationale:* No information about whether HWE was present in the original cohort, but only cases who were identified from 1970 onwards were included; controls were matched to cases by gender and age and at risk at the time of the case diagnosis, and were similar with respect to year of first employment, reducing the concern with HWSE. However, some concern remains given that sick workers hired at the same dates as healthy workers could move from higher to lower exposure groups. No analysis was conducted to account for this possibility. Participation rates were 94% for both cases and controls.	*Rating:* ++; ↑ Statistical analyses controlled for smoking (5 categories) and exposure to lung carcinogens (arsenic, asbestos, sulfuric acid mists and nickel). Due to the high correlation between cobalt and nickel exposure, categorical levels of cobalt variable could not be retained in the fully adjusted model. Only smoking adjusted exposure response estimates were available.
(Rogers *et al.* 1993)	*Rating:* +++ *Rationale:* Ascertained all cases of aerodigestive cancer from the Western Washington State SEER cancer registry and used random digit dialing to identify controls in the same defined area as controls.	*Rating:* +++ *Rationale:* Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol and tobacco use among cases and controls. Differences in education, not occupation reported. Food frequency questionnaire based on usual dietary habits 10 years prior to the interview.
(O'Rorke *et al.* 2012)	*Rating:* ++ *Rationale:* Methods for case and control selection vary by location. Northern Ireland cases identified systematically from electronic pathology records across the country; cases in the Republic were identified from "main" hospitals involved in the diagnosis and treatment of esophageal cancer. Potential for cases not referred to the major referral centers to be excluded. Very low participation rates and lower smoking rates in controls suggests the potential for selection bias is present due to lifestyle factors related to esophageal cancer.	*Rating:* +++ *Rationale:* Frequency matched controls to cases within 5-year age bands and sex strata; collected and reported information on a wide range of risk factors and potential confounders including past dietary data, alcohol, manual labor, and tobacco use among cases and controls. Dietary intake based on food frequency questionnaire assessing diet and alcohol use 5 years previously.

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.

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 because the window of exposure implied by measuring metals in nails

for cancer outcomes may not be valid. Toenail clippings likely reflect an integrated exposure that occurred 12 to 24 months prior to clipping, depending on age (with nails growing more slowly in older individuals) (Fleckman 1985) and are likely to not reflect the relevant period of exposure for esophageal and aerodigestive cancers, which are associated with long latency periods. Furthermore, reproducibility of cobalt 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). O'Rorke *et al.* (2012) reported that 76.7% of esophageal cancer cases had habitually clipped their toenails prior to admission to hospital, casting further doubt on the value of a sample at a single point in time. Finally, as nail samples were collected approximately 6.5 months after diagnosis and after participants were enrolled in the study (Rogers *et al.* 1993), it may be difficult to ascertain if the exposure changed as a consequence of the disease process itself (reverse causation). Given that some trace element deposition in nails is 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) the cancer process itself among cases could introduce bias. Overall, the direction of the bias is unknown since the study does not capture the most relevant time of exposure (which could be missed) or the possibility of reverse causality.

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'Rorke *et al*. 2012).

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

Study	Exposure misclassification	Outcome misclassification
(Tüchsen *et al.* 1996)	*Rating*: ++**,** Direction ↓ *Rationale:* Exposure designated by employment in a department considered to include exposure to cobalt or not; calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level or duration	*Rating;* +++ *Rationale*; Incident cases of lung cancer obtained through linkage with the Danish Cancer Registry
(Mur *et al.* 1987) (Moulin *et al.* 1993)	*Rating:* ++**,** ↓ *Rationale:* Exposure was assigned based on job location in various workshops including cobalt production, calendar periods of different exposures not incorporated into analysis; no information on intensity, frequency, level or duration.	*Rating;* ++ Some evidence that lung cancer cases may have been missed. Cause of death ascertained by physician interviews and medical records (Mur *et al.*), and only by death certificates in the re-analysis (Moulin *et al.*), resulting in one of four cases being dropped. Mortality may have missed cases certain cases with better survival (e.g., laryngeal, oral cavity and pharyngeal cancers), which were first reported by Mur et al., but not by Moulin et al.
(Moulin *et al.* 1998)	*Rating:* ++ to +++**,** ↓ *Rationale*: JEM based on expert judgment and limited data from records. Intensity scores (0-9), and frequency of work time exposed (<10%, 10-50%, >50%). JEM exposure scores for cobalt validated but did not include cobalt powder production areas.	*Rating;* +++ *Rationale*: Mortality data are adequate for lung cancer which has a low survival rate.
(Wild *et al.* 2000)	*Rating:* ++ to +++**,** ↓ *Rationale:* Similar JEM as Moulin 1998. As the focus of the study was on hard metals, exposures within other workshops (i.e., cobalt powder production) were assessed in less detail or were not precise as those for cobalt-tungsten carbide.	*Rating;* +++ *Rationale*: Mortality data are adequate for lung cancer which has a low survival rate.

Study	Exposure misclassification	Outcome misclassification
(Moulin *et al*. 2000)	*Rating:* ++, ↓ *Rationale:* JEM for specific job periods based on expert's subjective quantification which was based on interviews with former and current workers in each workplace and on measurements in other French factories and published results from the literature. No airborne exposure level measurements used to validate these judgments and little information on past exposures. Categories of exposure were based on frequency, intensity, duration, and probability.	*Rating;* +++ *Rationale*: Mortality data, which is adequate for lung cancer that has a low survival rate
(Grimsrud *et al.* 2005)	*Rating:* +++, ↓ *Rationale:* Quantitative JEM developed for nickel analysis using time- and department-specific exposure estimates plus a cobalt surrogate intensity measurement based on estimated levels of time- and department-specific periods from 3,500 personal samples of cobalt air concentrations from the breathing zone. Low level of concern for non-differential exposure misclassification as monitoring did not begin until 1973 and personnel files carried some degree of uncertainty concerning the earlier decades.	*Rating;* +++ *Rationale;* Incident cases of lung cancer obtained through linkage with the Norwegian Cancer Registry.
(Rogers *et al.* 1993)	*Rating:* +; direction not known *Rationale:* Window of exposure implied by a single measurement of cobalt in nails (12-18 week exposure) for cancer outcomes may not be valid for cancer induction of esophageal cancer. Reverse causation may be present as cobalt was measured post-diagnosis and deposition of minerals in nails can be affected by cancer process (reverse causation).	*Rating;* +++ *Rationale*: Cases identified through the local SEER cancer registry; diagnosis based on a positive histological finding or a positive cytology with follow-up to the attending physician to confirm the diagnosis. Potential controls with a history of any cancer were excluded.
(O'Rorke *et al.* 2012)	*Rating:* +; direction not known *Rationale:* Window of exposure implied by a single measurement of cobalt in nails (12-18 week exposure) for cancer outcomes may not be valid for cancer induction of esophageal cancer or Barrett's esophagus. Reverse causation may be present as cobalt was measured post-diagnosis and deposition of minerals in nails can be affected by cancer process (reverse causation).	*Rating;* +++ *Rationale*: Esophageal adenocarcinoma cases had a histologic confirmation of adenocarcinoma within the esophagus, and excluded *in situ* cancers. Available clinical and histologic records (surgical and radiological reports) were reviewed by 3 authors and a pathologist to confirm location of the tumor in the esophagus. Potential controls had no history of esophageal or any gastrointestinal cancer or Barrett's esophagus.

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

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. Rogers conducted stratification of the 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), which may provide some information to evaluate reverse causality.

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

Study	Selective reporting	Analysis
(Tüchsen *et al.* 1996)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* ++ *Rationale:* SMR external analysis only conducted; no internal analyses conducted to account for potential confounding, or induction period. No exposure-response analyses.
(Mur *et al.* 1987) (Moulin *et al.* 1993)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Conducted cobalt-specific external and internal analyses, and did not report exposure-response analyses or particular methods for analysis of the data (Mur); Moulin restricted analyses to French-born workers exposed to cobalt exclusively, to minimize bias from selection or confounding. Unclear whether adjustments for censoring or recent exposures (lagging) were considered to address the large number of electrochemical workers who worked fewer than 10 years, which would address potential bias from inclusion of short-term workers with potentially lower exposures.
(Moulin *et al.* 1998)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Conducted internal cobalt analysis with trend analyses for exposure to cobalt alone by levels of intensity, duration, and cumulative (weighted or unweighted) doses using a fit of the ranks of the recoded variables as a test for trend.
(Wild *et al.* 2000)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* ++ *Rationale:* Conducted external SMR analysis only for cobalt alone with little documentation of what the models included
(Moulin *et al.* 2000a)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Methods, assumptions, and statistical analysis is described in detail. Analyses using categorical and continuous variables were reported and methods of model fitting described
(Grimsrud *et al.* 2005)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Methods, assumptions, and statistical analysis is 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 *et al.* 1993)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Categorical analyses using unconditional logistic regression to calculate ORs as estimates of the relative risk of each cancer, adjusting for the effects of potentially confounding factors.
(O'Rorke *et al.* 2012)	*Rating:* +++ *Rationale:* No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.	*Rating:* +++ *Rationale:* Backwards elimination approach using multivariate logistic regression investigating the association between tertiles of toenail trace element concentrations (log transformed) and the risk of esophageal cancer

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

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 μ/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 (μg/g) of cobalt concentrations: O'Rorke *et al.* (Ireland) – < 0.004, 0.004 to < 0.011, and ≥ 0.011; Rogers *et al*. (Western Washington state U.S.A.) – < 0.05, 0.05 to 0.17, and > 0.17 μg/g. The range of levels in the O'Rorke *et al*. study are 0.002 to 0.60 μg/g (mean = 0.02 ± 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 ± SD = 0.042 ± 0.023.

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

Appendix D: Cancer Studies in Experimental Animals

D.1 Study quality 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.1.1 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

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).

D.3.1 Study quality tables: Carcinogenicity studies

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

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

Overall utility: High

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

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

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

Overall utility: High

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

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

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

Study duration	+ Treated animals were sacrified at 6 and 8 months; animal sacrificed at 8 rather than 12 months because of tumor growth from expousre to cobalt nanoparticles. Controls were sacrificed at 6 and 12 months. Duration may not be adquate for evaluating cobalt metal.

Overall utility: Moderate

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

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

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

Overall utility: Low

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

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

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

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

Overall utility: Low

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

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

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

Overall utility: Low

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

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

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

Overall utility: High

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

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

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

Overall utility: High

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

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

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

Study duration	+ 8 months (experiment 1) or 12 months (experiment 2). The 8-month study would not normally meet inclusion criteria, but since tumors were induced it was included.

Overall utility: Low

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

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

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

Overall utility: Moderate

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

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

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

Overall utility: Moderate

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

exposure was for less than one year and histological examination was only done on organs with gross tumors, all of which would limit the sensitivity to detect an effect.

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

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls were used.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
Dosing regimen	++ Only a single dose level was tested, but it was given at two intensities, either weekly or daily (1/5 the level) for 2 years. There was no reported basis for that dose level.
Survival	NR No survival information was reported.
Pathology	++ Only tumors observed from gross examination were evaluated by histological examination.
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated at "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical purity, animal husbandry, and rationale for selecting the dose level. Survival was not reported.
Sensitivity	
Animal model	++ Only male rats were tested.
Statistical power	+ There were only 10 animals per group and survival was not reported.
Study duration	+++ Observation duration was for lifespan.

Overall utility: Moderate

Duration of exposure and observation were sufficient; one dose level was tested, but it was tested at two intensity levels. However, few animals per group were used and only included males; histological exam was only done on organs with gross tumors, which would limit the senstivity

to detect an effect. Reporting of animal husbandry, including disease surveillance, and chemical purity were poor and no survival data was provided.

Table D-1m. Gilman and Ruckerbauer 1962 (rats): Cobalt oxide; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls (vehicle – aqueous suspension of pencillin G procaine) were used.
Historical data	No No historical controls were available..
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was "washed" to remove water soluble impurities and was < 5 µm particle size. Cobalt was administered in an aqueous suspension of pencillin G procaine.
Dosing regimen	+ Only a single dose given at one dose level, but preliminary tests using unwashed unwashed particles, which contained an unknown water-soluble toxin that had killed many mice. Rats tolerated dose.
Survival	++ No survival-related effects at 90 days, only time period reported.
Pathology	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
Confounding	+ Animals and bedding periodically dusted with rotenone powder; not clear if the same rats were used from the preliminary experiment using unwashed cobalt.
Reporting and analysis	+ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis. Tumor incidences were reported as both sexes combined.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic rats were used.
Statistical power	+ Only 10 animals were tested and they were reported as both sexes combined.
Study duration	++ 1.3 years.

Overall utility: Low

The duration of observation was sufficient and both sexes were tested. However, only a single dose was given at one dose level with results reported as both sexes combined; few animals per group were tested. Reporting was poor and lacked information on chemical purity or stability, animal husbandry, and necropsy methods. Animal bedding was periodically dusted with rotenone powder and half of the exposed group had been administered unwashed cobalt, which was known to contain additional chemicals.

Table D-1n. Gilman and Ruckerbauer 1962 (mice): Cobalt oxide; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent controls (vehicle – aqueuous suspension of pencillin G procaine) were used.
Historical data	No No historical controls were available..
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ No information provided on chemical purity, stability, or homogeneity, other than that the test material was "washed" to remove water soluble impurities and was < 5 µm particle size. Cobalt was administered in an aqueous suspension in pencillin G procaine.
Dosing regimen	+ Only a single dose given at one dose level, but preliminary tests using unwashed particles, which contained an unknown water-soluble toxin that had killed many animals, was reported. Half the animals died in this study between 2^{nd} and 6^{th} day.
Survival	++ No treatment-related survival effects at 90 days, only time period reported.
Pathology	++ Necropsy was not reported, but metastasis was reported, suggesting that necropsies were done.
Confounding	+ Animals and bedding periodically dusted with rotenone powder. Half of the animals were given washed particles and the other half were survivors of animals given unwashed particles in a preliminary experiment, which contained an unknown water-soluble toxin that had killed many animals.
Reporting and analysis	++ Methods were poorly reported and lacked chemical purity or stability, animal husbandry, necropsy methods, or statistical analysis.
Sensitivity	
Animal model	++ Only non-transgenic female mice were tested.
Statistical power	++ A large number of animals were tested; however, survival was only reported for 90 days.
Study duration	+++ 2 years.

Overall utility: Low

The duration of observation and the numbers of animals per group were sufficient. Survival was only reported for 90 days. However, only a single dose was given (without rationale for level), to females only; half of them received unwashed cobalt, which was known to contain other chemicals. Reporting was poor and lacked chemical purity or stability, animal husbandry, and necropsy methods, though metastasis was reported suggesting necropsies were performed. Bedding was periodically dusted with rotenone powder.

Table D-1o. Wehner 1977: Cobalt oxide; Inhalation

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Concurrent untreated controls (sham-smoked) were used.
Historical data	No No historical controls were available.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ There was no information on chemical purity or stability of the cobalt.
Dosing regimen	++ Single dose level with no justification for choosing that level, but administered for life.
Survival	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls although surival was low in both groups.
Pathology	+++ Detailed necropsies were performed.
Confounding	++ Animal conditions were partly reported, but chemical purity and disease surveillance were not reported.
Reporting and analysis	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as "carcinoma" or "polyp" without saying what tissue they originated from is meaningless.
Sensitivity	
Animal model	++ Only male hamsters were tested. Hamsters are less senstive for evaluating lung tumors.
Statistical power	+ A large number of animals was used; however, statstical power was reduced by poor survival (fewer than 10 animals were alive at 18 months) in both cobalt-exposed and the corresponding control animals (IARC 1999).
Study duration	+++ Duration of treatment and observation was for the animals' lifespans.

Overall utility: Moderate

Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported.

Only a single dose level was tested (with no justification for choosing that dose level) in a large number of male hamsters. There was relatively poor survival in both exposed and control groups.

Study quality tables: Co-carcinogen studies

Table D-2a. Finogenova 1973: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	++ Two dose levels (10-fold apart from each other) of cobalt were tested and were given twice a week for only 8 weeks, but this is a co-carcinogen study.
Survival	NR No survival information was reported.
Pathology	++ Only local skin tumors were reported and described as to histologic grade. Full necropsies were not conducted. Because it's a co-carcinogen study and tumors from the known carcinogen are what is of interest and the types of induced tumors are already known or expected, lack of a full necropsy is less critical than for a carcinogenicity study.
Confounding	NR No information about chemical purity or animal husbandry, including disease surveillance, was reported.
Reporting and analysis	+ Very poor reporting. There is no chemical purity, stability, or homogeneity reported and no information on animal husbandry disease surveillance, duration of observation, extent of necropsy, survival, or tumor incidence (only tumor latency was reported).
Sensitivity	
Animal model	++ Only females of non-transgenic mice were tested.
Statistical power	+++ Large number of animals used, but survival was not reported.
Study duration	++ The duration of observation was unreported, but was at least 24 weeks, however, this is a co-carcinogen study.
Assay utility	+

Co-Carcinogen study

Overall utility: Low

All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. A large number of female animals per group were given two dose levels. However, there was very poor reporting. Incidence was not reported, only tumor latency and onset were reported. Survival, chemical purity or stability, animal husbandry, duration of observation, and extent of necropsy were not reported.

Table D-2b. Kasirsky 1965: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Known carcinogen alone control, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	++ A single dose level of the known carcinogen and four dose levels of cobalt chloride were given for about 10 weeks, though the exact duration of exposure was not clearly reported.
Survival	+ Survival was only reported for three dose levels of cobalt chloride in the first trial, but not in any groups of the second trial or the carcinogen alone control of the first trial. The high cobalt chloride dose level of the first trial reported conflicting survival between Table 1 and the summary at the end of the paper.
Pathology	+ No necropsies were performed, just measurment of tumor size by external examination and histological exam of excised tumors.
Confounding	++ Very few details were reported about animal husbandry and no information was reported about chemical purity.
Reporting and analysis	++ Results were not reported per sex and the duration was not clearly reported. Survival for all groups, chemical purity, and animal husbandry were not reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	++ Sufficient number of animals of each sex, but results were not reported as per sex. Survival was not clearly reported.
Study duration	++ It was not clearly reported, but is thought to have been at least 72 days, though this is a co-carcinogen study.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogenicity studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Neither duration, survival, chemical purity, stability or animal hubandry were clearly reported. Results were not reported per sex. A full necropsy was not conducted, so this study is only relevant to skin tumors induced by the carcinogen.

Table D-2c. O'Hara 1971: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ There was no untreated group and no cobalt alone group, but it's similar to a co-carcinogen study and did have a known carcinogen alone group.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	Inadequate Cobalt was administered after tumors had already started to develop; this is more like a tumor treatment study. Only two very similar dose levels were tested: 50 and 60 mg/kg.
Survival	+++ The high dose level caused an increase in mortality, however low survival was caused by tumors. Statistical analysis was not done on survival.
Pathology	++ No full necropsies were reported; only local skin tumors were reported.
Confounding	++ Very few details reported about animal husbandry and no information reported about chemical characteristics.
Reporting and analysis	++ Chemical purity or stability or disease surveillance methods were not reported and animal husbandry conditions were poorly reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	+++ The high-dose level group had a decrease in survival, but statistics were not calculated for survival and the low survival was caused by tumors.
Study duration	++ 17 weeks, but this is a co-carcinogen study and neoplasms were induced before cobalt exposure started.
Assay utility	Inadequate Co-carcinogen study

Overall utility: Inadequate

This study has little utility for evaluation because the cobalt was not administered until after tumors had developed. No necropsies were performed; it only looked at local tumors. Only two, closely spaced dose levels were tested. A good number of mice per group was used. Chemical purity or stability and animal husbandry or disease surveillance were not reported. All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity.

Table D-2d. Zeller 1975: Cobalt chloride; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ A cobalt chloride alone group was said to have been tested, but no results of that group were reported. The reported control was the known carcinogen alone, which is appropriate for a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+ Only one dose level of cobalt, given for 43 weeks, was tested with no rationale for choosing that dose level.
Survival	+++ There was no significant effect on survival from cobalt.
Pathology	++ Pathology procedures not described but histopathological evaluations were done on the respiratory tract and the liver.
Confounding	NR No information on animal husbandry conditions, disease surveillance, or chemical purity were reported.
Reporting and analysis	+ Poor reporting of chemical purity or stability, animal husbandry, or necropsy methods. Tumor incidences were reported as both sexes combined, making it impossible to examine sex differences.
Sensitivity	
Animal model	++ Both sexes of non-transgenic rats were tested; however, tumor incidences were reported as both sexes combined.
Statistical power	+ Only 12 animals per group per sex were tested, but tumor incidences were reported as both sexes combined.
Study duration	+++ Duration of treatment was 43 weeks and duration of observation was for the animals' lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. The study had a long duration of observation. However, poor reporting of chemical purity/stability, animal husbandry, necropsy methods, or necropsy with histological descriptions only of the respiratory tract and liver. Only one dose level was tested, without a reported rationale, on a small number of males and females, with tumor incidences reported as both sexes combined.

Table D-2e. Orzechowski 1964: Sodium cobaltinitrite; Injection

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+++ Six dose levels were given over 72 days and maximum dose level was based on toxicity in preliminary studies.
Survival	+++ Survival was high and similar to known carcinogen only controls.
Pathology	+ Necropsies were not done, only histological examination of tumors were done.
Confounding	++ Very few details reported about animal husbandry and no information was reported about chemical purity.
Reporting and analysis	+ No chemical purity or animal husbandry were reported. Tumor incidences were reported as both sexes combined.
Sensitivity	
Animal model	++ Both sexes of non-transgenic mice were used, though tumor incidence were only reported as both sexes combined.
Statistical power	+++ Large number of animals per group and experiments were conducted in triplicate and survival was high and similar to controls.
Study duration	++ A very short duration of 75 days was used, but it's a co-carcinogenicity study and tumors were induced.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Tumor incidences were reported as both

sexes combined. No information on chemical purity or stability or animal husbandry were reported. Six dose levels were tested, which were based on preliminary studies and a high number of animals per group was used, with experiments conducted in triplicate. Necropsies were not performed; histological examination was conducted only on tumors.

Table D-2f. Thompson 1965: Sodium cobaltinitrite; Drinking water

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ There were no untreated controls or cobalt alone controls, but there was a known carcinogen alone group and this is a co-carcinogen study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	NR No information about chemical purity, stability, or homogeneity was provided.
Dosing regimen	+++ Three dose levels were tested. The duration of treatment was not reported, but assumed to be about 11 weeks, but this is a co-carcinogen study.
Survival	NR No survival information was reported.
Pathology	+ No full necropsies were performed, just histology of the tumors and hematology measurements.
Confounding	++ Very little information about animal husbandry and no information about chemical purity or disease surveillance.
Reporting and analysis	++ Nothing was reported for chemical purity or stability, disease surveillance, survival, or duration of treatment. Dosing regimen and duration of observation were not clearly reported.
Sensitivity	
Animal model	+++ Both sexes of non-transgenic mice were tested.
Statistical power	+++ Large number of animals per group, but survival was not reported.
Study duration	++ Not clearly reported, assumed to be about 11 weeks, but this is a co-carcinogen study.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Both sexes of mice were tested with

three dose levels. Nothing was reported for chemical purity or stability, survival, duration of treatment, and animal husbandry and the dosing regimen were not clearly reported. Full necropsies were not done; histology was only performed on tumors.

Table D-2g. Steinhoff and Mohr 1991: Cobalt oxide; Intratracheal

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ No untreated control was used, but a benzo[a]pyrene-only control was included, which is consistent with a co-carcinogenicity study.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure."
Dosing regimen	+ Only a single dose level was used and given for 47 weeks for cobalt and 13 wk for benzo[a]pyrene.
Survival	NR No survival information was reported.
Pathology	++ Only organs with gross lesions and the respiratory tract were examined histologically.
Confounding	++ The method of manufacture of the cobalt was reported, but the purity was only stated as "chemically pure." Animal husbandry was described, but disease surveillance was not reported.
Reporting and analysis	++ Methods were poorly reported and lacked details about the chemical and animal husbandry.
Sensitivity	
Animal model	++ Only female rats were tested.
Statistical power	+++ A large number of animals were used and survival was similar to controls.
Study duration	++ Treatment with cobalt was for 47 weeks, benzo[a]pyrene was given for 13 wk, while observation was lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. One dose level was tested on a high number of females for almost a year, with observations for their lifespan. However, only partial necropsies were performed. Details of the chemical, animal husbandry, and survival were not reported.

Table D-2h. Wehner 1977: Cobalt oxide; Inhalation

Study utility domain Question	Rating Rationale
Selection of animals	
Controls	+++ Controls were cigarette smoke alone.
Historical data	No No historical controls were used.
Randomization	NR No information about randomization was provided.
Exposure	
Chemical purity	+ There was no information on chemical purity or stability of the cobalt and the composition of the cigarettes is complex and variable. However, information on the type of research cigarette (Kentucky IRI research cigarettes) was provided.
Dosing regimen	++ Single dose level with no justification for choosing that level, but administered for life.
Survival	+++ Cobalt had no significant effect on survival or body weight compared to untreated controls.
Pathology	+++ Detailed necropsies were performed.
Confounding	+ Chemical purity and disease surveillance were not reported. The uncertainty of the composition of the cigarettes may contribute to confounding.
Reporting and analysis	+ Methods were not fully reported with no information on disease surveillance or chemical purity. Tumor sites were not always defined. Tumor incidence reported as "carcinoma" or "polyp" without saying from which tissue they originated is meaningless.
Sensitivity	
Animal model	++ Only male hamsters were tested.
Statistical power	+++ A large number of animals were used and survival was similar to controls.
Study duration	+++ Duration of treatment and observation was for the animals' lifespan.
Assay utility	+ Co-carcinogen study

Overall utility: Low

All co-carcinogen studies were categorically restricted to being ranked no higher than low utility to account for their indirect measure of carcinogenicity. Duration of exposure and observation were sufficient. However, methods and results were not fully reported; chemical purity or stability, animal husbandry, and randomized allocation into groups were not reported as well as the tissue sites of the tumors. Full necropsies were reported. Only a single dose level was tested (with no rationale for choosing that dose level).

Table D-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 *et al.* 1965)	Cobalt chloride	+++	No	NR	NR	++	+	+	++	++	+++	++	++	+	Low
(O'Hara *et al.* 1971)	Cobalt chloride Sodium cobaltinitrite	+++	No	NR	NR	0	+++	++	++	++	+++	+++	++	0	In-adequate
(Zeller 1975)	Cobalt chloride	+++	No	NR	NR	+	+++	++	NR	+	++	+	+++	+	Low
(Orzechowski *et al.* 1964)	Sodium cobaltinitrite	+++	No	NR	NR	+++	+++	+	++	+	++	+++	++	+	Low
(Thompson *et al.* 1965)	Sodium cobaltinitrite	+++	No	NR	NR	+++	NR	+	++	++	+++	+++	++	+	Low
(Steinhoff and Mohr 1991)	Cobalt oxide	+++	No	NR	++	+	NR	++	++	++	++	+++	++	+	Low
(Wehner *et al.* 1977)	Cobalt oxide	+++	No	NR	+	++	+++	+++	+	+	++	+++	+++	+	Low

+++ = high quality/little to no concerns, ++ = moderate quality/moderate concerns, + = low quality/high concerns, 0 = inadequate, NR = not reported.

Appendix E: Genotoxicity and Related Effects

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). There is some indication of weak mutagenicity for cobalt compounds in some tester strains, including those that detect mutations involving GC base pairs, although the evidence is not entirely consistent.

Cobalt chloride was reported to be mutagenic in only some of the studies in three *Salmonella* strains (TA97, TA98, and TA1537) (Pagano and Zeiger 1992, Wong 1988) but not in other strains (TA100, TA102, TA1535, TA1538, TA2637). It was also mutagenic in one of several studies in *Escherichia coli* (Ogawa *et al.* 1999) but not *Bacillus subtilis*. Cobalt sulfate heptahydrate was positive in TA100 but not in TA98 and TA1537. A growth inhibition assay for cobalt chloride in *B. subtilis* had mixed results in two studies; however, the test that showed growth inhibition (positive results) was conducted using preincubation, which is a more sensitive assay that the standard plate incorporation assay. Cobalt metal was positive in the *Salmonella* strains in which it was tested (TA98 and TA100) but not in *E. coli*.

Positive results in a particular set of *Salmonella typhimurium* and *E. coli* bacterial tester strains can suggest very specific types of mutations. Tester strains TA100, TA102 and TA1535 are generally considered indicators of base-pair substitution. The frameshift mutation detected by *S. typhimurium* strain TA98 is a disruption of a dinucleotide run of $(CG)_4$ residues, while TA100 detects base-pair mutations in a codon for proline (GGG sequence) in the *histidine G46* gene.

Reverse mutations at the trpE ochre (TAA) codon can be identified by positive results in the *E. coli* WP2 *uvrA*/pKM101 strain. The results for these strains in the studies identified in this review are not strong; however, sequencing of the *supF* tRNA mutational reporter gene in bacteria exposed to cobalt chloride showed that both frameshift and base-pair substitution occurred at G:C base pairs (Ogawa *et al.* 1999).

Overall, the results for cobalt-induced bacterial mutagenicity are considered mostly negative without the addition of S9 and are completely negative in all assays with S9. The negative results for the assays that included the addition of S9 may be due to the presence of radical-scavenging enzymes in the mixture, which could eliminate a mutagenic effect; alternatively, proteins in the S9 mixture could bind the cobalt ions, rendering them ineffective as a mutagen.

Some studies reported anti-mutagenic effects. Potent anti-mutagenic effects were observed for cobalt chloride on reverse mutations induced by 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) in *Salmonella* strains TA98 and TA1538. Cobalt chloride hexahydrate showed an anti-mutagenic effect (inhibition of spontaneous mutation) when tested in *B. subtilis* (Inoue *et al.* 1981).

Table E-1. *In vitro* mutagenicity and DNA-damage studies of cobalt compounds in bacteria

Compound	Reference	LED/HID	Results –S9	Results +S9	Comments and conclusions
Reverse mutation/ *Salmonella typhimurium*/ TA100					
Cobalt chloride	(Ogawa *et al.* 1986)*	NR	–		Negative –S9
Cobalt chloride hexahydrate	(Tso and Fung 1981)*	23,800 µg/mL	–		Negative –S9 in two studies
	(Arlauskas *et al.* 1985)*	NR	–		
Cobalt sulfate heptahydrate	(Zeiger *et al.* 1992, NTP1998)	100 µg/plate	+	(+)	Positive –S9; weak positive +S9
Cobalt metal	(NTP 2014b)	500 µg/plate –S9 7,500 µg/plate +S9	(+)	–	Weak positive –S9; negative +S9
Reverse mutation/ *Salmonella typhimurium*/ TA102					
Cobalt chloride	(Wong 1988)*	40 µg/mL [approx. 100 µg/plate]	–	–	Negative ±S9
Reverse mutation/ *Salmonella typhimurium*/ TA1535					
Cobalt chloride	(Arlauskas *et al.* 1985)*	NR	–		Negative –S9 in 2 of 2 studies; negative +S9
	(Wong 1988)*	40 µg/mL	–	–	
Cobalt sulfate heptahydrate	(Zeiger *et al.* 1992, NTP 1998	10,000 µg/plate	–	–	Negative ±S9
Reverse mutation/ *Salmonella typhimurium*/ TA97					
Cobalt chloride	(Pagano and Zeiger 1992)*	13 µg/mL [approx. 32 µg/plate]	+		Positive –S9; Preincubation assay (generally more sensitive than standard plate incorporation assay)
Reverse mutation/ *Salmonella typhimurium*/ TA98					
Cobalt chloride	(Arlauskas *et al.* 1985)*	NR	–		Positive –S9 in 1 of 3 studies; negative +S9
	(Ogawa *et al.* 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).

Compound	Reference	LED/HID	Results –S9	Results +S9	Comments and conclusions
Cobalt sulfate heptahydrate	(Zeiger *et al.* 1992, NTP 1998)	10,000 µg/plate	–	–	Negative ±S9
Cobalt metal	(NTP 2014b)	100 µg/plate –S9 7,500 µg/plate +S9	+	–	Positive –S9 (weak effect, not well-correlated with dose); negative +S9
Reverse mutation/ *Salmonella typhimurium*/ TA1537					
Cobalt chloride	(Arlauskas *et al.* 1985)* (Ogawa *et al.* 1986)* (Wong 1988)*	NR 130,000 µg/plate 40 µg/mL [approx. 100 µg/plate]	 – – +	 –	Positive –S9 in 1 of 3 studies; negative +S9 [note: recommended maximum dose for assay is generally 10,000 µg/plate]
Reverse mutation/ *Salmonella typhimurium*/ TA1538					
Cobalt chloride	(Arlauskas *et al.* 1985)*	NR	–		Negative –S9
Cobalt chloride hexahydrate	(Mochizuki and Kada 1982)*	20 µg/mL	–		Anti-mutagenic effect on Trp-P-1-induced reverse mutations; same effect in both strains, independent of plasmid pKM101, which is in TA98 but not TA1538
Reverse mutation/ *Salmonella typhimurium*/ TA2637					
Cobalt chloride	(Ogawa *et al.* 1986)*	130,000 µg/plate	–		Negative –S9; strain detects bulky DNA adduct formation
Mutation/ *Escherichia coli* strain WP2 *uvrA*/pKM101					
Cobalt chloride hexahydrate	(Arlauskas *et al.* 1985)*	NR	–		Negative –S9
Cobalt chloride hexahydrate	(Kada and Kanematsu 1978)* (Leitao *et al.* 1993)*	20 µg/mL 50 µg/mL	– –		Negative –S9 Induced anti-mutagenic effect (inhibition of mutagenesis induced by N-methyl-N′-nitrosoguanidine) in two studies
Cobalt metal	(NTP 2014b)	450 µg/plate	–	–	Negative ±S9
Mutation/ *Escherichia coli* strain SY1032/pKY241 *supF* tRNA locus					
Cobalt chloride	(Ogawa *et al.* 1999)*	2.6 µg/mL	+		Positive –S9
Prophage induction/ *Escherichia coli*					
Cobalt chloride	(Rossman *et al.* 1984)*	415 µg/mL [approx. 1037 µg/plate]	–		Negative –S9
Reverse mutation/ *Bacillus subtilis* strain NIG 1125					

Compound	Reference	LED/HID	Results –S9	Results +S9	Comments and conclusions
Cobalt chloride hexahydrate	(Inoue *et al.* 1981)*	30 µg/mL	–		Anti-mutagenic effect (inhibition of spontaneous mutation)
Growth inhibition/ *Bacillus subtilis rec* strain strains H17					
Cobalt chloride	(Nishioka 1975)*	325 µg/plate	–		Positive –S9 in 1 of 2 studies; positive study used 'cold preincubation' procedure
	(Kanematsu *et al.* 1980)*	325 µg/plate		+	

*As cited by (IARC 2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, (+) = weak positive, – = negative.

E.2 Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes

Cobalt water-soluble compounds (cobalt chloride, cobalt nitrate, and cobalt sulfate) and nanoparticles were tested for mutations, DNA damage and chromosomal damage, and apoptosis 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. Cobalt chloride caused apoptosis in nematodes. 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 which suggested that the genotoxic effect for the *mwh/flr3* is due to somatic recombination and not mutation (see study details in Table E-2).

Nematodes and fish have been used to assess genotoxic effects of cobalt compounds. A study using a knock-out strain of the nematode *Caenorhabditis elegans* to test cobalt chloride reported germline apoptosis, which was induced independently of DNA damage-response genes. Direct DNA damage was reported in sperm from exposed male zebrafish (*Danio rerio*) for both cobalt chloride and cobalt sulfate, showing a dose-dependent increase for both compounds.

Table E-2. Genotoxicity studies of cobalt compounds in non-mammalian eukaryotes

Compound	Reference	LED/HID		Results (–S9)[a]	Comments and conclusions
FUNGI (Yeast)					
Mutation/ *Saccharomyces cerevisiae*					
Cobalt chloride	(Prazmo *et al.* 1975)*	'Petite' mutation	260 µg/mL	+	Some positive results for mutation in yeast, especially respiratory deficiency type
	(Egilsson *et al.* 1979)*	SBTD-2B, respiratory deficiency	640 µg/mL	(+)	
	(Putrament *et al.* 1977)*	Respiratory deficiency	520 µg/mL	+	
	(Putrament *et al.* 1977)*	Strain 197/2d	520 µg/mL	–	
		Erythromycin-resistant mutation/*ilv* mutation DL7:			
	(Fukunaga *et al.* 1982)*	1,300 µg/mL		–	
	(Singh 1983)*	13,000 µg/ml		–	
	(Kharab and Singh 1985)*	3,000 µg/mL		(+)	
	(Kharab and Singh 1987)*	'Petite' mutation/DL7 respiratory deficiency: 750 µg/mL		+	
Gene conversion (*trp*)/*Saccharomyces cerevisiae* D7					
Cobalt chloride	(Fukunaga *et al.* 1982)*	1,300 µg/mL		+	Positive for 3 of 3 studies
	(Singh 1983)*	13,000 µg/mL		(+)	
	(Kharab and Singh 1985)*	1,500 µg/mL		(+)	
PLANTS (Onion or corn)					
DNA damage/ *Allium cepa* or *Zea mays*					
Cobalt chloride	(Yildiz *et al.* 2009)	5.5 ppm		+	Positive in comet assay in *Allium cepa* bulbs
Cobalt nitrate hexahydrate	(Erturk *et al.* 2013)	5 mM		+	Genomic template instability increases with cobalt exposure levels in *Zea mays* seedlings
Chromosomal aberrations/ *Allium cepa*					
Cobalt chloride	(Yildiz *et al.* 2009)	5.5 ppm		+	Positive in anaphase-telophase chromosome aberration assay in *Allium cepa* bulbs
Cobalt sulfate	(Gori and Zucconi 1957)*	3 µg/mL		+	Positive results

Compound	Reference	LED/HID		Results (–S9)[a]	Comments and conclusions
Aneuploidy/ *Allium cepa*					
Cobalt sulfate	(Gori and Zucconi 1957)*	15 µg/mL		+	Positive results/ dosed 5 d, then water 3d
INSECTS (*Drosophila melanogaster*; fruit fly)					
Mutation or mitotic recombination/ wing spot test					
Cobalt chloride	(Ogawa *et al.* 1994)*	*mwh/flr*	260 µg/mL	+	Positive results for single and total mutant spots at high doses (10 mM) of ionic cobalt indicate CoCl2 is more genotoxic than nanoparticles in this assay (see below).
	(Ogawa *et al.* 1994)*	*mwh/TM3*	1040 µg/mL	–	
	(Vales *et al.* 2013)	*mwh/flr*^{3} wings			
		small spots	10 mM	+	
		large spots	10 mM	i	
		twin spots	10 mM	i	
		total	10 mM	+	
Cobalt nitrate hexahydrate	(Yesilada 2001)*	Strain *mwh/flr*^{3} wings			Positive effects; additional details were not provided in review paper
		Mutations, chromosomal deletion, nondisjunction			
		291 µg/mL		+	
		Mitotic recombination			
		2,910 µg/mL		+	
Cobalt nanoparticles	(Vales *et al.* 2013)	*mwh/flr*^{3} wings			Dose-dependent induction of small, but not large, spots indicates slow progression of nanoparticles to reach the wing imaginal disks. Single mutant spots result from both somatic mutation and somatic recombination; twin spots only result from somatic mutation.
		small spots	1 mM	+	
		large spots	10 mM	–	
		twin spots	10 mM	i	
		total	5 mM	+	
		mwh/TM3 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	
NEMATODES *(Caenorhabditis elegans*; free-living roundworm)					
Apoptosis (germline)					

Compound	Reference	LED/HID	Results (–S9)[a]	Comments and conclusions
Cobalt chloride	(Chong *et al.* 2009)	0.01 mM	+	Knockout gene strains of *C. elegans* showed induction of apoptosis independent of DNA damage response genes.
FISH *(Danio rerio*; zebrafish)				
DNA damage				
Cobalt chloride	(Reinardy *et al.* 2013)	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water
Cobalt sulfate	(Reinardy *et al.* 2013)	5 mg/L	+	Concentration-dependent increase in DNA strand breaks in sperm from exposed male zebra fish for 13 d in water

*As cited by (IARC 2006).
[a]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 and related effects 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 damaged DNA in both human and animal cells and most (except cobalt metal) caused cellular transformation in animal cell lines. 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, apoptosis, 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 summarized in Table E-3.

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 (apoptosis and cellular transformation) with soluble cobalt salts (cobalt chloride) and some relatively insoluble forms or particles (cobalt oxide, cobalt sulfides and particles, cobalt metal and nanoparticles).

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).

Mutagenic effects were somewhat conflicting, which may have been explained in part by the type of loci in the various assays. Cobalt chloride and cobalt sulfide caused mutations in studies using V79 Chinese hamster fibroblast cells *Hprt* locus (Hartwig *et al.* 1990, Miyaki *et al.* 1979) and for the transgenic G12 *Gpt*, but not for the normal fibroblast *Gpt* locus (Kitahara *et al.* 1996). Both assays testing cobalt chloride hexahydrate were negative, but they tested different gene loci, one at the *Tk* locus of mouse lymphoma L5178Y cells (Amacher and Paillet 1980) and the 8AG locus of V79 cells (Yokoiyama *et al.* 1990). Thus, the disparity of results may be due to the specific locus tested in these assays; the *Hprt* locus was positive in the two studies where it was evaluated for cobalt chloride, while the other assays looked at different loci. Mixed results were also reported for cobalt sulfide tested in a Chinese hamster transgenic cell line; the *Gpt* locus for

G10 was negative while the G12 strain tested positive. These cells lines have different *gpt* locus insertion sites and differ in their response to clastogens. When compared with G10, the G12 strain has a lower spontaneous mutant frequency (30 compared with 100 per million cells) and is highly sensitive to insoluble metal (nickel) compounds, with mutant induction of 20 to 30 fold for G12, compared with only 2 to 3 times the number of spontaneous mutants induced for G10 (Klein *et al.* 1994).

In cytogenetic assays, cobalt chloride induced sister chromatid exchange (SCE) in mouse macrophage-like cells (Andersen 1983). SCE involves double-strand DNA breaks and is induced by agents that form DNA adducts or interfere with DNA replication and/or repair. Cobalt nanoparticles (Ponti *et al.* 2009), but not cobalt chloride (Ponti *et al.* 2009, Suzuki *et al.* 1993), caused micronucleus induction.

Cobalt compounds also caused apoptosis and cellular transformation, which may be related to genotoxicity but are not genotoxic effects *per se*. Exposure to cobalt chloride resulted in apoptosis in several studies using different cell lines including mouse J774 macrophages (Catelas *et al.* 2005, Huk *et al.* 2004) and rat PC12 neuronal cells, a tumor cell line derived from rat pheochromocytoma. It was negative in a mouse osteocyte cell line, but significant necrosis was reported at the same exposure level (Kanaji *et al.* 2014). 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). Thus, a positive result in a cell-transformation assay may indicate either a genotoxic or non-genotoxic mechanism. 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) for cobalt metal nanoparticles (Annangi *et al.* 2014, Sighinolfi *et al.* 2014, Ponti *et al.* 2009), and for cobalt sulfide (Abbracchio *et al.* 1982, Costa *et al.* 1982), but negative for the insoluble cobalt metal (Doran *et al.* 1998).

E.3.2 Human cells

Human cells were tested *in vitro* for genotoxicity (DNA strand breaks, sister chromatid exchange, micronuclei, chromosomal aberrations, and aneuploidy) and related effects (apoptosis) with soluble cobalt salts (cobalt acetate, cobalt chloride, cobalt nitrate, and some relatively insoluble forms or particles (cobalt oxide, cobalt metal, and nanoparticles).

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).

Cobalt chloride and cobalt powder have been shown to induce apoptosis in several human cell types, including resting lymphocytes, peripheral blood mononuclear cells, CD4+ T-cells (obtained from lymphoma Jurkat cell line), and alveolar macrophages (Akbar *et al.* 2011, Caicedo *et al.* 2007, Araya *et al.* 2002, Zou *et al.* 2001, Granchi *et al.* 1998).

Table E-3. *In vitro* studies of genotoxicity and related effects of cobalt compounds in mammalian cells

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
RODENT CELLS				
Mutation/ V79 Chinese hamster lung fibroblasts (V79) or Mouse lymphoma (MOLY), hamster transgenic cell line or BALB/3T3 mouse cells				
Cobalt chloride				V79 – mixed results
	(Miyaki *et al.* 1979)*	26 µg/mL	(+)	*Hprt* locus
	(Hartwig *et al.* 1990)*	13 µg/mL	+	*Hprt* locus
	(Kitahara *et al.* 1996)*	13 µg/mL	–	*Gpt* locus
	(Kitahara *et al.* 1996)*	6.5 µg/mL	+	Transgenic G12, *Gpt* locus
Cobalt chloride hexahydrate	(Yokoiyama *et al.* 1990)*	2 µg/mL	–	V79 – 8AG locus negative
	(Amacher and Paillet 1980)*	57.11 µg/mL	–	MOLY L5178Y cells, *Tk* locus negative
Cobalt sulfide (CoS_2 and CO_3S_4) particles				Chinese hamster transgenic cell lines (derived from V79)
	(Kitahara *et al.* 1996)*	1 µg/mL	–	G10, *Gpt* locus
		0.5 µg/mL	+	G12, *Gpt* locus
DNA damage/strand breaks/ alkaline elution, sucrose gradient, or Q-PCR/3T3 mouse cells, Chinese Hamster Ovary (CHO) cells, BALB/3T3 cells/neuronal cell mitochondria				
Cobalt chloride	(Hamilton-Koch *et al.* 1986)*	260 µg/mL (ASG)	+	CHO cells – positive using alkaline sucrose gradient but not nucleoid sedimentation in the same study
	(Hamilton-Koch *et al.* 1986)*	1,300 µg/mL (NS)	–	
	(Ponti *et al.* 2009)	1 µM	+	Positive in BALB/3T3 cells – 2 hr. exposure to sub-toxic dose
	(Wang *et al.* 2000)	100 µM	+	Positive in rat neuronal PC12 cell mitochondria
Cobalt (metal)	(Anard *et al.* 1997)*	1 µg/mL	+	BALB/3T3 mouse cells – positive for alkaline elution; used purified DNA
Cobalt sulfides (CoS_2 and CO_3S_4) particles	(Robison *et al.* 1982)*	10 µg/mL	+	CHO cells – Positive using sucrose gradient
Cobalt metal nanoparticles	(Ponti *et al.* 2009)	1 µM	+	Positive in BALB/3T3 cells – 2 hr. exposure to sub-toxic dose
Micronucleus formation/ mouse cells				
Cobalt chloride	(Ponti *et al.* 2009)	10 µM	–	Negative for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
Cobalt chloride hexahydrate	(Suzuki *et al.* 1993)*	50 µg/mL	–	Negative for MN induction in BALB/c mouse bone marrow

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
Cobalt metal nanoparticles	(Ponti *et al.* 2009)	1 µM	+	Positive for micronuclei induction (24 hr) in BALB/3T3 fibroblast cells
Sister chromatid exchange/ mouse macrophage-like cells P388D$_1$				
Cobalt chloride	(Andersen 1983)*	13 µg/mL	+	Positive for SCE induction
Cell transformation/ C3H10T1/2 mouse fibroblasts, Syrian hamster embryo cells (SHE), or BALB/3T3 cells				
Cobalt chloride	(Doran *et al.* 1998)*	5 µg/mL	+	Positive in C3H10T1/2 mouse fibroblast cells
	(Ponti *et al.* 2009)	70 µM	–	Negative in BALB/3T3 cells (72 hr exposure)
Cobalt sulfate monohydrate	(Kerckaert *et al.* 1996)*	0.125 µg/mL	+	Positive in SHE cells
Cobalt acetate	(Casto *et al.* 1979)*	0.2 mM (approx. 35.4 µg/mL)	+	Positive for cell transformation enhancement by simian adenovirus SA7/ SHE cells
Cobalt metal	(Doran *et al.* 1998)*	500 µg/mL	–	Negative in C3H10T1/2 mouse fibroblast cells, even at high exposure
Cobalt metal nanoparticles	(Ponti *et al.* 2009)	7 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	(Sighinolfi *et al.* 2014)	10 µM	+	Positive in BALB/3T3 cells (72 hr exposure)
	(Annangi *et al.* 2014)	0.05 µg/ml	+	Cell transformation in mouse embryo fibroblasts after 12 wk exposure to sub-toxic dose; *Ogg1*$^{+/+}$ and *Ogg1*$^{-/-}$ with knockout cells more sensitive
Cobalt sulfide (CoS, amorphous)	(Abbracchio *et al.* 1982)* and (Costa *et al.* 1982)*	10 µg/mL	(+)	Positive in Syrian hamster embryo cells
Cobalt sulfide (CoS_2, crystalline)	(Abbracchio *et al.* 1982)* and (Costa *et al.* 1982)*	1 µg/mL	+	Positive in Syrian hamster embryo cells
Apoptosis/ rat neuronal cells, mouse J774 macrophages, or mouse osteocyte cell line				
Cobalt chloride	(Zou *et al.* 2001)*	100 µM	+	Positive in dose-dependent manner (100 to 1000 µM) in PC12 neuronal cells by ROS formation
	(Huk *et al.* 2004)	10 ppm	+	Positive dose-dependent for ion concentration and incubation time in two studies in J774 macrophages
	(Catelas *et al.* 2005)	10 ppm	+	
	(Kanaji *et al.* 2014)	0.5 mM	–	Negative for apoptosis (but significant necrosis at same exposure) in mouse MLO-44 osteocyte cell line
HUMAN CELLS				
DNA damage - strand breaks/ several cell types/ alkaline elution, alkali-labile sites, or comet assay				

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
Cobalt chloride				Mostly positive results
	(McLean *et al.* 1982)*	6.5 µg/mL	+	White blood cells – fluorescence analysis of DNA unwinding
				Diploid fibroblasts
	(Hamilton-Koch *et al.* 1986)*	650 µg/mL	+	Alkaline sucrose gradient
	(Hamilton-Koch *et al.* 1986)*	1,300 µg/mL	+	Nick translation
	(Hamilton-Koch *et al.* 1986)*	1,300 µg/mL	–	Nucleoid sedimentation
	(Hartwig *et al.* 1990)*	65 µg/mL	+	Nucleoid sedimentation
	(De Boeck *et al.* 1998)*	0.3 µg/mL	+	Mononuclear leukocytes – comet assay
	(Alarifi *et al.* 2013)	10 µg/mL	+	HepG2 hepatocarcinoma cells (24 hr) – comet assay
	(Colognato *et al.* 2008)	100 µM	–	Negative in peripheral blood leukocytes – comet assay
	(Patel *et al.* 2012)	150 µM	+	Damage in H460 lung epithelial cells – comet assay
	(Caicedo *et al.* 2007)	5 mM	+	Damage in CD4+ T-cells obtained from lymphoma Jurkat cell line
	(Davies *et al.* 2005)	0.84 µM	+	Damage for artificial spiked fluids – comet assay
	(Jiang *et al.* 2012)	30 µM	–	Negative for DNA damage on T-cells – comet assay
Cobalt chloride hexahydrate	(Anard *et al.* 1997)*	25 µg/mL	–	Negative for lymphocytes using alkaline elution
Cobalt metal	(Anard *et al.* 1997)*	3.0 µg/mL	+	Positive for lymphocytes using alkaline elution
	(Anard *et al.* 1997)*	4.5 µg/mL	+	Positive in several studies, for mononuclear leukocytes for DNA single-strand breaks and alkali-labile sites, and alkaline comet assay
	(Van Goethem *et al.* 1997)*	0.6 µg/mL	+	
	(De Boeck *et al.* 1998)*	0.3 µg/mL	+	
	(De Boeck *et al.* 2003b)*	0.6 µg/mL	+	
	(Qiao and Ma 2013)	5µM	+	Positive for normal fetal fibroblast cells in single cell array assay
Cobalt nanoparticles	(Colognato *et al.* 2008)	50 µM	+	Positive in peripheral blood leukocytes – comet assay
	(Wan *et al.* 2012)	5µg/ml	+	Positive in exposed A549 lung epithelial cells – comet assay
	(Jiang *et al.* 2012)	3µM	+	Positive for DNA damage in T-cells– comet assay
Cobalt oxide nanoparticles	(Kain *et al.* 2012)	20 µg/mL	+	Positive in A549 lung cell line (DNA breaks)
	(Kain *et al.* 2012)	20 µg/mL	+	Positive in BEAS-2B lung cell (DNA breaks and oxidative damage)

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
	(Alarifi *et al.* 2013)	5 µg/mL	+	Positive in HepG2 hepatocarcinoma cells (24 hr) – comet assay
	(Cavallo *et al.* 2015)	A549		
		20 µg/mL	+	Positive in alveolar A549 and bronchial BEAS-2B cells, for both direct and oxidative damage – comet assay
			+	
		BEAS-2B	+	
		40 µg/mL	+	
		(direct) 5 µg/mL (oxidative)		
Micronucleus formation/ binucleates, cytochalasin-B assay/ lymphocytes or osteoblast-like cell line				
Cobalt chloride	(Colognato *et al.* 2008)	40 µM	+	Positive in peripheral blood leukocytes, with clear trend for increase, high variability in response of donors
Cobalt metal	(Van Goethem *et al.* 1997)*	0.6 µg/mL	+	Positive for micronuclei induction in three studies with different cell types
	(Miller *et al.* 2001)*	0.75 µg/ml	+	
	(De Boeck *et al.* 2003b)*	3 µg/mL	+	
Cobalt nanoparticles	(Colognato *et al.* 2008)	40 µM	+	Increase in peripheral blood leukocytes, high variability among donors, less effective than cobalt chloride in same study
Chromosomal aberrations/ lung fibroblast cells or lymphocytes				
Cobalt chloride hexahydrate	(Fairhall *et al.* 1949)	1.3 ppb	+	Induced significant increase of total aberrations in primary fibroblasts
	(Smith *et al.* 2014)	50 µM	+	In WTHBF-lung fibroblast cells – soluble cobalt induces more cytotoxicity and cell cycle arrest than particulate (cobalt oxide, see below) but both produced similar levels of genotoxicity; chromosomal damage significant ($p<0.05$).
Cobalt nitrate	(Paton and Allison 1972)*	0.015 µg/mL	–	Negative in diploid fibroblasts WI38 (derived from embryonic lung tissue) and MRC-5 (derived from fetal lung tissue) toxic dose
		0.15 µg/mL	–	Negative in mononuclear leukocytes (toxic dose)
Cobalt oxide	(Smith *et al.* 2014)	0.5 µg/mL	+	Lung fibroblast cells – significant chromosome damage at $P < 0.05$
	(Voroshilin *et al.* 1978)*	0.6 µg/mL	–	Negative in lymphocytes
Cobalt acetate tetrahydrate	(Voroshilin *et al.* 1978)*	0.6 µg/mL	–	Negative in lymphocytes
Sister chromatid exchange (SCE)/ lymphocytes				

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
Cobalt chloride	(Andersen 1983)*	1.3 µg/mL	+	Positive for SCE in lymphocytes
Aneuploidy/ lymphocytes				
Cobalt chloride	(Resende de Souza Nazareth 1976)*	3.7 µg/mL	+	Positive for aneuploidy in lymphocytes
Cobalt chloride hexahydrate	(Figgitt *et al.* 2010)	25 ppb	+	Induced significant increase in aneuploidy in primary fibroblasts
Apoptosis/ primary human lymphocytes or peripheral blood mononuclear cells (e.g., lymphocytes, monocytes, macrophages), macrophages or neuronal cells				
Cobalt chloride	(Zou *et al.* 2001)*	100 µM	+	Positive in dose-dependent manner (100 to 1000 µM) in PC12 cells neuronal cells by ROS formation
	(Araya *et al.* 2002)*	100 µM	+	Positive in U-937 alveolar macrophages
	(Caicedo *et al.* 2007)	5 mM	+	Positive in CD4+ T cells obtained from lymphoma Jurkat cell line
	(Akbar *et al.* 2011)	100 µM	+	Positive in resting lymphocytes, significant decrease in cell viability accompanied by significant increase in apoptosis
Cobalt powder (extract)	(Granchi *et al.* 1998)	Apoptotic cells: 50% cobalt (48 or 72 hr)	+	Positive in peripheral blood mononuclear cells; lower exposure levels produced apoptosis and high levels necrosis (at 24 hr = 50% cobalt; 48 hr = 25% and 72 hr = 1.5% cobalt)

*As cited by (IARC 2006).

[a]No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = 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-4 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 can induce DNA and chromosomal damage, i.e., micronucleus formation, chromosomal aberrations, and aneuploidy. The results of these studies are discussed below and summarized in Table E-5.

DNA damage was observed after i.p. exposure to cobalt acetate in the Fischer rat, with strongest results observed for kidney, liver, then lung cells (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-4. Studies of nucleic acid and protein binding and DNA damage/repair inhibition of cobalt compounds

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
Binding/ crosslinks				
Cobalt chloride	(Wedrychowski *et al.* 1986)*	130 µg/mL	+	Positive for DNA-protein crosslinks/ in rat Novikoff ascites hepatoma cells
	(Palecek *et al.* 1999)*	>13 µg/mL 78 µg/mL	+ (full) +	Inhibition of p53 protein-DNA binding for consensus sequence and supercoiled DNA
Cobalt chloride hexahydrate	(Sabbioni *et al.* 2014)	10µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) much lower (0.0019 ng/10^6 cells) than for microparticles or nanoparticles (see below)
Cobalt sulfate	(Lloyd *et al.* 1998)	20 µM	+	Salmon sperm DNA generative cross-links, induced single (not double) strand DNA damage
Cobalt metal ion	(Bal *et al.* 2013)	NTS 110 µM A 90 µM B 11 µM	+	Cobalt binds to human serum albumin at three sites: N-terminal site (NTS), A and B; they had different affinities –site B was the strongest
Cobalt nanoparticles	(Sabbioni *et al.* 2014)	10 µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) Binding 1.7 ng/10^6 cells
Cobalt microparticles	(Sabbioni *et al.* 2014)	10 µM	+	Radiolabelled cobalt binding to DNA (4 hr exposure) Binding 9.2 ng/10^6 cells
Cobalt ions (see comments)	(Alipázaga *et al.* 2008)	1.0 mM	+	Cobalt binds to O_2 in presence of glycylglycylhistidine, directly forming adducts; cobalt was prepared from cobalt carbonate reaction with perchloric acid
Inhibition of DNA repair				
Cobalt chloride hexahydrate	(Hartwig *et al.* 1991) and (Kasten *et al.* 1997)*	12 µg/mL (incision and polymerization step) 48 µg/mL (ligation step)	+ –	Positive for Inhibition of nucleotide excision repair of UV-induced DNA damage, alkaline unwinding/ repair VH16 fibroblasts; data shown for Kasten *et al.* (same research group, subsequent publication)
Cobalt chloride hexahydrate	(Kasten *et al.* 1997)*	86 µg/mL (incision step)	+	Inhibition of UV-induced cyclobutane pyrimidine dimers, alkaline unwinding + T4 endonuclease V/VH16 fibroblasts

Compound	Reference	Concentration (LED or HID)	Results (–S9)[a]	Comments and conclusions
Cobalt (metal)	(De Boeck *et al.* 1998)*	1.2 µg/mL (with 5.5 µg/mL MMS post-treatment) 1.2 µg/mL (with 5.5 µg/mL MMS co-exposure)	+ +	Positive for DNA repair inhibition, alkaline Comet assay/ human mononuclear leukocytes
Cobalt acetate	(Snyder *et al.* 1989)*	100 µg/mL	+	Inhibition of repair of UV-induced pyrimidine dimers, nucleoid sedimentation in HeLa S-3 cells
Inhibition/inactivation of protein				
Cobalt chloride	(Asmuss *et al.* 2000)*	6.5 µg/mL (XPA) 130 µg/mL (Fpg)	+ –	Positive for inhibition of xeroderma pigmentosum group A (XPA) protein (with Zn finger domain) binding to UV-irradiated oligonucleotide [XPA is a zinc finger protein involved in nucleotide excision repair, but no effect on bacterial Fpg protein (Zn finger domain)]
Cobalt nitrate hexahydrate	(Kopera *et al.* 2004)	10 µM	+	Reported substitution for zinc in the zinc finger derived from the DNA report protein XPA

*As cited by (IARC 2006).

[a]No studies reported testing with +S9.

LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, – = negative.

Table E-5. *In vivo* genotoxicity studies of cobalt compounds in rodents

Compound	Species/sex/#	Reference	Exposure	Results	Comments and conclusions
DNA damage					
Cobalt acetate	F344/CR rat male and female/12 per group	(Kasprzak *et al.* 1994)	i.p. one dose 50 or 100 µmol 2 or 10 d	Kidney + Liver + Lung +	Damage in kidney > liver > lung cells; retention of cobalt in kidney and liver, less in lung
Micronucleus formation/ peripheral blood lymphocytes					
Cobalt metal	B6C3F1/N mouse/male and female	(NTP 2014a)	Inhalation 3 months 10 mg/m^3	–	Negative for micronuclei induction in peripheral blood lymphocytes
Micronucleus formation/ bone marrow					
Cobalt chloride	Swiss albino mouse/male/5 per group	(Rasgele *et al.* 2013)	i.p. 24 hr 22.5 mg/kg 48 hr 11.2 mg/kg	 + +	Significant increase in micronucleated polychromatic erythrocytes; no toxicity observed; distilled water control
Cobalt chloride hexahydrate	BALB/c AnNCrj mouse/male	(Suzuki *et al.* 1993)*	i.p. 50 mg/kg bw	+; also enhanced formation with other mutagens	Micronuclei enhanced, compared with other mutagens used (20 mg/kg DMH, 50 mg/kg benzo(*a*)pyrene, and 200 mg/kg 2-napthylamine)
Chromosomal breaks and chromosomal aberrations (bone marrow)					
Cobalt chloride	Swiss mouse/male	(Palit *et al.* 1991d, Palit *et al.* 1991c, Palit *et al.* 1991b, Palit *et al.* 1991a)**	Oral Single dose 4.96 to 19.8 mg/kg	+	Dose response increase in chromosomal damage
Aneuploidy, pseudodiploidy, and hyperploidy/ bone marrow and testes (meiosis 1)					
Cobalt chloride	Hamster	(Farah 1983)*	i.p. 400 mg/kg bw total dose	+ (bone marrow and testes)	Exposure is total dose over 9 days
Cobalt chloride hexahydrate	Hamster	(Farah 1983)*	i.p. 400 mg/kg bw total dose	+ (bone marrow and testes)	Exposure is total dose over 9 days

*As cited by (IARC 2006), ** as cited by WHO (CICAD) (2006).
LED/HID = lowest effective dose/highest ineffective dose, NR = not reported, + = positive, – = negative.

E.6 Genotoxicity studies of occupational exposure to cobalt

The available database for evaluating occupational exposure to cobalt and genetic effects is inadequate because of the paucity of studies, exposure to other genotoxic agents, or small numbers of exposed workers. Two studies reported by IARC are not reviewed because they were not specific for cobalt exposure and a more recent study of occupational exposure was identified; however, a study of Brazilian copper smelter workers is also not reviewed because blood cobalt levels were similar among unexposed controls (De Olivera *et al.* 2012). Two studies are reviewed below, only one of which is of cobalt workers (De Boeck *et al.* 2000); however, the second study is briefly reviewed (Hengstler *et al.* 2003) because it conducted multivariate analyses.

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-6. Genotoxicity studies of occupational exposure to cobalt

Endpoint	Population	Reference	Exposure assessment	Results	Comments and conclusions
DNA damage Cobalt in urine and air	Workers in 10 facilities producing or recycling cadmium or its products in Germany (N = 78; 62 men and 16 women)	(Hengstler *et al.* 2003)	Cobalt in urine and air	+	Co-exposures to cadmium and lead contribute to DNA strand breaks in logistic regression models DNA single strand breaks correlated with cobalt concentration in air ($P < 0.001$, R = 0.401) Cobalt levels measured in air and worker urine
DNA damage 8-hydroxy-deoxy-guanosine (8-OHdG) Micronuclei	Refinery workers (three facilities – in Belgium, Norway and Finland) exposed to cobalt (analysis = 24); workers exposed to hard metal plants in one plant (analysis = 29) and unexposed workers from the four facilitates (analysis = 27) Workers and exposed workers from one plant were excluded from the analysis because of older population and higher 8-OHdG	(De Boeck *et al.* 2000)	Cobalt in urine	– (all three endpoints)	Negative for damage measured in lymphocytes – comet assay; cobalt levels measured in urine Exposure equivalent to 20 µg/m^3 of cobalt Exposure assessment from samples on Friday, and genetic damage assessed from samples the following Monday

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