OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Cobalt and Cobalt Compounds

Cancer Inhalation Unit Risk Factors

Technical Support Document for
Cancer Potency Factors
Appendix B

October 2020

Air, Community, and Environmental Research Branch
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Technical Support Document for Cancer Potency Factors Appendix B

Prepared by the

Office of Environmental Health Hazard Assessment

Lauren Zeise, Ph.D., Director

Authors

Daryn E. Dodge, Ph.D.

Rona M. Silva, Ph.D.

Technical Reviewers

David M. Siegel, Ph.D.

John D. Budroe, Ph.D.

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Introduction

This document summarizes the carcinogenicity and derivation of cancer inhalation unit risk factors (IURs) for cobalt and cobalt compounds. Cancer unit risk factors are used to estimate lifetime cancer risks associated with inhalation exposure to a carcinogen.

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). In implementing this requirement, OEHHA develops cancer IURs for carcinogenic air pollutants listed under the Air Toxics Hot Spots program. The cobalt and cobalt compounds IURs were developed using the most recent "Air Toxics Hot Spots Program Technical Support Document for Cancer Potency Factors", finalized by OEHHA in 2009. Literature summarized and referenced in this document covers the relevant published literature for cobalt and cobalt compounds through the spring of 2019. This document received external peer-review by the Scientific Review Panel on Toxic Air Contaminants on October 4, 2019.

Several government agencies or programs currently list cobalt metal and cobalt compounds as carcinogens. Under the California Proposition 65 program, cobalt metal powder, cobalt sulfate, cobalt sulfate heptahydrate, and cobalt(II) oxide are listed as chemicals known to the state to cause cancer (OEHHA, 2018a). Cobalt metal and soluble cobalt(II) salts are listed separately by the International Agency for Research on Cancer (IARC) as Group 2B carcinogens, *i.e.*, possibly carcinogenic to humans (IARC, 2006). The National Toxicology Program (NTP) listed cobalt and cobalt compounds that release cobalt ions *in vivo* in the 14th Report on Carcinogens, which identifies substances that either are known to be human carcinogens or are reasonably anticipated to be human carcinogens, and to which a significant number of persons residing in the United States are exposed (NTP, 2016).

NTP conducted inhalation carcinogenicity bioassays with cobalt sulfate heptahydrate, a soluble cobalt compound, in rats and mice of both sexes in 1998 (NTP, 1998). NTP subsequently conducted inhalation carcinogenicity bioassays with cobalt metal in rats and mice of both sexes in 2014 (NTP, 2014). These studies provided evidence of carcinogenicity for cobalt sulfate heptahydrate and for cobalt metal in rats and mice of both sexes. Due to chemical, physical, and toxicological differences between cobalt metal and various cobalt compounds, separate IURs were derived for water soluble cobalt compounds (based on studies with cobalt sulfate heptahydrate) and cobalt metal and insoluble cobalt compounds (based on studies with cobalt metal). The cobalt IURs do not apply to steel and metal alloys that contain cobalt. In addition, the alloy-like hard metals, particularly cobalt-tungsten carbide hard metal, are not included. Although cobalt-tungsten carbide dust is a probable human carcinogen (IARC, 2006), the physico-

chemical properties of hard metals are different from that of cobalt compounds, which warrant their own IURs. Most cobalt is used industrially in the form of cobalt metal powder as an alloying component and in the preparation of cobalt salts (NTP, 2016; HSDB, 2019). Cobalt salts and oxides are used as pigments in the glass and ceramics industries, as catalysts in the oil and chemical industries, as paint and printing ink driers, and as trace metal additives in agriculture and medicine. Other significant cobalt uses are as a catalyst or component in green energy technologies (*e.g.*, solar panels), and as a primary component in lithium- and nickel-based rechargeable batteries. The presence of cobalt in some electric and electronic devices may also result in exposure to cobalt in the E-waste recycling industry (Leyssens *et al.*, 2017).

Cobalt occurs naturally in the Earth's crust but is usually in the form of arsenides and sulfides (Baralkiewicz and Siepak, 1999). Natural levels of cobalt in air generally range from 0.0005 to 0.005 nanograms per cubic meter (ng/m^3). In major industrial cities, levels of cobalt may reach as high as 6 ng/m^3. The California Air Resources Board collects air monitoring data for numerous pollutants found in urban areas, including cobalt and other metals (CARB, 2018). In southern California, mean cobalt concentrations at air monitoring sites in 2017 ranged from 1.3 to 1.97 ng/m^3, with maximum levels between 2.9 and 5.6 ng/m^3. However, cobalt concentrations were often below the limit of detection (1.3 ng/m^3). As discussed below, the roughly 1000-fold increase in air levels of cobalt in urban areas, compared to wilderness areas, is the result of a number of human-related activities.

Emissions estimates of cobalt in California are collected and presented in the California Toxics Inventory, or CTI (CARB, 2017). Potential sources include stationary (point and aggregated point), area-wide, on-road mobile (gasoline and diesel), off-road mobile (gasoline, diesel, and other), and natural sources. For the most recent inventories (2008-2010), mobile sources emitted up to 6.05 tons of cobalt per year in California. A PM speciation profile for only gasoline-powered vehicle exhaust estimated the weight percentage of cobalt in $PM_{2.5}$ at 0.0021 (±0.0031) (Yang, 2011). Based on the most recent $PM_{2.5}$ emissions data, the amount of cobalt emitted from gasoline-powered vehicles in the $PM_{2.5}$ fraction was estimated at roughly 8.4 tons per year. However, there is a large variation in emitted cobalt in both reports.

The primary emission source for cobalt was area-wide sources, with up to 58 tons per year emitted (CARB, 2017). However, this amount may be overestimated, since many large area sources did not have measurable levels of cobalt, but were subsequently estimated at half the level of detection. Area-wide source emissions are related to human activities that take place over a wide geographic area. Such sources include residential fuel combustion and cooking, consumer products, farming operations, and paved and unpaved road-travel dust.

Stationary point sources released up to 2.2 tons of cobalt per year (CARB, 2017). Stationary sources include point sources provided by facility operators and/or districts pursuant to the Air Toxics “Hot Spots” Program (AB 2588). However, many facilities that would emit cobalt do not need to report their emissions, so this estimate is likely underestimated. Some of the main stationary sources in California that use and/or emit cobalt include the petroleum industry, concrete and cement manufacturing facilities, electronic computer manufacturers, semiconductor and electronic components facilities, military and commercial aerospace product and parts manufacturing, tungsten carbide and other super alloy manufacturing, mining and refining operations, lumber mills, and battery manufacturers (CARB, 2019; CBR, 2019).

List of Acronyms

8-OHdG	8-hydroxydeoxyguanosine	IARC	International Agency for Research on Cancer
AIC	Akaike Information Criterion	IUR	Inhalation unit risk
$BMDL_{05}$	The 95% lower confidence bound at the 5% response rate	IR	Inhalation rate
		LDH	Lactate dehydrogenase
BMD	Benchmark dose	MMAD	Mass median aerodynamic diameter
BMD_{05}	BMD 5% response rate		
BMDS	Benchmark dose modelling software	µg/L	Micrograms per liter
		µg/ml	Micrograms per milliliter
BMR	Benchmark response	µm	Micrometer
BNMN	Binucleated micronucleated	µM	Micromole per liter
BR	Breathing rate	mg/m^3	Milligrams per cubic meter
BW	Body weight	mg/kg-BW	Milligrams per kilogram of bodyweight
CEBS	Chemical effects in biological systems		
		mM	Millimole per liter
CF	Conversion factor	NCE	Normochromatic erythrocytes
CKE	Cystic keratinizing epithelioma	NP	Nanoparticle
		NTP	National Toxicology Program
Co	Cobalt	O_2^-	Superoxide radical
$CoSO_4 \cdot 7H_2O$	Cobalt sulfate heptahydrate	OECD	Organisation for Economic Co-operation and Development
CPF	Cancer potency factor		
CSF	Cancer slope factor		
CTI	California Toxics Inventory	OEHHA	Office of Environmental Health Hazard Assessment
DMSO	Dimethyl sulfoxide		
DNA	Deoxyribonucleic acid	PCE	Polychromatic erythrocytes
Fpg	Formamido-pyrimidine glycosylate	ROS	Reactive oxygen species
		SHE	Syrian hamster embryo
GSD	Geometric standard deviation	SIR	Standardized incidence rate
		SMR	Standardized mortality ratio
H_2O_2	Hydrogen peroxide	SPF	Specific pathogen free
HL	Human lymphocyte	TWA	Time-weighted average
hOOG1	Human 8-hydroxyguanine DNA-glycosylate 1	UV	Ultraviolet
		US EPA	United States Environmental Protection Agency

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COBALT AND COBALT COMPOUNDS

I. PHYSICAL AND CHEMICAL PROPERTIES

(Kyono *et al.*, 1992; Hillwalker and Anderson, 2014; NTP, 2016)

Molecular formula	Co (elemental form)
Molecular weight	58.93
Description	Gray, hard, magnetic, ductile, somewhat malleable metal
Density	8.92 g/cm^3
Boiling point	2927ºC
Melting point	1495ºC
Vapor pressure	Not applicable
Odor	Cobalt metal powder or fumes are odorless
Solubility	Metallic cobalt particles in the micrometer size range or larger are considered poorly water soluble. Soluble in dilute acids.
Conversion factor	Not applicable

II. HEALTH ASSESSMENT VALUES

Cobalt metal and water-insoluble cobalt compounds

Unit Risk Factor	7.7×10^{-3} $(\mu g/m^3)^{-1}$
Inhalation Slope Factor	27 $(mg/kg\text{-}day)^{-1}$

Water-soluble cobalt compounds (normalized to cobalt content)

Unit Risk Factor	8.6×10^{-4} $(\mu g/m^3)^{-1}$
Inhalation Slope Factor	3.0 $(mg/kg\text{-}day)^{-1}$

Insolubility of a cobalt compound in water is defined in this document as having a water solubility of ≤100 mg/L at 20˚C (MAK, 2007; USP, 2015). Cobalt compounds that have a water solubility of >100 mg/L at 20˚C are considered water-soluble. The cancer potency factors (unit risk and inhalation slope factors) for cobalt metal applies to insoluble cobalt compounds and the cancer potency factors for cobalt sulfate heptahydrate applies to soluble cobalt compounds. This definition of solubility is only applicable to this document for regulatory purposes, and does not apply to other OEHHA documents and programs.

III. CARCINOGENICITY

Bioaccessibility of the cobalt ion following inhalation is considered to be the primary factor for cancer risk (NTP, 2016). Thus, any inhaled cobalt compound that releases cobalt ion in pulmonary fluids presents an inhalation cancer risk. Water-soluble cobalt compounds reaching the alveoli following inhalation will dissolve in the alveolar lining

fluid and release the cobalt ion (Kreyling *et al.*, 1986; Stopford *et al.*, 2003). Water-insoluble cobalt compounds (*e.g.*, cobalt oxides) and cobalt metal reaching distal airways and alveoli are taken up by macrophages and other epithelial cells by endocytosis and dissolve intracellularly in the acidic environment (pH 4.5 to 5) of lysosomes (Kreyling *et al.*, 1990; Ortega *et al.*, 2014).

The IUR for insoluble cobalt (i.e., cobalt metal) is ninefold greater than the IUR for soluble cobalt sulfate heptahydrate, when normalized to cobalt content. Differences in cellular uptake between soluble and insoluble forms of cobalt have been proposed as a reason for differences in cancer potency (Smith et al. 2014). *In vitro* studies observed that insoluble cobalt nanoparticles interacted with proteins on the surface of cells and were readily taken up, resulting in a considerably greater intracellular concentration of cobalt ion (following release in lyosomal fluid) when compared to uptake of extracellular ions from soluble cobalt compounds (Ponti et al., 2009; Colognato et al., 2008). A similar mechanism for carcinogenic, insoluble nickel compounds has also been observed, in which insoluble Ni particles are phagocytized by cells with subsequent intracellular dissolution, whereupon the released Ni ions interact with chromatin causing DNA damage (Costa, 1991; Costa *et al.*, 1994). Soluble nickel salts are taken up more slowly by cells (as Ni ions) and do not produce the intracellular concentrations that insoluble nickel particles can produce.

The IUR values derived by OEHHA apply to metallic cobalt, water-soluble cobalt compounds, and water-insoluble cobalt compounds that have some solubility in lysosomal fluid. The IURs and cancer slope factors are intended for use in the evaluation of cancer risk due to the inhalation of cobalt and cobalt compounds. They are not intended to be used for the evaluation of cancer risk due to cobalt and cobalt compound exposure by the oral route. There is currently inadequate evidence for carcinogenicity of cobalt and cobalt compounds by the oral route of exposure. Commercially significant cobalt compounds include, but are not limited to, the oxide, hydroxide, chloride, sulfate, nitrate, carbonate, acetate, and oxalate forms (Table 1). The cobalt IURs do not apply to cobalt-containing alloys and steels, and to the alloy-like cobalt hard metals (*e.g.*, cobalt-tungsten carbide). In addition, cobalt aluminum spinel and the cobalt-containing essential nutrient vitamin B12 are not included with these IURs.

Table 1. Water solubility of some commercially important cobalt compounds
(IARC, 1991; Stopford *et al.*, 2003; Hillwalker and Anderson, 2014; NTP, 2016; Lison *et al.*, 2018; HSDB, 2019)

Molecular Formula	Molecular Weight	Form of Cobalt (Metal or Cobalt Compound)	CAS #	Water Solubility
Co	58.9	Cobalt metal particles/dust	7440-48-4	2.9 mg/L
$CoSO_4$	281.1	Sulfate (heptahydrate)	10026-24-1	604,000 mg/L
Co_3O_4	240.8	Oxide(II,III) [a]	1308-06-1	1.6 mg/L
$Co(OH)_2$	93.0	Hydroxide [a]	21041-93-0	3.2 mg/L
CoS	91.0	Sulfide [a]	1317-42-6	3.8 mg/L
CoO	74.9	Oxide(II) [a]	1307-96-6	4.9 mg/L
$CoCO_3$	118.9	Carbonate [a]	513-79-1	11.4 mg/L
CoC_2O_4	147.0	Oxalate [a]	814-89-1	32.2 mg/L
$C_8H_{16}O_2$:1/2Co	344.9	Octoate [b]	136-52-7	40,300 mg/L
$Co(C_2H_2O_2)_2$	249.1	Acetate (tetrahydrate) [b]	71-48-7	348,000 mg/L
$CoCl_2$	129.9	Chloride (hexahydrate) [b]	7646-79-9	450,000 mg/L
CoN_2O_6	182.9	Nitrate (hexahydrate) [b]	10141-05-6	670,000 mg/L

[a] The IUR value for cobalt metal applies to this cobalt compound (insoluble in water (≤100 mg/L at 20˚C)).

[b] The IUR value for cobalt sulfate heptahydrate (normalized to cobalt content) applies to this cobalt compound (soluble in water (>100 mg/L at 20˚C)).

The genotoxic and carcinogenic action of cobalt likely involves a number of mechanisms. Intracellular release of cobalt ions results in cobalt-mediated generation of free radicals, cellular oxidative stress, and oxidative damage to deoxyribonucleic acid (DNA) (Hanna *et al.*, 1992; Lison, 1996; Valko *et al.*, 2005). Cobalt and several other transition metals, such as nickel, copper, vanadium, and chromium, participate in ROS generation (*e.g.*, hydroxyl radical formation) through a Fenton-type reaction (Valko *et al.*, 2005). Cobalt has also been shown to inhibit DNA repair systems resulting in genomic instability and accumulation of genetic damage and mutations (Hartwig *et al.*, 1991; Kasten *et al.*, 1997). In addition, cobalt and other metals (e.g., nickel, cadmium and hexavalent chromium) have been shown to alter epigenetic homeostasis in cells that may result in

changed programs of gene expression, ultimately leading to carcinogenesis (Li *et al.*, 2009; Brocato and Costa, 2013).

A mechanistic study by Green *et al*. (2013) found that lung cells have a high tolerance (i.e., delayed apoptosis and cell death) for cobalt loading (as cobalt chloride), when compared to nickel (Ni^{2+}). High cobalt loading of the cells may promote an accumulation of genetic and epigenetic abnormalities. Exposure of lung cells to Ni^{2+} led to comparatively greater overall cell death and apoptosis and less genotoxicity. These investigators proposed that lung carcinogenicity may result from tolerance to cobalt cell loading, which allows cell replication and survival despite the presence of cobalt-mediated accumulation of genetic damage.

NTP Carcinogenicity Bioassays

Cobalt metal

NTP conducted lifetime rodent inhalation carcinogenicity studies for cobalt metal (NTP, 2014a). The mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) of the inhaled particles, recorded monthly, was in the range of 1.4-2.0 micrometers (μm) ± 1.6-1.9. This particle size was noted by NTP to be within the respirable range of the rodents. Groups of F-344/NTac rats and $B6C3F_1$/N mice (50/group/sex/species) were exposed to the cobalt metal aerosol via whole-body inhalation at concentrations of 0, 1.25, 2.5 or 5 milligrams per cubic meter (mg/m^3), for 6.2 hrs/day, 5 days/week for up to 105 weeks. These nominal concentrations were within 1% of the analytical concentrations. The daily exposures include the 6 hr exposure time at a uniform aerosol concentration plus the ramp-up time of 12 min (0.2 hrs/day) to achieve 90% of the target concentration after the beginning of aerosol generation. The decay time to 10% of the target concentration at the end of the exposures was about 9.4 min.

In rats, body weights of males and females in the 2.5 and 5 mg/m^3 groups were reduced (≥10%) compared to controls. In the 5 mg/m^3 groups, body weights were reduced starting after weeks 12 and 21 for males and females, respectively. In the 2.5 mg/m^3 groups, body weights were reduced after weeks 99 and 57 in males and females, respectively. Survival was significantly reduced in the mid-dose 2.5 mg/m^3 female rats compared to controls (p=0.038, life table pairwise comparison) (NTP, 2014a). However, significant differences in survival between the 2.5 mg/m^3 group and controls were not apparent until after week 85 of the study. Most of the female rats in the 2.5 mg/m^3 group had died with treatment-related tumors (42 of 50 (84%)), many of which were considered the primary cause of death (13 of 50 [26%]).

The statistically significant and/or biologically noteworthy tumor incidences in male and female rats are shown in Table 2. The incidences of pulmonary alveolar/bronchiolar

adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) were statistically significantly increased in nearly all cobalt-exposed groups. Positive trends for these tumors, both individually and combined, were observed in both males and females.

The rats also exhibited a generally increasing trend of multiple alveolar/bronchiolar adenoma and carcinoma with increasing exposure concentration. Squamous cell neoplasms of the lung, which were predominantly cystic keratinizing epitheliomas (CKE), were observed in several cobalt-exposed females and in two cobalt-exposed males, but did not reach statistical significance in either sex. CKE is a rare chemically-induced pulmonary tumor that has been observed in rats exposed to certain particulate compounds (Behl *et al.*, 2015). CKE originates from a different lung cell type from that of alveolar/bronchiolar adenoma and carcinoma, and is considered separately for tumor dose-response analysis (McConnell *et al.*, 1986; Brix *et al.*, 2010). One female rat in the high exposure group had a squamous cell carcinoma, which is believed to be part of the continuum of lesions progressing from CKE. NTP considered the increase in squamous cell neoplasms of the lung to be a treatment-related effect in female rats due to its rarity and exceedance in incidence when compared to the historical control range for all routes of administration. The incidence of lung squamous cell neoplasms in male rats was lower, resulting in an equivocal finding of carcinogenicity by NTP (2014a).

Increased incidences of benign and malignant pheochromocytoma, and benign or malignant pheochromocytoma (combined) of the adrenal medulla were observed in male and female rats. The incidences of these adrenal medulla neoplasms, both individually and combined, were statistically significantly increased at 2.5 and 5 mg/m^3 in male rats. The same was true for female rats, with the exception of a lack of increased incidence in malignant pheochromocytoma at 2.5 mg/m^3. NTP (2014a) also noted a trend-related increased incidence of bilateral pheochromocytoma, both benign and malignant, in male and female rats.

In male rats, a positive trend for pancreatic islet cell carcinoma, and pancreatic islet cell adenoma or carcinoma (combined), was observed following cobalt metal exposure. A positive trend (p=0.047, Cochran-Armitage trend test) for pancreatic islet cell adenoma was also observed. At 2.5 mg/m^3, the incidence of adenoma was significantly increased compared to controls. A significantly greater incidence of adenoma or carcinoma (combined) was observed at both 2.5 and 5 mg/m^3. In female rats the incidence of islet cell neoplasms was slightly increased at 5 mg/m^3 (two rats with a carcinoma, and one with an adenoma and a carcinoma), but was not statistically significant. A positive trend for these tumors was observed (p=0.044, Cochran-Armitage trend test), but islet cell tumor incidence in high exposure females did exceed the historical control incidences for all routes of administration. NTP concluded there was equivocal evidence of pancreatic islet cell carcinoma in female rats due to the absence of statistically significant

differences in pairwise comparisons and the absence of a significant positive trend (when the pairwise comparisons and trend were calculated by NTP using the poly-3 test). NTP stated this was the first time that the pancreas was a target organ of carcinogenicity in NTP inhalation studies.

Standard kidney evaluation, in which only one section of each kidney is microscopically examined, revealed a slightly increased incidence of renal tubule adenoma or carcinoma (combined) in 5 mg/m^3 male rats. Although not statistically significant, this finding suggested a treatment-related effect due to exceedance of historical control ranges for all routes of administration. An extended evaluation of the kidneys with step-sectioning at 1 mm intervals subsequently revealed more tumors in the 5 mg/m^3 rats but also more in the control group. Thus, pairwise test comparison was still not significant. In addition, no supporting nonneoplastic lesions were found in the kidneys. Nevertheless, NTP concluded that due to the relative rarity of these tumors, there is equivocal evidence that these tumors are related to cobalt exposure.

Lastly, female rats had an increased incidence of mononuclear cell leukemia in all exposure groups. NTP considered the increased incidence of this leukemia to be related to cobalt exposure.

Table 2. Unadjusted tumor incidence in rats exposed to cobalt metal for two years (NTP, 2014a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Male rat									
Lung: Alveolar/bronchiolar adenoma		2/50‡	10/50*	10/50*	14/50**	0.002	0.014	0.014	<0.001
Lung: Alveolar/bronchiolar carcinoma		0/50‡	16/50**	34/50**	36/50**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		2/50‡	25/50**	39/50**	44/50**	<0.001	<0.001	<0.001	<0.001
Lung: Cystic keratinizing epithelioma		*0/50*	*1/50*	*0/50*	*1/50*	*0.236*	*0.500*	*1.000*	*0.500*
Adrenal medulla: Benign pheochromocytoma		15/50‡	23/50	37/50**	34/50**	<0.001	0.074	<0.001	<0.001
Adrenal medulla: Malignant pheochromocytoma		2/50‡	2/50	9/50*	16/50**	<0.001	0.691	0.026	<0.001
Adrenal medulla: Benign or malignant pheochromocytoma		17/50‡	23/50	38/50**	41/50**	<0.001	0.154	<0.001	<0.001
Pancreatic islets: adenoma		0/50†	1/50	6/48*	3/49	0.047	0.500	0.012	0.117
Pancreatic islets: carcinoma		2/50†	1/50	5/48	6/49	0.023	0.500	0.201	0.128
Pancreatic islets: adenoma or carcinoma		2/50‡	2/50	10/48*	9/49*	0.003	0.691	0.011	0.023
Kidney: Adenoma or carcinoma, standard evaluation		*0/50‡*	*1/50*	*0/50*	*4/50*	*0.006*	*0.500*	*1.000*	*0.059*
Kidney: Adenoma or carcinoma, standard + extended evaluation		*3/50†*	*1/50*	*1/50*	*7/50*	*0.022*	*0.308*	*0.308*	*0.159*

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals examined. Tumor type and incidence data in italics represents equivocal finding for carcinogenicity by NTP (2014a).

(b) * = $p<0.05$, ** = $p<0.01$; *p*-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = $p<0.05$, ‡ = $p<0.01$, *p*-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical *p*-values for trend are in the statistical *p*-value control column.

Table 2 (continued). Unadjusted tumor incidence in rats exposed to cobalt metal for two years (NTP, 2014a)[a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Female rat									
Lung: Alveolar/bronchiolar adenoma		2/50‡	7/50	9/50*	13/50**	0.001	0.080	0.026	0.002
Lung: Alveolar/bronchiolar carcinoma		0/50‡	9/50**	17/50**	30/50**	<0.001	0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		2/50‡	15/50**	20/50**	38/50**	<0.001	<0.001	<0.001	<0.001
Lung: Squamous cell tumors (predominantly cystic keratinizing epithelioma)[c]		0/50	4/50	1/50	3/50	0.165	0.059	0.500	0.121
Adrenal medulla: Benign pheochromocytoma		6/50‡	12/50	22/50**	36/50**	<0.001	0.096	<0.001	<0.001
Adrenal medulla: Malignant pheochromocytoma		0/50‡	2/50	3/50	11/50**	<0.001	0.247	0.121	<0.001
Adrenal medulla: Benign or malignant pheochromocytoma		6/50‡	13/50	23/50**	40/50**	<0.001	0.062	<0.001	<0.001
Pancreatic islets: adenoma or carcinoma		*1/50*†	*0/50*	*0/50*	*3/50*	*0.044*	*0.500*	*0.500*	*0.309*
Immunologic system: Mononuclear cell leukemia		16/50†	29/50**	28/50*	27/50*	0.043	0.008	0.013	0.021

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals examined. Tumor type and incidence data in italics represents equivocal finding for carcinogenicity by NTP (2014a).

(b) * = $p<0.05$, ** = $p<0.01$; *p*-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = $p<0.05$, ‡ = $p<0.01$, *p*-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical *p*-values for trend are in the statistical *p*-value control column.

(c) Includes one squamous cell carcinoma in the 5 mg/m^3 group.

Nonneoplastic findings in the rats included various pulmonary lesions (alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation and bronchiole epithelium hyperplasia), which were observed in the animals at all exposure levels (data not shown). A spectrum of nonneoplastic nasal lesions was also observed in all exposed groups.

In mice exposed to cobalt metal for two years, body weights of males and females at the highest exposure were reduced ≥10% compared to controls. The body weights in these groups were reduced starting after weeks 85 and 21 for males and females, respectively. Survival of male mice was significantly reduced in the 2.5 and 5 mg/m^3 males compared to controls. However, most of the male mice in the two groups died late in the study resulting in mortality rates that were not significantly different than controls until after week 85. Most of the male mice in these two exposed groups died with treatment-related lung tumors (43/50 (86%) and 47/50 (94%) in the 2.5 and 5 mg/m^3 groups, respectively). For the males that died prior to terminal sacrifice, the primary cause of death were lung tumors in most cases (13 of 21 (62%) at 2.5 mg/m^3 and 25 of 28 (89%) at 5 mg/m^3).

The tumor incidences resulting from two-year exposure to cobalt metal in mice are presented in Table 3. Treatment-related tumors in mice were confined to the lungs. The incidences of pulmonary alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) were statistically significantly increased in both males and females in all cobalt-exposed groups, and showed positive trends with exposure in both sexes. Statistically significantly increased alveolar/bronchiolar adenomas were observed in male mice in the 2.5 mg/m^3 group, and in female mice in the 5 mg/m^3 group. The incidences of multiple alveolar/bronchiolar carcinomas were statistically significantly increased in both males and females in all cobalt-exposed groups.

Table 3. Unadjusted tumor incidence in mice exposed to cobalt metal for two years (NTP, 2014a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Male mouse									
Lung: Alveolar/bronchiolar adenoma		7/50	11/49	15/50*	3/50	0.896	0.204	0.045	0.159
Lung: Alveolar/bronchiolar carcinoma		11/50‡	38/49**	42/50**	46/50**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		16/50‡	41/49**	43/50**	47/50**	<0.001	<0.001	<0.001	<0.001
Female mouse									
Lung: Alveolar/bronchiolar adenoma		3/49†	9/50	8/50	10/50*	0.050	0.065	0.106	0.039
Lung: Alveolar/bronchiolar carcinoma		5/49‡	25/50**	38/50**	43/50**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		8/49‡	30/50**	41/50**	45/50**	<0.001	<0.001	<0.001	<0.001

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals examined.

(b) * = $p<0.05$, ** = $p<0.001$; *p*-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = $p<0.05$, ‡ = $p<0.001$, *p*-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical *p*-values for trend are in the statistical *p*-value control column.

Nonneoplastic findings in the mice were mainly confined to the lungs, including alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolization, alveolar epithelium hyperplasia, proteinosis, and infiltration of cellular histiocytes within alveolar spaces, which were observed at all exposure levels (data not shown). The incidences of bronchiole epithelium hyperplasia, bronchiole epithelium erosion, and suppurative inflammation occurred at mid- and/or high-exposure levels in one or both sexes. Additionally, nonneoplastic lesions in the nose, larynx and trachea were observed in males and females in all exposed groups.

Overall, NTP (2014a) concluded there was clear evidence of carcinogenic activity of cobalt metal in male and female rats and mice. The lung was the primary site for carcinogenicity in rats and mice exposed to cobalt metal, with concentration-related increases in alveolar/bronchiolar adenoma and carcinoma, including multiple adenomas and carcinomas, observed in males and females of both species.

Cobalt sulfate heptahydrate

Groups of F-344/N rats and $B6C3F_1$ mice (50 group/sex/species) were exposed to 0, 0.3, 1.0 or 3.0 mg/m^3 cobalt sulfate heptahydrate aerosol via whole-body inhalation for 6.2 hrs/day, 5 days/week, for 105 weeks (NTP, 1998a; Bucher *et al.*, 1999). The MMAD, recorded monthly, was within the range of 1 to 3 µm. Generation of the aerosol particles to which the rodents were exposed resulted in formation of primarily cobalt sulfate hexahydrate, although it is expected that environmental exposures to hydrated cobalt sulfate would be the heptahydrate form. The heptahydrate reportedly does not dehydrate to the hexahydrate until a temperature of 41.5º C is reached. The daily exposures included the 6 hr exposure time at a uniform aerosol concentration plus the ramp-up time of 12 min (0.2 hr/day) to achieve 90% of the target concentration after the beginning of aerosol generation. The decay time to 10% of the target concentration at the end of the exposures was in the range of 11-13 min.

In rats, survival and body weights of cobalt sulfate heptahydrate-exposed animals remained similar to that of controls throughout the studies. The statistically significant and/or biologically noteworthy tumor incidences in male and female rats are shown in Table 4. The tumor incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was statistically significantly increased in male rats exposed to 3.0 mg/m^3, and showed a positive trend with exposure. In addition, the incidence of alveolar/bronchiolar adenoma at 3.0 mg/m^3, and alveolar/bronchiolar carcinoma at 1.0 mg/m^3 exceeded historical control ranges in the males. Female rats at the two highest exposures showed statistically significantly increased incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined). A positive trend for these lung tumors was also present in the female rats.

One female rat in each of the 1.0 and 3.0 mg/m^3 exposure groups had a squamous cell carcinoma in the lungs at terminal necropsy. These tumors were included with the alveolar/bronchiolar adenoma or carcinoma (combined) for determination of the effective tumor incidence. Squamous cell carcinoma generally arises from a lung tissue different from that of alveolar/bronchiolar adenoma and carcinoma. However, NTP (1998a) noted that squamous lesion differentiation was a variable component of other alveolar/bronchiolar proliferative lesions, including the fibroproliferative lesions (some of which were diagnosed as alveolar/bronchiolar carcinomas) observed in this study. Therefore, NTP combined the two squamous cell carcinomas identified in cobalt-exposed female rats with the observed alveolar/bronchiolar adenomas and carcinomas in assessing treatment-related lung tumors.

A significant increase (p=0.045) in the incidence in the adrenal medulla of benign, complex or malignant pheochromocytoma (combined), was observed in 1.0 mg/m^3 male rats. There was also some evidence for an increased incidence of bilateral pheochromocytoma in the cobalt sulfate heptahydrate-exposed male rats. However, lack of increased severity of hyperplasia and lack of increased neoplasms in the 3.0 mg/m^3 group led to an equivocal finding of carcinogenicity in male rats by NTP. In female rats, statistically significantly increased incidences of benign pheochromocytoma, and benign, complex or malignant pheochromocytoma (combined) were observed in the 3.0 mg/m^3 exposure group. Positive trends were observed for both benign pheochromocytoma and for the combined adrenal medulla neoplasms.

Table 4. Unadjusted tumor incidence in rats exposed to cobalt sulfate heptahydrate for two years (NTP, 1998a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Male rat									
Lung: Alveolar/bronchiolar adenoma		1/50†	4/50	1/48	6/50	0.032	0.181	0.742	0.056
Lung: Alveolar/bronchiolar carcinoma		0/50	0/50	3/48	1/50	0.233	1.000	0.114	0.500
Lung: Alveolar/bronchiolar adenoma or carcinoma		1/50†	4/50	4/48	7/50*	0.023	0.181	0.168	0.030
Adrenal medulla: Benign pheochromocytoma[c]		*14/50*	*19/50*	*23/49**	*20/50*	*0.193*	*0.198*	*0.041*	*0.146*
Adrenal medulla: Benign, complex or malignant pheochromocytoma		*15/50*	*19/50*	*25/49**	*20/50*	*0.239*	*0.263*	*0.027*	*0.201*
Adrenal medulla: Benign bilateral pheochromocytoma		*1/50*	*4/50*	*6/49*	*5/50*	*0.133*	*0.181*	*0.053*	*0.102*
Female Rat									
Lung: Alveolar/bronchiolar adenoma		0/50‡	1/49	10/50**	9/50**	<0.001	0.495	<0.001	0.001
Lung: Alveolar/bronchiolar carcinoma		0/50†	2/49	6/50*	6/50*	0.012	0.242	0.013	0.013
Lung: Alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma		0/50‡	3/49	16/50**	16/50**	<0.001	0.117	<0.001	<0.001
Adrenal medulla: Benign pheochromocytoma		2/48‡	1/49	3/50	8/48*	0.002	0.492	0.520	0.045
Adrenal medulla: Benign, complex or malignant pheochromocytoma		2/48‡	1/49	4/50	10/48*	<0.001	0.492	0.359	0.014

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals examined. Tumor type and incidence data in italics represents equivocal finding for carcinogenicity by NTP (1998a).

(b) * = $p<0.05$, ** = $p<0.01$; *p*-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = $p<0.05$, ‡ = $p<0.01$, *p*-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical *p*-values for trend are in the statistical *p*-value control column.

(c) Includes benign bilateral pheochromocytoma.

Nonneoplastic pulmonary lesions (alveolar epithelium metaplasia, proteinosis, granulomatous inflammation, and interstitial fibrosis) were observed in nearly all cobalt sulfate heptahydrate-exposed rats of both sexes, and the severity generally increased with dose (data not shown). Squamous metaplasia of the larynx and a spectrum of nonneoplastic lesions in the nose were also observed in all cobalt-exposed groups.

In mice, two-year exposure to cobalt sulfate heptahydrate aerosol did not affect the survival rate. Body weights of 3.0 mg/m^3 males were slightly reduced compared to controls starting at week 96. Body weights of cobalt sulfate heptahydrate-exposed female mice were similar to, or slightly greater, than body weights of controls.

Neoplastic findings in mice included statistically significantly increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma in both 3.0 mg/m^3 males and females (Table 5). The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were statistically significantly increased in both 3.0 mg/m^3 males and females, and also in 1.0 mg/m^3 females. Positive trends were observed for these pulmonary neoplasms, both individually and combined.

The incidence of hemangiosarcoma was increased above the historical control range in all cobalt sulfate heptahydrate-exposed male mice, and was significantly increased (p=0.050) above control mice in the 1.0 mg/m^3 group. However, the presence of *Helicobacter hepaticus* infection in the males, and in some females, compromised the liver tumor findings in these studies, leading to equivocal findings of carcinogenicity by NTP.

Table 5. Unadjusted tumor incidence in mice exposed to cobalt sulfate heptahydrate for two years (NTP, 1998a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Male mouse									
Lung: Alveolar/bronchiolar adenoma		9/50†	12/50	13/50	18/50*	0.021	0.312	0.235	0.035
Lung: Alveolar/bronchiolar carcinoma		4/50†	5/50	7/50	11/50*	0.014	0.500	0.262	0.045
Lung: Alveolar/bronchiolar adenoma or carcinoma		11/50‡	14/50	19/50	28/50**	<0.001	0.322	0.063	<0.001
Liver: Hemangiosarcoma		*2/50*	*4/50*	*8/50**	*7/50*	*0.066*	*0.339*	*0.046*	*0.080*
Female mouse									
Lung: Alveolar/bronchiolar adenoma		3/50†	6/50	9/50	10/50*	0.031	0.243	0.061	0.036
Lung: Alveolar/bronchiolar carcinoma		1/50‡	1/50	4/50	9/50**	<0.001	0.753	0.181	0.008
Lung: Alveolar/bronchiolar adenoma or carcinoma		4/50‡	7/50	13/50*	18/50**	<0.001	0.262	0.016	<0.001
Liver: Hemangiosarcoma		*1/50*	*0/50*	*3/50*	*0/50*	0.713	0.50	0.308	1.000

a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals examined. Tumor type and incidence data in italics represents equivocal finding for carcinogenicity by NTP (1998a).

(b) * = p<0.05, ** = p<0.01; p-value indicators are from pairwise comparisons with controls using Fisher exact test performed by OEHHA. † = p<0.05, ‡ = p<0.001, p-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical p-values for trend are in the statistical p-value control column.

Non-neoplastic lesions of the bronchi, nasal tissue and larynx were observed either in the two highest exposure groups or in all exposed groups in both studies (data not shown). Similar to rats, squamous metaplasia of the larynx was observed in mice, and was considered one of the most sensitive tissue responses to cobalt sulfate heptahydrate exposure.

Overall, NTP (1998a) concluded that there is "clear evidence" for a treatment-related increase in carcinogenic activity in female rats exposed to cobalt sulfate heptahydrate due to the increased lung and adrenal tumors. The weaker tumor response in cobalt sulfate heptahydrate-exposed male rats resulted in a lower finding of "some evidence" for carcinogenic activity in male rats. In mice, NTP concluded there was "clear evidence" for treatment-related lung tumors in both males and females.

Other Supporting Cancer Bioassays

Inhalation

In an early chronic inhalation study, male Syrian golden hamsters (51/group) were exposed whole-body to 0 or 10.1 mg/m^3 aerosolized cobalt(II) oxide 7 hr/day, 5 days/week for their life span (Wehner *et al.*, 1979). The particle size was 0.45 µm ± 1.9 (MMAD ± GSD). Exposures began at 2 months of age. No difference in survival was observed between the two groups throughout the study. However, approximately 50% of the animals in both groups had died by 15-16 months of age, and the maximum survival was about 22 months. The normal average life span of Syrian golden hamsters is 2 to 2.5 years. Noncancer effects due to cobalt(II) oxide exposure included interstitial pneumonitis, diffuse granulomatous pneumonia, and emphysema. No differences were observed in the total incidence of neoplasms between cobalt(II) oxide-exposed animals (3/51) and control animals (3/51), which IARC (1991) suggested may be partly related to the overall poor survival rate. Only one of these tumors was specifically identified as a lung tumor (adenoma in the control group) by the authors. Compared to rats, Syrian golden hamsters appear to be more resistant to respiratory tract tumors following exposure to carcinogenic metals (*e.g.*, nickel) (Wehner *et al.*, 1979; NTP, 1996; 2014a).

Intratracheal instillation

Two additional sets of chronic exposure studies exposed the respiratory tract of animals via intratracheal instillation. Groups of male and female hamsters (25/sex/group) received weekly doses of 0 or 4 mg cobalt(II, III) oxide powder suspended in gelatin/saline vehicle via intratracheal administration for 30 weeks (Farrell and Davis, 1974). The animals were then observed for another 68 weeks. The size range of the particles were described as 0.5 to 1.0 µm. Two of 50 hamsters receiving cobalt oxide developed pulmonary alveolar tumors, and one of 50 hamsters receiving gelatin-saline control developed a tracheal tumor.

Steinhoff and Mohr (1991) administered cobalt(II) oxide to specific pathogen free (SPF)-bred male and female Sprague Dawley rats by intratracheal instillation every 2-4 weeks over a period of two years. Exposure groups in these studies consisted of 50 rats/sex/dose given either nothing (untreated control), saline (vehicle control), 2 mg/kg-body weight (BW) cobalt(II) oxide (total dose 78 mg/kg), or 10 mg/kg-BW cobalt(II) oxide (total dose 390 mg/kg). Approximately 80% of the cobalt particles instilled were said to be in the range of 5-40 µm. In males, no pulmonary tumors were found in the untreated controls or the saline controls, one benign squamous epithelial lung tumor was found in the low dose group, and 2 bronchioalveolar adenomas, 1 bronchioalveolar adenocarcinoma, and 2 adenocarcinomas (cell type not specified) were observed in the high dose group. The increase in combined pulmonary tumors in the high dose group was statistically significant (p=0.02) by pairwise comparison with controls. The authors concluded that under the conditions of this study, cobalt(II) oxide is weakly carcinogenic by the intratracheal instillation route. In females, no pulmonary tumors were found in the untreated controls or the saline controls, one bronchoalveolar adenoma was found in the low dose group and one bronchoalveolar carcinoma was found in the high dose group.

Subcutaneous, intraperitoneal and intramuscular administration

Subcutaneous and intraperitoneal injections of rats with cobalt(II) oxide resulted in local tumors (Steinhoff and Mohr, 1991). In SPF male Sprague Dawley rats (10/group), subcutaneous injection of saline (control), 2 milligrams per kilogram of bodyweight (mg/kg-BW) cobalt(II) oxide five times per week, or 10 mg/kg-BW cobalt(II) oxide once per week over a two-year period resulted in no tumors in controls and 9/20 malignant tumors in treated rats (p<0.001). In the intraperitoneal injection study, male and female SPF rats (10/sex/dose) were injected with saline (control) or 200 mg cobalt(II) oxide 3 times at intervals of 2 months. Tumors were reported for males and females combined at the end of two years: 1/20 control rats developed malignant tumors (1 malignant histiocytoma) compared to 14/20 cobalt-treated rats (10 histiocytomas, 3 sarcomas, 1 mesothelioma) (p<0.001).

Using a rodent implantation model, ten male Sprague-Dawley rats were implanted bilaterally with cobalt nanoparticles (NPs) (surface area to volume ratio: 5×10^4 mm^{-1}) intramuscularly, and bulk cobalt particles (surface area to volume ratio: 4.73 mm^{-1}) subcutaneously on the contralateral side (Hansen *et al.*, 2006). The specific cobalt compound was not identified by the authors, but was likely cobalt metal. On the cobalt NP side, malignant mesenchymal tumors were found in one of four rats sacrificed after six months of exposure, and in five out of six of the remaining rats sacrificed after eight months of exposure. On the cobalt bulk material side, inflammation was observed after six months, and one preneoplastic lesion out of six rats after eight months. The authors

concluded that the physical properties of cobalt (NP vs. bulk form) could have a significant influence on the acceleration of the neoplastic process.

Earlier non-inhalation studies summarized by IARC (1991) also suggest that cobalt metal and cobalt compounds are carcinogenic by the subcutaneous and intramuscular routes of administration, mainly producing local sarcomas.

Toxicokinetics

Human toxicokinetics and comparison to other mammalian species

For cobalt metal and salts, good correlations were found between the cobalt concentration in the breathing zone of workers and the concentration of cobalt in post-shift urine and blood (Swennen *et al.*, 1993; Lison *et al.*, 1994; Hutter *et al.*, 2016). For every 1 mg/m^3 cobalt in air, there was an excretion of approximately 200 micrograms per liter (µg/L) in urine of cobalt workers (Hutter *et al.*, 2016). In cobalt oxide workers, concentrations of cobalt in blood and urine were higher than in non-exposed subjects, but no correlation with air concentration was found with post-shift urine and blood concentrations (Lison *et al.*, 1994) The authors suggested the lack of correlation was due to lower pulmonary absorption of cobalt oxides compared to more soluble cobalt compounds.

Inhalation studies in workers and volunteers exposed to cobalt metal or cobalt oxides have shown that cobalt elimination from the lungs is multiphasic with reported half-lives of 2 to 44 hrs, 10 to 78 days, and a long-term phase lasting many months to years (Newton and Rundo, 1971; Foster *et al.*, 1989; Apostoli *et al.*, 1994; Beleznay and Osvay, 1994). These elimination phases likely involve an initial rapid elimination from the tracheobronchial region via mucociliary clearance, an intermediate phase of macrophage-mediated clearance, and long-term retention and clearance probably due to cobalt bound to cellular components in the lung. Approximately 1 to 10% of the inhaled cobalt deposited in lung is subject to long-term retention and is predominantly cleared by translocation to blood (Bailey *et al.*, 1989). The pattern of elimination appears to be independent of the level of exposure (Apostoli *et al.*, 1994).

In a study of human volunteers (n = 4) inhaling cobalt(II,III) oxide (as $^{57}Co_3O_4$), about 20% of the initial lung burden was eliminated after 10 days (Foster *et al.*, 1989). However, about 40% of the initial lung burden was still retained in the body 100 days following exposure. The clearance half-time of the slow phase, a result of lung to blood translocation of cobalt, was in the range of 150-250 days. Two volunteers each had inhaled cobalt particles with different MMADs of 0.8 and 1.7 µm. Fractional deposition averaged 52% for the 0.8 µm particles and 78% for the 1.7 µm particles. However, differences in the elimination rates and retention rates could not be detected.

Martin *et al.* (2010) estimated an average of 12% absorption of inhaled cobalt in a toxicokinetic study of 16 male workers exposed to cobalt metal dust (particle size not defined) at two carbide tungsten plants. The toxicokinetic model measured breathing zone cobalt concentrations during work shifts over 4-6 days, and collected urine samples for up to one week to determine urinary cobalt concentrations. The estimated absorption was based on a default respiratory rate of 500 L/hr for a man at rest. Most of the inhaled cobalt (88%) was assumed to be exhaled, or removed from the lungs via mucociliary clearance and then eliminated in feces without being absorbed in the gastrointestinal tract. The fraction of cobalt deposited in the lungs and other organs long-term was not determined or incorporated into the absorption estimate.

Oral intake of soluble cobalt, as cobalt chloride, by human volunteers resulted in intermediate half-times (32 days) and long-term half-times (80-720 days) that were consistent with intermediate and long half-times resulting from inhalation exposure to cobalt metal or cobalt oxides (Holstein *et al.*, 2015). An initial rapid half-time phase (mean = 0.71 days) following oral intake reflected loss through fecal excretion during the first week after ingestion.

Translocation rates of inhaled radiolabeled cobalt(II,III) oxide ($^{57}Co_3O_4$) from lung to blood show considerable interspecies variation (Bailey *et al.*, 1989). Excluding the initial rapid phase of mucociliary clearance from the tracheobronchial tree, rats, mice, dogs, hamsters, and guinea pigs exhibited 90% or greater lung clearance of cobalt six months after exposure. However, humans and baboons showed much slower lung clearance with only 50% and 70% cleared by six months, respectively. The translocation rate of dissociated ^{57}Co from the lung to the blood in humans and baboons was 0.2 to 0.6% day^{-1}. In other mammalian species, this translocation rate was greater (up to 2.4% day^{-1}) but varied considerably in some species over time. The maximum difference in the translocation rate was up to seven-fold between rats and humans for 0.8 µm particles (Bailey *et al.*, 1989; Kreyling *et al.*, 1991b). Kreyling *et al*. (1991b) considered that for cobalt oxide particles retained in the lung, the rate-determining process for translocation to blood is the intracellular particle dissolution in the macrophage since transfer of the dissociated material to blood is fast and almost quantitative. However, interspecies phago-lysosomal pH differences in alveolar macrophages were not found and do not appear to be the cause of translocation rate differences among mammalian species (Kreyling *et al.*, 1991a).

Lung retention is generally greater for larger cobalt particles (as Co_3O_4) than smaller particles (Kreyling *et al.*, 1986; Bailey *et al.*, 1989; Leggett, 2008). However, cobalt metal and radiolabeled cobalt oxide (^{57}Co) had whole body clearance times very similar to that of clearance times from the lung indicating cobalt does not translocate or accumulate appreciably in other tissues (Rhoads and Sanders, 1985; Bailey *et al.*, 1989; Patrick *et al.*, 1994; NTP, 2014a).

For soluble cobalt compounds (as $CoCl_2$ or $Co(NO_3)_2$), Patrick *et al*. (1994) found that the fraction of instilled or inhaled cobalt remaining in the lung of mammalian species (rat, dog, baboon, guinea pig, hamster) averaged 0.13-0.58% 100 days after exposure. This finding suggests lung clearance of cobalt may be faster with soluble compounds compared to poorly soluble or insoluble compounds such as Co_3O_4.

NTP tissue burden studies of cobalt metal in rats and mice

Tissue burden and concentration were assessed by NTP (2014a) in rats and mice exposed by inhalation to cobalt metal (1.25, 2.5 or 5 mg/m^3) for up to two years. Tissue burden (µg Co/tissue), rather than tissue concentration (µg Co/g tissue), was generally preferred to express levels of cobalt in the organs due to significant changes in organ weights caused by cobalt exposure. In the 2-year exposure studies, lung cobalt concentrations and burdens in rats and mice increased with increasing cobalt concentrations, but appeared to reach steady state by day 184. Little change in lung burden was observed through day 548, and then the burden steadily decreased following cessation of cobalt exposure. The modeled pulmonary clearance of cobalt showed a two-phase elimination. The half-life of the rapid phase ranged from 1.53 to 2.94 days in rats and 1.1 to 5.2 days in mice. The slow clearance phase in rats produced a half-life estimate ranging from 83 to 167 to days. In mice, the slow clearance half-life was 409, 172, and 118 days with increasing exposure concentration. The majority of the deposited lung cobalt was cleared during the fast elimination phase (>95% in rats and >82% in mice).

Cobalt concentrations and burdens in exposed rats and mice increased in all other tissues examined by NTP (2014a) indicating absorption and systemic distribution occurs by the inhalation route. In 13-week studies, blood cobalt levels increased proportionally to exposure concentration in rats and mice. Blood cobalt in exposed groups of animals reached steady state at the earliest time point (day 5) measured in rats, and at about day 12 in mice. In both rats and mice, blood cobalt then rapidly decreased below the level of detection following cessation of cobalt exposure. Cobalt burdens and concentrations in liver also increased with increasing cobalt concentration up to day 26 in both rodent species. However, cobalt burdens by day 40 were generally lower than at day 26. Liver cobalt burdens approached, or even exceeded, the lung cobalt burdens at days 26 and 40.

Overall, normalized lung tissue burdens (measured as µg Co/total lung per mg Co/m^3) did not increase with increasing exposure even though cobalt lung concentrations increased with increasing exposure (NTP, 2014a). Cobalt concentrations (µg Co/g tissue) in rats showed the following order: lung > liver >kidney > femur > heart > serum > blood. Tissue cobalt burdens (µg Co/tissue) showed similar order with the exception that liver accumulated more cobalt than lung, and the heart accumulated more cobalt than

the femur. With minor exceptions, the order for tissue concentration and burden were similar in mice.

Cellular toxicokinetics of cobalt nanoparticles

Cobalt oxide NPs are finding increasing use in commercial and industrial applications, leading to interest in conducting genotoxicity studies to examine their effects in various human and animal cells (Alarifi *et al.*, 2013). NPs have diameters of 0.1 µm or less. Compared to fine-scale particles, or micrometer-sized particles, NPs have a larger specific surface area, higher physical and chemical activity, and thus higher biological activity (Horie *et al.*, 2012). For poorly soluble cobalt oxide NPs, *in vitro* studies in human keratinocyte HaCaT cells suggested that the most important cytotoxic factor of these particles is cobalt ion (Co^{2+}) release. Horev-Azaria *et al*. (2011) and Cappellini et al. (2018) came to a similar conclusion following *in vitro* studies with cobalt metal NPs. Cappellini et al. (2018) found the high genotoxic activity of cobalt metal NPs was related to intracellular corrosion (i.e., oxidation) generating both Co^{2+} ions and ROS. However, when accounting for differences in surface area, the toxicity of Co (average primary size 25 nm) and CoO (primary size 43 nm) NPs was similar based on surface area dose rather than of mass dose.

The cellular uptake of radiolabeled cobalt(II) oxide NPs (^{60}Co) and cobalt chloride ($^{57}Co^{2+}$) were investigated *in vitro* in Balb/3T3 mouse fibroblasts (Ponti *et al.*, 2009) and human peripheral blood leukocytes (Colognato *et al.*, 2008). In both studies, cobalt NPs showed a 50- to 140-fold greater uptake compared to $^{57}Co^{2+}$. The authors postulated a "Trojan horse"-type mechanism was involved, in which cobalt NPs interacted with proteins on the surface of the cells and were more readily taken up (Ponti *et al.*, 2009). This led to the observed increase in cytotoxicity and genotoxicity. Further research suggests internalized cobalt metal nano- and micro-particles diffuse to subcellular organelles and release cobalt ion in millimolar concentrations in nuclei and mitochondria (Sabbioni *et al.*, 2014a; Sabbioni *et al.*, 2014b).

Epidemiological Studies

Limited information is available to assess the carcinogenic risk to workers exposed to cobalt and cobalt compounds.

In studies of workers primarily exposed to cobalt compounds, Mur *et al*. (1987) performed a retrospective mortality investigation of 1,143 workers at a French electrochemical plant producing cobalt and sodium. Of these, 110 workers had at least one year of service between 1950 and 1980 in the facility producing cobalt metal and some cobalt oxides and salts. Using male mortality in France as a reference, a Standardized Mortality Ratio (SMR) of 1.29 was found for the cobalt worker cohort. The

relative high death rate was attributed, in part, to higher lung cancer cases (SMR=4.66, $p<0.05$, 4 cases). However, this study had several limitations or confounders, including too small a number of lung cancer cases to reliably establish a link to occupational risk, no smoking assessment, possible (but not quantified) co-exposure to the carcinogenic metals arsenic and nickel, and no findings of nonneoplastic pulmonary diseases usually associated with cobalt exposure.

Follow-up by Moulin *et al*. (1993) at the French electrochemical plant extended mortality surveillance from 1981-1988. The study did not find excess mortality due to lung cancer in the cobalt worker cohort (SMR= 0.85 including all cobalt workers; SMR=1.16 including only French-born workers). This disparate finding was due to the lack of additional lung cancer deaths during the 1981-1988 follow-up and improved collection of causes of death by examining death certificates. Use of death certificates rather than medical records lowered the proportion of unknown causes from 20% observed by Mur *et al*. (1987) to 11% in the later study, though the number of lung cancer cases did not increase. Neither Mur *et al*. (1987) nor Moulin *et al*. (1993) provided an estimate of the airborne cobalt concentrations the workers were exposed to.

The incidence of lung cancer among Danish women plate painters was investigated in a retrospective study at two porcelain factories in which workers sprayed cobalt blue dye onto plates (Tuchsen *et al.*, 1996). Only trace exposure to other carcinogenic metals was said to have occurred. Participation for the study entailed employment between 1943 and 1987 at Factory 1 (n=382), and 1962 and 1987 at Factory 2 (n=492). The last year of follow-up occurred for both factories in 1992. A referent group consisted of 520 women working in another part of Factory 1 without exposure to cobalt. Cancer incidence rates for all Danish women were used to calculate the expected number of cancer cases.

Exposure at the porcelain factories was to insoluble cobalt-aluminate spinel with a cobalt content of 25%. The factories switched over to soluble cobalt silicate dye in 1972 (Factory 1) and 1989 (Factory 2). The authors reported the latency period was too short and number exposed too low to assess cancer risk for the soluble dye exclusively (Tuchsen *et al.*, 1996). Christensen and Poulsen (1994) observed that exposure of porcelain plate painters to the insoluble dye resulted in lower levels and slower excretion of cobalt in urine compared to painters exposed to the soluble dye. Limited personal sampling in 1982, before improvements in industrial hygiene, showed a mean airborne cobalt concentration of 1,356 nanomoles per cubic meter (0.08 mg/m^3) for painters exposed to the soluble silicate dye. Since 1982, personal exposures were 372-593 nmol/m^3 (0.03-0.04 mg/m^3).

Tuchsen *et al*. (1996) found a statistically significant increase in lung cancer incidence for the exposed group (8 observed, 3.41 expected, standardized incidence rate (SIR =

2.35, 95% confidence interval (95% CI) = 1.01 - 4.61) compared to all Danish women. Lung cancer in the reference group was also elevated, although not significantly (7 observed, 3.51 expected, SIR = 1.99, 95% CI 0.8 - 4.1), compared to all Danish women. Comparison of the exposed group with the reference group resulted in a relative risk of 1.2. No association was found between length of employment and lung cancer incidence. The authors noted that smoking information was incomplete, but suggested the increased risk was likely not due to differences in smoking. However, the women plate sprayers consisted of unskilled manufacturing workers who are known to have a higher lung cancer incidence rate compared to the general Danish population. The authors concluded follow-up is needed to determine if there is a true effect of lung cancer in the cobalt-exposed painters.

Stopford *et al*. (2003) found that *in vitro* bioaccessibility of cobalt aluminate spinel was very low in all physiological fluids tested, including artificial interstitial, alveolar and lysosomal fluids. Thus, the equivocal increased cancer risk noted by Tuchsen *et al*. may be related to the lack of significant *in vivo* release of cobalt ion from cobalt aluminate spinel. Presently, no cancer assessment for exposure exclusively to the soluble cobalt silicate dye has been performed.

In a recent retrospective study by Sauni *et al*. (2017), 995 male workers at a Finnish cobalt plant were assessed for cancer incidence during the period of 1968 to 2004. Workers were employed at the plant at least one year and the mean follow-up was 26.2 years. Follow-up began on the date at one year of work exposure and ended at death, emigration, or December 31, 2013. An average duration of exposure was not provided. Cancer incidence was determined as SIRs that compared the observed worker cancer incidence to the expected incidence of the population in the same region using the Finnish Cancer Registry, a population-based nationwide database. The cohort was also subdivided into low, moderate, high and variable exposure groups based on exposure by department. Respirators were available for use, but not mandatory, during the study period. Airborne levels of cobalt and other compounds were consistently measured several times per year over the study period (Linna *et al.*, 2003; Sauni *et al.*, 2017).

Highest cobalt exposures were in the reduction and powder production departments and sulfating-roasting department where mean cobalt levels during 1968-2003 were between 0.06 and 0.10 mg/m^3 (Sauni *et al.*, 2017). In the roasting department, dust in the ambient air contained 15-20% iron, 1% zinc, 0.4% cobalt and 0.2% nickel, with cobalt and nickel in the form of water-soluble sulfates. The concentration of nickel was usually ≤0.04 mg/m^3. In the reduction and powder production facility, cobalt was mainly in the form of cobalt powder and fine powder. Moderate exposures to cobalt (0.02-0.03 mg/m^3) as sulfates, carbonates, oxides and hydroxides occurred in the chemical department, whereas low exposure (≤0.02 mg/m^3) to cobalt sulfides and sulfates occurred in the leaching and solution purification building. Nickel compounds (as sulfates, carbonates,

oxides and hydroxides) were also present in the chemical department, but at lower levels compared to the cobalt compounds (Linna *et al.*, 2003).

Neither total cancer risk incidence (SIR 1.00; 95% CI 0.81-1.22) nor lung cancer incidence (SIR 0.50; CI 0.18-1.08) were increased in this cohort of Finnish cobalt workers (Sauni *et al.*, 2017). For workers with over five years of exposure, the total cancer risk (SIR 1.08; 95% CI 0.85-1.34) and lung cancer incidence (SIR 0.52; 95% CI 0.17-1.22) were likewise not significantly elevated. In addition, none of the exposure subgroups with over one year of employment had lung cancer SIRs significantly different from 1.0. Three cases of tongue cancer were observed in the cobalt worker group, which was significantly greater than expected (SIR 7.39; 95% CI 1.52-21.6). However, all were smokers. The authors suggested a synergistic action of cobalt exposure with smoking, although the excess may have occurred through chance alone. Bladder cancer among the workers was nearly twice the expected number (SIR 1.88; 95% CI 0.86-3.56), but not statistically significant. Six out of the nine total cases were in the low exposure group, only one of which was a non-smoker. The authors noted that the SIR of 0.5 for lung cancer was likely not a result of lower smoking prevalence because the cobalt worker smoking prevalence (31.8%) was greater than the regional control population prevalence (18 to 25%, depending on educational class). The authors concluded that at the cobalt levels measured, lung cancer risk and overall cancer risk is not increased in the cobalt workers. However, it was noted that the results should be interpreted with caution due to the low number of cancer cases.

OEHHA observes that the study did not provide the average age at end of the follow-up period. However, based on the person-years data provided in the study, the average age appears to be in the mid- to late-40s. Some of these workers may be too young to see an increased incidence of cancers, such as lung cancer. In addition, a mean follow-up period of 26 years may be too short if the latency period for lung cancer is 20 years or longer, as it is for some other occupational carcinogens.

Occupational exposure to combined cobalt-tungsten carbide powders in the hard metal refinery industry has resulted in excess lung cancer cases, and is also known to cause a severe noncarcinogenic lung disease known as hard metal lung disease (Hogstedt and Alexandersson, 1987; Lasfargues *et al.*, 1994; Lison, 1996; Moulin *et al.*, 1998; Wild *et al.*, 2000). Mixed tungsten carbide-cobalt hard metal powders are categorized by IARC (2006) in Group 2A (probably carcinogenic to humans). The cobalt metal powder content used in the presintering process usually ranges from 5-15% while tungsten carbide usually exceeds 80% (Keane *et al.*, 2002). Co-exposure to other pulmonary system carcinogens (nickel, hexavalent chromium, asbestos) in the hard metal industry has been reported. Nickel is sometimes added as a binding agent for the sintering of hard metal, but is normally found in only trace amounts in tungsten (Yamada *et al.*, 1987; Scansetti *et al.*, 1998).

Studies suggest an interaction between cobalt and tungsten carbide that produces activated oxygen species that is markedly greater than that produced by cobalt metal alone. Tungsten carbide alone appears to have no carcinogenic action or ability to generate ROS (Lison, 1996). The genotoxicity of tungsten carbide-cobalt powder is also considerably greater than cobalt metal alone (Anard *et al.*, 1997; Lloyd *et al.*, 1997; Van Goethem *et al.*, 1997; De Boeck *et al.*, 2003). Zanetti and Fubini (1997) suggest that the two metals together act like a new compound with different physico-chemical properties from those of cobalt and tungsten carbide alone. Clinical and epidemiological evidence support this interaction of cobalt and tungsten leading to pulmonary injury, while cobalt metal on its own is not as potent (Lison, 1996). Consequently, OEHHA recommends that a cancer potency factor for cobalt and cobalt compounds not be applied in estimating risks from cobalt-tungsten carbide exposure related to the hard metal refinery industry, as it may underestimate the cancer risk resulting from this metal-on-metal interaction.

Hard metal lung disease has also been observed in diamond polishers, in which there was concurrent exposure to cobalt metal dust, iron and diamond dust (Lison, 2015). The disease has not been found in workers exposed exclusively to cobalt metal dust or other cobalt compounds. However, cobalt exposure has been associated with other noncancer effects in workers, including cobalt-induced asthma and skin allergies following dermal contact. Oral exposure to cobalt well above normal daily intakes has resulted in myocardial degeneration, hypothyroidism, and neurotoxic effects including deafness, peripheral polyneuropathy, and optical atrophy. Systemic exposure from cobalt-containing metal prostheses has also been suspected in causing various neurotoxic effects.

Genotoxicity

Soluble and insoluble cobalt compounds, not including cobalt metal

Early studies examined the genotoxicity of soluble cobalt(II) compounds, since it was thought that bioavailable cobalt ions were a leading cause of genetic damage (IARC, 1991; Lison, 1996). More recent studies compared the genotoxicity of soluble and insoluble cobalt compounds (particularly NP cobalt). Thus, soluble and insoluble cobalt studies are presented together in this section. In *in vitro* mammalian cell systems, soluble and insoluble cobalt compounds were found to produce altered DNA bases, DNA strand breaks, DNA crosslinks, micronuclei, chromosomal aberrations, aneuploidy, and inhibition of DNA repair. However, *in vivo* studies show mixed results for induction of chromosomal aberrations in bone marrow cells, and bacterial and mammalian cell gene mutation tests have generally shown weak or negative results for mutagenesis. Alternatively, initial reports provide evidence that soluble cobalt compounds can alter epigenetic homeostasis in cells.

DNA strand-break and cross-linking tests

The comet assay is a commonly used method to identify DNA lesions (*e.g.*, breaks or alkali-labile sites) following exposure of an isolated cell culture with a genotoxin. When DNA lesions are present, this electrophoretic technique at high pH results in streaming of cellular DNA towards the anode giving the appearance of a comet. The comet effect is only seen when DNA contains breaks, or when DNA lesions are converted to breaks under alkaline conditions. This assay measures premutagenic lesions, which, in intact cells, can be removed by DNA repair processes if the repair occurs prior to DNA replication. Thus, positive assay data for a given compound do not necessarily indicate that the compound will induce mutations.

De Boeck *et al*. (1998) showed that cobalt chloride (0.3 to 6.0 micrograms per milliliter [µg/ml] Co-equivalents) induced DNA damage in isolated human lymphocytes (HLs) from three donors by the alkaline comet assay. DNA damage occurred in both a dose- and time-dependent manner.

Cobalt chloride induced DNA double strand breaks in a cancer-derived H460 human lung epithelial cell line (Patel *et al.*, 2012). Increased double strand break formation was determined by examining histone H2AX phosphorylation in Western blot analysis. The production of double strand breaks correlated with the intracellular generation of ROS; a 2.5-fold induction of ROS at 300 µM cobalt chloride resulted in a measurable increase in double strand break formation. Pretreatment of the cells with N-acetyl cysteine to inhibit ROS generation reduced the production of double strand breaks.

Cobalt sulfate produced DNA double strand breaks in *E. coli* as measured by the pulse field gel electrophoresis method (Kumar *et al.*, 2017). However, generation of ROS could not be detected using two different ROS-sensing dyes (2',7'-dichlorodihydrofluorescein diacetate and dihydroethidium) in *E. coli* cultured with cobalt sulfate, suggesting to the authors that oxidative stress did not cause the DNA damage.

The comet assay was also used to examine the genotoxicity of cobalt(II, III) oxide NPs (5 to 15 µg/ml) in human hepatocarcinoma (HepG2) cells (Alarifi *et al.*, 2013). A dose- and time-related increase in DNA damage, measured as increased percentage of tail DNA and increased olive tail moment, was observed in the HepG2 cells. The authors confirmed that a small percentage of Co^{2+} ions were released from cobalt(II, III) oxide in the suspensions, which is considered to be the factor responsible for genotoxicity. Similar levels of soluble cobalt chloride (10 and 15 µg/ml as Co^{2+}) in cell suspension also produced a statistically significant increase in DNA damage, although the genotoxic response was less than that of cobalt(II, III) oxide. The authors also observed that cobalt(II, III) oxide NPs caused a decrease in glutathione in HepG2 cells with a

concomitant increase in lipid hydroperoxides, ROS generation, and increased superoxide dismutase and catalase activity.

In a similar *in vitro* study using HLs, cobalt(II, III) oxide NPs caused a significant increase in percentage tail DNA damage in the comet assay (Rajiv *et al.*, 2016). The level of exposure used (100 µg/ml for 24 hrs) also led to a significant reduction in cell viability (<30% viability), and increases in cellular LDH leakage and ROS levels.

Cobalt NPs (likely as cobalt(II) oxide) and cobalt chloride were compared in their ability to cause DNA damage in human peripheral blood leukocytes by means of the comet assay (Colognato *et al.*, 2008). Incubation time was 2 hrs and subtoxic concentrations used were 10, 50 and 100 µM (0.6, 3 and 6 µg/ml as Co^{2+}). A dose-dependent increase in percent tail DNA was observed for cobalt NPs, which was significantly greater (p<0.05) than controls at the two highest doses. Cobalt chloride did not induce significant changes over control levels, which the authors thought could be a result of the short incubation time used and the longer uptake time needed for cobalt ions.

Ponti *et al*. (2009) compared cobalt(II) oxide NPs and cobalt chloride for induction of DNA damage in Balb/3T3 mouse fibroblast cells by the comet assay at doses of 1, 5, and 10 µM (0.075, 0.37 and 0.75 µg/ml, respectively) for cobalt(II) oxide, and 0.13, 0.65 and 1.3 µg/ml for cobalt chloride. Incubation time was 2 hrs. A comparable genotoxic response was observed for the two cobalt forms, including formation of single- and double-strand breaks. However, a dose-dependent increase in DNA damage was only seen for cobalt chloride, probably a result of increased cytotoxicity at the higher doses of cobalt NPs, which masked the genotoxic potential. Differences in results compared to work by Colognato *et al*. (2008) were suggested by the authors to be related to the sparse data on NP cobalt and different *in vitro* models used.

The genotoxicity of cobalt(II, III) oxide NPs was investigated by use of the comet assay in four different human cell lines: A549 lung carcinoma cells, HepG2 hepatocarcinoma cells, Caco-2 colorectal adenocarcinoma cells, and SH-SY5Y neuroblastoma cells (Abudayyak *et al.*, 2017). DNA damage was induced only in the A549 lung cell line, and was induced in a concentration-dependent manner over a range of 0.1 to 100 µg/ml. Additionally, cell viability was tested in all four cell types and only A549 cell viability was decreased by cobalt(II, III) oxide NPs (IC_{50} = 409.2 µg/ml). Oxidative damage was also demonstrated in A549, HepG2, and SH-SY5Y cell lines (but not in Caco-2 cells) resulting in increased malondialdehyde and 8-hydroxydeoxyguanosine levels and decreased GSH levels. The authors concluded that A549 lung carcinoma cells were the most sensitive cell line to DNA damage from cobalt(II, III) oxide NPs.

In isolated salmon sperm DNA exposed to a Fenton-type oxygen radical-generating system, including cobalt sulfate (25 micromoles per liter (µM) to 1 millimole per liter

(mM), or 1.5 to 59 µg Co/ml) with hydrogen peroxide, bulky DNA lesions were produced suggestive of free radical-mediated intrastrand cross-linking (Lloyd *et al.*, 1997). However, unlike other transition metals tested by the authors, cobalt sulfate did not cause DNA strand breaks up to 1 mM.

Oxidative DNA damage tests

Neither superoxide radical (O_2^-) nor hydrogen peroxide (H_2O_2) reacts chemically with DNA. However, a number of transition metal ions catalyze hydroxyl radical (•OH) formation in the presence of O_2^- and H_2O_2, which can modify purine and pyrimidine bases and cause strand breaks. In the presence of H_2O_2, cobalt sulfate (25 µM) was observed to cause DNA damage to isolated chromatin from human K562 cells (Nackerdien *et al.*, 1991). The altered base products (*e.g.*, cytosine glycol, formamidopyrimidines, 8-hydroxypurines) were typical of hydroxyl radical attack suggesting that the hydroxyl radical was generated in a Fenton-type reaction with cobalt and H_2O_2. Addition of scavengers of hydroxyl radical, mannitol and dimethyl sulfoxide (DMSO), led to a partial inhibition of DNA damage.

Human A549 alveolar adenocarcinoma cells and bronchial BEAS-2B normal cells were exposed to concentrations of 1 to 40 µg/ml cobalt(II, III) oxide NPs *in vitro* to investigate differences in cyto-genotoxic effects (Cavallo *et al.*, 2015). No cytotoxicity was found in A549 cells, whereas BEAS-2B cells showed reduced viability at 40 µg/ml. In A549 cells, direct and oxidative DNA damage occurred at 20 µg/ml and greater as measured by the Formamido-pyrimidine glycosylate (Fpg)-modified comet assay. The Fpg enzyme recognizes and cuts the oxidized DNA bases for evaluation of oxidative DNA damage. BEAS-2B cells showed peak oxidative DNA damage at a lower concentration of 5 µg/ml, but direct DNA damage only at 40 µg/ml. The authors suggested that the transformed A549 cells are more resistant to cytotoxicity, but have a lower capacity for DNA repair compared to BEAS-2B cells.

Induction of DNA strand breaks and oxidative DNA lesions by cobalt octoate (50, 200 and 800 µg/ml of original substance mass) and cobalt sulfate heptahydrate (800 µg/ml of original substance mass) were determined in A549 cells using the human 8-hydroxyguanine DNA-Glycosylate 1 (hOGG1) modified comet assay (Kirkland *et al.*, 2015). Oxidative DNA-base modifications can only be detected in the comet assay if lesion-specific repair enzymes (*e.g.*, hOGG1) are incorporated. For the assay, the cobalt compounds were suspended in artificial alveolar fluid to simulate dissolution in physiological environments. Cobalt octoate showed high solubility in this fluid. Both cobalt compounds induced a significant increase in mean tail intensity in the absence of hOGG1. In the presence of hOGG1, mean tail intensity was further enhanced indicating induction of oxidative DNA-base lesions. However, DNA strand breaks and oxidative DNA-base lesions seemed to coincide with cytotoxic activity (*i.e.*, reduced cell number).

The results suggested to the authors that cobalt solubility and the cobalt cations are important determinants of the observed DNA damaging effect.

Soluble cobalt acetate administered intraperitoneally in rats at a dose of 50 or 100 µmol/kg produced oxidative DNA damage in renal, hepatic and pulmonary chromatin (Kasprzak *et al.*, 1994). The altered DNA bases (*e.g.*, 5-hydroxycytosine) were the same as those found due to hydroxyl radical attack on DNA, supporting ROS generation by cobalt *in vivo*. Some of the altered bases, including 5-(hydroxymethyl)uracil and 7,8-dihydro-8-oxoguanine, have been shown to be promutagenic.

Tests for reduction in DNA replication and repair

Cobalt chloride was found to inhibit the removal of pyrimidine dimers in HeLa cells exposed to UV light, even though cobalt chloride by itself does not induce these DNA lesions (Hartwig *et al.*, 1991). This suggested to the authors that cobalt interferes with DNA repair processes. At a cobalt chloride concentration that did not cause strand breaks (100 µM), nucleoid sedimentation showed a greater accumulation of strand breaks when UV irradiation was combined with cobalt chloride treatment. Chromatin structures are repaired 3-5 hrs after UV alone, but the process was delayed with combined cobalt chloride-UV treatment indicating an interference with the completion of repair events.

Non-cytotoxic doses of cobalt chloride (50 to 200 µM as Co(II), or 3.0 to 12 µg Co/ml) were used to investigate DNA repair of lesions induced by low UVC rays (200 to 280 nm in wavelength) in cultured human fibroblasts (Kasten *et al.*, 1997). Employing the alkaline unwinding technique, cobalt was observed to inhibit both the incision and polymerization step of nucleotide excision repair, but did not interfere with the ligation step.

Kumar et al. (2017) observed that cobalt sulfate produced double strand breaks in *E. coli*, but could not demonstrate the generation of ROS and subsequent oxidative stress by the methods used. Rather, cobalt sulfate was found to reduce the rate of DNA replication in *E. coli* and inhibited the SOS repair pathway, which is the bacteria's response to DNA damage. The authors proposed that direct binding of cobalt to the DNA caused conformational changes in the DNA, as measured in a circular dichroism experiment, and led to replication fork stalling and the DNA damage observed.

Bacterial and mammalian cell gene mutation tests

A number of early prokaryotic assays were performed with soluble cobalt(II) salts and are reported in IARC (1991). Most of these studies were negative for mutagenicity, which in some cases may be related to poor bioavailability of cobalt(II) in these *in vitro* systems (Beyersmann and Hartwig, 1992; Kirkland *et al.*, 2015).

Mochizuki and Kada (1982) evaluated the mutagenicity of cobalt chloride in bacteria, using *Salmonella typhimurium* strains TA98 and TA1538 without S9 mix. The concentration of cobalt chloride added to plates ranged from 3 to 20 µg/ml. Cobalt was observed to have little effect on survival and was non-mutagenic in both *S. typhimurium* strains.

The mutagenicity of a number of metal salts, including cobalt chloride, was investigated by Arlauskas et al. (1985) by both the plate incorporation assay and the fluctuation assay. Tester strains used included *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and *Escherichia coli* WP2 uvr p*Km* 101 (fluctuation assay only). Half-log serial dilutions of cobalt chloride that was non-toxic to bacteria were used (concentrations not specified). A positive response was a reproducible, dose-related increase in revertant colonies with at least a doubling of revertant colonies compared to control values. Cobalt chloride was found to be non-mutagenic in both assays with all bacterial strains employed.

Cobalt chloride in a dose range of 0.1 to 1000 µmoles/plate (without S9 mix) was also observed to be non-mutagenic in *S. typhimurium* strains TA98, TA100, TA1537, and TA2637 (Ogawa *et al.*, 1986). However, when combined in equimolar amounts with the mutagen 4-aminopyridine, cobalt chloride enhanced the mutagenic activity of 4-aminopyridine in strains TA1537, and TA2637, but not TA98 or TA100. The increased mutagenicity was attributed to a complex formed between the two chemicals that acts as a carrier of cobalt ion in the transmembrane permeation process.

The mutagenicity of cobalt chloride, with and without S9 mix, was assessed in *S. typhimurium* strains TA98, TA102, TA1535 and TA1537, using the plate incorporation method (Wong, 1988). Concentrations used were between 40 and 120 ppm, corresponding to 50% to 90% growth inhibition, respectively. In the absence of S9 mix cobalt was considered mutagenic in the TA98 and TA1537 strains, which was described as 10.6 and 2.6 additional revertant colonies, respectively, over control values in the linear portion of the dose-response curve. Cobalt was non-mutagenic in all strains in the presence of S9 mix. The authors postulated that binding of cobalt to proteins in the S9 mix reduced the mutagenicity of cobalt.

NTP (1998a) evaluated the genotoxicity of cobalt sulfate heptahydrate in bacteria, using *S. typhimurium* strains (TA98, TA100, TA1535) either in buffer or S9 mix obtained from Arochlor 1254-induced liver of Sprague-Dawley rats or Syrian hamsters. A positive response was defined as a reproducible, dose-related increase in revertant colonies in any one strain/activation combination. Cobalt sulfate heptahydrate (3 to 10,000 µg/mL) was mutagenic in *S. typhimurium* TA100 with and without S9, but was not mutagenic in TA98 or TA1535 strains with or without S9.

To resolve inconsistencies in previous bacterial mutation assays, cobalt chloride was tested in strain TA97a and cobalt sulfate was tested in strain TA100 independently in three different laboratories using Organisation for Economic Co-operation and Development (OECD)-recommended guidelines (Kirkland *et al.*, 2015). A positive response was generally regarded as a reproducible increase (at least two-fold) in the number of revertants compared with the solvent control, which also exhibited a positive dose-response trend. Neither soluble cobalt compound produced a mutagenic response up to 5000 µg per plate, with and without S9, using either plate incorporation or pre-incubation methodology. Negative assay results in five strains of *S. typhimurium* (TA98, TA100, TA102, TA1535 and TA1537, with and without S9) were also found with two poorly soluble cobalt salts/compounds, cobalt acetyl acetonate and cobalt resinate.

Cobalt hydroxide and cobalt oxalate were tested for induction of *Hprt* mutations in mouse lymphoma L5178Y cells (Kirkland *et al.*, 2015). The cobalt compounds were incubated for 3 hr either in the absence or presence of S9. An extended 24 hr treatment in the absence of S9 was also performed with cobalt sulfate, cobalt sulfide and cobalt(II) oxide in order to detect any mutagenic effects that might only manifest after being in contact with the cells for a full cell cycle. *Hprt* enzyme activity is important for DNA synthesis. Incubation of the cells with a chemical that leads to mutations, which destroy the functionality of the *Hprt* gene and/or protein are detected by positive selection using a toxic analogue. *Hprt*-negative mutants are seen as viable colonies. Although some equivocal results were obtained, overall it was concluded by the authors that the cobalt compounds did not induce *Hprt* mutations when tested in the absence or presence of S9.

A number of other carcinogenic metals, including nickel, arsenic and cadmium, have shown little or no mutagenic activity in bacterial assays (Arita and Costa, 2009). However, mutagenesis is not the only pathway that can lead to carcinogenicity. Epigenetic mechanisms may also be involved in metal-induced carcinogenicity (see below).

Chromosomal damage

Micronucleus tests

The frequency of micronucleated cells was examined following exposure of human blood leukocytes *in vitro* to 0.01 to 0.5 µg/ml (0.0006 to 0.03 µM as Co) cobalt chloride (Capomazza and Botta, 1991). In general, the micronucleus test can detect DNA lesions that have survived at least one mitotic cycle. Micronuclei rates increased with cobalt chloride concentration up to a maximal level of 77 micronuclei per 1000 binucleated cells, corresponding to a dose of 0.1 µg/ml. This dose was considered subtoxic due to a marginal 10-20% decrease of the mitotic index. In the *in vitro* micronucleus test in Syrian hamster embryo (SHE) cells, cobalt sulfate heptahydrate also tested positive (Gibson *et*

al., 1997). A dose-dependent, significant increase in the percentage of binucleated micronucleated (BNMN) cells occurred at multiple concentrations (1.0 to 4.0 µg/ml) of cobalt sulfate heptahydrate.

Chromosomal aberrations

Human primary fibroblasts were examined for translocations and aneuploidy following 24-hour *in vitro* exposure to cobalt chloride at concentrations of 1.3, 25 and 50 ppb (0.005, 0.105 and 0.210 µM) (Figgitt *et al.*, 2010). Aneuploidy occurs during cell division when the chromosomes do not separate properly between the two cells. The lowest concentration tested was considered a physiological concentration, as it resulted in a cobalt ion concentration equivalent to that found in patients with well-functioning metal-on-metal cobalt chrome alloy hip implants. Cobalt chloride caused a dose-dependent increased incidence of total chromosomal aberrations that was statistically significant (p<0.05) at the lowest dose compared to controls. The types of chromosomal aberrations present were predominantly numerical (aneuploidy). Structural aberrations (translocations) were not observed.

Figgitt *et al.* (2010) also investigated the delayed effects of cobalt chloride up to 30 days post-exposure in order to monitor the repair of any lesions induced in human primary fibroblasts. Simple aneuploidy (cells displaying gains or losses involving only 3 chromosomes) was increased (p<0.001) one-day post-exposure in the 25 and 50 ppb groups, but had resolved by Day 10 post-exposure. Complex aneuploidy (numerical aberrations in excess of 49 chromosomes) was observed only at the highest dose one-day post-exposure, and none of the cobalt treatments led to chromosome fragments.

In human lung fibroblast cells, the genotoxic and cytotoxic potency of soluble cobalt chloride and insoluble cobalt(II) oxide (average size of 1 µm) was compared *in vitro* (Smith *et al.*, 2014). Genotoxicity was determined by treating cell cultures with varying concentrations of the soluble cobalt (50 to 500 µM, or 3 to 30 µg Co/ml) or insoluble cobalt (0.1 to 5 µg/cm^2), and then harvesting for metaphases to look for chromosome aberrations. Using intracellular cobalt ion levels for comparison, both soluble and insoluble cobalt induced similar levels of aberrations per 100 metaphases that were dose-dependent. The most common aberrations for both cobalt forms were simple chromatid lesions, with no complex lesions such as dicentrics and chromatid exchanges. However, soluble cobalt induced cell cycle arrest, indicated by a lack of metaphases, at much lower intracellular concentrations compared to insoluble cobalt. The authors concluded that both cobalt forms have similar levels of genotoxicity, but that soluble cobalt induces more cytotoxicity than insoluble cobalt.

Further *in vitro* research by Smith *et al*. (2014) observed that uptake of cobalt(II) oxide particulate by human lung fibroblast cells requires particle-cell contact, indicating that the primary mechanism for cobalt ion release is from the internal dissolution of phagocytized

particles rather than uptake of extracellular ions. The researchers concluded that solubility appears to play a role in cobalt-induced lung cell genotoxicity and suggests soluble and insoluble forms of cobalt may have different carcinogenicity potentials.

The genotoxicity and cytotoxicity of cobalt(II) oxide (0.1 to 5 μg/cm^2) and cobalt chloride (100 to 250 μM) were investigated in normal primary human bronchial epithelial cells (Xie *et al.*, 2016). Both cobalt compounds induced a concentration-dependent increase in cytotoxicity and chromosomal aberrations, most commonly seen as chromatid lesions. However, based on intracellular cobalt concentrations, cobalt chloride induced more chromosome damage than cobalt(II) oxide in the cells. In terms of cytotoxicity, intracellular levels of cobalt indicated no significant difference between the two cobalt compounds. The difference in genotoxicity between the two cobalt compounds was suggested by the authors to be a result of insoluble cobalt taking a longer time to reach genotoxic intracellular concentrations compared to soluble cobalt. In comparing similar work with human lung fibroblast cells (Smith *et al.*, 2014), the primary human bronchial epithelial cells were less efficient in taking up cobalt ions than fibroblasts. However, chromosome damage was similar after soluble cobalt treatment despite lower intracellular cobalt levels in the epithelial cells.

The chromosomal aberration test was conducted *in vitro* with cobalt acetyl acetonate, cobalt resinate, and cobalt oxyhydroxide suspended separately in cultures of HLs (Kirkland *et al.*, 2015). Cobalt acetyl acetonate induced a clastogenic response in the cells in both the absence (34 to 150 μg/ml) and presence (17 to 100 μg/ml) of S9. Cobalt resinate induced chromosomal aberrations with S9 (75 to 300 μg/ml), although cobalt precipitation may have been a confounding factor. The biological relevance of the results for cobalt oxyhydroxide was unclear due to the presence of a persistent cobalt compound precipitate on the cell layer resulting in cytotoxic and genotoxic effects.

Due to urinary elimination being the main route of excretion for absorbed cobalt, a human urothelial cell line (hTUI-38) was used by Speer *et al.* (2017) to investigate the genotoxicity and cytotoxicity of cobalt(II) oxide and cobalt chloride. Based on intracellular cobalt ion levels, both compounds induced similar levels of chromosomal aberrations primarily in the form of chromatid breaks and chromatid gaps. However, cobalt chloride was more cytotoxic at similar intracellular levels and induced cell cycle arrest that was not observed after treatment with cobalt(II) oxide. The authors concluded that both cobalt compounds were cytotoxic and genotoxic to human urothelial cells and solubility may play a role in cobalt-induced toxicity.

Cobalt chloride (10 to 100 μM, or 0.6 to 6 μg Co/ml) enhanced the number of UV-induced sister chromatid exchanges in V79 Chinese hamster cells (Hartwig *et al.*, 1991). The increase was significantly higher than the expected values from individual treatments of UV irradiation or cobalt alone.

Nanoparticle chromosomal damage tests

The frequency of BNMN cells was examined following exposure of human peripheral blood leukocytes to cobalt NPs (likely as cobalt(II) oxide) and cobalt chloride by means of the cytokinesis-block micronucleus assay (Colognato *et al.*, 2008). Both cobalt NPs and cobalt chloride increased the frequency of BNMN with increasing dose, which was statistically significant at 400 µM (24 µg Co/ml). Ponti *et al*. (2009) compared cobalt(II) oxide NPs and cobalt chloride *in vitro* with Balb/3T3 mouse fibroblast cells using the micronucleus test at doses of 1, 5, and 10 µM (0.06, 0.3 and 0.6 µg Co/ml), corresponding to 50% plating efficiency (a measure of cytotoxicity). Cobalt NPs caused chromosomal aberrations at all concentrations, although not dose-dependently, while cobalt chloride was not genotoxic under the conditions used.

Exposure of HLs to cobalt(II, III) oxide NPs (100 µg/ml) *in vitro* has also resulted in increased chromosomal aberrations in the form of greater numbers of chromosome breaks and deletions compared to controls (Rajiv *et al.*, 2016). Oxidative stress was observed in the cells, measured as increased ROS and lipid peroxidation, depletion of catalase, and reduced glutathione and superoxide dismutase. The authors concluded oxidative stress resulting from cobalt(II, III) oxide NP exposure led to DNA damage and chromosomal aberrations in the HLs.

In vivo chromosomal damage tests

Chromosomal aberrations in bone marrow cells of mice have been induced by cobalt chloride *in vivo* (Palit *et al.*, 1991). Mice orally administered cobalt chloride at high doses of 20, 40 and 80 (1/10 LD_{50}) mg/kg body weight resulted in a dose-related increase in aberrations, including chromosomes with and without gaps and breaks per cell.

Farah (1983) administered daily injections of cobalt chloride to male Syrian hamsters intraperitoneally over nine days (total dose: 0.04 g/100 g body weight). Bone marrow cells showed a significant increase (p<0.001) in pseudodiploidy and hyperdiploidy. An increased frequency (p<0.01) of meiotic cells with abnormal chromosome numbers during metaphase 1 was found in testicular preparations of the male hamsters.

Cobalt resinate and cobalt acetyl acetonate were tested for induction of micronuclei *in vivo* in mouse bone marrow cells (Kirkland *et al.*, 2015). Mice were administered the cobalt compounds up to the maximally tolerated dose by oral gavage (1500 and 500 mg/kg-d for cobalt resinate and cobalt acetyl acetonate, respectively) on two occasions 24 hr apart. Polychromatic erythrocytes (PCE) from bone marrow were counted to determine the micronucleus frequency, and total erythrocyte count was used to determine the ratio of PCE to normochromatic erythrocytes (NCE). Neither cobalt compound produced significant increases in micronucleus frequency up to the maximally tolerated dose, although cobalt resinate caused bone marrow toxicity with a significant decrease in PCE:NCE ratio.

Kirkland *et al*. (2015) also conducted an *in vivo* bone marrow chromosomal aberration study in rats with single-dose and multi-dose oral administration (3 dose levels each per sex) of cobalt sulfate, cobalt(II) oxide, and tricobalt tetroxide. In the single dose study, no increase in chromosomal aberrations was seen in the bone marrow with any cobalt compound up to the maximally tolerated dose (1000 or 2000 mg/kg-d). In the multi-dose phase of the study, rats were orally administered the same cobalt compounds daily for up to 5 days. The authors found no biologically significant induction of chromosome aberrations in bone marrow with any cobalt compound at or above the maximally tolerated dose. Kirkland *et al*. (2015) also orally administered daily doses of cobalt chloride (3, 10 and 30 mg/kg/day) to male rats for 28 days up to the maximally tolerated dose to look for chromosomal aberrations in spermatogonia. No reduction in the mitotic index was found and there was no increase in the frequency of chromosomal aberrations.

Table 6. Genotoxicity testing summary for soluble and insoluble cobalt compounds, not including cobalt metal

Cell type or species/strain	Cobalt compound	Without metabolic activation	With metabolic activation	Reference
DNA strand-break tests (comet assay, or other DNA damage assay)				
Human lymphocytes	Cobalt chloride	+	NA	De Boeck *et al.* (1998)
H460 human lung epithelial cells	Cobalt chloride	+	NA	Patel *et al*. (2012)
Human HepG2 cells	Cobalt chloride, and Cobalt(II, III) oxide NP	+ (all tested compounds)	NA (all tested compounds)	Alarifi *et al*., 2013
Human lymphocytes	Cobalt(II, III) oxide NP	+	NA	Rajiv *et al*. (2016)
Human peripheral blood leukocytes	Cobalt(II) oxide NP,	+	NA	Colognato *et al*. (2008)
As above	Cobalt chloride	-	NA	
Balb/3T3 mouse fibroblasts	Cobalt(II) oxide NP, and Cobalt chloride	+ (all tested compounds)	NA (all tested compounds)	Ponti *et al*. (2009)
A549 lung carcinoma	Cobalt(II, III) oxide NP	+	NA	Abudayyak *et al*. (2017)
HepG2 hepatocarcinoma	As above	-	NA	
Caco-2 colorectal	As above	-	NA	
SH-SY5Y neuroblastoma	As above	-	NA	
Salmon sperm DNA	Cobalt sulfate	+	NA	Lloyd *et al*. (1997)
Human K562 cells	Cobalt sulfate	+	NA	Nackerdien *et al*. (1991)
E.coli bacteria	Cobalt sulfate	+	NA	Kumar *et al*., 2017
Oxidative DNA Damage Tests				
Human A549 alveolar adenocarcinoma and bronchial BEAS-2B cells	Cobalt(II, III) oxide NP	+	NA	Cavallo *et al*. (2015)
Human A549 cells	Cobalt octoate, and Cobalt sulfate heptahydrate	+ (all tested compounds)	NA (all tested compounds)	Kirkland *et al.* (2015)
Rat *in vivo* IP - renal, hepatic and pulmonary chromatin	Cobalt acetate	+	NA	Kasprzak *et al*. (1994)
DNA Replication and Repair Inhibition tests				
HeLa cells; enhanced UV-caused strand breaks	Cobalt chloride	+	NA	Hartwig *et al*. (1991)
Human fibroblasts Inhibit DNA repair by UVC	Cobalt chloride	+	NA	Kasten *et al*. (1997)

+/-: equivocal NA: not applicable NP: nanoparticles

Table 6. Genotoxicity testing summary for soluble and insoluble cobalt compounds, not including cobalt metal (continued)

Cell type or species/strain	Cobalt compound	Without metabolic activation	With metabolic activation	Reference
Bacterial and mammalian cell gene mutation tests				
E.coli bacteria	Cobalt sulfate	+	NA	Kumar *et al*., 2017
S. typhimurium TA98, TA1538	Cobalt chloride	- (all strains)	NA (all strains)	Mochizuki & Kada, 1982
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538*; E.coli* WP2 uvrA p*Km* 101	Cobalt chloride	- (all strains)	NA (all strains)	Arlauskas *et al.* (1985)
S. typhimurium TA98, TA100, TA1537, and TA2637	Cobalt chloride	- (all strains)	NA (all strains)	Ogawa et al. 1986
S. typhimurium TA98	Cobalt chloride	+	-	
TA102	As above	-	-	
TA 1535	As above	-	-	
TA1537	As above	+	-	Wong (1988
S. typhimurium TA100	Cobalt sulfate $7H_2O$	+	+	
TA98	As above	-	-	
TA1535	As above	-	-	NTP (1998)
S. typhimurium TA97a	Cobalt chloride	-	-	
TA100	Cobalt sulfate	-	-	
S. typhimurium TA98, TA100, TA102, TA1535, TA1537	Cobalt acetyl acetonate and cobalt resinate	- (all strains)	- (all strains)	Kirkland *et al*. (2015)
L5178Y mouse lymphoma cells	Cobalt hydroxide, Cobalt oxalate, Cobalt sulfate, Cobalt sulfide, Cobalt(II) oxide	- (all tested compounds)	- (all tested compounds)	Kirkland *et al.* (2015)
Chromosomal Damage – Micronucleus test				
Human blood leukocytes	Cobalt chloride	+	NA	Capomazza and Botta (1991)
SHE cells	Cobalt sulfate heptahydrate	+	NA	Gibson *et al.* (1997)

+/-: equivocal NA: not applicable NP: nanoparticles

Table 6. Genotoxicity testing summary for soluble and insoluble cobalt compounds, not including cobalt metal (continued)

Cell type or species/strain	Cobalt compound	Without metabolic activation	With metabolic activation	Reference
Chromosomal Damage – Chromosomal Aberrations				
Human peripheral blood leukocytes	Cobalt(II) oxide NP, Cobalt chloride	+ (all tested compounds)	NA (all tested compounds)	Colognato *et al.* (2008)
Balb/3T3 mouse fibroblast cells	Cobalt(II) oxide NP,	+	NA	Ponti *et al.* (2009)
As above	Cobalt chloride	-	NA	
Human primary fibroblasts cells	Cobalt chloride	+	NA	Figgitt *et al.* (2010)
Human lung fibroblast cells	Cobalt chloride, Cobalt(II) oxide	+ (all tested compounds)	NA (all tested compounds)	Smith *et al.* (2014)
Human lung bronchial epithelial cells	Cobalt chloride, Cobalt(II) oxide	+ (all tested compounds)	NA (all tested compounds)	Xie *et al*. (2016)
Human lymphocytes	Cobalt acetyl acetonate	+	+	Kirkland *et al.* (2015)
As above	Cobalt resinate	-	+	
As above	Cobalt oxyhydroxide	+/-	+/-	
Human urothelial cells	Cobalt chloride, Cobalt(II) oxide	+ (all tested compounds)	NA (all tested compounds)	Speer *et al*. (2017)
Human lymphocytes	Cobalt(II, III) oxide NP	+	NA	Rajiv *et al.* (2016)
Mouse bone marrow cells (*in vivo*)	Cobalt chloride	+	NA	Palit *et al.* (1991)
Syrian hamster bone marrow cells (*in vivo*)	Cobalt chloride	+	NA	Farah (1983)
Rat bone marrow cells (*in vivo*)	Cobalt sulfate, Cobalt(II) oxide, Tricobalt tetroxide	- (all tested compounds)	NA (all tested compounds)	Kirkland *et al.* (2015)
Mouse bone marrow cells (*in vivo*)	Cobalt resinate, Cobalt acetyl acetonate	- (all tested compounds)	NA (all tested compounds)	Kirkland *et al.* (2015)
Male rat spermatogonia (*in vivo*)	Cobalt chloride	-	NA	Kirkland *et al.* (2015)
Chinese hamster V79 cells Enhanced UV-induced sister chromatid exchanges	Cobalt chloride	+	NA	Hartwig *et al*. (1991)

+/-: equivocal NA: not applicable NP: nanoparticles

Cobalt metal, including comparisons with soluble and insoluble cobalt compounds

Investigation of the metal form of cobalt for genotoxicity has been explored more recently. Some of these studies compared the genotoxicity of cobalt metal with soluble or insoluble cobalt compounds. Cobalt metal produced mixed results for mutations in bacterial tests. In *in vitro* mammalian cell systems, cobalt metal caused DNA strand breaks, chromosomal aberrations, gene mutations, and inhibition of DNA repair. Cobalt metal did not cause chromosomal aberrations *in vivo* in HLs or murine bone marrow.

DNA strand break tests

Employing the alkaline comet assay, cobalt metal (median particle size 4 μm) induced a dose-dependent increase in tail lengths and moments (0.6 to 6.0 μg/ml) in isolated HLs that were statistically significant at 4.5 μg/ml (Anard *et al.*, 1997). These changes occurred without a significant effect on cell viability. A modified alkaline elution assay also showed a dose-dependent increase (1.5 to 15 μg/ml) in production of DNA breaks in isolated lymphocytes exposed to cobalt metal. The increase in number of breaks was statistically significant starting at 3 μg/ml. The alkaline elution assay measures the rate of DNA elution through a filter membrane and the amount of DNA single strand breaks or lesions converted to breaks under alkaline conditions. DNA breaks are estimated by the increase in DNA elution rate. When cobalt chloride was substituted for cobalt metal in the alkaline elution assay, production of DNA breaks was not different from controls. Similar strand break results as that obtained with HLs were observed when mouse 3T3 fibroblast cells were exposed to cobalt metal in the alkaline elution assay.

In a similar study, De Brock *et al*. (1998) examined the ability of cobalt metal (median particle size: 4 μm) and cobalt chloride to induce DNA damage in isolated HLs from three donors using the alkaline comet assay. In this case, however, both cobalt compounds over a range of 0 to 6.0 μg Co-equivalent/ml showed comparable responses in inducing DNA damage in a dose-dependent and time-dependent manner. Relatively large inter-experimental and inter-donor variability in the response was observed. This finding plus differences in comet assay methodologies may explain the different results obtained by Anard *et al*. (1997).

Cobalt metal may also have an indirect genotoxic action through inhibition of DNA repair. Isolated HLs were exposed to methyl methanesulphonate (MMS) followed by a 2-hour post-incubation recovery period, or MMS followed by a 2-hour treatment with a non-genotoxic dose of 1.2 μg/ml cobalt metal particles (De Boeck *et al.*, 1998). Cobalt metal was observed to inhibit the repair of MMS-induced DNA damage.

The genotoxicity of cobalt metal, cobalt(II) oxide, and cobalt(II, III) oxide NPs were compared *in vitro* using human lung cells in culture (Cappellini *et al.*, 2018). All three

cobalt-containing NPs showed efficient uptake in A549 type II epithelial cells, although only cobalt metal NPs were cytotoxic after 24 hrs. Cobalt metal NPs significantly increased DNA damage in A549 (at 40 µg/ml) and HBEC (human bronchial epithelial cells) (20 and 40 µg/ml) cell types by the alkaline comet assay. Cobalt(II) oxide NPs also produced significant DNA damage in these cell types at the higher dose of 60 µg/ml. Cobalt(II, III) oxide NPs caused no DNA damage at any concentration. The Fpg assay used in A549 cells showed that DNA damage caused by cobalt metal and cobalt(II) oxide NPs was related to oxidative stress. The Fpg assay was negative for cobalt(II, III) oxide NPs.

Cappellini et al. (2018) also tested the three cobalt-containing NPs in the ToxTracker reporter assay to investigate the mechanisms of genotoxicity. The ToxTracker assay is a mouse embryonic stem cell-based genotoxicity assay employing six green fluorescent protein reporters specific for DNA damage, oxidative stress, protein damage, and cellular stress response. Cobalt metal NPs, and to a lesser extent cobalt(II) oxide NPs, caused an induction of the Srxn1-GFP reporter related to generation of ROS that can lead to DNA single strand breaks during the repair of oxidative DNA lesions. Cobalt metal and cobalt(II) oxide NPs also activated the Rtkn-GFP genotoxicity reporter that is associated with induction of DNA strand breaks. Cobalt(II, III) oxide NPs were inactive. Overall, the authors concluded that the primary mechanism of genotoxicity by cobalt metal and cobalt(II) oxide NPs, but not cobalt(II,III) oxide, was induction of oxidative stress that can lead to DNA strand breaks.

Wan et al. (2017) intratracheally instilled 50 µg cobalt NPs (85-90% metal cobalt and 10-15% cobalt(II, III) oxide) per mouse to determine if these NPs result in DNA damage and DNA mutation. Results on DNA mutation are discussed in “Gene Mutation Analysis”. DNA damage was measured four months after treatment by immunohistochemical staining for γ-H2AX, which is involved in DNA repair activities for specific types of DNA damage such as double-strand breaks, and as levels of 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage caused by ROS. At day 7 following exposure, during the acute inflammatory phase of pulmonary injury, treated mice showed a significant increase in γ-H2AX-positive nuclei as compared to saline-instilled controls. An increase in γ-H2AX-positive nuclei was still apparent at 4 months following exposure, during which the pulmonary injury in the treated mice progressed to pulmonary interstitial fibrosis and continued infiltration of inflammatory cells. The level of 8-OHdG was also significantly higher in genomic DNA of lung tissues of treated mice compared to saline controls. 8-OHdG formation was suggested by the authors to result in the G:C to T:A transversion, which was observed at higher frequencies in cobalt-treated tissues (also discussed in “Gene Mutation Analysis”).

Bacterial and mammalian cell gene mutation tests

NTP (2014a) evaluated the genotoxicity of cobalt metal particulate (1.7-1.8 µm) in bacteria, using *Salmonella typhimurium* strains (TA98, TA100) and *Escherichia coli* (WP2 uvrA/pKM101) either in buffer or S9 mix obtained from Arochlor 1254-induced liver of rats. Five doses of cobalt metal (100 to 5000 µg/plate) were examined, with the highest concentration resulting in toxicity. Without S9, cobalt produced an equivocal response with *S. typhimurium* TA100, but was weakly mutagenic with the TA98 strain. With S9, no mutagenic activity was observed in either *S. typhimurium* strain. No mutagenic activity was observed, with or without S9, in *E. coli*. Hong *et al*. (2015) suggested the lack of mutagenicity in *S. typhimurium* with S9 could be related to radical scavenging enzymes (*e.g.*, glutathione peroxidase) contained within the S9 mix and/or binding of cobalt to S9 proteins.

Kirkland *et al*. (2015) conducted the Ames test with cobalt metal following OECD-recommended guidelines. Cobalt metal powder (median diameter: 2.9 µm) was suspended in DMSO and tested in strain TA98 independently in three different laboratories up to maximum test concentrations of 1000 to 5000 µg/plate. A mutagenic response was not produced, with and without S9, using either plate incorporation or pre-incubation methodology.

Cobalt metal powder (median diameter: 3.4 µm) was tested for induction of *Hprt* mutations in mouse lymphoma L5178Y cells (Kirkland *et al.*, 2015). The metal was incubated for 3 hr both in the absence and presence of S9 at treatment concentrations ranging from 0 to 250 µg/ml. An extended 24 hr treatment in the absence of S9 was also performed with cobalt metal powder extract in order to detect any mutagenic effects that might only manifest after being in contact with the cells for a full cell cycle. Although some equivocal results were obtained, overall it was concluded by the authors that cobalt metal and the metal extract did not induce *Hprt* mutations when tested in the absence or presence of S9. Undissolved cobalt metal was present in the culture medium of the cobalt metal experiment, but it was unclear how this influenced the toxic and mutagenic responses.

Chromosomal damage

The ability of cobalt to induce micronuclei *in vivo* in normochromatic erythrocytes (NCEs) of male and female $B6C3F_1$/N mice was determined by NTP (2014a) following inhalation exposure to cobalt metal particulate (MMAD 1.7-1.8 µm) for 14 weeks. The percentage of circulating polychromatic erythrocytes (reticulocytes) was also scored as a measure of bone marrow toxicity. Peripheral blood samples were collected from mice (5 animals/sex/group) exposed to cobalt by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, and 10 mg/m^3 (6 hrs/day, 5 days/week). No increases in the frequencies of

NCEs, or significant alterations in the percentages of reticulocytes, were observed. Under the conditions examined, NTP concluded cobalt metal did not cause bone marrow toxicity.

The chromosomal damaging capacity of cobalt metal powder (median particle size: 4 µm) was assessed by the cytokinesis-blocked micronucleus test *in vitro* on isolated human leukocytes (Van Goethem *et al.*, 1997). The cytokinesis-block micronucleus assay is a sensitive and simple indicator of chromosome damage, both chromosome loss and chromosome breakage, and provides information on cell cycle progression and cytotoxicity. Cobalt metal induced a dose-dependent and statistically significant increase ($p<0.05$) in micronucleated cytokinesis-blocked cells at all concentrations tested (0.6 to 6.0 µg/ml). Cell cycle delay and/or cytotoxicity were also observed at all doses tested. A concurrently run alkaline Comet assay on the same cell type by the authors showed a dose-dependent increase of DNA breaks characterized by increased tail lengths and/or moments that was statistically significant from control at all doses (0.3 to 12.0 µg/ml). Combined, these two genotoxicity tests (i.e.,Comet assay and cytokinesis-block micronucleus assay) show that a significant amount of DNA breakage translated into chromosome damage and/or gene mutations.

Gene Mutation Analysis

A mutation analysis of the lung neoplasms observed in rats and mice in the 2-year cobalt metal NTP studies was performed to look for the most commonly altered genes, the *Kras*, *Egfr* and *Tp53* genes, which are found in human lung cancer (NTP, 2014a; Hong *et al.*, 2015). The most frequent mutation found in the mouse alveolar/bronchiolar carcinomas was in the *Kras* gene (67%, 46/69). None of these mutations (*Kras*, *Egfr* or *Tp53*) were present in alveolar/bronchiolar tumors examined in the control mice, although *Kras* gene mutations are observed in historical controls (27%, 34/124). The majority of the *Kras* mutations were within codon 12 of lung carcinomas in metal cobalt-exposed mice, where mutations are also frequently localized in spontaneous alveolar/bronchiolar carcinomas of mice. The difference is that G→T transversions were primarily found in cobalt metal-exposed mice (80%, 24/30), while G→A transversions are most common in historical spontaneous carcinomas examined (70%, 14/20). The G→T transversion is seen in chemically-induced lung tumors, including cobalt sulfate heptahydrate (NTP, 1998a) and is thought to be related to ROS generation.

In rats, the most frequent mutation found in alveolar/bronchiolar carcinomas from cobalt metal treated animals was also in the *Kras* gene (31%, 15/48) (Hong *et al.*, 2015). *Kras* mutations were not found in spontaneous lung tumors of controls (0/10). Similar to mice, the majority of *Kras* mutations in cobalt-exposed rats were within codon 12, with the most common being G→T transversions.

Cobalt metal NPs were instilled intratracheally (50 µg) in guanine phosphoribosyl-transferase (*gpt*) delta transgenic mice to determine if exposure results in DNA damage and DNA mutation in the lung (Wan et al., 2017). The transgenic mice carry about 80 copies of the transgene, lambda EG10 DNA, on each chromosome 17. Four months after exposure, mutation frequencies of *gpt* genes in the lungs were significantly greater compared with saline instilled controls. The most common mutation was G:C to T:A transversion, which can be increased due to oxidative stress.

Genotoxicity tests in workers exposed to cobalt metal

Genotoxic endpoints were evaluated in workers (n = 35) exposed exclusively to cobalt metal dust in hard metal refineries (De Boeck *et al.*, 2000). Matched control workers (n = 35) were recruited from the same plants. A third group consisted of workers (n = 29) exposed to both cobalt and tungsten carbide dust. Exposure to cobalt was characterized as moderate, with a mean cobalt urine concentration of 21.5 µg/g creatinine on Friday at the end of the work week. Based on previous epidemiological studies by the authors, a urine concentration at this level equates to a time-weighted average (TWA) exposure of 20 µg/m^3 of cobalt. Lymphocytes from the blood of the workers were examined for DNA damage by the comet assay and for chromosomal aberrations by the micronucleus test. In addition, the urine of the workers was examined for altered DNA (i.e., 8-OHdG). No significant increase in genotoxic effects could be detected in workers exposed to cobalt dust alone or combined cobalt-hard metal dusts. The authors suggested that the cobalt exposure may have been too low to detect genotoxic changes, and that analysis of respiratory cells that are in direct contact with inhaled particles may be more appropriate to examine for genotoxic effects.

In another study, sister chromatid exchange in blood lymphocytes was evaluated in 24 workers in a metal powder producing factory that were matched against 23 control workers by age and smoking status (Gennart *et al.*, 1993). Urinary cobalt levels in exposed workers were 23.6 µg/g creatinine, which was similar to that observed in hard metal refinery workers by De Boeck *et al*. (2000). However, it was not specified when the samples were collected during the week. The workers were also exposed to chromium and nickel powders and showed significantly higher levels of these metals in urine compared to controls. The mean sister-chromatid exchange score in lymphocytes was significantly greater (p<0.05) in the exposed workers. Considering the weak carcinogenic action of cobalt, the researchers believed that the small amounts of chromium and nickel that were solubilized and absorbed caused the increased score. The levels of serum tumor markers (carcinoembryonic antigen and polypeptide antigen) were also increased in exposed workers, but did not reach statistical significance.

Exfoliated cells were collected from the buccal and nasal mucosa of electroplate workers that were exposed to cobalt (specific cobalt compounds not discussed) and hexavalent

chromium to look for genotoxic effects (Wultsch *et al.*, 2017). The workers (n = 42) were matched with a control group (n = 43) with regard to gender, age, body mass index, alcohol consumption and smoking. No induction of micronuclei was detected in the collected cells that would suggest chromosomal aberrations. However, the electroplaters wore masks while working and blood levels of cobalt (0.85 µg/L plasma) were not statistically significantly elevated from the controls (0.80 µg/L plasma). Air levels of cobalt were below the detectable limit (0.12 ng/m^3) while the mean level of hexavalent chromium was 0.2 µg/m^3.

Table 7. Genotoxicity testing summary for cobalt metal, including comparisons with soluble and insoluble cobalt compounds

Cell type or species/strain	Cobalt metal/compound	Without metabolic activation	With metabolic activation	Reference
DNA strand-break tests (comet assay or other DNA damage assay)				
Human lymphocytes (HL) and mouse 3T3 fibroblasts (3T3)	Cobalt metal,	+ (all strains)	NA (all strains)	Anard *et al.* (1997)
As above	Cobalt chloride	- (HL only)	NA (HL only)	
Human lymphocytes	Cobalt metal, Cobalt chloride	+ (all tested compounds)	NA (all tested compounds)	De Broeck *et al.* (1998)
Human leukocytes	Cobalt metal	+	NA	Van Goethem *et al*. (1997)
Blood lymphocytes of exposed workers (*in vivo*)	Cobalt metal	-	NA	De Boeck *et al.* (2000)
Lung tissue of *gpt* delta transgenic mice (*in vivo*)	Cobalt metal NPs	+	NA	Wan *et al.* (2017)
Human A549 and HBEC cells	Cobalt metal NPs & Cobalt(II) oxide NPs	+ (all strains)	NA (all strains)	Cappellini et al. (2018)
As above	Cobalt(II, III) oxide NPs	- (all strains)	NA (all strains)	
Oxidative DNA damage test (8-hydroxy-deoxyguanosine)				
Lung tissue of *gpt* delta transgenic mice (*in vivo*)	Cobalt metal NPs	+	NA	Wan *et al.* (2017)
Blood lymphocytes of exposed workers (*in vivo*)	Cobalt metal	-	NA	De Boeck *et al.* (2000)
Bacterial and mammalian cell gene mutation tests				
S. typhimurium TA98,	Cobalt metal	+	-	NTP (2014a)
TA100	As above	+/-	-	
E. coli (WP2 uvrA/pKM101)	As above	-	-	
S. typhimurium TA98	Cobalt metal	-	-	Kirkland *et al.* (2015)
L5178Y mouse lymphoma cells	Cobalt metal, Cobalt metal extract	- (all tested compounds)	- (all tested compounds)	Kirkland *et al.* (2015)
Chromosomal damage				
Human leukocytes	Cobalt metal	+	NA	Van Goethem *et al.* (1997)
B6C3F1/N mouse reticulocytes (*in vivo*)	Cobalt metal	-	NA	NTP (2014a)

+/-: equivocal NA: not applicable NP: Nanoparticle

Table 7. Genotoxicity testing summary for cobalt metal, including comparisons with soluble and insoluble cobalt compounds (continued)

Cell type or species/strain	Cobalt metal/compound	Without metabolic activation	With metabolic activation	Reference
Chromosomal damage				
Blood lymphocytes of exposed workers (*in vivo*)	Cobalt metal	-	NA	De Boeck *et al.* (2000)
Buccal and nasal mucosa cells of exposed workers (*in vivo*)	Cobalt metal (likely)	-	NA	Wultsch *et al*. (2017)
Gene mutation analysis				
Kras gene mutations B6C3F$_1$/N mice and F-344/NTac rats (*in vivo*)	Cobalt metal	+ (all strains)	NA (all strains)	NTP (2014a); Hong *et al*. (2015)
gpt gene mutation Lung tissue of *gpt* delta transgenic mice (*in vivo*)	Cobalt metal NPs	+	NA	Wan et al. (2017)

+/-: equivocal NA: not applicable NP: Nanoparticle

Alteration of Epigenetic Homeostasis

The field of epigenetics focuses on the study of heritable alterations in gene expression that occur in the absence of changes in the DNA sequence. Mammalian gene expression at the chromatin level can be affected by two general categories of epigenetic mechanisms: altered DNA methylation and histone posttranslational modifications (Arita and Costa, 2009; Brocato and Costa, 2013). These types of changes may alter programs of gene expression and lead to carcinogenesis. Carcinogenic metals such as nickel, arsenic, cadmium, and hexavalent chromium have been shown to alter epigenetic homeostasis in cells even though they are mostly weak mutagens.

In one of the few studies that examined the potential for cobalt-induced epigenetic changes, Li *et al*. (2009) exposed both human lung carcinoma A549 cells and human bronchial epithelial Beas-2B cells to cobalt chloride to assess the potential for alteration of histone modifications. Exposure to cobalt ions resulted in changes in histone modifications in both lung cell types, including increased dimethylation (me2) or trimethylation (me3) at histone H3K4 (H3K4me3), H3K9 (H3K9me2), H3K9 (H3K9me3), H3K27 (H3K27me3), and H3K36 (H3K36me3), increased ubiquitination (u) at histone H2A (uH2A) and H2B (uH2B), but decreased acetylation (Ac) at histone H4 (AcH4). The increase in some histones (H3K4me3, H3K27me3) was a result of methylation process enhancement, while other histone increases (H3K9me3, H3K36me3) occurred due inhibition of the demethylation process. Regarding the latter two histones, the authors

showed that the competition of cobalt ions with iron for binding to JMJD2A protein directly inhibited JMJD2A demethylase activity, which likely led to the increase in these histones.

The increase in uH2A and uH2B histone ubiquitination was found to occur by prevention of deubiquitination, probably through inhibition of a histone-deubiquitinating enzyme (Li *et al.*, 2009). Ubiquitin is a small polypeptide that attaches to other proteins, including histones, resulting in the regulating of their activity or location or marking them for degradation. Finally, microarray analysis showed that A549 cells exposed to cobalt chloride both upregulated and downregulated expression of hundreds of genes involved in various cell functions, including tumorigenesis.

Morphological Cell Transformation and Tumor Suppressor Protein Induction

Positive cell transformation assays are suggestive of carcinogenic potential (Creton *et al.*, 2012). Cobalt sulfate was positive in the SHE cell transformation assay (Kerckaert *et al.*, 1996). SHE cells have been used to evaluate the potential carcinogenicity of a wide variety of chemical and physical agents. SHE cells display a multistage pattern of progression to cancer following acute (24 hr exposure in this study) carcinogen exposure that is similar to the multistage progression of *in vivo* carcinogenesis. At all concentrations tested (0.125 to 1 µg/ml), cobalt sulfate statistically significantly (p<0.05) increased the number of transformed colonies per total colonies, although a dose-response trend was not observed.

Crystalline cobalt sulfide (CoS_2) and amorphous cobalt sulfide (CoS) particles (1.25 to 2 µm) were observed to increase the incidence of morphological transformation in SHE cells (1 to 20 µg/ml), with the crystalline form showing a greater potency for cell-transforming activity (Costa *et al.*, 1982). Compared to findings of crystalline and amorphous nickel sulfides, the authors postulated that the crystalline cobalt sulfide form is more actively phagocytized by cells resulting in greater intracellular dissolution and ROS formation, and subsequently leading to greater cell transformation.

Balb/3T3 mouse fibroblast cells were used to evaluate the morphological transforming ability of cobalt(II) oxide NPs (1 to 30 µM) and cobalt chloride (1 to 70 µM) (Ponti *et al.*, 2009). Cobalt NPs were cytotoxic, but also increased morphological transformation at nearly all concentrations tested. Cobalt chloride was also cytotoxic but did not lead to morphological transformation at any concentration tested.

Wild-type mouse embryonic fibroblast cells (MEF *Ogg1*$^{+/+}$) and its isogenic *Ogg1 knockout* partner (MEF *Ogg1*$^{-/-}$) were exposed *in vitro* to low, subtoxic doses (0.05 and 0.1 µg/ml) of cobalt NPs for 12 weeks (Annangi *et al.*, 2015). MEF *Ogg1*$^{-/-}$ cells are unable to maintain genomic integrity by effectively repairing oxidative DNA damage lesions, such as 8-OH-dG lesions on DNA. At five weeks of exposure, there was an

increased number of colonies formed by MEF *Ogg1*$^{-/-}$ cells. After 10 weeks of exposure, significantly increased colony formation was observed for both cell types. Additionally, cancer-like phenotypic hallmarks were also observed in the exposed cells, including morphological cell changes, significant increases in the secretion of metalloproteinases, and anchorage-independent cell growth ability, with MEF *Ogg1*$^{-/-}$ cells showing greater sensitivity to these changes. The cobalt NP compound used was not specified, but was likely a cobalt oxide.

Balb/3T3 mouse fibroblast cells were used to evaluate the *in vitro* morphological transforming ability of cobalt metal NPs and microparticles and cobalt chloride (Sabbioni *et al.*, 2014a). Both cobalt NPs and microparticles were cytotoxic and significantly positive (p<0.05) at most concentrations tested (1 to 10 µM) for morphological transformation, measured as the increase of type III foci. Cobalt chloride added to the culture medium displayed lower cytotoxicity and did not cause an increase in morphological transformation in the cells. Cobalt microparticles were more efficient than NPs in inducing both morphological transformation and oxidative stress, which conforms with the finding of greater cellular uptake of cobalt microparticles by the cells. The authors concluded that a high degree of internalization of cobalt particles and/or dissolution within cells could play an important role in inducing morphological transformation. On the other hand, cobalt ions released from soluble cobalt chloride do not become bioavailable to cells until after saturation of binding with culture medium components (>40 µM).

The NCTC 929 cell line derived from mouse fibroblast cells was treated *in vitro* with cobalt sulfate (1 to 100 µg/ml) to determine if there is a resulting induction of p53 protein (Duerksen-Hughes *et al.*, 1999). The p53 protein is a tumor-suppressor protein that increases following DNA damage. The protein prevents replication of damaged DNA, either by causing the cell to undergo a reversible growth arrest or by initiating a cell's apoptotic pathway. Cobalt sulfate strongly induced p53 at 6 hrs (50 and 100 µg/ml) and 17 hrs (20 and 50 µg/ml) with subtoxic doses. Cytotoxicity was evident in cells exposed to 100 µg/ml cobalt sulfate for 17 hrs.

Toxicogenomics

Mateuca *et al*. (2005) evaluated polymorphisms responsible for reduced DNA repair capacity among cobalt-only exposed workers and hard metal workers to look for associations with genotoxic endpoints resulting from cobalt-generated ROS. The gene variations examined were involved in base-excision (*hOGGI*, *XRCC1*) and double strand break (*XRCC3*) DNA repair. Lymphocytes were collected for genotyping from 21 cobalt-exposed, 26 hard metal-exposed and 26 matched control male workers. The alkaline comet assay was used to look for DNA single strand breaks and the cytokinesis-block micronucleus test was used to look for chromosomal rearrangements. The presence of

8-OHdG in urine, suggestive of oxidative DNA damage, was also investigated. The only significant genotoxic endpoint found was a higher frequency of micronucleated mononucleates (p=0.01) in hard metal-exposed workers with the variant *hOGG1*^{326} genotype, which leads to a reduced ability to excise 8-OHdG and has been associated with increased risk of esophageal, lung and prostate cancers.

Multivariate analysis was also performed with a number of independent variables on cobalt and hard metal workers combined (Mateuca *et al.*, 2005). The presence of the variant *XRCC1*^{280} genotype was associated with higher Comet assay DNA breakage (p=0.053), and having both *XRCC3*^{241} and *hOGG1*^{326} variant genotypes was associated with greater micronucleated mononucleate frequency (p=0.020). Smoking status and type of plant (cobalt or hard metal) was also shown to have a significant impact on genotoxicity endpoints. The authors noted that the small number of subjects was a weakness of this study.

IV. CANCER HAZARD EVALUATION

The carcinogenicity of cobalt sulfate heptahydrate and cobalt metal were assessed by NTP in separate chronic inhalation rodent studies (NTP, 1998a; 2014a). *In vitro* studies suggest that different pathways of cellular uptake for soluble and insoluble forms of cobalt compounds are associated with differences in the intracellular concentration and distribution, which in turn may be reflected in distinct genotoxic and carcinogenic potencies (Colognato *et al.*, 2008; Ponti *et al.*, 2009; Smith *et al.*, 2014).

Based on the results of these NTP studies, cobalt exhibits carcinogenicity in multiple species, which corresponds with the greatest potential to induce tumors in other species including humans (Tennant and Spalding, 1996; NTP, 2014a; Behl *et al.*, 2015). Cobalt induced tumors at one or more sites in both rats and mice, and induced tumors at the same site (*i.e.*, lung) that are of the same histogenic type in both species. Similar toxicity results for cobalt metal and cobalt sulfate heptahydrate in the NTP studies point to a common mechanism of action.

Release of the cobalt ion in physiological fluids following inhalation is considered the primary factor for cancer risk. To compare cancer potencies of cobalt metal and cobalt sulfate heptahydrate, the exposure levels in the studies were calculated based on cobalt content alone. Thus, chamber concentrations of cobalt sulfate heptahydrate were normalized to the cobalt content. Since the rodents in the NTP study were actually exposed to the hexahydrate, the hydrated cobalt sulfate chamber concentrations of 0, 0.3, 1.0 and 3.0 mg/m^3 $CoSO_4 \cdot 6H_2O$ were normalized to 0, 0.067, 0.22 and 0.67 mg/m^3 Co, respectively. Thus, it might be expected that the lowest concentration of cobalt metal (1.25 mg/m^3 Co) would produce a greater incidence of tumors than the highest concentration of hydrated cobalt sulfate (0.67 mg/m^3 Co).

Comparing the two sets of NTP studies in this way, cobalt metal exposure at the lowest concentration (1.25 mg/m^3 Co) produced a greater incidence of pulmonary tumors in the mice and male rats, and proportionally more pulmonary carcinomas than adenomas, compared to the highest concentration of hydrated cobalt sulfate (0.67 mg/m^3 Co). In female rats, exposure to cobalt metal at the lowest concentration produced a similar incidence of pulmonary tumors compared to the highest concentration of cobalt sulfate hexahydrate.

Also in the lung, the rare chemically-induced squamous cell neoplasms (predominantly CKE neoplasms) were found only in rats exposed to cobalt metal. Pancreatic islet tumors in male rats were observed only with exposure to cobalt metal, although at comparatively higher Co concentrations (2.5 and 5 mg/m^3) than those used in the cobalt sulfate heptahydrate studies. In addition, an increased incidence of mononuclear cell leukemia in female rats was observed only with exposure to cobalt metal. On the other hand, cobalt sulfate in rats at the highest exposure (0.67 mg/m^3 Co) produced approximately the same number of benign, malignant and benign/complex/malignant pheochromocytomas (combined) as that produced by cobalt metal at the lowest exposure concentration (1.25 mg/m^3 Co).

Regarding the finding of pheochromocytomas in both studies, NTP has noted an association with the generation of these tumors in other inhalation studies that also produced extensive chronic non-neoplastic lung lesions (Ozaki *et al.*, 2002; NTP, 2014a; Behl *et al.*, 2015). However, it is unclear if pheochromocytoma is a secondary response to hypoxia, or a directly acting chemical response to cobalt exposure. It is hypothesized that large space-occupying tumors and nonneoplastic lesions, including fibrosis and chronic inflammation, may lead to systemic hypoxemia. This in turn chronically stimulates catecholamine secretion from the adrenal medulla causing endocrine hyperactivity. The result may be hyperplasia and neoplasia of adrenal gland tissue.

No conclusive inhalation carcinogenicity studies have been performed for water-insoluble cobalt particulate compounds (*e.g.*, cobalt oxides), although exposure to these compounds is prevalent in occupational settings. A cobalt(II) oxide carcinogenicity inhalation study in hamsters has been performed (Wehner *et al.*, 1979), but drawbacks with the experimental animal choice (*i.e.*, resistant to lung tumor development, unusually short life-span) prevent any conclusions regarding the carcinogenic potential for cobalt oxide. However, intratracheal instillation, subcutaneous injection and intraperitoneal injection studies with cobalt(II) oxide in animals suggest that this cobalt form is carcinogenic (IARC, 1991; Steinhoff and Mohr, 1991). In addition, cobalt oxide compounds have been shown to release cobalt ions in pulmonary fluids, which then reach the bloodstream (Bailey *et al.*, 1989; Foster *et al.*, 1989; Kreyling *et al.*, 1991b; Lison *et al.*, 1994). Therefore, water-insoluble cobalt compounds that release cobalt ion in pulmonary fluids are considered to be an inhalation cancer risk by OEHHA.

Several epidemiology studies have been conducted, but were too limited or inadequate to assess the carcinogenic risk of cobalt in humans. A recent retrospective study by Sauni *et al*. (2017) did not find an increased total cancer risk or lung cancer incidence among 995 workers exposed to cobalt metal powder and cobalt compounds. However, respiratory protection was available to the workers (the level of use was not specified), and the young age and short exposure period for some of the workers may preclude an observed increase in cancer. In a direct comparison (i.e., without adjustment parameters such as inhalation rate and body weight), the highest cobalt levels the workers were exposed to (0.06 to 0.10 mg/m^3) were below the lowest cobalt sulfate heptahydrate concentration (0.3 mg/m^3) used in the NTP rodent studies. This was a concentration that did not result in a measurable increase in tumor incidence in the rodents.

Overall, cobalt in its various forms has been found to be genotoxic, chiefly by *in vitro* DNA-breaking tests and chromosomal aberration tests. In particular, *in vitro* studies have shown cobalt oxide compounds to be genotoxic. Both cobalt(II, III) oxide and cobalt(II) oxide particles have been shown to cause DNA damage and chromosomal aberrations in human lung or lymphocyte cells (Alarifi *et al.*, 2013; Smith *et al.*, 2014; Rajiv *et al.*, 2016; Xie *et al.*, 2016; Abudayyak *et al.*, 2017; Cappellini *et al.*, 2018). Additionally, cobalt(II) oxide and cobalt sulfide particles have resulted in morphological cell transformation in mammalian cells (SHE cells and Balb/3T3 mouse fibroblast cells) *in vitro* (Costa *et al.*, 1982; Ponti *et al.*, 2009).

Several studies have pointed to ROS generation being involved in these types of genotoxicity studies. Positive morphological cell transformation findings in mammalian cells indicate a mutagenic action for cobalt metal and cobalt compounds. Recent rigorous *in vivo* studies (oral gavage and inhalation exposure) in cobalt-exposed rodents by Kirkland *et al*. (2015) and NTP (2014a) did not find evidence of chromosomal damage in bone marrow or erythrocytes, although *in vivo* chromosomal damage assays are regarded to be less sensitive than *in vitro* assays. The few genotoxicity tests conducted on blood lymphocytes of workers exposed to cobalt have been negative. Kirkland *et al*. (2015) suggest that protective processes that exist in whole animals compared to single cells are sufficient to prevent DNA damage resulting from ROS. Thus, other processes may be contributing (*e.g.*, inhibition of DNA repair; altered epigenetic homoestasis) to the genotoxicity of cobalt. However, cells exposed to cobalt at the point of contact (*i.e.*, pulmonary cells with inhalation exposure), as suggested by De Boeck *et al*. (2000), may be a better approach to investigate genotoxic damage caused *in vivo*. Cobalt metal NPs intratracheally instilled into lungs of mice have resulted in evidence of DNA damage in the lung cells (Wan *et al.*, 2017). In addition, the *in vivo* NTP (NTP, 1998a; 2014a) cobalt inhalation studies performed a mutation analysis of the lung neoplasms in the exposed rodents and observed a greater proportion of G→T transversions, which are thought to be chemically-induced and related to ROS generation.

In vitro and *in vivo* studies with cobalt NPs indicate that they are also genotoxic and possibly carcinogenic. However, the level of exposure to cobalt NPs in the general population is unclear since it appears to be largely limited to occupational exposure. In comparison studies with soluble cobalt compounds, cobalt NPs induced more cytotoxicity than cobalt ions while cobalt ions induced more micronuclei but fewer strand breaks than cobalt NPs (Colognato *et al.*, 2008; Ponti *et al.*, 2009). A separate *in vitro* study observed that soluble cobalt compounds induced more cytotoxicity than microparticles of water-insoluble cobalt compounds but with similar levels of genotoxicity (Smith *et al.*, 2014). Finally, an *in vitro* comparison of cobalt metal NPs and microparticles found that the cobalt metal microparticles are more efficient than NPs in inducing both morphological cell transformation and oxidative stress, which supported the finding of greater cellular uptake of cobalt metal microparticles compared to cobalt metal NPs (Sabbioni *et al.*, 2014a; Sabbioni *et al.*, 2014b). However, soluble cobalt compounds showed considerably lower cellular uptake than either cobalt metal NPs or microparticles, and induced no oxidative stress or morphological cell transformation.

The available carcinogenicity and genotoxicity data indicate that separate cancer slope factors (CSFs) and IURs should be used for water-soluble cobalt compounds and cobalt metal. Toxicity data are limited for poorly water-soluble cobalt compounds, but due to a similar particle uptake mechanism and intracellular distribution of cobalt ions released from these water-insoluble cobalt compounds, a CSF based on cobalt metal can also represent water-insoluble cobalt compounds. Similarities in how cells treat cobalt nano- and micro-particles indicate that a cobalt metal CSF based on microparticle exposure will also be relevant for exposure to cobalt metal NPs.

V. QUANTITATIVE CANCER RISK ASSESSMENT

Cobalt Metal

Effective tumor incidences

The effective tumor incidences in rats (Table 8) and mice (Table 9) were used to calculate the cancer potency factor (CPF) for cobalt metal. The effective tumor incidence is the number of tumor-bearing animals (numerator) over the number of animals alive at the time of first occurrence of the tumor (denominator). This method of tallying tumor incidence removes animals from the assessment that died before they are considered at risk for tumor development. For example, effective tumor incidences of tumor types that were only observed near the end of the rodents' lifespan will generally have smaller denominators as a result of early deaths occurring before first appearance of the tumor. The NTP individual animal pathology data from the cobalt inhalation studies were obtained from the Chemical Effects in Biological Systems (CEBS) database (NTP, 2014b).

After week 85 of the cobalt exposures, a reduction in female rat survival was observed in the 2.5 mg/m^3 exposure group compared to controls. A reduction in survival was not observed in other cobalt-exposed rat groups. When significant early mortality occurs in one or more treatment groups, OEHHA may apply the Poly-3 test to adjust the effective tumor incidence data to take survival differences into account (OEHHA, 2017). This procedure more closely approximates the total number of animal-months at risk. However, when reduced survival is not different from the control group until about week 85 or later, as occurred in the 2.5 mg/m^3 female rat group, a poly-3 adjustment is not applied. Thus, use of effective tumor incidences for cancer dose-response modeling were judged appropriate for the female rats.

Table 8a. Effective tumor incidence in rats exposed to cobalt metal for two years (NTP, 2014a) [a,b]

Tumor type in male rats		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Lung: Alveolar/bronchiolar adenoma		2/47‡	10/48*	10/50*	14/49**	0.003	0.015	0.018	0.001
Lung: Alveolar/bronchiolar carcinoma		0/47‡	16/48**	34/50**	36/49**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		2/47‡	25/48**	39/50**	44/49**	<0.001	<0.001	<0.001	<0.001
Adrenal medulla: Benign pheochromocytoma		15/46‡	23/48	37/49**	34/46**	<0.001	0.096	<0.001	<0.001
Adrenal medulla: Malignant pheochromocytoma		2/37‡	2/37	9/39*	16/38**	<0.001	0.693	0.029	<0.001
Adrenal medulla: Benign or malignant pheochromocytoma		17/46‡	23/48	38/49**	41/46**	<0.001	0.193	<0.001	<0.001
Pancreatic islets: Adenoma		0/38	1/39	6/40*	3/39	0.051	0.506	0.015	0.125
Pancreatic islets: Carcinoma		2/38†	1/39	5/40	6/39	0.025	0.490	0.237	0.140
Pancreatic islets: Adenoma or carcinoma		2/38‡	2/39	10/40*	9/39*	0.004	0.683	0.016	0.026

(a) The numerator represents the number of tumor-bearing animals; the denominator represents the number of animals alive at the time of first occurrence of the tumor.

(b) * = $p<0.05$, ** = $p<0.01$; *p*-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = $p<0.05$, ‡ = $p<0.01$, *p*-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical *p*-values for trend are in the statistical *p*-value control column.

Table 8b. Effective tumor incidence in rats exposed to cobalt metal for two years (NTP, 2014a) [a,b]

Tumor type in female rats		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	**0**	**1.25**	**2.5**	**5.0**	**0**	**1.25**	**2.5**	**5.0**
Lung: Alveolar/bronchiolar adenoma		2/45‡	7/47	9/46*	13/44**	<0.001	0.090	0.027	0.001
Lung: Alveolar/bronchiolar carcinoma		0/48‡	9/49	17/48	30/50	<0.001	0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		2/48‡	15/49**	20/48**	38/50**	<0.001	<0.001	<0.001	<0.001
Lung: Cystic keratinizing epithelioma[c]		0/45	4/43	1/40	3/40	0.134	0.053	0.471	0.100
Adrenal medulla: Benign pheochromocytoma		6/45‡	12/47	22/46	36/44	<0.001	0.112	<0.001	<0.001
Adrenal medulla: Malignant pheochromocytoma		0/36‡	2/27	3/25	11/30	<0.001	0.180	0.064	<0.001
Adrenal medulla: Benign or malignant pheochromocytoma		6/45‡	13/47	23/46	40/44	<0.001	0.074	<0.001	<0.001
Immunologic system: Mononuclear cell leukemia		16/50†	29/50**	28/50*	27/50*	0.043	0.008	0.013	0.021

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals alive at the time of first occurrence of the tumor.

(b) * = p<0.05, ** = p<0.01; p-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = p<0.05, ‡ = p<0.001, p-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical p-values for trend are in the statistical p-value control column.

(c) Includes one squamous cell carcinoma in the 5 mg/m^3 group.

In male mice, there was a significant reduction in survival after week 85 in the 2.5 and 5 mg/m^3 cobalt-exposed groups compared to the control group. However, similar to the situation with female rats, a statistically significant difference in mortality was not observed in these animal groups until late in the study (i.e., after week 85). Thus, the use of effective tumor incidences for cancer dose-response modeling was appropriate for the male mice.

Table 9. Effective tumor incidence in mice exposed to cobalt metal for two years (NTP, 1998a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Male mouse									
Lung: Alveolar/bronchiolar adenoma		7/49	11/48	15/43*	3/44	0.832	0.203	0.019	0.206
Lung: Alveolar/bronchiolar carcinoma		11/50‡	38/49**	42/49**	46/49**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		16/50‡	41/49**	43/49**	47/49**	<0.001	<0.001	<0.001	<0.001
Female mouse									
Lung: Alveolar/bronchiolar adenoma		3/46	9/49	8/49	10/48*	0.052	0.075	0.120	0.042
Lung: Alveolar/bronchiolar carcinoma		5/47‡	25/49**	38/50**	43/49**	<0.001	<0.001	<0.001	<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma		8/47‡	30/49**	41/50**	45/49**	<0.001	<0.001	<0.001	<0.001

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals alive at the time of first occurrence of the tumor.

(b) * = p<0.05, ** = p<0.001; p-value indicators are from pairwise comparisons with controls using Fisher exact test performed by OEHHA. ‡ = p<0.001, p-value indicator for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical p-values for trend are in the statistical p-value control column.

Calculation of single- and multi-site tumor cancer slope factors

For the derivation of the CSF, cobalt metal chamber concentrations of 0, 1.25, 2.5 and 5.0 mg/m^3 were time-adjusted (6.2 hrs/24 hrs × 5 days/7 days) to extrapolate from the intermittent chamber exposure conditions to a continuous exposure over the life span of the animals (*i.e.*, to simulate an annualized average air concentration). The time-adjusted concentrations were 0, 0.23, 0.46, and 0.92 mg/m^3.

The average daily dose, in mg/kg BW-day, is used for calculating the cancer potencies. To calculate the daily dose, the weighted average body weight of the control animals during the exposures (i.e., two-years of exposure starting at 5-6 weeks of age) is used to determine the inhalation rate (IR). The weighted average body weights were calculated from data of group mean body weights reported every 1 to 4 weeks during the 2-year exposure period. The average body weights were 453.8, 276.0, 48.5, and 52.7 g for the control male rats, female rats, male mice and female mice, respectively.

A comprehensive analysis of rat minute volume data was undertaken by OEHHA (2018b) to update the IR equation by Anderson (1983) and is shown below (Eq. 6-1a). The analysis incorporates studies since 1988 that more accurately reflect true resting IRs of rats. For mice, the IRs were determined using the equation (Eq. 6-1b) by Anderson (1983). These formulas reflect proportional differences of body weight (BW$^{2/3}$) on the respiratory rate within a species:

rats: $IR\ (m^3/day) = 0.702 \times (BW)^{2/3}$ Eq. 6-1a

mice: $IR\ (m^3/day) = 0.0345\ m^3/day \times (BW / 0.025\ kg)^{2/3}$ Eq. 6-1b

The calculated average daily IRs during the cobalt exposures are 0.4146, 0.2976, 0.05367, and 0.05672 m^3/day for male and female rats and male and female mice, respectively. The average daily doses (shown in Table 10) could then be calculated with the following equation:

$$\text{Dose (mg/kg BW-day)} = IR \times C / BW \qquad \text{Eq. 6-2}$$

Where: C = time-adjusted cobalt metal concentration (mg/m^3)

Table 10. Calculated average daily exposed dose (mg/kg-day) of cobalt metal in the rats and mice during the two-year exposures (rounded to two significant figures in the final assessment).

Species and sex	Cobalt Metal Chamber Concentration (mg/m^3)			
	0	1.25	2.5	5.0
	Daily Exposed Dose (mg/kg-day)			
Rats				
Males	0	0.21	0.42	0.84
Females	0	0.25	0.50	1.00
Mice				
Males	0	0.26	0.51	1.02
Females	0	0.25	0.50	0.99

The US Environmental Protection Agency's (US EPA's) Benchmark Dose Modeling Software (BMDS) version 2.7 (US EPA, 2017) was used to perform dose-response extrapolation. The multistage-cancer model in BMDS was applied for analysis of single-site tumors for tumor types considered by OEHHA to be treatment-related.

Where tumors of the same histological cell type (*e.g.*, alveolar/bronchiolar adenomas and carcinomas) were observed at a single site and benign tumors were considered to have the potential to progress to malignant tumors, the combined incidence was used for dose-response assessment (OEHHA, 2009). These tumor types included alveolar/bronchiolar adenoma and carcinoma for rats and mice (both sexes), benign and malignant pheochromocytoma in male and female rats, pancreatic islets adenoma and carcinoma in male rats, and mononuclear cell leukemia in female rats.

In the cancer dose-response analysis of the female rat study, OEHHA did not include tumor findings judged by NTP to be equivocal (i.e., pancreatic islet adenoma or carcinoma), or the CKE tumors. CKE in female rats was considered a treatment-related tumor by NTP (2014a). However, increases in the incidence of CKE were relatively small (0/45, 4/43, 1/40, 3/40 for control, low-, mid-, and high-dose groups, respectively) compared with increases in other treatment-related tumors, and were not statistically significant by trend test or pairwise comparison of cobalt-exposed group tumor incidence with controls.

NTP (2014a) observed a statistically significant increase in mononuclear cell leukemia in female rats at all exposure concentrations, and concluded that exposure to cobalt metal led to an increased incidence of this neoplasm. NTP also noted the lack of a clear exposure-concentration relationship, likely the result of a plateau response for all non-control cobalt exposures (16/50, 29/50, 28/50, 27/50 for control, low-, mid-, and high-

dose groups, respectively). A positive trend just within statistical significance (p=0.0426) was obtained by OEHHA for this neoplasm with the Cochran-Armitage trend test supplied in the BMD software (US EPA, 2017).

For large datasets such as those by NTP, OEHHA typically sets the benchmark response (BMR) equal to 5%, plus "extra risk" of a tumor response (OEHHA, 2008). The dose associated with this risk is defined as the BMD_{05} and the lower 95% confidence bound on that dose is defined as the $BMDL_{05}$. The multistage-cancer polynomial model was fit to the data, which fits most tumor data sets well. First- and second-degree polynomial multistage models were run for all exposure-related tumor incidence data shown in Tables 8 and 9 for each rodent species and each gender, e.g., in male rats polynomial models were run for alveolar and bronchiolar adenoma and carcinoma lung tumors (combined), for benign and malignant pheochromocytoma (combined), and for pancreatic islet cell adenoma and carcinoma (combined). The most appropriate polynomial model was chosen for each tumor data set based on BMD guidance (U.S. EPA, 2016). Briefly, a goodness-of-fit p-value > 0.05 indicates that the model fits the data well, and in cases where more than one model provides an adequate fit, the model with the lowest Akaike Information Criterion (AIC) value is often selected as the best fitting model. The BMD_{05} and $BMDL_{05}$ are shown in Table 11. The degree of polynomial chosen was 1 in all cases, except for adrenal medulla tumors in female rats where a 2nd degree polynomial provided the best fit to the data.

Male and female rats developed tumors in several organ systems following cobalt metal exposure. Basing cancer risk on only one tumor type may underestimate the carcinogenic potential of a chemical that induces tumors at multiple sites. Multisite tumor CSFs were calculated in both male and female rats using MS Combo Model (US EPA, 2017). The BMDS procedure for summing risks over several tumor sites uses the profile likelihood method. In this method, the maximum likelihood estimates (MLEs) for the multistage model parameters (q_i) for each tumor type are added together (i.e., Σq_0, Σq_1, Σq_2), and the resulting model is used to determine a combined BMD. A confidence interval for the combined BMD is then calculated by computing the desired percentile of the chi-squared distribution associated with a likelihood ratio test having one degree of freedom.

For male rats, multisite tumor analysis was conducted for lung (alveolar/bronchiolar adenoma or carcinoma combined), adrenal medulla (benign or malignant pheochromocytoma combined), and pancreatic islet tumors (pancreatic islets adenoma and carcinoma). In female rats, multisite tumor analysis was conducted for lung (alveolar/bronchiolar adenoma or carcinoma combined), adrenal medulla (benign or malignant pheochromocytoma combined), and mononuclear cell leukemia. Some evidence suggests that pheochromocytoma of the adrenal medulla may be dependent on tumor formation in the lungs (see Cancer Hazard Evaluation section), although NTP

(2014a) noted that the evidence is not clear. OEHHA therefore uses the health protective assumption that these two tumor types are independent and considered the lung and adrenal tumors as separate sites in the multi-site analysis. The treatment-related female rat CKE tumor data were not included in the dose-response analysis as they were judged not to contribute significantly to the CSF, based on the relatively small increased incidence (0/45, 4/43, 1/40, 3/40 for control, low-, mid-, and high-dose groups, respectively) compared with increases in other treatment-related tumors, and there was a lack of a dose-related trend.

For male and female mice, single-site tumor analyses were conducted for lung (alveolar/bronchiolar adenoma or carcinoma combined) tumors.

At the effective dose producing a 5% tumor response, the CSF is calculated as $0.05/BMDL_{05}$ and is in units of $(mg/kg\text{-}day)^{-1}$ (Table 11). The rodent CSFs (CSF_a) were then converted to human equivalents (CSF_h) using body weight ($BW^{3/4}$) scaling:

$$CSF_h = CSF_a \times (BW_h / BW_a)^{1/4} \qquad \text{Eq. 6-3}$$

Using this interspecies scaling factor is preferred by OEHHA because it is assumed to account not only for pharmacokinetic differences (*e.g.*, breathing rate, metabolism), but also for pharmacodynamic considerations, *i.e.*, tissue responses to chemical exposure (U.S. EPA, 2005). Lifetime body weights for control rats and mice of both sexes were calculated from the NTP (2014a) study as described above. The default body weight for humans is 70 kg. The body weight scaling factor assumes that mg/surface area/day is an equivalent dose between species (OEHHA, 2009).

Comparison of the single-site and multisite CSFs in Table 11 shows that the CSF_h of 27 $(mg/kg\text{-}day)^{-1}$ based on lung tumor incidence in male mice to be the most sensitive estimate of cancer risk (CSF rounded to two significant figures in the final assessment). Therefore, the cancer potency of cobalt metal will be based on this lung tumor response in male mice.

Table 11. BMD_{05}, $BMDL_{05}$, rodent CSFs (CSF_a), and human CSFs (CSF_h) for single-site and multi-site tumors in rats and mice resulting from 2-year inhalation exposure to cobalt metal

Tumor type	AIC[a]	*p*-value	BMD_{05} (mg/kg-day)[a]	$BMDL_{05}$ (mg/kg-day)	CSF_a - (mg/kg-day)$^{-1}$	CSF_h - (mg/kg-day)$^{-1}$
Rats						
Alveolar/bronchiolar						
Males	173.14	0.54	0.01647	0.01364	3.66	12.91
Females	202.56	0.54	0.04162	0.03363	1.49	5.96
Adrenal medulla						
Males	217.47	0.27	0.02451	0.01853	2.70	9.51
Females	187.45	0.75	0.1287	0.04931	1.01	4.03
Pancreatic Islet cell						
Males	126.34	0.16	0.1636	0.1029	0.49	1.71
Mononuclear cell leukemia						
Females	277.81	0.065	0.1297	0.06698	0.74	2.95
Multisite: lung-adrenal-pancreatic tumors combined						
Males	NA[b]	NA	0.009291	0.007947	6.29	22.17
Multisite: lung-adrenal-leukemia combined						
Females	NA	NA	0.02828	0.01867	2.68	10.70
Mice						
Alveolar/bronchiolar						
Males	167.47	0.12	0.01446	0.01122	4.46	27.49
Females	188.20	0.57	0.01868	0.01506	3.32	20.04

[a] Akaike Information Criterion

[b] Not applicable

The Multistage model fit to the data and the resulting BMD and BMDL are shown in Figure 1 for alveolar/bronchiolar lung tumors in male mice.

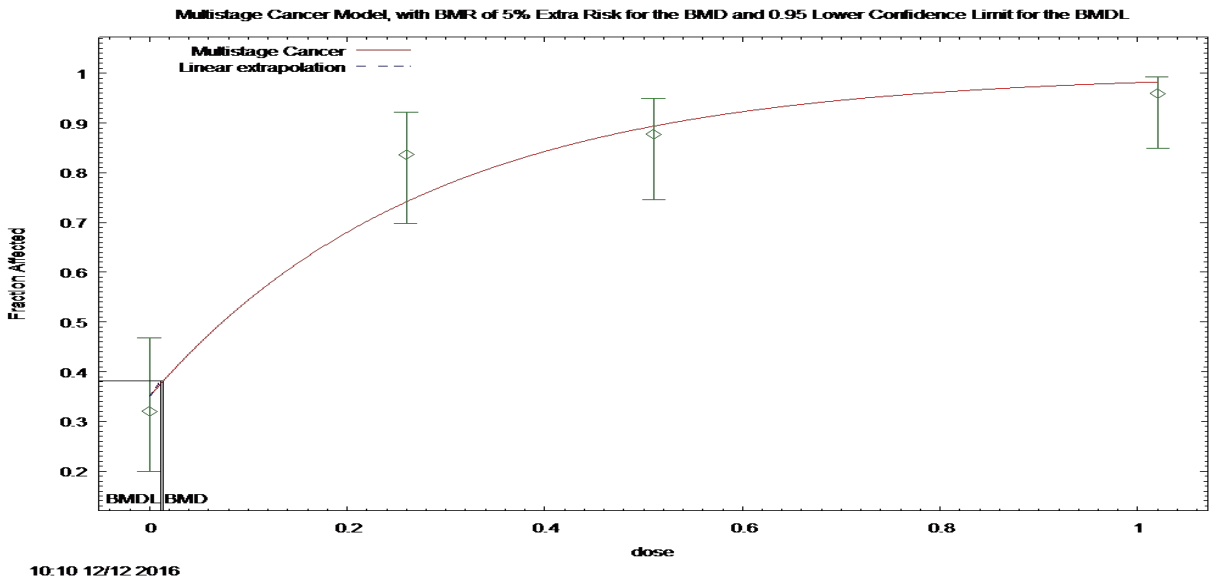

Figure 1. Multistage model fit to the male mouse lung tumor data for cobalt metal. (The benchmark used is the exposure concentration producing 5% tumor response (BMD) with the 95% lower confidence bound (BMDL) on the BMD.)

Figure 1 shows that the lowest non-zero dose is considerably greater than the BMD and BMDL, which is due to the high incidence of lung tumors found in the low-dose group. The lack of a data point nearer the control-dose group (and the BMR) generates uncertainty for the true shape of the curve at the low-dose end, which is important in establishing a low dose cancer potency value. Model and parameter uncertainty will therefore increase as the BMD and BMDL get further away from the lowest non-zero dose (U.S. EPA, 2005). BMDS version 3.0 (US EPA, 2018) provides a recommendation of "questionable" using a BMR of 5% with the male mouse lung tumor data. The "questionable" level occurs when the BMD is more than 3-fold lower than the lowest non-zero dose and the BMDL is more than 10-fold lower than the lowest non-zero dose. By sequentially raising the BMR and re-running the modeling program, a "viable" recommendation occurred when the BMR reached 13%. At this BMR, the BMDL (0.03045 mg/kg-day) is now within 10-fold of the lowest non-zero dose. The resulting CSF_a is 4.27 $(mg/kg\text{-}day)^{-1}$, and the corresponding CSF_h is 26 $(mg/kg\text{-}day)^{-1}$.

OEHHA notes that the CSF_h is adjusted only slightly lower from 27 to 26 $(mg/kg\text{-}day)^{-1}$ when raising the BMR from 5% to 13%, respectively. However, the high tumor incidence in all exposed groups resulted in a positive scaled residual at the low dose (+1.52), which indicates that the slope of the curve at a BMR of 5%, and the corresponding cancer potency, is likely underestimated. Choosing a BMR of 13% for BMD modeling will only increase this likely underestimation of the cancer potency. The small change in the CSF to achieve a "viable" recommendation (i.e., adjusting the BMR from 5% to 13%), combined with the likely increase in underestimation of the cancer risk with this

adjustment, strongly supports using a BMR of 5% for derivation of the CSF, as is recommended in OEHHA's cancer risk derivation methodology.

As a check on the probable underestimation of the CSF, the highest dose group can be removed and the BMD run using only the control, low- and mid-dose groups. Dropping the high dose group has been used in practice when a curve could not be fit to the data when the highest dose group is included, particularly if a 100% incidence response was reached at this dose. Since the high dose cobalt group is close to a 100% tumor response, this data point provides the least amount of information for estimating a BMR of 5%. However, removing this dose group weakens the power of the BMD model and may increase the uncertainty of the CSF.

The BMDL using three data points (control, low- and mid-dose groups) is 0.009478 mg/kg-day and provides a CSF_a of 5.28 $(mg/kg\text{-}day)^{-1}$ and a CSF_h of 33 $(mg/kg\text{-}day)^{-1}$. The resulting slope between the control and low-dose groups is slightly steeper with a smaller positive scaled residual (+1.10) on the low-dose group. That is to say, the curve is closer to the low-dose data point, indicating a better local fit, compared to the curve on the low-dose group when using all four dose groups. This check on the model supports the use of a BMR of 5% for deriving the CSF for cobalt metal.

Assuming 100% absorption of inhaled cobalt metal, as inferred in Equation 6-2, may also result in the underestimation of the cancer potency. This is because the derivation of the cancer slope factor could be based on an overestimation of the cobalt dose, if inhaled cobalt metal absorption is less than 100%. Few studies have attempted to determine the absorption of inhaled cobalt. Martin *et al.* (2010) estimated 12% absorption of cobalt metal dust in a toxicokinetic study of 16 workers in the carbide tungsten industry. Breathing zone concentrations and urinary clearance of cobalt were used to estimate absorption. However, cobalt remaining long-term in the body was not measured or incorporated into this estimate. In addition, the size (e.g., MMAD) of the cobalt metal particles were not presented, so it is unclear what proportion of the particles were too large to be inhalable. Nevertheless, the data suggests that absorption of inhaled cobalt metal will be less than 100%.

Calculation of inhalation unit risk

The Inhalation Unit Risk (IUR) describes the excess cancer risk associated with an inhalation exposure to a concentration of 1 $\mu g/m^3$ and is derived from the cobalt metal CSF. Using a human breathing rate (BR) of 20 m^3/day, an average human body weight (BW) of 70 kg, and a mg-to-µg conversion factor (CF) of 1,000, the IUR is calculated as:

$$IUR = (CSF \times BR) / (BW \times CF) \qquad \text{Eq. 6-4}$$

Use of the equation above with the cobalt metal CSF of 27 $(mg/kg\text{-}day)^{-1}$ results in a calculated IUR of 0.0077 $(\mu g/m^3)^{-1}$ or 7.7×10^{-3} $(\mu g/m^3)^{-1}$. Thus, the extra cancer risk associated with continuous lifetime exposure to 1 $\mu g/m^3$ cobalt metal is 77 in ten thousand, or 7700 in a million.

Cobalt Sulfate Heptahydrate

Effective tumor incidences

The effective tumor incidences (number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor) for treatment-related tumors observed in the NTP studies conducted in rats and mice are shown in Tables 12 and 13, respectively. The NTP individual animal pathology data used to determine the effective tumor incidences for cobalt sulfate heptahydrate were obtained from the CEBS database (NTP, 1998b).

Table 12. Effective tumor incidence in rats exposed to cobalt sulfate heptahydrate for two years (NTP, 1998a)[a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Male rat									
Lung: Alveolar/bronchiolar adenoma		1/43†	4/44	1/43	6/40*	0.022	0.187	0.753	0.044
Lung: Alveolar/bronchiolar carcinoma		0/24	0/28	3/34	1/25	0.209	1.000	0.194	0.510
Lung: Alveolar/bronchiolar adenoma or carcinoma		1/43†	4/44	4/43	7/40*	0.014	0.187	0.180	0.022
Female Rat									
Lung: Alveolar/bronchiolar adenoma		0/39‡	1/33	10/37**	9/35**	<0.001	0.458	<0.001	<0.001
Lung: Alveolar/bronchiolar carcinoma		0/44†	2/41	6/42*	6/46*	0.017	0.230	0.011	0.015
Lung: Alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma		0/44‡	3/41	16/42**	16/46**	<0.001	0.108	<0.001	<0.001
Adrenal medulla: Benign pheochromocytoma		2/39‡	1/37	3/38	8/38*	0.002	0.520	0.487	0.039
Adrenal medulla: Benign, complex or malignant pheochromocytoma		2/39‡	1/37	4/38	10/39	<0.001	0.520	0.325	0.012

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals alive at the time of first occurrence of the tumor.

(b) * = p<0.05, ** = p<0.001; p-value indicators are from pairwise comparisons with controls using Fisher exact tests performed by OEHHA. † = p<0.05, ‡ = p<0.01, p-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical p-values for trend are in the statistical p-value control column.

Table 13. Effective tumor incidence in mice exposed to cobalt sulfate heptahydrate for two years (NTP, 1998a) [a,b]

Tumor type		Incidence by concentration				Statistical *p*-values for pairwise comparison with controls (*p*-value for trend in control column)			
	mg/m^3	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Male mouse									
Lung: Alveolar/bronchiolar adenoma		9/49†	12/50	13/49	18/48*	0.016	0.331	0.234	0.030
Lung: Alveolar/bronchiolar carcinoma		4/50†	5/50	7/49	11/48*	0.010	0.500	0.251	0.037
Lung: Alveolar/bronchiolar adenoma or carcinoma		11/50‡	14/50	19/49	28/48**	<0.001	0.322	0.055	<0.001
Female mouse									
Lung: Alveolar/bronchiolar adenoma		3/40†	6/47	9/42	10/39*	0.015	0.330	0.069	0.029
Lung: Alveolar/bronchiolar carcinoma		1/49‡	1/49	4/49	9/45**	0.001	0.753	0.181	0.006
Lung: Alveolar/bronchiolar adenoma or carcinoma		4/49‡	7/49	13/49*	18/45*	<0.001	0.262	0.015	<0.001

(a) The numerator represents the number of tumor-bearing animals; the denominator represents number of animals alive at the time of first occurrence of the tumor.

(b) * = p<0.05, ** = p<0.01; p-value indicators are from pairwise comparisons with controls using Fisher exact test performed by OEHHA. † = p<0.05, ‡ = p<0.01, p-value indicators for trend in control incidence column determined using Cochran-Armitage trend test performed by OEHHA; numerical p-values for trend are in the statistical p-value control column.

Calculation of single- and multi-site tumor cancer slope factors

For the derivation of the CSF, cobalt sulfate heptahydrate chamber concentrations of 0, 0.3, 1.0 and 3.0 mg/m^3, were time-adjusted (6.2 hrs/24 hrs x 5 days/7 days) to extrapolate from the intermittent lab exposure conditions to a continuous exposure over the life span of the animals (*i.e.*, to simulate an annualized average air concentration). The time-adjusted cobalt sulfate heptahydrate concentrations of 0, 0.055, 0.18, and 0.55 mg/m^3 were used to calculate the average daily dose in mg/kg BW-day.

To calculate the daily dose, the average body weight of the rats and mice over the duration of the study is used to determine the IR. The weighted average lifetime body weights for control animals in each study were calculated from the data of group mean body weights reported every 1 to 4 weeks during the 2-year exposure period. The average body weights were 435.8, 263.3, 41.7 and 40.2 g for the control male rats, female rats, male mice and female mice, respectively.

The IRs were estimated the same as that shown in Eq 6-1a and 6-1b above, where:

For rats, IR (m^3/day) = 0.702 (BW)$^{2/3}$ (OEHHA, 2018b)

For mice, IR (m^3/day) = 0.0345 m^3/day (BW / 0.025 kg)$^{2/3}$ (Anderson, 1983)

The calculated average daily IRs during the cobalt exposures are 0.4035, 0.2884, 0.04852, and 0.04735 m^3/day for male and female rats and male and female mice, respectively. The IR multiplied by the time-adjusted exposure concentration and divided into the animal body weight gives the dose (Eq. 6-2) in mg/kg BW-day (Table 14).

Table 14. Calculated average daily exposed dose (mg/kg-day) of cobalt sulfate heptahydrate in the rats and mice during the two-year exposures (rounded to two significant figures in the final assessment)

Species and Sex	$CoSO_4 \cdot 7H_2O$ Chamber Concentration (mg/m^3)			
	0	0.3	1.0	3.0
	Daily Exposed Dose (mg/kg-day)			
Rats				
Males	0	0.051	0.17	0.51
Females	0	0.061	0.20	0.61
Mice				
Males	0	0.064	0.21	0.64
Females	0	0.065	0.22	0.65

US EPA (2017) BMD methodology (BMDS version 2.7) was applied for single-site tumors using the multistage-cancer model. US EPA BMD guidance (U.S. EPA, 2016) was used to choose the most appropriate model among multistage 1st, and 2nd degree polynomial models, similar as that described above for cobalt metal. Tumor incidences

in the low dose groups of both rats and mice were very near or below a 5% tumor response. Combined with the large group sizes (n = 48 to 50), a benchmark of 5% tumor response (BMD_{05}) is appropriate for determining the cancer potency (OEHHA, 2009).

The BMD_{05} and $BMDL_{05}$ were determined for treatment-related tumors in each of the studies. Specifically, these values were determined for lung alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma (combined) for rats of both sexes, for lung alveolar/bronchiolar adenoma or carcinoma (combined) for mice of both sexes, and for benign or malignant adrenal medulla tumors (combined) in female rats (Table 15). The incidence of these tumors showed a statistically significant increase above control values at one or more dose levels, and also exhibited a statistically significant positive trend across dose levels (See Tables 12 and 13).

Cobalt sulfate heptahydrate induced tumors at two sites in female rats (tumors in the lung and adrenal medulla). To avoid the potential underestimation of the true carcinogenic risk using a single tumor-site approach, a multi-site tumor risk analysis for female rats was performed, which included both alveolar/bronchiolar adenoma, carcinoma, or squamous cell carcinoma (combined) and benign, complex, or malignant pheochromocytoma (combined). The multi-site tumor CSF was calculated using MS Combo Model (US EPA, 2017). A description of the MS Combo Model is provided in the cobalt metal CSF derivation above.

Some evidence suggests that pheochromocytoma of the adrenal medulla may be dependent on tumor formation in the lungs (see Cancer Hazard Evaluation section), although NTP (2014a) noted that the evidence is not clear. OEHHA therefore uses the health protective assumption that these two tumor types are independent and considered separate tumor sites for multi-site analysis.

For modeling the single-site tumor incidence data, in all cases the selected models were first degree polynomials either because the model defaulted to polynomial = 1 or because the first degree polynomial provided the best fit to the data (*i.e.*, lowest AIC value). The multistage polynomial model fit the tumor data well (goodness of fit $p>0.05$), except for the lung tumor incidence in female rats. The female rat lung tumor data exhibited a plateau response at the two highest dose groups, resulting in the lack of model fit ($p=0.0065$). The high dose group was subsequently removed and the three remaining dose groups were rerun using the multistage model. An acceptable model fit to the data was achieved ($p=0.57$) with these three dose groups (Table 15 and Figure 2). For comparison, a plot showing the multistage model fit to the male mice lung tumor data is given in Figure 3.

At the effective dose producing a 5% tumor response, the cancer slope factor (CSF) is calculated as $0.05/BMDL_{05}$ and is in units of $(mg/kg\text{-}day)^{-1}$ (Table 15). The animal (a) CSFs were then converted to human (h) equivalents using body weight $(BW)^{3/4}$ scaling as shown above in Eq. 6-3. Lifetime body weights for control rats and mice of both sexes were calculated from NTP (2015) as described above. The default body weight for humans is 70 kg.

Comparison of the single-site and multi-site human CSFs in Table 15 shows the CSF_h of 13.41 $(mg/kg\text{-}day)^{-1}$ based on the female rat multi-site tumor data to be the most sensitive indicator of cancer risk for cobalt sulfate heptahydrate. Since the cobalt ion is considered to be the primary factor for cancer risk, the cobalt sulfate heptahydrate CSF is normalized to the content of cobalt. As discussed in Section III, generation of the aerosol particles to which the rodents were exposed resulted in formation of primarily cobalt sulfate hexahydrate, although it is expected that environmental exposures to hydrated cobalt sulfate would be to the heptahydrate form. Thus, the molecular weight of cobalt is divided by the molecular weight of cobalt sulfate hexahydrate (58.9 Co / 263.1 $CoSO_4 \bullet 7H_2O$ = 0.2239) and multiplied by 13.41 $(mg/kg\text{-}day)^{-1}$ to result in an adjusted CSF of 3.0 $(mg\ Co/kg\text{-}day)^{-1}$.

Table 15. BMD_{05}, $BMDL_{05}$, rodent CSFs (CSF_a), and human CSFs (CSF_h) for single-site and multi-site tumors in rats and mice resulting from 2-year inhalation exposure to cobalt sulfate heptahydrate

Tumor type	AIC[a]	*p*-value	BMD_{05} (mg/kg-day)[a]	$BMDL_{05}$ (mg/kg-day)	CSF_a $(mg/kg\text{-}day)^{-1}$	CSF_h $(mg/kg\text{-}day)^{-1}$
Rats						
Alveolar/bronchiolar						
Males	105.27	0.53	0.1644	0.08383	0.60	2.14
Females[b]	80.53	0.57	0.02456	0.01717	2.91	11.75
Adrenal medulla						
Females	100.07	0.60	0.1295	0.07852	0.64	2.58
Multisite: lung/adrenal tumors combined						
Females	NA[c]	NA	0.02064	0.01504	3.32	13.41
Mice						
Alveolar/bronchiolar						
Males	246.71	0.96	0.05161	0.03435	1.46	9.35
Females	189.87	0.70	0.07258	0.04819	1.04	6.72

[a] Akaike Information Criterion

[b] The high dose group was removed for benchmark dose modeling to achieve sufficient goodness of fit.

[c] Not applicable

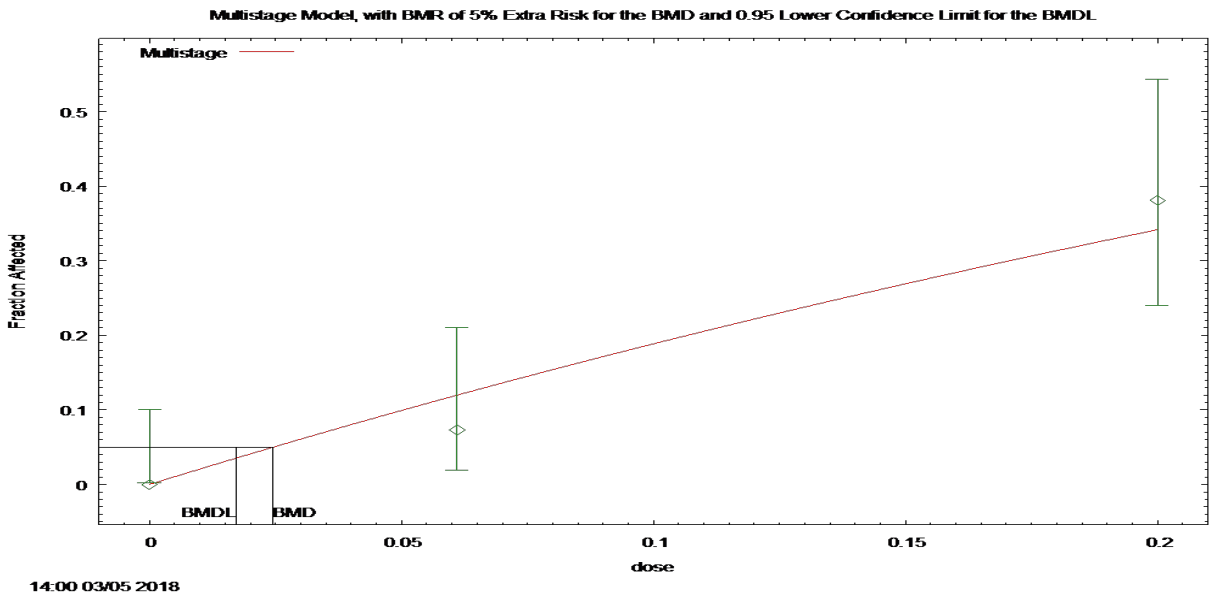

Figure 2. Multistage model fit to the female rat lung tumor data for cobalt sulfate heptahydrate (BMR = 0.05) (The benchmark used is the exposure concentration producing 5% tumor response (BMD) with the 95% lower confidence bound (BMDL) on the BMD.)

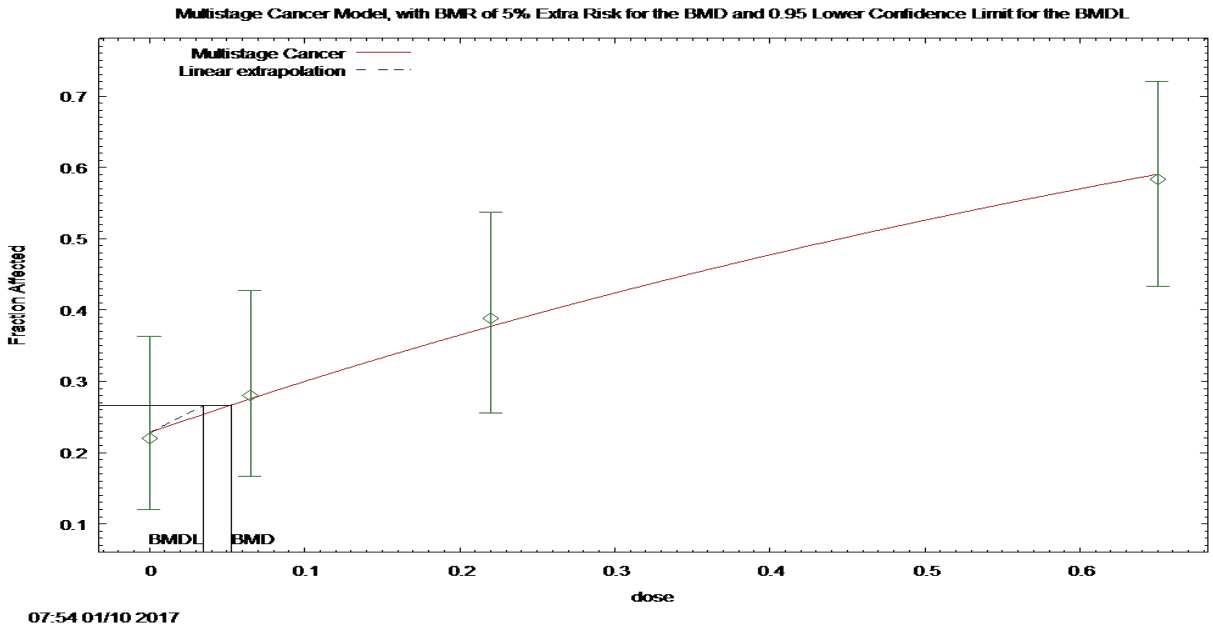

Figure 3. Multistage model fit to the male mice lung tumor data for cobalt sulfate heptahydrate (BMR = 0.05) (The benchmark used is the exposure concentration producing 5% tumor response (BMD) with the 95% lower confidence bound (BMDL) on the BMD.)

Calculation of inhalation unit risk

The Inhalation Unit Risk (IUR) describes the excess cancer risk associated with an inhalation exposure to a concentration of 1 μg/m^3 and is derived from the cobalt sulfate heptahydrate CSF. Using a human breathing rate of 20 m^3/day, an average human BW of 70 kg, and a mg-to-μg conversion factor of 1,000, the IUR was calculated as shown in Eq. 6-4 (see above).

Using the cobalt-normalized CSF of 3.0 (mg Co/kg-day)$^{-1}$ results in a calculated IUR of 0.00086 (μg Co/m^3)$^{-1}$ or 8.6 $\times$ 10^{-4} (μg Co/m^3)$^{-1}$. Thus, the extra cancer risk associated with continuous lifetime exposure to 1 μg/m^3 cobalt sulfate heptahydrate normalized to the cobalt content is 8.6 in ten thousand, or 860 in a million.

VI. CONCLUSIONS

Carcinogenicity studies conducted by NTP established clear evidence of carcinogenicity for cobalt metal and cobalt sulfate heptahydrate. Release of the cobalt ion in physiological fluids is considered the primary factor for cancer risk. The lungs were the primary site of tumor formation in both rats and mice, and both cobalt metal and cobalt sulfate heptahydrate induced tumors of the same histogenic type in lungs. Cobalt metal and cobalt sulfate heptahydrate exposure also induced tumors at multiple sites in rats. Carcinogens that produce tumors in more than one species have the greatest potential to induce tumors in other species, including humans. For each cobalt compound, the CSF was based on the most sensitive species and sex. Derivation of an IUR for cobalt metal (7.7 × 10^{-3} (μg/m^3)$^{-1}$) is based on lung tumor formation in male mice. The IUR derivation for cobalt sulfate heptahydrate (8.6 × 10^{-4} (μg Co/m^3)$^{-1}$) is based on a multi-site analysis of lung and adrenal medulla tumors observed in female rats.

Additionally, *in vitro* studies suggest differences in how the cells internalize cobalt metal particles and water-insoluble cobalt compounds compared to cobalt ions (released by water-soluble cobalt compounds), which are then distributed within the cells. The *in vitro* studies suggest that insoluble cobalt compounds, such as cobalt oxides, are internalized and distributed in cells in a manner similar to that of cobalt metal particles. This may explain some of the different genotoxicity results observed for cobalt metal and insoluble cobalt compounds as compared to those observed for soluble cobalt compounds.

With the information available, OEHHA recommends that the IUR derived from cobalt metal be used for cobalt metal exposure and for cobalt compounds, such as cobalt oxides, that are water insoluble (≤100 mg/L at 20˚C), but bioavailable in pulmonary fluids. The IUR derived for cobalt sulfate heptahydrate is recommended exclusively for water-soluble cobalt compounds (>100 mg/L at 20˚C), such as the chloride, acetate, and nitrate salts of cobalt.

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