FINAL

Report on Carcinogens Background Document for

Cobalt Sulfate

April 17, 2002

Prepared for the:
U.S. Department of Health and Human Services
Public Health Service
National Toxicology Program
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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Cobalt Sulfate. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Support to the National Toxicology Program for the preparation of this background document was provided by Technology Planning and Management Corporation through NIEHS Contract Number NO1-ES-85421

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

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

Known to be Human Carcinogens:

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

Reasonably Anticipated to be Human Carcinogens:

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

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

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

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

Executive Summary

Introduction

Cobalt sulfate is an inorganic salt of divalent cobalt that is used in the electroplating and electrochemical industries and as a coloring and drying agent. Cobalt sulfate heptahydrate is the hydrated form of cobalt sulfate. The behavior of the anhydrous and hydrated forms in solution is indistinguishable, as dissolution of either form results in a system containing hydrated ions and water. Cobalt sulfate was nominated by the National Institute of Environmental Health Sciences for possible listing in the Report on Carcinogens based on a National Toxicology Program (NTP) two-year inhalation study of cobalt sulfate heptahydrate which concluded that there was clear evidence of carcinogenicity in female F344/N rats and male and female B6C3F$_1$ mice and some evidence of carcinogenicity in male F344/N rats. Cobalt and cobalt compounds have been categorized by the International Agency for Research on Cancer as possibly carcinogenic to humans (Group 2B), based on sufficient evidence of carcinogenicity in experimental animals.

Human Exposure

Use. Cobalt sulfate is used in the electroplating and electrochemical industries; as a drier for lithographic inks, varnishes, paints, and linoleum; in storage batteries; and as a coloring agent in ceramics, enamels, glazes, and porcelain. In addition, cobalt sulfate has been used in animal feeds as a mineral supplement and used on pastures where the forage is cobalt deficient, to provide enough cobalt for ruminants to produce vitamin B_{12}. Past uses include addition to beers to improve the stability of the foam, use in veterinary medication to prevent and treat cobalt deficiency in ruminants, and use in humans to improve hematocrit, hemoglobin, and erythrocyte levels.

Production. Cobalt sulfate is formed by the interaction of cobalt oxide, hydroxide, or carbonate with sulfuric acid. Production of cobalt sulfate in the United States in 1983 was estimated at 450,000 lb. Import of cobalt sulfate in 1986 was reported to be 79,700 lb. United States imports for consumption of cobalt sulfates were 1,360 metric tons in 1999 and 1,040 metric tons in 1998.

Environmental exposure. No information was found that specifically identified environmental exposure to cobalt sulfate. Exposure to cobalt occurs through inhalation of ambient air and ingestion of food and drinking water. Cobalt is an essential trace element in humans, because a cobalt atom is present in each molecule of vitamin B_{12} (cobalamin). The National Health and Nutrition Examination Survey in 1999 reported that the geometric mean cobalt level in the urine of humans was 0.36 μg/L of urine (95% confidence interval = 0.32 to 0.40).

Occupational exposure. No information was found that specifically identified occupational exposure to cobalt sulfate. More than a million workers in the United States potentially are exposed to cobalt or cobalt compounds. Occupational exposure to cobalt occurs principally in refining processes, in production of alloys, and in the tungsten

carbide hard-metal industry. In addition, many workers are exposed to a limited degree when using cobalt-containing paint dryers. Occupational exposure is primarily dermal or through inhalation of cobalt metal dusts or fumes. A high degree of conformity between the concentration of cobalt in blood and urine and the average levels of cobalt in the air during a workweek has been reported for workers exposed to cobalt.

Regulations. No specific United States Environmental Protection Agency (EPA) regulations for cobalt sulfate were identified. Cobalt is regulated by the EPA, Food and Drug Administration, and Occupational Safety and Health Administration (OSHA). The current OSHA permissible exposure limit for cobalt metal, dust, and fume (as Co) is 0.1 mg/m^3 of air as an 8-hour time-weighted average (TWA) concentration. The National Institute for Occupational Safety and Health has established a recommended exposure limit for cobalt metal, dust, and fume of 0.05 mg/m^3 as a TWA for up to a 10-hour workday and a 40-hour workweek. The American Conference of Governmental Industrial Hygienists has assigned elemental cobalt and inorganic cobalt compounds (as Co) a threshold limit value of 0.02 mg/m^3 as a TWA for an 8-hour workday and a 40-hour workweek.

Human Cancer Studies

Although no human studies are available in which exposure to cobalt sulfate is specifically evaluated, some human studies have investigated carcinogenicity of cobalt and cobalt compounds as a class. Several studies suggest that exposure to cobalt in hard-metal production is associated with an increased risk of lung cancer. However, because the exposure considered in these studies is to metallic cobalt and tungsten carbide together, the results are of uncertain relevance for the evaluation of cancer due to cobalt exposure alone. Exposure to cobalt without co-exposure to tungsten carbide was found to be associated with a twofold increase in risk of lung cancer in two studies; however, the most likely source of this exposure is cobalt metal. Only one study (at an electrochemical factory) specifically mentioned exposure to cobalt salts. The small study size and unstable risk estimates reflected in the discrepancy between the findings of the initial study and the updated study limit the usefulness of these results for evaluation of the carcinogenic effects of cobalt salts in humans. A biomarker study showed a strong association between esophageal cancer and cobalt present in nails but did not provide any information on exposure to specific cobalt compounds. The human studies thus provide limited information for the specific evaluation of the carcinogenicity of cobalt sulfate.

Studies in Experimental Animals

Cobalt sulfate heptahydrate was found to be carcinogenic in $B6C3F_1$ mice and F344/N rats when administered by inhalation in a two-year study conducted by the NTP. There was clear evidence of carcinogenicity in male mice, female mice, and female rats, based on increased incidences of lung tumors. In addition, female rats had an increased incidence of pheochromocytoma of the adrenal medulla. Some evidence of carcinogenicity in male rats was reported, based on increased incidences of lung tumors at the highest exposure level.

Genotoxicity

The genotoxicity of cobalt compounds may depend on the ligand coordinated about the metal ion. Cobalt sulfate was mutagenic in *Salmonella typhimurium* strain TA100 but not in strains TA98 or TA1535. Cobalt sulfate induced cell transformation and micronuclei in Syrian hamster embryo cells and strongly induced p53 expression in mouse fibroblasts. In the presence of hydrogen peroxide, cobalt sulfate induced putative intrastrand cross-links in salmon sperm DNA and single-strand breaks in plasmid pBluescript K+ DNA. However, 8-hydroxy-2'-deoxyguanosine adducts were not induced in salmon sperm DNA. Sulfite in the presence of cobalt ions caused damage in DNA fragments derived from the human c-Ha-*ras*-1 protooncogene. Yields of DNA base products in human chromatin were increased by exposure to cobalt sulfate in the presence of hydrogen peroxide. Cobalt sulfate was not genotoxic to human lymphocytes.

Other Relevant Data

Absorption and excretion. Cobalt is absorbed from the gastrointestinal tract, lungs, and skin and is distributed throughout the body. The highest concentrations are found in the liver, kidney, and heart. It is excreted primarily in the urine, but fecal excretion also is important. There are two distinct elimination phases: the first is rapid and occurs within days of exposure, but the second phase may take several years.

Toxicity. Occupational exposure to cobalt has been associated with a severe and progressive respiratory disease known as hard-metal pneumoconiosis, as well as asthma and contact dermatitis. In the 1960s, several outbreaks of cardiomyopathy and polycythemia were reported in individuals who drank large quantities of beer containing added cobalt.

Potential mechanisms of carcinogenicity. Cobalt ions may mimic or replace other essential divalent metal ions (e.g., magnesium, calcium, iron, copper, or zinc), thus altering many important cellular reactions and functions. There is good evidence that cobalt ions can inhibit DNA repair processes or interact with hydrogen peroxide to form reactive oxygen species that can damage DNA. These mechanisms may contribute to the genotoxic and carcinogenic effects reported for cobalt sulfate and other cobalt compounds.

Table of Contents

List of Tables

List of Figures

1 Introduction

Cobalt sulfate is an inorganic salt of divalent cobalt. It is the usual source of water-soluble cobalt, because it is more economical and has less tendency to dehydrate than cobalt chloride or cobalt nitrate (Budavari *et al.* 1996). Cobalt sulfate is used in the electroplating and electrochemical industries, as a coloring agent for ceramics, as a drying agent in inks, paints, varnishes, and linoleum, and as a mineral supplement additive to animal feed. Cobalt sulfate heptahydrate is the hydrated form of cobalt sulfate. The behavior of the anhydrous and hydrated forms in solution is indistinguishable, as dissolution of either form results in a system containing hydrated ions and water (Davis *et al.* 1999).

Cobalt sulfate was nominated by the National Institute of Environmental Health Sciences (NIEHS) for possible listing in the Report on Carcinogens based on a National Toxicology Program (NTP) two-year inhalation study of cobalt sulfate heptahydrate which concluded that there was clear evidence of carcinogenicity in female F344/N rats (alveolar/bronchiolar neoplasms and pheochromocytoma of the adrenal medulla) and male and female $B6C3F_1$ mice (alveolar/bronchiolar neoplasms) and some evidence of carcinogenicity in male F344/N rats (alveolar/bronchiolar neoplasms) (NTP 1998). Cobalt sulfate heptahydrate also has been found to be mutagenic in *Salmonella typhimurium* strain TA100 with and without liver S9 metabolic activation enzymes. Cobalt and cobalt compounds have been categorized by the International Agency for Research on Cancer (IARC) as Group 2B, possibly carcinogenic to humans, based on sufficient evidence of carcinogenicity in experimental animals. The majority of the cancers in animals reported for cobalt and cobalt compounds in the publications reviewed by IARC (1991) were local sarcomas at injection sites. Data specific for carcinogenicity of cobalt sulfate in animals were not available at the time of the IARC review.

1.1 Chemical identification

Cobalt sulfate ($CoSO_4$, mol wt 155.0, CASRN 10124-43-3) occurs as red to lavender dimorphic, orthorhombic crystals. It also is known as cobalt monosulfate, cobaltous sulfate, and cobalt(II) sulfate. Its RTECS number is GG3100000. The structure of cobalt sulfate is illustrated in Figure 1-1.

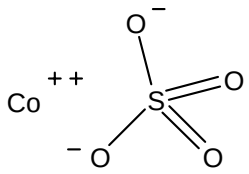

Figure 1-1. Structure of cobalt sulfate

In the majority of its compounds and complexes, cobalt exists in the +2 (cobaltous, cobalt[II]) and +3 (cobaltic, cobalt[III]) valence states. Evidence for cobalt(I) (Co^{+1}) was first obtained from the electrolytic reduction of cyano-compounds. Cobalt(I) also may be found in coordination compounds of the organo-metallic class carbonyl, isonitriles, and unsaturated hydrocarbon derivatives. Cobalt(II) forms numerous salts, most of which are octahedral and tetrahedral. Cobalt(II) forms more tetrahedral complexes than any other transition-metal ion. The octahedral and tetrahedral complexes of cobalt(II) differ little in stability. Octahedral cobalt(II) salts typically are pink to reddish brown (as in the case of cobalt sulfate), whereas most tetrahedral cobalt(II) salts are blue (Kirk and Othmer 1999). Although the cobalt(III) ion exists, only a few simple cobalt(III) salts are known. Examples of cobalt(IV) (Co^{+4}) compounds include cesium cobalt fluoride ($Cs_2[CoF_6]$) and cobalt (IV) fluoride (CoF_4) (Considine and Considine 1995, WebElements 2001).

1.2 Physical-chemical properties

Cobalt sulfate melts at 735°C. It is soluble in water (36.2 g/100 mL at 20°C), slightly soluble in methanol, and insoluble in ammonia. The physical and chemical properties of cobalt sulfate are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of cobalt sulfate

Property	Information	Reference
Molecular weight	155.00	Budavari *et al.* 1996
Color	red to lavender	Budavari *et al.* 1996
Physical state	dimorphic, orthorhombic crystals	Budavari *et al.* 1996
Melting point (°C)	735	HSDB 2000
Decomposition point (°C)	> 708	Budavari *et al.* 1996
Density/specific gravity (at 25°C/4°C)	3.71	Budavari *et al.* 1996
Solubility:		
water (at 20°C)	36.2 g/100 mL	HSDB 2000
water (at 100°C)	84 g/100 mL	HSDB 2000

Cobalt salts are soluble to varying degrees (Lide 1999, Jensen and Tüchsen 1990). Those more soluble in water than cobalt sulfate include cobalt chloride (52.9 g/100 mL at 20°C), cobalt chloride hexahydrate (76.7 g/100 mL at 20°C), and cobalt nitrate hexahydrate (133.8 g/100 mL at 20°C). Cobalt acetate tetrahydrate also is considered soluble. Other salts are much less soluble than cobalt sulfate; cobalt formate is slightly soluble in water (5.03 g/100 mL at 20°C), and cobalt hydroxide is very slightly soluble in water. Salts insoluble in water include cobalt carbonate (1.1 g/100 mL at 15°C), cobalt linoleate, and cobalt oxalate.

1.3 Role of cobalt in biological systems

Cobalt is considered an essential element for animals, including humans, because it is incorporated into the vitamin B_{12} molecule. Green plants do not synthesize vitamin B_{12}; microorganisms in ruminants (cud-chewing mammals with multi-chambered stomachs, such as cattle and sheep) are the only major producers of vitamin B_{12} in the food chain. The normal sources of this vitamin for humans are milk, cheese, meat, and eggs (Considine and Considine 1995).

Vitamin B_{12} contains about 4% cobalt by weight. Ruminants require 0.07 to 0.10 ppm cobalt in their feed, and lack of cobalt in the soil and feedstuffs prevents them from synthesizing enough B_{12} for their needs. To prevent cobalt deficiency in cattle and sheep, cobalt sulfate may be added to feedstuffs, or cobalt may be added to the soil to increase its levels in plants. Areas of low cobalt content in the United States include Florida, the New England area, much of New York, western Iowa, southwestern Minnesota, and a small area of Illinois around Peoria (Considine and Considine 1995).

2 Human Exposure

2.1 Use

Cobalt sulfate is used in the electroplating and electrochemical industries, where it is added to nickel plating baths in order to improve the smoothness, brightness, hardness, and ductility of the deposits. It also is used as a drier for lithographic inks, varnishes, paints, and linoleum and in storage batteries. Cobalt sulfate is employed as a coloring agent in ceramics, enamels, and glazes to prevent discoloring and as a co-pigment for decorating porcelain. In addition, cobalt sulfate has been used in animal feeds as a mineral supplement (Budavari *et al.* 1996, Kirk and Othmer 1999).

Cobalt sulfate has been mixed, in small quantities, with fertilizers for use on pastures where the forage is cobalt deficient, to provide enough cobalt for ruminants to produce vitamin $B_{12.}$ In the United States in 1996, the total amount of fertilizer consumed containing cobalt sulfate was two tons. All of the fertilizer use was in Washington State, where the highest concentrations of cobalt in fertilizers were 44.8 to 222 mg/kg (dry weight) (EPA 1999, Washington State 1999).

In the 1960s, some breweries added cobalt sulfate to their beers to improve the stability of the foam by counteracting the antifoaming activity of detergent residues left on poorly rinsed glasses. Although only a small amount (1 ppm) was used in the beer, this practice was stopped after an epidemic of “beer drinker’s cardiomyopathy” was linked to the cobalt (NTP 1998).

Cobalt sulfate has also been used in veterinary medication to prevent and treat cobalt deficiency in ruminants, which causes reduction in feed intake and body weight, accompanied by emaciation, anemia, and debility. Cobalt sulfate had been used in the past to improve hematocrit, hemoglobin, and erythrocyte levels in human patients with refractory anemia, including sickle-cell disease, thalassemia, chronic infection or renal disease, anemia associated with neoplastic disease, and various other refractory anemias of unknown cause. In 1985, cobalt was used clinically only in the treatment of normochromic, normocytic anemia associated with severe renal failure (HSDB 2000, Hillman and Finch 1985). There is no listing for cobalt or cobalt sulfate in the current *Goodman & Gilman's Pharmacological Basis of Therapeutics* (Goodman and Gilman 2001).

2.2 Production

Cobalt sulfate is formed by the interaction of cobalt oxide, hydroxide, or carbonate with sulfuric acid. Production of cobalt sulfate in the United States in 1983 was estimated at 450,000 lb (NTP 1998). Current production levels are not available. There are currently 11 U.S. suppliers of cobalt sulfate (ChemFinder 2001).

The United States did not mine or refine cobalt in 2000, although negligible amounts of cobalt were produced as a byproduct of mining operations. The U.S. supply of cobalt in 2000 included imports, stock releases, and secondary materials. Stock releases originated from the U.S. government reserve (National Defense Stockpile) for military, industrial,

and civilian use during a national emergency. Sales of the National Defense Stockpile of cobalt began in March of 1993. Seven companies were known to be active in the production of cobalt compounds. It was estimated that 45% of U.S. cobalt usage was in superalloys, 9% in cemented carbides, 9% in magnetic alloys, and the remaining 37% in various other metallic and chemical uses (USGS 2001). Table 2-1 summarizes recent patterns of cobalt production, import, export, and consumption in the United States.

Table 2-1. Cobalt production, consumption, import, and export

	Metric tons of cobalt	
Salient statistics	**1999**	**2000**
United States:		
Production:		
Mine	NR	NR
Secondary	2,720	2,800
Consumption:		
Reported	8,420	8,400
Apparent	10,700	10,900
Imports for consumption	8,150	8,000
Exports	1,550	2,300
World production:		
Mine	29,900	32,300
Refinery	31,200	NR

Sources: Shedd 1999, USGS 2001.

NR = not reported.

Import of cobalt sulfate in 1986 was reported to be 79,700 lb (HSDB 2000). U.S. imports for consumption of cobalt sulfates were 1,360 metric tons in 1999 and 1,040 metric tons in 1998, valued at $9,840,000 and $10,400,000, respectively. Reported 1999 U.S. cobalt consumption was 2,530 metric tons for chemical and ceramic uses and 64 metric tons for miscellaneous and unspecified uses. Reported 1999 U.S. consumption of cobalt chemical compounds (organic and inorganic) was 1,910 metric tons. Imports of cobalt sulfates and other cobalt salts (acetates, carbonates, and chlorides) from 10 countries totaled $12,400,000. Most imports were from Finland. The United States exported $49,700,000 of cobalt and cobalt compounds in 1999. No specific information on cobalt sulfate exports was identified (USGS 2001). Table 2-2 summarizes U.S. cobalt consumption patterns in early 2001.

Table 2-2. Patterns of cobalt consumption in the United States in 2001

Consumption information	Date	Metric Tons	Compounds and uses
Reported consumption of cobalt materials	Jan–May	669	oxide and other chemical compounds
Reported consumption of cobalt by end use	Jan–May	883	chemical uses including catalysts, driers in paints, feed or nutritive additive, glass decolorizer, ground coat frit, pigments, other uses
Reported consumption of cobalt by end use	Jan–May	127	miscellaneous and unspecified uses
Imports by consumption	Jan–April	498	salts and compounds including acetates, carbonates, chlorides, and sulfates
Exports	Jan–April	74	salts and compounds

Source: USGS 2001.

Chem Sources identified 15 suppliers of cobalt(II) sulfate, four suppliers of cobalt(II) sulfate monohydrate, and 16 suppliers of cobalt(II) sulfate heptahydrate in the United States (Chem Sources 2001). The Hazardous Substances Data Bank listed seven manufacturers of cobaltous sulfate (HSDB 2000).

2.3 Analysis

Determination of cobalt, especially in biological samples containing low levels of cobalt, is accurate only if samples are not contaminated. Contamination from disposable syringes and technical-grade anticoagulants was responsible for erroneous reports in earlier literature of grossly high levels of cobalt in biological specimens. The common classical methods used for determining cobalt concentration in biological samples are polarographic and colorimetric methods. However, these older methods are unsuitable for determining low levels of cobalt in many biological samples, and samples must be chemically pretreated before quantification. The most common single-element instrumental techniques used are electrothermal atomic absorption spectrometry (AAS) and voltammetric techniques (ATSDR 1992). Analytical methods for determining cobalt in biological matrices are summarized in Table 2-3. The samples analyzed in the studies presented in this table were primarily from cobalt-exposed and non-exposed workers (Heinrich and Angerer 1984, Ichikawa *et al.* 1985, Alexandersson 1988). However, one study used samples from laboratory volunteers (Bouman *et al.* 1986), and another used hospital patients with knee or hip prostheses (Sunderman *et al.* 1989). IARC (1991) reported that serum cobalt concentrations in humans were in the range of 0.1 to 0.3 μg/L. As shown in Table 2-3, the level of detection for cobalt in serum by direct injection into electrothermal AAS with Zeeman background correction is 0.02 μg/L.

Table 2-3. Analytical methods for determining cobalt in biological materials

Matrix	Analytical method	Detection limit
Urine	electrothermal AAS with Zeeman background correction — direct injection	0.3 μg/L 0.1 μg/L
	electrothermal AAS with Zeeman background correction — chemical preparation	2.4 μg/L
	electrothermal AAS with deuterium background correction — chemical preparation	0.1 μg/L
	differential pulse cathodic stripping voltametry (DPCSV) — chemical preparation	0.2 μg/L
Whole blood	electrothermal AAS with deuterium background correction	2 μg/L
	DPCSV — chemical preparation	0.8 μg/L
	colorimetry — chemical preparation	0.15 μg/L
Serum	electrothermal AAS with Zeeman background correction — direct injection	0.02 μg/L
Blood	inductively coupled plasma-atomic emission spectrometry (ICP–AES) — chemical preparation	10 μg/kg
Tissue	ICP–AES — chemical preparation	200 μg/kg

Source: ATSDR 1992.

Because of its rapidity, accuracy, and low detection limit, electrothermal AAS with Zeeman background correction is the method most commonly used to quantify cobalt levels in environmental samples. To meet detection limits of some of the analytical methods, preconcentration may be necessary for some environmental samples (e.g., seawater). As with biological samples, contamination of environmental samples during collection, storage, and treatment are concerns (ATSDR 1992). Analytical methods for determining cobalt in environmental samples are detailed in Table 2-4.

Table 2-4. Analytical methods for determining cobalt in environmental samples.

Matrix	Analytical method	Detection limit	Recovery[a]
Air (workroom)	τ-spectrometry with lithium-drifted germanium detector	0.17 μg/m^3	–
Air (occupational)	flame AAS with background correction	0.4 μg/m^3	98% with 12- to 96-μg spiked filter
	ICP–AES	0.5 μg/m^3	95%–100% with 2.5- to 1,000-μg spiked filter
Water (low ionic strength)	electrothermal AAS with Zeeman or deuterium background correction	< 0.5 μg/L	93%–113% at 8.5–30 μg/L
Lake water	ICP-AES	< 0.004 μg/L	–
Rainwater	photon-induced X-ray emission	0.08 μg/L	–
Seawater	electrothermal AAS with Zeeman background correction	0.0002 μg/L	90%
	DPCVS	0.0004 μg/L	103% at 0.02 μg/L
Water and waste water	flame AAS	0.05 mg/L	97%–98% at 0.2–5.0 mg/L
	electrothermal AAS with background correction	1 μg/L	–
Groundwater or leachate	flame AAS with background correction	0.05 mg/L	97%–98% at 0.2–5.0 mg/L
Groundwater or leachate	electrothermal AAS with background correction	1 μg/L	–
Food	electrothermal AAS with background correction	1.88 μg/L	100%–107% at 0.2–0.6 mg/kg in leaves and liver

Source: ATSDR 1992.

[a]– = no data available.

2.4 Environmental occurrence

Very limited information is available on the environmental occurrence of cobalt sulfate or other soluble forms of cobalt. Thus, this section summarizes the information available on the environmental occurrence of nonspecific cobalt and cobalt compounds. Where information on cobalt sulfate or other cobalt compounds was available, the specific compound is cited.

2.4.1 Air

Sources of cobalt in the atmosphere are both natural and anthropogenic. Natural sources include wind-blown continental dust, seawater spray, volcanoes, forest fires, and continental and marine biogenic emissions. The worldwide emissions from natural

sources have been estimated to range from 13 to 15 million pounds per year. Cobalt in the atmosphere probably exists in particulate form (ATSDR 1992).

In the United States, the average ambient atmospheric concentration of cobalt was reported to be 0.41 ng/m^3 (ATSDR 1992). Although the HSDB (2000) described the atmospheric concentration of cobalt in remote areas as being very low, the only value given was less than 1 ng/m^3 for the Antarctic. The same source reported that the air concentration of cobalt can reach or exceed 81 ng/m^3 in heavily industrialized cities. Near a beryllium-copper alloy facility, cobalt levels as high as 610 ng/m^3 were observed (HSDB 2000, ATSDR 1992).

Atmospheric cobalt concentrations are much higher near cobalt manufacturing and production facilities. In the ambient air of a facility that manufactured cobalt salts, cobalt concentrations measured by personal sampling ranged from 0.1 to 3.0 mg/m^3, with a mean of 0.2 mg/m^3, and mean concentrations measured by stationary sampling were 0.049 and 1.046 mg/m^3. The cobalt concentration in the ambient air during painting of pottery with soluble cobalt salts ranged from 0.07 to 8.61 mg/m^3 (HSDB 2000). More recent data on levels of cobalt in urban or rural areas were not located.

2.4.2 Water

Concentrations of cobalt in uncontaminated freshwater have been reported to range from 0.1 to 10 μg/L (IARC 1991). The average concentration in seawater has been estimated at 0.27 μg/L. Concentrations in surface water and groundwater can be elevated over the natural background levels as a result of industrial activities. In polluted river water, the concentration may be 27 μg/L. Cobalt levels in suspended material in rivers typically range from 7 to 94 mg/kg, but approach 500 mg/kg in highly polluted rivers (ATSDR 1992).

The National Community Water Supply Study found that cobalt concentrations in drinking water in the United States ranged from nondetectable to 19 μg/L, with 62% of the water samples containing a concentration greater than 1 μg/L. The average cobalt concentration in drinking water was 2.2 μg/L (ATSDR 1992).

2.4.3 Soil

The average concentration of cobalt is 25 mg/kg in the earth's crust, 18 mg/kg in igneous rocks, and 7.2 mg/kg in U.S. soils. Soils with cobalt concentrations less than 3 mg/kg are considered cobalt deficient, because plants that grow in these soils will not contain enough cobalt to meet the dietary needs of cattle and sheep (0.07 to 0.1 mg/kg). Soils near ore deposits, phosphate rocks, ore traffic sites, or industrial pollution sites have been reported to contain cobalt at concentrations of up to 800 mg/kg (ATSDR 1992).

2.5 Environmental fate

Very limited information is available on the environmental fate of cobalt sulfate or other soluble forms of cobalt. Thus, this section summarizes the information available on the environmental fate of nonspecific cobalt and cobalt compounds. Where information on cobalt sulfate or other cobalt compounds was available, the specific compound is cited.

2.5.1 Air

The Agency for Toxic Substances and Disease Registry (ATSDR 1992) proposed that cobalt originating from combustion sources would primarily be in the form of the oxide, whereas cobalt arsenide and sulfide could be released during ore extraction processes. Very few data, however, were available on the potential transformation of these forms to other chemical species, such as the sulfate. ATSDR speculated that chemical speciation of cobalt oxide in the air could lead to the formation of more-soluble cobalt sulfate, which would lead to a higher ratio of dissolved to particulate cobalt; however, no studies could be located on this subject in current literature.

2.5.2 Water

Many factors will affect the speciation and transport of cobalt in natural waters and sediments. Dissolved cobalt appears to be precipitated in the adsorbed state with oxides of iron and manganese and with crystalline sediments such as aluminosilicate and goethite. In addition, cobalt precipitates out as carbonate and hydroxide in water (ATSDR 1992). In freshwater, it is estimated that speciation may yield 76% free Co^{+2}, 19.4% carbonate or bicarbonate, 4% humic complexes, and 0.4% cobalt sulfate. Species of cobalt in seawater are $CoCl^{+}$, free Co^{+2}, carbonate, and humate. Seawater formation of cobalt sulfate is not estimated because of the high concentration of chloride ion. Organic waste concentration and pH play an important role in cobalt speciation (ATSDR 1992). Bioconcentration of cobalt in marine fish is expected to occur, with bottom-feeders accumulating high levels of cobalt (HSDB 2000, ATSDR 1992).

2.5.3 Soil

The speciation of cobalt is regulated primarily by pH, the concentration of chelating or complexing agents in the soil, and the redox potential of the soil. At low pH, cobalt is oxidized to trivalent cobalt and usually is associated with iron. In the process of weathering, cobalt is readily taken into solution. It also is adsorbed to a great extent by hydrolysate or oxidate sediments (HSDB 2000).

2.6 Environmental exposure

Very limited information is available on environmental exposure to cobalt sulfate or other soluble forms of cobalt. Thus, this section summarizes the information available on environmental exposure to nonspecific cobalt and cobalt compounds. Where information on cobalt sulfate or other cobalt compounds was available, the specific compound is cited.

Exposure to cobalt occurs through inhalation of ambient air and ingestion of food and drinking water (ATSDR 1992). The average intake of cobalt in foods by adults in the United States has been estimated at 300 μg per day. Daily intake from water is estimated at 6 μg, and intake from air is estimated at less than 0.1 μg. The major source of cobalt is food, in the form of green leafy vegetables, which may contain as much as 0.5 mg/kg dry weight (HSDB 2000). Cobalt is an essential trace element in humans, because a cobalt atom is present in each molecule of vitamin B_{12} (cobalamin). Cobalt's presence in vitamin B_{12} is its only known essential function in humans (Anderson 2000). An adult human body contains approximately 1.1 mg of cobalt (NTP 1998).

The National Health and Nutrition Examination Survey in 1999 measured cobalt levels in the urine of 1,007 participants aged 6 years or older, to provide physicians with a reference range of cobalt in the urine of the U.S. population for use in determining whether individuals have been exposed to cobalt (CDC 2001). The geometric mean was 0.36 μg/L of urine (95% CI = 0.32 to 0.40). The geometric mean of the creatinine-adjusted levels was 0.33 μg/g of creatinine (95% CI = 0.29 to 0.36).

No information was found that specifically identified environmental exposure to cobalt sulfate.

2.7 Occupational exposure

Very limited information is available on occupational exposure to cobalt sulfate or other soluble forms of cobalt. Thus, this section summarizes the information available on occupational exposure to nonspecific cobalt and cobalt compounds. Where information on cobalt sulfate or other cobalt compounds was available, the specific compound is cited.

It has been estimated by Jensen and Tüchsen (1990) that more than a million workers in the United States potentially are exposed to cobalt or cobalt compounds, though for many, the degree of potential exposure is limited (HSDB 2000, NTP 1998). Occupational exposure to cobalt occurs principally in refining processes, in production of alloys, and in the tungsten carbide hard-metal industry (Kazantzis 1981). In addition, many workers are exposed to a limited degree when using cobalt-containing paint dryers. Occupational exposure is primarily dermal or through inhalation of cobalt metal dusts or fumes (HSDB 2000, NTP 1998).

Cobalt metal has been reported in the air of metal manufacturing, welding, and grinding factories at concentrations ranging from 1 to 300 $\mu g/m^3$ and in the dust of an electric welding factory at 4.2 μg/g (ATSDR 1992). Occupational exposure to cobalt also has been assessed from the concentrations of cobalt in workers' tissues and body fluids. Alexandersson (1988) reported a high degree of conformity between the concentration of cobalt in blood and urine and the average levels of cobalt in the air during a workweek. The cobalt levels in the urine of workers exposed to cobalt in the air at concentrations of 0.005 to 0.15 mg/m^3 were almost 700 times those of the control group. In workers exposed to high levels (0.09 mg/m^3), cobalt concentrations in the blood were 20 times those of the control group, while in the low-exposure workers (0.01 mg/m^3), the concentrations were only slightly higher than in the controls. Other studies have shown that lungs from occupationally exposed workers, such as coal miners and metal-industry workers, contained from 2.5 to 6 times as much cobalt as lungs from control groups (ATSDR 1992).

2.8 Biological indices of exposure

Cobalt sulfate, like other water-soluble metallic salts, dissolves directly into blood serum (362 g/L at 20°C) (Jensen and Tüchsen 1990). Cobalt can be detected in urine, blood, and tissues; however, there currently is no way to correlate cobalt sulfate exposure with cobalt levels observed in these matrices. Based on reports of accidental exposure to

radioactive cobalt (^{60}Co) and intravenous or oral administration of ^{60}Co to volunteer human subjects (Smith *et al.* 1972), approximately 90% of inhaled, injected, or ingested cobalt is eliminated within a few days; however, the remaining 10% has a half-life in the body of two years after parenteral administration or 5 to 15 years after inhalation. No biological use of cobalt is known other than its presence in vitamin B_{12} (HSDB 2000).

2.9 Regulations

No specific U.S. Environmental Protection Agency (EPA) regulations for cobalt sulfate were identified. EPA regulates cobalt under the Clean Water Act (CWA), limiting effluent discharges of cobalt from facilities that produce cobalt from ore concentrate raw materials or process tungsten or tungsten carbide scrap raw materials. EPA also regulates cobalt and cobalt compounds under the Resource Conservation and Recovery Act (RCRA), establishing minimum criteria for all municipal solid waste landfills (MSWLFs). Under the Superfund Amendments and Reauthorization Act (SARA) of 1986, EPA mandates that all information regarding the release of toxic compounds, such as cobalt, be available to the public.

The Food and Drug Administration (FDA) regulates cobalt sulfate, barring its use in malted beverages as a foam stabilizer. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), section 503A(a), all drugs containing cobalt or cobalt sulfate have been withdrawn because they were deemed unsafe or ineffective. Cobalt preparations intended for use in humans are regulated under section 301(p) of the FFDCA. They must go through the new drug application process outlined in sections 314 and 505. Warning and caution statements are required on all drugs containing cobalt or cobalt sulfate. The FDA recognizes that cobalt sulfate and other cobalt compounds are generally recognized as safe when added to animal feeds as nutritional dietary supplements.

The Occupational Safety and Health Administration (OSHA) regulates cobalt under Sections 4, 6, and 8 of the Occupational Safety and Health Act of 1970. The current OSHA permissible exposure limit (PEL) for cobalt metal, dust, and fume (as Co) is 0.1 mg/m^3 of air as an 8-hour time-weighted average (TWA) concentration. The regulation requirements are exactly the same for shipyard and construction workers. The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit for cobalt metal, dust, and fume of 0.05 mg/m^3 as a TWA for up to a 10-hour workday and a 40-hour workweek. The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned elemental cobalt and inorganic cobalt compounds (as Co) a threshold limit value of 0.02 mg/m^3 as a TWA for an 8-hour workday and a 40-hour workweek (OSHA 1998). The ACGIH has established a biological exposure index of 15 μg of cobalt per liter of urine; this index is used to "generally indicate a concentration below which nearly all workers should not experience adverse health effects" (CDC 2001).

EPA regulations are summarized in Table 2-5, FDA regulations in Table 2-6, and OSHA regulations in Table 2-7.

Table 2-5. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR 60 – PART 60 – STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71. U.S. Codes: 42 U.S.C. 7401, 7411, 7413, 7414, 7416, 7601, and 7602.	The provisions of this part apply to the owner or operator of any stationary source that contains an affected facility, the construction or modification of which is commenced after the date of publication in this part of any standard applicable to that facility.
40 CFR 60.750ff. – Subpart WWW – Standards of Performance for Municipal Solid Waste Landfills. Promulgated: 61 FR 9919, 03/12/96.	This subpart describes methods that are applicable to the determination of cobalt emissions from stationary sources.
40 CFR 122 – PART 122 – EPA ADMINISTERED PERMIT PROGRAMS: THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM. Promulgated: 48 FR 14153, 04/01/83. U.S. Codes: 33 U.S.C. 1251 et seq., the CWA.	These regulations cover basic EPA permitting requirements for effluent discharges from point sources to waters of the United States. Appendix D lists pollutants that must be identified by dischargers if expected to be present. Cobalt is listed under Table IV — Conventional and nonconventional pollutants required to be tested by existing dischargers if expected to be present.
40 CFR 258 – PART 258 – CRITERIA FOR MUNICIPAL SOLID WASTE LANDFILLS. Promulgated: 56 FR 51016, 10/09/91. U.S. Codes: 33 U.S.C. 1345(d) and (e); 42 U.S.C. 6907(a)(3), 6912(a), 6944(a), and 6949a(c).	The provisions of this part establish minimum national criteria under RCRA, as amended, for all MSWLF units and under the CWA, as amended, for MSWLFs that are used to dispose of sewage sludge. The criteria ensure the protection of human health and the environment.
40 CFR 258 – APPENDIX II TO PART 258 – LIST OF HAZARDOUS AND ORGANIC CONSTITUENTS.	The practical quantitation limits (PQLs), which are the lowest concentrations of analytes in ground waters that can be reliably determined within specified limits of precision and accuracy by the indicated methods under routine laboratory operating conditions, for cobalt are 70 μg/L for method 6010, 500 μg/L for method 7200, and 10 μg/L for method 7201.
40 CFR 261 – PART 261 – IDENTIFICATION AND LISTING OF HAZARDOUS WASTES. Promulgated: 53 FR 13388, 04/22/88. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6921, 6922, and 6938.	Cobalt is listed as a hazardous waste with a concentration limit of 4.6 mg/kg.
	The requirements of this subpart apply to owners or operators who store munitions and explosive hazardous wastes. The PQL for cobalt is 70 μg/L for method 6010, 500 μg/L for method 7200, and 10 μg/L for method 7201.
40 CFR 372 – PART 372 – TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11013 and 11028. The effective date of this regulation for cobalt is 1/1/87.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and to aid in the development of regulations, guidelines, and standards.

Regulatory action	Effect of regulation or other comments
40 CFR 421 – PART 421 – NONFERROUS METALS MANUFACTURING POINT SOURCE CATEGORY. Promulgated: 49 FR 8790, 03/08/84. U.S. Codes: 33 U.S.C. 1311, 1314(b), (c), (e), and (g), 1316(b) and (c), 1317(b) and (c), 1318, and 1361.	The provisions of this part apply to facilities producing primary metals from ore concentrates and recovering secondary metals from recycled wastes which discharge pollutants to waters of the U.S. or which introduce or may introduce pollutants into a publicly owned treatment works.
40 CFR 421.230ff. – Subpart U – Primary Nickel and Cobalt Subcategory. Promulgated: 50 FR 38359, 09/20/85.	The provisions of this subpart are applicable to discharges resulting from the production of nickel or cobalt by primary nickel and cobalt facilities processing ore concentrate raw materials.
40 CFR 421.232 – Sec. 421-232. – Effluent limitations guidelines representing the degree of effluent reduction attainable by the application of the best practicable control technology currently available.	For raw material dust control, the cobalt maximum for any 1 day is 0.016, with a maximum monthly average of 0.007. For nickel wash water, the cobalt maximum for any 1 day is 0.007, with a maximum monthly average of 0.003. For nickel reduction decant, the cobalt maximum for any 1 day is 2.666, with a maximum monthly average of 1.143. For cobalt reduction recant, the cobalt maximum for any 1 day is 4.494, with a maximum monthly average of 1.926.
40 CFR 421.233 – Sec. 421-233. – Effluent limitations guidelines representing the degree of effluent reduction attainable by the application of the best available technology economically achievable.	For raw material dust control, the cobalt maximum for any 1 day is 0.011, with a maximum monthly average of 0.005. For nickel wash water, the cobalt maximum for any 1 day is 0.005, with a maximum monthly average of 0.002. For nickel reduction decant, the cobalt maximum for any 1 day is 1.777, with a maximum monthly average of 0.889. For cobalt reduction recant, the cobalt maximum for any 1 day is 2.996, with a maximum monthly average of 1.498.
40 CFR 421.234 – Sec. 421-234. – Standards of performance for new sources.	The requirements of this section are identical to those set forth in section 421-233.
40 CFR 421.236 – Sec. 421-236. – Pretreatment standards for new sources.	The requirements of this section are identical to those set forth in section 421-233.
40 CFR 421.310ff. – Subpart AC – Secondary Tungsten and Cobalt Subcategory. Promulgated: 50 FR 38386, 09/20/85.	The provisions of this subpart are applicable to discharges resulting from the production of tungsten or cobalt at secondary tungsten and cobalt facilities processing tungsten or tungsten carbide scrap raw materials.
40 CFR 421.312 – Sec. 421-312. – Effluent limitations guidelines representing the degree of effluent reduction attainable by the application of the best practicable control technology currently available.	For cobalt sludge leaching wet air pollution control, the cobalt maximum for any 1 day is 140.977, with a maximum monthly average of 61.901. For cobalt hydroxide filtrate, the cobalt maximum for any 1 day is 233.189, with a maximum monthly average of 97.999. For cobalt hydroxide filter cake wash, the cobalt maximum for any 1 day is 429.598, with a maximum monthly average of 188.631. Other maximum effluent limitations for various tungsten processes also are provided.

Regulatory action	Effect of regulation or other comments
40 CFR 421.313 – Sec. 421-313. – Effluent limitations guidelines representing the degree of effluent reduction attainable by the application of the best available technology economically achievable.	For cobalt sludge leaching wet air pollution control, the cobalt maximum for any 1 day is 98.756, with a maximum monthly average of 43.295. For cobalt hydroxide filtrate, the cobalt maximum for any 1 day is 156.346, with a maximum monthly average of 68.543. For cobalt hydroxide filter cake wash, the cobalt maximum for any 1 day is 300.094, with a maximum monthly average of 131.932. Other maximum effluent limitations for various tungsten processes also are provided.
40 CFR 421.314 – Sec. 421-314. – Standards of performance for new sources.	The requirements of this section are identical to those set forth in section 421-313.
40 CFR 421.316 Sec. 421-316 – Pretreatment standards for existing sources.	The requirements of this section are identical to those set forth in section 421-313.
40 CFR 716 – PART 716 – HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Codes: 15 U.S.C. 2607(d). The effective date for cobalt is 06/01/87, and the sunset date is 06/01/97.	The provisions of this part require the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of the Toxic Substances Control Act and on other chemicals for which EPA requires health and safety information in fulfilling the purposes of the Act.

Source: The regulations in this table have been updated through the Code of Federal Regulations 40 CFR, 1 July 2001.

Table 2-6. FDA regulations

Regulatory action	Effect of regulation or other comments
21 CFR 173 – PART 173 – SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14526 03/15/77. U.S. Codes: 21 U.S.C. 321, 342, and 348.	Cobalt sulfate may be safely used as a catalyst in boiler water additives in the preparation of steam that will contact food.
21 CFR 189 – PART 189 – SUBSTANCES PROHIBITED FROM USE IN HUMAN FOOD. Promulgated: 42 FR 14659, 03/15/77. U.S. Codes: 21 U.S.C. 321, 342, 348, and 371.	Cobalt sulfate has been used in fermented malt beverages as a foam stabilizer and to prevent "gushing." Food containing any added cobalt sulfate is deemed to be adulterated in violation of the act based upon an order published in the 31 FR 8788, 08/12/66.
21 CFR 216 – PART 216 – PHARMACY COMPOUNDING. Promulgated: 64 FR 10944, 03/08/99. U.S. Codes: 21 U.S.C. 351, 352, 353(a), 355, and 371.	All drug products containing cobalt salts, including cobalt sulfate (except radioactive forms of cobalt and its salts and cobalamin and its derivatives), were withdrawn or removed from the market because they were found to be unsafe or not effective.
21 CFR 310 – PART 310 – NEW DRUGS. Promulgated: 64 FR 401, 01/05/99. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 360(b)–360(f), 360(j), 361(a), 371, 374, 375, and 379(e); 42 U.S.C. 216, 241, 242(a), 262, and 263(b)–263(n).	Cobalt preparations intended for use by man have been determined by rulemaking procedures to be new drugs under the FFDCA. An approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing.

Regulatory action	Effect of regulation or other comments
21 CFR 369 – PART 369 – INTERPRETATIVE STATEMENTS RE WARNINGS ON DRUGS AND DEVICES FOR OVER-THE-COUNTER SALE. Promulgated: 39 FR 11745, 03/29/74. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, and 371.	Cobalt preparations must have the following warnings and caution statements: **Warning — Do not exceed the recommended dosage. Do not administer to children under 12 years of age unless directed by physician. Do not use for more than 2 months unless directed by physician.** This warning is not required on articles containing not more than 0.5 milligram of cobalt as a cobalt salt per dosage unit and which recommend administration of not more than 0.5 milligram per dose and not more than 2 milligrams per 24-hour period.
21 CFR 582 – PART 582 – SUBSTANCES GENERALLY RECOGNIZED AS SAFE. Promulgated: 41 FR 38657, 09/10/76. U.S. Codes: 21 U.S.C. 321, 342, 348, and 371.	Cobalt compounds, including cobalt sulfate, added to animal feeds as nutritional dietary supplements are generally recognized as safe when added at levels consistent with good feeding practice.

Source: The regulations in this table have been updated through the Code of Federal Regulations 21 CFR, 1 April 2001.

Table 2-7. OSHA regulations

Regulatory action	Effect of regulation or other comments
29 CFR 1910.1000 – TABLE Z-1 – Limits for Air Contaminants. Promulgated: 39 FR 23502, 06/27/74. U.S. Codes: 5 U.S.C. 553, 29 U.S.C. 653, 655, and 657.	Cobalt is identified as an air contaminant. The PEL for cobalt is 0.1 mg/m^3 as an 8-h TWA.
29 CFR 1915 – Subpart Z – Toxic and Hazardous Substances. Promulgated 58 FR 35514, 07/01/93.	The requirements applicable to shipyard employment under this section are identical to those set forth in section 1910.1000.
29 CFR 1926 – Subpart D – Occupational Health and Environmental Controls. Promulgated: 39 FR 22801, 06/24/74. U.S. Codes: 29 U.S.C. 653, 655, and 657.	The requirements applicable to construction employment under this section are identical to those set forth in section 1910.1000.

Source: The regulations in this table have been updated through the Code of Federal Regulations 29 CFR, 1 July 2001.

3 Human Cancer Studies

Although no human studies are available in which exposure to cobalt sulfate is specifically mentioned, some human studies have investigated carcinogenicity of cobalt and cobalt compounds as a class of chemicals. Most of these studies are cohort studies assessing occupational exposure to cobalt and cobalt compounds. They include studies of cobalt production workers, ceramics workers, hard-metal workers, and workers in nickel refineries. Studies on nickel refinery workers are not included in this discussion, because the main exposure is to nickel, which is a known human carcinogen.

3.1 IARC assessment

IARC (1991) reviewed the carcinogenicity of cobalt and cobalt compounds in 1991 and classified them as possibly carcinogenic to humans (Group 2B). The IARC evaluation included occupational studies and studies of patients with implanted medical devices that may have contained cobalt. Most of the investigations concerning implanted medical devices were case reports, 10 of which described single cases of malignant neoplasia, primarily sarcoma, at the site of implants made of cobalt-containing alloys. The only cohort study of implant patients reported an increased risk of tumors of the lymphatic and hematopoietic system among hip-replacement patients; however, this study did not describe the composition of the hip prosthesis and thus is not informative for the evaluation of cobalt.

The IARC (1991) evaluation discussed four cohort studies of occupational exposure to cobalt, two of which were considered informative; two studies of nickel refinery workers were considered not informative for the evaluation of cobalt and cobalt compounds. Both studies evaluated by IARC, a cohort at a French electrochemical plant producing cobalt and a cohort of Swedish hard-metal workers, reported an excess of lung cancer. The French study (Mur *et al*. 1987) is discussed below (Section 3.2) because an update of this report was published after the IARC review. The Swedish cohort of Hogstedt and Alexandersson (1990) consisted of 3,163 male workers employed at three hard-metal manufacturing plants from 1940 to 1982, with at least one year of exposure to cobalt-containing hard-metal dust, and followed until 1951 to 1982. A standardized mortality ratio (SMR) for lung cancer of 1.34 (95% CI = 0.77 to 2.13, 17 cases) was observed for the cohort, and a higher value was reported for workers with more than 10 years of exposure and more than 20 years since first exposure (SMR = 2.78, 95% CI = 1.11 to 5.72, 7 cases). Smoking habits among the cohort did not differ from those of the male Swedish population. Workers in both studies also were exposed to known carcinogens, such as nickel and arsenic (in the French study) or tungsten carbide present in hard-metal dust (in the Swedish study). IARC concluded that there was inadequate evidence of carcinogenicity in humans for cobalt and cobalt compounds.

3.2 Current human studies

Current studies on human exposure to cobalt are summarized in Table 3-1.

3.2.1 Occupational studies

Mur *et al*. (1987) conducted a retrospective cohort study of 1,143 workers who had been employed for at least one year between 1950 and 1980 at an electrochemical plant in France that produced cobalt and sodium. Cobalt was produced by etching of roasted ore, followed by neutralization, filtration, and electrolysis of the cobalt chloride solution. The manufacturing process also included production of cobalt salts and oxides. Vital status was assessed in 1981 by Mur *et al.* (1987) and in 1988 by Moulin *et al*. (1993). In the first report (Mur *et al.* 1987), fewer deaths from all causes were observed in the entire cohort (213) than expected from the French male population (SMR 0.8, 95% CI = 0.7 to 0.9). An increased risk of lung cancer was observed only for cobalt production workers (adjusted SMR = 4.7, 95% CI = 1.5 to 10.6, 4 cases), and not for sodium production workers or maintenance and general service workers at the plant. However, seven years later, lung cancer risk was no longer elevated in cobalt production workers; the SMR (Cohort II) was 1.2 (95% CI = 0.2 to 3.4, 3 cases) (Moulin *et al.* 1993). The SMR in this later study was based on 3 lung cancer cases, rather than 4 (as in the earlier study). The discrepancy in the number of observed cases is due to differences in how the cause of death was ascertained; the 1987 study used only physicians' records, whereas the later study used death certificates for the years 1968 to 1988. The use of death certificates decreased the proportion of unknown causes of death from 20% to 11%, and no additional lung cancer cases were observed in the extended follow-up period (1981 to 1988). [The small number of exposed cases and high percentage of unknown causes of death limit the power of these studies to detect an effect of exposure to cobalt salts.] The authors stated that the negative finding of the updated study could not be considered a definite conclusion. [Other limitations of these studies include their inability to consider smoking status.]

Lasfargues *et al.* (1994) conducted a cohort mortality study of 709 men employed for at least one year between January 1956 and December 1989 at a French plant producing hard-metal tools. Exposure was categorized into four degrees of hard-metal exposure (none, low, medium, and high) based on job histories and periods of employment. Elevated SMRs in the entire cohort were observed for esophageal cancer (nonsignificant), leukemia (nonsignificant), and lung cancer (significant; SMR = 2.1, 95% CI = 1.0 to 3.9, 10 cases). Risk of lung cancer was highest in the highest exposure category but was not related to duration of employment or time since first exposure. Smoking status was ascertained for 81% of the cohort and 69% of the deceased population; the proportions of smokers were similar to the proportion in a sample of the French adult male population.

Moulin *et al*. (1998) conducted a multicenter study of a cohort consisting of all male (5,777) and female (1,682) workers employed for at least three months in any of ten French factories that produced hard metal. Causes of death (684) were ascertained from death certificates and medical records. In addition to production of hard metal, activities at these factories included power metallurgy processes. Exposure to cobalt and other agents was assessed and semiquantified from a job-exposure matrix, which was validated

by atmospheric measurements of cobalt. A case-control study of 61 cases of lung cancer and 180 controls was nested within the cohort of all workers employed in this industry. An increased risk of lung cancer was associated with "other" cobalt exposure, which was defined as exposure to cobalt alone or simultaneous exposure to cobalt and agents that did not include tungsten carbide (odds ratio [OR] = 2.2, 95% CI = 1.0 to 4.9). A later study of the largest production plant (2,860 workers) in the multicenter cohort (Wild *et al*. 2000) reported that other industrial processes related to cobalt exposure included production of magnets and stainless steel made with cobalt, production of cobalt powders by calcination, and reduction of cobalt hydroxide. Thus, the "other" cobalt exposure probably was to metallic cobalt, but may have included exposure to ionized cobalt generated during the production of metallic cobalt. Wild *et al*. (2000) also reported an increased risk of lung cancer for "other" cobalt exposure that did not include co-exposure with tungsten carbide (OR = 2.0, 95% CI = 1.1 to 3.2) and was assessed from the job-exposure matrix.

Both studies reported an association between simultaneous exposure to cobalt and tungsten carbide (hard-metal production) and lung cancer. The case-control study nested in the multicenter cohort found exposure-response relationships for duration of exposure (test for trend, $P = 0.03$) and for the unweighted cumulative exposure to cobalt and tungsten carbide (test for trend, $P = 0.01$). Unweighted measures of cumulative exposure treat occasional and full-time exposure equally, thus favoring peak exposure (Moulin *et al.* 1998). Wild *et al.* (2000) reported that lung cancer risk was associated with hard-metal production before sintering (SMR = 2.9) and that little risk was associated with hard-metal production after sintering (SMR = 1.1). Exposure to hard metals is higher before than after sintering. Risk associated with exposure to hard-metal dust (cobalt and tungsten carbide) remained elevated and significant after controlling for smoking (Moulin *et al.* 1998) and in a regression model that included smoking and exposure to any IARC carcinogen, including asbestos, polycyclic aromatic hydrocarbons (PAH), certain chromium compounds, certain nickel compounds, and silica (Wild *et al.* 2000).

Tüchsen *et al.* (1996) studied a cohort of Danish porcelain workers exposed to cobalt-aluminate spinel and/or cobalt silicate at two factories (382 women in Factory 1 and 492 women in Factory 2). A significantly increased risk of lung cancer was observed in the exposed women, compared with the Danish population (standardized incidence ratio [SIR] = 2.4, 8 cases); however, an increased risk of lung cancer also was observed in a reference group of non-exposed workers at one of the factories (520 women). The authors recommended a longer follow-up, because of the small number of exposed cases and the need to assess the effects of exposure to cobalt silicate dye, which replaced the cobalt-aluminate spinel dye in Factory 1 in 1972 and Factory 2 in 1989.

3.2.2 Biomarker study

Rogers *et al*. (1993) conducted a population-based case-control study on levels of certain elements (cobalt, calcium, iron, zinc, and chromium) in toenail clippings and cancer of the upper aerodigestive tract. Cases (661) were identified by the local Surveillance, Epidemiology and End Results (SEER) cancer registry, and controls (466), matched on sex and age, were identified by random-digit dialing. Cobalt was measured from toenail samples (507 cases and 434 controls) with neutron activation analysis. Significantly

increased risks for esophageal (OR = 9.0, 95% CI = 2.7 to 30.0) and oral cancer (OR = 1.9, 95% CI = 1.0 to 3.6) were observed for individuals with the highest nail cobalt levels (highest 25%; see Table 3-1), and an exposure-response relationship was observed for esophageal cancer (test for trend, $P < 0.001$). These findings are in agreement with a small study conducted by Collecchi *et al*. (1986), which found higher plasma concentrations of cobalt in patients with laryngeal carcinoma (mean =18.27 ng/mL, N = 11) than in healthy subjects (mean = 0.73 ng/mL, N = 15) (see Section 6). [Strengths of the study by Rogers *et al.* (1993) include its large population size, the use of a biomarker to measure cobalt-specific exposure, and adjustment for potential confounders. The study is limited because it measures recent exposure (perhaps after the development of cancer) rather than past exposure.] Cobalt is deposited in nails during matrix formation, which usually occurs from eight months to two years after exposure, depending on the age of the individual. No differences in risks were observed after stratification by time from diagnosis to interview or stage of disease. This study does not provide any information on the source or type of cobalt exposure. The authors speculated that the cobalt exposure was unlikely to come from vitamin B_{12}, because the cancer patients tended to eat fewer animal products than controls, and there were no differences in the intake of vitamin B_{12} supplements between cases and controls.

3.3 Discussion and summary

The studies discussed in this section are not specific for cobalt sulfate. Whether studies on exposure to cobalt as a class are relevant for evaluation of the carcinogenicity of cobalt sulfate probably depends on the mechanism(s) of carcinogenicity. As discussed in Section 6.4, the proposed mechanisms of cobalt-induced carcinogenesis are based on exposure to cobalt ions. Although several studies suggest that exposure to cobalt in hard-metal production is associated with an increased risk of lung cancer, these studies involve exposure to metallic cobalt and simultaneous exposure to tungsten carbide. Lung toxicity of hard-metal particles may result from a specific interaction between cobalt metal and carbide particles that produces reactive oxygen species (Moulin *et al.* 1998). Thus, these studies are of uncertain relevance for the evaluation of cancer due to cobalt exposure alone.

Other studies discussed in this section include studies on exposure to cobalt as a class of compounds. In most, the types of cobalt present are not specified. The exception is the study of porcelain workers exposed to cobalt-aluminate spinel and cobalt silicate dyes. The small numbers, the increased risk of lung cancer among the non-exposed reference group, and the uncertain relevance of these dyes to cobalt sulfate make this study difficult to interpret. Two of the hard-metal studies reported a twofold increase in risk of lung cancer for “other” cobalt exposure, where “other” was defined as exposure to cobalt without co-exposure to tungsten carbide or hard metal. The most likely source of this exposure is cobalt metal; however, ionic cobalt could have been released during the production of cobalt. Because the focus of these studies was hard-metal exposure, characterization of “other” cobalt exposure and analyses controlling for confounders (co-exposure to other carcinogens) was less detailed than for exposure to hard metal.

Only one study, of the French electrochemical factory, specifically mentioned exposure to cobalt salts. The small study size and unstable risk estimates, reflected in the

discrepancy between the findings of the initial study (Mur *et al*. 1987) and the updated study (Moulin *et al.* 1998), limits its usefulness for evaluation of the carcinogenic effects of cobalt salts in humans.

The biomarker study showed a strong association between esophageal cancer and cobalt present in nails but did not provide any information on specific cobalt compounds. Moreover, the study assessed recent cobalt exposure, whereas past exposure is more likely important for cancer development.

In conclusion, the human studies provide limited information for the specific evaluation of the carcinogenicity of cobalt sulfate.

Table 3-1. Current studies of human exposure to cobalt

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Occupational studies				
Mur *et al*. 1987	Retrospective cohort study *Cohort:* 1,143 workers employed for at least 1 year between 1950 and 1980 at an electrochemical factory producing cobalt, sodium, and other chemicals. Vital status was assessed in 1981, and cause of death was ascertained from physicians' records.	Cobalt was produced by etching of the roasted ore, followed by neutralization, filtration, and electrolysis of the cobalt chloride solution. The manufacturing process included production of cobalt oxides and salts. Exposure was defined by the worker's occupation.	SMR (95% CI); number of cases *Entire cohort:* all causes 0.8 (0.7–0.9); 213 all cancer 0.8 (0.6–1.1); 44 lung cancer 0.9 (0.4–1.6); 9 *Cobalt production workers:* all causes 1.3 (0.9–1.9); 28 all cancers 1.7 (0.8–3.1); 8 lung cancer 4.7 (1.5–10.6); 4 oral cancer 3.4 (0.3–10.3); 2 *Sodium production workers:* all causes 0.8 (0.6–1.0); 62 all cancer 0.7 (0.4–1.2); 13 lung cancer 0.7 (0.1–2.2); 2 *Maintenance workers:* all causes 0.8 (0.6–1.1); 38 all cancer 1.0 (0.5–1.8); 8 lung cancer 0.5 (0–2.6); 1	*Confounders and limitations:* (1) There was co-exposure to nickel and arsenic. (2) Smoking was ascertained for only 30% of cohort and was not considered. (3) 20% of deaths were due to unknown causes. (4) Vital status assessment for foreign-born individuals was poor. (5) The small number of exposed cases limited the study's power to detect an effect.

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Moulin *et al.* 1993 France	Retrospective cohort study, update of Mur *et al*. (1987) (reported in IARC 1991) *Cohort:* 1,143 workers employed at least 1 year between 1950 and 1980 at an electrochemical factory producing cobalt, sodium, and other chemicals. Vital status was assessed in 1988. The cause of death was ascertained from death certificates in the French national file for 1968 to 1988 and from physicians' records for 1950 to 1967. The cohort was divided into 2 subcohorts because of differences in overall mortality according to birthplace: *Cohort I:* all members, but limited to age groups ≤ 74 for calculation of person-years for those born abroad. *Cohort II:* limited to workers born in France.	Cobalt was produced by etching of the roasted ore, followed by neutralization, filtration, and electrolysis of the cobalt chloride solution. The manufacturing process included production of cobalt oxides and salts. Exposure was defined by the worker's occupation.	SMR (95% CI); number of cases *Cohort I:* all causes 0.9 (0.8–1.0); 309 all cancer 0.8 (0.7–1.0); 84 *Cohort II:* all causes 1.0 (0.8–1.1); 247 all cancer 1.0 (0.8–1.3); 72 *Lung cancer in cobalt production workers:* cohort I 0.9 (0.2–2.5); 3 cohort II 1.2 (0.2–3.4); 3 *Duration/time since first exposure in cobalt production:* Cobalt production workers: No trend of increased risk for increasing duration or time since first exposure; however, there were only 3 exposed cases. Maintenance workers: Risk increased with increasing time since first exposure. Risk was elevated (SMR > 2 for cohort I and > 3 for cohort II) and significant for longest duration (> 30 years) and time since first exposure (> 30 years) in both cohort I and II.	*Confounders and limitations:* (1) There was co-exposure to nickel and arsenic; maintenance workers may have been exposed to asbestos in sodium production areas. (2) Smoking was ascertained for only 30% of cohort and was not considered. (3) 11% of deaths were due to unknown causes. (4) Vital status assessment for foreign-born individuals was poor; the SMR for workers over 75 was low, so these age groups were excluded. (5) The small number of exposed cases limited the study's power to detect an effect; the small number of cases among exposed maintenance workers may have led to chance findings.

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Lasfargues *et al.* 1994 France	Cohort mortality study *Cohort:* all men (709) employed for at least 1 year between 1/1/1956 and 12/31/1989 at a French plant producing hard-metal tools. The plant consisted of two workshops (A and B). Workers in A had the highest exposure (powders mixing, pressing, and soft carbide machining); it opened in 1956, and preventive measures were taken between 1973 and 1976. Workers in B had lower exposure (maintenance, hard carbide machining); preventive measures were taken since its opening in 1974. Vital status was assessed on 1/1/1990, and cause of death was ascertained from physicians' records.	Exposure was defined by workers' job histories and periods of employment (to assess preventive measures); job histories before 1970 often were missing. Four degrees of cobalt exposure: (1) no exposure (2) low exposure: < 10 μg/m^3 in 8 h (3) medium exposure: 15–40 μg/m^3 in 8 h (4) high exposure: > 50 μg/m^3 in 8 h	SMR (95% CI); number of cases *Entire cohort:* all causes 1.1 (0.8–1.3); 75 all cancer 1.3 (0.8–1.8); 26 esophagus 1.9 (0.4–5.6); 3 leukemia 3.1 (0.4–11.1); 2 lung 2.1 (1.0–3.9); 10 *Degree of exposure:* all cancers: increased risk with increasing exposure lung cancer: no 1.5 (0.0–8.5); 1 low 0.9 (0.0–5.2); 0 medium 1.4 (0.3–4.2); 3 high 5.0 (1.9–11.0); 6 *Duration of employment and time since first exposure:* no increase in SMR for lung cancer *Smoking and exposure:* highest risk in smokers with medium (SMR = 9.2) and high exposure (SMR = 15.1); no risk for smokers with no or low exposure; comparison group was non-exposed individuals who had never smoked	*Confounders and limitations:* (1) Smoking was ascertained for 81% of the workers and 69% of the deceased; the proportion of smokers was similar to that in a sample of the French male adult population. (2) The expected number of deaths was calculated from national rates; local rates for lung cancer were available from 1971 to 1978 and were lower than national rates, so risks based on national rates are conservative. (3) Misclassification of exposure may have been most pronounced between medium and high exposure; some low exposure may have been classified as a higher exposure. (4) The small number of exposed cases limited the study's power to detect an effect.

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Moulin *et al.* 1998 France	Historical (mortality) cohort and nested case-control study *Cohort:* all male (5,777) and female (1,682) workers employed at least 3 months in any of 10 factories of the hard-metal industry from the time the factory opened until 12/31/1991. Other production activities at the factories included powder metallurgy processes. Mortality was followed from 1968 (or first date of employment) to 12/31/1991. Cause of death was ascertained from death certificates and medical records. *Cases:* 61 cohort workers who died of lung cancer. *Controls:* 180 living cohort members who were under follow-up on the date the case died and had completed 3 months of employment (3 per case).	Exposure to hard metal was assessed from a job-exposure matrix developed by a panel of experts. The matrix consisted of 320 job periods with assigned semiquantitative estimates of cobalt and tungsten carbide exposure. Exposure to other carcinogens (e.g., PAH, asbestos) was considered. Atmospheric concentrations of cobalt previously measured by plasma emission spectrometry were used to validate the job-exposure matrix.	*Cohort study:* SMR (95% CI); number of cases all causes 0.9 (0.9–1.0); 684 all cancer 1.1 (0.9–1.2); 247 lung 1.3 (1.0–1.7); 63 *Case-control study – lung cancer:* OR (95% CI) for cobalt-related exposures other cobalt 2.2 (1.0–4.9) "Other cobalt" exposure refers to exposure to cobalt alone or simultaneously with agents other than tungsten carbide. Simultaneous cobalt and tungsten carbide exposure level: levels 0 to 1 1.0 (ref.) levels 2 to 9 1.9 (1.0–3.6) *Exposure-response* (test for trend): duration: $P = 0.03$ unweighted cumulative exposure: $P = 0.01$ frequency-weighted cumulative exposure: $P = 0.08$ The unweighted cumulative exposure measure assigns the same value for occasional and full-time workers, thus favoring peak exposure, whereas the frequency-weighted measure reduces the effects of occasional exposures.	*Confounders and limitations:* (1) Healthy worker effect: there were fewer deaths than in the general population. (2) Adjusting for smoking (50 cases and 143 controls) increased the crude OR slightly and did not affect trend relationships; the sources of information on smoking were different for cases and controls. (3) Other carcinogens were present in the factories. (4) 1,131 subjects were lost to follow-up (875 born abroad), lowering the study's power to detect an effect. *Validation of exposure assessment:* Linear relationship between cobalt levels assigned with job-exposure matrix and log-transformed atmospheric cobalt measurement: short-duration area samples ($P < 0.0001$) long-duration area samples ($P = 0.015$) long-duration personal samples ($P = 0.015$).

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Wild *et al.* 2000 France	Cohort study *Cohort:* 2,860 subjects who had worked at a hard-metal production site (the largest site in the multicenter study of Moulin *et al.* 1998) for at least 3 months between 1/1/1950 and 6/30/1992, still alive on 1/1/1968, and with available work histories. 14 workshops at the plant, identified by type of production, were regrouped into the various stages related to hard-metal production (e.g. powder production, hard metal before sintering, hard metal after sintering, other alloy production, maintenance, and non-exposed workshops). Cause of death was ascertained from death certificates and physicians' records.	Exposure to cobalt, tungsten carbide, hard metal, and other carcinogens was assessed from an industry-specific job-exposure matrix (Moulin *et al.* 1998) implemented by a subgroup of the panel of experts. The matrix was validated by atmospheric measurements of cobalt.	SMR (95% CI), number of cases *Entire cohort of women:* all causes 1.3 (1.0–1.6); 68 all cancers 1.3 (0.8–1.9); 22 *Entire cohort of men:* all causes 1.0 (0.9–1.1); 331 all cancers 1.1 (0.9–1.3); 118 *Lung cancer:* job exposure matrix: cobalt, not hard metal 2.0 (1.1–3.2); 15 smoking 2.3 (1.5–3.2); 29 any IARC carcinogen 2.1 (1.3–3.0); 26 hard metal 2.0 (1.3–3.0); 26 workshops (only employed): non-exposed 1.0 (0.4–2.0); 7 hard metal/sintering before 2.9 (1.1–6.3); 6 after 1.1 (0.3–2.9); 3 powder production 1.4 (0.2–5.0); 2 maintenance 2.8 (1.3–5.4); 9 Poisson regression* (RR): IARC carcinogen 1.5 (0.8–2.7) smoking 1.6 (0.7–3.6) unsintered dust 1.4 (1.0–2.0) sintered dust 0.8 (0.4–1.5) *Model included smoking and exposure to IARC carcinogens (asbestos, PAH, silica, nickel, and chromium compounds), unsintered hard-metal dust, and sintered hard-metal dust	*Confounders and limitations:* (1) Local death rates were used as the mortality reference. (2) Smoking was assessed from occupational records and co-workers. (3) Exposure to other carcinogens was not assessed in the same degree of detail as exposure to hard metal, which may have resulted in misclassification; however, job turnover was low, so the hard-metal exposure probably was not confounded by other industrial processes. (4) 21% of male subjects were lost to follow-up.

Reference	Study design and population	Exposure	Effect: SMR, RR, or OR	Comments
Tüchsen *et al.* 1996 Denmark	Retrospective cohort study *Cohort:* all women employed at any time in the plate underglazing departments in two porcelain factories, Factory 1 (382 women from 1943) and Factory 2 (492 women from 1962), and a reference group from a cobalt-free department in Factory 1 (520 women); these workers decorated glazed porcelain with small amounts of dye in a dust-protected room. The cohort was followed until 1992; mortality was identified from the population register, and incident cancer cases (1943–1992) were identified from the cancer registry.	Cobalt silicate dye replaced cobalt-aluminate spinel dye in 1972 in Factory 1 and 1989 in Factory 2. Cobalt content in both dyes was 25%, and nickel content was less than 0.5%. Airborne cobalt exposure measured in 19 workers in 6/1981 exceeded hygienic standards by a factor of 1.3 to 172.	SIR (95% CI); number of cases *All cancers:* all exposed 1.2 (0.9–1.5); 67 referents 1.0 (NR); 60 *Lung cancer:* all exposed 2.4 (1.1–4.6); 8 factory 1: 1.6 (NR); 3 factory 2: 3.3 (NR*), 5 referents 2.0 (0.8-4.1); 7 Comparison between exposed and reference, RR = 1.2 (0.4–3.8) *lower limit of 95% CI was reported to be > 1.0. *Other cancers with elevated significant SIRs:* exposed: cervical cancer SIR = 2.3 (1.2–4.0); 12 reference: corpus uteri cancer SIR = 3.0 (1.4–5.7); 9	*Confounders and limitations:* (1) Smoking habits were available from two small surveys; Factory 1 may have had more smokers than the general population, but this was unlikely to explain the increased risk relative to the general population of women. (2) The small study population limited the study's power to detect an effect.

Reference	Study design and population	Exposure	Effect: SMR, RR or OR	Comments
Biomarker study				
Rogers *et al.* 1993 Washington State, USA	Population-based case-control study on cancer of the upper aerodigestive tract (1983–1987) *Cases:* 507 cases with aerodigestive tract cancers (153 laryngeal, 73 esophageal, and 281 oral cancer) identified by the local SEER cancer registry, with available nail samples. *Controls:* 434 controls identified by random-digit dialing and matched by gender and age, with available nail samples.	Cobalt exposure was determined from nail samples by neutron activation analysis; subjects were divided into strata: lowest 25% (< 0.05 ppm), mid 50% (0.05–0.17 ppm), and highest 25% (> 0.17 ppm). Other elements to which exposure was assessed were iron, calcium, zinc, and chromium.	Adjusted OR for cobalt (ppm) and cancer (95% CI) *Larynx:* < 0.05 1.0 (ref.) 0.05–0.17 2.0 (1.0–3.8) > 0.17 1.0 (0.4–2.6) *Esophagus:* < 0.05 1.0 (ref.) 0.05–0.17 2.4 (0.8–7.2) > 0.17 9.0 (2.7–30.0)* *test for trend, $P < 0.001$. *Oral cavity:* < 0.05 1.0 (ref.) 0.05–0.17 1.5 (0.9–2.6) > 0.17 1.9 (1.0–3.6) *Significant associations between other element levels (highest dose) in nails and esophageal cancer:* iron 2.9 (1.1–7.5) calcium 2.6 (1.0–7.1)	*Confounders and limitations:* (1) ORs were adjusted for age, sex, cigarette use, alcohol use, energy intake, β-carotene intake, and ascorbic acid intake. (2) Exposure was assessed after diagnosis of disease, but no significant differences were observed in ORs by stage or time from diagnosis to interview. (3) Elements (Co) are deposited in nails during formation of the nail matrix (8 months to 2 years depending on age), so element levels probably represent recent exposure in most cases.

[a]NR = not reported; RR = relative risk.

4 Studies of Cancer in Experimental Animals

In its evaluation of the carcinogenicity of cobalt and cobalt compounds, IARC (1991) found that several cobalt compounds induced sarcomas at injection sites in animals. The limitations of the animal studies available to IARC for review were that they all were either injection or implantation studies and did not adequately evaluate the potential carcinogenicity of cobalt and cobalt compounds by other routes of exposure. After publication of the IARC monograph, the NTP (1998) completed a two-year inhalation carcinogenicity study of cobalt sulfate heptahydrate with $B6C3F_1$ mice and F344/N rats. Results are reported separately below for mice (Section 4.1) and rats (Section 4.2).

4.1 NTP carcinogenicity bioassay in mice

Groups of six-week-old $B6C3F_1$ mice (50 of each sex) were administered cobalt sulfate heptahydrate aerosols by inhalation at target concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3, 6 h/day, 3 days/week, for 105 weeks (NTP 1998, Bucher *et al.* 1999). The corresponding concentrations expressed as elemental cobalt were 0, 0.063 mg/m^3, 0.210 mg/m^3, and 0.628 mg/m^3. Exposure concentrations were based on previous subacute and subchronic studies (Bucher *et al.* 1990, NTP 1991). Cobalt sulfate heptahydrate was generated and delivered from an aqueous solution via a compressed-air-driven nebulizer, an aerosol charge neutralizer, and an aerosol distribution system. The aerosol was dried and mixed with humidified air before delivery to the inhalation chambers, thus allowing partial rehydration of the aerosol particles. The mass median aerosol particle diameter was 1 to 3 µm, and the aerosol consisted of 1 mole of cobalt, 1 mole of sulfate, and 5.9 moles of water per mole of aerosolized cobalt sulfate (Bucher *et al.* 1999). The overall chemical purity of the study material was reported to be 99%. Survival was not significantly affected by exposure (see Appendix B, pp. B-33 to B-34, Table 8 and Figure 3 in NTP 1998). Mean body weights were slightly higher in exposed females than in controls, and mean body weights were lower in the high-dose males than in controls from week 96 to the end of the study (see Appendix B, pp. B-35 to B-37, Figure 4 and Tables 9 and 10 in NTP 1998).

The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) showed a positive exposure-response trend in all groups. The incidences of these neoplasms were significantly higher in all the high-dose groups than in the controls, as was the incidence of adenoma or carcinoma (combined) in mid-dose female mice (Table 4-1). The NTP (1998) concluded that there was clear evidence of carcinogenic activity in both male and female mice, based on increased incidences of lung tumors.

Although the incidence of hemangiosarcoma was significantly increased in male mice in the mid-dose group (Table 4-1), *Helicobacter hepaticus* infection was present in these mice, making interpretation of this finding difficult. Liver sections from several male mice were positive for bacteria, and the spectrum of liver lesions in these mice was consistent with *H. hepaticus* infection.

Table 4-1. Tumor incidence in B6C3F$_1$ mice following inhalation exposure to cobalt sulfate heptahydrate for 105 weeks

Sex	Exposure conc. (mg/m^3)	Tumor incidence[a] (%)[b]			
		Alveolar/bronchiolar			Liver
		Adenoma	Carcinoma	Combined	Hemangiosarcoma
Male	0	9 (30.4%)	4 (13.2%)	11 (35.5%)	2 (9.1%)
	0.3	12 (30.9%)	5 (16.1%)	14 (36.5%)	4 (11.5%)
	1.0	13 (41.1%)	7 (25.3%)	19 (56.5%)	8 (23.5%)*[d]
	3.0	18 (54.6%)*	11 (43.7%)*	28 (78.8%)***	7 (25.0%)
	Trend[c]	$P = 0.018$	$P = 0.006$	$P < 0.001$	$P = 0.078$
Female	0	3 (8.8%)	1 (2.9%)	4 (11.8%)	1 (2.9%)
	0.3	6 (15.0%)	1 (2.7%)	7 (17.5%)	0
	1.0	9 (25.2%)	4 (9.2%)	13 (32.6%)*	3 (7.3%)
	3.0	10 (32.8%)*	9 (25.3%)**	18 (50.2%)***	0[e]
	Trend[c]	$P = 0.024$	$P < 0.001$	$P < 0.001$	$P = 0.431$N

Source: NTP 1998, Bucher *et al.* 1999.

*$P \leq 0.05$, **$P < 0.01$, ***$P<0.001$ (logistic regression test).

[a]The number of animals with the neoplasm, out of 50 animals per group unless otherwise noted.

[b]Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[c]Logistic regression test; lower incidence in an exposure group is indicated by N.

[d]Results were confounded by *H. hepaticus* infection.

[e]49 animals in the group.

In addition to the neoplastic lesions, exposure to cobalt sulfate induced a spectrum of inflammatory, fibrotic, and proliferative lesions in other portions of the respiratory tract that were consistent with results observed in the shorter-term studies (Table 4-2). These included hyperplasia of the olfactory epithelium (high-dose groups), squamous metaplasia of the larynx (all exposed groups), cytoplasmic vacuolization of the bronchi (all exposed groups), diffuse histiocytic cell infiltration (high-dose males), and focal histiocytic cell infiltration of the lung (high-dose females). Histiocytic infiltration was observed most often in lungs with alveolar/bronchiolar neoplasms and was attributed to the neoplasms, rather than to a direct effect of cobalt sulfate.

Table 4-2. Incidences and severity of nonneoplastic lesions in B6C3F$_1$ mice following inhalation exposure to cobalt sulfate heptahydrate for 105 weeks

	Incidence[a] (severity)[b]			
Exposure concentration:	**Controls**	**0.3 mg/m^3**	**1.0 mg/m^3**	**3.0 mg/m^3**
Male				
Lung				
Diffuse histiocytic cellular infiltration	1 (3.0)	2 (3.0)	4 (2.3)	10** (1.5)
Focal histiocytic cellular infiltration	10 (2.7)	5 (2.6)	8 (3.0)	17 (2.7)
Bronchiolar cytoplasmic vacuolization	0	18** (1.0)	34** (1.0)	38** (1.0)
Larynx				
Squamous metaplasia	0[c]	37**[d] (1.0)	48**[c] (1.0)	44**[d] (1.0)
Nose				
Atrophy of olfactory epithelium	0	0	29**[c] (1.2)	48**[d] (1.8)
Hyperplasia of olfactory epithelium	0	0	0[c]	10**[d] (1.0)
Suppurative inflammation	0	1 (3.0)	0[c]	6*[d] (2.2)
Female				
Lung				
Diffuse histiocytic cellular infiltration	0	0	0	4 (3.3)
Focal histiocytic cellular infiltration	2 (2.0)	5 (1.8)	7 (2.9)	10* (2.4)
Bronchiolar cytoplasmic vacuolization	0	6* (1.0)	31** (1.0)	43** (1.0)
Larynx				
Squamous metaplasia	0	45**[d](1.0)	40**[e] (1.0)	50** (1.1)
Nose				
Atrophy of olfactory epithelium	0	2 (1.5)	12**[d] (1.0)	46**[c] (1.5)
Hyperplasia of olfactory epithelium	0	0	0[d]	30**[c] (1.3)
Suppurative inflammation	0	1 (1.0)	5*[d] (1.6)	4[c] (1.5)

Source: NTP 1998.

*$P \leq 0.05$, **$P < 0.01$ (logistic regression test).

[a]The number of animals with the lesion, out of 50 animals per group unless otherwise noted.

[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

[c]48 animals examined.

[d]49 animals examined.

[e]47 animals examined.

4.2 NTP carcinogenicity bioassay in rats

Groups of six-week-old F344/N rats (50 of each sex) were administered cobalt sulfate heptahydrate aerosols by inhalation at target concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3, 6 h/day, 5 days/week, for 105 weeks (NTP 1998, Bucher *et al.* 1999). Exposure concentrations were based on previous subacute and subchronic studies (Bucher *et al.* 1990, NTP 1991). Survival of exposed rats did not differ significantly from that of controls. Among males, survival was 34%, 30%, 42%, and 30% in the control, low-exposure, mid-exposure, and high-exposure groups, respectively. Overall, survival was higher in females than in males, at 56%, 51%, 52%, and 60% in the control, low-exposure, mid-exposure, and high-exposure groups, respectively (see Appendix B, pp. B-21 and B-22, Table 2, and Figure 1 in NTP 1998). Mean body weights in all exposed groups did not differ significantly from those of controls throughout the study (see Appendix B, pp. B-23 to B-25, Tables 3 and 4, and Figure 2 in NTP 1998).

The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) showed a significant positive exposure-related trend in male rats and was significantly higher in the high-dose group than in the control group. A significant positive exposure-related trend for alveolar adenoma, carcinoma, and adenoma or carcinoma (combined) was observed in female rats, and incidences were significantly higher in the mid-dose and high-dose groups than in the controls (Table 4-3). In addition, squamous-cell carcinoma of the lung was observed in two female rats (one each in the mid-dose and high-dose groups). The incidence of benign adrenal pheochromocytoma was increased in high-dose females, and the incidence of benign, complex, or malignant pheochromocytoma (combined) was increased in mid-dose males and high-dose females (Table 4-3). The increased incidences in the high-dose females were considered to be exposure related. The NTP (1998) concluded that there was some evidence of carcinogenicity in male rats, based on increased incidences of alveolar/bronchiolar neoplasms. Marginal increases in adrenal medullary tumors in male rats may have been related to exposure to cobalt sulfate heptahydrate. There was clear evidence of carcinogenicity in female rats, based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytoma of the adrenal medulla.

Table 4-3. Tumor incidence in F344/N rats following inhalation exposure to cobalt sulfate heptahydrate for 105 weeks

Sex	Exposure conc. (mg/m^3)	Tumor incidence[a] (%)[b]				
		Alveolar/bronchiolar			Adrenal medulla	
		Adenoma	Carcinoma	Combined	Benign[c]	Total
Male	0	1 (2.3%)	0	1 (2.3%)	14 (51.0%)	15 (52.1%)
	0.3	4 (17.7%)	0	4 (17.7%)	19 (70.0%)	19 (70.0%)
	1.0	1[e] (2.4%)	3[e] (11.3%)	4[e] (13.4%)	23[f] (71.9%)	25[f] (74.1%)*
	3.0	6 (28.4%)	1 (6.7%)	7 (33.9%)*	20 (71.4%)	20 (71.4%)
	Trend[d]	*P* = 0.051	*P* = 0.360	*P* = 0.032	*P* = 0.172	*P* = 0.218
Female	0	0	0	0	2[e] (5.1%)	2[e] (5.1%)
	0.3	1[f] (3.4%)	2[f] (8.0%)	3[f] (11.2%)	1[f] (3.1%)	1[f] (3.1%)
	1.0	10 (36.4%)***	6(20.2%)*	15 (50.6%)***	3 (9.3%)	4 (11.7%)
	3.0	9 (30%)**	6 (17.5%)*	15 (46.1%)***	8[e] (26.4%)*	10[e] (31.5%)*
	Trend[d]	*P* = 0.001	*P* = 0.023	*P* < 0.001	*P* = 0.004	*P* < 0.001

Source: NTP 1998, Bucher *et al.* 1999.

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$ (logistic regression test).

[a]The number of animals with the neoplasm, out of 50 animals per group unless otherwise noted.

[b]Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[c]Pheochromocytoma.

[d]Logistic regression test.

[e]48 animals in the group.

[f]49 animals in the group.

Nonneoplastic lesions of the respiratory tract generally were more severe in rats than in mice (see Section 4.1). Significantly increased incidences of inflammatory, fibrotic, and proliferative lesions were observed in all dose groups in the lung (hyperplasia and metaplasia of the alveolar epithelium, granulomatous inflammation, interstitial fibrosis, and proteinosis), nose (lateral wall hyperplasia and olfactory epithelium atrophy), and larynx (squamous metaplasia of the epiglottis) (Table 4-4). The NTP characterized all fibroproliferative lesions as atypical hyperplasia. Several animals had malignant neoplasms with a very prominent fibrous component, some of which presumably had progressed from atypical hyperplasia. The NTP (1998) concluded that it was clear that all the morphologic variants of proliferative lesions represented a response to cobalt sulfate heptahydrate.

Table 4-4. Incidences and severity of nonneoplastic lesions in F344/N rats following inhalation exposure to cobalt sulfate heptahydrate for 105 weeks

	Incidence[a] (severity)[b]			
Exposure concentration:	**Controls**	**0.3 mg/m^3**	**1.0 mg/m^3**	**3.0 mg/m^3**
Male				
Lung				
Alveolar epithelial hyperplasia	9 (1.8)	20* (2.0)	20*[c] (2.1)	23** (2.0)
Alveolar epithelial metaplasia	0	50** (1.9)	48**[c] (3.1)	49** (3.7)
Granulomatous inflammation	2 (1.0)	50** (1.9)	48**[c] (3.1)	50** (3.7)
Interstitial fibrosis	1 (1.0)	50** (1.9)	48**[c] (3.1)	49** (3.7)
Proteinosis	0	16** (1.4)	40**[c] (2.3)	47** (3.4)
Larynx				
Squamous metaplasia of epiglottis	0	10**[d] (1.3)	37**[c] (1.8)	50** (2.8)
Nose				
Hyperplasia of lateral wall	2 (1.5)	14** (1.4)	21**[d] (1.5)	20** (1.6)
Squamous metaplasia of lateral wall	1 (1.0)	3 (1.3)	5[d] (1.4)	8* (2.0)
Atrophy of olfactory epithelium	8 (1.1)	24** (1.4)	42**[d] (1.5)	48** (2.5)
Metaplasia of olfactory epithelium	5 (1.2)	1 (3.0)	5[d] (1.8)	30** (1.9)
Female				
Lung				
Alveolar epithelial hyperplasia	15 (1.4)	7[d] (1.6)	20 (1.8)	33** (2.0)
Alveolar epithelial metaplasia	2 (1.0)	47**[d] (2.0)	50** (3.6)	49** (3.9)
Granulomatous inflammation	9 (1.0)	47**[d] (2.0)	50** (3.6)	49** (3.9)
Interstitial fibrosis	7 (1.0)	47**[d] (2.0)	50** (3.6)	49** (3.9)
Proteinosis	0	36**[d] (1.2)	49** (2.8)	49** (3.9)
Larynx				
Squamous metaplasia of epiglottis	1 (1.0)	22**[d] (1.1)	39** (1.4)	48** (2.6)
Nose				
Hyperplasia of lateral wall	1 (1.0)	8*[d] (1.3)	26** (1.4)	38** (1.7)
Squamous metaplasia of lateral wall	1 (1.0)	1[d] (3.0)	4 (1.3)	10** (1.4)
Atrophy of olfactory epithelium	5 (1.4)	29**[d] (1.2)	46** (1.6)	47** (2.9)
Metaplasia of olfactory epithelium	2 (2.0)	2[d] (1.5)	3 (1.7)	40** (2.3)

Source: NTP 1998.

*$P \leq 0.05$, **$P < 0.01$ (logistic regression test).

[a]The number of animals with the lesion, out of 50 animals per group unless otherwise noted.

[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

[c]48 animals examined.

[d]49 animals examined.

4.3 Summary

IARC (1991) concluded that there was sufficient evidence for the carcinogenicity of cobalt metal powder and cobalt(II) oxide and limited evidence for the carcinogenicity of metal alloys containing cobalt, cobalt(II) sulfide, and cobalt(II) chloride in experimental animals when exposure was by injection or implantation. However, evidence for the carcinogenicity of cobalt and cobalt compounds in experimental animals by other routes of administration were not available at that time. In a subsequent study, cobalt sulfate heptahydrate was found to be carcinogenic in B6C3F_1 mice and F344/N rats when administered by inhalation. There was clear evidence of carcinogenicity in male mice, female mice, and female rats, based on increased incidences of lung tumors. In addition, female rats had an increased incidence of pheochromocytoma of the adrenal medulla. Some evidence of carcinogenicity in male rats was described, based on increased incidences of lung tumors at the highest exposure level.

5 Genotoxicity

IARC (1991) reviewed the genotoxicity of cobalt and cobalt compounds. Although many studies investigated the genotoxicity of soluble cobalt(II) salts (e.g., cobalt chloride, cobalt acetate, and cobalt nitrate), none of them specifically addressed cobalt sulfate. In general, cobalt(II) compounds were not genotoxic in bacteria but induced DNA damage, mutations, sister chromatid exchange (SCE), and aneuploidy in some *in vitro* tests with animal and human cells (see Appendix A, pp. A-78 to A-80, Table 21 in IARC 1991). In addition, chlorophyll mutations, chromosomal aberrations, and aneuploidy were induced in plant cells.

Léonard and Lauwerys (1990) reviewed the mutagenicity, carcinogenicity, and teratogenicity of cobalt metal and cobalt compounds and concluded that cobalt and cobalt compounds were only weakly mutagenic. In another review, Beyersmann and Hartwig (1992) noted that the cobalt(II) ion is relatively inactive in prokaryotic systems, as are other metallic ions. Factors potentially contributing to this inactivity include precipitation of phosphates in bacterial media, a low rate of uptake or indirect mechanisms of interaction with DNA, and trapping of metal ions by proteins present in exogenous metabolic activating systems. Nevertheless, these authors concluded the following: (1) cobalt(II) salts generally are nonmutagenic in prokaryotic assays and were antimutagenic in some studies, (2) cobalt chloride is mutagenic to mitochondrial genes but only weakly mutagenic or nonmutagenic to chromosomal genes in *Saccharomyces cerevisiae*, (3) cobalt(II) salts induce gene mutations and chromosomal aberrations in plants, (4) cobalt(II) compounds cause DNA strand breaks, SCE, and aneuploidy in mammalian cells *in vitro*, and (5) cobalt(II) salts are comutagenic with ultraviolet light but not with gamma rays in mammalian cells.

5.1 Prokaryotic systems

Zeiger *et al.* (1992) presented the results of *Salmonella typhimurium* mutagenicity tests for 311 chemicals tested within the NTP's mutagenicity testing program. *S. typhimurium* strains TA98, TA100, and TA1535 were used with and without rat or hamster S9 metabolic activation. Each trial included triplicate plates of concurrent positive and negative controls and five exposure levels (between 10 and 10,000 µg/plate) of a test chemical. A positive response was defined as a reproducible, dose-related increase in revertant colonies in any one strain/activation combination. Cobalt sulfate heptahydrate was mutagenic in strain TA100 without metabolic activation and with either 5% hamster or rat liver S9. It was not mutagenic in strains TA98 or TA1535 with or without metabolic activation (NTP 1998).

5.2 Mammalian systems

5.2.1 Rodent cells

Kerckaert *et al.* (1996a) tested cobalt sulfate hydrate and other metal compounds in the Syrian hamster embryo (SHE) cell transformation assay. These authors noted that for heavy metals and heavy-metal compounds, the SHE transformation assay was a better predictor of rodent carcinogenicity than the *Salmonella* assay; concordance with the rodent bioassay was 92% for the SHE assay but only 33% for the *Salmonella* assay.

Cobalt sulfate hydrate caused a significant increase in SHE cell transformation at all five exposure levels tested (0.125 to 1 µg/mL) within 24 hours; however, no significant exposure-response trend was found. Nevertheless, the authors considered the results to be positive because significant cell transformation was observed for at least two exposure levels.

Gibson *et al.* (1997) tested 16 chemicals, including cobalt sulfate hydrate, in the SHE micronucleus assay. Cobalt sulfate hydrate was tested at 1.0, 2.0, and 4.0 µg/mL. All exposure levels significantly increased the percentage of micronucleated binucleated cells (MNBC) (Table 5-1).

Table 5-1. Effects of cobalt sulfate hydrate on micronucleus formation in SHE cells

Exposure level (µg/mL)	Relative cell number	Binucleated cells (%)	MNBC (%)	Fisher's exact *P* value
Control	100	42	25/1000 (2.5)	–
1.0	190	45	43/1000 (4.3)	0.0176
2.0	219	38	47/1000 (4.7)	0.0056
4.0	159	34	63/1000 (6.3)	< 0.001

Adapted from Gibson *et al.* 1997.

Cellular levels of the tumor-suppressor protein p53 increase following DNA damage. Therefore, Duerksen-Hughes *et al.* (1999) developed and tested a mammalian *in vitro* assay for genotoxicity based on p53 induction. NCTC 929 cells derived from mouse fibroblasts were exposed to 25 test chemicals being tested by the NTP for carcinogenicity in rodents. Cultured cells were exposed to cobalt sulfate heptahydrate at a concentration of 1, 10, 20, 50, or 100 µg/mL. Control plates were exposed to the vehicle alone (culture medium or dimethylsulfoxide [DMSO]). Cells were incubated at 37ºC and harvested at 6 hours (first series) or 17 hours (second series) post-treatment. Cobalt sulfate heptahydrate strongly induced p53 in NCTC 929 cells exposed at 50 or 100 µg/mL for 6 hours or at 20 or 50 µg/mL for 17 hours. A concentration of 100 µg/mL was cytotoxic to cells exposed for 17 hours.

Lloyd *et al.* (1997, 1998) investigated the generation of putative intrastrand cross-links, formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), and single- and double-strand breaks in DNA by Fenton-type reactions. In both studies, DNA was exposed to hydrogen peroxide at a concentration of 50 mM and to various transition-metal ions, including cobalt sulfate. In the first report (Lloyd *et al.* 1997), salmon sperm DNA exposed to cobalt sulfate developed putative intrastrand cross-links in a dose-dependent manner at concentrations of up to 1 mM cobalt. Six radioactive spots detected by thin-layer chromatography were thought to be hydroxyl radical–mediated oxidative DNA lesions; however, no DNA strand breaks were detected. The authors tentatively identified two of the adducts as products of a reaction between the metal ion and the purine dimers 2'-deoxyadenylyl-(3'-5')-2'-deoxyadenosine and 2'-deoxyadenylyl-(3'-5')-2'-deoxyguanosine,

which they interpreted as consistent with the formation of intrastrand cross-links. In the latter study (Lloyd *et al.* 1998), a more sensitive method for detecting DNA strand breaks was used. Double-stranded plasmid pBluescript K+ DNA was incubated with 1 mM hydrogen peroxide and each transition-metal ion for 15 minutes. No significant formation of 8-OHdG adducts was detected after incubation with cobalt sulfate; however, single-strand, but not double-strand, breaks were detected.

5.2.2 Human cells

Kawanishi *et al.* (1989) incubated ^{32}P-labeled DNA fragments obtained from human c-Ha-*ras*-1 protooncogene with 1 mM sodium sulfite and 20 µM cobalt(II) ion. Sulfite caused DNA damage in the presence of cobalt(II) and other metal ions; however, sulfite alone or metal ion alone did not cause damage. DNA damage was much greater in the presence of cobalt than with copper, manganese, or iron. Treatment with 3,5-dibromo-4-nitrobenzenesulfonate or primary or secondary alcohols inhibited DNA damage by sulfite plus cobalt(II), whereas treatment with superoxide dismutase, catalase, or tert-butyl alcohol did not. The authors noted that primary and secondary alcohols react readily with sulfate radicals but not sulfite radicals and that sulfate radicals react slowly with tert-butyl alcohol. They concluded that the DNA damage was caused by autooxidation of sulfite to the sulfate radical in the presence of cobalt(II).

Nackerdien *et al.* (1991) investigated the ability of mixtures of cobalt(II) and hydrogen peroxide to cause chemical changes in DNA bases in chromatin isolated from human K562 cells. Reaction mixtures consisted of chromatin (0.12 mg DNA/mL) alone, chromatin plus cobalt sulfate (25 µM), chromatin plus hydrogen peroxide (2.8 mM), and chromatin plus cobalt sulfate and hydrogen peroxide. In addition, the effects of adding ethylenediaminetetraacetic acid (EDTA) (120 µM), ascorbic acid (100 µM), glutathione (1 mM), mannitol (50 mM), DMSO (50 mM), or superoxide dismutase (200 units/mL) to the reaction mixture were measured. Yields of DNA base products were not increased in chromatin exposed to cobalt sulfate only or hydrogen peroxide only; however, yields of all base products were increased 2- to 18-fold in chromatin exposed to both cobalt sulfate and hydrogen peroxide for one hour. The major products included cytosine glycol, formamidopyrimidines, and 8-hydroxypurines. Addition of ascorbic acid had no effect, whereas addition of the hydroxyl radical scavengers mannitol and DMSO or chelation with EDTA inhibited product formation. Results for glutathione were mixed; yields of some products decreased moderately, while yields of others increased twofold. Superoxide dismutase increased product yields. The authors concluded that DNA damage in chromatin caused by cobalt ions in the presence of hydrogen peroxide might contribute to genotoxicity and carcinogenicity.

Olivero *et al.* (1995) compared the genotoxicity of cobalt chloride, cobalt sulfate heptahydrate, and cobalt nitrate hexahydrate in cultured human lymphocytes. The mitotic index, chromosomal aberrations, and micronuclei were measured in whole-blood samples obtained from a single healthy donor. Exposure to any of the three cobalt salts resulted in a dose-related decrease in the mitotic index; however, micronuclei increased significantly only in cells exposed to cobalt chloride. None of the cobalt salts increased the frequency of chromosomal aberrations. Results are summarized in Table 5-2.

Table 5-2. Genotoxic effects of cobalt chloride, cobalt nitrate hexahydrate, and cobalt sulfate heptahydrate in human lymphocytes

Compound	Conc. (µg/mL)	Conc. (mM)	Mitotic index	Micro-nucleated cells (%)	Aneuploidy (%)	Total structural aberrations (%)
Cobalt chloride	0	0	3.6	10	0	7
	0.0045	0.035	3.6	23*	0	11
	0.023	0.177	2.7	24*	1	12
	0.045	0.347	2.2	23*	2	12
	0.23	1.771	2.3	25**	2	14
	0.45	3.466	1.0	23*	0	8
Cobalt nitrate hexahydrate	0	0	3.5	5	0	16
	0.0045	0.015	3.4	4	0	10
	0.045	0.155	2.1	8	0	12
	0.45	1.546	1.2	8	0	16
Cobalt sulfate heptahydrate	0	0	3.5	5	1	8
	0.0045	0.016	2.4	1	2	8
	0.045	0.160	2.3	8	1	7
	0.45	1.601	1.3	7	1	9

Adapted from Olivero *et al.* 1995.

*$P < 0.05$, **$P \leq 0.01$ (chi-square test).

5.3 Summary

The genotoxicity of cobalt sulfate has been studied less extensively than that of other cobalt salts, especially cobalt chloride. There is evidence that the genotoxicity of cobalt compounds depends on the ligand coordinated about the metal ion. Overall, the data suggest that cobalt salts generally are not mutagenic in bacterial test systems. In one study, cobalt sulfate was mutagenic in *S. typhimurium* strain TA100 but not in strains TA98 or TA1535. Cobalt sulfate induced cell transformation and micronuclei in SHE cells and strongly induced p53 in mouse fibroblasts. In the presence of hydrogen peroxide, cobalt sulfate induced putative intrastrand cross-links in salmon sperm DNA and single-strand breaks in plasmid pBluescript K+ DNA. However, 8-OHdG adducts were not induced in salmon sperm DNA. Sulfite in the presence of cobalt ions caused damage in DNA fragments derived from the human c-Ha-*ras*-1 protooncogene. Yields of DNA base products in human chromatin were increased by exposure to cobalt sulfate in the presence of hydrogen peroxide. In a study of three cobalt salts, cobalt sulfate was not genotoxic to human lymphocytes.

6 Other Relevant Data

IARC (1991) reviewed the carcinogenicity of cobalt and cobalt compounds. Although very little information specific to cobalt sulfate was presented in the IARC monograph, general information on cobalt(II) was considered relevant to the potential carcinogenicity of cobalt sulfate. IARC (1991) reached the following conclusions:

- There was sufficient evidence for the carcinogenicity of cobalt metal powder and cobalt(II) oxide in experimental animals.
- There was limited evidence for the carcinogenicity of metal alloys containing cobalt, chromium, and molybdenum and of cobalt(II) sulfide and cobalt(II) chloride in experimental animals.
- There was inadequate evidence for the carcinogenicity of cobalt-aluminium-chromium spinel, cobalt(II,III) oxide, cobalt naphthenate, and cobalt(III) acetate in experimental animals.
- There was inadequate evidence for the carcinogenicity of cobalt and cobalt compounds in humans.

This section summarizes the toxicity, toxicokinetics, and possible mechanisms of carcinogenesis of cobalt sulfate and similar cobalt compounds.

6.1 Toxicity of cobalt sulfate

As a component of vitamin B_{12}, cobalt is an essential nutrient in humans. No other physiological function of cobalt has been identified. A daily intake of about 50 µg of cobalt, with about 80% (40 µg) as vitamin B_{12}, is sufficient to meet the nutritional requirement (Léonard and Lauwerys 1990). However, excessive exposure to cobalt can result in many adverse effects. Cobalt can replace other essential divalent cations, such as magnesium and calcium ions; bind to sulfhydryl groups; inhibit heme synthesis; and reduce cytochrome P450 concentrations (Bucher *et al.* 1999).

The oral 50% lethal dose (LD_{50}) of various inorganic cobalt(II) compounds in rats ranges from about 150 to 500 mg/kg body weight (b.w.) For cobalt sulfate, the oral LD_{50} is 424 mg/kg b.w. in rats and 584 mg/kg b.w. in mice. Acute effects in animals include sedation, diarrhea, weight loss, tremor, and convulsions (IARC 1991, RTECS 2001). Rats and mice exposed to cobalt sulfate heptahydrate aerosols for 13 weeks at 0.3 to 30 mg/m^3 developed lesions in the respiratory tract, which included degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, inflammation in the nose, epithelial hyperplasia in the alveoli, squamous metaplasia of the larynx, and other effects. In addition, polycythemia occurred in rats, and reproductive effects (e.g., abnormal sperm, decreased sperm motility, and decreased testis and epididymal weights) occurred in mice. Two of the 10 male mice exposed to the highest concentration died during the study (Bucher *et al.* 1990).

In humans, hard-metal pneumoconiosis and occupational asthma are considered the primary effects of occupational exposure to cobalt-containing dust. Hard-metal pneumoconiosis is a severe and progressive disease marked by interstitial fibrosis that may develop after a few months to several years of exposure to dust containing cobalt and other metals (e.g., titanium and tantalum) or tungsten carbide. Cobalt hypersensitivity has been associated with hard-metal asthma and allergic dermatitis in workers. In the 1960s, several outbreaks of cardiomyopathy, with mortality rates as high as 50%, were reported in individuals who drank large quantities of cobalt-fortified beer. At that time, cobalt sulfate, cobalt acetate, or cobalt chloride was added to some beers as a foaming agent. Polycythemia also was reported in some beer drinkers. Although the cobalt intake by the beer drinkers was a few milligrams per day, which is much higher than normal daily intakes, the exposure was much lower than the 25 to 300 mg/day once used to treat patients with anemia (IARC 1991). Therefore, the beer-drinkers' cardiomyopathy may have resulted from a synergistic effect with alcohol and poor nutrition (Lauwerys and Lison 1994).

6.2 Mammalian absorption, distribution, metabolism, and excretion of cobalt

Cobalt is absorbed from the gastrointestinal tract, lungs, and skin. Normal levels in blood and urine in the general population are 0.2 to 2 µg/L, but concentrations greater than 200 µg/L have been reported in the urine of workers occupationally exposed to cobalt (IARC 1991, NTP 1998). Gastrointestinal tract absorption is highly variable depending on the compound, concentration, and other factors, but is estimated to range from 5% to 45% (Lauwerys and Lison 1994) and may be higher in females than in males (Christensen and Poulsen 1994). There is evidence that iron and cobalt share the same transport mechanism in the duodenum (Léonard and Lauwerys 1990). The degree of respiratory absorption in humans is unknown but varies with concentration. Some studies have shown a good correlation between concentrations in air and concentrations in urine of workers (Christensen and Poulsen 1994). Respiratory absorption of cobalt inhaled as cobalt oxide was about 30% (Lauwerys and Lison 1994). Scansetti *et al.* (1994) demonstrated substantial absorption of cobalt through the skin.

Once absorbed, cobalt is preferentially distributed to the liver, kidney, and heart (Léonard and Lauwerys 1990, Christensen and Poulsen 1994). Without occupational exposure, the cobalt content in the adult human body is about 1 to 2 mg. The cobalt content of bone and muscle account for 14% and 13%, respectively, of the total body burden, with the rest occurring in soft tissues (Léonard and Lauwerys 1990, IARC 1991). The highest cobalt concentrations are in the liver, because vitamin B_{12} is stored there; IARC (1991) reported that the cobalt concentration in the liver at autopsy ranged from 6 to 151 µg/kg, with a median value of 30 µg/kg. Patients dying of cardiomyopathy from excessive intake of cobalt-fortified beer had 10 times the normal amount of cobalt in the heart (IARC 1991).

Concentrations of arsenic and cobalt were evaluated in tissue and plasma of patients with laryngeal carcinoma (Collecchi *et al.* 1986). Plasma and histologically nonmalignant and malignant laryngeal tissues were obtained from each of 15 male patients with no known exposure to toxic amounts of cobalt. The cobalt concentrations in malignant laryngeal tissue (68.7 ± 7.3 ng/g dry weight, mean ± SD) were significantly higher ($P < 0.01$,

paired *t*-test and Wilcoxon's test) than those in nonmalignant laryngeal tissue (39.6 ± 7.0). The plasma cobalt concentrations were 25-fold higher in the 15 patients with laryngeal carcinoma than in 11 apparently normal male individuals (18.27 ± 2.10 and 0.73 ± 0.10 ng/mL, respectively; $P < 0.001$, Student's *t*-test and Mann-Whitney U-test). Similar significant differences were reported for plasma and tissue arsenic levels. The authors reported that further studies were in progress to ascertain the clinical significance of the changes in tissue and plasma cobalt and arsenic concentrations; however, no additional publications on this subject were identified in a search of the literature since 1986.

Cobalt is excreted in the urine and, to a lesser degree, in the feces. In experimental animals, 70% or more is eliminated in the urine (IARC 1991). In humans, 28% to 56% of radiolabelled cobalt chloride was eliminated in the urine and 2% to 12% in the feces within eight days after parental administration. Between 9% and 16% of the administered dose was eliminated very slowly, with a biological half-life of about two years (Smith *et al.* 1972). Thus, cobalt excretion has two distinct phases: a rapid initial phase, with a half-life of a few days, followed by a slow second phase, with a half-life of a year or more (Léonard and Lauwerys 1990, Lauwerys and Lison 1994). Cobalt concentrations in the urine of workers in the Italian hard-metal industry were 10 to 100 μg/L at the beginning of the work shift, increasing to 16 to 210 μg/L at the end of the shift (Sabbioni *et al.* 1994). Clearance from the lungs has not been studied but is expected to be rapid for soluble cobalt salts (NTP 1998).

6.3 Syrian hamster embryo cell transformation assay

Kerckaert *et al*. (1996a, b) tested five heavy-metal compounds (cobalt sulfate hydrate, gallium arsenide, molybdenum trioxide, vanadium pentoxide, and nickel sulfate heptahydrate) in the SHE cell transformation assay. The cobalt compound induced morphological transformation in a 24-hour exposure at five concentrations from 0.125 to 1 μg/mL ($P < 0.05$, Fisher's exact test); the highest concentration caused 66% cytotoxicity. The exposure-response trend test was not significant ($P = 0.0739$, unstratified binomial exact permutation trend test); however, the authors concluded that the overall SHE assay results were positive, based on significant results for at least two concentrations.

Positive results ($P < 0.05$, Fisher's exact test) also were reported for nickel sulfate heptahydrate at concentrations of 20 to 50 μg/mL in a 24-hour exposure (Kerckaert *et al.* 1996a).

6.4 Possible mechanisms of cobalt-induced carcinogenesis

The mechanisms of cobalt-induced carcinogenesis are not well understood. IARC (1991) did not address the possible mechanism(s) for carcinogenicity of cobalt ions beyond proposing that cobalt(II) ions could decrease the fidelity of DNA polymerase and could damage DNA through generation of reactive oxygen species, to explain the genotoxicity of cobalt compounds.

Lison *et al.* (2001) published an updated review of the information on genotoxicity and carcinogenicity of cobalt compounds, including both ionic and metallic cobalt. They discussed several potential mechanisms for DNA damage specific to cobalt(II) ions, which fell into two general categories: direct mechanisms (induction of DNA breaks) and indirect mechanisms (inhibition of DNA repair systems). Several of the reviewed studies demonstrated that micromolar concentrations of cobalt(II) ions in the presence of hydrogen peroxide could damage isolated DNA through a Fenton-like mechanism with generation of hydroxyl radicals. In addition, cobalt ions were shown to substitute for zinc ions in protein-zinc-finger domains that control the transcription of specific genes, and it was suggested that this substitution could generate DNA-damaging free radicals close to the DNA molecule. Mechanisms proposed for the indirect genotoxic effects of cobalt(II) ions were (1) inhibition of binding of the mammalian damage-recognition protein xeroderma pigmentosum group A protein to DNA by inhibition of binding of magnesium ions to the enzyme or (2) binding of cobalt(II) ions to zinc finger domains of the repair proteins themselves. In addition, binding of p53 protein to DNA is a zinc-dependent process that can be modulated by cobalt (II) ions. Although few of the data on the effects of cobalt(II) ion on DNA damage or inhibition of DNA repair were from studies of cobalt sulfate, Lison (1996) concluded that "it seems reasonable to consider that all soluble cobalt(II) salts (chloride, sulphate, acetate) share this carcinogenic potential."

Kawanishi *et al.* (1989) demonstrated that cobalt(II) ion catalyzed the autooxidation of sulfite to the sulfate radical that caused DNA damage. Several researchers have reported that the interaction of divalent cobalt and other metal ions with hydrogen peroxide may form oxygen radical species that react with DNA (Nackerdien *et al.* 1991, Beyersmann and Hartwig 1992, Kawanishi *et al.* 1994, Lloyd *et al.* 1998). Nackerdien *et al.* (1991) demonstrated that the DNA base products formed in isolated human chromatin exposed to cobalt sulfate and hydrogen peroxide were consistent with hydroxyl radical formation and concluded that the DNA base damage may contribute to the genotoxicity and carcinogenicity of the divalent cobalt ion. Although both hydroxyl and superoxide radicals were formed by the interaction of divalent cobalt ions and hydrogen peroxide, their role in causing DNA breaks in intact cells was not established (Beyersmann and Hartwig 1992).

Other possible mechanisms of carcinogenesis include effects on DNA synthesis, DNA repair inhibition, oxidative stress, and gene expression changes. Divalent cobalt ions may decrease the fidelity of DNA synthesis by replacing magnesium in DNA polymerases; however, it is not clear whether the high concentrations used *in vitro* are relevant *in vivo*. Cobalt may inhibit DNA repair by replacing magnesium in the polymerization step or by binding to the DNA template and interfering with the polymerase-DNA interaction. (Beyersmann and Hartwig 1992).

Both nickel and cobalt mimic the effects of hypoxia by inducing several genes that are under transcriptional control by hypoxia-inducible factor-1 (HIF-1). Following hypoxia, or exposure to transition metals, HIF-1α protein is stabilized and accumulates in cells. If HIF-1 transcriptional activity is not induced under hypoxic conditions, tumor cells fail to grow and metastasize (Salnikow *et al.* 1999a, 2000).

Although cobalt exposure produces oxidative stress in cells, which can be substantial, as measured by dichlorofluorescein fluorescence (Salnikow *et al.* 2000), cobalt compounds are only weakly mutagenic (see Section 5 and Kitahara *et al.* 1996). Furthermore, human A549 lung cells exposed to cobalt chloride showed a time- and concentration-dependent increase in reactive oxygen species, which were much lower in A549 cells exposed to nickel chloride. Nevertheless, both cobalt chloride and nickel chloride equally increased upregulation of *Cap43*, an HIF-1-dependent gene (Salnikow *et al.* 2000). Another study showed that increased intracellular calcium levels were essential for *Cap43* upregulation in nickel-exposed cells (Salnikow *et al.* 1999b). The free-radical scavenger 2-mercaptoethanol did not block the increased expression of *Cap43* mRNA induced by cobalt chloride or nickel chloride, even though generation of reactive oxygen species was completely suppressed (Salnikow *et al.* 2000). Therefore, oxidative stress apparently is not involved in HIF-1 induction. These researchers suggested that the signaling cascade responsible for HIF-1α stabilization and upregulation of *Cap43* could be activated if the iron in the oxygen sensor protein was replaced by cobalt or nickel (Salnikow *et al.* 2000). Carcinogenesis could be related to metal-induced hypoxia-like conditions with subsequent selection for increased HIF-1-dependent transcription (Salnikow *et al.* 1999a).

6.5 Cocarcinogenicity of cobalt and Rauscher leukemia virus

Gainer (1973) showed that cobalt sulfate may exert cocarcinogenic effects by activating an oncogenic virus. He studied the interaction between several metal salts and Rauscher leukemia virus (RLV) infection in mice. RLV disease was determined by the development of large spleens containing high titers of virus. Fifteen male CD-1 mice were given drinking water containing a 0.01 M solution of cobalt sulfate beginning at four weeks of age. A control group of 15 mice was not given cobalt sulfate. At six weeks of age, 10 mice in the treatment and control groups were inoculated with RLV. Treatment with cobalt sulfate induced RLV splenomegalies in male CD-1 mice. Spleen weights in the uninoculated mice exposed to cobalt sulfate were not significantly different from those in unexposed controls. Spleens from mice exposed to cobalt sulfate also contained high titers of virus, whereas spleens from virus-injected control mice did not contain virus. The authors speculated that exposure to cobalt sulfate might reduce interferon activity and permit easier replication of virus.

6.6 Summary

Cobalt is part of the vitamin B_{12} complex. A daily intake of about 50 µg is sufficient to meet the nutritional requirement. Occupational exposure to cobalt has been associated with a severe and progressive respiratory disease known as hard-metal pneumoconiosis, as well as asthma and contact dermatitis. In the 1960s, several outbreaks of cardiomyopathy and polycythemia were reported in individuals who drank large quantities of beer containing added cobalt.

Cobalt is absorbed from the gastrointestinal tract, lungs, and skin and is distributed throughout the body. The highest concentrations are found in the liver, kidney, and heart. It is excreted primarily in the urine, but fecal excretion also is important. There are two

distinct elimination phases: the first is rapid and occurs within days of exposure, but the second phase may take several years.

Cobalt ions may mimic or replace other essential divalent metal ions (e.g., magnesium, calcium, iron, copper, or zinc), thus altering many important cellular reactions and functions. There is good evidence that cobalt ions can inhibit DNA repair processes or interact with hydrogen peroxide to form reactive oxygen species that can damage DNA. These mechanisms may contribute to the genotoxic and carcinogenic effects reported for cobalt sulfate and other cobalt compounds.

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Appendix A: IARC (1991). Monographs on the Evaluation of Carcinogenic Risks to Humans. Chlorinated Drinking-Water: Chlorination By-products; Some Other Halogenated Compounds: Cobalt and Cobalt Compounds. V 52. pp 363 - 472.

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