FINAL

Report on Carcinogens Background Document for

Cobalt Sulfate

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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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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.

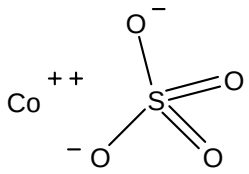

Figure 1-1. Structure of cobalt sulfate

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

Salient statistics	Metric tons of cobalt	
	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.
40 CFR 264.1200ff. – Subpart EE – Hazardous Waste Munitions and Explosives Storage. Promulgated: 62 FR 6652, 02/12/97.	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

Exposure concentration:	Incidence[a] (severity)[b]			
	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

Exposure concentration:	Incidence[a] (severity)[b]			
	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.

COBALT AND COBALT COMPOUNDS

The agents considered herein include (a) metallic cobalt, (b) cobalt alloys (including cobalt-containing medical implants) and (c) cobalt compounds. Organic cobalt-containing agents (e.g., vitamin B_{12}) are not covered comprehensively in this monograph.

1. Chemical and Physical Data

1.1 Synonyms, trade names and molecular formulae

Synonyms, trade names and molecular formulae for cobalt, cobalt alloys and cobalt compounds are presented in Table 1. The cobalt alloys and compounds given in Table 1 are not an exhaustive list, nor are they necessarily the most commercially important cobalt-containing substances; the list indicates the range of cobalt alloys and compounds available.

Table 1. Synonyms (Chemical Abstracts Service names are given in bold type), trade names and atomic or molecular formulae of cobalt and cobalt compounds

Chemical name	Chem. Abstr. Services Reg. No.[a]	Synonyms and trade names	Formulae
Metallic cobalt			
Cobalt	7440-48-4	C.I. 77320; cobalt element; cobalt-59	Co
Cobalt alloys			
Cobalt-chromium alloy[b]	11114-92-4 (91700-55-9)	**Cobalt ally (nonbase), Co, Cr**; chromium alloy (nonbase), Co, Cr	CoCr
Nickel-based cobalt alloy[b]	11068-91-0 (12604-26-1; 12616-60-3; 12616-61-4; 12624-82-7; 12630-37-4; 12636-02-1; 12672-01-4; 12774-12-8; 37323-85-6; 64941-39-5)	**Nickel alloy (base), Ni 47-59, Co 17-20, Cr 13-17, Mo 4.5-5.7, Al 3.7-4.7, Ti 3-4, Fe 0-1, C 0-0.1 (AISI 687)** APK 1; Astroloy; Cabot 700; NiCo18Cr15MoAlTi; Nimonic AP 1; NK17CADT; PM-ATS 380; PWA 1013; R 77; Rene 77; U 700; U 700m; U700PM; Udimet 700	C·Al·Co·Cr·Fe·Mo·Ni·Ti

Table 1 (contd)

Chemical name	Chem. Abstr. Services Reg. No.[a]	Synonyms and trade names	Formulae
Metallic cobalt (contd)			
Cobalt-chromium-nickel-tungsten alloy	12638-07-2 (12618-75-6; 12748-86-6; 37329-48-9; 52827-91-5; 62449-84-7)	**Cobalt alloy (base), Co 48-58, Cr 24-26, Ni 9.5-12, W 7-8, Fe 2, Mn 0-1, Si 0-1, C 0.4-0.6 (ASTM A567-2)** AFNOR K-C25NW; AMS 5382; Co X-40; G-X 55; CoCrNiW 55 25; Haynes Stellite 31; HS 31; 31H114; K-C25NW; MAS 5382; PN 31H114; S-31; Stellite 31; Stellite 31 X 40; Stellite X40; 45VF; X 40	C·Co·Cr·Fe·Mn·Ni·Si·W
Cobalt-chromium-molybdenum alloy[b]	12629-02-6 (8064-15-1; 11068-92-1; 12618-69-8; 55345-18-1; 60382-64-1; 83272-15-5; 85131-98-2; 94076-26-3 115201-64-4)	**Cobalt alloy (base), Co 56-68, Cr 25-29, Mo 5-6, Ni 1.8-3.8, Fe 0-3, Mn 0-1, Si 0-1 C 0.2-0.3 (AST A567-1)** Akrit CoMo35; AMS 5385D; Celsit 290; F 75; Haynes Stellite 21; HS 21; Protasul-2; Stellite 21; Vinertia; Vitallium; X25CoCrMo62 28 5; Zimaloy	C·Co·Cr·Fe·Mn·Mo·Ni·Si
Cobalt compounds			
Cobalt(II) acetate	71-48-7 (33327-32-1; 68279-06-1; 73005-84-2)	**Acetic acid; cobalt(2 +) salt;** bis(acetato)cobalt; cobalt acetate; cobalt(2 +) acetate; cobalt diacetate; cobaltous acetate; cobaltous diacetate	$Co(CH_3CO_2)_2$
Cobalt(II) acetate tetrahydrate	6147-53-1	**Bis(acetato)tetraquacobalt**	$Co(CH_3CO_2)_2{\cdot}4H_2O$
Cobalt(III) acetate	917-69-1	**Acetic acid, cobalt(3 +) salt**; cobalt(3 +) acetate; cobaltic acetate; cobalt triacetate	$Co(CH_3CO_2)_3$
Cobalt(II) carbonate	513-79-1	**Carbonic acid, cobalt(2 +) salt (1:1)**; C.I. 77353; cobalt carbonate (1:1); cobalt(2 +) carbonate; cobalt monocarbonate; cobaltous carbonate	$CoCO_3$

Table 1 (contd)

Chemical name	Chem. Abstr. Services Reg. No.[a]	Synonyms and trade names	Formulae
Cobalt compounds **(contd)**			
Cobalt(II) carbonate hydroxide (1:1)	12069-68-0	Basic cobalt carbonate; carbonic acid, cobalt complex; cobalt carbonate hydroxide; cobalt, (carbonato)dihydroxydi-; **cobalt, [.mu.-[carbonato-(2-)-0:0']]dihydroxydi-**	$CoCO_3 \cdot Co(OH)_2$
Cobalt(II) carbonate hydroxide (2:3)	12602-23-2	**Cobalt, bis(carbonato(2-))-hexahydroxypenta-**; cobalt, bis(carbonato)hexahydroxypenta-; cobalt carbonate hydroxide; cobalt hydroxide carbonate	$2CoCO_3 \cdot 3Co(OH)_2$
Cobalt(II) carbonate hydroxide (2:3) monohydrate	51839-24-8	Basic cobalt carbonate; carbonic acid, cobalt(2 +) salt, basic; **cobalt, bis(carbonato-(2-))hexahydroxypentamonohydrate**; cobaltous carbonate, basic	$2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$
Cobalt(II) chloride	7646-79-9 (1332-82-7)	**Cobalt chloride ($CoCl_2$)**; cobalt dichloride; cobaltous chloride	$CoCl_2$
Cobalt(II) chloride hexahydrate	7791-13-1	**Cobalt chloride, hexahydrate**; cobalt dichloride hexahydrate; cobaltous chloride hexahydrate	$CoCl_2 \cdot 6H_2O$
Cobalt(II) hydroxide	21041-93-0 (1307-85-3)	Cobalt dihydroxide; **cobalt hydroxide (Co(OH)2)**; cobalt(2 +) hydroxide; cobaltous hydroxide	$C0(OH)_2$
Cobalt(III) hydroxide	1307-86-4	**Cobalt hydroxide ($Co(OH)_3$)**; cobaltic hydroxide; cobalt trihydroxide	$Co(OH)_3$
Cobalt(II) naphthenate	61789-51-3	Cobalt naphthenates; naftolite; naphthenic acid, cobalt salt; **naphthenic acids, cobalt salts** Cobalt Nap-All; Naphthex Co; 8SN-Co	Unspecified
Cobalt(II) nitrate	10141-05-6 (14216-74-1; 19154-72-4)	Cobalt bis(nitrate); cobalt(2 +) nitrate; cobaltous nitrate; **nitric acid, cobalt(2 +) salt**	$Co(NO_3)_2$

Table 1 (contd)

Chemical name	Chem. Abstr. Services Reg. No.[a]	Synonyms and trade names	Formulae
Cobalt compounds **(contd)**			
Cobalt(II) nitrate hexahydrate	10026-22-9 (13478-32-5)	Cobalt dinitrate hexahydrate; cobalt nitrate hexahydrate; cobalt(2 +) nitrate hexahydrate; cobalt(II) nitrate hydrate; cobaltous nitrate hexahydrate; **nitric acid, cobalt(2 +) salt, hexahydrate**	$Co(NO_3)_2 \cdot 6H_2O$
Cobalt(II) molybdenum(VI) oxide	13762-14-6 (12205-99-1; 14566-03-1; 63511-60-4)	Cobalt molybdate; cobalt molybdate(VI); cobalt(2 +) molybdate; **cobalt molybdenum oxide ($CoMoO_4$)**; cobaltous molybdate; cobalt monomolybdate; molybdenum cobaltate; molybdenum cobalt oxide; molybdic acid (H_2MoO_4), cobalt(2 +) salt (1:1)	$CoMoO_4$
Cobalt(II) oxide	1307-96-6	C.I. 77322; C.I. Pigment Black 13; cobalt black; cobalt monoxide; cobalt monooxide; cobaltous oxide; **cobalt oxide (CoO)**; cobalt(2 +) oxide; monocobalt oxide Zaffre	CoO
Cobalt(II,III) oxide	1308-06-1 (12314-25-9; 25729-03-7)	Cobaltic-cobaltous oxide; cobalto-cobaltic oxide; cobalto-cobaltic tetroxide; cobaltosic oxide; **cobalt oxide (Co_3O_4)**; cobalt tetraoxide; tricobalt tetraoxide; tricobalt tetroxide	Co_3O_4
Cobalt(III) oxide	1308-04-9 (12314-25-9; 25729-03-7)	C.I. 77323; cobaltic oxide; **cobalt oxide (Co_2O_3)**; cobalt(3 +) oxide; cobalt peroxide; cobalt sesquioxide; cobalt trioxide; dicobalt oxide; dicobalt trioxide	Co_2O_3
Cobalt(III) oxide monohydrate	12016-80-7 (61864-72-0)	**Cobalt hydroxide oxide (Co(OH)O)**; cobalt(III) hydroxide oxide; cobalt oxide hydroxide; cobalt oxyhydroxide	Co(OH)O or $Co2O_3 \cdot H2O$

Table 1 (contd)

Chemical name	Chem. Abstr. Services Reg. No.[a]	Synonyms and trade names	Formulae
***Cobalt compounds* (contd)**			
Cobalt(II) sulfate	10124-43-3 (10393-49-4)	Cobalt monosulfate; cobaltous sulfate; cobalt sulfate (1:1); cobalt(2 +) sulfate; cobalt sulphate; **sulfuric acid, cobalt(2 +) salt (1:1)**	$CoSO_4$
Cobalt(II) sulfide	1317-42-6	Cobalt monosulfide; cobaltous sulfide; cobalt(2 +)sulfide	CoS
Dicobalt octacarbonyl	10210-68-1 (12553-61-6; 14525-26-9; 19998-88-0; 24917-04-2; 90043-99-5)	Cobalt, di-.mu.-carbonylhexacarbonyldi-; **cobalt tetracarbonyl dimer**	$[Co(CO)_4]_2$ or $Co_2(CO)_8$
Tetracobalt dodecacarbonyl	17786-31-1 (12083-62-9; 19212-11-4; 19478-05-8; 19495-98-8; 20623-64-7; 28963-39-5)	**Cobalt, tri-.mu.-carbonylnonacarbonyltetra-**	$[Co(CO)_3]_4$ or $Co_4(CO)_{12}$

[a]Replaced CAS Registry Numbers are given in parentheses.
[b]Approximately 5000 alloys of cobalt with other metals are listed by the Chemical Abstracts Registry Service, of which cobalt is the base metal for approximately 2000. Chromium is contained in approximately 1400 of these alloys and nickel in approximately 1500. An example of each is listed here.

1.2 Chemical and physical properties of the pure substances

Selected chemical and physical properties of cobalt and cobalt compounds covered in this monograph are presented in Table 2.

Metallic cobalt

Cobalt metal was isolated by the Swedish scientist G. Brandt in 1735; in 1780, T.O. Bergman established cobalt as an element (Donaldson, 1986).

Cobalt exists in two allotropic forms. The hexagonal close-packed form is more stable at temperatures below 417°C, and the face-centred cubic form at higher temperatures (from

Table 2. Physical properties of cobalt and cobalt compounds[a]

Chemical name	Atomic/molecular weight	Melting-point (°C)	Typical physical description	Solubility
Metallic cobalt				
Cobalt	58.93	1495 (boiling point, 2870)	Silver-grey, hard, magnetic, ductile, somewhat malleable metal	Practically insoluble in water Readily soluble in dilute nitric acid Readily soluble in hydrofluoric acid and readily in sulfuric and hydrochloric acids[b]
Cobalt compounds				
Cobalt(II) acetate (tetrahydrate)	177.03 249.08	- Loses four H_2O at 140	Light-pink crystals Red-violet monoclinic, deliquescent	Readily soluble in water Soluble in water, dilute acids, pentyl acetate and alcohols
Cobalt(III) acetate	236.07	100 (decomposes)	Dark-green, very hygroscopic powder or green crystals	Soluble in water, acetic acid, ethanol, *n*-butanol Aqueous solutions hydrolyse slowly at room temperature, rapidly at 60-70°C
Cobalt(II) carbonate	118.94	Decomposes	Red, trigonal	Practically insoluble in water, ammonium hydroxide, ethanol or methyl acetate Soluble in acids
Cobalt(II) carbonate hydroxide (2:3)	516.73	Decomposes[c]	Pale-red powder, usually containing some H_2O	Practically insoluble in water Soluble in dilute acids and ammonium carbonate solution
(monohydrate)	534.74	Decomposes[d]	Violet-red crystals	Insoluble in cold water Decomposes in hot water Soluble in acid and ammonium carbonate solution
Cobalt(II) chloride	129.84	724 (in HCl gas) decomposes at 400 on long heating in air	Pale-blue, hygroscopic leaflets; colourless in very thin layers; turns pink on exposure to moist air	Soluble in water (450 g/L at 7°C; 1050 g/L at 96°C), ethanol (544 g/L), acetone (86 g/L), methanol (385 g/L), glycerol and pyridine Slightly soluble in diethyl ether

Table 2 (contd)

Chemical name	Atomic/molecular weight	Melting-point (°C)	Typical physical description	Solubility
(hexahydrate)	237.93	86; loses four H_2O at 52-56, an additional H_2O by 100 and another H_2O at 110	Pink to red, slightly deliquescent, monoclinic, prismatic; turns blue when heated or when hydrochloric or sulfuric acid is added; slight odour[e]	Soluble in ethanol and in water (767 g/L at 0°C; 1907 g/L at 100°C), acetone, diethyl ether (2.9 g/L) and glycerol
Cobalt(II) hydroxide	92.95	Decomposes	Blue-green or rose-red powder or microscopic crystals	Very slightly soluble in water (0.0032 g/L) Soluble in acid and ammonium salts Insoluble in aqueous hydroxide solutions
Cobalt(III) hydroxide (trihydrate)	219.91	Decomposes; loses H_2O at 100	Black-brown powder	Practically insoluble in water and ethanol Soluble in nitric acid[f], sulfuric acid and hydrochloric acid
Cobalt(II) molybdenum oxide	218.87	-	Grey-green powder	-
Cobalt naphthenate	-[g]	140[h]	Brown, amorphous powder or bluish-red solid[d]	Practically insoluble in water Soluble in ethanol, diethyl ether and oils
Cobalt(II) nitrate	182.96	100-105 (decom-poses)	Pale-red powder	Soluble in water
(hexahydrate)	291.03	55-56; loses three H_2O at 55	Red, monoclinic; liquid becomes green and decomposes to the oxide above 74°C	Soluble in water (1338 g/L at 0°C; 2170 g/L at 80°C), ethanol (1000 g/L at 12.5°C), acetone and most organic solvents Slightly soluble in ammonium hydroxide

Table 2 (contd)

Chemical name	Atomic/molecular weight	Melting-point (°C)	Typical physical description	Solubility
Cobalt(II) oxide	74.93	1795±20	Powder or crystals; colour varies from olive-green to red, depending on particle size, but the commercial material is usually dark-grey	Practically insoluble in water, ethanol and ammonium hydroxide Soluble in acids (hydrochloric, sulfuric, nitric[f])
Cobalt(II,III) oxide	240.80	895[i], transition-point to CoO is 900-950	Black or grey crystals	Practically insoluble in water, aqua regia, hydrochloric or nitric acid Soluble in sulfuric acid and fused sodium hydroxide[d]
Cobalt(III) oxide	165.86	895 (decomposes)	Black-grey crystals	Insoluble in water and ethanol Soluble in acids
Cobalt(II) sulfate	154.99	735 (decomposes)	Dark-bluish crystals	Soluble in water (362 g/L at 20°C; 830 g/L at 100°C) and methanol (10.4 g/L at 18°C) Insoluble in ammonium hydroxide
(heptahydrate)	281.10	96.8; loses H_2O at 41.5, six H_2O at 71 and seven H_2O at 420	Pink-to-red monoclinic, prismatic	Soluble in water (604 g/L at 3°C; 670 g/L at 70°C), ethanol (25 g/L at 3°C) and methanol (545 g/L at 18°C)
Cobalt(II) sulfide	90.99		Exists in two forms: ß-CoS-reddish, silver-white crystals or grey powder;	Practically insoluble in water (0.0038 g/L at 18°C) and soluble in acids
	>1116		α-CoS-black amorphous powder	Soluble in hydrochloric acid

Table 2 (contd)

Chemical name	Atomic/molecular weight	Melting-point (°C)	Typical physical description	Solubility
Dicobalt octacarbonyl	341.95	Decomposes above 52	Orange crystals or dark-brown microcrystals	Practically insoluble in water Slightly soluble in ethanol Soluble in carbon disulfide and diethyl ether
Tetracobalt dodeca-carbonyl	571.86	-	Black crystals	Slightly soluble in cold water Soluble in benzene

[a]From Weast (1988); Budavari (1989), unless otherwise specified

[b]From Considine (1974)

[c]From CP Chemicals (1989a)

[d]From Sax & Lewis (1987)

[e]From Hall Chemical Co. (undated a)

[f]From Brauer (1965)

[g]The molecular weight of cobalt naphthenate varies, depending on the source of naphthenate and the method of preparation, ranging between 239-409 (6-10.5% cobalt) (US Environmental Protection Agency, 1983).

[h]From Bennett (1974)

[i]From Aldrich Chemical Co. (undated a)

417°C to the melting-point; Considine, 1974). The free energy change is low, however, so that transformation from the face-centred cubic back to the hexagonal close-packed form is slow and may be inhibited by physical form (e.g., grain size or presence of other metals) (Donaldson, 1986).

The main oxidation states of cobalt are Co(2+) and Co(3+). Cobalt is stable to atmospheric oxygen, but when it is heated it is oxidized to the mixed oxide, Co(II,III) oxide (Co_3O_4); at temperatures above 900°C, Co(II) oxide (CoO) is the end-product. Cobalt metal does not combine directly with hydrogen or nitrogen but combines with sulfur, phosphorus and carbon when heated. Cobalt forms a protective layer of sulfide scale when reacted with sulfur at temperatures below 877°C or in an atmosphere of hydrogen sulfide. It forms a mixed oxide-sulfide scale in air containing sulfur dioxide (Donaldson *et al.*, 1986a).

Cobalt also has magnetic properties. Hexagonal cobalt is ferromagnetic. The cubic form is magnetically anisotropic up to about 1000°C and becomes paramagnetic at 1121°C. Single crystals show marked magnetic anisotropy up to about 250°C (Donaldson, 1986).

Cobalt compounds

With the exception of the mixed oxide (Co_3O_4), the major commercial cobalt chemicals are all compounds of cobalt in its stable +2 oxidation state. A few simple salts of cobalt in its +3 oxidation state have been used commercially (e.g., Co_2O_3), and many Co(III) complexes with ligands such as NH_3, CN^-, No^{2-}, ethylenediaminetetraacetic acid, phthalocyanines and azo dyes have been studied extensively. These electron-donor ligands strongly stabilize Co^{3+} in solution, usually forming octahedral complexes, many of which can be isolated as stable salts. In acid solution, in the absence of such complexing ligands, Co^{2+} is the stable form and Co^{3+} is so unstable that it is reduced rapidly and spontaneously to Co^{2+}, oxidizing water to molecular oxygen. In contrast, in an alkaline solution containing ammonium hydroxide or cyanide, Co^{2+} is readily oxidized by air or hydrogen peroxide to the more stable Co^{3+} complex. The $Co^{2+} \leftrightarrows Co^{3+}$ interconversion is important in many applications of cobalt compounds, including their use as catalysts and as paint driers and in the reactions of vitamin B_{12} (National Research Council, 1977; Donaldson, 1986; Donaldson *et al.*, 1986a,b).

1.3 Technical products and impurities

(a) *Cobalt metal and cobalt alloys*

Cobalt metal is available for industrial use as 'broken' or 'cut' cathodes or electrolytic coarse powder. The cathodes measure 10-25 mm and weigh 20-50 g, with a purity greater

than 99.5%. The 'fine', 'extrafine' and 'superfine' cobalt powders manufactured from the cathodes have a submicrometre mean particle size and contain both allotropic crystal forms in varying proportions for different applications. Electrolytic coarse powder has a mean particle size of 4-10 μm (Cobalt Development Institute, 1989). Cobalt is also available as briquets, granules (99.5% cobalt), rondelles, powder (99.995% cobalt or 99.8% cobalt, < 2 μm), ductile strips (95% cobalt, 5% iron), high purity strips (99% cobalt), foil (99.95 or 99.99% cobalt, 0.1-1 mm), rods (99.998% cobalt, 5.0 mm) and wire (> 99.9% cobalt, 0.25-2 mm) (Sax & Lewis, 1987; American Chemical Society, 1988; Aldrich Chemical Co., 1990).

Cobalt alloys can be categorized into six broad types: superalloys (high-temperature alloys), magnetic alloys, hard-metal alloys, high-strength steels, electrodeposited alloys and alloys with special properties (Donaldson, 1986).

Elements used in cobalt alloys are classified in terms of their effect on the transition from the cubic to the hexagonal form. Enlarged-field components, which lower the transition temperature, include aluminium, boron, carbon, copper, iron, manganese, niobium, nickel, tin, titanium and zirconium. Restricted-field components, which raise the transition temperature, include antimony, arsenic, chromium, germanium, iridium, molybdenum, osmium, platinum, rhenium, rhodium, ruthenium, silicon, tantalum and tungsten (Donaldson, 1986).

Cobalt *superalloys*, a term generally applied to immensely strong, hard, wear and corrosion-resistant alloys, were first introduced in the 1930s. They were developed for use at high temperatures where relatively severe mechanical stressing is encountered and where high surface stability is required. Their superior strength at high temperatures arises from a close-packed face-centred cubic, austentitic lattice system, which can maintain better tensile, rupture and creep properties at elevated temperatures than a body-centred cubic system (Donaldson & Clark, 1985; Donaldson, 1986).

Superalloys are usually either cobalt- or nickel-based. Cobalt-based superalloys typically consist of a cobalt-chromium face-centred cubic solid solution matrix with the following ranges of composition: chromium, 15-29.5%; nickel, ≤ 28%; tungsten, ≤ 15%; tantalum, ≤ 9%; molybdenum, ≤ 5.5%; aluminium, ≤ 4.3%; titanium, ≤ 4%; zirconium, ≤ 2.25%; carbon, 0.04-1%; and boron, ≤ 0.11%. Small quantities of niobium, yttrium, lanthanum, iron, manganese, silicon and rhenium are present; and the balance is cobalt. Chromium is added to improve resistance to hot corrosion and oxidation. Nickel is added to stabilize the face-centred cubic structure by offsetting the tendency of the refractory metals to initiate transformation to the hexagonal close-packed structure (Donaldson & Clark, 1985).

Nickel-based superalloys were developed from the nickel-chromium alloys that had been used for over 50 years for electrical resistance, which often contain cobalt. They consist of a face-centred cubic, solid solution matrix with the following ranges of composition: chromium, 1.6-28.5%; cobalt, 1.1-22%; tungsten, 0-12.5%; molybdenum, 0-10%; aluminium, 0-6%; titanium, 0-5%; boron, 0-0.62%; carbon, 0.04-0.35%; zirconium, 0-0.13%; small amounts of tantalum, hafnium, iron, manganese, silicon, vanadium, niobium, magnesium and rhenium; and the balance as nickel (Donaldson & Clark, 1985).

Vitallium (CAS No. 12629-02-6), a cobalt-chromium alloy containing 56-68% cobalt with additions of chromium (25-29%), molybdenum (5-6%) and nickel (1.8-3.8%) was developed in 1936 (ASTM A567-1; Planinsek & Newkirk, 1979; Donaldson *et al.*, 1986b; Johnston, 1988; Roskill Information Services, 1989).

Some representative analyses of cobalt-containing alloys are given in Table 3.

Magnetic alloys. Cobalt is the only element capable of increasing the saturation magnetization of iron and is an important constituent of permanent magnets, commercial magnet steel (35% cobalt) and soft-magnet alloys. Representative analyses of some Alnico magnetic alloys (cobalt added to alloys of aluminium, nickel and iron) are given in Table 4. Magnets combining cobalt with rare-earth minerals were developed in 1967. Rare-earth cobalt alloys contain 60-65% cobalt and have the composition RCo_5, where R represents a rare-earth metal (Donaldson, 1986). A samarium-cobalt magnet was commercially available in the early 1970s, and a series of magnets with the composition R_2Co_{17} was marketed in 1980.

In *'hard-metal' alloys* (cemented carbides), cobalt powder is used as a matrix or bonding agent. The most commonly used cemented carbide, tungsten carbide, contains 80-90% by weight of hard metal and 5-10% cobalt, although up to 30% cobalt may be used for certain purposes. The properties of cemented tungsten carbides are sometimes enhanced by addition of the carbides of niobium, tantalum or titanium (Donaldson, 1986).

Cobalt-containing high-strength steels. Although cobalt is not a common alloying element in steel, it can be an important component when high strength is required (Donaldson, 1986). Maraging steels, used in the fabrication of tools and other applications requiring high strength-to-weight ratios, typically contain 8-18% cobalt alloyed with iron, nickel (8-19%), molybdenum (1-14%) and small amounts of aluminium and titanium (Roskill Information Services, 1989).

Cobalt-containing martensitic stainless maraging steels, especially designed for corrosion resistance and high tensile strength, typically contain 5-20% cobalt, 10-15.5% chromium, 0-8.2% nickel, 2-5.5% molybdenum and small amounts of carbon and titanium (Roskill Information Services, 1989).

Table 3. Examples of superalloys containing cobalt (values in weight %)[a]

Trade name	Co	Cr	Ni	Fe	Mo	W	Ta	Nb	Al	Ti	Mn	Si	C	B	Zn
Nimocast alloy 263	20.0	20.0	55.0	0.5	5.8	-	-	-	0.5	2.2	0.5	-	0.06	0.008	0.04
Udimet 500	19.0	18.0	52.0	-	4.2	-	-	-	3.0	3.0	-	-	0.07	0.007	0.05
Hastelloy alloy X	1.5	22.0	47.0	18.5	9.0	0.6	-	-	-	-	0.5	0.5	0.10	-	-
Inconel alloy 617	12.5	22.0	54.0	-	9.0	-	-	-	1.0	-	-	-	0.07	-	-
Haynes alloy 1002	Balance	22.0	16.0	1.5	-	7.0	3.75	-	0.3	0.2	0.7	0.4	0.6	-	0.3
WI-52	63.0	21.0	-	2.0	-	11.0	-	2.0	-	-	0.25	0.25	0.45	-	-
Haynes alloy 188	39.0	22.0	22.0	3.0 max	-	14.0	-	-	-	-	1.25 max	0.4	0.1	-	-
Haynes alloy 556	20.0	22.0	20.0	29.0	3.0	2.5	0.9	0.1	0.3	-	1.5	0.4	0.1	-	-

[a]From Nickel Development Institute (1987)

Table 4. Composition and magnetic properties of Alnico alloys[a]

Compisition (%)						Method of manufacture	Coercive force (kA/m)
Co	Ni	Al	Cu	Ti	Nb		
3-5	21-28	11-13	2-4	0-1	-	Cast	36-56
12-14	16-20	9-11	3-6	0-1	-	Cast	40-50
17-20	18-21	8-10	2-4	4-8	-	Cast	60-72
23-25	12-15	7.8-8.5	2-4	0-0.5	-	Field treated	46-52
32-36	14-16	7-8	4	4-6	-	Field treated	110-140
24-25	13-15	7.8-8.5	2-4	-	0-1	Columnar	56-62
32-36	14-16	7-8	4	4-6	0-1	Columnar	110-140

[a]From Donaldson (1986)

The uses and composition of *electrodeposited alloys* and *alloys* with special properties are described below. Typical specifications for one class of special purpose alloys, those used in surgical implants, are given in Table 5.

Table 5. Composition of some cobalt-containing alloys used for surgical implants (%)[a]

Element	Alloy			
	A	B	C	D
Cobalt	Balance	Balance	Balance	Balance
Chromium	27.0-30.0	19.0-21.0	18.0-22.0	26.0-30.0
Molybdenum	5.0-7.0	9.0-10.5	3.0-4.0	5.0-7.0
Nickel	1.0 max	33.0-37.0	15.0-25.0	1.0 max
Iron	0.75 max	1.0 max	4.0-6.0	0.75 max
Carbon	0.35 max	0.025 max	0.05 max	0.35 max
Silicon	1.0 max	0.15 max	0.50 max	1.0 max
Manganese	1.0 max	0.15 max	1.0 max	1.0 max
Nitrogen	NA	NA	NA	0.25 max
Phosphorus	NA	0.015 max	NA	NA
Sulfur	NA	0.010 max	0.010 max	NA
Titanium	NA	1.0 max	0.50-3.50	NA
Tungsten	NA	NA	3.0-4.0	NA

[a]From American Society for Testing and Materials (1984, 1987a,b 1988)
NA, not applicable

(b) *Cobalt compounds*

Cobalt(II) acetate is sold by one company as a reddish-pink solution containing 6-9% cobalt and 2% acetic acid (Hall Chemical Co., undated b).

Cobalt(II) *acetate tetrahydrate* is available at purities up to 100% from several companies as pink to red-violet crystals (BDH Ltd, 1989a; CP Chemicals, 1989b; J.T. Baker, 1989a; Mallinckrodt, 1989a; Hall Chemical Co., undated c). Technical-grade cobalt(II) acetate tetrahydrate, offered by one US company as red crystals, contains a minimum of 23.5% cobalt and small amounts of impurities (iron, 0.005% max; copper, 0.005% max; chlorine, 0.01% max; sulfate ion, 0.05% max; insolubles in acetic acid, 0.03% max; Shepherd Chemical Co., 1987a, 1989a).

Cobalt carbonate is offered by one US company as a reddish-purple powder containing a minimum of 45.5% cobalt and small amounts of impurities (iron, 0.005% max; copper, 0.005% max; lead, 0.005% max; chlorine, 0.01% max; sodium, 0.6% max; insolubles in dilute hydrochloric acid, 0.05% max; cadmium, 0.005% max; sulfate ion, 0.2% max; Shepherd Chemical Co., 1987b, 1989b). Several companies offer cobalt carbonate as a pink powder or red crystals at 90-100% purity (CP Chemicals, 1989c; J.T. Baker, 1989b; Hall Chemical Co., undated d). Basic cobalt carbonate, the primary commercial product, typically contains 45-47% cobalt (Donaldson *et al.*, 1986a).

Cobalt chloride is sold commercially mainly as the hexahydrate or other hydrated form. Cobalt chloride hexahydrate is available from several companies as red crystals in purities up to approximately 100% (BDH Ltd, 1989b; CP Chemicals, 1989c; Mallinckrodt, 1989b; Aldrich Chemical Co., undated b,c; Hall Chemical Co., undated a). Technical-grade cobalt chloride hexahydrate, available from one US company as red crystals, contains a minimum of 24% cobalt and small amounts of impurities (iron, 0.02% max; copper, 0.02% max; sulfate ion, 0.1% max; water insolubles, 0.05% max; Shepherd Chemical Co., 1987c, 1989c). The hexahydrate is also available as a pink-to-red powder at 98-100% purity (J.T. Baker, 1989c) and as a clear reddish aqueous solution containing 14.5% cobalt (Hall Chemical Co., undated e). Cobalt chloride is also available commercially as a clear, purple aqueous solution containing approximately 6% cobalt chloride (Mallinckrodt, 1989c) and as essentially pure (99.999%) hydrated red-violet powder and chunks (Aldrich Chemical Co., undated d).

Anhydrous cobalt chloride is available from two companies as a blue powder at purities up to 97% (BDH Ltd, 1989c; Aldrich Chemical Co., undated e) and from another at a purity of 100% (Hall Chemical Co., undated f).

Cobalt(II) hydroxide is available commercially as a solid containing 62% cobalt and an antioxidant (Donaldson *et al.*, 1986a), as a blue-green, moist press cake (E grade) containing 68% cobalt hydroxide and less than 500 ppm ammonia (Hall Chemical Co., undated g), as a technical grade (95% cobalt hydroxide; Aldrich Chemical Co., 1990) and as a pink powder containing a minimum of 61% cobalt and small amounts of impurities (chlorine, 0.02% max; acetic acid insolubles, 0.2% max; copper, 0.01% max; iron, 0.01% max; manganese, 0.03% max; nickel, 0.3% max; sulfate ion, 0.3% max; Shepherd Chemical Co., 1988a, 1989d).

Cobalt molybdenum oxide is produced by one company in the USA (Chemical Information Services Ltd, 1988).

Commercial grade *cobalt naphthenate* is available as a solution of 65% cobalt naphthenate (6% cobalt) in white spirits (Nuodex, 1986; Hall Chemical Co., undated h). One US company offers 6 and 8% liquid grades; another offers liquid, flake and solid forms (American Chemical Society, 1988). One Canadian company and one US company offer 6% cobalt naphthenate in solution with white spirits and 10.5% flaked cobalt naphthenate (Dussek Campbell Ltd, 1989a,b; Shepherd Chemical Co., 1989e,f).

One US company offers *cobalt nitrate hexahydrate* as a red-brown crystalline powder at 99.999% purity or as red chips in reagent grade or at 99% purity. The reagent grade is 98% pure and contains small amounts of impurities (insolubles, < 0.01%; chloride ion, < 0.002%; copper, < 0.002%; iron, < 0.001%; ammonium, ≤ 0.2%; nickel, ≤ 0.15%; and sulfate ion, ≤ 0.005%) (Aldrich Chemical Co., 1990, undated f,g,h). The hexahydrate is available as pink-to-red crystals at 90-100% purity from three US companies and from one company in the UK (BDH Ltd, 1989d; J.T. Baker, 1989d; Mallinckrodt, 1989d; Hall Chemical Co., undated i). Technical-grade cobalt nitrate hexahydrate is available from one US company as small, red flakes with a slight odour of nitric acid and contains a minimum of 19.8% cobalt, with small amounts of impurities (iron, 0.002% max; copper, 0.005% max; lead, 0.005% max; zinc, 0.05% max; chlorine 0.005% max; sulfate ion, 0.01% max; water insolubles, 0.02% max; Shepherd Chemical Co., 1986a, 1989g). Aqueous cobalt nitrate ($Co(No_3)2xH_2o$) is available from one US company as a dark-red solution containing approximately 14% cobalt (Hall Chemical Co., undated j).

Cobalt nitrate is also available in 1-2% aqueous nitric acid solution as a laboratory standard containing 1000 ppm cobalt (0.1% w/v; J.T. Baker, 1989e; Aldrich Chemical Co., 1990).

Cobalt(II) oxide is available as a laboratory reagent from one US company as a green, red, grey or black powder at 90-100% purity (70-74% as cobalt), with small amounts of impurities (chloride, 0.02% max; nitrogen compounds as nitrogen, 0.02% max; sulfur compounds as sulfate ion, 0.1% max; iron, 0.1% max; nickel, 0.2% max; insolubles in

hydrochloric acid, 0.05%; J.T. Baker, 1989f,g). One company in the UK offers cobalt oxide as a fine, black powder (BDH Ltd, 1989e). Cobalt(II) oxide is also available in ceramic grade (70-71% cobalt), metallurgical grade (76% cobalt) and high-purity powder grade (99.5%; may contain 10 ppm metallic impurities; American Chemical Society, 1988). Cobalt(II) oxide is produced by only a few companies (Chemical Information Services Ltd, 1988) and is not of major commercial importance.

Cobalt(*II,III*) *oxide* is available as a black powder at 99.995% purity (Aldrich Chemical Co., undated a), as a black powder with a cobalt content of 72-73% (Aldrich Chemical Co., 1990, undated i) and as a black-grey powder with 71-72% cobalt as cobalt oxide and less than 1% nickel as nickel monoxide (Hall Chemical Co., undated k). Another mixed oxide, containing a ratio of 3:1 cobalt(III) oxide:cobalt(II) oxide, is available at 99.999% purity (Chemical Dynamics Corp., 1989). It is produced by many companies throughout the world.

Cobalt(*III*) *oxide* is available in small quantities for laboratory use from one US company as a powder at 99.9996% purity (72.3% as cobalt) with small amounts of impurities (chloride, 80 μg/g; nitrate, 35 μg/g; silicon, 2 μg/g; aluminium, < 1 μg/g; copper, < 0.5 μg/g; iron, 1 μg/g; magnesium, 0.7 μg/g; nickel, 2 μg/g; J.T. Baker, 1989g).

Cobalt sulfide (form unspecified) is sold by one company in the USA (Chemical Information Services Ltd, 1988).

Cobalt sulfate heptahydrate is available from several companies as pink-to-dark-red crystals in purities of 90-100% (BDH Ltd, 1989f; J.T Baker, 1989h; Mallinckrodt, 1989e; Aldrich Chemical Co., undated j,k; Hall Chemical Co., undated l). Technical-grade cobalt sulfate heptahydrate available from one US company as red-pink crystals contains a minimum of 20.8% cobalt and small amounts of impurities (iron, 0.005% max; copper, 0.002% max; water insolubles, 0.05% max; Shepherd Chemical Co., 1986b, 1989h). The monohydrate is available as pink-to-red crystals with a minimum of 33% cobalt and with small amounts of impurities (iron, 0.007% max; copper, 0.003% max; water insolubles, 0.1% max; Shepherd Chemical Co., 1987d, 1988b), and with a purity of 100% (Hall Chemical Co., undated m).

Cobalt sulfate is also available commercially as a rose-to-dark-red aqueous solution containing approximately 8% cobalt (CP Chemicals, 1989d; Hall Chemical Co., undated n).

2. Production, Use, Occurrence and Analysis

2.1 Production

(a) *Cobalt and cobalt alloys*

Cobalt, a major constituent of about 70 naturally occurring oxide, sulfide, arsenide and sulfoarsenide minerals, is produced primarily as a by-product of the mining and processing of copper and nickel ores and, to a lesser extent, of silver, zinc, iron, lead and gold ores.

Commercial cobalt production began in Canada in 1905. In 1924, a company in Zaire (then the Belgian Congo) started recovering cobalt during the mining of copper ores, and that country has been the world's largest producer since 1926 (Roskill Information Services, 1989). World mine production of cobalt peaked in the mid-1980s, but the production of refined cobalt metal has been decreasing since the early 1980s because beneficiation and extractive metallurgy are not designed for maximizing the recovery of cobalt (Roskill Information Services, 1989). World mine and metal production of cobalt in 1970-88 is presented in Table 6.

Table 6. World mine and metal production of cobalt, 1970-88 (tonnes)[a]

Year	Mine production	Metal production	Year	Mine production	Metal production
1970	28 985	25 909	1980	37 873	36 720
1971	26 405	27 203	1981	37 363	31 325
1972	30 177	24 645	1982	24 567	19 292
1973	35 746	28 113	1983	37 875	18 084
1974	39 453	30 745	1984	41 075	23 627
1975	37 479	25 275	1985	48 304	26 906
1976	26 024	22 827	1986	48 903	30 673
1977	26 303	25 227	1987	46 382	26 939
1978	32 817	24 780	1988	43 900[b]	25 286[c,d]
1979	36 148	34 317	1989	38 700[b,c]	NA

[a]From Roskill Information Services (1989), unless otherwise specified
[b]From Shedd (1990)
[c]Estimate
[d]From Shedd (1988)
NA, Not available

Between 1983 and 1987, cobalt was mined in amounts greater than 100 tonnes in 16 countries and was refined in 12. The cobalt-producing countries or regions in those years were Albania, Australia, Botswana, Brazil, Canada, China, Cuba, Finland, Morocco, New Caledonia, the Philippines, South Africa, the USSR, Zaire, Zambia and Zimbabwe. The countries that refined cobalt during this period were Belgium, Canada, China, Finland, France, Japan, Norway, South Africa, the USSR, Zaire, Zambia and Zimbabwe (Johnston, 1988; Shedd, 1988).

(i) *Cobalt mining, refining and/or production by country*

Australia: Cobalt is mined but not refined in Australia (Shedd, 1988). In 1986, one company ceased supplying nickel-cobalt sulfides to Japanese refineries and began to supply all of their by-products to a refinery in Finland (Kirk, 1986).

Belgium: Small quantities of partly processed materials containing cobalt have been imported, but information is inadequate to estimate the recovery of cobalt (Kirk, 1986). About one-third of the cobalt exported by Zaire is processed in Belgium, and about half of this production is exported to the USA (Kirk, 1985).

Botswana: One company in Botswana began mining for cobalt in 1973 (Kirk, 1985). The cobalt-containing nickel-copper matte is sent to Norway (74%) and Zimbabwe (26%) for refining (Shedd, 1988); previously, it was refined in the USA (Kirk, 1985).

Brazil: One company began production of electrolytic cobalt in late 1989 at a nickel plant with an initial production capacity of 300 tonnes. It produced a cobalt concentrate which was sent to a Norwegian refinery for processing. Previously, Brazil depended on imports from Canada, Norway, Zaire and Zambia (Kirk, 1987; Shedd, 1988, 1989).

Bulgaria: Bulgaria is known to produce ores that contain cobalt, but information is inadequate to estimate output (Kirk, 1985).

Canada: Cobalt production in Canada began in 1905 (Roskill Information Services, 1989). Three companies currently mine cobalt, and one of these refines it (Shedd, 1988). The intermediate metallurgical product cobalt oxide has been shipped to the UK for further processing, and a nickel-copper cobalt matte has been shipped to Norway (Kirk, 1986, 1987).

China: A primary cobalt deposit mine was equipped in 1986 and has a reported annual output of 45 thousand tonnes of ore (Kirk, 1986). Cobalt mine production in 1987 was estimated to be 270 tonnes (Johnston, 1988). A large deposit of nickel-copper-cobalt was discovered in China in 1988 (Shedd, 1988).

Czechoslovakia: Czechoslovakia is believed to recover cobalt from Cuban nickel-cobalt oxide and oxide sinter (Kirk, 1985; Shedd, 1988).

Finland: In 1986, a company in Finland began processing nickel-cobalt sulfide from Australian nickel oxide production into cobalt and nickel salts (Kirk, 1986). In 1987; a

mining and metallurgical cobalt and nickel producing company in Finland suspended production of standard-grade cobalt powder and briquets to focus on producing extra-fine powder and cobalt chemicals. In 1988, the copper-cobalt mine was closed and cobalt concentrates were no longer produced (Kirk, 1987; Shedd, 1988).

Germany: Ores that contain cobalt are produced in Germany, but information is inadequate to estimate output (Kirk, 1985).

Greece: Ores that contain cobalt are produced in Greece, but information is inadequate to estimate output (Roskill Information Services, 1989).

India: A plant projected to open in 1990 can recover approximately 27 tonnes of cobalt per year from a lead-zinc ore mine in India. In addition, recovery of cobalt from lateritic overburden in chromite mines is being studied (Shedd, 1988).

Indonesia: One company in Indonesia produces ores that contain cobalt, but information is inadequate to estimate output (Shedd, 1988).

Japan: Mining of cobalt in Japan ceased in 1986. Two Japanese refiners have received nickel-matte from a Canadian facility in Indonesia and feedstock from Australia and the Philippines (Shedd, 1988).

Morocco: Mining of cobalt was begun in Morocco in the late 1930s (Roskill Information Services, 1989); mining of cobalt as a primary product ceased in 1982, but mining from cobalt-iron-nickel arsenides was resumed in 1988. Beginning in 1988, Morocco agreed to provide China with cobalt concentrate (Shedd, 1988).

New Caledonia: Ores and intermediate metallurgical products have been exported to France, Japan and the USA (Kirk, 1987; Shedd, 1988).

Norway: One company in Norway refines cobalt mostly from nickel-cobalt-copper matte imported from Canada (60%) and Botswana (30%) (Shedd, 1988).

Philippines: Cobalt was recovered as a by-product of nickel mining by a state-owned company in the Philippines until 1986, when the mine was closed. Production of cobalt from the mine peaked at about 1360 tonnes in 1979 (Kirk, 1987; Shedd, 1988).

Poland: Ores that contain cobalt are produced in Poland, but information is inadequate to estimate output (Kirk, 1985).

South Africa: Cobalt is mined and refined in South Africa (Shedd, 1988), and a foreign-owned company produced cobalt as a by-product of platinum mining operations (Kirk, 1987).

Spain: Ores that contain cobalt are produced in Spain, but information is inadequate to estimate output (Kirk, 1985).

Uganda: Construction of a cobalt refinery is planned in conjunction with the rehabilitation of copper mines, which ceased operation in 1979 (Shedd, 1988).

UK: Products of Canadian origin are processed in the UK (Kirk, 1986, 1987).

USA: The USA began mining cobalt in the late 1930s but ceased domestic mine production at the end of 1971. Refining of imported nickel-cobalt matte by the sole US cobalt refinery was discontinued in late 1985. In 1985-88, the USA imported 31% of its cobalt from Zaire, 21% from Zambia, 21% from Canada, 10% from Norway (originating in Canada and Botswana) and 17% from other countries (Shedd, 1990), which include Belgium, Finland, France, Germany, Japan, the Netherlands, South Africa and the UK (Kirk, 1987).

Two companies in the USA produce extra-fine cobalt powder: one is a foreign-owned company that uses imported primary metal; the other is a domestically controlled company that uses cobalt recovered from recycled materials. Seven companies produce cobalt compounds (Shedd, 1990).

USSR: Cobalt is mined and refined in the USSR (Shedd, 1988); in addition, nickel-cobalt sulfide concentrate from Cuba is refined (Kirk, 1985).

Zaire: Cobalt recovery from the mining of copper ores began in 1924, and since 1926 Zaire has been the world's largest producer of cobalt (Roskill Information Services, 1989). Sulfide and oxide concentrates are processed to cobalt metal in the form of cathodes and granules. About one-third of their exports go to Belgium for further processing (Kirk, 1985).

Zambia: Mining of cobalt began in Zambia in the late 1930s (Roskill Information Services, 1989). Cobalt is also mined and refined as a by-product of copper mining (Kirk, 1985; Shedd, 1988).

Zimbabwe: Cobalt is mined and refined in Zimbabwe and is also recovered from nickel-copper matte imported from Botswana (Shedd, 1988).

Mine and metal production of cobalt by country or region with reported outputs for 1984 to 1988 are presented in Tables 7 and 8.

(ii) *Metallurgy*

Cobalt-containing ores vary widely in composition but usually contain less than 1% cobalt. Although each type of ore (arsenide, sulfide or oxide) is processed differently, six general metallurgical processes can be distinguished; depending on the ore's composition, recovery of cobalt may require one or a combination of these techniques. It is important to note that in nearly all cases cobalt is a by-product of the refining of other metals (Roskill Information Services, 1989), especially copper and nickel. Refinery methods therefore are generally not designed to maximize cobalt recovery (Anon., 1990a).

The main sources of cobalt (in decreasing ease of recovery) are ores of copper-cobalt oxides (Zaire) and sulfides (Zaire and Zambia), copper-nickel sulfides (Canada), cobalt-iron-nickel arsenides (Morocco and China) and nickel-cobalt oxides (lateritic nickel

Table 7. World mine production of cobalt by country or region, 1984-88[a]

Country	Mine output, metal content (tonnes)				
	1984	1985	1986	1987	1988[b]
Albania	590	590	590	590	590
Australia	938	1 136	1 218	1 200	1 100
Botswana	259	222	162	182	292[c]
Brazil	100	100	150	150	150
Canada	2 330	2 071	2 491	2 495	2 770
Cuba[d]	1 400	1 491	1 500	1 590	2 000
Finland	862	1 094	628	190	182
Morocco	NA	NA	NA	NA	253
New Caledonia[b]	500	677	700	750	800
Philippines	64	913	92	NA	NA
South Africa[b]	682	682	682	727	727
USSR[b]	2 590	2 725	2 815	2 815	2 860
Zaire	25 997	29 226	33 403	29 056[b]	25 425
Zambia	4 625	5 812[b]	5 770[b]	5 950[b]	6 675
Zimbabwe[b]	77	100	76	109	126
Total	41 014	46 838	50 277	45 804	43 950

[a]From Shedd (1988), unless otherwise specified
[b]Estimates
[c]Reported figure
[d]Estimates from reported nickel-cobalt content of granular and powder oxide, oxide sinter and sulfide production
NA, not available

ore from most other sources) (Planinsek & Newkirk, 1979; Donaldson *et al.*, 1986a; Shedd, 1988).

After crushing and grinding, the first stage of cobalt recovery from ore involves the physical separation of cobalt-containing minerals from other nickel ores and gauge, usually by gravity (arsenide ores) or froth flotation (sulfoarsenide and sulf de ores). Flotation is also used for separating cobalt in oxide and mixed oxide-sulfde ores. Flotation is frequently aided by the addition of xanthates, oils or cyanide to depress cobalt flotation (Donaldson, 1986; Donaldson *et al.*, 1986a); the amount of cobalt in the concentrate is usually enhanced four to eight fold by these operations (Roskill Information Services, 1989).

Cobalt is extracted from ore and concentrated by pyrometallurgical, hydrometallurgical and electrolytic processes alone or in combination. Arsenic-free cobalt concentrates can be mixed with lime and coal and smelted in a reducing environment to give copper-cobalt

Table 8. World metal production of cobalt by country, 1984-88 (tonnes)[a]

Country	1984	1985	1986	1987	1988[b]
Canada	2 218	2 027	1 994	2 205[b]	2 205
Finland	1 456	2 235	1 350	498	220
France	116	123	100[b]	109[b]	50
Japan	907	1 279	1 340	124	109
Norway	1 193	1 640	1 583	1 603	1 605
South Africa[b]	500	500	500	523	523
USSR[b]	4 725	4 815	5 315	5 315	5 315
Zaire	9 083	10 690	14 513	11 911	10 150
Zambia	3 475	4 365	4 348	4 483	4 995
Zimbabwe	78	92	76	110	126
Total	23 751	27 766	31 119	26 881	25 298

[a]From Shedd (1988)
[b]Estimates

alloys. The alloy is further processed to separate copper and cobalt. The most commonly used hydrometallurgical processes involve roasting and leaching of ore concentrates (with acid or alkali solutions), fractional separation of cobalt from other metals in the leachate (by differential sulfide or hydroxide precipitation) and reduction of the cobalt ions to metal (by chemical or electrochemical means) (Donaldson, 1986; Donaldson *et al.*, 1986a; Roskill Information Services, 1989).

The three main processes for leaching cobalt from ores and concentrates are described below.

Acid sulfate leaching can be done by one of four methods: (a) treating oxide ore concentrates with sulfuric acid and reducing agents (SO_2); this is the primary process used in Zaire; (b) water extraction of cobalt sulfate from ores following an oxidizing roast; (c) cobalt sulfate extraction of sulfide ore concentrate following a sulfatizing roast; this method is used in Zaire, Zambia and Finland; or (d) pressure leaching with sulfuric acid, which has recently been introduced in Canada and is useful for arsenic-containing ores. The cobalt is separated from copper, iron, nickel and zinc (when present) by alkalinization and fractional dissolution with sulfide. Cobalt is precipitated as the hydroxide, redissolved and refined by electrolysis or hydrogen reduction to cobalt metal cathode or powder, respectively (Roskill Information Services, 1989).

Acid chloride leaching of ore mattes and recyclable materials is used as an alternative to acid sulfate leaching on oxides, sulfides, arsenides and alloys. This method is usually

followed by solvent extraction or ion exchange purification. The soluble chloride complexes are often formed by reaction with chlorine or hydrogen chloride gas or a metal chloride. This method is used in Japan.

Ammoniacal solution leaching gives rise to the hexammine cobalt complex $[Co(NH_3)_6]^{2+}$. This method has been used to treat alloy scrap and laterite or arsenide ores. It is used in Canada for processing lateritic nickel ores. The soluble extract is treated with hydrogen sulfide to produce mixed nickel-cobalt sulfides, which are redissolved in sulfuric acid. Cobalt powder is recovered after the introduction of ammonia and hydrogen under high pressure.

Metallic cobalt can also be recovered directly from purified leachate by electrolysis (electrowinning) after nickel has been removed as the carbonyl. Some cobalt salts can be formed by dissolution of the metal in the corresponding acid. Some refineries utilize cobalt hydroxide to form the oxide and other cobalt compounds directly (Donaldson, 1986; Donaldson *et al.*, 1986a; Roskill Information Services, 1989; Anon., 1990a).

(iii) *Production processes*

Refined cobalt is available to the industrial market primarily as broken or cut cathodes (92%) and to a lesser extent as electrolytic coarse powder (3%) and in other forms. The cathode form is further processed to alloys, chemicals and oxide or used in the manufacture of special cobalt powders for cemented carbide by chemical and pyrometallurgical processes. About 2000 tonnes of cobalt cathode are converted to a distinct allotropic mixture, called 'fine powder' or 'extrafine powder', by specialist producers for cemented carbide and diamond polishing. The process involved is a chemical reaction that results in a submicrometre powder with a high proportion of face-centred cubic crystal retained in the mixture. This special material differs from electrolytic coarse powder and from cobalt powders generated during industrial attritive operations, which are predominantly hexagonal crystals (Cobalt Development Institute, 1989).

Cobalt alloys are usually manufactured from broken or cut cathodes by electric arc or by induction melting techniques, although vacuum induction melting is required for some alloys containing metals such as aluminium, titanium, zirconium, boron, yttrium and lanthanum. The resultant master alloy is then remelted and cast into moulds (Donaldson & Clark, 1985; Donaldson, 1986).

An important use of cobalt is in the production of cemented tungsten carbide, also called 'hard metal'. Hard metals are used to tip the edges of drills and cutting tools and for dies, tyre studs and stamping machines (Kipling, 1980). Hard metal is made by a process in which precise weights of tungsten carbide (80-90% by weight) and cobalt metal powder (5-10%) and, in some grades, small amounts of other carbides (titanium, tantalum, niobium and molybdenum) are added and thoroughly mixed in mills. The cobalt thus acts as a

matrix; nickel is also used with cobalt as a matrix in some grades. Organic solvents, such as acetone and *n*-hexane, are added for mixing; the mixture is dried, and the organic solvents are evaporated off. The powder is put into frames made of steel or rubber and then pressed into the desired shapes; the pieces are placed on graphite plates and embedded in nitrous aluminium powder; and the pressed material is presintered in hydrogen furnaces at 500-800°C. After presintering, the material has the consistency of chalk, and it is cut, ground, drilled or shaped into the configurations required. The shaped material is finally sintered at temperatures of 1550°C. After sintering, the product approaches the hardness of diamond. Hard-metal products are sand blasted or shot blasted, brazed into holders made of iron using fluoride-based fluxes and then ground with diamond or carborundum wheels. These processes are illustrated in Figure 1 (Kusaka *et al.*, 1986).

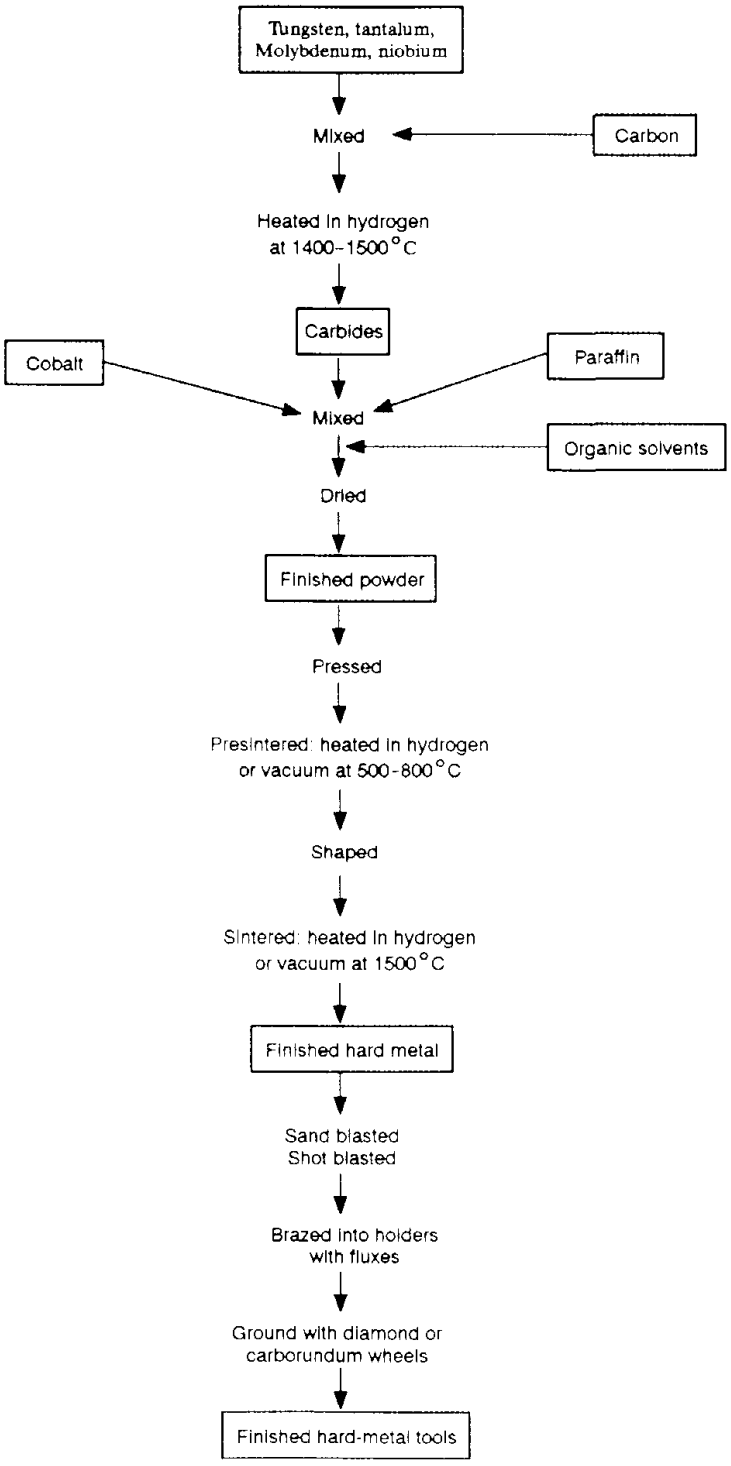
The manufacture of some alloys containing cobalt and their further fabrication into engineering parts can be assumed to take place to some extent in almost all industrialized countries. Manufacture specifically of superalloys for aircraft engines is concentrated in the USA, the UK, France, Germany and Japan, but small volumes of manufacture and specialist manufacture occur in several other regions. Use of cobalt in magnetic applications occurs mainly in Japan, but the USA and European countries (particularly Germany, France and the UK) also have large production capacities (Johnston, 1988).

(b) *Cobalt compounds*

Europe produces 50% of the global amount of cobalt chemicals and 70% of fine cobalt powders (Johnston, 1988). Most cobalt chemicals (75-80%) are produced by six companies in Belgium, Germany, Finland and the USA. A further 6-8% is made by three Japanese companies; minor quantities are made directly from concentrates in France and South Africa; and the balance is shared by a number of small manufacturers serving local markets or specializing in perhaps one group of cobalt products, such as naphthenates for the paint (Sisco *et al.*, 1982) and ink industries.

Most countries—industrialized or not—have a ceramics industry of some kind or size, many of them very ancient, and in each there is some use of cobalt oxide or some manufacture of cobalt pigment. The major world suppliers of cobalt pigments are, however, located in Germany, the USA, the Netherlands and the UK.

Fig. 1. Steps in the manufacture of hard-metal tools[a]



[a]From Kusake *et al.* (1986)

Cobalt(*II*) *acetate* is prepared commercially (a) by concentrating solutions of cobalt powder in acetic acid in the presence of oxygen or (b) from cobaltous hydroxide or carbonate and an excess of dilute acetic acid. Preparation of the tetrahydrate involves treatment of cobalt powder in acetic acid solution with hydrogen peroxide (Donaldson *et al*., 1986a; Budavari, 1989).

Cobalt(*III*) *acetate* can be prepared by electrolytic oxidation of cobalt(II) acetate tetrahydrate in glacial acetic acid containing 2% (v/v) water. Another method is oxidation of solutions of cobaltous salts by alkaline persulfates in the presence of acetic acid (Budavari, 1989).

Cobalt(II) *chloride* can be produced by several processes: (a) from cobalt powder and chlorine, (b) from the acetate and acetyl chloride, (c) by dehydration of the hexahydrate with thionyl chloride and (d) by dissolving cobalt metal, oxide, hydroxide or carbonate in hydrochloric acid (Considine, 1974; Donaldson *et al.*, 1986a; Budavari, 1989). The hexahydrate is prepared by treating an aqueous solution of a cobaltous salt with hydrochloric acid (Budavari, 1989). Solutions of high-purity cobalt chloride and its hexahydrate can be manufactured by dissolving high-purity cobalt metal electrolytically using a dilute hydrochloric acid electrolyte at about 60°C (Donaldson *et al.*, 1986a).

Cobalt(*II*) *carbonate* is prepared by heating cobalt sulfate with a solution of sodium bicarbonate. Basic cobalt carbonate (cobalt(II) carbonate hydroxide (2:3) monohydrate) is prepared by adding sodium carbonate to a solution of cobaltous acetate followed by filtration and drying (Sax & Lewis, 1987).

Cobalt(*II*) *hydroxide* is prepared commercially as a pink solid by precipitation from a cobalt(II) salt solution with sodium hydroxide. Precipitation at higher temperatures (55-70°C) causes partial oxidation of cobalt(II) to cobalt(III) and yields the pink form, whereas precipitation at lower temperatures yields the blue form. Cobalt(II) hydroxide is prepared *in situ* during the manufacture of secondary batteries: typically, a spongy nickel foam plate is impregnated with an acidic solution of cobalt chloride, nitrate or sulfate, and cobalt(II) hydroxide is precipitated by alkali treatment (Donaldson *et al.*, 1986a).

Cobalt(*III*) *hydroxide* can be produced by several methods, e.g., addition of sodium hydroxide to a solution of cobaltic salt, action of chlorine on a suspension of cobaltous hydroxide, or action of sodium hypochlorite ion on a cobaltous salt (Brauer, 1965; Sax & Lewis, 1987).

Cobalt(*II*) *molybdenum*(*VI*) *oxide* is obtained by raising the pH to 6.4 to coprecipitate the hydroxides of cobalt and molybdenum from mixed solutions of cobalt nitrate and ammonium molybdate. The product is dried at 120°C and calcined at 400°C to give the mixed metal oxide (Donaldson *et al.*, 1986a). This is invariably also mixed with aluminium oxide in commercial manufacture and use.

Cobalt(II) naphthenate is prepared by treating cobalt hydroxide or cobalt acetate with naphthenic acid (Sax & Lewis, 1987), which is recovered as a by-product of petroleum refining. Commercial naphthenic acids used in the production of cobalt naphthenate differ widely in properties and impurities, depending upon the crude oil source and refining processes. All contain 5-25 wt % hydrocarbons, the composition of which corresponds to the petroleum fraction from which the naphthenic acids are derived; and all contain impurities (e.g., phenols, mercaptans and thiophenols) in small quantities (Sisco *et al.*, 1982).

Cobalt(II) nitrate hexahydrate is produced by dissolving cobalt metal, the oxide, hydroxide or carbonate in dilute nitric acid and concentrating the solution (Considine, 1974; Donaldson *et al.*, 1986a).

Cobalt(II) oxide (CoO) containing 78.7% cobalt is usually manufactured by controlled oxidation of the metal at above 900 ° C, followed by cooling in a protective atmosphere to prevent partial oxidation to cobalt(II,III) oxide (Donaldson *et al.*, 1986a).

Cobalt(II) oxide can also be prepared by additional processing of the white alloy formed during the processing of arsenic-free cobalt-copper ores to remove copper and iron as sulfates and calcining cobalt as the carbonate (Morral, 1979) or by calcination of cobalt carbonate or its oxides at high temperatures in a neutral or slightly reducing atmosphere (Sax & Lewis, 1987).

Another method for preparing cobalt(II) oxide is dissolution of a cobalt salt that is unstable at high temperatures (e.g., cobalt sulfate) in molten sodium sulfate or potassium fluoride. The cobalt salt decomposes, leaving the cobalt(II) oxide, which crystallizes out at high temperatures. The water-soluble salts are then dissolved, leaving cobalt(II) oxide crystals (Wilke, 1964).

Cobalt(II,III) oxide (Co_3O_4) containing 73.44% cobalt can be prepared by the controlled oxidation of cobalt metal or cobalt(II) oxide or by thermal decomposition of cobalt(II) salts at temperatures below 900°C. It absorbs oxygen at room temperature but is not transformed to cobalt(III) oxide (Co_2O_3) (Donaldson *et al.*, 1986a).

Pyrohydrolysis of cobalt chloride has also been used to manufacture cobalt(II,III) oxide. The reaction is performed in a spray roaster by heating a fine spray of aqueous solution of cobalt(II) chloride in a countercurrent heating gas stream. The hydrogen chloride gas produced is removed with the exhaust gases, and the cobalt(II,III) oxide falls to the bottom of the furnace (Donaldson *et al.*, 1986a).

Cobalt(III) oxide (Co_2O_3) is derived by heating cobalt compounds (e.g., hydroxides) at low temperature with an excess of air (Sax & Lewis, 1987).

Cobalt(*II*) *sulfate heptahydrate* is prepared commercially by dissolving cobalt metal in sulfuric acid (Donaldson *et al.*, 1986a).

α-*Cobalt*(II) *sulfide* can be precipitated from cobalt nitrate hexahydrate by reaction with hydrogen sulfide and dried for 90 h, the temperature being raised slowly from 100 to 540°C (Brauer, 1965). β-*Cobalt*(*II*) *sulfide* can be synthesized by heating fine cobalt powder mixed with fine sulfur powder at 650° C for two to three days. It can also be derived by treating a solution of cobalt chloride with acetic acid, precipitating with hydrogen sulfide and drying for 90 h, the temperature being raised slowly from 100 to 540°C (Brauer, 1965). Cobalt sulfides are normally produced *in situ* as needed, as mixed metal catalysts with molybdenum (Roskill Information Services, 1989).

Dicobalt octacarbonyl is prepared commercially by heating cobalt metal with carbon monoxide at high pressure (200-300 atm) [20.2-30.3 x 10^3 kPa] or by heating a mixture of cobalt(II) acetate with cyclohexane at about 160°C and 300 atm (30.3 x 10^3 kPa) in the presence of a 1:1 mixture of carbon monoxide:hydrogen (Donaldson *et al.*, 1986a). Dicobalt octacarbonyl is frequently prepared *in situ* as needed.

2.2 Use

Cobalt compounds have been used as blue colouring agents in ceramic and glass for thousands of years, although most of the blue colour of ancient glasses and glazes has been found to be due to copper. Cobalt has been found in Egyptian pottery dated at about 2600 BC, in Persian glass beads dating from 2250 BC, in Greek vases and in pottery of Persia and Syria from the Christian era, in Chinese pottery from the Tang (600-900 AD) and Ming (1350-1650 AD) dynasties and in Venetian glass from the early fifteenth century. Leonardo Da Vinci was one of the first artists to use cobalt as a brilliant blue pigment in oil paints. The pigment was probably produced by fusing an ore containing cobalt oxide with potash and silica to produce a glass-like material (a smalt), which was then reduced to the powdered pigment. In the sixteenth century, a blue pigment called zaffre was produced from silver-cobalt-bismuth-nickel-arsenate ores in Saxony (Young, 1960; Donaldson, 1986).

It was not until the twentieth century, however, that cobalt was used for industrial purposes. In 1907, a US scientist, E. Haynes, patented a series of cobalt-chromium alloys known as stellites that were very resistant to corrosion and wear at high temperatures (Kirk, 1985). Cobalt was added to tungsten carbide in 1923 to produce cemented carbides (Anon., 1989) and to permanent magnet alloys known as Alnicos (cobalt added to alloys of aluminium, nickel and iron) in 1933 (Johnston, 1988).

(i) *Cobalt*

Cobalt has many important uses in industry today, and in some major applications there is no suitable replacement. The most important use of metallic cobalt is as an alloying element in superalloys, magnetic and hard-metal alloys, such as stellite and cemented carbides, cobalt-containing high-strength steels, electrodeposited alloys and alloys with special properties. Cobalt salts and oxides are used as pigments in the glass and ceramics industries, as catalysts in the oil and chemical industries, as paint and printing ink driers and as trace metal additives for agricultural and medical uses (Donaldson, 1986).

Most cobalt is used industrially in the form of cobalt metal as an alloying component and in the preparation of cobalt salts. Estimated consumption as primary raw materials, such as cobalt metal, cobalt oxide and cobalt salts, in selected countries in 1979-87, is presented in Table 9. These countries represented approximately 59% of total consumption in the western world in 1979, 71.5% in 1980, 65% in 1981, 65.5% in 1982, 59.4% in 1983, 53% in 1984, 53.6% in 1985 and 62.5% in 1986. Consumption of cobalt in the western world represented approximately 85% of total world consumption from 1983 to 1988 (Roskill Information Services, 1989).

Table 9. Consumption of cobalt in selected countries, 1979-87 (thousand tonnes)[a]

Country	1979	1980	1981	1982	1983	1984	1985	1986	1987[b]
USA	7.9	6.9	5.3	4.3	5.1	5.4	6.1	6.6	6.9
Japan	2.2	1.9	1.5	1.4	1.5	1.8	1.7	1.7	1.8
UK	2.5	2.3	2.00	1.1	1.2	0.91	0.96	1.6	1.33
France	0.95	1.0	0.75	1.5	0.51	0.62	0.48	0.74	NA
Italy	0.23	0.23	0.19	0.23	0.30	0.38	0.36	0.57	0.56
Sweden	0.29	0.39	0.21	0.21	0.17	0.31	0.36	0.36	0.26
Canada	0.12	0.11	0.10	0.09	0.10	0.11	0.16	0.1[c]	NA

[a]From Roskill Information Services (1989)
[b]Preliminary
[c]Estimated

Industrial consumption of cobalt in the western world averaged 4000 tonnes in 1936-46, 7000 in 1947-52, 10 000 in 1953-62, 16 800 in 1963-72, 19 500 in 1973-78, 21 000 in 1979-81 and 17 500 in 1982-84. Recently, less cobalt has been used in alloys and more in chemical applications. Table 10 presents overall estimates of cobalt consumption in western economies by end use.

Table 10. Evolution of cobalt consumption in selected countries (thousand tonnes)[a]

End product	1950	1960	1970	1981	1987
Alloys	2.85	5.95	6.98	8.74	6.83
Hard metals	0.30	0.73	0.78	1.43	2.02
Magnets	2.10	3.77	3.41	2.47	2.15
Ceramics	0.90	1.60	1.55	1.81	2.04
Chemicals	1.35	2.46	2.79	4.56	6.77
Total	7.50	14.50	15.5	19.01	19.81

[a]From Johnston (1988)

(ii) *Cobalt alloys*

Superalloys are used primarily in the manufacture of components for gas turbine and jet engines. Their combined properties of resistance to hot corrosion and high strength at elevated temperatures contribute to their great commercial and strategic importance. They are used in turbine components that operate at temperatures above 540°C, including ducts, cases and liners, as well as the major turbine blade, vane, disc and combustion-can components. Nickel-based superalloys are usually used for gas turbine components such as discs because they are more workable than cobalt-based superalloys; the latter have excellent resistance to thermal shock and hot corrosion and are used for combustor tubes, stator vanes and diaphragms. Superalloys designed to operate for long periods at temperatures above 900°C sacrifice some of their resistance to oxidation and hot corrosion for increased strength. The nickel-based superalloys are more resistant to oxidation than the cobalt-based superalloys because they have a higher aluminium content and form a better aluminium oxide coating on the alloy. The cobalt-based superalloys primarily form a chromium oxide coating which is not as stable, and when they are used in components subject to extremely high operating temperatures, such as turbine blades and nozzle guide vanes, oxidation-resistant protective coatings are required. Two types of coating can be used: intermetallic and overlay coatings. Intermetallic coatings are applied by heat treatment of the surface of the alloy with cement powders containing aluminides or, less often, silicides. Overlay coatings, which are applied by hot vapour deposition methods, are alloys containing aluminium, chromium and yttrium together with nickel, cobalt or iron. Other applications of the superalloys include airframes, chemical reactors, natural gas transmission pipelines, marine equipment and hazardous waste incineration equipment (Donaldson & Clark, 1985, Donaldson, 1986, Kirk, 1987, Cobalt Development Institute, 1989).

Magnetic alloys. Cobalt is used in a wide variety of magnetic applications, including telecommunication systems, magnetic couplings, electromagnets, meters, loudspeakers, permanent magnet motors and repulsion devices. Alnico magnets, invented in the mid-1930s, are used for heavy-duty applications such as automobile anti-skid braking systems. Consumption of Alnicos declined through the 1960s and 1970s due to the introduction in the 1960s of the less powerful but cheaper and smaller ferrite-ceramic combinations of barium and strontium with iron (Kirk, 1985; Donaldson, 1986; Cobalt Development Institute, 1989; Anon., 1990b).

Magnets combining cobalt with rare-earth minerals were developed in 1967 (Johnston, 1988). The first such magnets were samarium-cobalt alloys, but limited supplies of samarium led to the development of competitive neodymium-iron-boron magnets, which became available commercially in 1983. Rare-earth cobalt magnets have remained important because of their power/size advantages in certain applications. In the 1980s, they contributed to the miniaturization of electrical and electronic equipment. They are used as focusing magnets in travelling wave tubes, as magnetic bearings in ultra-high-speed centrifugal separators and inertia wheels, and in actuators, motors, and generators of various sizes, from watches to 100-hp [74.6-kw] motors (Kirk, 1985; Donaldson, 1986; Anon., 1990b).

Magnetic alloys are also used in medicine to provide an external attractive force. For instance, Alnicos have been used to operate a reed switch in implanted heart pacemakers; samarium-cobalt magnets have been used to hold dental plates in mouth reconstruction, to correct funnel chest and to remove magnetic fragments from the posterior portion of the eye. Magnetic cobalt alloys attached to flexible tubes have also been used to remove iron-containing material from the intestinal and bronchial tubes. Platinum-cobalt and samarium-cobalt magnetic alloys are also used as prostheses, to provide a mechanical closing device in situations where muscle function is impaired. They have been used in the treatment of urinary incontinence in women, to close eyelids in patients with facial paralysis and as colostomy closure devices. In addition, rare-earth-cobalt magnets are used in hearing aids (Donaldson *et al.*, 1986b).

Use of cobalt in magnetic alloys in western countries declined from 28% in 1950, 26% in 1960, 22% in 1970 and 13% in 1981 to 10.8% in 1987 (Johnston, 1988).

Hard-metal alloys (cemented carbides) have essential applications in wear-related engineering because of their high strength, corrosion resistance and ability to retain hardness at elevated temperature. 'Fine', 'extrafine' and 'superfine' special cobalt powders are used as the metal matrix or bonding agent in cemented carbides used in cutting, grinding and drilling tools destined for use on hard materials, such as metals and rocks, and in diamond polishing. Annual industrial consumption of these special powders is approximately 2000 tonnes. Applications of cemented carbides include grinding wheels, moulds, seal rings,

dies, valves, nozzles, pump liners, wear parts subject to severe shock, hot mill rolls, extrusion and can tooling, cutters and slitters, mining, drilling and tunnelling (Kirk, 1985; Donaldson, 1986; Anon., 1989; Cobalt Development Institute, 1989).

Consumption of cobalt for hard-metal alloys in the western world rose from 4% in 1950 to 10.2% in 1987. The tungsten carbide industry accounted for the majority of use in 1987 and diamond polishing for the rest (Johnston, 1988).

Cobalt-containing high-strength steels (maraging steels) are used in the aerospace industry for the manufacture of helicopter drive shafts, aircraft landing gear components and hinges for swing-wing aircraft. Machine component uses include timing mechanisms in fuel injection pumps, index plates for machine tools, bolts and fasteners, barrels for rapid-firing guns and components for cryogenic applications. They also find use in marine equipment, such as deep-submergence vehicles and foil assemblies on hydrofoil ships. In addition, they are used in the manufacture of tools, especially hot forging and stamping dies, close tolerance plastic moulds and die holders (Roskill Information Services, 1989).

Cobalt-containing martensitic stainless maraging steels have been developed for a variety of applications, including in machine construction, the aerospace industry, the chemical industry and naval engineering (Roskill Information Services, 1989).

Electrodeposited nickel-cobalt alloys have good corrosion resistance in many environments and have been used as protective coatings in the production of mirrors and decorative coatings and for electroforming. Electrodeposited cobalt-tungsten alloys retain their hardness at high temperature and are used to improve the wear resistance of hot forging dies. Electrodeposited cobalt alloys containing iron, nickel, platinum or phosphorus have magnetic properties suitable for use in recording systems and computer applications (Donaldson, 1986).

Alloys with special properties. Some cobalt-containing alloys have special applications as dental material, surgical implants, low expansion alloys and springs. Properties that are suitable for dentistry include ease of casting, resistance to tarnish, compatibility with mouth tissues, high strength and stiffness, and low density. Vitallium, a cobalt-chromium alloy, was used for cast denture bases, complex partial dentures and some types of bridgework. A modified alloy is used to fuse porcelain coatings to crowns via a metal bridge.

Cobalt-chromium surgical implant alloys were first used in the 1940s for femoral head cups because of their resistance to corrosion by body fluids; they were subsequently developed for use in bone replacement and bone repair (Donaldson, 1986). The use of metallic implants has played an increasingly important role in orthopaedy: about 500 000 knee, hip and other joint replacements were manufactured in the 1970s (Donaldson *et al.*, 1986b). Total joint arthroplasty using artificial prostheses has become a common surgical technique in the treatment of severely injured or diseased hip joints; other applications

include plates, screws and nails. The implantation of each metallic device is associated with the release of metal, either by corrosion, dissolution or wear or some combination of these processes. Although different materials have been used in the fabrication of prostheses, the preferred material for clinically acceptable knee or hip prostheses is the cobalt-chromium-molybdenum alloy (Donaldson *et al.*, 1986b; Cobalt Development Institute, 1989).

A range of iron-nickel-cobalt alloys is used by the electronics industry for sealing metals in glasses (Donaldson, 1986).

A new chemical use of cobalt is in the manufacture of video tapes. Cobalt is used to coat the basic ferric oxide particles to increase coercivity and reconcile opposing properties of erasability and control of stray magnetic effects. Manufacturers of high-quality audio tapes have also applied this development. Thin films containing cobalt phosphate and cobalt-nickel alloy particles are the most important metallic recording materials. The introduction of cobaltchromium film for perpendicular recording is a potentially very important use of cobalt. Normally, magnetic particles are orientated horizontally on the tape surface; but by getting them to orientate vertically, much closer packing of information is allowed. Magnetic optical recording (using gadolinium-cobalt and terbium-cobalt alloys) and, to a much smaller extent, bubble memory applications also involve cobalt. Another use of cobalt is as an additive in dry electric cells (Donaldson *et al.*, 1988).

(iii) *Cobalt compounds*

Table 11 summarizes the uses of a number of compounds of cobalt. The commercially significant compounds are the oxides, hydroxide, chloride, sulfate, nitrate, phosphate, carbonate, acetate, oxalate and other carboxylic acid derivatives (Donaldson, 1986).

The compounds of cobalt have a variety of end uses. Cobalt oxides and organic compounds are used in paints, ceramics and allied products as decolorizers, dyes, dryers, pigments and oxidizers. Cobalt oxide, used as a ground-coat frit, promotes the adherence of enamel to steel. In the rubber industry, organic cobalt compounds are used to promote the adherence of metal to rubber in steel-belted radial tyres. Cobalt is also used in chemical processes. It is used in the petroleum industry principally as a catalyst for hydrodesulfurization, oxidation, reduction and synthesis of hydrocarbons. The artificial isotope cobalt 60 provides a controllable source of gamma-radiation and is used in physical, chemical and biological research, the treatment of cancer, and in industrial radiography for the investigation of physical strains and imperfections in metals (Kirk, 1985).

Table 11. Industrial uses of cobalt compounds[a]

Compound	Formula	Uses
Acetate(III)	$Co(C_2H_2O_2)_3$	Catalyst
Acetate(II)	$Co(C_2H_2O_2)_2 \cdot 4H_2O$	Driers for lacquers and varnishes, sympathetic inks, catalysts, pigment for oil-cloth, mineral supplement, anodizer, stabilizer for malt beverages
Acetylacetonate	$Co(C_5H_7O_2)_3$	Vapour planting of cobalt
Aluminate	$CoAl_2O_4$	Pigment, catalysts, grain refining
Ammonium sulfate	$CoSo_4(NH_4)_2SO_4 \cdot 6H_2O$	Catalysts, planting solutions
Arsenate	$Co_3(AsO_4)_2 \cdot 8H_2O$	Pigment for paint, glass and porcelain
Bromide	$CoBr_2$	Catalyst, hydrometers
Carbonate	$CoCO_3$	Pigment, ceramics, feed supplements, catalyst
Carbonate (basic)	$2CoCO_3Co(OH)_2 \cdot H_2O$	Chemicals
Carbonyl	$Co_2(CO)_8$	Catalyst
Chloride	$CoCl_2 \cdot 6H_2O$	Chemicals, sympathetic inks, hydrometers, plating baths, metal refining, pigment, catalyst
Chromate	$CoCrO_4$	Pigment
Citrate	$Co_3(C_6H_5O_7)_2 \cdot 2H_2O$	Therapeutic agents, vitamin preparations
Dicobalt manganese tetroxide	$MnCo_2O_4$	Catalyst
Dicobalt nickel tetroxide	$NiCo_2O_4$	Catalyst, anode
Dilanthanum tetroxide	La_2CoO_4	Catalyst, anode
2-Ethylhexanoate	$Co(C_8H_{15}O_2)_2$	Paint and varnish drier
Ferrate	$CoFe_2O_4$	Catalyst, pigment
Fluoride(II)	CoF_2	Fluorinating agent
Fluoride(III)	CoF_3	Flourinating agent
Fluoride	$CoF_2 \cdot 4H_2O$	Catalyst
Fluorosilicate	$CoSiF_6 \cdot 6H_2O$	Ceramics
Formate	$Co(CHO_2)_2 \cdot 2H_2O$	Catalysts
Hydroxide	$Co(OH)_2$	Paints, chemicals, catalysts, printing inks
Iodide	CoI_2	Moisture indicator
Lanthanum trioxide	$LaCoO_3$	Electrode
Linoleate	$Co(C_{18}H_{31}O_2)_2$	Paint and varnish drier
Lithium oxide	$LiCoO_2$	Battery electrode
Maganate	$CoMn_2O_4$	Catalyst, electrocatalyst

Table 11 (contd)

Compound	Formula	Uses
Naphthenate	$Co(C_{11}H_{10}O_2)_2$	Catalyst, paint and varnish drier
Nitrate	$Co(NO_3)_2 \cdot 6H_2O$	Pigments, chemicals, ceramics, feed supplements, cataylst
Oleate	$Co(C_{18}H_{33}O_2)_2$	Paint and varnish drier
Oxalate	CoC_2O_4	Catalysts, cobalt powders
Oxide(II)	CoO	Chemicals, catalysts, pigments
Oxide(II,III)	Co_3O_4	Enamels, semiconductors
Oxides	Mixed metal	Pigments
Phosphate	$Co_3(PO_4)_2 \cdot 8H_2O$	Glazes, enamels, pigments, steel pretreatment
Potassium nitrite	$K_3Co(NO_2)_6 \cdot 1.5H_2O$	Pigment
Resinate	$Co(C_{44}H_{62}O_4)_2$	Paint and varnish drier, catalyst
Sodium oxide	$NaCoO_2$	Battery electrode
Stearate	$Co(C_{18}H_{35}O_2)_2$	Paint and varnish drier, tyre cord adhesives
Succinate	$Co(C_4H_4O_4) \cdot 4H_2O$	Therapeutic agents, vitamin preparations
Sulfamate	$Co(NH_2SO_3) \cdot 3H_2O$	Plating baths
Sulfate	$CoSO_4 \cdot xH_2O$	Chemical, ceramics, pigments
Sulfide	CoS	Catalysts
Tricobalt tetralanthanum decaoxide	$La_4Co_3O_{10}$	Catalysts
Tungstate	$CoWO_4$	Drier for paints and varnishes

[a]From Donaldson (1986); Donaldson *et al.* (1986a)

Cobalt is an effective catalyst for many organic reactions. Its major use in this way is in hydrotreating catalysts, the active components of which are molybdenum and cobalt sulfides. This type of catalyst is used in the synthesis of fuels (Fischer-Tropsch process). The reactions catalysed by cobalt also include the oxo synthesis, in which olefins and carbon monoxide are combined to form aldehydes. The basic catalyst is cobalt carbonyl ($CO_2(CO)_8$), although other cobalt carbonyls can be used. In both the Fischer-Tropsch and the oxo process, the catalysts are normally generated *in situ* in the reactor. Cobalt catalysts are also used in hydrogenation reactions, such as the hydrogenation of nitriles to amines. Cobalt salts are valuable oxidation catalysts, e.g., for the production of terephthalic acid by the oxidation of para-xylene, and the manufacture of phenol by the oxidation of toluene. Cobalt-containing catalysts have also been used for polymerization reactions, e.g.,

polyethylene production by the Amoco process (Morral, 1979; Donaldson, 1986; Donaldson *et al.*, 1986a; Johnston, 1988; Schrauzer, 1989).

Combinations of the oxides of cobalt and those of aluminium, magnesium, zinc and silicon are constituents of blue and green ceramic glazes and pigments (Donaldson, 1986). Cobalt zinc silicate is used in a blue underglaze paint for porcelain articles; the pigment is specially developed to withstand intense heat (Raffn *et al.*, 1988). Cobalt is also used in the glass industry to impart blue colours and to mask the greenish tinge in glass or porcelain caused by iron impurities (Donaldson, 1986).

Spinels are mixed metal oxides with a special crystal structure, based on magnesium and aluminium oxides ($MgAl_2O_4$). These two metals may be partially replaced in the crystal structures by other metals, such as cobalt(II) and chromium(III). Spinels occur naturally and are also produced synthetically. Some cobalt spinels, such as the cobalt-magnesium-aluminium and cobalt-aluminium oxide spinels, are used as pigments (Donaldson *et al.*, 1986a; Sax & Lewis, 1987).

An important use of cobalt is as a drying agent for paints, varnishes, lacquers and printing inks. In these processes, cobalt oleate, resinate and linoleate have been used, but cobalt naphthenate is the more common ingredient (Buono & Feldman, 1979). Cobalt naphthenate is also added to polyester and silicone resins to promote hardening (Bedello *et al.*, 1984).

Consumption in ceramics was relatively stable from 1950 to 1987, ranging from a low of 9.5% to a high of 12% of the total annual cobalt consumption. Use of cobalt in chemicals in 1987 was almost equal to the amount used in alloys. Consumption in chemicals was 17-18% during 1950-70, 24% in 1981 and 34.2% in 1987; use in chemicals during 1987 represented 42.6% of consumption in Europe and 34.4% in the USA. In 1987, applications were: chemicals, catalysts, paint, ink and rubber additives, 24.9%; unspecified, 3.7%; electronics and magnetic tape, 2.8%; medical and veterinary, 1.5%; and plating and anodizing, 1.3% of total cobalt consumption (see Table 10; Johnston, 1988).

Cobalt(*III*) *acetate* has been used as a catalyst in cumene hydroperoxide decomposition (Budavari, 1989). *Cobalt*(*II*) *acetate* is used much more commonly, in the manufacture of drying agents for inks and varnishes, as dressings for fabrics, as catalysts and pigments, and in anodizing and agricultural applications. Mixed metal acetates such as cobalt-tin acetate can also be prepared (Donaldson, 1986; Donaldson *et al.*, 1986a; Budavari, 1989). During the 1960s, cobalt(II) acetate, cobalt chloride and cobalt sulfate (see below) were used as foam stabilizers in malt beverages in Canada, Belgium and the USA. In 1964-66, US breweries reportedly added up to 1.5 μg/ml of cobalt in 20-25% of all beer sold (Morral, 1979; Budavari, 1989; Cobalt Development Institute, 1989).

Cobalt(II) *carbonate* is used in ceramics, as a trace element added to soils and animal feed, as a temperature indicator, as a catalyst and in pigments (Morral, 1979; Sax & Lewis, 1987). Basic cobalt carbonate is often used as a starting material in the manufacture of other chemicals, such as cobalt oxide, cobalt pigments and cobalt salts. It is also used in ceramics and in agriculture (Donaldson, 1986; Donaldson *et al.*, 1986a; Budavari, 1989).

The main use of *cobalt*(*II*) *chloride* hexahydrate is as an intermediate in the manufacture of other cobalt salts. It has been used in invisible inks because, when it is heated, the crystal water is liberated and the almost invisible colour changes to dark blue (Suvorov & Cekunova, 1983). Because of its hygroscopie nature, anhydrous cobalt chloride has been used in barometers and as a humidity indicator in hygrometers; the anhydrous form turns from blue to pink when hydrated. Other uses include the absorption of military poison gas and ammonia, as electroplating flux for magnesium refining, as a solid lubricant and dye mordant, in the preparation of catalysts, for painting on glass and porcelain, as a temperature indicator in grinding, as a fertilizer additive, as a trace mineral supplement in animal feed and in magnetic recording materials (Morral, 1979; Donaldson *et al.*, 1986a; Budavari, 1989). The hexahydrate is used to prepare a standard solution of cobalt for analytical purposes (National Library of Medicine, 1989).

Cobalt chloride is also used in the ceramic and glass industries, in pharmaceuticals for the manufacture of vitamin B_{12} and as catalysts for the oxidation in air of toxic waste solutions containing sulfites and antioxidants (Considine, 1974). It was used as a foam stabilizer in malt beverages in the 1960s (see under cobalt acetate above). Cobalt chloride has been used as an adjunct to iron therapy (if cobalt deficiency is suspected) in patients with refractory anaemia to improve haematocrit, haemoglobin and erythrocyte values. Although cobalt stimulates erythropoietin production, it also blocks certain enzymes involved in iron transport and may stimulate erythrocyte production by causing intracellular hypoxia. Therapeutic doses of 20-300 mg per day orally have been used (Goodman & Gilman, 1975; Goodman-Gilman *et al.*, 1985; Berkow, 1987). According to Reynolds (1989), its general therapeutic use is unjustified.

Cobalt(*II*) *hydroxide* is used in the manufacture of other cobalt compounds, as a starting material to make driers for paints and printing inks, as a catalyst or starting material for catalysts and in solutions for impregnating electrodes in storage batteries (Morral, 1979; Donaldson *et al.*, 1986a; Budavari, 1989).

Cobalt(*III*) *hydroxide* is used as an oxidation catalyst (Sax & Lewis, 1987; Budavari, 1989).

Cobalt molybdenum oxide is used with aluminium oxide as a desulfurization and reforming catalyst in oil refining (Considine, 1974; Donaldson, 1986; Sax & Lewis, 1987).

Cobalt(*II*) *naphthenate* is used primarily as a drying agent in paints, inks and varnishes. Additionally, it is used to enhance the adhesion of sulfur-vulcanized rubber to steel and other metals (i.e., in tyres), as a dressing for fabrics, as a catalyst and as an antistatic adhesive (Buono & Feldman, 1979; Donaldson *et al.*, 1986a; Sax & Lewis, 1987).

Cobalt(*II*) *nitrate hexahydrate* is used mostly in the preparation of catalysts, in pigments, chemicals, ceramics, feed supplements, battery materials, invisible inks, hair dyes and vitamin B_{12} preparations. It serves as an important source of high-purity cobalt for use in the electronics industry (Considine, 1974; Morral, 1979; Donaldson, 1986; Donaldson *et al.*, 1986a; Budavari, 1989).

Cobalt(*II*) *oxide* (CoO) is used as a starting material for the manufacture of other chemicals and catalysts, in pigments such as colour reagents and in ceramics, gas sensors and thermistors (Donaldson *et al.*, 1986a).

Cobalt(*II,III*) *oxide* (Co_3O_4) is used in ceramics and enamels as a colorizer and decolorizer, in semiconductors, as a catalyst, in solar collectors, in grinding wheel coolants and as an implant into the oesophagus of cobalt-deficient ruminants (Morral, 1979; Donaldson, 1986; Donaldson *et al.*, 1986a; Sax & Lewis, 1987).

Lilac pigments containing 22-33 wt % *cobalt*(*III*) *oxide* (CO_2O_3) and bluegreen pigments containing 8-20 wt % cobalt(III) oxide are used in ceramics. A prime enamel has been prepared that contains 0.8 wt % cobalt(III) oxide (Donaldson *et al.*, 1986a). Cobalt(III) oxide monohydrate is used as an oxidation catalyst (Budavari, 1989).

Cobalt(*II*) *sulfate* is the preferred source of water-soluble cobalt salts used in the manufacture of other cobalt chemicals and in electroplating, because it has less tendency to deliquesce or dehydrate than the chloride or nitrate. The monohydrate and heptahydrate are used in plating, feed supplements, to make catalysts, magnetic recording materials, anodizing agents and corrosion protection agents (Morral, 1979; Donaldson *et al.*, 1986a; Budavari, 1989). Cobalt sulfate is also used in the manufacture of vitamin B_{12} during the biological fermentation of molasses by *Pseudoneras denitrificans* (Cobalt Development Institute, 1989). Treating cobalt-deficient soil with 100-150 g/acre [247-371 g/ha] of cobalt sulfate prevents cobalt deficiency in ruminant animals (Jones *et al.*, 1977); injection of cobalt sulfate solution through rumenal fistulas and subcutaneous implantation of slow-release cobalt glasses have been used as alternative methods of supplying cobalt (Donaldson *et al.*, 1986b). In the 1960s, cobalt sulfate was used in various countries as a foam stabilizer in beer (see under cobalt acetate above).

Both α- and β-*cobalt sulfides* are used as catalysts for hydrodesulfurization of organic compounds in petroleum refining. The sulfide is generated as needed by passing hydrogen sulfide over mixed cobalt-molybdenum-aluminium oxides in refinery reactors to form catalytic cobalt sulfide *in situ* (Brauer, 1965; Donaldson *et al.*, 1986a; Budavari, 1989).

2.3 Occurrence

(a) *Geological occurrence*

Cobalt is widely distributed throughout the environment. It is thirty-third in abundance among the elements in the earth's crust, accounting for 0.001-0.002%. The largest concentrations of cobalt are found in mafic (igneous rocks rich in magnesium and iron and comparatively low in silica) and ultramafic rocks; the average cobalt content in ultramafic rocks is 270 mg/kg, with a nickel:cobalt ratio of 7. Sedimentary rocks contain varying amounts of cobalt; average values are 4 mg/kg for sandstone, 6 mg/kg for carbonate rocks and 40 mg/kg for clays or shales. Levels of cobalt in metamorphic rock depend on the amount of the element in the original igneous or sedimentary source. Cobalt has also been found in meteorites (Donaldson, 1986; Donaldson *et al.*, 1986b; Weast, 1988; Budavari, 1989).

Cobalt minerals occur in nature as a small percentage of other metal deposits (particularly copper), generally as sulfides, oxides or arsenides, which are the largest mineral sources. Smaltite ($CoAs_2$) has a cobalt content of 25% and is the most important arsenide found in the USA, Canada and Morocco; other arsenides include safflorite $(CoFe)As_2$, skutterudite ($(Co,Fe)As_3$) and the arsenosulfide (CoAsS; cobaltite), which contains up to 35% cobalt and is found in Cobalt City, Australia, and in Burma. Carrollite ($(Co,Ni)_2CuS_4$) and linnaeite (CO_3S_4) are sulfides which contain 40-50% cobalt and are found in the African copper belt; siegenitc ($(Co,Ni)_3S_4$), which contains 25% cobalt, is found in the mines of Missouri, USA. The supplies of oxides that have the greatest economic importance are heterogenite (CoO(OH)) and sphaerocobaltite (containing 50% cobalt) from Katanga, Zaire, and asbolite (obtained from manganese copper) from New Caledonia (Kipling, 1980; Merian, 1985; Donaldson, 1986; Budavari, 1989; Schrauzer, 1989).

(b) *Occupational exposure*

The main route of absorption during occupational exposure to cobalt is via the respiratory tract, due to inhalation of dusts, fumes or mists containing cobalt or inhalation of gaseous cobalt carbonyl. Occupational exposures occur during the production of cobalt powder, in hard-metal production, processing and use, and in the use of cobalt-containing pigments and driers. Workers who regenerate spent catalysts may also be exposed to cobalt sulfides.

Occupational exposure to cobalt can be measured by analysis of ambient air levels and by biological monitoring, i.e., analyses of cobalt concentrations in blood or urine (for reviews see Ferioli *et al.*, 1987; Alessio & Dell'Orto, 1988; Angerer, 1989; Angerer *et al.*, 1989). (See also Table 19 and p. 419).

Data on exposure to cobalt measured by air and biological monitoring in various industries and occupations are summarized in Table 12. Where possible, the correlations between the concentrations of cobalt in air and biological body fluids are given. Information available to date on blood and urinary concentrations of cobalt indicates that these tests are suitable for assessing exposure on a group basis. The determination of urinary levels of cobalt seems to offer more advantages than that of blood levels. The biological indicator levels are influenced by the chemical and physical properties of the cobalt compound studied and by the time of sampling. It should be noted that the type of compound, the timing of collection of biological samples (normally at the end of a shift) and the analytical methods differ among the studies.

Using biological indicators, the concentration of cobalt in air was related to that in biological fluids; an exposure to 50 µg/m^3 cobalt in air was found to be equivalent to a level of 2.5 µg/L cobalt in blood and 30 µg/L cobalt in urine (Angerer, 1989).

Lehmann *et al.* (1985) took stationary and personal air samples at workplaces during dry grinding (with exhaust facilities) in the mechanical processing of cobalt alloys containing 5-67% cobalt. They found the following airborne concentrations: stationary sampling—total dust, 0.1-0.85 mg/m^3 (median, 0.55 mg/m^3; 13 samples); cobalt in total dust, 0.06-23.3 µg/m^3 (median, 0.4 µg/m^3; 13 samples); personal sampling—total dust, 0.42-2.05 mg/m^3 (median, 0.55 mg/m^3; six samples); cobalt in total dust, 0.2-69.1 µg/m^3 (median, 3.2 µg/m^3; seven samples).

In dental laboratories, concentrations of cobalt were measured during the preparation and polishing of cobalt-chromium alloys and ranged from 30 to 190 g/m^3 (Kempf & Pfeiffer, 1987).

Kusaka *et al.* (1986) carried out extensive personal air monitoring at different stages of hard-metal (cemented carbide) manufacturing and processing; the results, by group of workers, are given in Table 13. A similar study was performed by Lehmann *et al.* (1985), who took stationary and personal air samples during various grinding operations involving hard metal (Table 14). The airborne concentrations of cobalt were mainly below 100 µg/m^3; higher concentrations were observed mainly during dry and wet grinding operations without ventilation or exhaust facilities. Exposure to cobalt during wet grinding presumably originates not only in the workplace but also from cobalt dissolved in coolants. After one

Table 12. Occupational exposures to cobalt in various industries and activities[a]

Industry/activity	No. of samples	Sex	Concentration of cobalt in ambient air	Concentration of cobalt in blood and urine	Comments	References
Hard-metal production (two subgroups)	10	M	a. Mean, 0.09 mg/m^3 b. Mean, 0.01 mg/m^3 (personal samples)	Blood: a. Mean, 10.5 µg/L b. Mean, 0.7 µg/L Urine: a. Mean, [106] µg/L b. Mean, [~3] µg/L Sampling on Friday pm	Significant correlations: air:urine (r = 0.79); air:blood (r = 0.87); blood:urine (r = 0.82)	Alexandersson & Lidums (1979); Alexandersson (1988)
Hard-metal grinding (seven subgroups)	153	-	Up to 61 µg/m^3 (stationary samples)	Median values for all subgroups: serum, 2.1 µg/L; urine, 18 µg/L	Significant correlation: serum (x)/urine (y) y = 2.69x + 14.68	Hartung & Schaller (1985)
Hard-metal tool production (11 subgroups)	170 5	M F	Mean, 28-367 µg/m^3 (personal samples)	Blood: mean, 3.3-18.7 µg/L; urine, 10-235 µg/L Sampling on Wednesday or Thursday at end of shift	Significant correlations (based on mean values): air (x)/urine (y): y = 0.67x + 0.9; air (x)/blood (y): y = 0.004x +0.23; urine (x)/blood (y): y = 0.0065x + 0.23	Ichikawa *et al*. (1985)
Hard-metal production (six subgroups)	27	-	Breathable dust range, 0.3-15 mg/m^3 with 4-17% cobalt	Serum: mean, 2.0-18.3 µg/L; urine, 6.4-64.3 µg/g creatinine	Significant correlation: serum/urine, r = 0.93	Posma & Dijstelberger (1985)
Hard-metal production	26	M	Range, approx. 0.002-0.1 mg/m^3; median, approx. 0.01 mg/m^3 (personal samples)	Urine: Monday at end of shift (a) up to 36 µg/L; Friday at end of shift (b) up to 63 µg/L	Significant correlations: air (x)/urine (y): (a) y = 0.29x + 0.83; (b) y = 0.70x + 0.80	Scansetti *et al*. (1985)
Cobalt powder production Presintered tungsten carbide production Hard-metal use	6 15 7	- - - -	a. Range, 0.675-10 mg/m^3 b. Range, 0.120-0.284 mg/m^3 c. Range, 0.180-0.193 mg/m^3	Urine: a. mean, 35.1 µg/L b. mean, 9.6 µg/L c. mean, 11.7 µg/L Sampling on Sunday (24 h)	Times of sampling: Monday am for basic exposure level; Friday evening for cumulative exposure level	Pellet *et al*. (1984)

Table 12 (contd)

Industry/activity	No. of samples	Sex	Concentration of cobalt in ambient air	Concentration of cobalt in blood and urine	Comments	References
Cobalt powder and cobalt salt production (seven subgroups)	40	M	Mean, 46-1046 µg/m^3 (stationary samples)	Blood: mean, 5-48 µg/L; urine: mean, 19-438 µg/L Post-shift sampling	Significant correlations: air/urine; air/blood; blood (x)/urine (y): y = 7.5x + 11.2	Angerer *et al*. (1985)
Cobalt oxide processing and cobalt salt manufacture	49	M	Median, 0.52 mg/m^3; range, 0.1-3.0 mg/m^3 (personal samples)	Urine: mean, 0.34 mg/L; range, 0.1-0.9 mg/L	Poor correlation air:urine	Morgan (1983)
Painting porcelain with soluble cobalt salts	46	F	a. Range, 0.07-8.61 mg/m^3	Blood: a. Mean, 2.16 µg/L; b. Mean, 0.63 µg/L;	Significant correlation: blood/urine (r = 0.88)	Christensen & Mikkelsen (1986)
Painting porcelain with slightly soluble cobalt salts	15	F	b. Range, 0.05-0.25 mg/m^3 (personal samples)	Urine a. Mean, 8.35 µg/ mmol creatinine; b. Mean, 0.13 µg/ mmol creatinine		

[a]From Angerer & Heinrich (1988)

Table 13. Airborne concentrations of cobalt at various stages in the manufacture and processing of hard metals[a]

Activity	No. of workers	No. of samples	Concentration of cobalt ($\mu g/m^3$)	
			Mean ± SD	Range
Powder	12	38	688 ± 1075	6-6388
Press				
Rubber	4	19	473 ± 654	48-2905
Machine	25	27	85 ± 95	4-407
Sintering	21	38	28 ± 26	2-145
Shaping	47	129	126 ± 191	6-1155
Grinding				
Wet	131	205	53 ± 106	11-1247
Dry	1	2	1292 ± 179	1113-1471
Electron discharging	5	5	4 ± 1	1-5
Blasting	2	5	3 ± 1	1-4

[a]From Kusaka *et al.* (1986)

Table 14. Concentrations of cobalt in total dust during hard-metal grinding[a]

Type of grinding/ type of sample	No. of workplaces	No. of companies	Sampling time (h)	Concentration of cobalt ($\mu g/m^3$)	
				Median	Range
Dry grinding with exhaust facilities					
Stationary	16	6	2	3.1	0.1-203.5
Personal	16	5	2	12.3	0.5-223.8
Wet grinding without exhaust facilities					
Stationary	9	5	2	12.8	2.4-90.4
Personal	14	5	2	42.5	7.9-208.0
Wet grinding with exhaust facilities					
Stationary	8	1	2	6.9	1.1-11.8
Personal	7	1	2	13.7	1.3-29.9

[a]From Lehmann *et al.* (1985)

week of use, levels of up to 118 mg/kg were found in the coolant; after four weeks, up to 182 mg/kg were observed (Lehmann *et al.*, 1985). This finding was confirmed by Hartung (1986). Einarsson *et al.* (1979) studied the dissolution of cobalt in nine commercial cutting fluids one to five days after use in the grinding of hard-metal alloys. After one day, most of the cobalt liberated by grinding was found in solution; this percentage decreased when grinding was continued using the same coolant fluid. Only a small fraction of the cobalt was found as particles in the circulating fluid. The authors concluded that the bulk probably remains in the sediment in the storage tank.

The concentration of cobalt dust was measured in the air of a Danish porcelain factory in 1981. In personal air samples taken for 19 female plate painters, the levels were 0.07-8.61 mg/m^3. The cobalt levels in blood and urine were measured in 1982 in 46 female plate underglaze painters exposed to soluble cobalt silicate and in 51 female plate overglaze painters with no exposure to cobalt. The mean levels in the blood of exposed persons (longer than four weeks) were 2.16 µg/L (range, 0.2-24; median, 1.0) compared with 0.24 µg/L in the controls (range, 0.05-0.6; median, 0.2). Mean levels in urine were: 77 µg/L (median, 26; range, 2.2-848) in exposed workers and 0.94 µg/L (median, 0.3; range, 0.05-13.8) in unexposed workers (Mikkelsen *et al.*, 1984; Christensen & Mikkelsen, 1986). In 1984, after conditions in the workplace had been improved, the concentration of cobalt in air had decreased to about 0.05 mg/m^3. The mean urinary level of cobalt in 38 of the 46 workers investigated originally who were selected for urine analysis was 2.6 µg/mmol creatinine (range, 0.16-16.1) compared to 4.2 µg/mmol creatinine (range, 0.24-29.1) in 1982. A significant correlation was observed between blood cobalt and creatinine-corrected urinary cobalt levels ($p < 0.001$). In 1982, in a factory using a slightly soluble cobalt silicate, the mean cobalt levels in blood and urine from 15 female plate painters were 0.63 µg/L (median, 0.60; range, 0.37-1.58) and 0.13 µg/mmol creatinine (median, 0.11; range 0.02-0.37), respectively (Christensen & Mikkelsen, 1986; see also Table 12).

(c) *Air*

Levels of cobalt in the ambient air are a function of the extent to which particles of soil are dispersed by the wind. They are higher near factories in which cobalt is used, and atmospheric concentrations of cobalt in remote areas are very low: less than 1 ng/m^3 in the Antarctic. In other areas, ambient air concentrations are usually around 1 ng/m^3. Levels exceeding 10 ng/m^3 have been reported in heavily industrialized cities (Elinder & Friberg, 1986). Combustion of organic materials containing cobalt is reported to be an additional source of emission (Lange, 1983; Angerer & Heinrich, 1988). Coal contains up to 40 mg/kg

(average, 1 mg/kg) cobalt (Angerer & Heinrich, 1988), and hard coal contains about 8 mg/kg (Schrauzer, 1989). Merian (1985) estimated a global annual generation of about 5000 tonnes of cobalt from the burning of coal.

A survey of atmospheric trace elements in the UK in 1977 showed ambient concentrations of cobalt in the range of 0.04-6.5 ng/kg at seven stations sampled. Around 57% of the cobalt content was in a soluble form (Cawse, 1978).

(d) *Tobacco smoke*

The content of cobalt in cigarettes has been studied by means of neutron activation; different brands of tobacco were found to contain < 0.01-2.3 mg/kg dry weight (Wyttenbach *et al*., 1976; Iskander, 1986; Iskander *et al*., 1986). When cigarettes were smoked in a standard smoking machine, 0.5% of the cobalt content of the cigarette was transferred into smoke condensate (Nadkarmi & Ehmann, 1970).

(e) *Water and sediments*

Uncontaminated samples of fresh water generally contain low concentrations of cobalt, ranging from 0.1 to 10 μg/L (Schrauzer, 1989). Concentrations of 0.1-5 μg/L have been found in drinking-water (Elinder & Friberg, 1986).

Approximately 20 000 tonnes of cobalt are transported annually by rivers to oceans, where they are precipitated (Merian, 1985). A cobalt content of 74 mg/kg has been measured in sediments (Schrauzer, 1989). Natural transport is not significantly affected by mining activities or industrial use. The concentration of cobalt in seawater is normally quite low, at 0.002-0.007 μg/L, the level decreasing with increased depth (Knauer *et al*., 1982).

(f) *Foods and beverages*

Human dietary intake of cobalt is highly variable; Table 15 summarizes estimated total intake of cobalt from food in various countries. Most of the cobalt ingested is inorganic: vitamin B_{12}, which occurs almost entirely in food of animal origin, accounts for only a very small fraction. Vegetables contain inorganic cobalt but little or no vitamin B_{12} (Friedrich, 1984; Donaldson *et al*., 1986b).

Values for the cobalt content of foods vary widely between reports, even among analyses of the same foods, probably owing as much to differences in environmental cobalt levels as to analytical diffculties or inadequate analytical techniques. Green leafy vegetables and fresh cereals are the richest and most variable sources of cobalt (0.2-0.6 μg/g dry mass), while dairy products, refined cereals and sugar contain the least cobalt (0.01-0.03 μg/g dry mass; Donaldson *et al*., 1986b). Plant products have been estimated to contribute up to 88% of the total cobalt in the Japanese diet (Yamagata *et al*., 1963). Normal cows' milk contains

Table 15. Total daily intake of cobalt from food *per caput*

Country	Daily intake *per caput* (μg)	Reference
Canada	45-55	Kirkpatrick & Coffin (1974)
Finland	13	Varo & Koivistoinen (1980)
Germany	17	Pfannhauser (1988)
	15	Pfannhauser (1988)
	5-10 (vitamin B_{12} only)	Schormüller (1974)
Hungary	100	Lindner-Szotyori & Gergely (1980)
Italy	9	Pfannhauser (1988)
Japan	19.5	Yamagata *et al*. (1963)
Netherlands	5-7	Pfannhauser (1988)
Spain	25	Barberá & Farré
UK (vitamin B_{12} only)	7.0	Spring *et al*. (1979)
USA	5-6	Harp & Scoular (1952)
USSR	1.7	Reshetkina (1965)
	31	Nodiya (1972)

very little cobalt (average, about 0.5 μg/L); shelled eggs have been reported to contain 0.03 μg/g (Donaldson *et al.*, 1986b). Varo and Koivistoinen (1980) found concentrations of 30-50 μg/kg dry weight in fish and vegetables; that in meat and dairy products was 10 μg/kg. The daily diet of the 70-kg 'reference man' contains cobalt at 0.01-0.02 mg/kg fresh weight (based on 20-40 μg/day intake) (Donaldson *et al.*, 1986b).

In 15 commercial beers analysed in 1965 using a colorimetric method, the levels of cobalt were well below 0.1 mg/L. When cobalt salts had been added during processing, values of up to 1.1 mg/L were recorded (Elinder & Friberg, 1986).

The cobalt content of five brewed teas averaged 0.2 μg/g (range, 0.16-0.34) and that of seven brewed coffees, 0.75 μg/g (range, 0.42-2.0 μg/g; Horwitz & Van der Linden, 1974).

(g) *Soils and plants*

In one study, the cobalt content of soils ranged from 1 to 40 mg/kg (Merian, 1985) with an average of 8 mg/kg (Schrauzer, 1989). In general, cobalt tends to be deficient in areas where there is granite, sand or limestone and in volcanic and peaty soils. Good drainage may reduce cobalt content (Kipling, 1980). The solubilities of cobalt compounds are pH-dependent, and cobalt is more mobile in acid soils than in alkaline soils (Schrauzer, 1989).

In industrialized areas, up to 75 mg/kg cobalt have been found in the soil around factories using cobalt powders, and higher concentrations may occur in waste-metal dumps (Kipling, 1980).

The uptake of cobalt by plants is species-dependent: cobalt is hardly detectable in green beans and the level is exceedingly low in radishes (Schrauzer, 1989). Leafy plants, such as lettuce, cabbage and spinach, have a relatively high cobalt content, whereas the content is low in grasses and cereals (Kipling, 1980). It is as yet unknown whether cobalt is essential for plants. In some cases, small amounts of cobalt produce positive growth effects, but these are dose-dependent and may be indirect (Schrauzer, 1989). It has been suggested that the element is necessary for the fixation of nitrogen in vegetables that are relatively rich in cobalt. Cobalt concentrations in pastures vary according to season and the presence of fertilizers (Kipling, 1980).

(h) *Human tissues and bodyfluids*

Over the years, there has been a progressive downward adjustment in the reported normal levels of cobalt in human tissues and body fluids as a result of improvements in analytical methodology. Concentrations of cobalt observed in the blood and urine of the general population are summarized in Table 16. The concentrations in body fluids are well below the microgram per litre level; mean concentrations reported in serum range from 0.1 to 0.3 µg/L.

Alexandersson (1988) found that smokers with no occupational exposure had a significantly higher mean cobalt concentration in urine (0.6 µg/L; SD, 0.6) than nonsmokers (0.3 µg/L; SD, 0.1). There was no difference between smokers and nonsmokers in the cobalt levels in blood.

Patients in various stages of renal failure showed a significantly higher serum concentration of cobalt than a control group, but there was no correlation to the degree of renal insufficiency. Haemodialysis did not influence the levels, whereas kidney transplantation reduced them (Lins & Pehrsson, 1984). Values for whole blood were a little higher than serum concentrations but were not well documented (Iyengar & Woittiez, 1988). In urine samples obtained from normal adults, the concentrations of cobalt were reported to be approximately 0.1-2 µg/L (see Table 16). Greatly increased urinary levels have been reported for persons taking multivitamin pills containing cobalt (Reynolds, 1989).

Considerable differences have been found in the levels of cobalt in hair, ranging from 0.4 to 500 µg/kg (Iyengar & Woittiez, 1988).

In autopsy studies, the liver has been shown to contain the highest concentration of cobalt, with individual values ranging from 6 to 151 µg/kg (median, 30 µg/kg) in seven studies. This may be attributed, at least in part, to differences in food intake, since this

Table 16. Concentrations of cobalt in urine, serum and whole blood of persons not exposed occupationally to cobalt

Urine		Serum (or plasma) (µg/L)	Whole blood (µg/L)[a]	Reference
Mean	Range			
-	-	0.108 ± 0.06	-	Versieck *et al*. (1978)
-	-	-	0.5 ± 0.1	Alexandersson & Swensson (1979)
0.4 µg/L	0.2-1.2	-	0.5	Alexandersson & Lidums (1979)
-	-	0.195 ± 0.015 (plasma)	-	Kasperek *et al*. (1981)
0.18 µg/creatinine	-	-	-	Kennedy *et al*. (1981)
0.38 µg/L	0.1-0.75 µg/L	-	-	Schumacher-Wittkopf & Angerer (1981)
1.3 µg/L	-	-	-	Hartung *et al*. (1982)
-	-	0.01-1.9	-	Masiak *et al*. (1982)
-	-	-	0.09 ± 0.02	Ostapczuk *et al*. (1983)
-	-	0.15 ± 0.07 (plasma)	-	Andersen & Høgetveit (1984)
0.94 µg/L	0.05-13.8 µg/L	-	-	Mikkelsen *et al*. (1984)
-	4.6 µg/g creatinine	-	-	Posma & Dijstelberger (1985)
0.41 µg/L or 0.28 µg/g creatinine	-	-	-	Scansetti *et al*. (1985)
2.0 µg/L	-	-	1.9 ± 1.1	Ichikawa *et al*. (1985)
-	-	0.28	-	Lewis *et al*. (1985)
0.09 µg/mmol creatinine	0.004-1.21 µg/ mmol creatinine	-	0.24 (0.05-0.6)	Christensen & Mikkelsen (1986)
-	-	0.1	-	Hartung (1986)
-	-	0.73 ± 0.10 (plasma)	-	Collecchi *et al*. (1986)
0.4 µg/L	0.1-2.2 µg/L	-	0.5 (0.1-1.2)	Alexandersson (1988)
0.01 µg/L	-	-	0.2-1.3	Angerer *et al*. (1989)

[a]Range, or mean ± standard deviation
-, not given

organ stores vitamin B_{12} (Iyengar & Woittiez, 1988). In New Zealand, 96 human liver samples showed a mean concentration of 120 µg/kg wet weight cobalt, with no significant difference between sex, age or regional district (Pickston *et al.*, 1983). Levels of cobalt were lower in liver carcinoma tissue than in normal hepatocytes from the same liver samples (Kostić *et al.*, 1982). The total cobalt content of a 70-kg, unexposed man was estimated to be about 1.5 mg. The total amount of vitamin B_{12} in the body of an adult is about 5 mg, corresponding to 0.25 mg cobalt, of which 50-90% is localized in the liver (Schrauzer, 1989).

Cobalt concentrations in the hearts of patients dying from myocardiopathy associated with the consumption of beer containing cobaltous salts were found to be 10 times higher than in normal cardiac muscle (Sullivan *et al.*, 1%8).

(i) *Iatrogenic exposure*

Cobalt is the major constituent (approximately 62%) of porous-coated cobalt-chromium alloys used in surgical implants; therefore, body levels of cobalt (urine, serum) have been used as an index of the wear rate of the prostheses. Table 17 summarizes the results of several investigations on trace metal concentrations in the body fluids of patients with total knee and hip arthroplasty with metal prostheses. Cobalt-containing particles have also been identified by microscopic examination of tissues adjacent to prostheses (Hildebrand *et al.*, 1988; Sunderman *et al.*, 1989).

Certain authors observed significant increases in mean concentrations of cobalt in the serum or urine from patients with various metal implants (especially those with metal-to-metal contact), while others found that the concentrations of this metal were only sporadically elevated. These discordant results may reflect greater rates of release of metals from implants with metal-to-metal versus metal-to-polyethylene articular surfaces, as well as differences among the cobalt-containing alloys used (e.g., porous-coated versus non-porous surfaces and cemented versus cementless implants). Analytical limitations may also play a major role, since the concentrations of cobalt in the serum and urine specimens from control subjects far exceeded the currently accepted ranges. Analytical inaccuracies in previous studies probably resulted from metal contamination during specimen collection, inattention to quality assurance techniques and/or inadequate instrumental sensitivity and specificity (Sunderman *et al.*, 1989).

Raithel *et al.* (1989) investigated the cobalt content in tissues surrounding hip arthroplasties and in distant muscle samples. From 10 patients with loosening of prostheses, tissue samples were taken from the implanted cup (polyethylene surface to avoid metal-to-metal friction), from the implanted shaft and from the musculus vastus lateralis, and the patients received new hip prostheses. The old cobalt-chromium-molybdenum types

Table 17. Cobalt concentrations is body fluids of patients with total hip or knee arthroplasty[a]

Study	No. of patients	Period of observation	Type of implant	Observations	Concentration of cobalt		
					Urine	Blood	Synovial fluid
Coleman *et al*. (1973)	12	3 weeks to 32 months	Hip, cobalt-molybdenum-chromium alloy, cemented, nonporous, with or without polyethylene component (C cast alloy)	Increased cobalt and chromium in blood and urine, only with metal-to-metal contact (no polyethylene)	15-73 µg/L after 1 year	4.5-16 µg/L after 1 year	
Jones *et al*. (1975)	4	Not given	Hip, cobalt-chromium-molybdenum alloy, cemented, nonporous, with metal-to-metal contact (C cast alloy)	Increased cobalt in urine and (in one case) in synovial fluid and liver, bone and brain tissues	22-55 µg/L	-	250 µg/L 0.5-3 mg/kg
Miehlke *et al*. (1981)	30	6 months to 10 years	Knee, colbalt-chromium alloy, cemented, nonporous, with or without polyethylene component	Increased colbalt and chromium in synovial fluid and serum, especially with metal-to-metal contact	-	0.16-79 µg/L	0.36-7200 µg/L
Jorgensen *et al*. (1983)	10	Not given	Hip, cobalt-chromium-molybdenum alloy, porous-coated or nonporous cementless	Increased cobalt in urine,especially in patients with porous-coated implants	Porous: mean, 14.2 µg/L; nonporous: mean, 8.4 µg/L		
Black *et al*. (1983)	15	1 day to 6 months	Hip, cobalt-chromium-nickel alloy, cemented, nonporous, polyethylene cup (cobalt-chromium/UMHWPE THRs[a]	Increase in serum chromium (peak at 15 days), serum nickel (peak at 6 months); normal serum cobalt	-	-	-
Bartolozzi & Black (1985)	14	1 to > 30 days	Hip, cobalt-chromium alloy, cemented, nonporous, polyethylene cup	Increase in chromium (serum peak at 10 days, urine peak at 15 days)	Peak, 26.2 ng/mg creatinine	Peak, 39.9 pg/mg protein	
Pazzaglia *et al*. (1986)	17	7-15 years	Hip, cobalt-chromium-molybdenum alloy, cemented, nonporous, with or without polyethylene cup	Increased cobalt and chromium in urine and chromium in plasma	0.9-1.05 µg/L	-	
Jones & Hungerford (1987)	14	1 week to 1 year	Hip, cobalt-chromium alloy, cementless, porous-coated, polyethylene cup (PCA®)	Increased urinary nickel in 2 of 14 patients at 6 months; increased urinary nickel and cobalt in 3 of 4 measured at 1 year	-	-	-

Table 17 (contd)

Study	No. of patients	Period of observation	Type of implant	Observation	Concentration of cobalt		
					Urine	Blood	Synovial fluid
Braun *et al*. (1986)	22	5 months to 3 years	Hip, cobalt-chromium-molybdenum alloy, cementless, porous-coated, polyethylene cup, fixed	Increased urinary chromium	-	-	-
Raithel *et al*. (1989)	15	2 years	Fixed hip, cobalt-chromium-nickel-molybdenum alloy, cemented, nonporous with polyethylene cup	Increased serum cobalt	-	1.8 µg/;	
	10	5-15.5 years (mean, 12.5)	Loose hip, coblat-chromium-nickel molybdenum alloy, cemented, nonporous with polyethylene cup; old hip replaced	Increased urinary chromium, nickel and cobalt, increased serum nickel	3.8 µg/L	-	
Sunderman *et al*. (1989)	28	1 day to 2.5 years	Knee or hip, cobalt-chromium alloy (ASTM F-75-82); porous-coated, 10 cemented, 18 cementless with polyethylene	Slight increase in serum and urinary cobalt in knee prostheses. 2 patients, substantially elevated levels (7 weeks and 22 months post-arthroplasty, with loosening of prostheses); serum and urinary chromium levels also elevated in one patient	1 µg/g creatinine (6-120 weeks) 7.7 µg/g creatinine and 5.6 µg/L in the 2 patients	0.15 µg/L (6-120 weeks) 1 and 1.15 µg/L in the 2 patients	

[a]Ultrahigh molecular weight polyethylene (total hip replacements)

(ASTM F 75-74) were replaced after 5-15.5 years (median, 12.5 years). The concentrations of cobalt in the tissues surrounding the shaft ranged from 367 to 6510 µg/kg (median, 868 µg/kg), and those in tissues surrounding the cup, from 98 to 16 293 µg/kg (median, 1080 µg/kg). Muscle tissue contained 24-151 µg/kg (median, 124 µg/kg) cobalt.

Hildebrand *et al.* (1988) also found extremely high concentrations of cobalt, up to three orders of magnitude (140 µg/g dry weight) above the normal values, in connective tissue taken on a Vitallium plate.

(j) *Others*

The total concentration of cobalt in cement made in Asia ranged from 8.1 to 14.2 µg/g. The metal existed mainly as insoluble salts; the concentration of water-soluble cobalt was 0.39-0.65 µg/g (Goh *et al.*, 1986). The cobalt content in 42 US cement samples was < 0.5 µg/g (Perone *et al.*, 1974).

The cobalt content of 30 household cleaning products sold in Spain in 1985 ranged from 0.1 to 14 mg/L; the highest levels were found in two bleaches, containing 1.1 and 1.4 mg/L (Vilaplana *et al.*, 1987).

2.4 Regulatory status and guidelines

Occupational exposure limits and guidelines established in different parts of the world are given in Table 18.

Table 18. Occupational exposure limit values for cobalt[a]

Country or region	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1985	0.1 cobalt, metal fumes and dust	TWA
Belgium	1989	0.05 cobalt, metal dust and fumes (as Co)	TWA
Bulgaria	1985	0.5 cobalt and compounds (as Co); cobalt, metal dust and fumes (as Co)	TWA
Canada	1980	0.1 cobalt as metal dust and fume	TWA
Czechoslovakia	1985	0.05 cobalt and compounds (as Co)	TWA
		0.1 cobalt and compounds (as Co)	max
Denmark	1988	0.1 cobalt carbonyl (as Co); cobalt hydro-carbonyl (as Co)	TWA
		0.05 cobalt in the form of powder, dust and fumes and inorganic compounds (as Co)	TWA
Finland	1987	0.05 cobalt and inorganic compounds (as Co)	TWA

Table 18 (contd)

Country or region	Year	Concentration (mg/m^3)	Interpretation[b]
Hungary	1987	0.1 cobalt and compounds (as Co)	TWA
		0.2 cobalt and compounds (as Co)	STEL
Indonesia	1987	0.1 cobalt and compounds (as Co)	TWA
Italy	1987	0.1 cobalt, metal dust and fumes (as Co)	TWA
Mexico	1987	0.1 cobalt, metal dust and fumes (as Co)	TWA
Netherlands	1986	0.1 cobalt, metal dust and fume (as Co)	TWA
Norway	1981	0.05 cobalt and compounds (as Co)	TWA
Poland	1985	0.5 cobalt and compounds (as Co); cobalt, metal dust and fumes (as Co)	TWA
Romania	1985	0.2 cobalt and cobalt oxide and cobalt, metal dust and fumes (as Co)	TWA
		0.5 cobalt and cobalt oxide and cobalt, metal dust and fumes (as Co)	max
Sweden	1988	0.05 cobalt and inorganic compounds (as Co)	TWA
Switzerland	1987	0.1 cobalt dust and compounds (as Co)	TWA
Taiwan	1987	0.1 cobalt, metal dust and fumes (as Co)	TWA
UK	1987	0.1 cobalt and compounds (as Co)	TWA
USA			
ACGIH	1989	0.05 cobalt (as Co) metal dust and fumes	TWA
		0.1 cobalt carbonyl (as Co); cobalt hydrocarbonyl (as Co)	Guide-lines
OSHA	1988	0.1 cobalt (as Co) metal dust and fume	TWA
USSR	1987	0.5 cobalt and compounds (as Co); cobalt, metal dust and fumes (as Co)	max
		0.01 cobalt hydrocarbonyl and decomposition production (as Co)	
Venezuela	1987	0.1 cobalt, metal dust and fumes (as Co)	TWA
Yugoslavia	1985	0.1 cobalt and compounds (as Co); cobalt, metal dust and fumes (as Co)	TWA

[a]From Direktoratet for Arbeidstilsynet (1981); Arbeidsinspectie (1986); Cook (1987); Health and Safety Executive (1987); National Swedish Board of Occupational Health (1987); Arbejdstilsynet (1988); National Institute for Occupational Safety and Health (1988); American Conference of Governmental Industrial Hygienists (ACGIH) (1989); US Occupational Safety and Health Administration (OSHA) (1989); United Nations Environment Programme (1990).
Guidelines and standards are generally prepared by scientific bodies and sometimes become official standards, or they are recognized and applied in practice on a voluntary basis as a guide for monitoring the working environment or for technical prevention.
[b]TWA, 8-h time-weighted average; STEL, 10-15-min short-term exposure limit

2.5 Analysis

Typical methods for the analysis of cobalt in air, water, various working materials, food and biological materials are summarized in Table 19.

Table 19. Methods for the analysis of cobalt

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Urine	Digestion with nitric/sulfuric acid; ion-exchange separation	GF/AAS	0.1 µg/L	Lidums (1979)
	Chelatization, extraction	GF-AAS	0.1 µg/L	Schumacher-Wittkopf & Angerer (1981)
	Dilution with nitric acid	GF-AAS	Not given	Hartung *et al*. (1983)
	Dilution with nitric acid	GF-AAS	2 µg/L	Pellet *et al*. (1984)
	Digestion with sulfuric, nitric, perchloric acid; chelation, extraction	F-AAS	1 µg/L	Ichikawa *et al*. (1985)
	Direct analysis	GF-AAS	6 µg/L	Bouman *et al*. (1986)
	N,*N*-Hexamethyleneammonium-hexamethylenedithiocarbamic acid/xylene extraction	GF-AAS (Z)	1 µg/L (0.2 µg/L for 6 ml urine)	Bouman *et al*. (1986)
	Magnesium nitrate modifier	GF-AAS (Z)	2.6 µg/L	Kimberley *et al*. (1987)
Blood, urine	Dilution with nitric acid	GF-AAS (Z)	0.1 µg/L	Christensen et al. (1983)
	Protein precipitation; dilution with nitric acid	GF-AAs (Z)	0.1 µg/L	Christensen & Mikkelsen (1986)
Blood	Digestion with nitric, sulfuric acid	GF-AAS	0.1 µg/L	Lidums (1979)
	Dilution and matrix modification	GF-AAS	0.2 µg/L	Delves *et al*. (1983)
	Freeze-dried, low-temperature ashing; resolved in nitric acid	GF-AAS (Z)	0.8 µg/L	Ichikawa *et al*. (1985)
Blood, tissues	Digestion with nitric, sulfuric, perchloric acid	ICP	10 µg/kg blood 0.2 µg/kg tissue	National Institute for Occupational Safety and Health (1985)
Blood serum	Wet digestion with nitric, sulfuric, perchloric acid; chelation, extraction	GF-AAS	0.1 µg/L	Barfoot & Pritchard (1980)
Serum	Dry ashing at 450ºC	NAA	Not given	Versieck *et al*. (1978)

Table 19 (contd)

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Plasma, urine	Palladium matrix modificaiton	GF-AAS (Z)	0.15 µg/L	Sampson (1988)
Biological materials	Wet digestion with nitric, sulfuric acid	ADPV	1 ng/L in the analyte solution	Ostapczuk *et al*. (1983)
Air	Digestion with nitric acid	GF-AAS	Not given	Hartung *et al*. (1983)
	Digestion with nitric, perchloric acid	ICP	1 µg/sample	National Institute for Occupational Safety and Health (1984a)
	Digestion with aqua regia	F-AAS	0.6 µg/ sample	National Institute for Occupational Safety and Health (1984b)
	Digestiion with hydrochloric, nitric acid	GF-AAS	1 µg/m^3	Ichikawa *et al*. (1985)
	Digestion with nitric acid	GF-AAS	20 ng/m^3 (sample volume 1.5 m^3	Kettrup & Angerer (1988)
Seawater	Direct analysis	DPCSV	6 pmol (0.4 ng)	Donat & Bruland (1988)
Water	Chelation with ammonium pyrrolidinedithiocarbamate; preconcentration on activated charcoal	F-AAS, ICP	< µg/L	Berndt *et al*. (1985)
Food	Dry digestion; triethanolamine electrolyte	Adsorption voltammetry	Not given	Meyer & Neeb (1985)
	Dry digestion; chelation with sodium di(trifluoroethyl)dithiocarbamate	GC	50 ng/ sample	Meyer & Neeb (1985)
	Digestion with nitric acid; extraction with cupferron, chloroform	F-AAS	1.4 ng/ml	Barberá *et al*. (1986)
Milk	Ashing in muffle furnace	GF-AAS (Z)	Not given	Gunshin et al. (1985)

[a]Abbreviations: GF-AAS, graphite furnace-atomic absorption spectrometry; F-AAS, flame atomic absorption spectrometry; Z, background correction for Zeeman effect; ICP, inductively coupled plasma emission spectrometry; NAA, neutron activation analysis; ADPV, adsorption differential pulse voltammetry; DPCSV, differential pulse cathodic stripping voltammetry; GC, gas chromatography

Methods for quantitative analysis include graphite furnace-atomic absorption spectrometry (GF-AAS), inductively coupled plasma emission spectrometry (ICP), neutron activation analysis and electrochemical methods such as differential pulse anodic stripping voltammetry (DPASV). ICP and X-ray fluorescence appear to be too insensitive for the determination of cobalt in environmental and biological matrices; this is also true of the older photometric methods, which also showed lack of specificity.

With NAA, cobalt can be determined at the nanogram per kilogram level. This method offers the advantage that it requires little sample preparation, but its application is restricted to a few highly specialized laboratories. Voltammetry and, in particular, GF-AAS are much more common and permit determination of cobalt at the nanogram per kilogram level. GF-AAS, in comparison to voltammetry, does not usually require complete digestion of the sample, which makes the technique more practicable.

Air samples are collected on cellulose ester membrane filters, wet-digested with nitric and perchloric acids or aqua regia and analysed by AAS or ICP (National Institute for Occupational Safety and Health, 1984a,b; Kettrup & Angerer, 1988). The routine procedures do not permit identification of individual cobalt compounds.

Analysis of cobalt in soil, food, industrial samples and human tissues also requires complete digestion of the matrices. The US Environmental Protection Agency (1983) established standard methods using ICP and GF-AAS for the chemical analysis of water and wastes. An extremely low detection limit of 1.2 ng/1 natural water was obtained using cation-exchange liquid chromatography with luminol chemiluminescence (Boyle *et al.*, 1987). A similarly high sensitivity, 0.64 ng/kg, is obtained by photoacoustic spectroscopy after extraction with 2-nitroso-1-naphthol/*meta*-xylene (Kitamori *et al.*, 1986).

Determination of cobalt in whole blood, plasma, serum and urine is used as a biological indicator of exposure to cobalt (Ichikawa *et al.*, 1985; Ferioli *et al.*, 1987; Angerer *et al.*, 1989). Choice of specimen, sampling strategies, specimen collection, transport, storage and contamination control, as well as quality control and quality assurance procedures (Schaller *et al.*, 1987), are of fundamental importance for an adequate monitoring programme. GF-AAS and DPASV are practical and reliable techniques that furnish the requisite sensitivity for measuring cobalt concentrations in biological samples. The detection limits for cobalt determination by GF-AAS analysis with Zeeman background collection are below 0.6 μg/L of body fluids, depending on the type of sample preparation.

Greater sensitivity in DPASV analysis can be achieved by using a dimethylglyoxime-sensitized mercury electrode, which provides detection limits down to 1 ng/L for cobalt in biological media (Ostapczuk *et al.*, 1983, 1984).

Koponen *et al.* (1982) analysed cobalt-containing airborne dusts from hard-metal manufacturing and grinding processes by AAS and instrumental NAA. The structure of the dusts was studied by scanning electron microscopy with an energy dispersive X-ray. Cobalt was found to exist as separate particles in the dust from the mixing of raw material powders only. In the dusts from the pressing, forming and grinding of hard metal, cobalt appeared mainly in contact with tungsten carbide particles.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Inhalation exposure[1]

Hamster: As part of a larger study, groups of 51 male Syrian golden hamsters (ENG:ELA strain), two months of age, were exposed by inhalation to 0 or 10 mg/m^3 *cobalt*[*II*] *oxide* dust (with a mass median diameter of 0.45 μm) for 7 h per day on five days per week for life. Median survival was 16.6 months in treated hamsters compared to 15.3 months in controls. No difference in the incidence of any tumour was observed between the cobalt oxide-treated and untreated hamsters (Wehner *et al.*, 1977). [The Working Group noted the poor survival of the treated and control animals.]

(b) Intratracheal instillation

Rat: Groups of 50 male and 50 female Sprague-Dawley rats, ten weeks of age, received intratracheal instillations of 2 or 10 mg/kg bw *cobalt*[*II*] *oxide* powder (derived from thermal decomposition of *cobalt*[*II*] *nitrate*; approximately 80% of particles 5-40 μm [purity unspecified]) or 10 mg/kg bw of a *cobalt-aluminium-chromium spinel* (a blue powder [purity unspecified], with the empirical formula Co[II] 0.66, A1 0.7, Cr[III] 0.3, O 3.66, made of a mixture of CoO, $Al(OH)_3$ and Cr_2O_3 ignited at 1250°C; 80% of particles < 1.5

[1] The Working Group was aware that an inhalation study of cobalt sulfate heptahydrate was planned in mice and rats (IARC, 1990)

μm) in saline every two weeks (then every four weeks from the nineteenth to the thirtieth treatment) for two years (total doses, 78 and 390 mg/kg bw cobalt oxide and 390 mg/kg bw cobalt spinel). Control groups of 50 males and 50 females received instillations of saline only or remained untreated. Animals were allowed to live until natural death or were sacrificed when moribund. No appreciable difference in body weights or survival times was observed between the treated and control groups [exact survival data not given]. Bronchoalveolar proliferation was observed in 0/100 untreated controls, 0/100 saline controls, 51/100 low-dose cobalt oxide-treated rats and 70/100 high-dose cobalt oxide-treated rats, and in 61/100 rats treated with the spinel. [The Working Group noted that the nature of the bronchoalveolar proliferation or possible association with inflammation was not described.] No pulmonary tumour was observed in 100 untreated or 100 saline controls. In the groups treated with the low dose of cobalt oxide, one male and one female developed benign lung tumours; in the groups treated with the high dose of cobalt oxide, one bronchoalveolar carcinoma occurred in a female and three adenocarcinomas and two bronchoalveolar adenomas were observed in males; in the groups receiving the spinel, one squamous-cell carcinoma was observed in males and two squamous-cell carcinomas were observed in females (Steinhoff & Mohr, 1991).

In a smaller experiment by the same authors, groups of 20 female Sprague-Dawley rats, 10 weeks of age, received weekly intratracheal instillations of 10 mg/kg bw *cobalt*[*II*] *oxide* for seven weeks and 20 mg/kg bw once every two weeks for 20 treatments (total dose, 470 mg/kg bw), and 20 mg/kg bw benzo[*a*]pyrene following the same dose regimen (total dose, 200 mg/kg bw), with a four-day interval between the two treatments. A further group of 20 females received treatment with benzo[*a*]pyrene alone. Animals were allowed to live their natural lifespan or were sacrificed when moribund [exact survival not stated]. Eight rats treated with cobalt oxide and benzo[*a*]pyrene had squamous-cell carcinomas and one had an adenocarcinoma of the lung. One animal given benzo[*a*]pyrene had a squamous cell carcinoma of the lung (Steinhoff & Mohr, 1991).

Hamster: In a large experiment to study the effects of particulates on *N*-nitrosodiethylamine (NDEA)-induced respiratory tract carcinogenesis, groups of 25 male and 25 female hamsters [strain unspecified], seven weeks old, were given subcutaneous injections of 0.5 mg NDEA in saline or saline alone once a week for 12 weeks. One week later and once a week thereafter for 30 weeks, 4 mg *cobalt*[*II,III*] *oxide* powder (particle size, 0.5-1.0 μm [purity unspecified]) suspended in a gelatin and saline vehicle were administered by intratracheal instillation. Groups of 25 male and 25 female hamsters receiving subcutaneous injections of NDEA or saline and intratracheal instillations of the gelatin-saline vehicle served as controls. At the end of treatment (42 weeks), 39, 43, 33 and 43 animals were still alive in the four groups, respectively. Animals were observed for an

additional 43-68 weeks following the last intratracheal instillation. Two of 50 hamsters receiving injections of saline and cobalt oxide by intratracheal instillation developed pulmonary alveolar tumours; 1/50 hamsters receiving injections of saline and gelatin-saline intratracheally developed a tracheal tumour. The incidences of tumours at various sites in hamsters given NDEA with cobalt oxide in gelatin-saline were similar to those in animals receiving NDEA and gelatin-saline alone (Farrell & Davis, 1974).

(c) *Subcutaneous injection*

Rat: In a study designed to monitor cobalt-induced hyperlipidaemia, 20 male Wistar rats, about four weeks of age, received two courses, separated by a nine-day interval, of five daily subcutaneous injections of 40 mg/kg bw *cobalt*[*II*] *chloride* [purity unspecified] dissolved in saline, and were observed for 12 months. A control group of 20 males received injections of saline alone. At the end of the observation period, 8/11 surviving treated rats had developed subcutaneous fibrosarcomas (four of which were reported to be distant from the injection site), whereas none of the 19 surviving controls developed a tumour [$p < 0.001$, Fisher's exact test]. Post-mortem examinations were not made on the nine rats that died during the experiment. In a second experiment, 20 male Wistar rats received the same treatment but were observed for eight months. No control group was provided. At the end of this observation period, six of the 16 survivors had subcutaneous fibrosarcomas, including one tumour distant from the site of injection. Four rats that died during the observation period were not autopsied (Shabaan *et al.*, 1977).

Groups of 10 male Sprague-Dawley rats, 10 weeks of age, received subcutaneous injections of saline (two groups) or 2 mg/kg bw *cobalt*[*II*] *oxide* [purity unspecified] suspended in saline, five times a week, or subcutaneous injections of 10 mg/kg bw *cobalt*[*II*] *oxide* in saline once a week over a period of two years (total dose, 1000 mg/kg bw). Animals were allowed to live their natural lifespan or were sacrificed when moribund [survival data not given]. Malignant tumours (histiocytomas or sarcomas) developed at the injection site in 0/10, 0/10, 5/10 and 4/10 rats in the four groups, respectively (Steinhoff & Mohr, 1991).

(d) *Subcutaneous implantation*

Rat: Groups of five male and five female Wistar rats, four to six weeks of age, received subcutaneous implants of four pellets (approximately 2 mm in diameter) of either a *cobalt-chromium-molybdenum* (and lesser amounts of nickel) alloy (Vitallium; see p. 374 of this monograph), nickel metal, copper metal, nickel-gallium alloy (60% nickel, 40% gallium) or one of seven other implant materials not known to contain nickel, chromium or cobalt. Animals were observed for up to 27 months [survival of animals receiving cobalt-chromium-molybdenum alloy not given]. Sarcomas (mostly fibrosarcomas and

rhabdomyosarcomas) developed around the implants in 5/10 rats that received nickel pellets and in 9/10 rats that received nickel-gallium alloy pellets; no sarcoma developed in rats that received the cobalt-chromium-molybdenum pellets or in any of the other groups (Mitchell *et al.*, 1960).

(e) *Intramuscular injection*

Mouse: A group of 50 female Swiss mice, two to three months of age, received single intramuscular injections of 10 mg/site of unwashed powdered *cobalt*[*II*]*oxide* (particle size, ≤ 5 μm [purity unspecified]) in 10% aqueous penicillin G procaine in each thigh. Within two to six days, 25 mice had died. A further group of 25 females received similar injections of the powdered cobalt oxide that had been washed repeatedly in distilled water; this washed cobalt oxide did not induce acute mortality. The 25 survivors of the first group and the 25 mice from the second group were combined, and 46 were still alive 13 weeks after injection. A control group of 51 female mice similarly received intramuscular injections of penicillin G procaine vehicle (60 000 IU/site) into each thigh; 48 survived 13 weeks after injection. Animals were observed for up to 110 weeks [survival unspecified]. No tumour developed at the injection site in any of the cobalt oxide-treated or control mice. Incidences of tumours at other sites were similar in the treated and control groups (Gilman & Ruckerbauer, 1962).

A group of 30 mice [sex, strain and age unspecified] received intramuscular injections of 0.2 mg cobalt as cobalt naphthenate [purity, dosage, schedule, vehicle and duration unspecified] into the right hind limb. Tumours of the muscle in the hind leg developed in eight of the mice (Nowak, 1966). [The Working Group noted the incomplete reporting.]

Rat: A group of 10 male and 10 female hooded rats, two to three months old, received a single intramuscular injection of 28 mg *cobalt metal powder* (spectrographically pure, 400 mesh; 3.5 μm x 3.5 μm to 17 μm x 12 μm with large numbers of long narrow particles of the order of 10 μm x 4 μm) in 0.4 mL fowl serum into the thigh; a control group of ten males and ten females received fowl serum only. Average survival times were 71 weeks in treated males and 61 weeks in treated females; survival of controls was not specified. During the observation period of up to 122 weeks, 4/10 male and 5/10 female treated rats developed sarcomas (mostly rhabdomyosarcomas) at the injection site compared to 0/20 controls. A further group of ten female rats received a single intramuscular injection of 28 mg *cobalt metal powder* in 0.4 mL fowl serum; others received injections of 28 mg zinc powder (five rats) or 28 mg tungsten powder (five rats). Average survival time for cobalt-treated rats was 43 weeks. During the observation period of up to 105 weeks, sarcomas (mostly rhabdomyosarcomas) developed in 8/10 cobalt powder-treated rats; none occurred in the zinc powder- or tungsten powder-treated rats. No other tumour occurred in

any of the cobalt-treated or other rats, except for one malignant lymphoma in a zinc-treated rat (Heath, 1954a, 1956).

In a supplementary study, a group of 30 male hooded rats, two to three months of age, received a single intramuscular injection of 28 mg *cobalt metal powder* (spectrographically pure [particle size unspecified]) in 0.4 mL fowl serum into the right thigh; a control group of 15 males received a single injection of fowl serum only. The rats were killed at intervals of one to four weeks after injection or at fortnightly intervals up to 20 weeks after injection, when the first tumour appeared. The author described leukocyte infiltration, muscle fibre necrosis and regeneration and the development of a tumour nodule in one rat (Heath, 1960).

Groups of 10 male and female Wistar rats [sex ratio unspecified], two to three months old, received a single intramuscular injection of 30 mg/site of powdered, reagent-grade *cobalt[II] oxide* (particles ground to ≤ 5 μm and washed repeatedly in distilled water) suspended in 10% aqueous penicillin G procaine or penicillin G procaine (90 000 IU/site) alone into the thigh muscle and were observed for 74 weeks [number of survivors unspecified]. No tumour occurred at the site of injection in the 10 control rats during the study, whereas rhabdomyosarcomas developed at the injection site in 5/10 cobalt oxide-treated rats. Metastases were seen in four of the five tumour-bearing rats. No other neoplasm was noted in control or treated rats (Gilman & Ruckerbauer, 1962).

A group of 30 male and female Wistar rats [sex ratio unspecified], two to three months of age, received simultaneous intramuscular injections of 20 mg/site of powdered *cobalt*[*II*] *sulfide* [purity unspecified] (ground to ≤ 5 μm diameter and washed repeatedly in water) suspended in penicillin G procaine into each thigh. A total of 35 sarcomas were observed at the 58 injection sites in the 29 rats that survived 13 weeks after treatment, with a mean latency of 28 weeks. Metastases were noted in 16/29 rats with tumours; no other neoplasm was seen. No control was reported (Gilman, 1962).

Groups of male and female Wistar rats [sex ratio unspecified], two to three months of age, received two simultaneous intramuscular injections (five rats) in each thigh or single injections (19 rats) of *cobalt*[*II*] *oxide* (20 mg/site; particle size ≤ 5 μm; washed repeatedly in water) suspended in aqueous procaine G penicillin. No control group was reported. A total of 13 sarcomas (mostly rhabdomyosarcomas) were noted at the 29 injection sites of the 24 rats that survived 13 weeks of treatment (mean latency, 25 weeks). Metastases were noted in 3/12 rats with tumours (Gilman, 1962).

In a series of three experiments, a total of 80 female hooded rats, seven to nine weeks of age, received an intramuscular injection of 28 mg/rat of wear particles, obtained by working in Ringer's solution *in vitro* of artificial hip or knee prostheses made from

cobalt-chromium-molybdenum alloy (66.5% cobalt, 26.0% chromium, 6.65% molybdenum, 1.12% manganese; particle diameter, down to 0.1 μm [mostly 0.1-1 μm]), in 0.4 mL horse serum and were observed for up to 29 months [survival not specified]. No control group was reported. Sarcomas developed at the injection site in 3/16, 4/14 and 16/50 rats in the three series, respectively. Approximately half of the tumours were rhabdomyosarcomas; the remainder were mostly fibrosarcomas (Heath *et al.*, 1971; Swanson *et al.*, 1973).

(f) *Intramuscular implantation*

Rat: As a follow-up to the studies by Heath and Swanson (see above), groups of female Wistar and hooded rats, weighing 190-310 and 175-220 g, respectively, received intramuscular implants of 28 mg of coarse (100-250 μm diameter; 51 Wistar rats) or fine (0.5-50 μm diameter, 85% 0.5-5 μm; 61 Wistar and 53 hooded rats) particles as a dry powder, obtained by grinding a *cobalt-chromium-molybdenum* alloy (68% cobalt, 28% chromium, 4% molybdenum), and were observed for life. A sham-operated control group of 50 female Wistar rats was available. Survival at two years was 11/51 rats receiving the coarse particles, 7/61 Wistar rats receiving the fine particles, 0/53 hooded rats receiving the fine particles and 5/50 Wistar controls. No tumour was noted at the implantation site of rats treated with either of the alloy particles or in sham-operated control animals (Meachim *et al.*, 1982).

Groups of 15 male and 15 female Sprague-Dawley rats, aged 20-30 days, received intramuscular implants of polished rods (1.6 mm diameter, 8 mm length) of one of three alloys (wrought Vitallium: 19-20% chromium, 14-16% tungsten, 9-11% nickel, < 0.15% carbon, < 2% [manganese], < 1% silicium, < 3% iron, balance cobalt; cast Vitallium: 27-30% chromium, 5-7% molybdenum, < 2.5% nickel, < 0.3% carbon, < 1% [manganese], < 1% silicium, < 0.75% iron, balance cobalt; $MP_{35}N$ alloy: 19-21% chromium, 33-37% nickel, < 0.025% carbon, < 1% iron, < 0.15% manganese, 9.5-10.5% molybdenum, < 0.15% silicium, 0.65-1% titanium; balance cobalt) and were observed for up to two years [survival unspecified]. Groups of 15 male and 15 female untreated and sham-operated control animals were available. No benign or malignant tumour developed at the implant site in any of the groups receiving metal implants or in either control group. The incidences of malignant tumours at distant sites did not differ significantly among the treated and control groups (Gaechter *et al.*, 1977).

Guinea-pig: A group of 46 female Dunkin-Hartley guinea-pigs, weighing 550-930 g, received intramuscular implants of 28 mg of a powdered *cobalt-chromium-molybdenum* alloy (68% cobalt, 28% chromium, 4% molybdenum; particle diameter, 0.5-50 gm) and were observed for life; 12/46 animals were alive at three years. No control group was

reported. No tumour was observed at the implantation site of any guinea-pig; nodular fibroblastic hyperplasia was observed at the implantation site in eight animals (Meachim *et al.*, 1982).

(g) *Intra-osseous implantation*

Rat: Groups of 10-17 male and 8-15 female Sprague-Dawley rats, 30-43 days of age, received implants of one of seven test materials containing *cobalt alloyed with chromium and nickel, molybdenum, tungsten and/or zirconium*, with traces of other elements (as small rods, 1.6 mm diameter and 4 mm length, powders or porous compacted wire), in the femoral bone and were observed for up to 30 months. Groups of 13 male and 13 female untreated and sham-operated controls were available. Average survival was longer than 22 months. Sarcomas at the implant site were observed in 1/18 rats (males and females given cobalt-based alloy powder containing 41% Co), 3/26 rats (males and females given $MP_{35}N$ powder containing 33% Co) and 3/32 rats (males and females given porous compacted wire containing 51% Co). No tumour was observed in two groups of 25 rats given rods containing 69 or 47% cobalt, in two groups of 26 rats given rods containing 0.11 or 33% cobalt, in two groups of 25 and 26 untreated rats, or in a group of 26 sham-treated control rats (Memoli *et al.*, 1986).

(h) *Intraperitoneal injection*

Mouse: In a screening study based on the enhanced induction of lung tumours, groups of 10 male and 10 female strain A mice, six to eight weeks of age, received intraperitoneal injections of *cobalt*[*III*] *acetate* (> 97% pure) in saline three times per week for eight weeks (total doses, 95, 237 and 475 mg/kg bw). After 30 weeks, lung tumours were found in 8/20, 8/20 and 10/17 mice in the respective treatment groups, and in 7/19 saline-treated controls (not significant) (Stoner *et al.*, 1976).

Rat: Groups of 10 male and 10 female Sprague-Dawley rats, 10 weeks of age, received three intraperitoneal injections at two-month intervals of saline or 200 mg/kg bw *cobalt*[*II*] *oxide* [purity unspecified] or *cobalt-aluminium-chromium spinel powder* (see above) in saline (total dose, 600 mg/kg bw). Animals were allowed to live their natural lifespan or were sacrificed when moribund [survival not given]. Malignant peritoneal tumours occurred in 1/20 controls (histiocytoma), 14/20 cobalt oxide-treated rats (10 histiocytomas, three sarcomas, one mesothelioma) and 2/20 spinel-treated animals (one histiocytoma, one sarcoma) (Steinhoff & Mohr, 1991).

(i) *Intrarenal administration*

Rat: Two groups of 20 and 18 female Sprague-Dawley rats, weighing 120-140 g, received a single injection of 5 mg *cobalt*[*II*] *sulfide* [reagent grade; purity and particle size

unspecified] or 5 mg *metallic cobalt powder* [purity unspecified] suspended in 0.05 mL glycerine into each pole of the right kidney. A group of 16 female rats receiving injections of 0.05 mL glycerine into each pole of the kidney served as controls. After 12 months, all rats were necropsied; no tumour was observed in the kidneys of treated or control rats (Jasmin & Riopelle, 1976). [The Working Group noted the short duration and inadequate reporting of the experiment.]

(i) *Other*

Rat: Two groups of 10 female hooded rats, two to three months of age, received intrathoracic injections of 28 mg cobalt metal powder (spectrographically pure; particle size, < 400 mesh; 3.5 µm x 3.5 µm to 17 µm x 12 µm, with many long narrow particles of the order of 10 µm x 4 µm) in serum [species unspecified] through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group) and were observed for up to 28 months. Death occurred within three days of the treatment in 6/10 rats injected through the diaphragm and in 2/10 rats injected through the intercostal space. The remaining rats in the first group (diaphragm) survived 11-28 months and in the second group (intercostal space), 7.5-17.5 months. Of the 12 rats that survived the injection, four developed intrathoracic sarcomas (three of mixed origin, including rhabdomyosarcomatous elements, one rhabdomyosarcoma arising in the intercostal muscles) (Heath & Daniel, 1962).

Rabbit: Twelve male rabbits [strain unspecified], weighing 2-2.5 kg, were given intramuscular, intravenous, intrapleural or intrahepatic injections of *cobalt naphthenate* [purity and dose unspecified]. Within two to six months, tumours developed at the site of injection in eight rabbits, including one pleural mesothelioma, one haemangioendothelioma of the liver, one osteochondroma of the ear and five skeletal muscle tumours (Nowak, 1961). [The Working Group noted the lack of controls, the small number of animals and the incomplete reporting of the experiment.]

A summary of most of these studies is given in Table 20.

3.2 Other relevant data

The metabolism and toxicity of cobalt have been reviewed (Taylor & Marks, 1978; Elinder & Friberg, 1986). Recent interest has centred on the biological monitoring of cobalt, i.e., the determination of cobalt in human biological materials such as blood and urine, and how such data may be used to assess absorption, exposure and possible health risks (Alessio & Dell'Orto, 1988).

Table 20. Summary of animal carcinogenicity studies by form of cobalt

Reference	Species/ strain	Sex	Dose schedule	Experimental parameter/ observation	Group				Comments
					0	1	2	3	
Cobalt metal powder									
Heath (1954a, 1956)	Rat Hooded	M	i.m., single inj., fowl serum	Dose (mg) Survival (122 weeks) Local sarcoma	0 Not given 0/10	28 4/10			
		F		Dose (mg) Survival (122 weeks) Local sarcoma	0 Not given 0/10	28 5/10	28 8/10		
Health & Daniel (1962)	Rat Hooded	F	intrathoracic in serum	Dose (mg) Survival (3 days) Thoracic tumour	0	28 12/20 4/12			
Jasmin & Riopelle (1976)	Rat Sprague-Dawley	F	intrarenal	Dose (mg) Survival (12 months) Kidney tumour	0 Not given 0/16	5 0/18			Inadequate
Cobalt alloys									
Heath *et al*. (1971); Swanson *et al*. (1973)	Rat Hooded	F	i.m., single inj., wear particles from Co/Cr/Mo, in horse serum	Dose (mg) Survival (29 months) Local sarcoma	0 Not given	28 23/80			
Gaechter *et al*. (1977)	Rat Sprague-Dawley	M+F	i.m. impl. Co/Cr/ W/Ni/C/Mn/Si/Fe (1.6 x 8 mm)	Dose (polished rod) Survival (2 years) Local tumour	0[a] Not given 0/30	0[a] 0/30	1 0/90		No significant difference in distant tumours
Memoli *et al*. (1986)	Rat Sprague-Dawley	M+F	Intraoss. Impl., Co/Cr/Ni/Mo/W/ Zr	Dose (powder, wire, rod) Survival (30 months) Local sarcoma	0[a] Not given 0/51	0[a] 0/26	1 7/76[b]		
Mitchell *et al*. (1960)	Rat Wistar	M+F	s.c. impl. Co/Cr/ Mo/Ni	Dose (pellets – 2-mm diam) Survival (27 months) Local tumour	 Not given	 0/10			
Meachim *et al*. (1982)	Rat Wistar and hooded	F	i.m. impl. Co/Cr/ Mo fine and coarse particles	Dose (mg) Survival (2 years) Local tumour	0 5/50 0	28 11/51 0	28 7/61 0	28 0/53 0	

Table 20 (contd)

Reference	Species/ strain	Sex	Dose schedule	Experimental parameter/ observation	Group				Comments
					0	1	2	3	
Cobalt alloys (contd)									
Steinhoff & Mohr (1991)	Rat Sprague-Dawley	M+F	3 i.p. inj., Co/Al/Cr spinel powder	Dose (mg/kg/bw) Survival (2 years) Local tumour	0 Not given 1/20	200 2/20			
Steinhoff & Mohr (1991)	Rat Sprague-Dawley	M+F	Intratracheal inst. 1 x 2 weeks Co/Al/Cr spinel 2 years	Dose (mg/kg bw) Survival (2 years) Squamous-cell tumour of the lung	0 Not given 0/200	10 3/100			
Meachim *et al*. (1982)	Guinea-pig	F	i.m. impl. Co/Cr/Mo powder	Dose (mg) Survival (3 years) Local tumour Local fibroblastic hyperplasia		28 12/46 0/46 8/46			
Cobalt[II] oxide									
Gilman & Ruckerbauer (1962)	Mouse Swiss	F	i.m. inj. in each thigh	Dose (mg/site) Survival (13 weeks) Local sarcoma	0 48/51 0/48	10 46/75 0/46			
Steinhoff & Mohr (1991)	Rat Sprague-Dawley	M	Intratracheal inst. 1 x 2 weeks 2 years	Dose (mg/kg bw) Survival (2 years) Benign squamous pulmonary tumour Bronchioalveolar adenoma Pulmonary adenocarcinoma Bronchoalveolar adenocarcinoma	0 Not given 0/100 0/100 0/100 0/100	2 1/50 0/50 0/50 0/50	10 0/50 2/50 2/50 1/50		
		F		Dose (mg/kg bw) Survival Bronchoalveolar adenoma Bronchoalveolar carcinoma	0 Not given 0/100 0/100	2 1/50 0/50	10 0/50 1/50		

Table 20 (contd)

Reference	Species/ strain	Sex	Dose schedule	Experimental parameter/ observation	Group				Comments
					0	1	2	3	
Cobalt[II] oxide (contd)									
Gilman & Ruckerbauer (1962)	Rat Wistar	M+F	i.m. inj.	Dose (mg/site) Survival (90 days) Local sarcoma	0 10/10 0/10	30 10/10 5/10			
Gilman (1962)	Rat Wistar	M+F	i.m. inj.	Dose (mg/site) Survival (13 weeks) Local sarcoma		20 24/32 13/29 sites			
Steinhoff & Mohr (1991)	Rat Sprague-Dawley	M	s.c. inj. 2 mg/kg bw 5/week or 10 mg/kg bw 1/week for 2 years	Dose (mg/kg bw) Survival (2 years) Local malignant tumour	0 Not given 0/20	2 5/10	10 4/10		
Steinhoff & Mohr (1991)	Rat Sprague-Dawley	M+F	3 i.p. inj. at 2-month intervals	Total dose (mg/kg bw) Survival (2 years) Local malignant tumour	0 Not given 1/20	200 14/20			
Wehner *et al*. (1977)	Hamster ENG:ELA	M	Inhalation 7 h/day 5 d/week for life	Dose (mg/m^3) Survival (18 months) Reticulum-cell sarcoma Carcinoma Lymphosarcoma Leukaemia Plasma-cell tumour	0 7/51 0/51 0/51 0/51 0/51 1/51	10 9/51 1/51 1/51 0/51 0/51 0/51			No statistical difference
Cobalt[II] sulfide									
Gilman (1962)	Rat Wistar	M+F	i.m. inj.	Dose (mg/site) Survival (13 weeks) Local sarcoma		20 29/30 35/58 sites			
Jasmin & Riopelle (1976)	Rat Sprague-Dawley	F	Intrarenal	Dose (mg) Survival (12 months) Kidney tumours	0 Not given 0/16	5 0/20			Inadequate

Table 20 (contd)

Reference	Species/ strain	Sex	Dose schedule	Experimental parameter/ observation	Group				Comments
					0	1	2	3	
Cobalt[II] chloride									
Shabaan *et al*. (1977)	Rat Wistar	M	s.c. inj. 2 x 5 d, 9-d interval	Dose (mg/kg bw) Survival[c] Subcutaneous sarcoma	0 19/20 0/19	40 11/20 8/11	40 16/20 6/16		 $p < 0.001$ (Fisher exact test)
Cobalt naphthenate									
Nowak (1966)	Mouse NS	NS	i.m. inj. NS	Dose (mg) Survival Tumour of the striated muscle	0	0.2 8/30			Inadequate
Nowak (1961)	Rabbit	M	i.m. i.v. i. pleural i. hepatic	Dose unspecified	0	5 1 1 1			Inadequate
Cobalt[III] acetate									
Stoner *et al*. (1976)	Mouse strain A	M+F	i.p. inj. 3/week, 24 doses	Total dose (mg/kg bw) Survival (30 weeks) Pulmonary tumour	0 19/20 7/19	95 20/20 8/20	237 20/20 8/20	475 17/20 10/17	Not significant

[a]Group 0, untreated; group 1, sham-treated

[b]Powder, 1/18 sarcoma; $MP_{35}N$, 3/26 sarcomas; compacted wire, 3/32 sarcomas

[c]12 months for groups 0 and 1; at 8 months for group 2

NS, not specified

(a) *Experimental systems*

(i) *Absorption, distribution, metabolism and excretion*

Cobalt compounds

The gastrointestinal absorption of radiolabelled cobalt chloride in rats was found to vary between 11 and 34%, depending on the administered dose (0.01-1000 μg/rat). The relative absorption decreased with increasing dose (Taylor, 1962). However, less than 0.5% of cobalt oxide given at an oral dose of 5 mg was absorbed by hamsters (Wehner & Craig, 1972).

The pulmonary absorption of inhaled cobalt(II) oxide (particle size, 1.0-2.5 μm) by hamsters was both rapid and high: about 25% was recovered in the carcass, lung, liver and kidney 24 h after inhalation of 0.8 mg cobalt oxide; essentially all of the cobalt oxide was eliminated by the sixth day after exposure (Wehner & Craig, 1972). Intratracheally instilled cobalt(II) oxide (1.5 μg) was cleared slowly from the rat lung (half-time, 15 days), and only very low concentrations were found in extrapulmonary tissues (Rhoads & Sanders, 1985). After inhalation or instillation of cobalt oxides in dogs and rats, the highest concentrations of cobalt were found in the lungs (Barnes *et al.*, 1976; Rhoads & Sanders, 1985). After rapid initial elimination (half-time, 0.7 days), the half-time of cobalt oxides deposited in the lungs of dogs was 36-86 days (Barnes *et al.*, 1976).

Kreyling *et al.* (1986) exposed beagle dogs by inhalation to radioactive cobalt[II,III] oxide particles of different size (0.3-2.7 μm) and found that small particles were cleared more rapidly from the lungs. Brune *et al.* (1980) exposed rats by inhalation to chromium-cobalt-containing abrasive dust obtained from dental laboratories. The concentration of cobalt in the lung increased with the length of exposure, indicating slow elimination of deposited metal. Histological examination revealed macrophages containing metal particles. The concentration of cobalt was also elevated in liver and kidney, showing that some systemic uptake of cobalt had taken place. Animals given cobalt chloride orally or by injection showed highest concentrations in the liver, with lower concentrations in kidney, pancreas and spleen (Taylor & Marks, 1978; Stenberg, 1983). Relatively high concentrations were also found in myocardium (Stenberg, 1983; Clyne *et al.*, 1988) and in cartilage and bone (Soremark *et al.*, 1979).

The major proportion of parenterally administered cobalt is cleared rapidly from the body, mainly via urine: 63% of radioactive cobalt chloride was recovered in the urine of rats within 24 h (Taylor, 1962). After a single intravenous injection of cobalt chloride to rats, about 70 and 7% were recovered in the urine and faeces, respectively, during the first three days (Onkelinx, 1976). Similarly, 73 and 15% of an intravenous dose of cobalt chloride (0.3 mg/kg bw) to rats was eliminated via urine and faeces, respectively, within four days

(Gregus & Klaassen, 1986). Dogs injected intravenously with 20 µg/kg bw radioactive cobalt sulfate eliminated 40-70% of the label in urine and bile (90% in urine) over a period of 7-13 h (Lee & Wolterink, 1955). In rats, only 2-7% of intravenously injected cobalt chloride was eliminated in the bile (Cikrt & Tichy, 1981; Gregus & Klaassen, 1986).

Autoradiographic examination of pregnant mice injected intravenously with radioactive cobalt chloride revealed high activity in maternal liver, kidney, pancreas and cartilage and in the fetal skeleton and other tissues (Flodh, 1968; Söremark *et al.*, 1979).

Metal alloy implants

In an experiment *in vitro* simulating mechanical stress on four different types of metallic hip prostheses, three of which contained cobalt, more than 1 mg/L cobalt was found in solution, and metal particles with a size down to 0.1 µm were formed as a result of frictional movement (Swanson *et al.*, 1973).

(ii) *Toxic effects*

Cobalt compounds

The oral LD_{50}s for different inorganic cobalt(II) compounds (cobalt fluoride, oxide, phosphate, bromide, chloride, sulfate, nitrate and acetate) in rats ranged from 150 to 500 mg/kg bw anhydrous compound (Speijers *et al.*, 1982). When the amounts were expressed in moles, the variability in toxicity between different compounds ranged from 1.5 to 3 mmol/kg cobalt. Acute effects recorded in the animals included sedation, diarrhoea and decrease in body temperature. All hamsters died after 6-h exposures by inhalation to 100 mg/m^3 cobalt oxide (Wehner & Craig, 1972). Pulmonary haemorrhagia and oedema and death were observed in guinea-pigs exposed by inhalation to cobalt chloride [dose unclear] (Höbel *et al.*, 1972).

Life-time exposure of hamsters to cobalt oxide by inhalation (10 mg/m^3, 7 h per day, five days a week) resulted in emphysema and in hyperplastic and hypertrophic changes in the alveolar epithelium and distal bronchi (Wehner *et al.*, 1977). Exposure of rabbits by inhalation to concentrations of 0.4 or 2 mg/m^3 cobalt chloride for 6 h per day on five days a week for 14-16 weeks produced nodular aggregation of alveolar type II cells, abnormal accumulation of enlarged, vacuolated alveolar macrophages and interstitial inflammation (Johansson *et al.*, 1987).

Daily doses of 2.5-10 mg/kg bw cobalt(II) salts given orally or parenterally caused polycythaemia in rats (Orten & Bucciero, 1948; Hopps *et al.*, 1954; Oskarsson *et al.*, 1981); reduced weight gain was seen as an early sign of general toxicity in some of these studies. Parenteral administration of 10-60 mg/kg bw cobalt chloride caused hyperlipidaemia in rabbits (Caplan & Block, 1963), induction of hepatic haemoxygenase and a decrease in

activity of δ-aminolaevulinic synthase and certain cytochrome P450-dependent drug metabolizing enymes in rats (Maines & Kappas, 1975; Maines *et al.*, 1976; Numazawa *et al.*, 1989).

Myocardial toxicity of cobalt salts has been reported in rats (Grice *et al.*, 1969; Lin & Duffy, 1970; Rona, 1971), guinea-pigs (Mohiuddin *et al.*, 1970; Desselberger & Wegener, 1971), rabbits (Hall & Smith, 1968) and dogs (Sandusky *et al.*, 1981) following long-term dietary (10-100 mg/kg bw) or parenteral (5-30 mg/kg bw) administration. Observed toxic effects included noninflammatory myocardial degeneration, alterations in mitochondria and myofibrils and abnormal electrocardiographic traces.

Metallic cobalt

Intratracheal instillation of metallic cobalt (50 mg/animal; sterile suspension [particle size not given]) caused pulmonary haemorrhage and oedema and death in rats (Harding, 1950).

In miniature swine exposed to 0.1-1 mg/m^3 metallic cobalt particles (0.4-3.6 μm) for 6 h per day on five days per week for three months by inhalation, a progressive decrease in lung compliance was observed. In addition collagenization of alveolar septa in lung biopsies and electrocardiographic changes indicative of cardiomyopathy were observed (Kerfoot *et al.*, 1975).

In contrast to findings with cobalt chloride, exposure of rabbits by inhalation to metallic cobalt dust (0.2- 1.3 mg/m^3,6 h per day, five days per week for four weeks) had no profound effect on alveolar macrophages (Johansson *et al.*, 1980, 1986).

Cobalt released from cobalt metal, alloys or dissolved salts was cytotoxic to chick primary cultures and rodent fibroblast cell lines, inducing cell death, growth inhibition and mitotic abnormalities at concentrations greater than 7.5 μg/ml (Heath, 1954b; Daniel *et al.*, 1963; Bearden, 1976; Bearden & Cooke, 1980; Takahashi & Koshi, 1981).

(iii) *Effects on reproduction and prenatal toxicity*

Reproductive effects: Ingested cobalt chloride (265 mg/kg diet for 98 days, providing an initial dose of 20 mg/kg bw cobalt) induced degenerative and necrotic changes in the seminiferous tubules of rats. Cyanosis and vascular engorgement of the testes were seen on day 35 of treatment, and necrosis, degenerative and necrotic changes in the germinal epithelium and Sertoli cells by day 70. Damaged tubules were present side by side with normal ones. Multinucleated giant cells containing cellular debris were observed in the damaged tubules. Loss of sperm-tail filaments and degeneration of sperm mitochondria were also observed (Corrier *et al.* 1985a; Mollenhauer *et al.*, 1985). The same group of investigators did not find the lesion in sheep treated with 3.0-15.0 mg/kg bw cobalt for 109 days (Corrier *et al.* 1985b).

Intraperitoneal injection of cobalt chloride (1 mg/kg bw cobalt) 16 and 6 days before sacrifice stimulated spermiogenesis and spermatogenesis in the mouse testis (Niebrój, 1967). Intraperitoneal administration of 200 μmol [47.6 mg]/kg bw cobalt chloride for three days to male mice resulted in small but significant decreases in fertility two to three weeks later in an acute study. Similarly, in a chronic study, 100, 200 and 400 mg/1 cobalt chloride given in drinking-water *ad libitum* for 7-13 weeks decreased fertility, sperm concentration, sperm mobility and testicular weight in a time-dose-dependent manner (Pedigo *et al.*, 1988). [The Working Group noted that the apparent differences in the results described above may be due to differences in dose and duration of observation.]

Developmental toxicity: Embryonic death was reported following administration to rats of cobalt chloride in the drinking-water either before and during pregnancy (0.05-5 mg/L) or during pregnancy only (0.005-0.05 mg/L) (Nadeenko *et al.*, 1980). In contrast, no developmental toxicity was observed in the offspring of rats given daily doses of 0, 25, 50 or 100 mg/kg bw cobalt chloride by gavage on days 6-15 of gestation, except for a nonsignificant increase in the incidence of stunted fetuses in the groups given 50 and 100 mg/kg (Paternain *et al.* 1988).

Numbers of litters as well as growth and survival of the offspring were reduced in rats that received 12, 24 and 48 mg/kg bw per day cobalt chloride by gavage from day 14 of gestation through day 21 of lactation (Domingo *et al.*, 1985).

Delay in ossification of the skeleton during embryonic and fetal development was observed at gestation day 17 in the offspring of six- to eight-week-old female mice (24-26 g) administered cobalt chloride (0.1 ml of a 5 mM solution [4.8 mg/kg bw]) intravenously on day 8; the effect was not seen when the cobalt was administered on day 3 of pregnancy. There was no change in fetal body weight on day 17 of pregnancy, and no increase in the frequency of resorption or implantation sites compared with controls (Wide, 1984).

In CF-1 mice, cobalt chloride was reported to protect against cleft lip and palate induced by cortisone (Kasirsky *et al.*, 1967).

As reported in an abstract, fetal damage was detected on gestation day 15 in hamsters administered cobalt acetate (40, 60, 80, 100 or 160 mg/kg bw) subcutaneously on day 8 of pregnancy. The resorption rate ranged from 6% at the low dose to 100% at the high dose. Central nervous system defects were reported at the median doses. Similarly, resorptions and central nervous system defects were observed after intraperitoneal injections of 40-70 mg/kg (Gale, 1980). [The Working Group noted that no information on maternal toxicity was reported.]

Studies on the effects of cobalt salts on chick embryos have produced conflicting results, perhaps due to differences in dose and routes of administration. Degeneration of the brain (Ridgway & Karnofsky, 1952), neural tube malformations (Adhikari, 1967), lethality, eye

abnormalities and structural defeets (Kury & Crosby, 1968; Gilani & Alibai, 1985, abstract) have been reported.

(iv) *Genetic and related effects*

The results of tests for genetic and related effects of cobalt and cobalt compounds, with references, are given in Table 21. Other studies are described in the text.

The genetic toxicology of cobalt and cobalt eompounds has been reviewed (Léonard & Lauwerys, 1990). With few exceptions, only soluble cobalt[II] salts have been tested. Only two reports were available on genetic effects of insoluble cobalt sulfide, and no data have been reported on genetic effects of metallic cobalt.

Like other metallic compounds, cobalt compounds are known to be relatively inactive in prokaryotic systems (Rossman, 1981; Swierenga *et al.*, 1987). The precipitation of metal as phosphates in bacterial culture media may contribute to this inactivity (Rossman, 1981; Arlauskas *et al.*, 1985). However, four of 15 cobalt[III] complexes with aromatic ligands were active in a DNA repair assay and were mutagenic to *Salmonella typhimurium* (Schultz *et al.*, 1982). Several other studies of cobalt salts with positive results have been reported in prokaryotes.

Cobalt[II] chloride was inactive in the λ prophage induction assay, and it gave conflicting results in the *Bacillus subtilis* $rec^{+/-}$ growth inhibition assay. In the study with positive results, a preincubation procedure was used. Cobalt[II] chloride was inactive in all but one bacterial mutagenicity test. One study gave positive results in the absence but not in the presence of an exogenous metabolic system.

In bacteria, cobalt[II] chloride was reported to reduce the incidence of spontaneous mutations and to inhibit mutations induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine and Trp-P-1 (Kada & Kanematsu, 1978; Inoue *et al.*, 1981; Mochizuki & Kada, 1982). It was comutagenic with several heteroaromatic compounds (Ogawa *et al.*, 1986, 1987, 1988).

In *Saccharomyces cerevisiae*, cobalt[II] chloride induced gene conversion and mitochondrial but not other types of mutation. Cobalt[II] salts induced chlorophyll mutations, chromosomal aberrations and aneuploidy in plant cells.

In cultured mammalian cells *in vitro*, predominantly positive results were obtained, with induction of DNA-protein cross-linkage, DNA strand breakage and sister chromatid exchange. Chromosomal aberrations were not observed in cultured human cells. [The Working Group noted the low concentrations employed.] Cobalt[II] chloride induced aneuploidy in cultured human lymphocytes. It also induced mutations at the *hprt* locus in Chinese hamster V79 cells, but not, in a single study, at the *tk* locus in mouse lymphoma L5178Y cells.

Cobalt[II] acetate enhanced viral transformation in Syrian hamster embryo cells, and

cobalt sulfide induced morphological transformation in Syrian hamster embryo cells; the crystalline form of cobalt sulfide was more active than the amorphous form.

Cobalt[II] chloride administered *in vivo* to Syrian hamsters by intraperitoneal injection induced aneuploidy in bone marrow and testes. In an assay for dominant lethal mutation in mice, reported as an abstract, significant increases in early embryonic losses were observed (Pedigo, 1988).

A mechanism for the genetic effects of soluble Co[II] salts may involve decreased fidelity of DNA polymerase (Sirover & Loeb, 1976). Cobalt[II] chloride caused extensive cleavage of isolated DNA in the presence of hydrogen peroxide; this effect was attributed to the generation of reactive oxygen species at those sites of DNA bound to cobalt ions (Yamamoto *et al.*, 1989).

(b) *Humans*

(i) *Absorption, distribution, excretion and metabolism*

The normal concentrations of cobalt in blood and urine from nonoccupationally exposed persons are about 0.1-2 µg/L. The levels of cobalt in blood, and particularly in urine, increase in proportion to the level of occupational exposure and can be used for biological monitoring in order to assess individual exposure (Elinder *et al.*, 1988). Increased levels of cobalt have also been found in blood (serum) from uraemic patients (Curtis *et al.*, 1976; Lins & Pehrsson, 1984).

In a patient who died three months after treatment with cobalt[II] chloride (50 mg per day for three months), the myocardial concentration of cobalt was 1.65 mg/kg wet weight, which was 25-80 times higher than that in control samples (0.01-0.06 mg/kg) (Curtis *et al.*, 1976). Increased levels of cobalt were also reported in lung and mediastinal lymph nodes from hard-metal workers with lung disease; concentrations of cobalt were about 100-1000 µg/kg in two lung tissue samples compared to 5 µg/kg wet weight in controls, and 3280 µg/kg in mediastinal lymph nodes compared to > 2 µg/kg in controls (Hillerdal & Hartung, 1983).

The mean urinary excretion within 24 h of radioactive cobalt chloride given orally at 20 µM was estimated to be about 18% (Sorbie *et al.*, 1971). When healthy persons and uraemic patients were given 50 mg cobalt chloride orally, the two healthy volunteers eliminated between 5.7 and 8.3% of the dose via the urine within one week; elimination was considerably slower in uraemic patients, confirming the importance of renal clearance (Curtis *et al.*, 1976). High concentrations of radiolabelled cobalt were found in the liver shortly after parenteral administration of cobalt chloride to humans. After eight days, 28-56% and 2-12% of the dose were eliminated via the urine and faeces, respectively. A significant component (9-16% of the administered dose) was cleared very slowly, with a biological half-time of about two years (Smith *et al.*, 1972). Similar results, suggesting that

Table 21. Summary of studies on genetic and related effects of cobalt

Test system	Result		Dose LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt(II) salts				
PRB, Prophage induction in *Escherichia coli*	-	0	4.0000	Rossman *et al*. (1984)
BSD, *Bacillus subtilis rec* strains H17/M45, growth inhibition	-	0	325.0000	Nishioka (1975)
BSD, *Bacillus subtilis rec* strains H17, growth inhibition	+	0	325.0000	Kanematsu *et al*. (1980)
BSD, *Bacillus subtilis rec* strains H17, growth inhibition	(+)	0	325.0000	Kanematsu *et al*. (1980)
BSD, *Bacillus subtilis rec* strains H17, growth inhibition	(+)	0	325.0000	Kanematsu *et al*. (1980)
???, *Bacillus subtilis* strain NIG 1125, reverse mutation	-[a]	0	0.0000	Inoue *et al*. (1981)
SAO, *Salmonella typhimurium* TA100, reverse mutation	-	0	130.0000	Tso & Fung (1981)
SAO, *Salmonella typhimurium* TA100, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
SAO, *Salmonella typhimurium* TA100, reverse mutation	-	0	0.0000	Ogawa *et al*. (1986)
SA2, *Salmonella typhimurium* TA102, reverse mutation	-	-	40.0000	Wong (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	40.0000	Wong (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	0	65000.0000	Ogawa *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	-	0.0000	Wong (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	0	20.0000	Mochizuki & Kada (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-[a]	0	20.0000	Mochizuki & Kada (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	0.0000	Ogawa *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	-	0.0000	Wong (1988)
SAS, *Salmonella typhimurium* TA2637, reverse mutation	-	0	65000.0000	Ogawa *et al*. (1986)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	-	0	0.0000	Arlauskas *et al*. (1985)
EC2, *Escherichia coli* WP2, reverse mutation	-[a]	0	20.0000	Kada & Kanematsu (1978)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	0	1300.0000	Fukunaga *et al*. (1982)

Table 21 (contd)

Test system	Result		Dose LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt(II) salts (contd)				
SCG, *Saccharomyces cerevisiae* D7, gene conversion	(+)	0	0.0000	Singh (1983)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	0	1500.0000	Kharab & Singh (1985)
SCF, *Saccharomyces cerevisiae* petite mutation	+	0	130.0000	Lindegren *et al*. (1958)
SCF, *Saccharomyces cerevisiae* SBTD-2B, petite mutation	+	0	260.0000	Prazmo *et al*. (1975)
SCF, *Saccharomyces cerevisiae* petite mutation	(+)	0	640.0000	Egilsson *et al*. (1979)
SCF, *Saccharomyces cerevisiae* D7, petite mutation	+	0	750.0000	Kharab & Singh (1987)
SCR, *Saccharomyces cerevisiae* S/M 13-D, erythromycin-resistant mut.	-	0	1300.0000	Putrament *et al*. (1977)
SCR, *Saccharomyces cerevisiae* D7, *ilv* gene mutation	-	0	1300.0000	Fukunaga *et al*. (1982)
SCR, *Saccharomyces cerevisiae* D7, *ilv* gene mutation	-	0	0.0000	Singh (1983)
SCR, *Saccharomyces cerevisiae* D7, *ilv* gene mutation	(+)	0	3000.0000	Kharab & Singh (1985)
PLM, *Pisum abyssinicum*, chlorophyll mutation	+	0	0.0000	Von Rosen (1964)[b]
ACC, *Allium cepa*, chromosomal aberration	+	0	3.0000	Gori & Zucconi (1957)
???, *Allium cepa*, aneuploidy	+	0	0.0000	Gori & Zucconi (1957)
DIA, DNA strand breaks, Chinese hamster CHO cells	+	0	260.0000	Hamilton-Koch *et al*. (1986)
DIA, DNA cross-links, Novikoff hepatoma cells	(+)	0	130.0000	Wedrychowski *et al*. (1986)
G9H,Gene mutation, Chinese hamster V79 cells, *hprt* locus	(+)	0	26.0000	Miyaki *et al*. (1979)
G9H,Gene mutation, Chinese hamster V79 cells, *hprt* locus	+	0	0.0000	Hartwig *et al*. (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	-	0	57.0000	Amacher & Paillet (1980)
SIM, Sister chromatid exchange, mouse macrophage P388D1 cell line	+	0	13.0000	Andersen (1983)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	+	0	35.0000	Casto *et al*. (1979)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	+	0	55.0000	Casto *et al*. (1979)
DIH, DNA strand breaks, human white blood cells	+	0	6.5000	McLean *et al*. (1982)
DIH, DNA strand breaks, human diploid fibroblasts	+	0	650.0000	Hamilton-Koch *et al*. (1986)
DIH, DNA strand breaks, HeLa cells	+	0	0.0000	Hartwig *et al*. (1990)
SHL, sister chromatid exchanges, human lymphocytes	+	0	1.3000	Andersen (1983)

Table 21 (contd)

Test system	Result		Dose LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt(II) salts (contd)				
CHF, Chromosomal aberrations, human fibroblasts	-	0	0.0150	Paton & Allison (1972)
CHL, Chromosomal aberrations, human lymphocytes	-	0	0.6000	Voroshilin *et al*. (1978)
CIH, Chromosomal aberrations, human leukocytes	-	0	0.1500	Paton & Allison (1972)
AIH, Aneuploidy, human lymphocytes	+	0	3.7000	Resende de Souza-Nazareth (1976)
AVA, Aneuploidy, bone marrow and testes of male hamsters	+	0	400.0000	Farah (1983)[c]
Cobalt sulfides				
DIA, DNA strand breaks, Chinese hamster CHO cells	+	0	10.0000	Robison *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells	+	0	5.0000	Costa *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells	(+)	0	10.0000	Costa *et al*. (1982)
Cobalt(III) salts				
BSD, *Bacillus subtilis rec* strain H17, growth inhibition	(+)	0	1375.0000	Kanematsu *et al*. (1980)

[a]Antimutagenic effect

[b]Or as EDTA chelate

[c]Injected intraperitoneally over nine days

a small proportion of the cobalt (from either the metal or the oxide) retained after inhalation has a biological half-time in the order of years, were obtained by other investigators (Newton & Rundo, 1970; Hedge *et al.*, 1979).

Measurements of cobalt, chromium and nickel in blood and urine from persons with metallic hip replacements containing a high proportion of these metals have repeatedly shown elevated levels of one or several of them compared to controls or prior to surgery (Coleman *et al.*, 1973; Jones *et al.*, 1975; Hildebrand *et al.*, 1985; Braun *et al.*, 1986; Hildebrand *et al.*, 1988). [The Working Group noted that the analytical accuracy of several of the earlier studies was not confirmed.]

Sunderman *et al.* (1989) measured the concentrations of chromium, cobalt and nickel in serum and urine samples collected from patients who had undergone bone surgery and had received metallic hip or knee prostheses. Patients were followed for up to two years. The concentration of chromium in serum and urine remained essentially unchanged, whereas the concentration of nickel was markedly increased in both urine and serum collected shortly after the operation (1-14 days). The cobalt concentration, however, displayed a relatively small, slow increase in serum and blood. The highest concentrations were seen after two and 22 months in two patients who had loosening of their prosthesis.

(ii) *Toxic effects*

Pulmonary effects have been regarded as the major occupational problem in relation to cobalt, particularly in the hard-metal industry where cobalt-containing dust is generated. Two types of lung lesions may develop—interstitial fibrosis (so-called 'hard-metal pneumoconiosis') and occupational asthma (Demedts & Ceuppens, 1989). Hard-metal pneumoconiosis is a severe and progressive type of pneumoconiosis which may develop after several years of exposure to cobalt containing dust at concentrations of 0.1-2 mg/m^3 (for reviews, see Elinder & Friberg, 1986; Sprince *et al.*, 1988). As the dust in the hard-metal industry always contains agents in combination with cobalt (tungsten carbide and sometimes other metals such as titanium and tantalum), it has been questioned whether cobalt is solely responsible for the observed health effects (Brooks, 1981). Diamond polishers exposed to fine dust containing cobalt and diamond had severe lung fibrosis (Demedts *et al.*, 1984).

Symptoms and signs of obstructive lung disease can develop as a result of occupational exposure to cobalt-containing dust during the production of hard metal (Coates & Watson, 1971, 1973; Bech, 1974; Scherrer & Maillard, 1982), but these were also observed in workers in a porcelain factory using cobalt dye (Raffn *et al.*, 1988) and among diamond polishers (Gheysens *et al.*, 1985). This condition, which usually improves after cessation of exposure, is considered to be of allergic origin (Sjögren *et al.*, 1980). Provocation tests with cobalt usually induce a typical asthmatic reaction (Hartmann *et al.*, 1982). Shirakawa *et al.*

(1989) examined eight workers who developed asthma after having worked in a Japanese hard-metal plant. The total number of workers was about 400. The eight asthmatic workers all reacted with a drop in peak expiratory flow rate after an inhalation challenge with cobalt chloride. In four of them, it was possible to identify specific IgE antibodies towards cobalt-conjugated human albumin. This finding supports the hypothesis that cobalt hypersensitivity has a role in hard-metal asthma.

Histopathological findings in lung biopsies from workers with fibrosis (hard-metal pneumoconiosis) and/or obstructive problems (hard-metal asthma) have been published (Coates & Watson, 1971, 1973; Davison *et al.*, 1983; Demedts *et al.*, 1984; Anttila *et al.*, 1986; Cugell *et al.*, 1990). Typical microscopic findings include advanced fibrosis and desquamative interstitial pneumonia of the giant-cell type (Coates & Watson, 1971; Anttila *et al.*, 1986).

Cobalt has an erythropoietic effect and has been used for the treatment of anaemia (Berk *et al.*, 1949; Duckham & Lee, 1976). Berk *et al.* (1949) gave patients about 100 mg cobalt in the form of cobalt chloride three times a day for several weeks and recorded vomiting and anorexia in some patients, but only mild symptoms in the alimentary tract were seen as side-effects of the treatment in others. Duckham and Lee (1976) used a lower dose of cobalt chloride (25-50 mg cobalt per day) and observed fewer side effects. Polycythaemia has also been reported in heavy drinkers of cobalt-fortified beer (Morin *et al.*, 1971; Alexander, 1972).

Endemic outbreaks of cardiomyopathy with mortality rates of up to 50% were described among heavy consumers (up to 10 l per day) of cobalt-fortified beer (Morin & Daniel, 1967; Kesteloot *et al.*, 1968; Morin *et al.*, 1971; Alexander, 1972). As the daily dose of cobalt ingested by heavy beer drinkers (a few milligrams) was certainly excessive compared to the normal daily intake of cobalt (around 5-50 µg/day), but considerably lower than the doses prescribed to patients with anaemia, it was suggested that the cardiomyopathy had a multicausal origin (Morin & Daniel, 1967; Balazs & Herman, 1976). Three cases of cardiomyopathy, two of which were fatal, were described in workers exposed industrially to cobalt (Barbořík & Dusek, 1972; Kennedy *et al.*, 1981; Alušík *et al.*, 1982).

There are some indications that workers in hard-metal plants have increased morbidity and mortality from cardiovascular disease. Alexandersson and Atterhög (1980) examined workers exposed to cobalt-containing dusts at concentrations of 0.01-0.06 mg/m^3. Symptoms of dyspnoea, 'heavy breathing' and 'tightness in chest' were more prevalent in exposed workers than in controls, but no pulmonary dysfunction was found. In a recent study of 3163 workers exposed to cobalt-containing dusts at concentrations ranging from 0.001 to up to 11 mg/m^3 for at least one year, Hogstedt and Alexandersson (1990) found an

excess of deaths from ischaemic heart disease (standardized mortality ratio (SMR), 169; 95% confidence interval (CI), 96-275) among workers who had been exposed to 0.02-11 mg/m^3 cobalt for at least 10 years (see also p. 445).

Cobalt may provoke allergic dermatitis (Camarasa, 1967). Of 853 patch-tested workers, about 7% showed allergic reactions to 1% cobalt chloride (Fischer & Rystedt, 1983). Cobalt allergy, which is usually found in people who suffer from other skin allergies and/or eczema (Rystedt & Fischer, 1983), is also seen in other occupational groups, such as offset printers and construction workers handling cobalt-containing cement (Goh *et al.*, 1986).

Cobalt and nickel released from orthopaedic or dental prostheses may precipitate allergic reactions, with local effects and inflammation (Jones *et al.*, 1975; Fernandez *et al.*, 1986; Thomas *et al.*, 1987).

(iii) *Effects on reproduction and prenatal toxicity*

The spontaneous abortion rate appeared to be increased in women who either worked in metal smelting or had spouses working in the metallurgical industry. Exposure to cobalt, arsenic, copper, zinc and sulfur was considered possible in the work setting (Hemminki *et al.*, 1983). [The Working Group noted that the contribution of cobalt, if any, to the increase in abortion rate was not separately identified.]

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3. Case reports and epidemiological studies of carcinogenicity in humans

(a) *Implanted medical devices*

The first report of development of a sarcoma at the site of a stainless-steel plate prosthesis for a fracture of the humerus was made in 1956 (McDougall, 1956). There have been 17 further reports of single cases of malignant neoplasia at the site of implants of metal-containing fracture plates or joint prostheses. The metal material used was unknown in four cases, stainless-steel in three cases and cobalt-containing alloys in 10 cases. The period between implantation and tumour development ranged from one to 30 years. The tumours described were various types of sarcoma in 14 cases (Delgado, 1958; Castleman & McNeely, 1965; Dube & Fisher, 1972; Arden & Bywaters, 1978; Tayton, 1980; Bagó-Granell *et al.*, 1984; Lee *et al.*, 1984; Penman & Ring, 1984; Swann, 1984; Weber, 1986; Hughes *et al.*, 1987; Ryu *et al.*, 1987; Martin *et al.*, 1988; Ward *et al.*, 1990), one carcinoma (Mazabraud *et al.*, 1989) and lymphoma in two cases (McDonald, 1981; Dodion *et al.*, 1983).

Incident cancers were recorded for a cohort of 1358 persons who received a total hip replacement in New Zealand in the period 1966-73 and were followed up for six months to

17 years (mean, 10.5 years) to the end of 1983 (Gillespie *et al.*, 1988). Total cancer incidence was similar to that expected (164 observed *versus* 179.4 expected on the basis of general population rates; SMR, 91 [95% CI, 78-107]). While the overall cancer risk within 10 years of hip replacement was significantly low (SMR, 74; 95% CI, 61-90, based on 107 observed cases), the risk after 10 or more years was significantly high (SMR, 160; 95% CI, 122-209, based on 57 cases). There was a significant overall increase in the incidence of tumours of the lymphatic and haematopoietic system (21 observed *versus* 12.5 expected; SMR, 168; 95% CI, 106-260). When the five lymphatic and haematopoietic malignancies diagnosed within two years of hip replacement were excluded, this SMR fell to 151 (16 *versus* 10.6 expected [95% CI, 86-245]). There were significant deficits of breast cancer (six observed *versus* 16.6 expected; SMR, 36; 95% CI, 14-82) and of colorectal cancer (21 observed *versus* 33.8 expected; SMR, 62; 95% CI, 39-96). [No specific information on the composition of the hip prostheses was provided.]

(b) *Occupational exposure*

Schulz (1978) reported a cobalt-containing giant-cell tumour of the buccal membrane in a mineral-oil refinery employee five months after a single accidental exposure to dust containing cobalt[II] phthalocyanine.

Sakuyn and Shabynina (1970, 1973) examined mortality rates among workers at four nickel plants in the USSR in 1955-67. The workers were exposed to cobalt, but also to various nickel and arsenic compounds. A two- to four-fold increase in the risk for lung cancer was reported. The risks relative to those of inhabitants in the towns in which the plants were located were increased in various parts of the plants, including the cobalt shops (relative risks, 5-13), where there was exposure to cobalt dust but also to nickel sulfates, nickel chlorides and arsenic compounds. A 1.5-3.3-fold increase in stomach cancer risk was also noted. [The observed numbers of deaths were not given, and no allowance was made for potential confounding factors.]

Cuckle *et al.* (1980) studied mortality in 297 men employed in two departments opened in 1937 and 1938 at a nickel refinery in the UK. In one department, a wet treatment plant, nickel sulfate, copper sulfate, 'cobaltic hydrate' and precious metal concentrates were manufactured; in the other, a chemical production department, a range of compounds of nickel, cobalt and selenium were produced. The men had all been first employed in the refinery in or after 1933 and had worked in one or other of the departments for at least 12 months before 1960. They were followed up to 30 June 1980. Overall, there were 105 deaths (SMR, 109 [95% CI, 89-132]). There were 13 deaths from lung cancer (SMR, 131 [95% CI, 70-224]); six of the men who died from lung cancer [SMR, 154; 95% CI, 57-336] had been employed in the precious metal concentration section of the wet treatment plant. When the expected number of lung cancer deaths was estimated from death rates in rural

districts of Glamorganshire (where the refinery was located), rather than in the population of England and Wales as a whole, the SMR was [172; 95% CI, 92-295]. Excess mortality from lung cancer occurred mainly less than 20 years from first employment in the refinery (SMR, 178 [95% CI, 65-387]) and among men who had been employed for six or more years (SMR, 138 [95% CI,55-283]). Among the 1173 workers employed in the whole refinery in or after 1930 (International Committee on Nickel Carcinogenesis in Man, 1990), those first employed in 1930-39 had a SMR for lung cancer of 154 (95% CI, 97-233), those first employed in 1940-49 a SMR of 130 (95% CI, 71-218) and those first employed after 1950 a SMR of 77 (95% CI, 33-152). Cuckle *et al.* (1980) did not attribute any increase in risk in this cohort to exposure to cobalt.

Mur *et al.* (1987) followed up 1143 workers with at least one year of employment betwen 1950 and 1980 in an electrochemical plant producing cobalt and sodium in France. Altogether, 24.9% of the cohort were migrants (mainly North Africans and Italians). Vital status was established for 99.5% of the French-born workers and for 81.3% of the migrants. A total of 213 deaths occurring before 1981 was identified; cause of death was determined for 80% by interview with attending physicians and from hospital records. After adjustment for unknown causes of death (assuming that the distribution by cause of death was similar to that of cases with known cause of death), a SMR of 90 (95% CI, 44-159, based on nine cases) was observed for the total cohort for cancer of the lung, using mortality rates for France as a reference. For workers employed only in cobalt production, the SMR for lung cancer was 466 (95% CI, 146-1064, based on four observed cases). [The migrants may have had different rates of lung cancer from French-born workers, but the proportion of migrant workers in the different departments of the plants was not reported.] A case-control analysis was performed of lung cancer cases and controls, matched for year of birth, year of death and smoking habits. [The quality and manner of collection of information on smoking is unclear.] An odds ratio of 4.0 [95% CI, 1.6-9.9; calculated by the Working Group using an unmatched analysis] was associated with ever having worked in cobalt production. Workers in cobalt production were also exposed to unknown levels of forms of nickel and arsenic. [It is not known whether workers in other areas also had such exposure. No analysis based on latency or duration of exposure was presented.]

Hogstedt and Alexandersson (1990) reported on 3163 male Swedish workers with at least one year of exposure to cobalt-containing hard-metal dust at one of three hard-metal manufacturing plants in 1940-82 who were followed up during the period 1951-82. There were four categories of exposure (with estimated ambient air concentrations prior to 1970): occasionally present in rooms where hard metal was handled (less than 2 $\mu g/m^3$ Co); continuously present in rooms where hard metal was handled, but own work not involving

hard metal (1-5 μg/m^3 Co); manufacturing hard-metal objects (10-30 μg/m^3 Co); and exposed to cobalt in powder form when manufacturing hard-metal objects (60-11 000 μg/m^3 Co). No specific information was given on exposure to other substances in this cohort, but the workers were exposed to a number of substances that are used in the production of hard metal, such as tungsten carbide. There were 292 deaths among persons under 80 years of age during the study period; the SMRs relative to that of the male Swedish population were 96 (95% CI, 85-108) for mortality from all causes and 105 (95% CI, 82-132) for all incident tumours (73 cases). There were 17 cases of lung cancer *versus* 12.7 expected (SMR, 134; 95% CI, 77-213). With more than 10 years of exposure time and more than 20 years since first exposure, there were seven cases of lung cancer *versus* 2.5 expected (SMR, 278; 95% CI, 111-572); there were three cases of cancer of the lung *versus* 1.3 expected in the two lower exposure groups, and four cases of lung cancer *versus* 1.2 expected in the two higher exposure groups. A survey carried out at the end of the 1970s among hard-metal workers in Sweden showed that their smoking habits were not different from those of the male Swedish population (Alexandersson, 1979).

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Cobalt is widely distributed in the environment; it is the thirty-third most abundant element in the earth's crust. Cobalt is obtained primarily as a by-product of the mining and processing of copper and nickel ores and is a constituent of about 70 naturally occurring oxide, sulfide, arsenide and sulfoarsenide minerals. Cobalt is extracted from ore and concentrated by pyrometallurgical, hydrometallurgical and electrolytic processes alone or in combination. Refined metallic cobalt is available to the industrial market as cathodes and to a lesser extent as powders; oxides and other compounds are also available.

Cobalt compounds have been used as pigments in glass and ceramics in many countries for thousands of years. Since the beginning of the twentieth century, the major uses of cobalt have been in the production of metal alloys, such as superalloys and magnetic alloys, as well as high-strength steels and hard-metal cemented carbides. At the end of the 1980s, about one-third of the cobalt used was in the production of cobalt chemicals, which are used primarily as catalysts and pigments.

The main route of occupational exposure is *via* the respiratory tract by inhalation of dusts, fumes and mists containing cobalt. Exposures have been measured in hard-metal production, processing and use and in porcelain painting. Occupational exposure to cobalt is regulated in many countries.

Cobalt occurs in vegetables *via* uptake from soil, and vegetables account for the major part of human dietary intake of cobalt. Animal-derived foods, particularly liver, contain cobalt in the form of vitamin B_{12}. Cobalt is also found in air, water and tobacco smoke. Human tissues and fluids normally contain low levels of cobalt, which may be increased as a result of occupational exposures. Cobalt concentrations in tissue, serum and urine can be increased in patients with implants made of cobalt-containing alloys. Cobalt-containing particles have been detected in tissues immediately adjacent to such prostheses.

4.2 Experimental carcinogenicity data

Cobalt metal powder was tested in two experiments in rats by intramuscular injection and in one experiment by intrathoracic injection, producing sarcomas at the injection site.

A finely powdered *cobalt-chromium-molybdenum alloy* was tested in rats by intramuscular injection, producing sarcomas at the injection site. In two other experiments in rats, coarsely or finely ground cobalt-chromium-molybdenum alloy implanted in muscle or pellets of cobalt-chromium-molybdenum alloy implanted subcutaneously did not induce sarcomas. Implantation in the rat femur of three different *cobalt-containing alloys*, in the form of powder, rod or compacted wire, resulted in a few local sarcomas. In another experiment, intramuscular implantation of polished rods consisting of three different cobalt-containing alloys did not produce local sarcomas. In an experiment in guinea-pigs, intramuscular implantation of a *cobalt-chromium-molybdenum alloy* powder did not produce local tumours.

Intraperitoneal injection of a *cobalt-chromium-aluminium spinel* in rats produced a few local malignant tumours, and intratracheal instillation of this spinel in rats was associated with the occurrence of a few pulmonary squamous-cell carcinomas.

In two experiments in rats, intramuscular injection of *cobalt*[*II*] *oxide* powder produced sarcomas at the injection site. In an experiment in mice, intramuscular injection of cobalt oxide powder did not produce local tumours. Intratracheal instillation of cobalt oxide powder in rats was associated with a few benign and malignant pulmonary tumours. In a study limited by poor survival, hamsters administered a cobalt oxide dust by inhalation showed no increase in the incidence of pulmonary tumours. In two experiments in rats by subcutaneous and intraperitoneal injection, cobalt oxide powder produced local malignant tumours.

Cobalt[*II*] *sulfide* powder was tested in one study in rats by intramuscular injection, producing a high incidence of local sarcomas.

Cobalt[II] *chloride* was tested in one study in rats by repeated subcutaneous injection, producing many local and a few distant subcutaneous sarcomas.

Cobalt[*II,III*] *oxide* was tested in one experiment in hamsters to determine the effects of various particulates on carcinogenesis induced by *N*-nitrosodiethylamine. Intratracheal instillation of cobalt[II,III] oxide did not increase the incidence of pulmonary tumours over that in appropriate control groups.

Studies in mice and rabbits with *cobalt naphthenate* could not be evaluated.

In a screening test for lung adenomas by intraperitoneal injection, *cobalt*[*III*] *acetate* did not increase the incidence of lung tumours in strain A mice.

Interpretation of the available evidence for the carcinogenicity of cobalt in experimental animals was difficult because many of the reports failed to include sufficient details on results of statistical analyses, on survival and on control groups. Further, statistical analyses could not be performed by the Working Group in the absence of specific information on survival and on whether the neoplasms were fatal. Nevertheless, weight was given in the evaluation to the consistent occurrence of tumours at the site of administration and to the histological types of tumours observed.

4.3 Human carcinogenicity data

A number of single cases of malignant tumours, mostly sarcomas, have been reported at the site of orthopaedic implants containing cobalt. In one cohort study of people with a hip prosthesis, there was a significant increase in the incidence of lymphatic and haematopoietic malignancies, and significant deficits of breast and colorectal cancers. Overall cancer incidence was significantly lower than expected in the first 10 years after surgery, but significantly higher than expected after 10 or more years. No data were provided on the composition of the prostheses in this study.

Four cohort studies on the association between industrial exposure to cobalt and death from cancer were reviewed, two of which provided information for the evaluation. In a French electrochemical plant, there was a significant increase in the risk for lung cancer among workers in cobalt production, who were also exposed to nickel and arsenic, but not among workers in other departments of the factory. In a study in Sweden of hard-metal workers with documented exposure to cobalt-containing dusts, a significant increase in lung cancer risk was seen in people exposed for more than 10 years whose exposure had begun more than 20 years previously.

Interpretation of the available evidence on the possible association between occupational exposure to cobalt and cancer in humans is made difficult by the fact that in three of the four studies there was concurrent exposure to other potentially carcinogenic substances,

including forms of nickel and arsenic. In the Swedish study, there was concurrent exposure to other components of hard-metal dust.

4.4 Other relevant data

Occupational exposure to cobalt-containing dusts can cause fibrotic changes in the lung and can precipitate asthma. Cardiotoxic effects have been reported in exposed humans; in particular, cardiomyopathy can occur after prolonged oral intake.

Cobalt[II] chloride reduced fertility in male mice.

Cobalt[II] compounds had weak or no genetic effect in bacteria; some cobalt[III] complexes with heterocyclic ligands were active.

In single studies with an extensive range of eukaryotes, including animal and human cells *in vitro*, cobalt[II] compounds induced DNA damage, mutation, sister chromatid exchange and aneuploidy. Gene conversion and mutation in eukaryotes and DNA damage in human cells were observed in several studies. There was some evidence that these compounds can also induce aneuploidy in hamsters *in vivo*. In single studies, cobalt[II] sulfide induced DNA damage and transformation in cultured mammalian cells.

4.5 Evaluation[1]

There is *inadequate evidence* for the carcinogenicity of cobalt and cobalt compounds in humans.

There is *sufficient evidence* for the carcinogenicity of cobalt metal powder in experimental animals.

There is *limited evidence* for the carcinogenicity of metal alloys containing cobalt, chromium and molybdenum in experimental animals.

There is *sufficient evidence* for the carcinogenicity of cobalt[II] oxide in experimental animals.

There is *limited evidence* for the carcinogenicity of cobalt[II] sulfide in experimental animals.

There is *limited evidence* for the carcinogenicity of cobalt[II] chloride in experimental animals.

There is *inadequate evidence* for the carcinogenicity of cobalt-aluminium-chromium spinel, cobalt[II,III] oxide, cobalt naphthenate and cobalt[III] acetate in experimental animals.

[1] For definition of the italicized terms, see Preamble.

Overall evaluation

Cobalt and cobalt compounds *are possibly carcinogenic to humans* (*Group 2B*).

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

COBALT SULFATE HEPTAHYDRATE

(CAS NO. 10026-24-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

August 1998

NTP TR 471

NIH Publication No. 98-3961

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

ABSTRACT

$CoSO_4 \cdot 7H_2O$

COBALT SULFATE HEPTAHYDRATE

CAS No. 10026-24-1

Molecular Weight: 281.13

Synonyms: Bieberite; cobalt(II) sulfate (1:1) heptahydrate; cobalt monosulfate heptahydrate; cobalt(II) sulphate heptahydrate; sulfuric acid, cobalt(2+) salt (1:1) heptahydrate

Cobalt sulfate is used in the electroplating and electrochemical industries. It is also used as a coloring agent for ceramics and as a drying agent in inks, paints, varnishes, and linoleum. Cobalt sulfate may be added to animal feed as a mineral supplement and has been used as a top dressing on pasture lands. Cobalt sulfate was nominated by the National Cancer Institute for study based on a lack of information on the toxicity of soluble salts. Male and female F344/N rats and $B6C3F_1$ mice were exposed to cobalt sulfate heptahydrate (approximately 99% pure) by inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*. The results of prechronic inhalation toxicity studies were reported previously (Bucher *et al.*, 1990; NTP, 1991).

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt sulfate heptahydrate 6 hours per day, 5 days per week, for 105 weeks.

Survival and Body Weights

Survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Pathology Findings

The incidences and severities of proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation, and interstitial fibrosis were markedly greater in all exposed groups of male and female rats than in the chamber controls. The incidences of alveolar epithelial hyperplasia in all groups of exposed males and in females exposed to 3.0 mg/m^3 were significantly greater than those in the chamber control groups, as were the incidences of squamous metaplasia in 1.0 mg/m^3 females and atypical alveolar epithelial hyperplasia in 3.0 mg/m^3 females. In 3.0 mg/m^3 males, the combined incidence of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) was significantly greater than in the chamber controls. In female rats exposed to 1.0 or 3.0 mg/m^3, the

incidences of alveolar/bronchiolar neoplasms were significantly greater than those in the chamber control group and exceeded the NTP historical control ranges. A squamous cell carcinoma was observed in one 1.0 mg/m^3 and one 3.0 mg/m^3 female.

The incidences of benign, complex, or malignant pheochromocytoma (combined) in 1.0 mg/m^3 males and in 3.0 mg/m^3 females were significantly greater than those in the chamber controls and exceeded the historical control ranges.

Hyperplasia of the lateral wall of the nose, atrophy of the olfactory epithelium, and squamous metaplasia of the epiglottis were observed in all exposed groups of males and females, and the severities of these lesions increased with increasing exposure concentration. The incidences of squamous metaplasia of the lateral wall of the nose and metaplasia of the olfactory epithelium were increased in 3.0 mg/m^3 males and females.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt sulfate heptahydrate 6 hours per day, 5 days per week, for 105 weeks.

Survival and Body Weights

Survival of exposed males and females was similar to that of the chamber controls. Mean body weights of 3.0 mg/m^3 male mice were less than those of the chamber controls from week 96 until the end of the study. The mean body weights of all exposed groups of female mice were generally greater than those of the chamber controls from week 20 until the end of the study.

Pathology Findings

The incidences of diffuse histiocytic cell infiltration in 3.0 mg/m^3 males and of focal histiocytic cell infiltration in 3.0 mg/m^3 females were significantly greater than those in the chamber controls. The incidences of alveolar/bronchiolar neoplasms in 3.0 mg/m^3 males and females were significantly greater than those in the chamber control groups. The combined incidences of alveolar/bronchiolar adenoma or carcinoma and the incidences of alveolar/bronchiolar carcinoma in 3.0 mg/m^3 males and females and the incidence of alveolar/bronchiolar adenoma in 3.0 mg/m^3 females exceeded the NTP historical control ranges for inhalation studies.

The incidences of atrophy of the olfactory epithelium in 1.0 and 3.0 mg/m^3 males and females and hyperplasia of the olfactory epithelium in 3.0 mg/m^3 males and females were significantly greater than in the chamber controls. Squamous metaplasia of the larynx was observed in all exposed groups of males and females.

Male mice had a pattern of nonneoplastic liver lesions along with silver-staining helical organisms within the liver, characteristic of an infection with *Helicobacter hepaticus*. In NTP studies with *H. hepaticus*-associated hepatitis, increased incidences of heman-giosarcoma were seen in the liver of male mice. In this study of cobalt sulfate heptahydrate, incidences of hemangiosarcoma were increased in exposed groups of male mice. Because of the above association, interpretation of the increased incidences of hemangiosarcoma in the livers of male mice was confounded. Incidences of lesions at other sites in this study of cobalt sulfate heptahydrate were not considered to have been significantly impacted by the infection with *H. hepaticus* or its associated hepatitis.

GENETIC TOXICOLOGY

Cobalt sulfate heptahydrate was mutagenic in *S. typhimurium* strain TA100 with and without liver S9 metabolic activation enzymes; no mutagenic activity was detected in strain TA98 or TA1535, with or without S9.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of cobalt sulfate heptahydrate in male F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms. Marginal increases in incidences of pheochromocytomas of the adrenal

medulla may have been related to exposure to cobalt sulfate heptahydrate. There was *clear evidence of carcinogenic activity* in female F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytomas of the adrenal medulla in groups exposed to cobalt sulfate heptahydrate. There was *clear evidence of carcinogenic activity* of cobalt sulfate heptahydrate in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to cobalt sulfate heptahydrate caused a spectrum of inflammatory, fibrotic, and proliferative lesions in the respiratory tract of male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cobalt Sulfate Heptahydrate

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations	Chamber control, 0.3, 1.0, or 3.0 mg/m^3	Chamber control, 0.3, 1.0, or 3.0 mg/m^3	Chamber control, 0.3, 1.0, or 3.0 mg/m^3	Chamber control, 0.3, 1.0, or 3.0 mg/m^3
Body weights	Exposed groups similar to chamber controls	Exposed groups similar to chamber controls	3.0 mg/m^3 group slightly less than chamber controls	Exposed groups slightly greater than chamber controls
Survival rates	17/50, 15/50, 21/50, 15/50	28/50, 25/49, 26/50, 30/50	22/50, 31/50, 24/50, 20/50	34/50, 37/50, 32/50, 28/50
Nonneoplastic effects	Lung: proteinosis (0/50, 16/50, 40/48, 47/50); alveolar epithelial metaplasia (0/50, 50/50, 48/48, 49/50); granulomatous alveolar inflammation (2/50, 50/50, 48/48, 50/50); interstitial fibrosis (1/50, 50/50, 48/48, 49/50); alveolar epithelial hyperplasia (9/50, 20/50, 20/48, 23/50) Nose: lateral wall hyperplasia (2/50, 14/50, 21/49, 20/50); olfactory epithelial atrophy (8/50, 24/50, 42/49, 48/50); lateral wall squamous metaplasia (1/50, 3/50, 5/49, 8/50); olfactory epithelial metaplasia (5/50, 1/50, 5/49, 30/50) Larynx: epiglottis squamous metaplasia (0/50, 10/49, 37/48, 50/50)	Lung: proteinosis (0/50, 36/49, 49/50, 49/50); alveolar epithelial metaplasia (2/50, 47/49, 50/50, 49/50); granulomatous alveolar inflammation (9/50, 47/49, 50/50, 49/50); interstitial fibrosis (7/50, 47/49, 50/50, 49/50); alveolar epithelial hyperplasia (15/50, 7/49, 20/50, 33/50); squamous metaplasia (0/50, 1/49, 8/50, 3/50); atypical alveolar epithelial hyperplasia (0/50, 0/49, 3/50, 5/50) Nose: lateral wall hyperplasia (1/50, 8/49, 26/50, 38/50); olfactory epithelial atrophy (5/50, 29/49, 46/50, 47/50); lateral wall squamous metaplasia (1/50, 1/49, 4/50, 10/50); olfactory epithelial metaplasia (2/50, 2/49, 3/50, 40/50) Larynx: epiglottis squamous metaplasia (1/50, 22/49, 39/50, 48/50)	Lung: diffuse histiocytic cell infiltrate (1/50, 2/50, 4/50, 10/50) Nose: olfactory epithelial atrophy (0/50, 0/50, 29/48, 48/49); olfactory epithelial hyperplasia (0/50, 0/50, 0/48, 10/49) Larynx: squamous metaplasia (0/48, 37/49, 48/48, 44/49)	Lung: focal histiocytic cell infiltrate (2/50, 5/50, 7/50, 10/50) Nose: olfactory epithelial atrophy (0/50, 2/50, 12/49, 46/48); olfactory epithelial hyperplasia (0/50, 0/50, 0/49, 30/48) Larynx: squamous metaplasia (0/50, 45/49, 40/47, 50/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cobalt Sulfate Heptahydrate

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Lung: alveolar/ bronchiolar adenoma (1/50, 4/50, 1/48, 6/50); alveolar/ bronchiolar carcinoma (0/50, 0/50, 3/48, 1/50); alveolar/ bronchiolar adenoma or carcinoma (1/50, 4/50, 4/48, 7/50)	Lung: alveolar/ bronchiolar adenoma (0/50, 1/49, 10/50, 9/50); alveolar/ bronchiolar carcinoma (0/50, 2/49, 6/50, 6/50); alveolar/ bronchiolar adenoma, alveolar/bronchiolar carcinoma, or squamous cell carcinoma (0/50, 3/49, 16/50, 16/50) Adrenal medulla: benign, complex, or malignant pheochromocytoma (2/48, 1/49, 4/50, 10/48)	Lung: alveolar/ bronchiolar adenoma (9/50, 12/50, 13/50, 18/50); alveolar/ bronchiolar carcinoma (4/50, 5/50, 7/50, 11/50); alveolar/ bronchiolar adenoma or carcinoma (11/50, 14/50, 19/50, 28/50)	Lung: alveolar/ bronchiolar adenoma (3/50, 6/50, 9/50, 10/50); alveolar/ bronchiolar carcinoma (1/50, 1/50, 4/50, 9/50); alveolar/ bronchiolar adenoma or carcinoma (4/50, 7/50, 13/50, 18/50)
Uncertain findings	Adrenal medulla: benign, complex, or malignant pheochromocytoma (15/50, 19/50, 25/49, 20/50)	None	None	None
Level of evidence of carcinogenic activity	Some evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive in strain TA100 with and without S9 Negative in strains TA98 and TA1535 with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on cobalt sulfate heptahydrate on 11 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 December 1996, the draft Technical Report on the toxicology and carcinogenesis studies of cobalt sulfate heptahydrate received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of cobalt sulfate heptahydrate by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on the chemical-related neoplastic and nonneoplastic lesions in male and female rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* in male F344/N rats and *clear evidence of carcinogenic activity* in female F344/N rats and male and female B6C3F$_1$ mice.

Dr. Tyson, a principal reviewer, agreed with the proposed conclusions. Concerning the genetic mechanisms involved in murine lung tumorigenesis, he said that although a comprehensive study of K-*ras* activation was done in lung neoplasms, other molecular markers could have been assessed as well. Loss of heterozygosity or homozygous deletions on regions of chromosome 4, which are syntenic to regions of human chromosome 9p21 where frequent deletions are observed in human lung cancer, could have been studied to determine if similar mechanisms are at work in both murine and human lung tumorigenesis via exposure to this chemical. Dr. R.C. Sills, NIEHS, reported that further studies were planned with the next step being to look at loss of heterozygosity not only on chromosome 4, but also to look at chromosomes 6 and 11, where the p53 genes are located.

Dr. Ward, the second principal reviewer, agreed with the proposed conclusions. He agreed with the rationale for the exposure concentrations chosen for the 2-year studies but because there was no concentration-related body weight gain depression, he thought that rats and mice could have tolerated higher concentrations. With regard to the extensive lesions in the nasal cavity and larynx, he stated that this was a classic case showing the association between toxic and regenerative/reparative lesions resulting in no neoplasms.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions.

Dr. Tyson moved that the Technical Report on cobalt sulfate heptahydrate be accepted with the revisions discussed and with the conclusions as written for male F344/N rats, *some evidence of carcinogenic activity* and for female F344/N rats and male and female B6C3F$_1$ mice, *clear evidence of carcinogenic activity*. Dr. Russo seconded the motion, which was accepted unanimously with eight votes.

INTRODUCTION

$CoSO_4 \cdot 7H_2O$

COBALT SULFATE HEPTAHYDRATE

CAS No. 10026-24-1

Molecular Weight: 281.13

Synonyms: Bieberite; cobalt(II) sulfate (1:1) heptahydrate; cobalt monosulfate heptahydrate; cobalt(II) sulphate heptahydrate; sulfuric acid, cobalt(2 +) salt (1:1) heptahydrate

CHEMICAL AND PHYSICAL PROPERTIES

Cobalt sulfate is a reddish, crystalline, water-soluble powder. It is usually produced as cobalt(II) sulfate but can also exist in the cobalt(III) sulfate form with a formula of $Co_2(SO_4)_3 \cdot 18H_2O$. The heptahydrate salt is reported to have a structure of $[Co(H_2O)_6] \cdot [H_2SO_5]$ (*Merck Index*, 1983). Cobalt(II) salts are stable to autoxidation in air or in solution (Smith and Carson, 1981).

PRODUCTION, USE, AND HUMAN EXPOSURE

The production of cobalt sulfate in the United States in 1983 was estimated to be 450,000 pounds (204,000 kg) (J.V. Gandhi, Hall Chemical Co., personal communication); more recent production estimates are not available. Seven companies were listed as producing or handling cobalt sulfate at 10 facilities in the United States (USDHHS, 1992). Cobalt sulfate has been widely used in the electroplating and electrochemical industries. It is used as a coloring agent for ceramics and as a drying agent in inks, paints, varnishes, and linoleum. Cobalt sulfate may be added to animal feed as a mineral supplement and has been used as a top dressing on pasture lands (De Bie and Doyen, 1962).

Cobalt is an essential trace element because it is an integral part of vitamin B_{12}. The human body burden is approximately 1.1 mg, and the daily intake is about 0.3 mg, primarily via food (Hammond and Beliles, 1980). Cobalt is found in urban air (0.5 to 60 ng/m^3) (Morgan *et al.*, 1970) and has been identified in trace amounts in natural waters; concentrations in excess of 10 µg/L are rare (NRC, 1977). Ocean water contains about 0.3 µg/L (Hamilton, 1994). Cobalt has been identified in chemical waste dumps (Barrett, 1983).

In the 1960s, several breweries added cobalt sulfate to beer at a level of about 1 ppm to counteract the antifoaming activity of detergent residues left on poorly rinsed glasses (Morin and Daniel, 1967). Soon after this, an epidemic of "beer-drinkers' cardiomyopathy" occurred, and cobalt was identified as the causative agent. The addition of cobalt salts to beer was discontinued, and the epidemic ceased. Doses of cobalt chloride of up to 200 to 300 mg per day were given orally to patients as treatment for various types of anemia in the 1950s (Finch, 1980). This practice

has largely stopped because of associated toxicity (gastrointestinal upset, goiter, cardiomyopathies) and the development of less hazardous therapies.

It has been estimated that over 1 million workers in the United States are exposed to cobalt or cobalt compounds (Jensen and Tüchsen, 1990). Occupational exposure to cobalt occurs principally in refining processes, in the production of alloys, and in the tungsten carbide hard metal industry (Kazantzis, 1981). Exposure under these conditions is primarily dermal or via inhalation of cobalt metal dusts or fumes, often in combination with other elements such as nickel, arsenic, or tungsten; adverse respiratory effects (such as pneumoconiosis) have been reported at cobalt concentrations between 0.1 and 2 mg/m^3 (Domingo, 1989). The threshold limit value-timeweighted average for elemental cobalt is 0.02 mg/m^3 (ACGIH, 1996). Airborne levels of cobalt dust from spray painting in a Danish porcelain factory in 1981 were as high as 8.6 mg/m^3 (Jensen and Tüschen, 1990).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The absorption of cobalt salts after oral administration is variable and is influenced by the nature of the salt, the size of the dose, and the presence of food in the gastrointestinal tract (Murdock, 1959; Smith *et al.*, 1972). Clearance of inhaled soluble cobalt salts from the lung has not been studied but is expected to be rapid (Kerfoot *et al.*, 1975). Several processes could contribute to this effect. The water-soluble salts dissolve directly, and certain insoluble salts and cobalt metal powder appear to have an appreciable solubility in protein-containing fluids (Harding, 1950). Clearance by phagocytic alveolar macrophages may also occur (Kerfoot *et al.*, 1975). Cobalt is distributed to all tissues after administration by the oral or inhalation route or by injection (Smith and Carson, 1981). Tissue retention is not marked, but higher concentrations have been noted in the liver, kidney, spleen, and heart than in other organs (Domingo *et al.*, 1984a,b; Llobet *et al.*, 1986).

Experimental Animals

In an unspecified strain of rabbits administered cobalt sulfate at doses of 0.25 mg/kg per day orally or by injection for 2 months, some accumulation of cobalt occurred in the liver, small intestine, lung, blood, kidney, and stomach (Kichina, 1974). Excretion is primarily via the urine and secondarily via the feces. The cobalt content of bile collected for 2 hours after intravenous administration of [^{57}Co] cobalt chloride to Sprague-Dawley rats totaled about 2% to 5% of the dose over a thirty-fold dose range (0.03 mg/kg to 1 mg/kg of Co^{2+}) (Gregus and Klaassen, 1986). Several studies have shown that a small portion of cobalt, given in several forms by parenteral or inhalation routes, is retained in tissues with a biological half-time of several years (IARC, 1991). The form of these materials has not been determined, but this could represent uptake into vitamin B_{12} (Edel *et al.*, 1990).

Humans

A recent report has demonstrated significant dermal absorption of cobalt by humans exposed to mixed cobalt-tungsten carbide powders (Scansetti *et al.*, 1994). The concentration of cobalt in the blood and urine of nonoccupationally exposed humans is 0.2 to 2.0 µg/L (Hamilton, 1994). Cobalt concentrations in the urine of workers in the Italian hard metal industry were between 10 and 100 µg/L at the beginning of the work shift and increased to between 16 and 210 µg/L at the end of the work shift (Sabbioni *et al.*, 1994).

TOXICITY

Experimental Animals

Exposure to cobalt results in a wide spectrum of toxicities in mammals. The ionic radius of cobalt is between that of Mg^{2+} and Ca^{2+}, so cobalt can replace or mimic these ions and also may influence reactions normally involving Fe^{2+}, Zn^{2+}, Cu^{2+}, or Mn^{2+} (Jennette, 1981). For example, cobalt can bind to Ca^{2+}-binding proteins in or near microtubules (Phillips, 1980) and has been shown to block Ca^{2+} channels in squid axons (Baker *et al.*, 1973). Cobalt promotes aberrant microtubule assembly (Buttlaire *et al.*, 1980) and can alter the activity of metalloenzymes such as carboxypeptidase (Jennette, 1981). Cobalt also inhibits the activity of DNA polymerase I from *Micrococcus luteus* (Korman *et al.*, 1978). Cobalt binds to sulfhydryl groups, including those of glutathione and cysteine, and through its binding to lipoic acid inhibits pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, effectively stopping oxidative metabolism (Dingle *et al.*, 1962).

A 250 µmol/kg (approximately 60 mg/kg) dose of cobalt chloride heptahydrate administered by subcutaneous injection to male Sprague-Dawley rats caused a rapid increase in biliary excretion of both reduced and oxidized glutathione, but total hepatic glutathione tended to increase after cobalt exposure (Stelzer and Klaassen, 1985).

A dose of 60 mg cobalt/kg body weight given to an unspecified strain of rats was found to inhibit heme synthesis in the liver (De Matteis and Gibbs, 1977). This apparently results from the formation of cobalt protoporphyrin by ferrochelatase and feedback inhibition of δ-aminolevulinic acid synthetase activity by the abnormal protoporphyrin (Sinclair *et al.*, 1982). Cobalt also induces heme oxygenase (Maines and Kappas, 1976), and the combined effect of these actions is to rapidly decrease the cytochrome P_{450} concentrations in the liver. Other cytochromes appear to be less affected (Tephly and Hibbeln, 1971).

In contrast to its actions on heme synthesis in the liver, cobalt administration promotes polycythemia. This effect is more pronounced in humans than in rodents (Smith and Carson, 1981) and is the basis for the use of cobalt chloride to treat anemia. The oral administration of 10 mg cobalt/kg body weight given as cobalt chloride to male rats of unspecified strain five times per week for 150 days resulted in an increase in the erythrocyte count, hematocrit value, and hemoglobin concentration of the blood; however, the mean cell volume and hemoglobin concentration per cell were unchanged, indicating a simple polycythemic effect (Murdock, 1959). This response is mediated by an increase in circulating erythropoietin, postulated to be a secondary response to a central nervous system effect of cobalt which results in respiratory alkalosis. Alkalosis increases the affinity of heme for oxygen, which is interpreted by tissue "sensors" as hypoxia (Miller *et al.*, 1974).

A second effect of cobalt administration on the blood is an increase in triglycerides, cholesterol, and free fatty acids (Taylor and Marks, 1978). This may be caused by inhibition of tissue lipoprotein lipase, resulting in failure to clear very low-density lipoprotein (Taylor and Marks, 1978), and perhaps by stimulation of lipoprotein synthesis in the liver (Eaton, 1972).

A single injection of 35 mg/kg cobalt chloride caused degranulation and disintegration of the α cells of the pancreatic islets in rabbits (Telib, 1972). This was followed by degranulation of the ß cells.

Although exposure to cobalt affects a wide variety of enzymatic processes, the acute toxicity of cobalt is not as great as might be expected. The oral LD_{50} for anhydrous cobalt sulfate is 420 mg/kg in male and female Wistar rats (Speijers *et al.*, 1982).

Krasovskii and Fridlyand (1971) administered 0.5 or 2.5 mg/kg cobalt chloride by gavage to rats six times per week for 7 months. These investigators found polycythemia and a suppression of leukocyte function. Myocardial histologic changes were seen in 26 of 30 rats given 26 mg/kg cobalt sulfate by gavage once daily for 8 weeks (Grice *et al.*, 1969). This study is representative of a large number of animal studies designed to examine beer-drinkers' cardiomyopathy (cited in Smith and Carson, 1981, and USDHHS, 1992). Overall, these studies indicated that rather large doses of cobalt could mimic the cardiomyopathy caused by cobalt-treated beer, but that cobalt probably acted synergistically in humans with thiamine deficiency and an insufficient intake of sulfur-containing amino acids. Deficits in thyroid function have been shown in l-day-old chicks and guinea pigs but not in young chicks, rats, mice, or rabbits given cobalt (Sederholm *et al.*, 1968).

A variety of cobalt dusts and aerosols have been administered to animals via inhalation. Results of these studies indicate that lung compliance is decreased and that electrical properties of the heart are affected as in beer-drinkers' cardiomyopathy (Kerfoot *et al.*, 1975; Smith, 1980). In general, similar toxicity has been elicited by cobalt whether administered orally or by inhalation. These effects have been seen after exposure of rats to atmospheres containing 0.05 or 0.5 mg/m^3 cobalt for 3 months (Popov, 1977). In addition, specific pulmonary effects in male rabbits exposed to 0.5 mg/m^3 cobalt (as cobalt chloride) by inhalation for 6 hours per day, 5 days per week, for 4 to 6 weeks included a change in the growth pattern of alveolar type II cells, resulting in clusters of cells projecting into the alveolar lumen, and changes in oxidative metabolism of lung macrophages (Johansson *et al.*, 1984, 1986).

Sixteen-day and 13-week inhalation studies with cobalt sulfate heptahydrate in F344/N rats and B6C3F$_1$ mice have been reported (Bucher *et al.*, 1990; NTP, 1991). In the 13-week studies, groups of 10 male and 10 female rats and mice were exposed to cobalt sulfate heptahydrate concentrations ranging from 0 to 30 mg/m^3, 6 hours per day, 5 days per week. Two male mice exposed to 30 mg/m^3 died. All groups at this concentration initially lost weight, but then gained weight at rates similar to controls. At the end of the studies, lung weights were generally increased in rats and mice exposed to 1.0 mg/m^3 and higher, and polycythemia was observed in exposed rats but not in mice. Lesions observed in the respiratory tract of rats and mice included degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, and inflammation in the nose; inflammation, necrosis, squamous metaplasia, ulcers (rats), and inflammatory polyps (rats) of the larynx; squamous metaplasia of the trachea (mice); and histiocytic infiltrates, bronchiolar regeneration, peribronchiolar and septal fibrosis, and epithelial hyperplasia in the alveoli of the lung. A no-observed-adverse-effect-level (NOAEL) was not reached in these studies as lesions, particularly in the larynx, were observed at the lowest exposure (0.3 mg/m^3) used.

In other NTP studies (unpublished, available upon request), cobalt sulfate elicited contact hypersensitivity. Female Hartley guinea pigs received dermal applications of 100 µL of an aqueous 6% solution once per day for 14 days. A dose-related increase in contact hypersensitivity, as measured by retention of labeled inflammatory cells in the skin, was observed upon challenge application of solutions of 0.3%, 1%, or 3% aqueous cobalt sulfate to a site distant from the induction site 7 days after the last induction dose. Erythema and edema in the ears and paws of rats resulted from the administration of 5 mg cobalt sulfate by injection (Jasmin, 1974).

Humans

Besides myocardial toxicity, as noted above, a second effect of cobalt observed in victims of beer-drinkers' cardiomyopathy was hypothyroidism (Taylor and Marks, 1978). Thyroid function tests, including uptake of [^{131}I]iodide, were also depressed in patients receiving 0.17 to 3.9 mg/kg cobalt per day for treatment of anemia (Paley *et al.*, 1958). It has been proposed that cobalt interferes with binding of inorganic iodide to tyrosine in the thyroid gland.

Hypersensitivity reactions have been observed in patients who received prosthetic implants made of a cobalt alloy and in industrial workers exposed to cobalt dusts (Smith and Carson, 1981). Asthma related to cobalt exposure has also been described (Cirla, 1994).

Most inhalation of cobalt is by workers in the refining and alloy production industries (NIOSH, 1981). The dusts may be in the form of the metal, its alloys, or its salts, but most often the oxide form is present. Consequently, no toxicity studies exist on exposure to pure cobalt metal or to cobalt sulfate. Exposure appears to cause pulmonary fibrosis, splenic enlargement, dermatitis, and losses of appetite and sense of smell (Dorsit *et al.*, 1970). Cobalt is used in the cemented tungsten carbide industry and is thought to be primarily responsible for pulmonary "hard metal disease," consisting of upper respiratory tract irritation, pneumonitis, and pulmonary fibrosis (NIOSH, 1981). However, the actual role of inhaled cobalt versus an interaction of cobalt and other inhaled particles remains a subject of debate (Swennen *et al.*, 1993).

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Experimental Animals

Sprague-Dawley rats maintained on diets containing 265 ppm cobalt for 98 days showed degenerative changes in the testis; these changes were considered secondary to hypoxia (Mollenhaur *et al.*, 1985). Decreases in sperm motility and/or increased abnormal sperm were noted in mice, but not in rats, exposed to 3 mg/m^3 or higher in 13-week inhalation studies with cobalt sulfate (NTP, 1991). Following 13 weeks of chronic exposure to 100 to 400 ppm cobalt chloride in drinking water, male CD-1 mice showed marked dose-related decreases in fertility, testicular weight, and sperm concentration and motility, and increases in circulating levels of testosterone (Pedigo *et al.*, 1988).

Cobalt has been shown to cross the placenta; cobalt chloride and nitrite salt solutions induced fetal cleft

palates when injected alone into mouse dams, but inhibited cleft formation caused by cortisone or phenytoin (Kasirsky *et al.*, 1969; Mitala *et al.*, 1978). Oral exposure of rats to cobalt chloride at daily doses of 5.4 or 21.8 mg cobalt/kg body weight from gestation day 14 through lactation day 21 resulted in stunted growth and/or decreased pup survival, although adverse effects were also evident in the dams at both doses (Domingo *et al.*, 1985). In contrast, Paternain *et al.* (1988) reported that doses of up to 100 mg/kg cobalt chloride administered by gavage to pregnant Sprague-Dawley rats once per day on days 6 to 15 of gestation did not result in significant fetotoxicity or teratogenicity. Similarly, Seidenberg *et al.* (1986) reported no effect on mouse fetal growth or mortality in dams given daily doses of 81.7 mg cobalt/kg on days 8 to 12 of pregnancy.

Humans

Cobalt has not been shown to cause significant teratogenic or reproductive effects in humans (Smith and Carson, 1981). No clinical effects were noted in the babies of women who had taken cobalt chloride to counter anemia while pregnant (Jacobziner and Raybin, 1961).

CARCINOGENICITY

Experimental Animals

There have been no reports of adequate chronic inhalation toxicity or carcinogenicity studies with soluble or insoluble cobalt salts or metal powders (IARC, 1991). Wehner *et al.* (1977) found no increase in tumors in Syrian golden hamsters exposed to 10 mg/m^3 cobalt oxide dust for 7 hours per day, 5 days per week, for life; however, the study was faulted for poor survival (IARC, 1991). Cobalt oxide has been studied by intratracheal administration to groups of 50 male and 50 female Sprague-Dawley rats (Steinhoff and Mohr, 1991). Doses of 2 or 10 mg/kg were given in 19 treatments at 2-week intervals and in 10 treatments at 4-week intervals over 2 years. Two groups of 50 male and 50 female controls received saline or no treatment. Approximately 80% of the material was within the particle size range of 5 to 40 µm. At the end of the study an unspecified bronchioalveolar proliferation was noted in 51 of 100 low-dose rats (male and females combined), in 70 of 100 high-dose rats, and in no controls. One male and one female from the low-dose groups developed a benign lung tumor, and one high-dose female had a bronchioalveolar carcinoma. Three adenocarcinomas and two bronchioalveolar adenomas were observed in high-dose males. No lung tumors occurred in the controls. In a similar but smaller study by the same group, cobalt oxide was found to enhance the lung tumor yield of benzo[a]pyrene treatment (Steinhoff and Mohr, 1991).

Sarcomas in rats have been observed at the site of injection of cobalt salts or cobalt metal powder (IARC, 1991). Heath (1956, 1960) gave an unspecified strain of rats a single injection of 0.28 mg cobalt metal powder in fowl serum into the thigh muscle. Within 2 weeks, atypical myoblasts were observed (Heath, 1960), and between 5 and 12 months, malignant neoplasms developed at the injection site in 17 of 30 rats; 11 were rhabdomyosarcomas (Heath, 1956). Gilman (1962) reported a similar neoplastic response to injections of cobalt sulfide and cobalt oxide in an unspecified strain of rats but saw no neoplasms in an unspecified strain of mice. These materials are relatively insoluble, and Abbracchio *et al.* (1982) suggested that intracellular solubilization of relatively insoluble cobalt salts would favor cellular transformation. Heath and Webb (1967) determined that cobalt is bound intracellularly in primary rhabdomyosarcomas induced by intramuscular injection of metallic cobalt, with 70% to 90% of the bound cobalt found in the nucleus. Further fractionation studies demonstrated that 50% of the nuclear cobalt is bound in the nucleolus (Webb *et al.*, 1972). Similar injection studies have given little evidence of cobalt-induced cancer in mice, hamsters, or guinea pigs (Christensen and Poulsen, 1994).

There is only one report of the formation of neoplasms after injection of a soluble cobalt salt. Shabaan *et al.* (1977) observed fibrosarcomas in 14 of 40 male Wistar rats 8 months to 1 year after administration of 40 mg/kg cobalt chloride by subcutaneous injection once per day for 10 days. Four of these neoplasms were not at the site of injection.

Humans

Cobalt has been used in hundreds of patients as part of an alloy with chromium and molybdenum in prosthetic implants. During the first 14 years of its use for this purpose, no fibrosarcomas were identified in the recipients (McKee, 1971); however, a number of cases of malignant neoplasia have been reported

since that time at the sites of metal-containing fracture plates or joint prostheses, some of which contained cobalt (IARC, 1991).

The IARC (1991) considered the available data inadequate to establish an association between cancer and cobalt exposure to humans. At that time there were two epidemiological studies that were considered adequate for evaluation (Mur *et al.*, 1987; Hogstedt and Alexandersson, 1990). The Mur *et al.* (1987) cohort study was composed of 1,143 workers who were employed for at least a year between 1950 and 1980 in a French electrochemical plant producing cobalt and sodium. For workers employed only in cobalt production, the standard mortality ratio for lung cancer was 466 (95% confidence interval from 146 to 1,064) based on four cases. Hogstedt and Alexandersson (1990) studied a cohort of 3,163 male Swedish workers with at least 1 year of exposure to cobalt-containing, hard-metal dust ore between 1940 and 1982. There were 17 cases of lung cancer versus 12.7 expected (SMR, 134; 95% CI 77 to 213). Interpretation of both studies was made difficult by concurrent exposures to other substances including arsenic and nickel in the French plant and tungsten carbide in the Swedish facility.

Since the IARC evaluation, a follow-up study of the French electrochemical plant workers was completed which extended the period of observation from 1981 to 1988. No additional lung cancers were observed. Based on this and other factors, the authors concluded that the data no longer supported an association of cobalt exposure with lung cancer (Moulin *et al.*, 1993). In contrast, Lasfargues *et al.* (1994) reported on a cohort mortality study carried out on workers at a French hard-metal plant. The study specifically addressed lung cancer risks in relation to cobalt exposure and included 709 male workers who had at least 1 year of employment at the plant and who died between the years 1956 and 1989. While overall mortality was not increased, death due to lung cancer was significantly elevated (SMR=213), with 10 cases observed. This excess was associated with high cobalt exposure, but no effect of employment duration was noted. Smoking did not account for the observed incidence of lung cancer.

GENETIC TOXICITY

Genetic toxicity data for cobalt sulfate heptahydrate are limited to a single publication. Zeiger *et al.* (1992) reported the results of a mutagenicity study with cobalt sulfate heptahydrate which showed a weakly positive response in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation as well as with hamster or rat liver S9; the authors reported no induction of mutations in strain TA98 or TA1535, with or without S9.

Few studies with other cobalt compounds have been reported. The literature on genetic and related effects of cobalt compounds was reviewed by Beyersmann and Hartwig (1992). Most of the bacterial mutagenicity test results included in this review were negative. However, some positive results were reported for mammalian cell DNA damage studies, including the observation of DNA strand breaks in human cells (McLean *et al.*, 1982; Hamilton-Koch *et al.*, 1986; Hartwig *et al.*, 1990) and sister chromatid exchange induction in human (Anderson, 1983) and hamster cells (Hartwig *et al.*, 1991) treated *in vitro* with cobalt chloride in the absence of exogenous metabolic systems. The authors discussed the possible role of hydroxyl and superoxide radical formation in the generation of DNA breaks (Beyersmann and Hartwig, 1992). Morita *et al.* (1991) reported a weak response in an *in vitro* test designed to detect increased frequencies of 6-thioguanine-resistant mutant FM3A cell colonies. At a concentration of 2×10^{-4} M cobalt chloride (which induced a 50% decrease in cell survival), an increased number of mutant colonies (approximately four to five times the control number) was observed. At concentrations higher and lower than 2×10^{-4} M, the mutagenic response was weaker. The authors suggested, based upon results from the testing of other known mutagens in this assay, that metal ions such as cobalt require relatively high concentrations and long exposure periods to induce an effect and that the induced mutagenic response obtained is weak and seen over a narrow dose range. In the *Drosophila* wing spot test, cobalt chloride was demonstrated to induce a significant, dose-dependent increase in somatic recombination in third instar larvae exposed to cobalt chloride concentrations of 2 to 10 mM during development to the adult stage (Ogawa *et al.*, 1994).

STUDY RATIONALE

Cobalt sulfate was nominated by the National Cancer Institute for study based on a lack of information on the toxicity of soluble cobalt salts. The more common cobalt(II) form and the inhalation route were selected for study to mimic worker exposure. Prechronic studies were previously reported (Bucher *et al.*, 1990; NTP, 1991) with a spectrum of lesions noted in the respiratory tract of rats and mice. Polycythemia was also observed in rats. A NOAEL was not reached in these studies using doses as low as 0.3 mg/m^3. This report documents the findings of 2-year inhalation exposure studies with cobalt sulfate heptahydrate in F344/N rats and $B6C3F_1$ mice.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF COBALT SULFATE HEPTAHYDRATE

Cobalt sulfate heptahydrate was obtained from Curtin Matheson Scientific (Kansas City, MO) in one lot (412092). Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix F). Reports on analyses performed in support of the cobalt sulfate heptahydrate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a red, crystalline solid, was identified as cobalt sulfate heptahydrate by infrared, ultraviolet, and/or visible spectroscopy. The purity of lot 412092 was determined by elemental analysis, Karl Fischer water analysis, and spark source mass spectroscopy. Elemental analyses for sulfur and hydrogen were in agreement with the theoretical values for cobalt sulfate heptahydrate, but results for cobalt were slightly low. Karl Fischer water analysis indicated 44.6% ± 0.5% water. Spark source mass spectroscopy indicated 140 ppm nickel present as an impurity; all other impurities had a combined total of less than 175 ppm. The overall purity was determined to be approximately 99%.

Literature references indicate that cobalt sulfate heptahydrate is stable as a bulk chemical when stored protected from light at normal temperatures. The heptahydrate dehydrates to the hexahydrate at 41.5° C and to the monohydrate when heated to 71° C, with no further changes expected below the decomposition temperature (708° C). Therefore, an accelerated stability study was not conducted. To ensure stability, the bulk chemical was stored in its original shipping containers, metal cans, at room temperature. Stability was monitored during the studies using elemental analysis by inductively coupled plasma/atomic emission spectroscopy (ICP/AES) normalized against a cobalt standard (National Institute of Standards and Technology, Gaithersburg, MD); no degradation of the bulk chemical was detected.

AEROSOL GENERATION AND EXPOSURE SYSTEM

Cobalt sulfate heptahydrate was generated and delivered from an aqueous solution by a system composed of three main components: a compressed-air-driven nebulizer (Model PN7002; RETEC Development Laboratory, Portland, OR), an aerosol charge neutralizer, and an aerosol distribution system. Cobalt sulfate heptahydrate in deionized water was siphoned from the bulk reservoir to the nebulizer reservoir and then aspirated into the nebulizer chamber and expelled as a stream. Shear forces broke the stream into droplets that were evaporated to leave dry particles of cobalt sulfate heptahydrate. The aerosol generation and delivery system included primary and secondary compressed-air-driven nebulizers. The aerosol generated by the compressed-air-driven nebulizer was passed through the aerosol charge neutralizer to remove static charge that formed on the aerosol particles during generation. Detailed descriptions of the inhalation chambers and the vapor generation system are provided in Appendix F.

A distribution line carried aerosol to the Hazleton 2000 inhalation exposure chambers (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) on both sides of the exposure room. At each chamber, aerosol moving through the chamber inlet was further diluted with HEPA-filtered air to the appropriate concentration for the chamber.

AEROSOL CONCENTRATION MONITORING

The chamber concentrations of cobalt sulfate heptahydrate were monitored by computer-controlled real-time aerosol monitors (Model RAM-1; MIE, Inc.,

Bedford, MA). Chamber aerosol concentrations were sampled at least once per hour during each exposure day. Throughout the studies, the background concentrations of total suspended particles in the control chambers were less than the limit of detection. The RAM-1 voltage output was calibrated against cobalt sulfate heptahydrate concentrations of chamber filter samples. Solutions of filter samples in 2% nitric acid were analyzed quantitatively for cobalt sulfate heptahydrate by ICP/AES. The ICP/AES was calibrated with a solution of standard cobalt diluted with nitric acid. Stability studies performed with X-ray diffraction analyses of samples from the 0.3 and 3.0 mg/m^3 chambers indicated that cobalt sulfate hexahydrate was the primary species delivered to the chambers. Chamber concentration uniformity was maintained throughout the 2-year studies. A summary of chamber concentrations is presented in Table F1.

CHAMBER ATMOSPHERE CHARACTERIZATION

The time required for the chamber concentration to reach 90% of the target value following the beginning of exposure (T_{90}) and the time required for the chamber concentration to reach 10% of the target value following termination of the exposure (T_{10}) were determined for each exposure chamber. Without animals present, T_{90} values ranged from 9 to 11 minutes for rats and from 7 to 12 minutes for mice; T_{10} ranged from 8 to 9 minutes for rats and mice. With animals present, T_{90} values ranged from 11 to 16 minutes for rats and from 8 to 12 minutes for mice; T_{10} ranged from 12 to 13 minutes for rats and from 11 to 12 minutes for mice. A T_{90} of 12 minutes was selected for the 2-year studies.

Aerosol size distribution was determined monthly for each exposure chamber with a Mercer-style seven-stage impactor (In-Tox Products, Albuquerque, NM). Samples were analyzed for cobalt sulfate heptahydrate with ICP/AES. The relative mass on each impactor stage was analyzed by probit analysis; the mass median aerodynamic diameter for the aerosol was within the specified range of 1 to 3 µm (Tables F2 and F3).

Studies of cobalt sulfate heptahydrate degradation and monitoring for impurities were conducted throughout the 2-year studies with ICP/AES. No degradation of cobalt sulfate heptahydrate was observed during the studies. Cageboards were used after the first 8 weeks of the studies to control ammonia in the exposure chambers.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to aqueous aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt sulfate heptahydrate for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks.

The exposure concentrations for the 2-year cobalt sulfate heptahydrate studies were based on the findings of 16-day and 13-week studies reported previously (NTP, 1991). The most sensitive tissue was the larynx, with squamous metaplasia observed in rats and mice at the lowest exposure concentration of 0.3 mg/m^3. A NOAEL was not reached for this tissue. Inflammatory polyps, some nearly obstructing the esophagus, were observed at 10 and 30 mg/m^3 in rats, while these lesions at the 0.3 and 1.0 mg/m^3 exposure concentrations were composed of mild or minimal squamous metaplasia and/or chronic inflammation in both rats and mice. The severity of the laryngeal changes and other lesions in the respiratory tract at 3.0 mg/m^3 was not considered life threatening, and, therefore, exposure concentrations of 0.3, 1.0, and 3.0 mg/m^3 were chosen for the 2-year study with rats and mice.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 14 days before the beginning of the studies. Five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. Rats and mice were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix H).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Cages and racks

were rotated weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix G.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings and body weights were recorded initially, at weeks 5, 9, and 13 (clinical findings) or weekly for 13 weeks (body weights), monthly through week 92, every 2 weeks thereafter, and at the end of the studies.

A complete necropsy and microscopic examination were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs which included the adrenal medulla, lung, larynx, nose, and all neoplasms in all groups except testicular neoplasms for male and female rats. For male and female mice, the quality assessment pathologist evaluated slides from all tumors and all potential target organs which included the larynx, liver, lung, nose, and trachea, and all neoplasms in all organs. Additionally, all thyroid glands were reviewed for incidences of proliferative lesions of the follicular cells. Renal and iliac lymph nodes of male mice were reviewed when the diagnosis of lymphoid hyperplasia occurred. Ovaries of female mice were reviewed when the diagnoses of cyst, bilateral cyst, or corpus luteum cyst occurred.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues usually without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al*. (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al*. (1986).

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Inhalation Studies of Cobalt Sulfate Heptahydrate

Study Laboratory
Battelle Pacific Northwest Laboratories
(Richland, WA)

Strain and Species
Rats: F344/N
Mice: B6C3F_1

Animal Source
Simonsen Laboratories
(Gilroy, CA)

Time Held Before Studies
14 days

Average Age When Studies Began
6 weeks

Date of First Exposure
Rats: 30 August 1990
Mice: 23 August 1990

Duration of Exposure
6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks

Date of Last Exposure
Rats: 28 August 1992
Mice: 21 August 1992

Necropsy Dates
Rats: 1-4 September 1992
Mice: 24-27 August 1992

Average Age at Necropsy
111 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights; cages were distributed randomly into groups from another computer-generated list of random numbers.

Animals per Cage
1

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Inhalation Studies of Cobalt Sulfate Heptahydrate

Method of Animal Identification
Tail tattoo

Diet
NIH-07 open formula pellet diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* except during exposure periods, changed weekly

Water Distribution
Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Stainless-steel wire-bottom (Hazleton System, Inc., Aberdeen, MD), changed weekly

Bedding
Cageboard (Bunzl Cincinnati Paper Co., Cincinnati, OH), changed daily (15 October 1990 to study termination)

Chamber Air Supply Filters
Single HEPA (Flanders Filters, Inc., San Rafael, CA)

Chambers
Stainless-steel with excreta pan suspended below each cage unit (Harford System Division of Lab Products, Inc., Aberdeen, MD), changed weekly

Chamber Environment
Temperature: 21.3°–26.6° C (rats); 19.5°–27.1° C (mice)
Relative humidity: 31%–89% (rats); 28%–93% (mice)
Room fluorescent light: 12 hours/day
Chamber air changes: 9-23/hour

Exposure Concentrations
0, 0.3, 1.0, or 3.0 mg/m^3

Type and Frequency of Observation
Observed twice daily; animals were weighed and clinical findings were recorded initially, at weeks 5, 9, and 13 (clinical findings) or weekly for 13 weeks (body weights), monthly through week 92, every 2 weeks thereafter, and at the end of the studies.

Method of Sacrifice
CO_2 anesthetization

Necropsy
Necropsy performed on all animals

Histopathology
Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), harderian gland (rats), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs/bronchi, lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland (except male mice), nose, oral cavity (rats), ovary, pancreas, pancreatic islets, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord, spleen, stomach (forestomach and glandular), testes/epididymides, thymus, thyroid gland, trachea, urinary bladder, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or pregnant were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time.

Analysis of Continuous Variables

Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of cobalt sulfate heptahydrate was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of cobalt sulfate heptahydrate are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the molecular structure and the effects of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemical-induced DNA damage and to predict carcinogenicity in animals, based on the electrophilicity theory of chemical mutagenesis and the somatic mutation theory of cancer (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone.

RESULTS

RATS

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 1). Survival of exposed males and females was similar to that of the chamber controls.

Body Weights and Clinical Findings

Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study (Figure 2 and Tables 3 and 4). Irregular breathing was observed more frequently in female rats exposed to 3.0 mg/m^3 than in the chamber controls or other exposed groups.

TABLE 2
Survival of Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	30	34	26	34
Natural deaths	3	1	3	1
Animals surviving to study termination	17	15	21	15
Percent probability of survival at end of study[a]	34	30	42	30
Mean survival (days)[b]	648	655	663	643
Survival analysis[c]	P=0.723	P=1.000N	P=0.292N	P=0.876
Female				
Animals initially in study	50	50	50	50
Moribund	19	20	20	17
Natural deaths	3	4	4	3
Pregnant[d]	0	1	0	0
Animals surviving to study termination	28	25	26	30
Percent probability of survival at end of study	56	51	52	60
Mean survival (days)	699	677	691	684
Survival analysis	P=0.642N	P=0.583	P=0.756	P=0.959N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.
[d] Censored from survival analyses

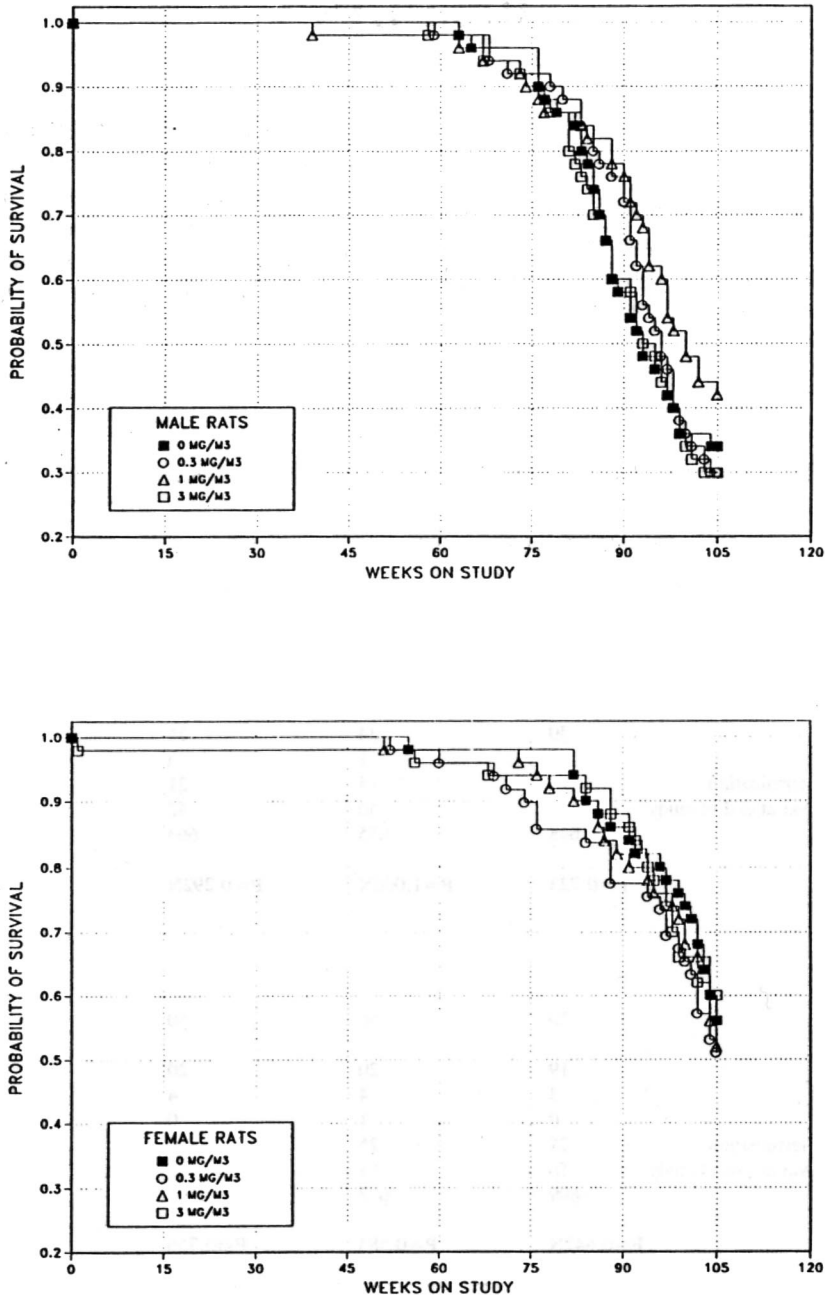
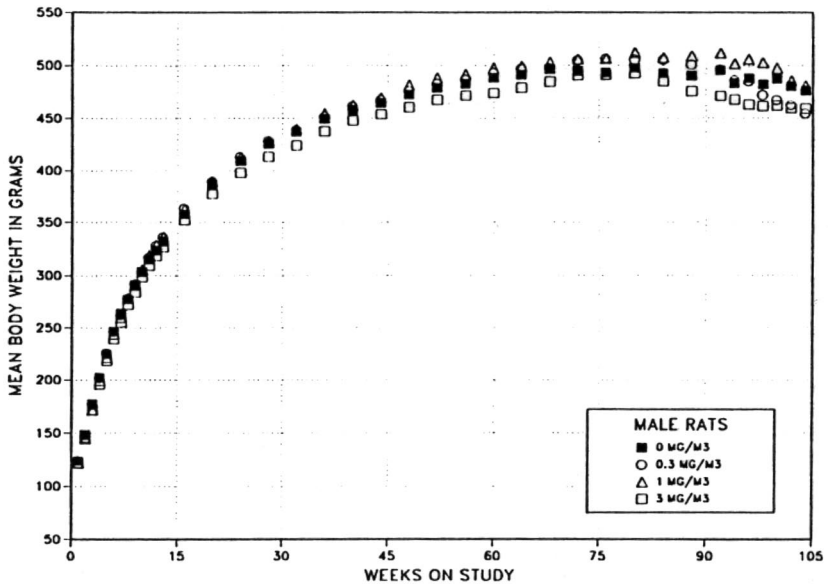


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Cobalt Sulfate Heptahydrate by Inhalation for 2 Years



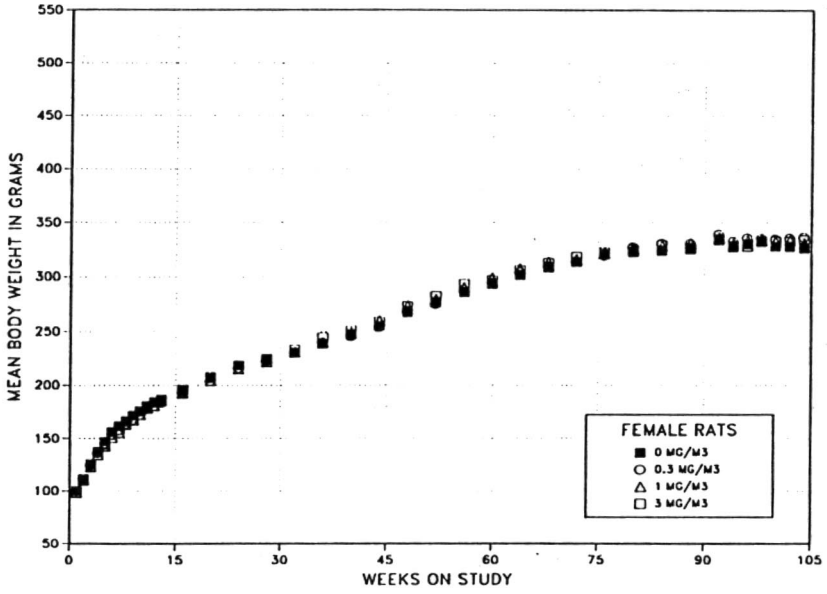


FIGURE 2
Growth Curves for Male and Female Rats
Exposed to Cobalt Sulfate Heptahydrate by Inhalation for 2 Years

TABLE 3
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

Weeks on Study	Chamber Control		0.3 mg/m^3			1.0 mg/m^3			3.0 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	124	50	124	100	50	122	99	50	122	99	50
2	149	50	148	100	50	145	97	50	146	98	50
3	177	50	177	100	50	172	97	50	172	97	50
4	202	50	202	100	50	200	99	50	196	97	50
5	225	50	226	101	50	221	99	50	219	97	50
6	247	50	247	100	50	244	99	50	240	97	50
7	264	50	263	99	50	260	98	50	255	97	50
8	277	50	278	100	50	279	101	50	273	98	50
9	291	50	292	100	50	291	100	50	284	98	50
10	303	50	304	100	50	306	101	50	299	98	50
11	315	50	317	101	50	320	102	50	310	98	50
12	324	50	328	101	50	329	102	50	319	99	50
13	332	50	336	101	50	336	101	50	327	98	50
16	358	50	364	102	50	363	101	50	352	98	50
20	387	50	390	101	50	390	101	50	377	98	50
24	409	50	413	101	50	412	101	50	398	97	50
28	425	50	428	101	50	429	101	50	413	97	50
32	437	50	439	100	50	440	101	50	424	97	50
36	450	50	452	101	50	455	101	50	438	97	50
40	458	50	462	101	50	463	101	49	448	98	50
44	464	50	468	101	50	470	101	49	454	98	50
48	473	50	476	101	50	482	102	49	461	98	50
52	479	50	483	101	50	488	102	49	468	98	50
56	483	50	487	101	50	492	102	49	472	98	50
60	489	50	493	101	49	498	102	49	474	97	49
64	491	49	497	101	49	499	102	48	479	98	49
68	497	48	498	100	49	503	101	47	485	98	47
72	495	48	505	102	46	506	102	47	491	99	47
76	493	48	506	103	46	506	103	45	491	100	46
80	498	43	505	102	45	512	103	43	493	99	43
84	493	40	505	103	42	507	103	42	485	98	38
88	490	32	501	102	39	509	104	41	476	97	33
92	495	27	497	100	33	512	103	36	471	95	29
94	483	24	486	101	28	502	104	34	468	97	25
96	488	23	485	99	26	506	104	31	463	95	24
98	482	21	472	98	23	503	104	27	462	96	21
100	487	18	467	96	19	498	102	26	462	95	18
102	481	18	462	96	17	486	101	24	460	96	16
104	476	18	454	95	16	481	101	22	459	96	15
Mean for weeks											
1-13	248		249	100		248	100		243	98	
14-52	434		438	101		439	101		423	97	
53-104	489		489	100		501	102		474	97	

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

Weeks on Study	Chamber Control		0.3 mg/m^3			1.0 mg/m^3			3.0 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	100	50	100	100	50	99	98	50	99	98	50
2	112	50	112	100	50	110	99	50	111	99	49
3	126	50	125	99	50	123	98	50	123	98	49
4	138	50	136	99	50	134	98	50	134	98	49
5	147	50	145	99	50	143	97	50	144	98	49
6	156	50	154	99	50	151	97	50	154	99	49
7	162	50	158	98	50	156	97	50	158	98	49
8	166	50	164	99	50	163	98	50	166	100	49
9	171	50	168	98	50	168	98	50	170	99	49
10	176	50	173	98	50	173	98	50	176	100	49
11	179	50	178	99	50	179	100	50	181	101	49
12	184	50	181	99	49	181	99	50	184	100	49
13	186	50	184	99	49	186	100	50	187	100	49
16	195	50	193	99	49	193	99	50	196	101	49
20	207	50	204	99	49	205	99	50	208	101	49
24	219	50	216	99	49	215	99	50	219	100	49
28	224	50	223	99	49	222	99	50	225	100	49
32	230	50	230	100	49	231	100	50	233	101	49
36	238	50	240	101	49	241	101	50	244	103	49
40	247	50	246	100	49	249	101	50	251	102	49
44	255	50	254	100	49	260	102	50	259	101	49
48	267	50	269	101	49	273	102	50	273	102	49
52	276	50	275	100	49	279	101	49	282	102	49
56	286	49	288	101	48	290	102	49	293	103	49
60	293	49	294	100	48	299	102	49	297	101	48
64	302	49	304	101	47	307	102	49	306	101	48
68	308	49	310	100	47	313	102	49	312	101	48
72	314	49	314	100	45	316	101	49	318	102	47
76	321	49	320	100	44	322	100	48	323	101	47
80	323	49	328	102	42	326	101	46	325	101	47
84	324	47	331	102	42	329	101	45	329	102	47
88	326	44	331	102	41	331	102	42	327	101	46
92	334	42	339	102	38	337	101	40	335	100	43
94	327	41	333	102	38	331	101	40	328	100	41
96	330	41	336	102	37	334	101	38	328	99	39
98	333	39	336	101	34	334	100	38	333	100	37
100	328	38	335	102	33	333	102	36	333	102	33
102	328	36	336	103	31	334	102	34	333	102	33
104	326	32	337	103	27	331	102	32	334	102	31
Mean for weeks											
1-13	154		152	99		151	98		153	99	
14-52	236		235	100		237	100		239	101	
53-104	319		323	101		323	101		322	101	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, adrenal medulla, nose, and larynx. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Lung: In all exposed groups of male and female rats, the incidences of proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation, and interstitial fibrosis were significantly greater than those in the chamber controls (Tables 5, A5, and B5). In general, these lung lesions increased in incidence and severity with increased exposure to cobalt sulfate heptahydrate. The incidence of squamous metaplasia in 1.0 mg/m^3 females was significantly greater than in the chamber control group. Multifocally, throughout the lungs, pulmonary architecture was distorted by a combination of inflammatory cells, fibrosis, and epithelial metaplasia. Lesions tended to be subpleural, peripheral, and/or along larger blood vessels and airways. Granulomatous inflammation was characterized by accumulations of alveolar macrophages with foamy cytoplasm, occasional multinucleated giant cells and cholesterol clefts, cell debris and few neutrophils. In these areas, the alveolar interstitium and occasionally the overlying pleura were variably thickened by dense fibrous connective tissue which often effaced alveoli (Plates 1 and 2). Although a diffuse change, aggregates of homogeneous to granular eosinophilic material within alveolar lumens (alveolar proteinosis) were often pronounced within the areas of chronic inflammation. Metaplasia of the alveolar epithelium in alveoli within and at the periphery of foci of inflammation was characterized by replacement of normal Type I epithelial cells with plump cuboidal or ciliated columnar epithelial cells. The incidences of alveolar epithelial hyperplasia in all groups of exposed males and in females exposed to 3.0 mg/m^3 and atypical alveolar epithelial hyperplasia in 3.0 mg/m^3 females were significantly greater than those in the chamber control groups.

The combined incidence of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) was significantly greater in 3.0 mg/m^3 males than that in the chamber controls and exceeded the historical control range (Tables 5 and A3). In females exposed to 1.0 or 3.0 mg/m^3, the incidences of alveolar/bronchiolar neoplasms were significantly greater than those in the chamber control group and exceeded the historical control ranges (Tables 5, B3, and B4a). Although the incidences of alveolar/bronchiolar adenoma in 3.0 mg/m^3 males and alveolar/bronchiolar carcinoma in 1.0 mg/m^3 males were not significantly increased, they exceeded the historical control ranges for inhalation studies (Tables 5, A3, and A4a).

The spectrum of alveolar/bronchiolar neoplasms and nonneoplastic proliferative lesions observed within the lungs of exposed rats was broad. While many of these lesions were highly cellular and morphologically similar to those observed spontaneously, others were predominantly fibrotic, squamous, or mixtures of alveolar/bronchiolar epithelium and squamous or fibrous components. Hyperplasia generally represented an increase in numbers of epithelial cells along alveolar walls with maintenance of normal alveolar architecture (Plates 3 and 4). Multiple hyperplastic lesions were often observed in animals receiving higher concentrations of cobalt sulfate heptahydrate. The benign neoplasms typical of those observed spontaneously were generally distinct masses that often compressed surrounding tissue (Plates 5 and 6). Component epithelial cells were often arranged in acinar and/or irregular papillary structures and occasionally in a solid cellular pattern. These epithelial cells were typically uniform and similar to hyperplastic counterparts. Malignant alveolar/bronchiolar neoplasms had similar cellular patterns but were generally larger and had one or more of the following histologic features: heterogeneous growth pattern, cellular pleomorphism and/or atypia, and local invasion or metastasis (Plate 7).

In addition to these more typical proliferative lesions, there were "fibroproliferative" lesions ranging from less than 1 mm to greater than 1 cm in diameter. Generally, these lesions had a rounded outline and a central fibrous core containing dispersed glandular (alveolar) structures lined by uniformly cuboidal epithelial cells. Aggregates of mostly necrotic

TABLE 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Number Examined Microscopically	50	50	48	50
Alveolar Epithelium, Hyperplasia[a]	9 (1.8)[b]	20* (2.0)	20* (2.1)	23**(2.0)
Alveolar Epithelium, Hyperplasia, Atypical	0	1 (2.0)	2 (3.0)	2 (4.0)
Metaplasia, Squamous	0	1 (1.0)	4 (2.0)	2 (3.0)
Alveolar Epithelium, Metaplasia	0	50**(1.9)	48**(3.1)	49**(3.7)
Inflammation, Granulomatous	2 (1.0)	50**(1.9)	48**(3.1)	50**(3.7)
Interstitium, Fibrosis	1 (1.0)	50**(1.9)	48**(3.1)	49**(3.7)
Proteinosis	0	16**(1.4)	40**(2.3)	47**(3.4)
Cyst	0	0	0	1 (4.0)
Alveolar/bronchiolar Adenoma[c]				
Overall rate[d]	1/50 (2%)	4/50 (8%)	1/48 (2%)	6/50 (12%)
Adjusted rate[e]	2.3%	17.7%	2.4%	28.4%
Terminal rate[f]	0/17 (0%)	2/15 (13%)	0/21 (0%)	2/15 (13%)
First incidence (days)	568	589	611	638
Logistic regression test[g]	P=0.051	P=0.179	P=0.753	P=0.055
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	0/50 (0%)	0/50 (0%)	3/48 (6%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	11.3%	6.7%
Terminal rate	0/17 (0%)	0/15 (0%)	1/21 (5%)	1/15 (7%)
First incidence (days)	—[i]	—	652	734 (T)
Logistic regression test	P=0.360	—	P=0.136	P=0.475
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	1/50 (2%)	4/50 (8%)	4/48 (8%)	7/50 (14%)
Adjusted rate	2.3%	17.7%	13.4%	33.9%
Terminal rate	0/17 (0%)	2/15 (13%)	1/21 (5%)	3/15 (20%)
First incidence (days)	568	589	611	638
Logistic regression test	P=0.032	P=0.179	P=0.163	P=0.029
Female				
Number Examined Microscopically	50	49	50	50
Alveolar Epithelium, Hyperplasia	15 (1.4)	7 (1.6)	20 (1.8)	33**(2.0)
Alveolar Epithelium, Hyperplasia, Atypical	0	0	3 (3.7)	5* (3.2)
Metaplasia, Squamous	0	1 (2.0)	8**(2.3)	3 (1.7)
Alveolar Epithelium, Metaplasia	2 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Inflammation, Granulomatous	9 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Interstitium, Fibrosis	7 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Proteinosis	0	36**(1.2)	49**(2.8)	49**(3.9)
Cyst	0	0	1 (4.0)	0
Alveolar/bronchiolar Adenoma[k]				
Overall rate	0/50 (0%)	1/49 (2%)	10/50 (20%)	9/50 (18%)
Adjusted rate	0.0%	3.4%	36.4%	30.0%
Terminal rate	0/28 (0%)	0/25 (0%)	9/26 (35%)	9/30 (30%)
First incidence (days)	—	714	692	735 (T)
Logistic regression test	P=0.001	P=0.480	P < 0.001	P=0.003

TABLE 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Female (continued)				
Alveolar/bronchiolar Carcinoma[l]				
Overall rate	0/50 (0%)	2/49 (4%)	6/50 (12%)	6/50 (12%)
Adjusted rate	0.0%	8.0%	20.2%	17.5%
Terminal rate	0/28 (0%)	2/25 (8%)	4/26 (15%)	4/30 (13%)
First incidence (days)	—	735 (T)	694	610
Logistic regression test	P=0.023	P=0.213	P=0.015	P=0.017
Alveolar/bronchiolar Adenoma or Carcinoma[m]				
Overall rate	0/50 (0%)	3/49 (6%)	15/50 (30%)	15/50 (30%)
Adjusted rate	0.0%	11.2%	50.6%	46.1%
Terminal rate	0/28 (0%)	2/25 (8%)	12/26 (46%)	13/30 (43%)
First incidence (days)	—	714	692	610
Logistic regression test	P < 0.001	P=0.096	P < 0.001	P < 0.001
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	1/50 (2%)
Alveolar/bronchiolar Adenoma, Alveolar/bronchiolar Carcinoma, or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	16/50 (32%)	16/50 (32%)
Adjusted rate	0.0%	11.2%	54.1%	49.2%
Terminal rate	0/28 (0%)	2/25 (8%)	13/26 (50%)	14/30 (47%)
First incidence (days)	—	714	692	610
Logistic regression test	P < 0.001	P=0.096	P < 0.001	P < 0.001

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year inhalation studies with chamber controls (mean ± standard deviation): 17/654 (2.6% ± 3.6%); range 0%-10%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.
[h] Historical incidence: 6/654 (0.9% ± 1.0%); range 0%-2%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 23/654 (3.5% ± 3.7%); range 0%-10%
[k] Historical incidence: 7/650 (1.1% ± 1.6%); range 0%-4%
[l] Historical incidence: 0/650
[m] Historical incidence: 7/650 (1.1% ± 1.6%); range 0%-4%

inflammatory cells were also present in adjacent alveoli and often within the glandular structures. Peripherally, the fibroproliferative lesions had one to several layers of epithelium which coursed along and often extended into adjacent alveoli, frequently forming papillary projections (Plates 8, 9, and 10). These epithelial cells were often slightly pleomorphic with occasional mitotic figures. The smallest of these lesions were usually observed adjacent to areas of chronic inflammation. Small lesions with modest amounts of peripheral epithelial proliferation were diagnosed as atypical hyperplasia, while larger lesions with florid epithelial proliferation, marked cellular pleomorphism, and/or local invasion were diagnosed as alveolar/bronchiolar carcinomas (Plate 11).

While squamous epithelium is not normally observed within the lung, squamous metaplasia of alveolar/ bronchiolar epithelium is a relatively common response to pulmonary injury and occurred in a number of rats in this study (Table 5). Squamous metaplasia was a minor change consisting of a small cluster of alveoli in which the normal epithelium was replaced by multiple layers of flattened squamous epithelial cells (Plate 12) that occasionally formed keratin. One 3.0 mg/m^3 male and one 1.0 mg/m^3 female had a large cystic squamous lesion rimmed by a variably thick (a few to many cell layers) band of viable squamous epithelium with a large central core of keratin (Plate 13). These were diagnosed as cysts. In one 1.0 mg/m^3 and one 3.0 mg/m^3 female, proliferative squamous lesions had cystic areas but also more solid areas of pleomorphic cells and invasion into the adjacent lung; these lesions were considered to be squamous cell carcinomas (Plate 14). In general, diagnoses of squamous lesions were made only when the lesion composition was almost entirely squamous epithelium. However, squamous metaplasia/ differentiation was a variable component of other alveolar/bronchiolar proliferative lesions (Plate 15), including the fibroproliferative lesions, and was clearly a part of the spectrum of lesions resulting from exposure to cobalt sulfate heptahydrate.

Adrenal Medulla: The incidence of benign pheochromocytoma in 3.0 mg/m^3 females was significantly greater than that in the chamber controls and exceeded the historical range for inhalation studies (Tables 6, B3, and B4b). The incidences of benign, complex, or malignant pheochromocytoma (combined) in 1.0 mg/m^3 males and in 3.0 mg/m^3 females were significantly greater than those in the chamber controls and exceeded the historical control ranges (Tables 6, A3, A4b, B3, and B4b).

The incidences of bilateral pheochromocytoma in exposed males slightly exceeded that in the chamber control group. The incidence of hyperplasia was not significantly increased in exposed males or females. Focal hyperplasia and pheochromocytoma are considered to constitute a morphological continuum in the adrenal medulla. Focal hyperplasia consisted of irregular, small foci of small- to normal-sized medullary cells arranged in packets or solid clusters slightly larger than normal; compression of surrounding parenchyma was minimal or absent. Benign pheochromocytomas were well-delineated masses often with altered architecture and variable compression of surrounding parenchyma. Neoplastic cells were arranged in variably sized aggregates, clusters, and/or variably thick trabecular cords. Larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller neoplasms. Malignant pheochromocytomas were identified when there was invasion of or beyond the adrenal capsule or when distant metastases were observed. Although a very common spontaneous neoplasm in male F344/N rats, pheochromocytomas have a lower spontaneous occurrence in females. In this study, the incidence of pheochromocytoma in 3.0 mg/m^3 females was considered related to the administration of cobalt sulfate heptahydrate. The marginally increased incidence of pheochromocytoma in males was considered an uncertain finding because it occurred only in the 1.0 mg/m^3 group and was not supported by increased incidence or severity of hyperplasia.

TABLE 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Number Examined Microscopically	50	50	49	50
Hyperplasia[a]	34 (2.0)[b]	23* (2.5)	29 (2.1)	30 (2.1)
Benign Bilateral Pheochromocytoma				
Overall rate	1/50 (2%)	4/50 (8%)	6/49 (12%)	5/50 (10%)
Benign Pheochromocytoma (includes benign bilateral pheochromocytoma)[c]				
Overall rate[d]	14/50 (28%)	19/50 (38%)	23/49 (47%)	20/50 (40%)
Adjusted rate[e]	51.0%	70.0%	71.9%	71.4%
Terminal rate[f]	6/17 (35%)	8/15 (53%)	13/21 (62%)	8/15 (53%)
First incidence (days)	534	541	526	526
Logistic regression test[g]	P=0.172	P=0.226	P=0.069	P=0.126
Benign, Complex, or Malignant Pheochromocytoma (includes benign bilateral pheochromocytoma)[h]				
Overall rate	15/50 (30%)	19/50 (38%)	25/49 (51%)	20/50 (40%)
Adjusted rate	52.1%	70.0%	74.1%	71.4%
Terminal rate	6/17 (35%)	8/15 (53%)	13/21 (62%)	8/15 (53%)
First incidence (days)	534	541	526	526
Logistic regression test	P=0.218	P=0.295	P=0.045	P=0.180
Female				
Number Examined Microscopically	48	49	50	48
Hyperplasia	8 (1.6)	7 (2.3)	11 (2.1)	13 (2.0)
Benign Pheochromocytoma[i]				
Overall rate	2/48 (4%)	1/49 (2%)	3/50 (6%)	8/48 (17%)
Adjusted rate	5.1%	3.1%	9.3%	26.4%
Terminal rate	0/27 (0%)	0/25 (0%)	1/26 (4%)	7/29 (24%)
First incidence (days)	666	702	694	709
Logistic regression test	P=0.004	P=0.498N	P=0.512	P=0.043
Benign, Complex, or Malignant Pheochromocytoma[j]				
Overall rate	2/48 (4%)	1/49 (2%)	4/50 (8%)	10/48 (21%)
Adjusted rate	5.1%	3.1%	11.7%	31.5%
Terminal rate	0/27 (0%)	0/25 (0%)	1/26 (4%)	8/29 (28%)
First incidence (days)	666	702	685	663
Logistic regression test	P < 0.001	P=0.498N	P=0.323	P=0.014

* Significantly different ($P \leq 0.05$) from the chamber control by the logistic regression test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year inhalation studies with chamber controls (mean ± standard deviation): 163/623 (26.2% ± 13.2%); range 0%-50%
[d] Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by **N**.
[h] Historical incidence: 176/623 (28.3% ± 12.0%); range 8%-50%
[i] Historical incidence: 35/608 (5.8% ± 4.9%); range 0%-14%
[j] Historical incidence: 39/608 (6.4% ± 4.4%); range 2%-14%

Nose: The incidences of hyperplasia of the lateral wall of the nose and atrophy of the olfactory epithelium in all exposed groups of males and females were significantly greater than those in the chamber controls, and the severities of these lesions increased with increasing exposure concentration (Tables 7, A5, and B5). The incidences of squamous metaplasia of the lateral wall of the nose and metaplasia of the olfactory epithelium in 3.0 mg/m^3 males and females were significantly greater than those in the chamber controls.

Although the incidence and severity of nasal lesions increased with increased exposure to cobalt sulfate heptahydrate, they involved limited portions of nasal epithelium and none were severe. Hyperplasia and squamous metaplasia were minimal to mild, unilateral or bilateral, and involved the transitional epithelium along the walls and turbinates of the anterior nasal passage. Hyperplasia was characterized by an increase in thickness of the epithelium from the normal one to two layers to two or more layers, while squamous metaplasia represented areas where the normal transitional epithelium was replaced by multiple layers of flattened epithelial cells. More posterior in the nose, along the dorsal meatus, atrophy of the olfactory epithelium was characterized by loss of cell layers and disorganization of remaining epithelium, and in some instances, increased prominence of sensory cell nuclei. Metaplasia was characterized by replacement of olfactory epithelium with respiratory-type ciliated columnar epithelium.

TABLE 7
Incidences of Nonneoplastic Lesions of the Nose and Larynx in Rats in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Nose[a]	50	50	49	50
Lateral Wall, Hyperplasia[b]	2 (1.5)[c]	14**(1.4)	21**(1.5)	20**(1.6)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	3 (1.3)	5 (1.4)	8* (2.0)
Olfactory Epithelium, Atrophy	8 (1.1)	24**(1.4)	42**(1.5)	48**(2.5)
Olfactory Epithelium, Metaplasia	5 (1.2)	1 (3.0)	5 (1.8)	30**(1.9)
Larynx	50	49	48	50
Epiglottis, Metaplasia, Squamous	0	10**(1.3)	37**(1.8)	50**(2.8)
Female				
Nose	50	49	50	50
Lateral Wall, Hyperplasia	1 (1.0)	8* (1.3)	26**(1.4)	38**(1.7)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	1 (3.0)	4 (1.3)	10**(1.4)
Olfactory Epithelium, Atrophy	5 (1.4)	29**(1.2)	46**(1.6)	47**(2.9)
Olfactory Epithelium, Metaplasia	2 (2.0)	2 (1.5)	3 (1.7)	40**(2.3)
Larynx	50	49	50	50
Epiglottis, Metaplasia, Squamous	1 (1.0)	22**(1.1)	39**(1.4)	48**(2.6)

* Significantly different ($P \le 0.05$) from the chamber control by the logistic regression test
** $P \le 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Larynx: The incidences of squamous metaplasia of the epiglottis in all exposed groups of males and females were significantly greater than those in the chamber controls, and the severity of this lesion increased with increasing exposure concentration (Tables 7, A5, and B5). Squamous metaplasia was limited to the base of the epiglottis and was not a severe lesion in exposed rats. It was characterized by replacement of the ciliated respiratory epithelium by one or more layers of flattened epithelial cells overlying a basal layer of cuboidal cells. Keratinization was sometimes observed.

MICE

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 3). Survival of exposed males and females was similar to that of the chamber controls.

Body Weights and Clinical Findings

Mean body weights are given in Figure 4 and Tables 9 and 10. Mean body weights of 3.0 mg/m^3 male mice were less than those of the chamber controls from week 96 until the end of the study. The mean body weights of all exposed female mice were generally greater than those of the chamber controls from week 20 until the end of the study. Irregular breathing was observed slightly more frequently in female mice exposed to 1.0 mg/m^3 than in the chamber controls or other exposed groups.

TABLE 8
Survival of Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	1	0	0	1
Moribund	19	16	17	23
Natural deaths	8	3	9	6
Animals surviving to study termination	22	31	24	20
Percent probability of survival at end of study[b]	46	62	48	42
Mean survival (days)[c]	662	695	670	643
Survival analysis[d]	P=0.104	P=0.088N	P=0.861N	P=0.577
Female				
Animals initially in study	50	50	50	50
Moribund	11	10	13	16
Natural deaths	5	3	5	6
Animals surviving to study termination	34	37	32	28
Percent probability of survival at end of study	68	74	64	56
Mean survival (days)	694	713	685	680
Survival analysis	P=0.102	P=0.529N	P=0.855	P=0.327

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.

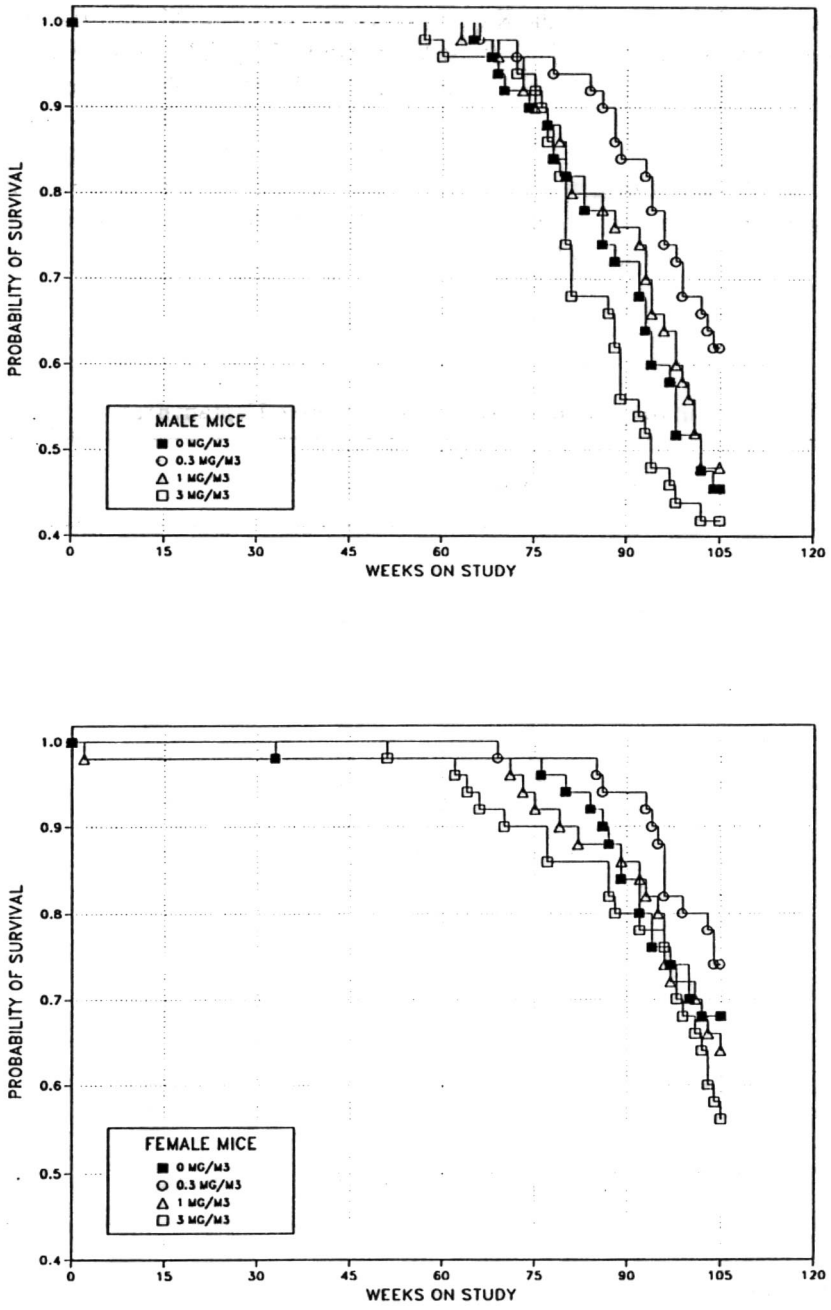
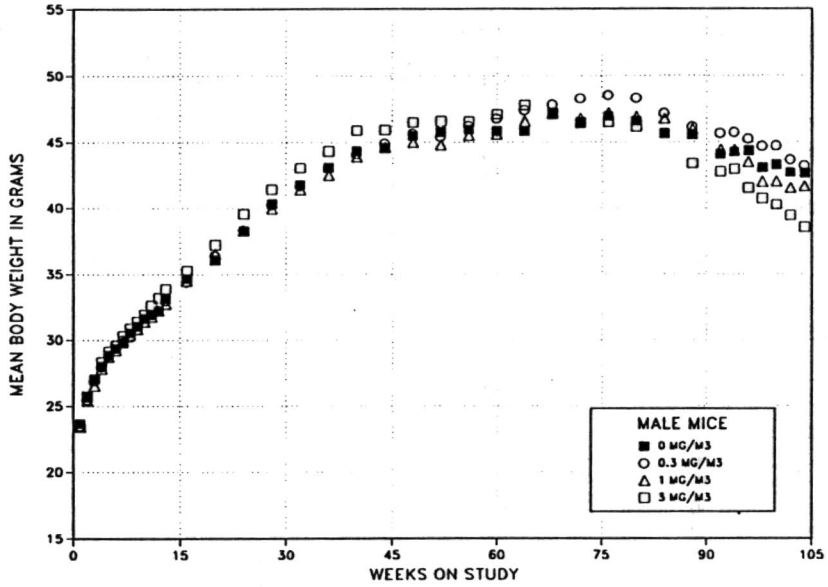


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Cobalt Sulfate Heptahydrate by Inhalation for 2 Years



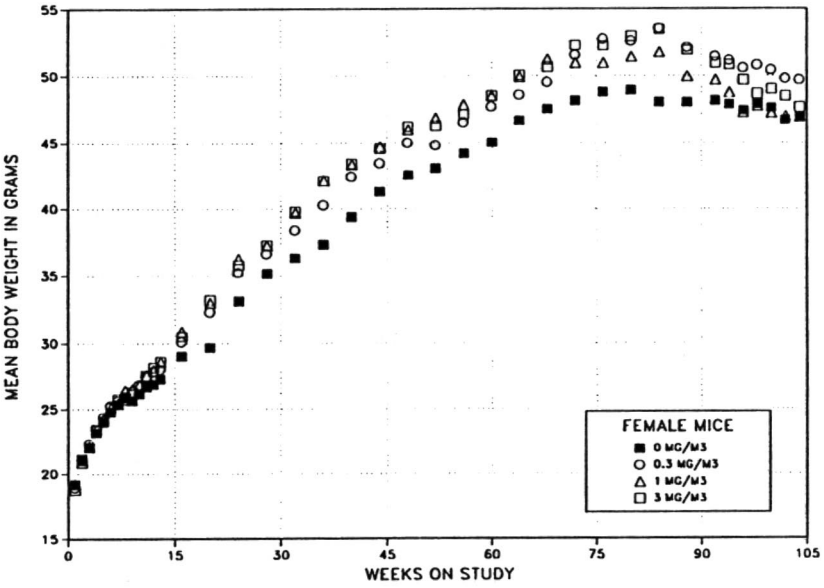


FIGURE 4
Growth Curves for Male and Female Mice Exposed to Cobalt Sulfate Heptahydrate by Inhalation for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

Weeks on Study	Chamber Control		0.3 mg/m^3			1.0 mg/m^3			3.0 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.7	50	23.5	99	50	23.4	99	50	23.4	99	50
2	25.8	50	25.5	99	50	25.4	98	50	25.7	100	50
3	27.0	50	26.9	100	50	26.6	99	50	27.1	100	50
4	28.0	50	27.9	100	50	27.8	99	50	28.3	101	50
5	28.8	50	28.8	100	50	28.7	100	50	29.1	101	50
6	29.3	50	29.6	101	50	29.2	100	50	29.6	101	50
7	29.8	50	30.0	101	50	30.0	101	50	30.3	102	50
8	30.5	50	30.2	99	50	30.4	100	50	30.9	101	50
9	31.0	50	30.8	99	50	30.8	99	50	31.4	101	50
10	31.6	50	31.3	99	50	31.4	99	50	31.9	101	50
11	32.0	50	31.9	100	50	31.8	99	50	32.7	102	50
12	32.2	50	32.2	100	50	32.2	100	50	33.2	103	50
13	33.1	50	33.0	100	50	32.7	99	50	33.9	102	50
16	34.7	50	34.4	99	50	34.5	99	50	35.3	102	50
20	36.1	50	36.4	101	50	36.6	101	50	37.2	103	50
24	38.3	50	38.4	100	50	38.3	100	50	39.6	103	50
28	40.3	50	40.3	100	50	40.0	99	50	41.4	103	50
32	41.8	50	41.7	100	50	41.4	99	50	43.1	103	50
36	43.1	50	43.1	100	50	42.5	99	50	44.3	103	50
40	44.3	50	44.1	100	50	43.9	99	50	45.9	104	50
44	44.6	50	44.9	101	50	44.7	100	50	45.9	103	50
48	45.5	50	45.6	100	50	45.0	99	50	46.5	102	50
52	45.8	50	45.4	99	50	44.8	98	50	46.6	102	50
56	45.9	50	46.2	101	50	45.5	99	50	46.5	101	50
60	45.8	50	46.8	102	50	45.6	100	50	47.1	103	48
64	45.8	50	47.4	104	50	46.6	102	49	47.8	104	48
68	47.1	48	47.8	102	49	47.2	100	49	47.2	100	48
72	46.4	46	48.3	104	48	46.8	101	48	46.5	100	48
76	46.9	45	48.5	103	48	47.2	101	45	46.5	99	46
80	46.6	42	48.3	104	47	46.9	101	43	46.1	99	40
84	45.6	39	47.2	104	47	46.7	102	40	45.7	100	34
88	45.5	37	46.1	101	45	46.0	101	38	43.4	95	32
92	44.1	36	45.6	103	42	44.4	101	38	42.8	97	28
94	44.2	32	45.7	103	41	44.4	101	35	42.9	97	26
96	44.3	30	45.2	102	39	43.5	98	33	41.5	94	24
98	43.0	29	44.7	104	37	42.0	98	32	40.7	95	22
100	43.3	25	44.7	103	34	42.0	97	29	40.3	93	21
102	42.7	25	43.7	102	34	41.5	97	26	39.5	93	21
104	42.6	23	43.2	101	32	41.6	98	24	38.5	90	20
Mean for weeks											
1-13	29.4		29.4	100		29.3	100		29.8	101	
14-52	41.5		41.4	100		41.2	99		42.6	103	
53-104	45.0		46.2	103		44.9	100		43.9	98	

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

Weeks on Study	Chamber Control		0.3 mg/m^3			1.0 mg/m^3			3.0 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.2	50	18.9	98	50	19.2	100	50	18.7	97	50
2	21.2	50	21.1	100	50	21.1	100	50	20.8	98	50
3	22.0	50	22.3	101	50	22.2	101	49	22.1	101	50
4	23.2	50	23.5	101	50	23.4	101	49	23.5	101	50
5	24.0	50	24.4	102	50	24.3	101	49	24.1	100	50
6	24.8	50	25.3	102	50	25.1	101	49	25.1	101	50
7	25.4	50	25.6	101	50	25.5	100	49	25.8	102	50
8	26.0	50	25.9	100	50	26.5	102	49	25.7	99	50
9	25.6	50	26.2	102	50	26.6	104	49	26.4	103	50
10	26.2	50	26.9	103	50	26.9	103	49	26.8	102	50
11	26.7	50	27.5	103	50	27.6	103	49	27.6	103	50
12	26.9	50	27.9	104	50	28.0	104	49	28.2	105	50
13	27.3	50	28.0	103	50	28.6	105	49	28.6	105	50
16	29.1	50	30.1	103	50	30.9	106	49	30.5	105	50
20	29.7	50	32.3	109	50	33.0	111	49	33.2	112	50
24	33.1	50	35.3	107	50	36.3	110	49	35.8	108	50
28	35.2	50	36.6	104	50	37.3	106	49	37.3	106	50
32	36.3	50	38.4	106	50	39.7	109	49	39.8	110	50
36	37.3	49	40.3	108	50	42.1	113	49	42.1	113	50
40	39.4	49	42.4	108	50	43.3	110	49	43.4	110	50
44	41.3	49	43.4	105	50	44.7	108	49	44.5	108	50
48	42.5	49	45.0	106	50	46.0	108	49	46.2	109	50
52	43.0	49	44.8	104	50	46.9	109	49	46.3	108	49
56	44.2	49	46.5	105	50	47.9	108	49	47.1	107	49
60	45.0	49	47.7	106	50	48.5	108	49	48.5	108	49
64	46.7	49	48.6	104	50	49.9	107	49	50.1	107	47
68	47.5	49	49.5	104	50	51.3	108	49	50.7	107	46
72	48.2	49	51.6	107	49	51.0	106	48	52.3	109	45
76	48.8	49	52.8	108	49	51.0	105	46	52.3	107	45
80	48.9	48	52.6	108	49	51.4	105	45	53.0	108	43
84	48.1	46	53.5	111	49	51.8	108	44	53.5	111	43
88	48.1	44	52.1	108	47	50.0	104	44	51.9	108	41
92	48.2	42	51.5	107	47	49.7	103	43	51.0	106	40
94	47.9	40	51.2	107	46	48.8	102	41	50.8	106	39
96	47.4	38	50.6	107	44	47.3	100	40	49.7	105	39
98	47.9	37	50.8	106	41	47.8	100	36	48.7	102	37
100	47.6	37	50.5	106	40	47.2	99	36	49.0	103	34
102	46.7	35	49.8	107	40	47.0	101	35	48.5	104	33
104	46.9	34	49.7	106	39	46.9	100	33	47.7	102	30
Mean for weeks											
1-13	24.5		24.9	102		25.0	102		24.9	102	
14-52	36.7		38.9	106		40.0	109		39.9	109	
53-104	47.4		50.6	107		49.2	104		50.3	106	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, nose, larynx, thyroid gland, and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: In all exposed groups of males and females, the incidences of cytoplasmic vacuolization of the bronchi were significantly greater than those in the chamber control groups (Tables 11, C5, and D5). The incidences of diffuse histiocytic cell infiltration in 3.0 mg/m^3 males and of focal histiocytic cell infiltration in 3.0 mg/m^3 females were significantly greater than those in the chamber controls.

Cytoplasmic vacuolization of the bronchial epithelium was a minimal change of unknown biological significance confined to the epithelial cells lining the apex of the bronchial bifurcation. The affected cells were somewhat larger than normal with a diffusely clear to finely vacuolated cytoplasm. Histiocyte infiltration was characterized by one or more histiocytes with foamy cytoplasm within variable numbers of alveolar lumens. Focal infiltrate was a localized accumulation of histiocytes, while diffuse infiltrate was more widely scattered. The histiocyte infiltrate was very commonly seen in lungs with alveolar/bronchiolar neoplasms, and the increased incidences of infiltrate in the lungs of exposed animals were considered to reflect the higher incidences of lung neoplasms in these animals rather than a primary effect of cobalt sulfate heptahydrate exposure.

The incidences of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) in 3.0 mg/m^3 males and females and the combined incidence of alveolar/ bronchiolar neoplasms in 1.0 mg/m^3 females were significantly greater than those in the chamber control groups and generally exceeded the historical control ranges for inhalation studies (Tables 11, C3, C4a, D3, and D4a). In exposed males and females, the incidences of all lung neoplasms occurred with positive trends.

Unlike in the rat, all the alveolar/bronchiolar proliferative lesions observed within the lungs of exposed mice were typical of those observed spontaneously. Hyperplasia generally represented an increase in numbers of epithelial cells along alveolar walls which retained normal alveolar structure. Adenomas generally were distinct masses that often compressed surrounding tissue (Plate 16). Component cells were arranged in acinar and/or irregular papillary structures and occasionally in a solid cellular pattern. These cells were typically uniform and similar to hyperplastic counterparts. Malignant alveolar/bronchiolar neoplasms had similar cellular patterns but were generally larger (Plate 17) and had one or more of the following: heterogeneous growth pattern, cellular pleomorphism, and/or atypia and local invasion or metastasis.

Although similar in appearance to "spontaneous" lung neoplasms in chamber controls, alveolar/ bronchiolar neoplasms in mice exposed to cobalt sulfate heptahydrate had different molecular lesions in the K*ras* gene (Appendix I). Of the K-*ras* mutations detected at the second base of codon 12, a higher frequency (5/9, 55%) of G to T transversions was detected compared to concurrent (0/1) and historical control lung neoplasms (1/24, 4%). K-*ras* codon 61 CTA or CGA mutations were not present in cobalt sulfate heptahydrate-induced lung neoplasms.

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Number Examined Microscopically	50	50	50	50
Infiltration Cellular, Diffuse, Histiocyte[a]	1 (3.0)[b]	2 (3.0)	4 (2.3)	10**(1.5)
Infiltration Cellular, Focal, Histiocyte	10 (2.7)	5 (2.6)	8 (3.0)	17 (2.7)
Bronchus, Cytoplasmic Vacuolization	0	18**(1.0)	34**(1.0)	38**(1.0)
Alveolar Epithelium Hyperplasia	0	4 (2.3)	4 (1.8)	4 (2.3)
Alveolar/bronchiolar Adenoma[c]				
Overall rate[d]	9/50 (18%)	12/50 (24%)	13/50 (26%)	18/50 (36%)
Adjusted rate[e]	30.4%	30.9%	41.1%	54.6%
Terminal rate[f]	4/22 (18%)	6/31 (19%)	7/24 (29%)	7/20 (35%)
First incidence (days)	600	460	548	524
Logistic regression test[g]	P=0.018	P=0.353	P=0.256	P=0.027
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	4/50 (8%)	5/50 (10%)	7/50 (14%)	11/50 (22%)
Adjusted rate	13.2%	16.1%	25.3%	43.7%
Terminal rate	2/22 (9%)	5/31 (16%)	4/24 (17%)	7/20 (35%)
First incidence (days)	449	733 (T)	687	552
Logistic regression test	P=0.006	P=0.528	P=0.273	P=0.033
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	11/50 (22%)	14/50 (28%)	19/50 (38%)	28/50 (56%)
Adjusted rate	35.5%	36.5%	56.5%	78.8%
Terminal rate	5/22 (23%)	8/31 (26%)	10/24 (42%)	13/20 (65%)
First incidence (days)	449	460	548	524
Logistic regression test	P < 0.001	P=0.345	P=0.071	P < 0.001
Female				
Number Examined Microscopically	50	50	50	50
Infiltration Cellular, Diffuse, Histiocyte	0	0	0	4 (3.3)
Infiltration Cellular, Focal, Histiocyte	2 (2.0)	5 (1.8)	7 (2.9)	10* (2.4)
Bronchus, Cytoplasmic Vacuolization	0	6* (1.0)	31**(1.0)	43**(1.0)
Alveolar Epithelium Hyperplasia	2 (1.5)	3 (1.3)	0	5 (2.0)
Alveolar/bronchiolar Adenoma[j]				
Overall rate	3/50 (6%)	6/50 (12%)	9/50 (18%)	10/50 (20%)
Adjusted rate	8.8%	15.0%	25.2%	32.8%
Terminal rate	3/34 (9%)	4/37 (11%)	6/32 (19%)	8/28 (29%)
First incidence (days)	734 (T)	664	649	706
Logistic regression test	P=0.024	P=0.287	P=0.057	P=0.024
Alveolar/bronchiolar Carcinoma[k]				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	9/50 (18%)
Adjusted rate	2.9%	2.7%	9.2%	25.3%
Terminal rate	1/34 (3%)	1/37 (3%)	1/32 (3%)	4/28 (14%)
First incidence (days)	734 (T)	734 (T)	495	536
Logistic regression test	P < 0.001	P=0.743N	P=0.201	P=0.009

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Female (continued)				
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	4/50 (8%)	7/50 (14%)	13/50 (26%)	18/50 (36%)
Adjusted rate	11.8%	17.5%	32.6%	50.2%
Terminal rate	4/34 (12%)	5/37 (14%)	7/32 (22%)	11/28 (39%)
First incidence (days)	734 (T)	664	495	536
Logistic regression test	P < 0.001	P=0.318	P=0.016	P < 0.001

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 141/947 (14.9% ± 7.0%); range 6%-36%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by **N**.
[h] Historical incidence: 75/947 (7.9% ± 5.7%); range 0%-16%
[i] Historical incidence: 205/947 (21.7% ± 8.0%); range 10%-42%
[j] Historical incidence: 61/939 (6.5% ± 3.2%); range 0%-14%
[k] Historical incidence: 38/939 (4.1% ± 3.2%); range 0%-12%
[l] Historical incidence: 97/939 (10.3% ± 3.7%); range 0%-16%

Nose: The incidences of atrophy of the olfactory epithelium in 1.0 and 3.0 mg/m^3 males and females and hyperplasia of the olfactory epithelium in 3.0 mg/m^3 males and females were significantly greater than those in the chamber controls. The incidences of suppurative inflammation in 3.0 mg/m^3 males and in 1.0 mg/m^3 females were significantly greater than those in the chamber controls (Tables 12, C5, and D5). The nasal lesions in mice were less severe than in the rats and involved limited segments of the olfactory epithelium located further back in the nasal passage. Atrophy of the olfactory epithelium was characterized by loss of cell layers (sensory cells) and a decrease in the number of axons in the lamina propria. Hyperplasia of the olfactory epithelium was observed only in animals exposed to 3.0 mg/m^3 and was characterized by increased numbers of sensory cells that were usually arranged in nests or rosettes. The suppurative inflammation involved only a few animals and was a very mild change. It primarily involved animals that died prior to the end of the study and consisted of a focal aggregate of inflammatory cells.

Larynx: The incidences of squamous metaplasia in all exposed groups of males and females were significantly greater than those in the chamber controls (Tables 12, C5, and D5). Squamous metaplasia was limited to the base of the epiglottis and was not a severe lesion in exposed mice. It was characterized by replacement of the ciliated respiratory epithelium by one or more layers of flattened epithelial cells overlying a basal layer of cuboidal cells. Keratinization was sometimes observed.

TABLE 12
Incidences of Nonneoplastic Lesions of the Nose and Larynx in Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Nose[a]	50	50	48	49
Olfactory Epithelium, Atrophy[b]	0	0	29**(1.2)[c]	48**(1.8)
Olfactory Epithelium, Hyperplasia	0	0	0	10**(1.0)
Inflammation, Suppurative	0	1 (3.0)	0	6* (2.2)
Larynx	48	49	48	49
Metaplasia, Squamous	0	37**(1.0)	48**(1.0)	44**(1.0)
Female				
Nose	50	50	49	48
Olfactory Epithelium, Atrophy	0	2 (1.5)	12**(1.0)	46**(1.5)
Olfactory Epithelium, Hyperplasia	0	0	0	30**(1.3)
Inflammation, Suppurative	0	1 (1.0)	5* (1.6)	4 (1.5)
Larynx	50	49	47	50
Metaplasia, Squamous	0	45**(1.0)	40**(1.0)	50**(1.1)

* Significantly different ($P \leq 0.05$) from the chamber control by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Thyroid Gland: The incidences of follicular cell hyperplasia in all exposed groups of males were significantly greater than the incidence in the chamber controls (chamber control, 3/49; 0.3 mg/m^3, 17/50; 1.0 mg/m^3, 11/50; 3.0 mg/m^3, 10/50; Table C5). Minimal hyperplasias are commonly observed in untreated male and female mice, suggesting that the rate in the concurrent chamber control group is low. The severity of most hyperplasias in these mice was minimal to mild and did not differ between chamber control and exposed groups. The incidence of hyperplasia did not increase with exposure to cobalt sulfate heptahydrate, nor was the incidence of neoplasms of the follicular cells increased.

Liver: High incidences of chronic inflammation, karyomegaly, oval cell hyperplasia, and regeneration occurred in all groups of male mice and were usually observed together in the same liver (Tables 13 and C5). These changes were generally mild to moderate in severity and observed throughout the liver (usually not within proliferative lesions), but they appeared most pronounced in the portal regions. Similar lesions were observed in only a few females, and the severity was also much less than that observed in most males (Tables 13 and D5). This spectrum of lesions is consistent with those observed with *Helicobacter hepaticus* infection (Appendix J). Liver sections from four of five male mice with liver lesions were positive for bacterial organisms consistent with *H. hepaticus* when examined using Steiner's modification of the Warthin Starry silver stain.

The incidences of hemangiosarcoma in all exposed groups of male mice and in 1.0 mg/m^3 in female mice exceeded the range observed in historical controls for inhalation studies (Tables 13, C3, and C4b). In addition, the incidence of hemangiosarcoma in 1.0 mg/m^3 males was significantly greater than that in

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Mice in the 2-Year Inhalation Study of Cobalt Sulfate Heptahydrate

	Chamber Control	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Male				
Number Examined Microscopically	50	50	50	50
Inflammation, Chronic[a]	33 (1.3)[b]	36 (1.6)	40 (1.7)	39 (1.3)
Karyomegaly	39 (2.3)	35 (2.8)	39 (2.7)	43 (2.7)
Regeneration	32 (2.3)	30 (2.7)	35 (2.4)	38 (2.8)
Bile Duct, Hyperplasia	0	3 (1.3)	6* (1.7)	4 (2.5)
Oval Cell, Hyperplasia	38 (2.6)	36 (2.8)	40 (2.7)	44 (2.7)
Hemangiosarcoma[c]				
Overall rate[d]	2/50 (4%)	4/50 (8%)	8/50 (16%)	7/50 (14%)
Adjusted rate[e]	9.1%	11.5%	23.5%	25.0%
Terminal rate[f]	2/22 (9%)	2/31 (6%)	2/24 (8%)	3/20 (15%)
First incidence (days)	733 (T)	685	523	502
Logistic regression test[g]	P=0.078	P=0.441	P=0.050	P=0.069
Female				
Number Examined Microscopically	50	50	50	49
Inflammation, Chronic	6 (1.7)	1 (1.0)	1 (1.0)	2 (2.0)
Karyomegaly	4 (2.8)	2 (1.5)	0	1 (2.0)
Oval Cell, Hyperplasia	2 (2.0)	1 (2.0)	0	0
Hemangiosarcoma[h]				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/49 (0%)
Adjusted rate	2.9%	0.0%	7.3%	0.0%
Terminal rate	1/34 (3%)	0/37 (0%)	1/32 (3%)	0/28 (0%)
First incidence (days)	734 (T)	—[i]	524	—
Logistic regression test	P=0.431N	P=0.483N	P=0.318	P=0.539N

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 12/947 (1.3% ± 1.7%); range 0%-6%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by **N**.
[h] Historical incidence: 5/937 (0.5% ± 1.0%); range 0%-3%
[i] Not applicable; no neoplasms in animal group

the chamber controls. Hemangiosarcomas were morphologically similar to those observed spontaneously and consisted of multiple variably sized blood-filled spaces that were separated by cords of hepatocytes and lined by plump endothelial cells.

GENETIC TOXICOLOGY

Cobalt sulfate heptahydrate (3 to 10,000 µg/mL) was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of S9 metabolic activation, and with 5% hamster or rat liver S9; no mutagenicity was detected in strain TA98 or TA1535, with or without S9 (Zeiger *et al.*, 1992; Table E1).

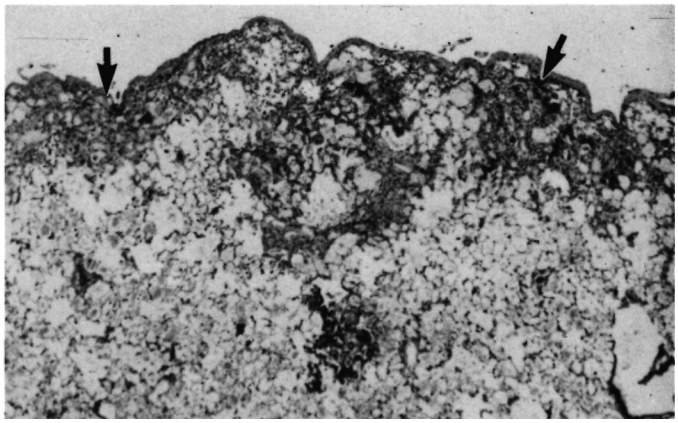


PLATE 1
Low magnification of a typical area of chronic inflammation (arrows) in the lung of a female F344/N rat exposed to 3.0mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 20×

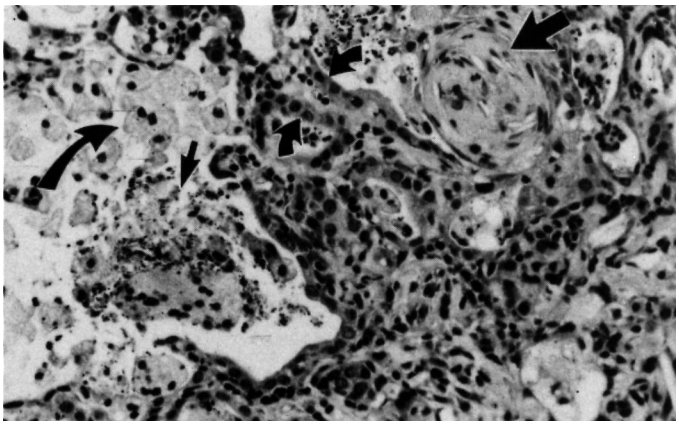


PLATE 2
Higher magnification of an area of chronic inflammation. Note the areas of fibrosis (large arrow), foamy alveolar macrophages (large curved arrow), necrotic cellular debris (small arrow), and epithelial hyperplasia (curved arrows) in the lung of a female F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 160×

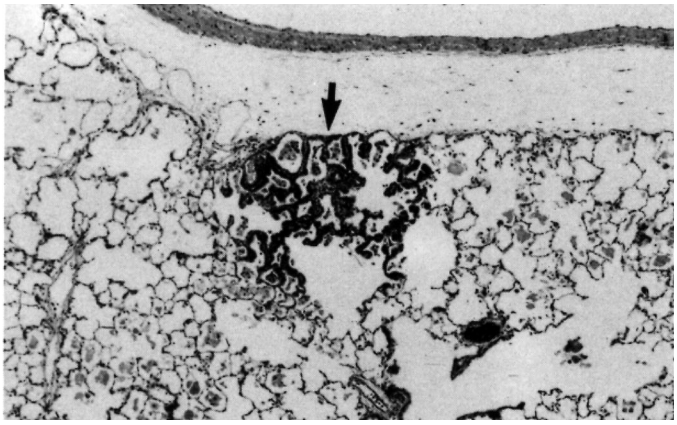


PLATE 3
Hyperplasia (arrow) in the lung of a female F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 20×

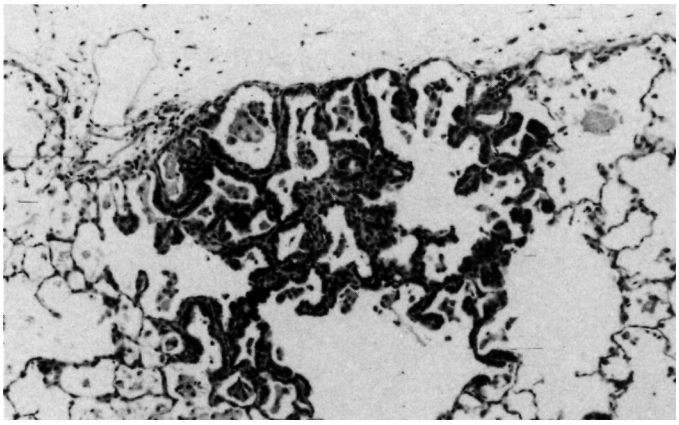


PLATE 4
Higher magnification of Plate 3. Note the proliferation of cells along the alveolar walls, but normal alveolar strucvture is maintained. H&E; 100×

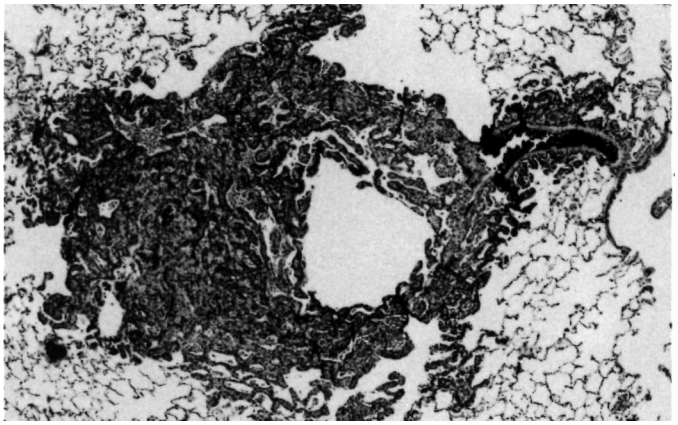


PLATE 5
Alveolar/bronchiolar adenoma in the lung of a male F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 26×

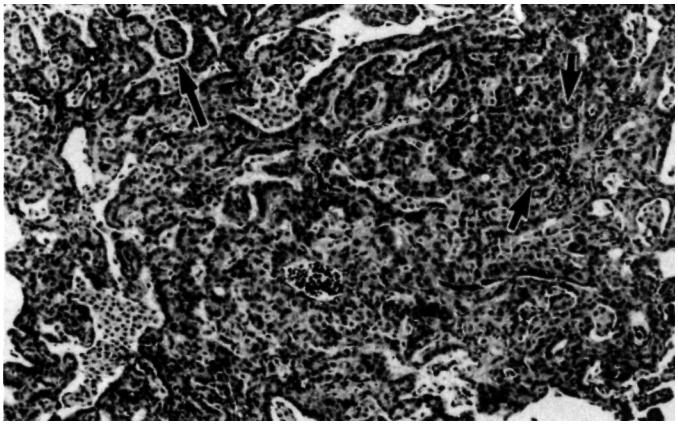


PLATE 6
Higher magnification of Plate 5. Component cells are arranged in acini (small arrows) and papillary projection s (large arrow). H&E; 66×

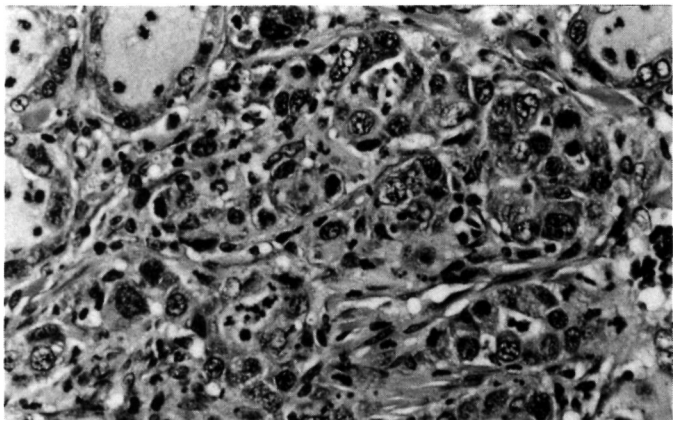


PLATE 7
Alveolar/bronchiolar carcinoma in the lung of a female F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. Note the variation in the size of the cells comprising acini at this high magnification. H&E; 200×

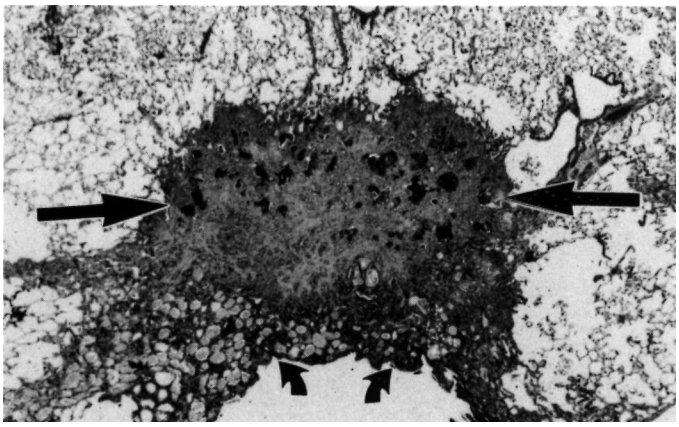


PLATE 8
Atypical hyperplasia (arrows) in the lung of a female F344/N rat exposed to 1.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. The lesion is located within an area of chronic inflammation (curved arrows). H&E; 16×

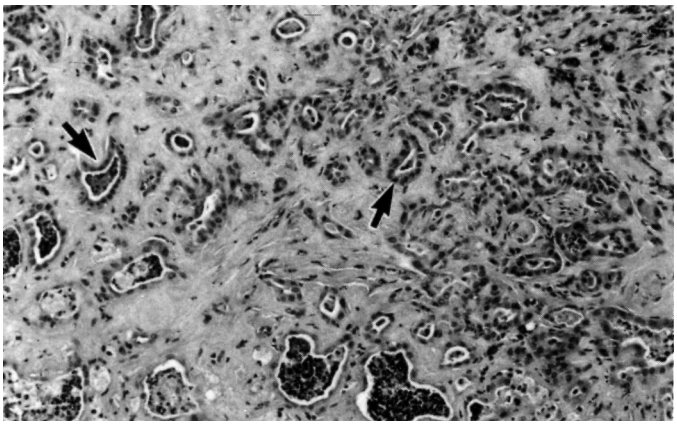


PLATE 9
Higher magnification of Plate 8. Note the glandular structures (arrows) lined by cuboidal epithelium within the fibrotic core. H&E; 80×

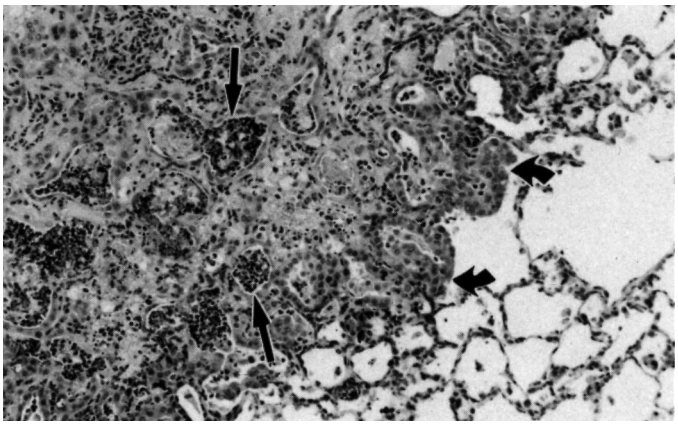


PLATE 10
High magnification of the border of an atypical hyperplasia in the lung of a male F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. Note the necrotic debris within the glandular structure (arrows) and the proliferative epithelium at the periphery (curved arrows). H&E; 80×

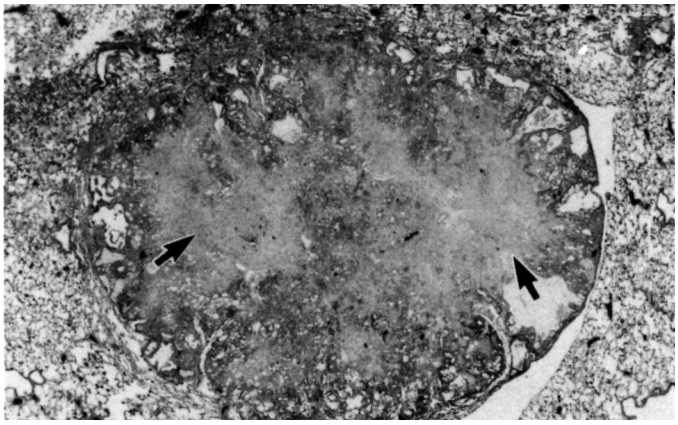


PLATE 11
Alveolar/bronchiolar carcinoma with abundant fibrous connective tissue (arrows) in the lung of a male F344/N rat exposed to 1.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 10×

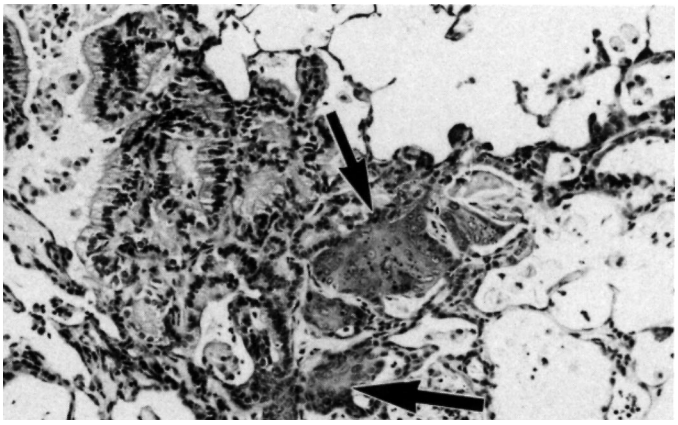


PLATE 12
Squamous metaplasia along the alveolar wall consisting of several layers of squamous epithelium (arrows) in the lung of a female F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 100×

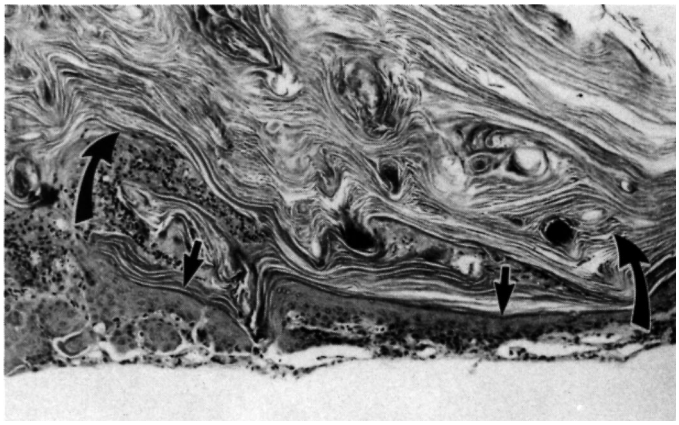


PLATE 13
Squamous cyst rimmed by a variably thick wall of squamous epithelium (large arrows) and filled with keratinous material (curved arrows) in the lung of a male F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 66×

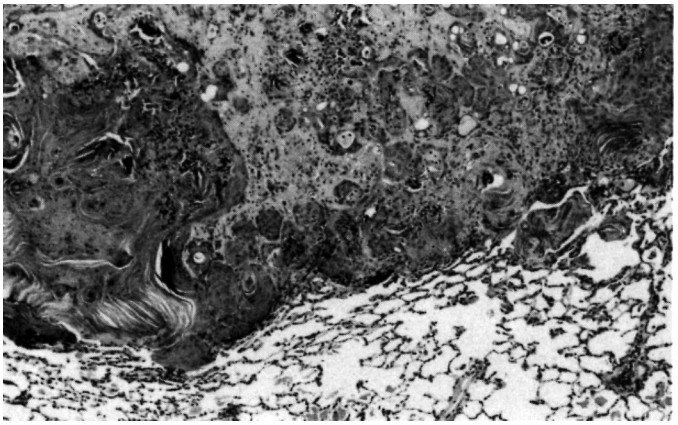


PLATE 14
High magnification of a squamous cell carcinoma in the lung of a female F344/N rat exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 40×

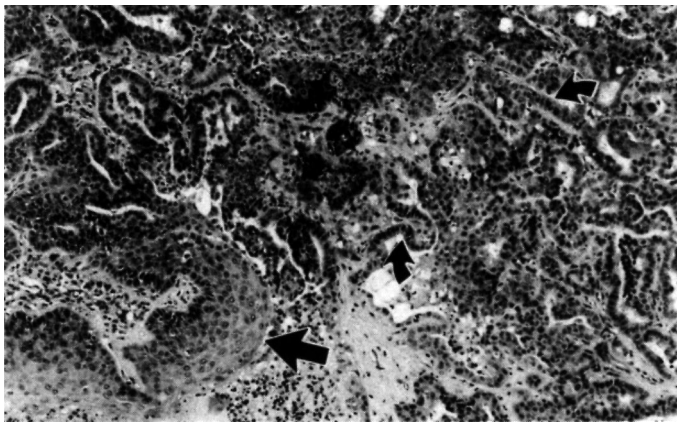


PLATE 15
Alveolar/bronchiolar carcinoma with an area of alveolar/bronchiolar epithelium to the right (curved arrows) and squamous differentiation to the left (arrow) in the lung of a male F344/N rat exposed to 1.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 66×

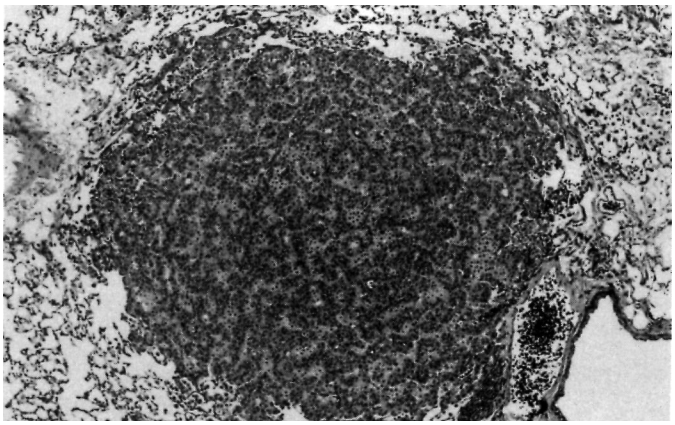


PLATE 16
Alveolar/bronchiolar adenoma in the lung of a female B6C3F$_1$ mouse exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 40×

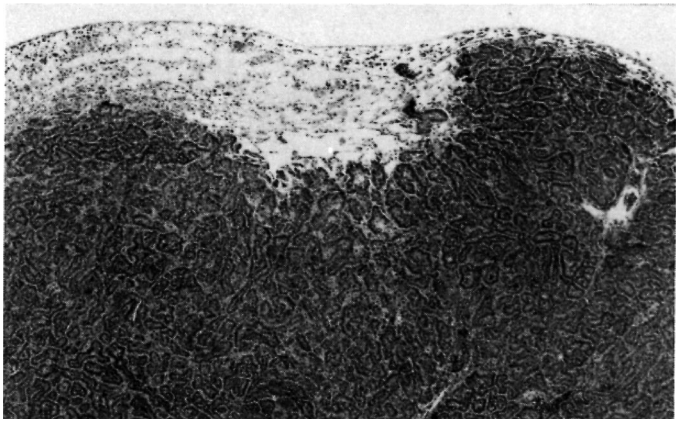


PLATE 17
Section of an alveolar/bronchiolar carcinoma with irregular and variably sized acinar structures in a female B6C3F$_1$ mouse exposed to 3.0 mg/m^3 cobalt sulfate heptahydrate by inhalation for 2 years. H&E; 26×

DISCUSSION AND CONCLUSIONS

This report presents the findings and conclusions of 2-year inhalation studies with cobalt sulfate heptahydrate. A companion report (NTP, 1991) discusses the findings of 16-day and 13-week inhalation studies conducted prior to the 2-year studies at the same laboratory. In all studies, the respiratory tract was the primary site of nonneoplastic lesions and neoplasms. In the 13-week studies, laryngeal lesions ranged from mild squamous metaplasia with or without chronic inflammation at concentrations ultimately selected for the 2-year studies, to large inflammatory polyps present in rats exposed to higher concentrations. Although other respiratory tract lesions were present, the larynx appeared to be the most sensitive to cobalt sulfate heptahydrate exposure, and lesions in this tissue were the determining factor in exposure concentration selection for the 2-year studies.

The highest concentration (3.0 mg/m^3) chosen for the 2-year studies did not affect survival or body weight gains of rats or survival of mice in either the 13-week or 2-year studies. The polycythemia noted in rats in the 13-week study was very mild at 3.0 mg/m^3, and there was no indication that this effect worsened to the point of causing clinical effects with longer exposure, although no hematologic measures were performed during the 2-year study. Similarly, there was no indication that the lesions observed in rats and mice in the 13-week studies in the larynx progressed in extent or changed in character with the prolonged exposures. There was no evidence of laryngeal polyp formation in rats, and the metaplastic and inflammatory changes in rats remained greater than in mice.

In contrast to the findings in the larynx, prolonged exposure to cobalt sulfate heptahydrate aerosol appeared to cause a progressive injury to the nose of rats and mice and to the lung of rats. Olfactory epithelial degeneration occurred primarily in rats and mice exposed to 10 and 30 mg/m^3 in the 13-week studies, but olfactory epithelial atrophy was increased at even the lowest concentration (0.3 mg/m^3) in rats and at 1.0 mg/m^3 in mice in the 2-year studies. Lesions in the lungs of rats changed markedly in character with the prolonged exposure in the 2-year study. Inflammation in the alveoli of rats was much more severe and occurred at lower concentrations than in the prechronic studies, and proteinosis was moderate to marked in the 2-year study rats and not noted in the prechronic study. Interstitial fibrosis is known to be a rather slowly developing lesion, but the extent of this lesion and its occurrence in essentially all rats at all exposure concentrations was not predicted based on the findings of the 13-week study. The alveolar epithelium of rats also displayed a spectrum of proliferative changes ranging from metaplasia through hyperplasia and atypical hyperplasia, and extending to neoplasia.

The spectrum of proliferative lung lesions observed in rats in the 2-year study ranged from highly cellular proliferations (typical of spontaneous lesions) to fibroproliferative and squamous lesions not typical of spontaneous lesions, and morphologic variants in between. The biological behavior of "typical" lung lesions, and to a lesser extent, squamous lesions, is fairly well documented. However, little is known about the biology of fibroproliferative lesions. In this study, many of the smaller lesions were identified within and/or adjacent to areas of chronic inflammation and fibrosis; however, it was clear that these lesions represented proliferative lesions distinct from the inflammation. Based upon the morphologic spectrum observed, it appears that their growth is progressive. There was, however, no clear morphologic correlate signaling autonomy of growth (i.e., consistent with a benign neoplasm) for these fibroproliferative lesions. Therefore, unless growth alterations consistent with a malignant neoplasm were present, all fibroproliferative lesions were diagnosed as atypical hyperplasia. There were several animals that had malignant neoplasms with a very prominent fibrous component; presumably, some of these progressed from atypical hyperplasias. In many respects, the range of proliferative lesions within the lungs of exposed rats resembled those observed in NTP studies of particulates (talc and the nickel compounds; NTP, 1993, 1996a,b,c), and it is clear that all the morphologic variants of proliferative lesions represent a response to cobalt sulfate heptahydrate.

Nonneoplastic lesions in the lungs of mice exposed to cobalt sulfate heptahydrate did not appear to differ appreciably from those expected in mice based on the results of the prechronic study. The lesions were confined primarily to histiocytic infiltration, and there was an absence of fibrosis and only minimal evidence of the nonneoplastic proliferative lesions noted in exposed rats. Most of the diagnoses of histiocytic infiltration were noted in animals that also had an alveolar/bronchiolar neoplasm; this is a frequent observation in mice with lung neoplasms and is not necessarily related to exposure to cobalt sulfate heptahydrate. Thus, it is not possible to clearly attribute the presence of histiocytic infiltration to cobalt sulfate heptahydrate exposure. Nonetheless, the lung changes were clearly much less severe than those seen in rats and differed markedly in character.

While rats and mice exhibited quite different nonneoplastic pulmonary responses to cobalt sulfate heptahydrate, exposed male and female rats and mice developed alveolar/bronchiolar adenomas and carcinomas. The distinction between these neoplasms is largely based on size, and both categories of this neoplasm were increased in exposed male and female rats and mice. In all groups, the neoplasms appeared with a significant positive trend, and the incidences in the 3.0 mg/m^3 groups exceeded the historical control ranges in the respective groups. The magnitude of the neoplastic response was somewhat less in male rats than in the other groups.

The incidences of follicular cell hyperplasia of the thyroid gland were moderately increased in all exposed groups of male mice, although no dose response was observed. Hypothyroidism has been noted in humans who also exhibited cardiomyopathy associated with consumption of cobalt-contaminated beer (Taylor and Marks, 1978).

Incidences of pheochromocytoma of the adrenal medulla were increased in female rats exposed to cobalt sulfate heptahydrate. Pheochromocytomas are relatively common in male F344/N rats, occurring with an historical rate of about 30% in inhalation studies. The historical inhalation chamber control rate in females is much lower (6%), and the incidence in the concurrent chamber control was 4%. While the incidences of this neoplasm were increased in exposed males and females, the strength of the response was much greater in females, and the increase in males was judged equivocal. In the NTP database of chemical carcinogenesis studies of nearly 450 chemicals, pheochromocytomas were part of a carcinogenic response in only 13 rat studies, five of which were inhalation studies. Although the historical control rates of pheochromocytomas do not appreciably differ between inhalation and dosed feed studies, a positive response is more likely to occur in inhalation studies than in studies using other routes of exposure. The reasons for this are not clear. Of the five other positive inhalation studies, two were with nickel compounds (oxide and subsulfide) and one with the particulate, talc.

Although the mechanisms responsible for induction of pheochromocytomas in rats are not understood, it is worth considering whether the adrenal gland and the pulmonary responses to cobalt sulfate heptahydrate in the rat might represent nonspecific responses to the physical inhalation and pulmonary accumulation of a particle, rather than a chemical-specific response. Measures of the possible accumulation of cobalt in the lung were not taken during these studies, although urinary cobalt concentrations have demonstrated dose-related absorption in the prechronic studies. Nickel sulfate hexahydrate is a highly water-soluble salt, as is cobalt sulfate heptahydrate. In similar studies, nickel sulfate hexahydrate did not show evidence of exposure-concentration-related accumulation in the lung of rats or mice exposed to concentrations as high as 30 mg/m^3 (NTP, 1996c). In contrast, the less soluble nickel subsulfide (NTP, 1996b) and the highly insoluble nickel oxide (NTP, 1996a) did accumulate in the lung. Thus, given the similar solubility and use of exposure concentrations ten-fold lower than those used with nickel sulfate hexahydrate, it is unlikely that cobalt would accumulate in the lung unless there was specific toxicity to pulmonary clearance mechanisms. The absence of nonneoplastic changes associated with cobalt sulfate heptahydrate inhalation by mice would argue against impaired clearance. The rather extensive and progressive pulmonary toxicity in the rat could have resulted in impaired clearance of cobalt, but it is unlikely that the toxicity represented a simple inflammatory and fibrotic response to an "overload" situation as has been postulated with chemically inert particles (Morrow *et al.*, 1991). The fact that the entire respiratory tract demonstrated a toxic response to cobalt sulfate heptahydrate argues convincingly that

the chemical has inherent toxicity and is not acting through secondary mechanisms related to its inhalation as a particle.

A number of factors need to be considered to properly address the relationship of these findings to typical human exposures to cobalt. The segments of the human population with the highest potential exposure to significant airborne cobalt concentrations are workers in the hard metal industry, coal mining, and those involved in ore processing (USDHHS, 1992). In these situations cobalt may exist in various forms, primarily as cobalt powder or cobalt oxide. These agents are less soluble than cobalt sulfate heptahydrate, and the toxic response of the respiratory system would likely depend on the combination of inherent toxicity, solubility in biological fluids, and residence time in the tissue. The carcinogenic potential of various cobalt compounds has been perhaps best demonstrated in injection studies in experimental animals (reviewed in IARC, 1991), and both insoluble and soluble forms have been shown to produce injection-site neoplasms.

The present demonstration of alveolar/bronchiolar neoplasms in rats and mice exposed to cobalt sulfate heptahydrate by inhalation confirms the findings of the injection studies and suggests that cobalt is inherently carcinogenic. These findings also lend credence to the epidemiological investigations of Mur *et al.* (1987), Hogstedt and Alexandersson (1990), and Lasfargues *et al.* (1994) that reported increased risks for lung cancer among workers producing cobalt and exposed to cobalt in the hard metal industry. Cobalt concentrations in the urine of workers in the Italian hard metal industry were found to be as high as 0.21 µg/mL at the end of the work shift (Sabbioni *et al.*, 1994). Ichikawa *et al.* (1985) reported even higher concentrations (0.39 µg/mL) in Japanese workers. In prechronic inhalation studies reported previously (NTP, 1991), average urinary cobalt concentrations in rats exposed to 0.3, 1.0, and 3.0 mg/m^3, respectively, were 0.14, 0.32, and 1.77 µg/mL. If urine cobalt concentrations roughly approximate relative inhalation exposures to cobalt, then the results from the current 2-year rat and mouse studies appear similar to occupational exposure levels and suggest that humans and rodents may be similarly sensitive to cobalt carcinogenesis.

The mechanisms of cobalt-induced carcinogenesis are not well understood. The genotoxicity of cobalt compounds has been established in a variety of eukaryotic test systems (reviewed in IARC, 1991), and cobalt has been shown under certain conditions to catalyze the production of oxygen-based free radicals that may underlie some of the observed adverse genetic events (Shi *et al.*, 1993). The observation of a larger than usual number of G to T transversions at the second base of codon 12 of those mouse lung neoplasms carrying a mutated K-*ras* gene (Appendix I) is also consistent with oxidative injury. Similar increases in G to T transversions were seen in lung neoplasms from mice exposed to ozone (NTP, 1994)

The potential contribution of the sulfate moiety to the carcinogenic response is worthy of consideration in that exposures of humans to concentrated inorganic acid mists are recognized as causing respiratory tract neoplasms, primarily in the larynx (IARC, 1992). There are no experimental animal carcinogenicity studies with sulfuric acid mists *per se* (IARC, 1992), but nickel sulfate hexahydrate was studied by inhalation as mentioned earlier (NTP, 1996c). In this instance, there was no evidence of carcinogenicity of nickel sulfate hexahydrate to the respiratory tract or other tissues despite the fact that other nickel salts are carcinogenic. Additionally, nickel sulfate hexahydrate was studied at an equivalent exposure concentration to that which caused significant increases in lung neoplasms in mice exposed to cobalt sulfate heptahydrate (1.0 mg/m^3). Thus, there seems to be little evidence to suggest that the sulfate moiety contributed significantly to the carcinogenic response.

Based on retrospective analyses, *Helicobacter hepaticus* was determined to have infected mice in 12 recent NTP 2-year studies (Appendix J). Of the 12 studies, mice (primarily males) from nine studies (including this study of cobalt sulfate heptahydrate) had a *H. hepaticus*-associated hepatitis. Qualitatively, the hepatitis and silver-staining organisms within the liver were similar among the nine studies. In a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based assay, *H. hepaticus* was identified in studies from which adequately preserved (frozen) liver tissue was available. In general, efforts to identify *H. hepaticus* from tissue fixed in formalin for over a week were not successful

(Malarkey *et al.*, 1997), which was the case for this study of cobalt sulfate heptahydrate. However, because of the presence of the typical liver lesions and silver-staining helical organisms, mice from the study were presumed to be infected with *H. hepaticus*.

Increases in the incidences of hepatocellular neoplasms in male mice have been shown to be associated with *H. hepaticus* infection when hepatitis is also present (Ward *et al.*, 1994; Fox *et al.*, 1996; Appendix J). Additionally, in NTP studies with *H. hepaticus*-associated hepatitis, increased incidences of hemangiosarcoma were seen in the livers of male mice (Appendix J). Because of the latter association, interpretation of the increased incidences of hemangiosarcoma in the liver of male mice was confounded. Incidences of lesions at other sites in this study of cobalt sulfate heptahydrate were not considered to have been significantly impacted by the infection with *H. hepaticus* or its associated hepatitis (Appendix J).

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of cobalt sulfate heptahydrate in male F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms. Marginal increases in incidences of pheochromocytomas of the adrenal medulla may have been related to exposure to cobalt sulfate heptahydrate. There was *clear evidence of carcinogenic activity* in female F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytomas of the adrenal medulla in groups exposed to cobalt sulfate heptahydrate. There was *clear evidence of carcinogenic activity* of cobalt sulfate heptahydrate in male and female B6C3F_1 mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to cobalt sulfate heptahydrate caused a spectrum of inflammatory, fibrotic, and proliferative lesions in the respiratory tract of male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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