Provisional Peer Reviewed Toxicity Values for

Cobalt
(CASRN 7440-48-4)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268
Acronyms and Abbreviations

bw  body weight
cc  cubic centimeters
CD  Caesarean Delivered
CERCLA Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS  central nervous system
cu.m  cubic meter
DWEL  Drinking Water Equivalent Level
FEL  frank-effect level
FIFRA  Federal Insecticide, Fungicide, and Rodenticide Act
g  grams
GI  gastrointestinal
HEC  human equivalent concentration
Hgb  hemoglobin
i.m.  intramuscular
i.p.  intraperitoneal
IRIS  Integrated Risk Information System
IUR  inhalation unit risk
i.v.  intravenous
kg  kilogram
L  liter
LEL  lowest-effect level
LOAEL  lowest-observed-adverse-effect level
LOAEL(ADJ)  LOAEL adjusted to continuous exposure duration
LOAEL(HEC)  LOAEL adjusted for dosimetric differences across species to a human
m  meter
MCL  maximum contaminant level
MCLG  maximum contaminant level goal
MF  modifying factor
mg  milligram
mg/kg  milligrams per kilogram
mg/L  milligrams per liter
MRL  minimal risk level
MTD  maximum tolerated dose
MTL  median threshold limit
NAAQS  National Ambient Air Quality Standards
NOAEL  no-observed-adverse-effect level
NOAEL(ADJ)  NOAEL adjusted to continuous exposure duration
NOAEL(HEC)  NOAEL adjusted for dosimetric differences across species to a human
NOEL  no-observed-effect level
OSF  oral slope factor
p-IUR  provisional inhalation unit risk
p-OSF  provisional oral slope factor
p-RfC  provisional inhalation reference concentration
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>p-RfD</td>
<td>provisional oral reference dose</td>
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<tr>
<td>PBPK</td>
<td>physiologically based pharmacokinetic</td>
</tr>
<tr>
<td>ppb</td>
<td>parts per billion</td>
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<tr>
<td>ppm</td>
<td>parts per million</td>
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<tr>
<td>PPRTV</td>
<td>Provisional Peer Reviewed Toxicity Value</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cell(s)</td>
</tr>
<tr>
<td>RCRA</td>
<td>Resource Conservation and Recovery Act</td>
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<tr>
<td>RDDR</td>
<td>Regional deposited dose ratio (for the indicated lung region)</td>
</tr>
<tr>
<td>REL</td>
<td>relative exposure level</td>
</tr>
<tr>
<td>RfC</td>
<td>inhalation reference concentration</td>
</tr>
<tr>
<td>RfD</td>
<td>oral reference dose</td>
</tr>
<tr>
<td>RGDR</td>
<td>Regional gas dose ratio (for the indicated lung region)</td>
</tr>
<tr>
<td>s.c.</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SCE</td>
<td>sister chromatid exchange</td>
</tr>
<tr>
<td>SDWA</td>
<td>Safe Drinking Water Act</td>
</tr>
<tr>
<td>sq.cm.</td>
<td>square centimeters</td>
</tr>
<tr>
<td>TSCA</td>
<td>Toxic Substances Control Act</td>
</tr>
<tr>
<td>UF</td>
<td>uncertainty factor</td>
</tr>
<tr>
<td>μg</td>
<td>microgram</td>
</tr>
<tr>
<td>μmol</td>
<td>micromoles</td>
</tr>
<tr>
<td>VOC</td>
<td>volatile organic compound</td>
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</table>
PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR COBALT (CASRN 7440-48-4)

Background

On December 5, 2003, the U.S. Environmental Protection Agency’s (EPA’s) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA’s Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA’s Superfund Program.

3. Other (peer-reviewed) toxicity values, including:
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA’s Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically
updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development’s National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development’s National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The Integrated Risk Information System (IRIS) does not report a Reference Dose (RfD) for cobalt (U.S. EPA, 2007). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997a) and Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) likewise do not contain an RfD for cobalt. The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991, 1994a) report a Health Effect Assessment (HEA) for cobalt (U.S. EPA, 1987). The 1987 HEA derived a chronic RfD of 0.005 mg cobalt/kg-day based on a no-observed-adverse-effect level (NOAEL) of 5 mg cobalt/kg-day for testicular effects in a subchronic rat study (Nation et al., 1983). The Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for cobalt and its compounds reports an oral Minimal Risk Level (MRL) for intermediate exposure of 1x10^{-2} mg/kg-day (ATSDR, 2004), based on a lowest-observed-adverse-effect level (LOAEL) of approximately 1 mg cobalt/kg-day for polycythemia in humans (Davis and Fields, 1958). ATSDR (2004) did not derive an oral MRL for chronic exposure. This MRL for intermediate exposure was based on the polycythemic effect of cobalt exposure (1 mg cobalt/kg-day, Davis and Fields, 1958) by application of an UF of 10 for a LOAEL and an UF of 10 for human variability. The World Health Organization (WHO, 2005) has not published an Environmental Health Criteria (EHC) document on cobalt. An International Agency for Research on Cancer (IARC) Monograph on cobalt and its compounds (IARC, 2006) and the National Toxicology Program (NTP) Status Reports (NTP, 2005) were searched for relevant information.

IRIS (U.S. EPA, 2007) does not report a Reference Concentration (RfC) for cobalt. The HEAST (U.S. EPA, 1997a) likewise does not list an RfC for cobalt. The cobalt HEA (U.S. EPA, 1987) derived a subchronic inhalation RfC of 9x10^{-5} mg/m^3 based on a LOAEL of 0.1 mg/m^3 for
respiratory effects in a 3-month study in swine (Kerfoot et al., 1975). A chronic inhalation RfC of $9 \times 10^{-6}$ mg/m$^3$ was derived from the same study. The ATSDR Toxicological Profile for cobalt and its compounds reports an inhalation MRL for chronic exposure of $1 \times 10^{-4}$ mg/m$^3$ (ATSDR, 2004), based on a NOAEL of 0.0053 mg cobalt/m$^3$ for decreased pulmonary function in humans (Nemery et al., 1992). The American Conference of Governmental Industrial Hygienists (ACGIH, 2004) has set a Threshold Limit Value-Time-Weighted Average (TLV-TWA) of 0.02 mg/m$^3$ for cobalt and inorganic cobalt compounds, expressed as cobalt, based on respiratory and cardiovascular effects. The National Institute for Occupational Safety and Health (NIOSH, 2005) Recommended Exposure Limit (REL) TWA for cobalt is 0.05 mg/m$^3$, based on effects in the respiratory system. The Occupational Safety and Health Administration (OSHA, 2005) Permissible Exposure Limit (PEL) is 0.1 mg/m$^3$.


Literature searches for studies relevant to the derivation of provisional toxicity values for cobalt were conducted initially through 2000 in TOXLINE (supplemented with BIOSIS and NTIS updates), MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT and subsequently from 2000 to August 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents. An updated literature search was performed in MEDLINE from 2005 to June 2008.

**REVIEW OF PERTINENT DATA**

**Human Studies**

*Overview*

Indicators of adverse health effects in humans following oral exposure to cobalt include increased erythrocyte number and hemoglobin (Taylor et al., 1977; Duckham and Lee, 1976; Davis and Fields, 1958), cardiomyopathy (Morin et al., 1971; Alexander, 1969, 1972) and decreased iodine uptake by the thyroid (Roche and Layrisse, 1956). Cardiomyopathy is an endpoint of concern for cobalt in humans; however, it is highly likely that alcohol consumed in “beer-cobalt cardiomyopathy,” as well as other factors, such as smoking, played a role in the effects that were observed. Cobalt is a sensitizer in humans by any route of exposure. Sensitized individuals may react to inhalation of cobalt by developing asthma; ingestion or dermal contact with cobalt may result in development of dermatitis. Several studies have suggested that
cross-sensitization may occur between cobalt and nickel (Shirakawa et al., 1990; Lammintausta et al., 1985; Bencko et al., 1983; Rystedt and Fisher, 1983).

Respiratory effects, including respiratory irritation, wheezing, asthma, pneumonia and fibrosis, have been widely reported in humans exposed to cobalt by inhalation (for review, see Barceloux, 1999; Lison, 1996). Epidemiology studies show decreased pulmonary function in workers exposed to inhaled cobalt (Nemery et al., 1992; Gennart and Lauwerys, 1990). Results of studies investigating cancer incidence in workers exposed to inhaled cobalt are suggestive of a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983).

Oral Exposure

In humans, cobalt stimulates production of red blood cells through increased production of the hormone erythropoietin and has been explored for use in the treatment of anemia (Smith and Fisher, 1973; Duckham and Lee, 1976). Increases in red blood cell counts and blood hemoglobin have been reported in non-anemic volunteers (Davis and Fields, 1958) and in anephric anemic patients (Taylor et al., 1977; Duckham and Lee, 1976).

Reversible polycythemia (increase in blood cell number) was reported (see Table 1) in six healthy adult males following treatment with 150 mg cobalt chloride per day for 22 days (Davis and Fields, 1958). Five subjects received 150 mg cobalt chloride/day for the entire exposure period and a sixth subject initially received 120 mg cobalt chloride/day, which was later increased (time not specified) to 150 mg/day. Cobalt chloride was administered as a 2% solution diluted in either water or milk. Assuming an average body weight of 70 kg, 150 mg cobalt chloride/day corresponds to approximately 1 mg cobalt/kg-day. Outcomes assessed in this study were red blood cell count, hemoglobin percentage, leukocyte count, reticulocyte percentage and thrombocyte count. Polycythemia was observed in all six patients within 7 to 22 days of treatment as demonstrated by increases in red blood cell counts ranging from 0.5 to 1.19 million (approximately 16-20% increase above pre-treatment levels) and increases in hemoglobin levels ranging from 6 to 11% above pretreatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts were not significantly different from pretreatment values. Erythrocyte counts returned to pre-treatment levels within 9 to 15 days after cobalt administration was discontinued. The fact that leucocyte counts remained relatively constant throughout the experiment supports the concept that this is a true polycythemia. As such, based on the results of this study, 1 mg cobalt/kg-day was identified as a LOAEL for cobalt-induced polycythemia in humans.
Table 1. Hematopoietic, Thyroid and Developmental Effects of Cobalt (Co) via Oral Route

<table>
<thead>
<tr>
<th>Target Organ</th>
<th>Species</th>
<th>Effect</th>
<th>Dosage (mg Co/kg-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic Effects</td>
<td>Human</td>
<td>Reversible Effect (Polycythemia)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>↑ Hemoglobin and ↑ RBC</td>
<td>0.16 – 0.32*</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Hematopoietic effect</td>
<td>0.5 – 32.0</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Human</td>
<td>↓ Iodine uptake</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>Histopathological changes in thyroid</td>
<td>48.0</td>
</tr>
<tr>
<td>Fetus</td>
<td>Rat</td>
<td>Developmental toxicity</td>
<td>5.2 – 21.0</td>
</tr>
<tr>
<td>Heart</td>
<td>Rat</td>
<td>↓ Myocardial function</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Therapeutic doses for anemic patients

Duckham and Lee (1976) treated 12 anephric patients on dialysis with 25 to 50 mg cobalt chloride daily for approximately 12 weeks. Assuming an average body weight of 70 kg, doses of 25 and 50 mg cobalt chloride/day are equivalent to 0.16 and 0.32 mg cobalt/kg-day, respectively. During the exposure period, patients also received daily treatment with 100 mg ferrous sulfate and 50 mg ascorbic acid. Within approximately 2 months of initiation of treatment with cobalt, an increase in hemoglobin of 26-70% was observed in patients treated with 0.32 mg cobalt/kg-day. Serum cobalt levels appeared to reach steady state within 2 months of exposure (approximately 40-100 µg cobalt/100 mL). In a subgroup of three patients, continuation of treatment with 0.16 mg cobalt/kg-day for approximately 3 months maintained elevated hemoglobin levels. Hemoglobin levels decreased rapidly when cobalt therapy was discontinued. The authors did not report whether therapy with ferrous sulfate and ascorbic acid was discontinued at the same time. Results of this study are difficult to interpret because patients were anephric and on dialysis, which may have altered cobalt pharmacokinetics and dose-effect relationships. Furthermore, since it is well established that treatment with ferrous sulfate alone increases hemoglobin concentration (Hillman, 2001), concomitant therapy with iron is a confounding factor. Since this study did not evaluate the response of patients treated with ferrous sulfate alone, it is not possible to determine the relative contributions of iron and cobalt to the observed increases in hemoglobin. Thus, adverse effect levels cannot be confidently determined for cobalt. In a separate study, a group of eight anephric patients with refractory anemia were treated with 25 to 50 mg cobalt chloride daily for 12 to 36 weeks (Taylor et al., 1977). Increased hemoglobin concentration and decreased requirement for blood transfusions were observed (Taylor et al., 1977). Data on hemoglobin concentrations (or other indicators of polycythemia) were not reported.

Pregnant women given 75 to 100 mg cobalt chloride/day with no other treatment for 90 days to 6 months did not experience pregnancy-induced reductions in hematocrit and hemoglobin levels, compared to untreated controls (Holly, 1955). However, daily treatment with 1 g ferrous sulfate alone or combined daily treatment with 60 to 90 mg cobalt chloride and 0.8 to 1.2 g ferrous sulfate prevented pregnancy-related decreases in hematocrit and hemoglobin levels. The response to combined cobalt chloride and iron therapy was more pronounced than the
response to iron therapy alone. In patients treated with iron only, decreases in hemoglobin and hematocrit were prevented in approximately 80% of patients, compared to 100% of patients treated with combined cobalt chloride and iron.

Cardiomyopathy has been observed in association with consumption of large quantities of beer containing cobalt chloride (introduced into the beer to stabilize the foam) (Alexander, 1969, 1972; Morin et al., 1971). Exposure estimates in reported cases range from 0.04 to 0.14 mg cobalt/kg-day (corresponding to approximately 8-30 pints of beer daily) over a period of years (Alexander, 1969, 1972; Morin et al., 1971). The cardiomyopathy in the beer drinkers, referred to in the literature as “beer-cobalt cardiomyopathy,” was fatal to 43% of the subjects within several years, with approximately 18% of these deaths occurring within the first several days following diagnosis. Beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi; however, the onset of the beer-cobalt cardiomyopathy was much more abrupt. The practice of adding cobalt to beer to stabilize the foam has been discontinued.

It should be noted, however, that the cardiomyopathy may also have been due to the fact that the beer drinkers had protein-poor diets and may have had prior or concurrent cardiac and hepatic damage from alcohol abuse. Due to the potential adverse effects of poor nutrition and/or chronic ethanol exposure on cardiovascular health, it is difficult to delineate the contribution of oral cobalt exposure to the observed cardiomyopathy. As such, no adverse effects levels can be determined for cobalt-induced cardiotoxicity.

The thyroid also appears to be a target organ for cobalt (see Table 1). Treatment of 12 euthyroid (normal thyroid) patients with 150 mg cobalt chloride/day (equivalent to 1 mg cobalt/kg-day, assuming a body weight of 70 kg) for 2 weeks resulted in a greatly reduced uptake of 48-hour radioactive iodine by the thyroid when measured after 1 week of exposure to cobalt, with uptake nearly abolished completely by the second week of exposure to cobalt (Roche and Layrisse, 1956). It should be noted that when cobalt treatment was discontinued, iodine uptake returned to pre-treatment reported values. No other clinical details were provided for the human subjects. Therefore, based on the results of this study, a LOAEL of 1 mg cobalt/kg-day was identified for decreased radioactive iodine uptake in human thyroid following oral cobalt exposure. In another small clinical study (Paley et al., 1958), decreased radioactive iodine uptake was reported in two of four (3 males, 1 female) euthyroid patients orally administered 37.5 mg cobalt/day as cobalt chloride (equivalent to 0.54 mg cobalt/kg-day, assuming a body weight of 70 kg) for 10 to 14 days. One of the two subjects with reported decreased iodine uptake had received i.v. cobalt in addition to oral cobalt intake, and had been previously diagnosed with hyperthyroidism (although was clinically euthyroid at the time of study). The i.v. dosing may have raised the internal cobalt concentration to a level greater than the reported 0.54 mg dosage based upon oral dosing of 37.5 mg/day in other subjects that did not receive i.v. cobalt. Of the remaining three subjects, 24-hour iodine uptake was not significantly decreased following oral cobalt exposure compared to corresponding pre-treatment values (based on pairwise t-test). The oral cobalt dose of 0.54 mg cobalt/kg-day represents a NOAEL for thyroid effects in humans. It should be noted that the Roche and Layrisse (1956) and Paley et al. (1958) studies lack details pertinent to other clinical conditions (e.g. including effects on thyroid stimulating hormone [TSH]) of these patients; thus the mechanism for the effect of cobalt on thyroidal iodine uptake cannot be ascertained. However, cobalt appears to increase thiocyanate-
induced release of radioiodine from the thyroid, suggesting a possible effect on binding of iodine (e.g., iodination of thyroglobulin) in the thyroid gland.

Cobalt has been found to be a sensitizer in humans. Individuals are sensitized following dermal or inhalation exposure, but flares of dermatitis may be triggered following cobalt ingestion. In a small clinical study, several patients with eczema of the hands were challenged orally with 1 mg cobalt sulfate (0.005 mg cobalt/kg-day, assuming a body weight of 70 kg) in tablet form once per week for 3 weeks; this translates to an estimated average daily dose of 0.0007 mg cobalt/kg-day (1 day a week/7 days a week × 0.005 mg cobalt/kg-day). 28/47 patients had a flare of dermatitis following the oral challenge (Veien et al., 1987). All 47 patients had positive dermal patch tests to cobalt (13 to cobalt alone and 34 to nickel and cobalt) and 7 of the 13 patients who had patch-tested positive to cobalt alone reacted to the oral challenge. These results suggest that cobalt allergy can be induced from oral ingestion exposures to cobalt. Although the exposure levels associated with sensitization to cobalt following inhalation or dermal exposure have not been established, interrelationships have been found to exist between cobalt and nickel sensitization (Bencko et al., 1983; Rystedt and Fisher, 1983; Veien et al., 1987). In guinea pigs, nickel and cobalt sensitization appear to be interrelated and mutually enhancing (Lammintausta et al., 1985). Therefore, it is possible that in people sensitized by nickel, exposure to cobalt may result in an allergic reaction.

Inhalation Exposure

Numerous studies have investigated health effects in workers occupationally exposed to cobalt-bearing dust (Linna et al., 2003; Swennen et al., 1993; Auchincloss et al., 1992; Cugell, 1992; Nemery et al., 1992; Prescott et al., 1992; Gennart and Lauwerys, 1990; Meyer-Bisch et al., 1989; Raffh et al., 1988; Shirakawa et al., 1988, 1989; Sprince et al., 1988; Kusaka et al., 1986a,b; Demedts et al., 1984; Davison et al., 1983). However, many of these studies are of limited utility for risk assessment due to inadequate characterization of exposure and/or effects. Four studies were considered to be potentially suitable for RfC derivation. Two of these focused exclusively on respiratory effects (Nemery et al., 1992; Gennart and Lauwerys, 1990); one studied only thyroid effects (Prescott et al., 1992) and one considered multiple endpoints (Swennen et al., 1993). The populations studied included diamond-cobalt saw manufacturers, diamond polishers, plate painters and cobalt production workers. All four studies were cross-sectional design.

Several studies have examined the effects of hard metal, a mixture containing approximately 20% cobalt with the remainder being primarily tungsten carbide. Exposure of humans to hard metal has been shown to result in an increase in cancer mortality (Moulin et al., 1998; Lasfargues et al., 1994) as well as a number of other diseases, including asthma and pulmonary fibrosis (for reviews, see Barceloux, 1999; Lison, 1996). There is substantial evidence from animal studies that tungsten, although it acts as an inert dust by itself, can potentiate the effects of cobalt on the respiratory tract (Lasfargues et al., 1995; Lison et al., 1995, 1996; Swennen et al., 1993). For this reason, studies of hard metal were not given further consideration.
Gennart and Lauwerys (1990) studied ventilatory function in workers at a plant producing diamond-cobalt circular saws. The form of cobalt used in diamond polishing is primarily metallic cobalt powder; specific cobalt species contained in this powder were not identified. The exposed population consisted of 48 workers (34 males and 14 females) who agreed to participate in the study (an additional 27 workers declined). Exposure duration for these workers ranged from 0.1 to 32 years, with an average of approximately 6 years. The work involved weighing and mixing cobalt powder and microdiamond particles (and possibly small amounts of other undisclosed substances), cold pressing, heating and hot pressing. After sintering, the pieces were welded onto steel disks. These operations were performed in two rooms called the mixing room and the oven room, where all the examined workers spent most of their time. Controls consisted of 23 workers (11 males and 12 females) from other factories in the same area who were not exposed to known pneumotoxic chemicals. Personal air samples were collected at different workplaces during half a workshift. Subjects filled out a questionnaire regarding occupational and medical histories, smoking habits and pulmonary symptoms; gave a urine sample for cobalt determination; and participated in lung function tests. Cobalt concentrations varied from 9.4 to 2875 μg/m³ in the mixing room (geometric mean=135.5 μg/m³) and from 6.2 to 51.2 μg/m³ in the oven room (geometric mean=15.2 μg/m³). The prevalence of respiratory symptoms, such as cough, sputum and dyspnea, were significantly increased in the exposed workers compared to the control group (numeric data not reported). Mean predicted values of FEV₁ (forced expiratory volume in 1 second adjusted for body size) and FVC (forced vital capacity) were significantly lower, and the prevalence of abnormal values was higher in the cobalt exposed workers (both smokers and non-smokers) compared to the control group. In controls, FEV₁ and FVC were 95.4 and 101.6 percent of predicted values, respectively. Mean percent predicted FEV₁ and FVC in exposed non-smokers were 87.1 and 92.3, respectively, and in exposed smokers were 83.9 and 93.4, respectively. Among non-smokers, all measures of pulmonary function were lower in workers exposed for 5 years or more than in those exposed to cobalt for a shorter period of time.

Nemery et al. (1992) conducted a cross-sectional study of cobalt exposure and respiratory effects in diamond polishers who were primarily exposed to metallic cobalt-containing dust; species of cobalt in the dust samples were not identified. The study group was composed of 194 polishers working in 10 different workshops. In two of these workshops (#1, 2), the workers used cast iron polishing disks almost exclusively, and in the others, they primarily used cobalt-containing disks. The number of subjects from each workshop varied from 6 to 28 and the participation rate varied from 56 to 100%. The low participation in some workshops reflects the fact that only workers who used cobalt disks were initially asked to be in the study; low participation is not due to a high refusal rate (only eight refusals were documented). More than a year after the polishing workshops were studied, an additional three workshops with workers engaged in sawing diamonds, cleaving diamonds or drawing jewelry were studied as an unexposed control group (n=59 workers). Subjects were asked to fill out a questionnaire regarding employment history, working conditions, medical history, respiratory symptoms and smoking habits; to give a urine sample for cobalt determination; and to undergo a clinical examination and lung function tests. Both area air samples and personal air samples were collected (always on a Thursday). Sampling for area air determinations started 2 hours after work began and continued until 1 hour before the end of the work day. Personal air samples were collected from the breathing zone of a few workers per workshop for four successive
1-hour periods. Air samples were analyzed for cobalt and iron. In addition, personal air samplers were used to sample the air 1 cm above the polishing disks. These samples were analyzed for the entire spectrum of mineral and metallic compounds. Air samples were not obtained at one of the polishing workshops (#4); however, this workshop was reported to be almost identical to an adjoining workshop (#3) for which samples were obtained. Urinary cobalt levels were similar between workers in these two workshops, so exposure was considered to be similar as well.

Results of area and personal air sampling were strongly correlated (R=0.92), with area air sampling reporting lower concentrations than personal air samples in all workshops except one (#9) (Nemery et al., 1992). In this workshop, personal air samples appeared to be artificially low in comparison to area air samples and urinary cobalt levels of the workers. When this workshop was excluded, a strong correlation (R=0.85-0.88) between urinary cobalt and cobalt in the air was observed. Based on urinary cobalt levels, the predicted concentration of cobalt expected in personal air samples from workshop #9 was approximately 45 μg/m³ (the mean value actually reported was 6 μg/m³). The polishing workshops were divided into two groups: those with low exposure to cobalt (#1-5, n=102) and those with high exposure to cobalt (#6-10, n=91). Mean cobalt exposure concentrations were 0.4, 1.6 and 10.2 μg/m³ by area air sampling and 0.4, 5.3 and 15.1 μg/m³ by personal air sampling in the control, low-exposure and high-exposure groups, respectively. The inclusion of the apparently biased personal air samples from workshop #9 means that the reported mean cobalt exposure in the high-exposure group obtained by personal air sampling (15.1 μg/m³) may be lower than the true value. Air concentrations of iron were highest in the two polishing workshops that used iron disks and the sawing workshop (highest value=62 μg/m³), and were not correlated with cobalt levels. Analysis of samples taken near the disks showed the presence of cobalt, with occasional traces of copper, zinc, titanium, manganese, chromium, silicates and silicon dioxide. No tungsten was detected. Some workers may have previously been exposed to asbestos since pastes containing asbestos had been used in the past to glue the diamonds onto holders. However, since the asbestos was in its non-friable form, exposure was insufficient to produce functional impairment. Smoking habits were similar in workers from the high-exposure, low-exposure and control groups. Duration of exposure was not discussed.

Workers in the high-exposure group were more likely than those in the other groups to complain about respiratory symptoms; the prevalences of eye, nose and throat irritation and cough, and the fraction of these symptoms related to work, were significantly increased in the high-exposure group (Nemery et al., 1992). Workers in the high-exposure group also had significantly lower lung function compared to controls and low-exposure group workers, as assessed by FVC, FEV₁, MMEF (forced expiratory flow between 25 and 75% of the FVC) and mean PEF (peak expiratory flow rate), although the prevalence of abnormal values did not differ significantly between exposure categories. In controls, FVC, FEV₁ and MMEF were approximately 110, 107 and 94 percent of predicted values, respectively, compared to approximately 105, 104 and 87 percent of predicted values, respectively, in the high-exposure group workers. Results in the low-exposure group did not differ from controls. The effect on spirometric parameters in the high exposure group was present in both men and women. Women seemed to be affected more than men; however, the interaction between exposure and sex was not significant (two-way analysis of variance). Smoking was found to exert a strong effect on
lung function; however, lung function level remained negatively correlated with exposure to cobalt, independent of smoking.

A cobalt dose-effect relationship is evident from the Nemery et al. (1992) study, based on a multivariate regression analysis of urinary cobalt and lung function measurements. Increasing urinary cobalt concentration (approximate range <1-70 µg cobalt/g creatinine) was significantly \( (p<0.05) \) associated with co-variate-adjusted decreasing forced expiratory volume (FEV1%) and forced vital capacity (FVC%). Significant co-variates retained in the regression analysis included gender and smoking. The model predicted 3% and 4% decreases in FEV1% and FVC%, respectively, in association with a 10-fold increase in urinary cobalt concentration. The approximate mean urinary cobalt levels of the control and high exposure groups were 2 and 20 µg cobalt/g creatinine, respectively. The magnitude of the cobalt effect was similar to the predicted effect of smoking, approximately 3-4% decrease in FEV1% and FVC%. Cobalt concentration determined from personal air sampling may be more representative of airborne cobalt exposure than area sampling. As such, 5.3 µg/m³ and 15.1 µg/m³ represent a NOAEL and LOAEL, respectively, for decreased pulmonary function and increased symptoms of airway irritation.

Swennen et al. (1993) conducted a cross-sectional study of workers exposed to metallic cobalt and various inorganic cobalt salts and oxides (specific species not identified) at a cobalt plant producing these materials from cobalt metal cathodes and scrap metal. The study group included 82 male workers from the cobalt plant who had no history of lung disease prior to employment and who had never been exposed to other pneumotoxic chemicals. Methods for selection or exclusion of subjects in constructing the cohort and participation were not reported. The control group comprised 82 age-matched workers from the mechanical workshop of a nearby plant owned by the same company. Workers filled out a questionnaire regarding occupational history, respiratory complaints and smoking habits; received a routine clinical examination; participated in lung function tests; had a chest radiograph taken; and gave blood and urine samples (before and after working on Monday and Friday of one week) for determination of cobalt content as well as hematological and serum chemistry analyses. Exposure was monitored by personal air samplers worn by each cobalt worker for 6 hours on both Monday and Friday.

Workers in the cobalt plant were exposed to cobalt concentrations ranging from 1 to 7772 µg/m³ (Swennen et al., 1993). The geometric mean exposure concentration was 125 µg/m³. Exposure duration ranged from 0.3 to 39.4 years, with an average exposure of 8.0 years. A significantly higher number of exposed workers reported dyspnea than did controls. The increase occurred primarily among smokers although no significant interaction was found between smoking and exposure to cobalt. Based on a logistic regression model, the probability of dyspnea during exercise was significantly associated with increasing cobalt concentration in the air or urine. The parameters of the model were not reported. The clinical examinations detected significantly increased prevalence of skin disorders (eczema, erythema) (51 vs. 25%) and wheezing (16 vs. 6%) in the exposed group compared to controls. Lung function tests did not differ between the two groups; however, a few significant trends were noted: the FEV1/VC (forced expiratory volume in one second/vital capacity) ratio decreased with increasing concentration of cobalt in the air and urine, and the RV (residual volume) and TLC (total lung
capacity) increased with increasing duration of exposure. No lung abnormalities were found by chest radiographs in either group. Blood analyses did not show polycythemia, and in fact, there were slight, but significant, decreases in red blood cell count, hemoglobin and hematocrit in the exposed workers. White blood cell counts were significantly increased. Serum levels of the thyroid hormone T3 (triiodothyronine) were slightly (7%), but significantly, decreased in the exposed group, while T4 (thyroxine) and TSH (thyrotropin) were not affected. Serum markers for cardiomyopathy (i.e., myocardial creatine kinase) were unchanged.

Prescott et al. (1992) conducted a cross-sectional study to investigate the effects of cobalt exposure on thyroid volume in female plate painters. The test group included 61 female plate painters exposed to cobalt blue dyes in two porcelain factories. The control group consisted of 48 unexposed women working at the same factories. The dyes used in the two factories differed; factory I (36 workers) used cobalt aluminate, which is insoluble, and factory II (25 workers) used cobalt-zinc silicate, which was reported to be “semi-soluble.” Workers were exposed to cobalt during the painting procedure when the plates were spray-painted (under a fume hood) two or three times with the water-based cobalt blue underglaze and when the excess color was removed with a brush after drying. Cobalt concentrations were reported to be approximately 0.05 mg/m³ in the workplaces (no further details on air levels were reported). The average duration of exposure was 14.6 years in group I workers and 16.2 years in group II workers. Subjects filled out a questionnaire regarding health, use of medicines, day of menstrual cycle, employment information and smoking habits and agreed to give blood and urine samples for determination of thyroid hormone levels (e.g. thyroxine (T4), triiodothyronine (T3), and thyroid stimulating hormone) and cobalt concentration, respectively, and to undergo ultrasonography to determine volume of the thyroid gland.

Urinary cobalt levels were similar in group I exposed workers and controls (Prescott et al., 1992). Group II workers exposed to semi-soluble cobalt-zinc silicate had urinary cobalt levels that were approximately 10-fold higher than controls. Group I workers did not differ from controls for any of the thyroid parameters measured; however, Group II workers had a significant 22% increase in serum T4 (thyroxine) levels. Mean thyroid volume was lower in this group as well, although the difference from controls (16.1 mL in group II vs. 19.2 mL in controls and 18.7 mL in group I) was not statistically significant. The occurrence of respiratory effects in these workers was not reported.

Results of three studies investigating cancer incidence in workers exposed to cobalt by the inhalation route (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983) are suggestive of a possible association between exposure to cobalt and respiratory tumors. Morgan (1983) investigated the health and causes of death of 49 men occupationally exposed to cobalt salts and oxides (specific species not identified) in a manufacturing plant in South Wales. During the study period, 33 men died (five with lung cancer and three with cancer at other sites). The expected number of deaths was 3.0 for lung cancer and 4.1 for cancers at other sites, based on national statistics, resulting in mortality ratios of 1.7 and 0.73, respectively (statistical analysis of data not reported).

Mur et al. (1987) analyzed the mortality of a cohort of 1143 workers in a plant that refined and processed cobalt and sodium. The plant workers may have been involved in multiple processing applications utilizing different forms of cobalt including cobalt chloride, oxides and
other salts (specific species not identified). An increase in deaths [Standard Mortality Ratio (SMR) = 4.66; 95% confidence interval (CI) = 1.46-10.64] resulting from lung cancer was observed in workers based on four cases observed in the exposed group and one case expected based on French national statistics. In a study within the cohort that controlled for age and smoking habits, 44% (four workers) in the exposed group and 17% (three workers) in the control group died of lung cancer. The authors indicated that the differences were not statistically significant and that the workers were exposed to arsenic and nickel in addition to cobalt. The exposure levels of cobalt were not reported.

Tuchsen et al. (1996) analyzed the cancer incidence of a cohort of 874 women who worked in one of two factories (382 from one factory, 492 from a second factory) applying a cobalt-based (cobalt-aluminate spinel) plate underglaze. From unexposed areas of factory I, 520 referents were selected. Both groups were compared to statistics for all Danish women in the same calendar year. During the 5-year follow-up period, the overall cancer incidence was only slightly elevated in exposed workers, while the incidence of lung cancers was significantly increased [Standard Incidence Ratio (SIR) = 2.35; 95% CI = 1.01-4.6]. The incidence of lung cancers in the referents (not exposed to cobalt) was greater than that of all Danish women, but the difference was not statistically significant. Exposure characterization prior to 1980 was not described, while exposures after 1980 were variable and reported as a mean concentration for a given year. Exposures were generally in the range of 0-1 mg cobalt/m³ except for 2 years, during which they were greater.

Animal Studies

Overview

Studies in animals show that oral exposure to cobalt produces effects similar to those observed in humans, including increases in red blood cells and hemoglobin (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdoch, 1959; Holly, 1955; Stanley et al., 1947), thyroid effects (Shrivastava et al., 1996) and cardiac effects (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970). Other findings in animals not reported in humans include neurobehavioral changes (Singh and Junnarkar, 1991; Bourg et al., 1985; Krasovskii and Fridlyand, 1971) and testicular toxicity (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Developmental toxicity studies in rats and mice provide evidence that high oral doses of cobalt may produce developmental effects in animals, in some cases in the absence of overt maternal toxicity (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985).

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic inhalation exposure to cobalt resulted in cytotoxicity and reparative proliferation in all regions of the respiratory tract in rats and mice (NTP, 1991; Bucher et al., 1990). Available chronic animal studies have demonstrated the carcinogenic potential of inhaled cobalt in male and female rats and mice, with alveolar and bronchiolar tumors being the most prevalent (Bucher et al., 1999; NTP, 1998).
Oral Exposure

Studies in rats show that subchronic oral exposure to cobalt chloride increases red blood cell counts and hemoglobin levels with NOAELs ranging from 0.05 to 0.62 mg cobalt/kg-day (Krasovskii and Fridlyand, 1971; Stanley et al., 1947) and LOAELs ranging from 0.5 to 32 mg cobalt/kg-day (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955; Stanley et al., 1947). In general, effects in animal studies were observed at higher exposure levels than those reported in humans.

Effects of cobalt on red blood cells and hemoglobin were investigated in Sprague-Dawley rats treated with 2.5, 10, and 40 mg cobalt chloride hexahydrate/kg-day (equivalent to 0.62, 2.5, and 9.9 mg cobalt/kg-day, respectively) for 8 weeks (Stanley et al., 1947). After 8 weeks of exposure, increases in hemoglobin and red blood cell number were observed in the 2.5 and 9.9 mg cobalt/kg-day treatment groups. Statistical significance was not reported.

Hemoglobin and hematocrit were significantly increased in male Sprague-Dawley rats exposed to 500 ppm cobalt chloride in drinking water, equivalent to approximately 32 mg cobalt/kg-day (assuming a water intake of 0.139 L/kg-day for male Sprague-Dawley rats; U.S. EPA, 1988), for 3 months (Domingo et al., 1984). Compared to controls, hematocrit and hemoglobin were both increased by approximately 30% at the end of the 3-month exposure period, with increases observed within the first 2 weeks of exposure (numeric data not presented). Following the 3-month exposure period, histopathological examination showed no treatment-related morphological or ultrastructural changes to any organ. Increased tissue weights were observed for spleen, heart and lungs, and testicular weight was decreased compared to controls. Based on the results of this study, 32 mg cobalt/kg-day was identified as a subchronic LOAEL for increased hematocrit and hemoglobin and decreased testicular weight in rats.

In rats exposed to 40 mg cobalt chloride/kg-day (equivalent to 18 mg cobalt/kg-day) for 4 months, hemoglobin and red blood cell count were increased by 37 and 21%, respectively, compared to controls (Holly, 1955). Similar effects were observed following concomitant administration of 40 mg cobalt chloride/kg-day and 200 mg ferrous sulfate, with increases of 30% for hemoglobin and 32% for red blood cell count, compared to controls. Statistical significance was not reported.

Oral exposure of rats to 10 mg cobalt/kg-day (as cobalt chloride) for 5 months resulted in increases in hemoglobin, hematocrit and red blood cell count compared to untreated controls, with effects reaching a plateau after approximately 60 days of exposure (Murdock, 1959). Statistical significance was not reported. No changes were observed for mean corpuscular hemoglobin concentration and mean cell volume compared to untreated controls, indicating that stimulation of erythropoiesis by cobalt did not result in the production of abnormal red blood cells.

The effects of exposure to 0.05, 0.5, and 2.5 mg cobalt/kg-day (as cobalt chloride) for 7 months were examined in rats (Krasovskii and Fridlyand, 1971). Treatment with 0.5 and 2.5 mg cobalt/kg-day, but not 0.05 mg cobalt/kg-day, for 7 months increased red blood cells and hemoglobin. Stimulation of hematopoiesis was more pronounced in the 2.5 mg cobalt/kg-day
group than in the 0.5 mg cobalt/kg-day group, with polycythemia in the 0.5 mg cobalt/kg-day group described as mild and transient. Results of this study are difficult to evaluate since numeric data and statistical analyses were not reported.

Studies in animals have noted cardiac effects following cobalt (cobalt sulfate) exposure (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970), although at higher exposure levels than observed in human studies. The effect of cobalt on myocardial function was examined in rats exposed to 8.4 mg cobalt/kg-day for 16 or 24 weeks (Haga et al. 1996). After 24 weeks of exposure, decreased left ventricular systolic and diastolic function was observed. An increase in the ventricular weight to body weight ratio indicates that left ventricular hypertrophy is a contributory factor in cobalt-induced myocardial dysfunction although a mechanism was not identified. Significant effects on cardiac function were not observed following 16 weeks of exposure. In guinea pigs, exposure to 20 mg cobalt/kg-day as cobalt sulfate in the diet for 5 weeks resulted in decreased absolute and relative heart weights and a greater incidence of abnormal electrocardiograms compared to animals fed on diets not supplemented with cobalt (Mohiuddin et al., 1970). Cardiac arrhythmias, including bradycardia, and repolarization abnormalities, were observed in 65% of cobalt-treated animals compared to 5% of control animals. Cellular alterations, observed at the light and electron microscopic levels, in cardiac tissues included pericardial thickening and inflammation, myocardial degeneration and vacuolization, endocardial thickening and myofibrillar damage. In contrast, no effects on cardiac function were observed in male rats (12/group) exposed to protein-restricted diets containing 8.4 mg cobalt/kg-day for 8 weeks (Pehrsson et al., 1991). Treated rats showed a significant decrease in body weight but no differences in left ventricular function relative to animals treated with protein-restricted diets without added cobalt. Although the results from the Pehrsson et al. (1991) and Haga et al. (1996) rat studies conflict, it appears that oral cobalt-induced myocardial injury/dysfunction may have a significant time-dependence. Oral cobalt (as cobalt sulfate) at the same dose level (8.4 mg cobalt/kg-day) did not appear to alter cardiac structure or function following exposure for up to 16 weeks (Pehrsson et al., 1991; Haga et al., 1996). However, ventricular hypertrophy with a concomitant decrease in left ventricular systolic and diastolic function was observed in rats after 24 weeks of oral cobalt (Haga et al., 1996). Thus, based on the results of this study, 8.4 mg cobalt/kg-day represents a subchronic LOAEL for myocardial toxicity in rats; Based on the results of the Mohiuddin et al. (1970) study, a LOAEL of 20 mg cobalt/kg-day was identified for myocardial toxicity in guinea pigs.

Histopathological changes in the thyroid gland have been observed following exposure of female mice to 400 ppm cobalt chloride (~48 mg cobalt/kg-day, assuming an average water intake of 0.265 L/kg-day for female mice; U.S. EPA, 1988) in drinking water for 15 to 45 days (Shrivastava et al., 1996). The severity of effect increased with exposure duration. After 15 days of exposure, a reduction in thyroid epithelial cell height with degenerated nuclei and reduced amount of colloid with peripheral resorption vacuoles was observed, with more pronounced effects after 30 days of exposure. More significant degenerative changes were observed after 45 days of exposure, including necrotic epithelial cells, reduced connective tissue between follicles, lymphocytic infiltrate and larger amounts of colloid within the lumen. Based upon significant thyroid toxicity observed in this study, a LOAEL of 48 mg cobalt/kg-day was identified in mice.
Developmental effects of orally administered cobalt have been studied in rats, rabbits and mice (Szakmary et al., 2001; Pedigo and Vernon, 1993; Paternain et al., 1988; Seidenberg et al., 1986; Domingo et al., 1985; Elbetieha et al., 2008). Szakmary et al. (2001) evaluated the developmental effects of oral cobalt sulfate exposure in rats, mice and rabbits. Exposure of pregnant rats to 5.2-21.0 mg cobalt/kg-day (oral gavage) decreased perinatal growth and survival, retarded skeletal development and produced skeletal and urogenital malformations, with a LOAEL of 5.2 mg cobalt/kg-day. Maternal toxicity (increased relative liver, adrenal, spleen weights; increased BUN, serum creatinine) was only observed at the highest dose (21.0 mg cobalt/kg-day). Thus, embryotoxicity in rats was observed at exposure levels below the LOAEL for maternal toxicity. In pregnant mice exposed to 10.5 mg cobalt/kg-day, retarded skeletal development and malformations of the eye, kidney and skeleton were observed in the absence of maternal toxicity. In pregnant rabbits exposed to 4.2 mg cobalt/kg-day, 20% mortality was observed in dams. Fetal resorptions were observed in 30% of surviving dams. Results of the studies in rats and mice provide evidence that adverse developmental effects can occur in the absence of maternal toxicity, and that rabbits are more sensitive to oral cobalt.

Domingo et al. (1985) treated pregnant female rats (15 animals/group) with 5.4 to 21.8 mg cobalt/kg-day as cobalt chloride from gestation day 14 through lactation day 21. Offspring were examined for mortality, body weight, body and tail length and general signs of toxicity after 1, 4 and 21 days of nursing. In contrast to the study by Szakmary et al. (2001), results of the Domingo et al. (1985) study reported maternal toxicity at all doses that produced adverse developmental effects (specific maternal effects observed were not reported). Fetal effects at 5.4 mg cobalt/kg-day included stunted growth of the pups of both sexes, decreased body length and tail length in male offspring and decreased spleen and liver weight in female offspring. Effects at the 10.9 mg cobalt/kg-day dose included decreased body weight in female pups, while at 21.8 mg cobalt/kg-day, decreased number of living young and decreased survival were seen. Blood parameters (liver enzymes, bilirubin, total protein, uric acid, urea, creatinine, hemoglobin and hematocrit) in pups did not show any treatment-related changes. No signs of toxicity were observed in surviving pups in any of the cobalt exposure groups.

No significant effects on fetal growth or survival were found in rats exposed to 6.2 to 24.8 mg cobalt/kg-day as cobalt chloride (oral gavage) during gestation days 6-15 (Paternain et al., 1988). The incidence of stunted fetuses was higher in the animals treated with 12.4 or 24.8 mg cobalt/kg-day (0.3 stunted fetuses per litter in the 12.4 mg cobalt/kg-day group; 1.0 stunted fetuses per litter in the 24.8 mg cobalt/kg-day group) compared to the control group (0 stunted fetuses per litter); however, the differences were not statistically significant. No treatment-related effects were observed for the number of corpora lutea, total implants, resorptions, the number of dead and live fetuses or fetal size parameters. No gross external abnormalities, skeletal malformations or other signs of fetal toxicity were observed. Maternal effects, including reduced body weight gain and food consumption and altered hematological parameters (increased hematocrit, hemoglobin and reticulocytes), were reported at all exposure levels. No fetal effects were reported in mice exposed to 81.7 mg cobalt/kg-day (oral gavage) during gestation days 8-12 (Seidenberg et al., 1986), but a significant ($p<0.05$) decrease in maternal weight was found. Additional details were not reported.
Pedigo and Vernon (1993) exposed male B6C3F1 mice to 400 ppm cobalt chloride (~45 mg cobalt/kg-day, assuming a water intake of 0.247 L/kg-day for male B6C3F1 mice; U.S. EPA, 1988) in the drinking water for 10 weeks, after which the males were mated with control females to examine for dominant lethal effects. Relative to the control group, the cobalt treatment group had a lower percentage of pregnant females (control, 29/32; cobalt, 18/31), lower number of implantations per female (control, 8.3; cobalt, 6.5) and higher preimplantation losses (control, 0.43; cobalt, 2.4). At the end of the 10-week treatment period, sperm concentration was decreased to 15.3% and motility decreased to 18.3% of controls. Several measures of sperm velocity were also depressed relative to controls. All sperm parameters, except sperm concentration, returned to control levels 8 weeks after the cobalt exposure was terminated. The increase in preimplantation losses in the dominant lethal assay appears related to adverse effects on spermatogenesis rather than to effects on preimplantation development of embryos.

Several studies reported testicular degeneration and atrophy in rats exposed to 11.7 to 46.9 mg cobalt/kg-day as cobalt chloride for 2-3 months in the diet or in the drinking water (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Pedigo et al. (1988) exposed male CD-1 mice to 100, 200 or 400 ppm of cobalt chloride (~11.7, 23.4 or 46.9 mg cobalt/kg-day, respectively, assuming an average water intake of 0.258 L/kg-day for male mice; U.S. EPA, 1988) in the drinking water for 13 weeks. High-dose animals showed a significantly decreased testicular weight beginning at week 9 of treatment and a decreased epididymal sperm concentration by week 11 of treatment. All dose groups showed significantly decreased testicular weight and epididymal sperm concentration and increased serum testosterone levels by week 12 of exposure, with the magnitude increasing with dose. Effects on serum testosterone levels may be secondary to effects on spermatogenesis and related to inhibition of local inhibitory feed-back mechanisms. Based on the results of this study, 11.7 mg cobalt/kg-day was identified as a subchronic LOAEL for decreased testicular weight and epididymal sperm concentration in male rats.

Anderson et al. (1992, 1993) exposed groups of male CD-1 mice to 400 ppm of cobalt chloride (~46.9 mg cobalt/kg-day, assuming an average water intake of 0.258 L/kg-day for male mice; U.S. EPA, 1988) in the drinking water for up to 13 weeks. A decrease in testicular weight and a progressive degeneration of the seminiferous tubules were seen beginning at 9 weeks of exposure. Initial changes were vacuolization of Sertoli cells and abnormal spermatid nuclei, followed by sloughing of cells, shrinkage of tubules and thickened endothelium. No recovery was reported after a 20-week non-exposure recovery period. Co-administration of 800 ppm of zinc chloride provided a partial protection against the effects of cobalt. Based on the results of this study, 46.9 mg cobalt/kg-day was identified as a subchronic LOAEL for decreased testicular weight and degeneration of seminiferous tubules in male mice. Similar histology (degeneration of the testes, particularly the seminiferous tubules) was noted in Sprague-Dawley rats exposed to 20 mg cobalt/kg-day in the diet for up to 98 days (Corrier et al., 1985; Mollenhauer et al., 1985). Decreased testicular weight was seen in Sprague-Dawley rats exposed to 500 ppm cobalt chloride (~32 mg cobalt/kg-day, assuming a water intake of 0.139 L/kg-day for male Sprague-Dawley rats; U.S. EPA, 1988) for 3 months (Domingo et al., 1984).
Elbetieha et al. (2008) examined the potential effects of cobalt on male fertility in forty adult (60 day-old) male Swiss mice exposed to cobalt chloride hexahydrate via drinking water at concentrations of 200, 400, or 800 ppm for 12 weeks. Based on daily water intake reported in the study, daily average doses of cobalt chloride were estimated at 26, 47, or 93 mg/kg-day (equivalent to 6.5, 11.7, or 23 mg cobalt/kg-day); control animals received untreated tap water. Mice were observed daily for signs of clinical toxicity during the exposure period. At the end of the 12-week cobalt exposure period, male mice were separated into individual cages containing two virgin Swiss female mice and given ad libitum access to food and untreated tap water. Mice were cohabitated for 10 days during which it was estimated that the females completed two estrus cycles. Male control and cobalt-treated mice were necropsied after day 10 of cohabitation and testes, seminal vesicles, epididymides and preputial glands were harvested, weighed, and prepared for analysis. The left testis and epididymis from each male mouse was processed for determination of sperm count, while the right testis was processed for histopathology. Ten days later, female mice were necropsied and examined for number of pregnancies, number of implantation sites, number of viable fetuses, total number of resorptions, and incidence rate of resorptions.

Ingestion of cobalt chloride was associated with 1/10 and 2/10 deaths in the mid- and high-dose treatment groups, respectively, during week 10 of exposure. Average body weight gain was significantly reduced in all cobalt treatment groups \((p < 0.01)\). No other signs of clinical toxicity were observed in surviving male mice. Relative to the control group, the number of pregnant females mated with male mice from the mid- and high-dose groups was significantly \((p < 0.05)\) reduced (control, 19/20; mid-dose, 12/18; high-dose, 7/16). The number of implantation sites was significantly \((p < 0.01)\) reduced in females mated with low- and mid-dose males (control, 7.89; low-dose, 5.67; mid-dose, 5.42), and the number of viable fetuses was significantly \((p < 0.05)\) reduced in females mated with males from all cobalt treatment groups (control, 7.74; low-dose, 5.0; mid-dose, 4.67; high-dose, 5.83). In addition, the total number of resorptions (control, 3/150; low-dose, 9/81; mid-dose, 9/65; high-dose, 10/45) and the number of animals with resorptions (control, 3/19; low-dose, 10/15; mid-dose, 10/16; high-dose, 5/7) were significantly \((p < 0.05)\) increased in females mated with males from all three cobalt-treatment groups. Analysis of male reproductive organs revealed a significant \((p < 0.005)\) decrease in absolute epididymal weight in mice of the high-dose treatment group. Testes weights were significantly \((p < 0.01)\) reduced in males at all doses of cobalt, and a significant \((p < 0.005)\) increase in the absolute weight of seminal vesicles of the mid- and high-dose males only. Compared to controls, testicular sperm counts and daily sperm production were decreased in the mid- and high-dose males, but not in the low-dose animals. Epididymal sperm counts were decreased in male mice from all three cobalt treatment groups. Histopathological examination of testis tissue from males of the mid- and high-dose revealed a number of abnormalities including necrosis of the seminiferous tubules and interstitium, congested blood vessels, hypertrophy of the interstitial Leydig cells, and degeneration of the spermatogonial cells; incidence rate of these observations was not reported. These testicular histopathologies were not observed in the testes of control and low-dose treated males. Based on the results of this study, a LOAEL of 6.5 mg/kg-day was identified for decreased testicular weight, epididymal sperm counts, and associated reproductive abnormalities in pregnant females. A NOAEL was not identified.
Nation et al. (1983) exposed groups (n=6) of male Sprague-Dawley rats (weighing 200-210 g) to diets containing 0, 5 or 20 mg cobalt/kg-day as cobalt chloride for a total of 69 days. Following 14 days of exposure, animals were trained for scheduled (operant) or conditioned suppression neurobehavioral tests. Other than two seizures in the same high-dose animal, no overt signs of neurotoxicity were reported at any exposure level. A trend toward a decreased response rate in the schedule training behavior was observed in both the exposed groups but only attained statistical significance in the high-dose animals near the end of the operant testing period (sessions 28-35, on exposure days 44-51). A trend toward decreased conditioned suppression behavior did not attain statistical significance in either group. Animals exposed to 20 mg cobalt/kg-day, but not 5 mg cobalt/kg-day, showed a significantly decreased weight of the testes following 69 days of exposure. Based on the results of this study, a NOAEL of 5 mg cobalt/kg-day and a LOAEL of 20 mg cobalt/kg-day was identified for decreased testicular weight and changes in operant behavior in male Sprague-Dawley rats.

Several other studies have examined the effects of cobalt on neurobehavioral parameters (Singh and Junnarkar, 1991; Krasovskii and Fridlyand, 1971; Bourg et al., 1985). In groups of male Sprague-Dawley rats (n=8) exposed to 20 mg cobalt/kg-day as cobalt chloride for 57 days in the drinking water, cobalt enhanced behavioral reactivity to stress (the animals were less likely to descend from a safe platform to an electrified grid) (Bourg et al., 1985). Singh and Junnarkar (1991) reported a moderate reduction in spontaneous activity and mild hypothermia in rats exposed orally to cobalt chloride (approximately 8 mg cobalt/kg-day) or cobalt sulfate (approximately 35 mg cobalt/kg-day). Krasovskii and Fridlyand (1971) exposed groups of rats (number and sex not specified) to 0.05, 0.5 or 2.5 mg cobalt/kg-day as cobalt chloride for up to 7 months. Neurobehavioral tests showed that treatment with cobalt resulted in a significant ($p<0.05$) increase in the latent reflex period at 0.5 mg cobalt/kg and above, and a pronounced neurotropic effect (disturbed conditioned reflexes) at 2.5 mg cobalt/kg.

**Inhalation Exposure**

In a subchronic inhalation study, groups of 10 F344/N rats and 10 B6C3F1 mice of each sex were exposed to cobalt sulfate hexahydrate aerosol (MMAD=0.83-1.10 μm; $\sigma_g$ not reported) at concentrations of 0, 0.3, 1, 3, 10 or 30 mg/m$^3$ (equivalent to 0, 0.067, 0.22, 0.67, 2.2 or 6.7 mg cobalt/m$^3$) 6 hours/day, 5 days/week for 13 weeks (Bucher et al., 1990; NTP, 1991). Although this report indicates that exposure was to cobalt sulfate heptahydrate aerosol, detailed analysis of the cobalt aerosol in the 2-year continuation study (Bucher et al., 1999; NTP, 1998) reports that the aerosol was actually composed of cobalt sulfate hexahydrate; thus, exposure to the hexahydrate form is assumed for the 13-week study. Animals were monitored for body weight and observed for clinical signs during the exposure period. Urine samples for urinalysis and cobalt determination were collected from rats prior to sacrifice. Following termination of exposure, all animals were sacrificed and necropsied. Blood samples were collected and analyzed for hematological parameters (rats and mice) and serum chemistry and thyroid function parameters (rats only). The major organs were weighed. Animals from the control and high-dose groups received comprehensive histopathological examinations, while those from the lower dose groups received more limited examinations focused on the respiratory tissues.
All rats survived until scheduled necropsy (NTP, 1991; Bucher et al., 1990). Gross evidence of toxicity was noted only in rats exposed to 6.7 mg cobalt/m$^3$, and they displayed clinical signs of toxicity (ruffled fur, hunched posture) and reduced body weights. Polycythemia, indicated by significant increases in red blood cell count, hemoglobin and hematocrit, was noted in males exposed to $\geq 0.67$ mg cobalt/m$^3$ and females exposed to $\geq 2.2$ mg cobalt/m$^3$. In addition, platelets were significantly reduced in rats of both sexes at $\geq 2.2$ mg cobalt/m$^3$ and reticulocytes were increased in females at 6.7 mg cobalt/m$^3$. Leukocyte counts and differentials were unaffected. Serum cholesterol was significantly reduced in males at $\geq 2.2$ mg cobalt/m$^3$ and females at 6.7 mg cobalt/m$^3$. No other serum chemistry parameters were affected, including creatine kinase isozymes indicative of damage to cardiac muscle cells. Among the thyroid hormones, T3 (triiodothyronine) was significantly reduced in females at 2.2 mg cobalt/m$^3$ (83% of control) and males at 6.7 mg cobalt/m$^3$ (62% of control) and TSH (thyrotropin) was significantly reduced in males at 6.7 mg cobalt/m$^3$ (30% of control), but T4 (thyroxine) was not affected in either sex at any dose and the researchers concluded that thyroid function was not consistently affected in this study. Urinalysis revealed a dose-related increase in the number of epithelial cells and granular casts in the urine of many exposed male rats (3–7 per group exposed to $\geq 0.67$ mg cobalt/m$^3$) but not in the urine of control male rats. The researchers interpreted this finding as indicating minimal nephropathy in exposed male rats although histopathological lesions were not detected in the kidney. No effects on sperm counts, sperm motility or the incidence of abnormal sperm were noted. Average estrus cycle of females exposed to 6.7 mg cobalt/m$^3$ was slightly longer than controls, but the difference was not significant. Absolute and relative lung weights were significantly increased in both male and female rats at $\geq 0.22$ mg cobalt/m$^3$. Other organ weights were not affected by treatment. Compound-related lesions were found only in the respiratory tissues of exposed rats. Degenerative, inflammatory and regenerative lesions were found throughout the respiratory tract (see Table 2). Incidence and severity of lesions were similar in males and females. The most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels.

Among mice, 2/10 males exposed to 6.7 mg cobalt/m$^3$ died during the study (NTP, 1991; Bucher et al., 1990). The only clinical signs of toxicity observed were rapid breathing and skin discoloration in one of the mice that died. Body weights were reduced throughout the study in both males and females exposed to 6.7 mg cobalt/m$^3$. No dose-related hematological effects were found. Absolute and relative lung weights were significantly increased in male and female mice exposed to $\geq 2.2$ mg cobalt/m$^3$. Respiratory lesions were similar to those observed in rats. As with rats, the most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels. Reproductive system effects were more prominent in mice than rats. Males had significantly decreased testicular weight (48% compared to control), decreased epididymal weight (81% compared to control), testicular atrophy consisting of loss of germinal epithelium in the seminiferous tubules and foci of mineralization and an increased percentage of abnormal sperm at 6.7 mg cobalt/m$^3$ (295% compared to control). Significant reductions in sperm motility of 90, 87 and 54% were observed in the 0.67, 2.2 and 6.7 mg cobalt/m$^3$ exposure groups, respectively (lower doses were not tested). Females had a significantly increased length of the estrus cycle at 6.7 mg cobalt/m$^3$ (119% longer compared to control).
Table 2. Rats with Selected Lesions in the 13-Week Cobalt Sulfate Inhalation Study\(^a\)

<table>
<thead>
<tr>
<th>Site</th>
<th>Lesion</th>
<th>Control</th>
<th>0.067 mg Co/m(^3)</th>
<th>0.22 mg Co/m(^3)</th>
<th>0.67 mg Co/m(^3)</th>
<th>2.2 mg Co/m(^3)</th>
<th>6.7 mg Co/m(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M: 0</td>
<td>M: 2</td>
<td>M: 8(^c)</td>
<td>M: 9(^c)</td>
<td>M: 9(^c)</td>
<td>M: 9(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 1</td>
<td>F: 2</td>
<td>F: 7(^c)</td>
<td>F: 10(^c)</td>
<td>F: 10(^c)</td>
<td>F: 10(^c)</td>
</tr>
<tr>
<td>Larynx</td>
<td>Inflammation</td>
<td>M: 9(^c)</td>
<td>M: 10(^c)</td>
<td>M: 10(^c)</td>
<td>M: 10(^c)</td>
<td>M: 10(^c)</td>
<td>M: 10(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 7(^c)</td>
<td>F: 15(^c)</td>
<td>F: 15(^c)</td>
<td>F: 15(^c)</td>
<td>F: 15(^c)</td>
<td>F: 15(^c)</td>
</tr>
<tr>
<td></td>
<td>Squamous metaplasia</td>
<td>M: 0</td>
<td>M: 0</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 1</td>
<td>F: 2</td>
<td>F: 2(^b)</td>
<td>F: 2(^b)</td>
<td>F: 2(^b)</td>
<td>F: 2(^b)</td>
</tr>
<tr>
<td>Lung</td>
<td>Inflammation</td>
<td>M: 0</td>
<td>M: 0</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 0</td>
<td>F: 0</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>M: 0</td>
<td>M: 0</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 0</td>
<td>F: 0</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
</tr>
<tr>
<td></td>
<td>Bronchiolar epithelium</td>
<td>M: 0</td>
<td>M: 0</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
<td>M: 0(^b)</td>
</tr>
<tr>
<td></td>
<td>regeneration</td>
<td>F: 0</td>
<td>F: 0</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
<td>F: 0(^b)</td>
</tr>
</tbody>
</table>

M: number of males with lesions out of 50 animals.
F: number of females with lesions out of 50 animals.
\(^a\)NTP, 1991; Bucher et al., 1990
\(^b\)\(p<0.05\) vs controls by Fisher exact test
\(^c\)\(p<0.01\) vs controls by fisher exact test

Other studies in animals have also reported respiratory lesions and altered respiratory function following inhalation exposure to cobalt. Kyono et al. (1992) observed mild pulmonary lesions in rats exposed to 2.12 mg/m\(^3\) of cobalt aerosols (generated from an aqueous suspension of ultrafine metallic cobalt particles) 5 hours/day for 4 days. Lesions were characterized by focal hypertrophy of the epithelium, abnormal macrophages, vacuolization of type I epithelial cells and proliferation of type II epithelial cells, which are indicative of an initial inflammatory response. Kerfoot et al. (1975) exposed groups of five miniature swine to 0, 0.1 or 1.0 mg/m\(^3\) of pure cobalt metal powder for 6 hours/day, 5 days/week for 3 months. Wheezing was observed in animals from both cobalt groups after 4 weeks of exposure (numeric data not reported). Tidal volume was decreased to 73% and 64% of controls in the low and high dose groups, respectively, and total respiratory compliance was decreased relative to controls (low dose, 66% of control; high dose, 56% of control). Statistical significance was not reported. Examination of lung tissue by electron microscopy revealed septa thickened by collagen, elastic tissue and fibroblasts in both exposure groups, with more pronounced effects in the high dose group. Johansson et al. (1987) exposed rabbits (8/group) to 0.4 or 2 mg cobalt/m\(^3\) as cobalt chloride, 6 hours/day, 5 days/week for 14-16 weeks. Nodular accumulation of alveolar type II cells (8/8 rabbits in both cobalt groups), abnormal accumulation of enlarged, vacuolated alveolar macrophages (5/8 in the low dose group and 8/8 in the high dose group) and interstitial inflammation (4/8 rabbits in the low dose group and 8/8 rabbits in the high dose group) were observed, with more pronounced effects in the high dose group.

The carcinogenicity of inhaled cobalt was investigated in groups of 50 F344/N rats and 50 B6C3F1 mice of each sex exposed to cobalt sulfate hexahydrate aerosol (MMAD=1.4-1.6 \(\mu\)m; \(\sigma_g=2.1-2.2\)) at concentrations of 0, 0.3, 1 or 3 mg/m\(^3\) (equivalent to 0,
0.067, 0.22 or 0.67 mg cobalt/m$^3$) 6 hours/day, 5 days/week for 105 weeks (Bucher et al., 1999; NTP, 1998). Animals were monitored for body weight and observed for clinical signs during the exposure period. Following termination of exposure, all animals were sacrificed and necropsied. At necropsy, all organs and tissues were examined for gross lesions, trimmed and examined histologically.

In F344 rats, there were no changes in survival or mean body weights in males or females of any exposure group (Bucher et al., 1999; NTP, 1998). Irregular breathing was noticed more frequently in female rats exposed to 0.67 mg cobalt/m$^3$ than in controls or other treatment groups; no changes in clinical signs were noted in any of the treated male rats. Incidence of selected neoplasms and nonneoplastic lesions of the lung in rats is summarized in Table 3. Both male and female rats in all exposure groups showed a high incidence (94% or greater) of squamous metaplasia of the alveolar epithelium, fibrosis of the pulmonary interstitium and granulomatous inflammation, with all lesions increasing in severity with increasing exposure level. Significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in high-dose male rats, while significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in the mid- and high-dose female rats. The combined incidence of alveolar/bronchiolar neoplasms (adenoma and carcinoma) in male rats and female rats was significantly greater than that in control animals, and a significant linear trend occurred in both sexes. Rats of both sexes showed treatment-related increases in hyperplasia of the lateral nasal wall, atrophy of the olfactory epithelium and squamous metaplasia of the larynx. A significant increase in the incidence of pheochromocytoma in 0.67 mg cobalt/m$^3$ dosed females was also noted (2/48, 1/49, 4/50 and 10/50 in control, 0.067, 0.22 and 0.67 mg cobalt/m$^3$ groups, respectively). A marginally increased incidence of pheochromocytoma in males exposed to 0.22 mg cobalt/m$^3$, but not in those exposed to 0.67 mg cobalt/m$^3$, was considered by the study authors not to be related to treatment.

In B6C3F1 mice, no changes in survival were observed in any exposure group (Bucher et al., 1999; NTP, 1998). Male mice exposed to 0.67 mg cobalt/m$^3$ showed a decreased mean body weight relative to controls from week 96 through the end of the study (105 weeks). Mean body weights of exposed female mice were generally greater than those of controls throughout the study. Irregular breathing was noted slightly more frequently in female mice exposed to 0.22 mg cobalt/m$^3$ than in controls or other exposed groups. Incidence of selected neoplasms and nonneoplastic lesions of the lung in mice is summarized in Table 4. A dose-related increase in the occurrence of cytoplasmic vacuolization of the bronchus was seen in both sexes of mice, with incidences at all exposure levels being significantly different from controls. As in rats, both sexes of mice showed a significant linear trend toward increased alveolar/bronchiolar tumors, with the 0.67 mg cobalt/m$^3$ male and the 0.22- and 0.67 mg cobalt/m$^3$ female groups attaining statistical significance. Mice of both sexes showed significantly increased incidences of squamous metaplasia of the larynx ($p<0.05$) at all exposure levels examined. In male mice, but not in females, the incidence of hemangiosarcoma was significantly elevated in animals exposed to 0.22 mg cobalt/m$^3$, but not in other exposure groups (2/50, 4/50, 8/50 and 7/50 in the control, 0.067, 0.22 and 0.67 mg cobalt/m$^3$ groups, respectively).
Table 3. Incidence of Selected Neoplasms and Nonneoplastic Lesions in the Respiratory Tract of Rats in the 2-Year Inhalation Study of Cobalt Sulfate

<table>
<thead>
<tr>
<th>Site</th>
<th>Lesion Type</th>
<th>Exposure Group (mg Cobalt (Co) per m³)</th>
<th>Control</th>
<th>0.067 mg Co/m³</th>
<th>0.22 mg Co/m³</th>
<th>0.67 mg Co/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M: 0 F: 2</td>
<td>M: 50 c F: 47 c</td>
<td>M: 48 c F: 50 c</td>
<td>M: 49 c F: 49 c</td>
</tr>
<tr>
<td></td>
<td>Inflammation granulomatous</td>
<td>M: 2 F: 9</td>
<td>M: 50 c F: 47 c</td>
<td>M: 48 c F: 50 c</td>
<td>M: 50 c F: 49 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alveolar/bronchiolar adenoma</td>
<td>M: 1 F: 0</td>
<td>M: 4 F: 1</td>
<td>M: 1 F: 10 c</td>
<td>M: 6 F: 9 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alveolar/bronchiolar carcinoma</td>
<td>M: 0 F: 0</td>
<td>M: 0 F: 2</td>
<td>M: 3 F: 6 b</td>
<td>M: 1 F: 6 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/B adenoma or carcinoma</td>
<td>M: 1 F: 0</td>
<td>M: 4 F: 3</td>
<td>M: 4 F: 15 c</td>
<td>M: 7 b F: 15 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>M: 0 F: 0</td>
<td>M: 0 F: 0</td>
<td>M: 0 F: 1</td>
<td>M: 0 F: 1</td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>Squamous metaplasia</td>
<td>M: 0 F: 1</td>
<td>M: 10 c F: 22 c</td>
<td>M: 37 c F: 39 c</td>
<td>M: 50 c F: 48 c</td>
<td></td>
</tr>
</tbody>
</table>

M: Incidence of lesions in male rats out of 50 animals.
F: Incidence of lesions in female rats out of 50 animals.

a Bucher et al., 1999; NTP, 1998

b p<0.05 compared to control by logistic regression test

c p<0.01 compared to control by logistic regression test
Table 4. Incidence of Selected Neoplasms and Nonneoplastic Lesions in the Respiratory Tract of Mice in the 2-Year Inhalation Study of Cobalt Sulfate

<table>
<thead>
<tr>
<th>Site</th>
<th>Lesion Type</th>
<th>Exposure Group (mg cobalt (Co) per cubic meter)</th>
<th>Control</th>
<th>0.067 mg Co/m³</th>
<th>0.22 mg Co/m³</th>
<th>0.67 mg Co/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/B adenoma or carcinoma</td>
<td></td>
<td></td>
<td>M: 0</td>
<td>F: 0</td>
<td>M: 0</td>
</tr>
<tr>
<td>Nose</td>
<td>Olfactory epithelium atrophy</td>
<td></td>
<td></td>
<td>M: 0</td>
<td>F: 0</td>
<td>M: 0</td>
</tr>
</tbody>
</table>

M: Incidence of lesions in male mice out of 50 animals.  
F: Incidence of lesions in female mice out of 50 animals.  
\(^a\) Bucher et al., 1999; NTP, 1998  
\(^b\) \(p<0.05\) compared to control by logistic regression test  
\(^c\) \(p<0.01\) compared to control by logistic regression test

Wehner et al. (1977, 1979) exposed 2-month-old male Syrian golden hamsters to inhaled cobalt oxide at 0 or 10 mg/m³ (51 animals/group), 7 hours/day, 5 days/week for approximately 15 months. The incidence of tumors in treated hamsters was not statistically different from controls. There was “limited” histopathologic and ultrastructural examination in the study. No developmental toxicity studies were located following inhalation exposure to cobalt.

Other Studies

Parenteral Administration

Heath (1956) injected groups of 10 male and 20 female rats with a single intramuscular 28 mg dose of powdered cobalt in the thigh. Injection-site sarcomas appeared in 18 (60%) of the treated rats within 5-12 months. Similar results were observed in Wistar rats by Gilman (1962) and Gilman and Ruckerbauer (1962), with single intramuscular doses of 20 mg of cobalt oxide and cobalt sulfide. Cobalt oxide and cobalt sulfide given intramuscularly at doses twice those used in rats did not induce sarcomas in mice (Gilman and Ruckerbauer, 1962). Shabaan et al. (1977) observed a high incidence of fibrosarcomas in rats given subcutaneous injections of cobalt chloride at 40 mg/kg-day for 10 days. Tumors developed in 8-12 months. Stoner et al. (1976) tested cobalt acetate in the strain A mouse pulmonary tumor test. Groups of 20 mice/sex...
received three times per week intraperitoneal injections for a total of 19 cumulative doses of 0, 95, 237 or 475 mg/kg. Survival was high over the 30-week observation period, and the incidence of lung tumors in treated mice was not statistically different from controls.

Genotoxicity Studies

The genetic toxicity of cobalt was reviewed by Beyersman and Hartwig (1992) and more recently by De Boeck et al. (2003b), Hartwig and Schwerdtle (2002) and Lison et al. (2001). Cobalt compounds have generally tested negative in bacterial mutagenicity assays, with occasional positive results occurring with the addition of an exogenous metabolic system. In contrast, cobalt compounds have generally tested positive in yeast and plant cells. In mammalian cell systems, cobalt has been shown to induce DNA strand breaks, sister-chromatid exchanges and morphological cell transformation.

Results of in vitro studies using human peripheral blood mononucleated cells show that cobalt metal and cobalt chloride induced DNA strand breaks at non-cytotoxic concentrations (De Boeck et al., 1998, 2003a). Evidence demonstrating mutagenic activity of cobalt in vivo in humans is lacking. No significant change in DNA strand breaks were observed in lymphocytes from nonsmoking workers who had been occupationally exposed to cobalt or hard metal dust although a positive association was observed between DNA strand breaks and smoking (De Boeck et al., 2000).

Experimental data in animals provide evidence of genotoxicity following in vivo exposure to cobalt. Single oral exposure of male Swiss mice to 0, 4.96, 9.92 or 19.8 mg cobalt/kg-day, as cobalt chloride, resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells, with significant linear trends toward increasing aberrations with increased exposure (Palit et al., 1991a,b,c,d). Thirty hours following single intraperitoneal injection of cobalt chloride at doses of 6.19, 12.4, or 22.3 mg cobalt/kg in BALB/c mice, an increase in micronucleus formation was seen in the mid- and high-dose mice but not in low-dose mice (Suzuki et al., 1993). Single injection of 12.4 mg cobalt/kg resulted in significantly increased micronucleus formation at 24 hours post-injection but not at 12, 48, 72 or 96 hours. Pedigo and Vernon (1993) reported that treatment with 400 ppm cobalt chloride (~45 mg cobalt/kg-day, assuming a water intake of 0.247 L/kg-day for male B6C3F1 mice; U.S. EPA, 1988) in the drinking water of male B6C3F1 mice for 10 weeks resulted in an increase in dominant lethal effects as indicated by changes in the number of pregnant females, percentage of live embryos and number of pre-implantation losses per female.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR COBALT

Indicators of human health effects following oral exposure to cobalt (Co) include increased erythrocyte production and hemoglobin levels, decreased iodine uptake by the thyroid gland, elicitation of dermatitis in sensitized individuals and cardiomyopathy. Observations in humans for effects on the heart, blood and the thyroid gland are supported by results of studies in animals. Other effects, including neurobehavioral, developmental and testicular toxicity were
observed in animals and at relatively high doses; these endpoints were not considered further for the development of the subchronic or chronic provisional RfD (p-RfD).

Cardiomyopathy was considered as an endpoint of concern for cobalt exposure in humans; however, it is probable that alcohol consumed in “beer-cobalt cardiomyopathy,” as well as other associated factors such as nutritional deficiency, played a role in the cardiotoxic effects observed. Therefore, a dose-response relationship could not be determined for cobalt exposure from these studies. Studies in animals have noted cardiac effects following cobalt exposure at higher exposure levels than observed in human studies of “beer-cobalt cardiomyopathy.” On this basis, cardiomyopathy was not selected as the critical endpoint for p-RfD derivation.

Allergic response in cobalt-sensitized workers was considered as a potential critical endpoint for the derivation of an oral p-RfD. However, the available data provide no information on the dose-response relationship of cobalt sensitization, nor is a no observable adverse effect level (NOAEL) for the elicitation of an allergic response in humans defined. Interrelationships also exist between cobalt and nickel (Ni) sensitization so that people sensitized by (Ni) may have an allergic reaction following cobalt exposure. Allergic response was, therefore, not chosen as the critical effect for p-RfD derivation.

Cobalt has been shown to induce polycythemia which is characterized by an increase in erythrocyte number and hemoglobin levels through stimulation of erythropoietin, a hormone produced primarily in the kidney. The hematological effects of cobalt treatment have been reported in healthy, non-anemic adults (Davis and Fields, 1958) and in anephric anemic dialysis patients (Taylor et al., 1977; Duckham and Lee, 1976). However, the effects observed in healthy adults were reversible and erythrocyte counts returned to pre-treatment levels within 9 to 15 days after cobalt administration was discontinued. In anephric dialysis patients, treatment with cobalt resulted in an increase in hemoglobin from levels clinically described as “anemic” to levels at or near “normal.” Thus, the effect of cobalt administration in these patients was clinically beneficial. Furthermore, the results of this study are difficult to interpret due to confounding factors, including the anephric status of patients and the concomitant administration of iron. Hematologic effects of cobalt were also found in several studies in rats (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955, Stanley et al., 1947), supporting the plausibility for the effects observed in humans. However, the effects in animals were generally observed at higher doses than that used in the Davis and Fields (1958) human study. It is not known whether cobalt exposure in humans at higher dose levels would increase erythrocytes sufficiently above normal physiological levels to significantly increase the risk of cardiovascular effects. Therefore, polycythemia was not chosen as the critical effect for p-RfD derivation.

Effects of cobalt on thyroidal iodine uptake were identified as an endpoint of concern in humans, based on a preliminary report by Roche and Layrisse (1956). This report showed that oral exposure to cobalt (1 mg cobalt/kg-day) for 2 weeks markedly inhibited radioactive iodine uptake in the human thyroid. In a smaller human clinical study, reduced iodine uptake was reported in 2 of 4 euthyroid patients exposed to 0.54 mg cobalt/kg-day by the oral route for up to 14 days (Paley et al., 1958). A confounding factor in this study is that one of the two subjects reported to have reduced iodine uptake had received intravenous (i.v.) cobalt in addition to oral cobalt intake. The i.v. loading dose regimen may have raised the internal concentration of cobalt
to a level greater than the estimated 0.54 mg cobalt/kg-day based on oral intake alone, rendering the Paley et al. (1958) study inappropriate for consideration. Importantly, long-term cobalt exposure (up to 7 months) at 2-4 mg/kg-day in anemic children has been reported to cause goiter (Gross et al., 1954; Kriss et al., 1955; Little and Sunico, 1958). Therefore, while reduced iodine uptake is reported in humans following short-term exposures at low doses (Roche and Layrisse, 1956; Paley et al., 1958), potentially more severe thyroid lesions may occur as a function of increased duration or dose. Based on observations from rodent models of cobalt exposure, the severity of thyroid toxicity appears to be related to duration of exposure. Indeed, necrosis and inflammation of the thyroid has been reported in mice exposed to approximately 48 mg cobalt/kg-day with an increase in severity over a period of 15-45 days (Shrivastava et al., 1996).

**Subchronic provisional RfD**

Although cobalt exposure induces decreased radioactive iodine uptake in the thyroid (Roche and Layrisse, 1956), and polycythemia (Davis and Fields, 1958) in humans at similar daily exposure levels (1 mg/kg-day and 0.97 mg/kg-day, respectively), thyroid toxicity is chosen as the critical effect for derivation of provisional oral reference values. Cobalt-induced polycythemia and decreased iodine uptake by the thyroid were reversible following relatively short-term exposure in humans, however supporting studies indicate the potential for more severe thyroid effects (e.g., Kriss et al., 1955). The point of departure (POD) of 1 mg cobalt/kg-day for decreased iodine uptake in human thyroid is the LOAEL; dividing this POD by a composite uncertainty (UF) of 300 yields a **subchronic p-RfD of 3E-3 mg/kg-day** as follows:

\[
\text{Subchronic p-RfD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{1 \text{ mg/kg-d}}{300} = 0.003 \text{ or } 3E-3 \text{ mg/kg-day}
\]

The composite UF of 300 is composed of three uncertainty factors: An UF of 10 for LOAEL to NOAEL extrapolation was applied because the POD is based on a LOAEL. An UF of 10 was applied due to the lack of data regarding inter-individual human variability or information on sensitive subpopulations. Specifically, because the critical study (Roche and Layrisse, 1956) for oral cobalt was based on healthy (euthyroid) adults, an UF of 10 was applied to protect sensitive human populations. The available database includes several short-term human studies, multiple developmental studies in animals and animal studies investigating hematological, cardiac, neurological, neurobehavioral, and thyroid endpoints. The lack of a multi-generation reproductive toxicity study is of particular concern because the database includes several animal studies indicating effects on sperm function and testicular degeneration which raises concerns that cobalt exposure may affect reproductive capability. Therefore, an UF of 3 was applied to account for lack of a multi-generation toxicity study.

**Chronic provisional RfD**

Using the same LOAEL of 1 mg/kg-day for decreased iodine uptake in humans, and an additional UF of 10 for extrapolating from subchronic to chronic duration (composite UF of 3000), a **chronic p-RfD of 3E-4 mg/kg-day** is derived as follows:
p-RfD  = LOAEL ÷ UF  
= 1 mg/kg-d ÷ 3000  
= 0.0003 or 3E-4 mg/kg-day

An UF of 10 for extrapolation from subchronic to chronic duration was applied because the critical effect was chosen from a principal study of a relatively short duration (2 weeks) of oral exposure in humans. The temporal relationship between cobalt-induced decreased radioactive iodine uptake and more severe thyroid toxicity should be considered carefully. One postulated temporal relationship is that chronic exposure may have no greater effect than that resulting from short-term exposure, because if the precursor event of inhibition of iodine uptake does not occur, then there may be no change in thyroid function in the short- or long-term. Prolonged cobalt exposure could have less of an effect because of the compensatory response of the pituitary-thyroid axis to iodine deficiency, via increasing iodine uptake. However, although plausible, there are no data to suggest that this postulated temporal relationship exist for cobalt-induced thyroid toxicity. Indeed, a limited number of clinical observations primarily in children exposed to oral cobalt at doses of 2-4 mg/kg-day for up to 7 months suggest the potential for more severe thyroid toxicity (e.g., Kriss et al., 1955). In addition, cobalt may not be readily eliminated from the body; for example, the biological half-life of cobalt chloride in rats is 25 hours (Rosenberg, 1993). Therefore, an UF of 10 for extrapolation from subchronic to chronic duration was applied.

Confidence in the principal study is low-to-medium. Roche and Layrisse (1956) examined twelve subjects over a two-week exposure period. Since only a single dose level was evaluated, a NOAEL for decreased iodine uptake was not identified. Other human and animal studies support the plausibility of cobalt producing thyroid toxicity (Paley et al., 1958; Prescott et al., 1992; Shirivistava et al., 1996). Confidence in the database is low-to-medium. Although some studies (Gross et al., 1954; Kriss et al., 1955; Little and Sunico, 1957) of longer duration reported increased severity of thyroid effects (e.g., goiter) in children exposed to cobalt at higher doses (2-4 mg cobalt/kg-day), critical details of these studies are unavailable for assessment. Therefore, a temporal relationship between prolonged oral cobalt exposure and increased severity of thyroid effects in humans (or experimental animals) is not clear, based upon available data. As such, a low confidence in the provisional subchronic and chronic RfDs results.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR COBALT

The human and animal database indicates that respiratory effects are sensitive endpoints of inhaled cobalt. Symptoms of respiratory tract irritation and altered pulmonary function have been widely reported in workers exposed to cobalt-containing airborne media. Of the four human epidemiology studies discussed above, the study by Nemery et al. (1992) provides the strongest basis for derivation of a provisional RfC (p-RfC). Workers in this study were exposed to lower air concentrations of metallic cobalt dust than in the studies by Gennart and Lauwerys (1990), Prescott et al. (1992) and Swennen et al. (1993). The values obtained from personal air samples from the Nemery et al. (1992) study, indicate a NOAEL of 5.3 μg/m³ and a LOAEL of 15.1 μg/m³. Furthermore, the Nemery et al. (1992) study demonstrated a dose-effect relationship
on lung function which correlated with urinary cobalt-levels, after adjusting for effects of smoking and gender.

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic and chronic inhalation exposure to cobalt resulted in inflammation, fibrosis, and bronchiolar regeneration in all regions of the respiratory tract in both rats and mice (NTP, 1991, 1998; Bucher et al., 1990, 1999) at doses higher than those identified in the Nemery et al. (1992) study. The NTP (1991) study further demonstrated that cobalt can produce testicular effects in male mice following inhalation exposure, but the effects were produced only at high dose levels. Oral studies have also identified the testes as a target for cobalt toxicity. Multi-generation reproduction studies following inhalation or oral exposure to cobalt are not available. Although developmental toxicity studies following inhalation exposure to cobalt are not available, oral studies provide evidence that high oral doses of cobalt may produce developmental effects in animals (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985).

Decreased pulmonary function and respiratory tract irritation were identified as the co-critical effects for derivation of the subchronic and chronic p-RfCs. Assuming the personal air samples to be more representative of worker exposure than the area air samples, the study by Nemery et al. (1992) identified a NOAEL of 5.3 μg/m³ and a LOAEL of 15.1 μg/m³ for metallic cobalt for effects on pulmonary function (e.g. forced expiratory volume (FEV), forced vital capacity (FVC) and forced expiratory flow [referred to as MMEF]) and an increased prevalence of symptoms of respiratory tract irritation (e.g. nose/throat irritation, cough, phlegm, dyspnea). Although the LOAEL may be biased low due to inclusion of data from workshop #9, this does not affect the p-RfC derivation. A NOAEL/LOAEL approach is taken for the derivation of inhalation RfC values because the critical effect data are not amenable to benchmark dose modeling. For example, workers in the low cobalt exposure group experienced a slight but non-statistically significant increase in ventilatory function compared to controls, whereas a significant decrease in ventilatory function was observed in the high cobalt exposure group compared to both the control and the low cobalt exposure groups. The NOAEL for occupational exposure was adjusted to continuous exposure as follows:

\[ 5.3 \, \mu g/m^3 \times (10 \, m^3/day / 20 \, m^3/day) \times (5 \, days / 7 \, days) = 1.9 \, \mu g/m^3 \]

Using the NOAEL$_{ADJ}$ of 1.9 μg/m³ as the POD, the subchronic p-RfC and chronic p-RfC for cobalt was derived as shown below.

Subchronic p-RfC

Dividing the NOAEL$_{ADJ}$ of 1.9 μg/m³ by a composite UF of 100 yields a subchronic p-RfC of 2E-5 mg/m³ for metallic cobalt as follows:

\[
\text{Subchronic p-RfC} = \frac{\text{NOAEL}_{ADJ}}{\text{UF}} = \frac{1.9 \, \mu g/m^3}{100} = 0.00002 \text{ or } 2E-5 \, \text{mg/m}^3
\]
The composite UF of 100 is composed of two uncertainty factors: 10 for database insufficiencies and 10 for inter-individual variability. Nemery et al. (1992) did not report exposure duration for any worker in this study; an assumption is made that worker exposure was at least of subchronic duration. A factor of 10 was applied to account for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. A factor of 10 was applied to account for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This subchronic p-Rfc may not be protective for people with hypersensitivity to cobalt.

**Chronic p-Rfc**

Dividing the NOAEL\textsubscript{ADJ} of 1.9 μg/m\textsuperscript{3} by a composite UF of 300 yields a **chronic p-Rfc of 6E-6 mg/m\textsuperscript{3}** for metallic cobalt as follows:

\[
\text{Chronic p-Rfc} = \frac{\text{NOAEL}_{\text{ADJ}}}{\text{UF}} = \frac{1.9 \ \mu g/m^3}{300} = 0.000006 \text{ or } 6E-6 \text{ mg/m}^3
\]

The composite UF of 300 is composed of three uncertainty factors: 3 to account for extrapolating from an assumed subchronic exposure duration to a chronic exposure duration, 10 for database insufficiencies and 10 for human inter-individual variability. A factor of 3 is applied to account for extrapolating from an assumed subchronic to chronic exposure duration. Since Nemery et al. (1992) did not report duration for any worker in this study, it is possible that exposure duration may have been subchronic or longer for some workers. A factor of 10 is applied to account for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. A factor of 10 is applied to account for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This chronic p-Rfc may not be protective for people with hypersensitivity to cobalt.

Confidence in the key study (Nemery et al., 1992) is low because this cross-sectional study:

- looked at only respiratory endpoints;
- included a control group that was studied more than 1 year after the exposed population;
- included a study group exposed to iron and diamond dust in addition to cobalt (and possibly to asbestos in the past);
- did not report duration of exposure; and
- encountered a number of procedural difficulties during its course (e.g., construction of control group).

Confidence in the database is medium. The choice of the critical endpoint is well supported by other studies in humans and animals. Subchronic exposure studies in rats and mice (NTP, 1991) found histopathological changes in the upper respiratory tract. Other studies in animals support these findings. Reproductive and developmental effects have not been adequately studied. Furthermore, oral studies reported large doses were required to produce reproductive or
developmental effects. It would be difficult to get a large enough internal dose via inhalation to produce these effects. For these reasons, there is medium-to-low confidence in the subchronic and chronic p-RfCs.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR COBALT

Weight-of-Evidence Descriptor

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), cobalt sulfate (soluble) is described as “likely to be carcinogenic to humans by the inhalation route,” based on both the limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals as shown by a statistically significant increased incidence of alveolar/bronchiolar tumors in both sexes of rats and mice, pheochromocytomas in female rats, and hemangiosarcomas in male mice (Bucher et al., 1999). While available studies in humans have suggested a possible association between exposure to cobalt and respiratory tumors in cobalt workers (Tuchsen et al., 1996; Mur et al., 1987; Morgan et al., 1983), limitations within these studies, including small numbers of subjects, inadequate exposure assessment and potential exposure to other chemicals make them inadequate for assessing the carcinogenic potential of cobalt. Studies for evaluation of the oral carcinogenic potential for cobalt were not located.

Mode-of-Action Discussion

The U.S. EPA (2005a) Guidelines for Carcinogen Risk Assessment defines mode of action as “a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation.” Examples of possible modes of carcinogenic action, in general, include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immunologic suppression.

While the mode of action of cobalt-induced carcinogenicity has not been determined, data suggests a number of potential biological events that might be involved including non-mutagenic genotoxicity (e.g. clastogenicity). A recent review by Lison et al. (2001) of in vitro and in vivo experiments in animal models indicates that two different mechanisms of genotoxicity may contribute to the carcinogenic potential of cobalt compounds: DNA strand breakage and inhibition of DNA repair. DNA strand breaks have been reported at non-cytotoxic concentrations in human peripheral blood monocytes (De Boeck et al., 1998, 2003a). Furthermore, oral exposure of mice to cobalt chloride resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells (Palit et al., 1991a,b,c,d). Mechanistic studies suggest cobalt-induced oxidative stress may be involved. Exposure to cobalt compounds increases indices of oxidative stress, including diminished levels of reduced glutathione, increased levels of oxidized glutathione, increased levels of oxygen radicals and increased free-radical-induced DNA damage (Kawanishi et al., 1994; Lewis et al., 1991; Kadiiska et al., 1989; Zhang et al., 1998; Moorehouse et al., 1985). To compound the potential DNA strand breaking effects of cobalt, it appears that cobalt may also inhibit the repair of such genetic damage. A review by Hartwig and Schwerdtle (2002)
concluded that cobalt may specifically target zinc finger structures in DNA repair proteins, interfering with base and nucleotide excision repair. Collectively, while data indicate that cobalt induces DNA damage and repair inhibition, there is weak evidence to suggest direct or indirect mutagenicity in bacterial or mammalian systems.

Potential for a Mutagenic Mode of Action

Key events

The precise mechanism of cobalt-induced carcinogenicity has not been fully determined. There is evidence that cobalt is capable of eliciting genotoxic effects. While evaluations for mutagenic effects in bacteria have generally yielded negative results, results in several mammalian cell systems have suggested that cobalt is genotoxic in mammalian cells. Limited data from in vivo animal studies show that cobalt induces genotoxic effects, including chromosomal breaks, chromosomal aberrations and micronucleus formation. The most likely mechanisms for the genotoxic effects of cobalt are DNA strand breakage and the inhibition of DNA repair.

Strength, consistency, specificity of association

Although the carcinogenic potential of inhaled cobalt has been demonstrated in rats and mice by increased incidence of alveolar/bronchiolar tumors (Bucher et al., 1999; NTP, 1998), direct evidence demonstrating that cobalt can induce mutagenic changes in cells of the respiratory tract is lacking. In vivo exposure to hard metal dust containing 6.3% cobalt, 84% tungsten and 5.4% carbon induced DNA strand breaks in rat type II epithelial lung cells (De Boeck et al., 2003c). Chromosome/genome mutations were observed within 12 hours of exposure to a single intratracheal instillation of 16.6 mg hard metal dust/kg body weight. Since the mutagenic potential of cobalt alone was not evaluated in this study, a causal relationship between type II epithelial cell mutations and cobalt exposure could not be established. Potential mutagenic changes in respiratory tract cells could also be mediated through activated oxygen species released by inflammatory cells (e.g., macrophages, polymorphonuclear neutrophils), rather than directly by cobalt (Lison et al., 2001).

Dose-response concordance

A dose-response concordance has not been established between the development of bronchoalveolar tumors and mutagenesis following inhalation exposure to cobalt. Dose-response information on mutagenicity is available for acute oral and parenteral exposure to cobalt in mice (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). No carcinogenicity data are available for the oral or parenteral routes upon which to base a dose-response concordance. Furthermore, no data are available on the mutagenic potential of cobalt in respiratory tract cells following in vitro or in vivo exposure.
Temporal relationships

*In vivo* studies in animals show that acute oral and parenteral exposure to cobalt produces genotoxicity to bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Due to the lack of data on the mutagenic potential of cobalt in respiratory tract cells, the temporal relationship between potential mutagenic mechanisms and the development of bronchoalveolar tumors cannot be assessed. Development of lung tumors in animals exposed to cobalt occurred following chronic exposure (NTP, 1998).

Biological plausibility and coherence

*In vivo* mutagenicity studies in mice show that oral and intraperitoneal exposure to single doses of cobalt chloride induced mutagenic changes in bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Although it has been hypothesized that the bronchoalveolar tumors are the result of genotoxicity (De Boeck et al., 2003b; Hartwig and Schwerdtle, 2002; Lison et al., 2001), no direct evidence is available linking cobalt-induced mutagenesis to the development of cancer. Carcinogenicity through an indirect mutagenic mode of action may be mediated by activated inflammatory cells (macrophages, polymorphonuclear neutrophils) (Lison et al., 2001).

Other Potential Mode(s) of Action: Cytotoxicity and Cellular Regeneration

Subchronic and chronic inhalation studies (Bucher et al., 1990, 1999; NTP, 1991, 1998) in rodents provide some evidence that cobalt causes cell injury with subsequent reparative cell proliferation, which may be involved in the development of bronchoalveolar tumors. Following inhalation exposure to cobalt sulfate hexahydrate aerosol at concentrations of 0.3 to 30 mg/m$^3$ (equivalent to 0.067 to 6.7 mg cobalt/m$^3$) for 3 months, rats and mice developed several lesions indicative of cell damage and proliferation throughout the entire respiratory tract, including nasal epithelial degeneration and metaplasia, laryngeal inflammation and metaplasia, bronchiolar epithelial regeneration and ectasia, alveolar hyperplasia and lung fibrosis (NTP, 1991; Bucher et al., 1990). Squamous hyperplasia of the larynx was the most sensitive effect (LOAEL=0.067 mg cobalt/m$^3$). The results of the 2-year carcinogenesis study (Bucher et al., 1999; NTP, 1998) in rats and mice revealed a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas in the 0.67 mg cobalt/m$^3$ group, but not in the 0.067 and 0.22 mg cobalt/m$^3$ groups for male rats and mice. In female rats and mice, a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas was observed in the 0.22 and 0.67 mg cobalt/m$^3$ groups, but not in the 0.067 mg cobalt/m$^3$ group. In this same study, granulomatous inflammation of the lung was observed at all exposure levels (0.067, 0.22 and 0.67 mg cobalt/m$^3$) in rats. Other markers of cell damage and proliferation, including hyperplasia, metaplasia and fibrosis, were observed in the 0.22 and 0.67 mg cobalt/m$^3$ exposure groups. Compared to rats, mice appeared to be less sensitive to cobalt-induced cytotoxic changes. Results of this study show that bronchoalveolar tumors develop at exposure levels that also produce cell damage and reparative proliferation, although cell damage and repair are also observed at lower exposure levels than tumorigenesis. These observations suggest the possibility that cell injury in the respiratory tract may have preceded the development of cancers although direct evidence for this assertion is lacking.
Although limited evidence of carcinogenicity in humans is available, results of several epidemiologic studies suggest a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983). Subchronic exposure studies in cobalt workers show an association between cobalt exposure and diminished pulmonary function (Nemery et al., 1992; Gennart and Lauwerys, 1990). Taken together, results of studies in rodents and humans suggest that inhaled cobalt may produce a cytotoxic response in the respiratory tract that may contribute to decreases in pulmonary function and the development of bronchoalveolar tumors.

Sustained cell proliferation, in response to cytotoxicity, can be a significant risk factor for cancer (Correa, 1996). Sustained cytotoxicity and regenerative cell proliferation may result in the perpetuation of mutations (spontaneous or directly or indirectly induced by the chemical), resulting in uncontrolled growth. It is also possible that continuous proliferation may increase the probability that damaged DNA will not be repaired. No data on cobalt are available to directly evaluate the relationship between cell damage and reparative proliferation and the development of bronchoalveolar tumors.

**Conclusions Regarding Cancer Mode of Action**

Limited evidence supports genotoxicity and cytotoxicity followed by cellular regeneration as potential modes of action for cobalt tumorigenicity. *In vitro* and *in vivo* studies provide evidence that cobalt is capable of eliciting genotoxic effects in mammalian cells; however, two key uncertainties remain:

1. No direct evidence linking cobalt-induced mutagenesis to the development of cancer is available and (2) the mutagenic potential of cobalt in respiratory cells has not been evaluated.

Results of the 3-month and 2-year inhalation studies in rats and mice (Bucher et al., 1990, 1999; NTP, 1991, 1998) are also consistent with the hypothesis that cobalt acts through a mode of action involving cytotoxicity and cellular regeneration, based on the observations that these effects occur following subchronic exposure and bronchoalveolar tumors develop at exposure levels that produce cytotoxicity and reparative proliferation. These observations suggest the possibility that cell injury in the respiratory tract may have preceded the development of cancers although direct evidence for this assertion is lacking. No mode of action data are available to explain the statistically significant increases in the incidences of pheochromocytomas and hemangiosarcomas that were observed in female rats and male mice, respectively.

Because a mutagenic mode of action is plausible, but cannot be clearly established for carcinogenicity of inhaled cobalt, it is recommended that an age-dependent adjustment factor not be applied to the unit risk to account for possible age-dependence of carcinogenic potency as described in U.S. EPA (2005b).
Quantitative Estimates of Carcinogenic Risk

Oral Exposure

Human or animal studies examining the carcinogenicity of cobalt following oral exposure were not located. Therefore, derivation of an oral slope factor is precluded.

Inhalation Exposure

As available human inhalation studies were not sufficiently detailed, particularly with regards to analysis of exposure, the NTP (1998; Bucher et al., 1999) 2-year carcinogenicity study in rats and mice was chosen as the principal study for the derivation of an inhalation unit risk, based on the dose-response relationship for statistically significant increased incidences of alveolar/bronchiolar (A/B) neoplasms (adenoma and carcinoma). Although statistically significant increases in the incidences of pheochromocytomas and hemangiosarcomas were observed in female rats and male mice, respectively, these tumors were not considered for the derivation of the inhalation unit risk because a higher and more consistent response across species was observed for alveolar/bronchiolar tumors. The exposure concentrations in this study were adjusted to continuous exposure as follows:

\[
\text{Conc}_{\text{ADA}} = \text{Conc} \times \frac{5 \text{ days/week}}{7 \text{ days/week}} \times \frac{6 \text{ hours/day}}{24 \text{ hours/day}}
\]

This adjustment resulted in duration-adjusted concentrations of 0, 0.012, 0.040 and 0.120 mg cobalt/m$^3$, respectively, for exposure to cobalt sulfate hexahydrate at 0.0, 0.3, 1.0 and 3.0 mg/m$^3$ exposure levels. Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), human equivalent concentrations (HECs, in mg cobalt/m$^3$) were calculated at each exposure level for each species and sex using body weight default values (U.S. EPA, 1994b), assuming exposure to particulates (MMAD=1.5 μm, $\sigma_g$=2.2) with effects occurring in the thoracic region of the respiratory tract. Table 5 shows the resulting HECs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Male Rat</th>
<th>Female Rat</th>
<th>Male Mouse</th>
<th>Female Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDDR Multiplier</td>
<td>0.83</td>
<td>0.79</td>
<td>1.48</td>
<td>1.44</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low</td>
<td>0.010</td>
<td>0.0095</td>
<td>0.018</td>
<td>0.017</td>
</tr>
<tr>
<td>Medium</td>
<td>0.033</td>
<td>0.032</td>
<td>0.059</td>
<td>0.058</td>
</tr>
<tr>
<td>High</td>
<td>0.10</td>
<td>0.095</td>
<td>0.18</td>
<td>0.17</td>
</tr>
</tbody>
</table>
All models for quantal data in the U.S. EPA Benchmark Dose (BMD) software (version 1.3.2) were fit to incidence for tumors (combined A/B adenomas and carcinomas), in rats and mice; males and females were modeled separately. All data sets modeled showed a statistical trend for increased tumor incidence with increasing exposure concentration. In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMD, with the BMDL represented by the 95% lower confidence limit on the BMD. Models were run using the default restrictions on parameters built into the BMD software. Table 6 shows the exposure concentration and incidence data that were modeled.

<table>
<thead>
<tr>
<th>Animal/Strain/Site</th>
<th>Incidence of Neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human Equivalent Concentration of Cobalt (mg/m³)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1/50</td>
</tr>
<tr>
<td>F-344 Rats (male)</td>
<td>Human Equivalent Concentration of Cobalt (mg/m³)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0/50</td>
</tr>
<tr>
<td>F-344 Rats (female)</td>
<td>Human Equivalent Concentration of Cobalt (mg/m³)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11/50</td>
</tr>
<tr>
<td>B6C3F1 Mice (male)</td>
<td>Human Equivalent Concentration of Cobalt (mg/m³)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4/50</td>
</tr>
<tr>
<td>B6C3F1 Mice (female)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7 summarizes the BMD modeling results. BMDLs shown were derived from acceptable model fits ($p>0.5$). As is shown in Table 7, BMDLs were similar across study groups (range: 0.011-0.035 mg/m³). Lung tumors in female rats were chosen as the endpoint for use as a point of departure for derivation of the inhalation unit risk. The BMDL for this endpoint was the lowest for all study groups (i.e., male and female rats and mice) and was based on a model that showed a good fit to the data ($p=0.84$), as reflected in the proximity of the BMDL to the BMD, after dropping the high exposure group. Dropping the high exposure group is recommended according to U.S. EPA (2000) procedure when no models achieve adequate fit using all exposure levels. Although this left only two exposure levels (in addition to the control), these exposure levels are in the low-dose portion of the curve within the region of the dose-response relationship in which response is increasing with exposure level (i.e., the region of interest for deriving the point of departure) and bracket the derived BMD. Appendix A presents the results from all model runs used to support this toxicity assessment.
### Table 7. Summary of BMD Modeling Results for Cobalt Cancer Data

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Species</th>
<th>Sex</th>
<th>BMD (mg/m³)</th>
<th>BMDL (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung: A/B adenoma or carcinoma</td>
<td>rat</td>
<td>male</td>
<td>0.085</td>
<td>0.035</td>
</tr>
<tr>
<td>Lung: A/B adenoma or carcinoma</td>
<td>rat</td>
<td>female</td>
<td>0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.011&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lung: A/B adenoma or carcinoma</td>
<td>mouse</td>
<td>male</td>
<td>0.026</td>
<td>0.015</td>
</tr>
<tr>
<td>Lung: A/B adenoma or carcinoma</td>
<td>mouse</td>
<td>female</td>
<td>0.038</td>
<td>0.023</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on control, low and middle exposure levels; high exposure level was dropped due to failure of models to achieve adequate fit using all exposure levels.

In the absence of mode of action data to inform the low dose extrapolation for cobalt, an inhalation cancer unit risk was calculated by linear extrapolation of the BMDL to zero exposure level (U.S. EPA, 2005a). The provisional inhalation unit risk of 9 (mg/m³)<sup>-1</sup> for cobalt sulfate (soluble) was calculated as follows:

\[
\text{Provisional Unit Risk} = \frac{\text{BMR}}{\text{BMDL}} \\
= \frac{0.1}{0.011} \\
= 9 \text{ (mg/m}^3\text{)}^{-1}
\]

Table 8 shows continuous life-time exposure concentrations that correspond with specified risk levels (i.e., 1x10<sup>-4</sup>, 1x10<sup>-5</sup>, 1x10<sup>-6</sup>).

### Table 8. Continuous Life-time Exposure Concentrations Corresponding to Specified Cancer Risk

<table>
<thead>
<tr>
<th>Exposure Concentration at 1x10&lt;sup&gt;-4&lt;/sup&gt; Risk</th>
<th>1.1x10&lt;sup&gt;-5&lt;/sup&gt; mg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure Concentration at 1x10&lt;sup&gt;-5&lt;/sup&gt; Risk</td>
<td>1.1x10&lt;sup&gt;-6&lt;/sup&gt; mg/m³</td>
</tr>
<tr>
<td>Exposure Concentration at 1x10&lt;sup&gt;-6&lt;/sup&gt; Risk</td>
<td>1.1x10&lt;sup&gt;-7&lt;/sup&gt; mg/m³</td>
</tr>
</tbody>
</table>

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http://www.epa.gov/raf.


www.epa.gov/iris.


http://www.who.int/dsa/cat97/zehc.htm and  
http://www.who.int/dsa/justpub/add.htm.

APPENDIX A: SUMMARY OF BMD MODELING OF TUMOR INCIDENCE DATA IN MALE AND FEMALE RATS AND MICE (NTP, 1998; BUCHER ET AL., 1999)

Male rat – A/B adenoma or carcinoma:

All models show acceptable fit (p > 0.1)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.035 mg/m³

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>AIC</th>
<th>BMD mg/m³</th>
<th>BMDL mg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma (power ≥1)</td>
<td>0.502</td>
<td>111.12</td>
<td>0.087</td>
<td>0.043</td>
</tr>
<tr>
<td>logistic</td>
<td>0.446</td>
<td>111.52</td>
<td>0.099</td>
<td>0.066</td>
</tr>
<tr>
<td>log logistic (slope ≥1)</td>
<td>0.510</td>
<td>111.07</td>
<td>0.085</td>
<td><strong>0.035</strong></td>
</tr>
<tr>
<td>2 degree polynomial (pos betas)</td>
<td>0.502</td>
<td>111.12</td>
<td>0.087</td>
<td>0.043</td>
</tr>
<tr>
<td>1 degree polynomial (pos betas)</td>
<td>0.502</td>
<td>111.12</td>
<td>0.087</td>
<td>0.043</td>
</tr>
<tr>
<td>probit</td>
<td>0.453</td>
<td>111.47</td>
<td>0.098</td>
<td>0.063</td>
</tr>
<tr>
<td>log probit (slope ≥1)</td>
<td>0.357</td>
<td>112.11</td>
<td>0.104</td>
<td>0.064</td>
</tr>
<tr>
<td>quantal linear</td>
<td>0.502</td>
<td>111.12</td>
<td>0.087</td>
<td>0.043</td>
</tr>
<tr>
<td>quantal quadratic</td>
<td>0.373</td>
<td>111.99</td>
<td>0.010</td>
<td>0.069</td>
</tr>
<tr>
<td>weibull (power ≥1)</td>
<td>0.502</td>
<td>111.12</td>
<td>0.087</td>
<td>1.043</td>
</tr>
</tbody>
</table>

Output from BMD v1.3.2 is shown below:

-----------------------------------------------
Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\PROJECTS\COBALT\BMDS\RAMALULOG.(D)
Gnuplot Plotting File: C:\PROJECTS\COBALT\BMDS\RAMALULOG.plt
Fri Sep 09 11:46:38 2005

-----------------------------------------------

BMDS MODEL RUN

-----------------------------------------------

The form of the probability function is

P[response] = background+(1-background)/(1+EXP(-intercept-slope*Log(dose)))

Dependent variable = INRM
Independent variable = ECRM
Slope parameter is restricted as slope ≥ 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.02
intercept = 0.683504
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -slope
have been estimated at a boundary point or have been specified by the user
and do not appear in the correlation matrix )

background   intercept
background   1   -0.63
intercept   -0.63   1

Parameter Estimates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>0.0398603</td>
<td>0.0231667</td>
</tr>
<tr>
<td>intercept</td>
<td>0.272287</td>
<td>0.592931</td>
</tr>
<tr>
<td>slope</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

<table>
<thead>
<tr>
<th>Model</th>
<th>Log(likelihood)</th>
<th>Deviance</th>
<th>Test DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>-52.8567</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitted model</td>
<td>-53.5353</td>
<td>1.35715</td>
<td>2</td>
<td>0.5073</td>
</tr>
<tr>
<td>Reduced model</td>
<td>-55.5862</td>
<td>5.45902</td>
<td>3</td>
<td>0.1411</td>
</tr>
</tbody>
</table>
AIC:  111.071

Goodness of Fit

<table>
<thead>
<tr>
<th>Dose</th>
<th>Est._Prob.</th>
<th>Expected</th>
<th>Observed</th>
<th>Size</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>0.0399</td>
<td>1.993</td>
<td>1</td>
<td>50</td>
<td>-0.7178</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.0523</td>
<td>2.615</td>
<td>4</td>
<td>50</td>
<td>0.8797</td>
</tr>
<tr>
<td>0.0330</td>
<td>0.0797</td>
<td>3.827</td>
<td>4</td>
<td>48</td>
<td>0.09207</td>
</tr>
<tr>
<td>0.1000</td>
<td>0.1513</td>
<td>7.565</td>
<td>7</td>
<td>50</td>
<td>-0.2228</td>
</tr>
</tbody>
</table>

Chi-square = 1.35     DF = 2        p-value = 0.5099

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.0846262
BMDL = 0.0394914
Female rat – A/B adenoma or carcinoma:

Most models showed poor fit \((p < 0.05)\) with highest exposure level included (no increase in incidence at the highest exposure level. The log-logistic model showed the best fit \((p=0.11, \text{ lowest AIC})\)

<table>
<thead>
<tr>
<th>Model</th>
<th>(p)</th>
<th>AIC</th>
<th>BMD mg/m(^3)</th>
<th>BMDL mg/m(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma (power (\geq 1))</td>
<td>0.025</td>
<td>155.21</td>
<td>0.018</td>
<td>0.043</td>
</tr>
<tr>
<td>logistic</td>
<td>0.000</td>
<td>167.02</td>
<td>0.045</td>
<td>0.036</td>
</tr>
<tr>
<td>log logistic (slope (\geq 1))</td>
<td>0.090</td>
<td>152.86</td>
<td>0.015</td>
<td>0.011</td>
</tr>
<tr>
<td>2 degree polynomial (pos betas)</td>
<td>0.025</td>
<td>155.21</td>
<td>0.018</td>
<td>0.014</td>
</tr>
<tr>
<td>1 degree polynomial (pos betas)</td>
<td>0.025</td>
<td>155.21</td>
<td>0.018</td>
<td>0.014</td>
</tr>
<tr>
<td>probit</td>
<td>0.000</td>
<td>166.25</td>
<td>0.042</td>
<td>0.033</td>
</tr>
<tr>
<td>log probit (slope (\geq 1))</td>
<td>0.000</td>
<td>166.51</td>
<td>0.032</td>
<td>0.023</td>
</tr>
<tr>
<td>quantal linear</td>
<td>0.025</td>
<td>155.21</td>
<td>0.018</td>
<td>0.014</td>
</tr>
<tr>
<td>quantal quadratic</td>
<td>0.000</td>
<td>170.16</td>
<td>0.052</td>
<td>0.040</td>
</tr>
<tr>
<td>weibull (power (\geq 1))</td>
<td>0.025</td>
<td>155.21</td>
<td>0.018</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Omitting the data from the highest exposure level improved fit of all models \((p > 0.1)\)

Log-probit model yielded best fit (highest \(p\)-value and lowest AIC)

Best estimate of BMDL=0.011 mg/m\(^3\)

<table>
<thead>
<tr>
<th>Model</th>
<th>(p)</th>
<th>AIC</th>
<th>BMD mg/m(^3)</th>
<th>BMDL mg/m(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma (power (\geq 1))</td>
<td>1.000</td>
<td>87.66</td>
<td>0.013</td>
<td>0.0077</td>
</tr>
<tr>
<td>logistic</td>
<td>0.242</td>
<td>89.69</td>
<td>0.020</td>
<td>0.0164</td>
</tr>
<tr>
<td>log logistic (slope (\geq 1))</td>
<td>1.000</td>
<td>87.66</td>
<td>0.013</td>
<td>0.0071</td>
</tr>
<tr>
<td>2 degree polynomial (pos betas)</td>
<td>0.710</td>
<td>87.66</td>
<td>0.014</td>
<td>0.0077</td>
</tr>
<tr>
<td>1 degree polynomial (pos betas)</td>
<td>1.000</td>
<td>86.40</td>
<td>0.011</td>
<td>0.0073</td>
</tr>
<tr>
<td>probit</td>
<td>0.289</td>
<td>89.31</td>
<td>0.019</td>
<td>0.0152</td>
</tr>
<tr>
<td>log probit (slope (\geq 1))</td>
<td>0.843</td>
<td>85.98</td>
<td>0.014</td>
<td>0.0110</td>
</tr>
<tr>
<td>quantal linear</td>
<td>0.710</td>
<td>86.40</td>
<td>0.011</td>
<td>0.0073</td>
</tr>
<tr>
<td>quantal quadratic</td>
<td>0.535</td>
<td>86.70</td>
<td>0.017</td>
<td>0.0139</td>
</tr>
<tr>
<td>weibull (power (\geq 1))</td>
<td>1.000</td>
<td>87.66</td>
<td>0.014</td>
<td>0.0077</td>
</tr>
</tbody>
</table>

Output from BMD v1.3.2 (all data included) is shown below:

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.(D)
Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.plt
BMDS MODEL RUN

The form of the probability function is

\[ P[\text{response}] = \text{background} + \frac{(1-\text{background})}{[1+\exp(-\text{intercept}-\text{slope}\times \log(\text{dose}))]} \]

Dependent variable = INRF
Independent variable = ECRF
Slope parameter is restricted as slope \( \geq 1 \)

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 1.93572
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )

intercept

intercept 1

Parameter Estimates
Variable          Estimate           Std. Err.
background          0                NA
intercept           1.98253         0.20995
slope               1                NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

<table>
<thead>
<tr>
<th>Model</th>
<th>Log(likelihood)</th>
<th>Deviance</th>
<th>Test DF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>-72.3723</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitted model</td>
<td>-75.4299</td>
<td>6.1152</td>
<td>3</td>
<td>0.1061</td>
</tr>
<tr>
<td>Reduced model</td>
<td>-89.3929</td>
<td>34.0413</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

AIC: 152.86

Goodness of Fit

<table>
<thead>
<tr>
<th>Dose</th>
<th>Est. Prob.</th>
<th>Expected</th>
<th>Observed</th>
<th>Size</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>0.0000</td>
<td>0.000</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>0.0095</td>
<td>0.0645</td>
<td>3.162</td>
<td>3</td>
<td>49</td>
<td>-0.09415</td>
</tr>
<tr>
<td>0.0320</td>
<td>0.1885</td>
<td>9.427</td>
<td>15</td>
<td>50</td>
<td>2.015</td>
</tr>
<tr>
<td>0.0950</td>
<td>0.4082</td>
<td>20.411</td>
<td>15</td>
<td>50</td>
<td>-1.557</td>
</tr>
</tbody>
</table>

Chi-square = 6.49    DF = 3    p-value = 0.0900

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.0153022
BMDL = 0.0109172
Output from BMD v1.3.2 (highest exposure level excluded) is shown below:

The form of the probability function is:

\[ P[\text{response}] = \text{Background} + (1-\text{Background}) \times \text{CumNorm(Intercept+Slope*Log(Dose))}, \]

where \text{CumNorm(.)} is the cumulative normal distribution function

Dependent variable = INRF

53
Independent variable = ECRF
Slope parameter is restricted as slope ≥ 1

Total number of observations = 4
Total number of records with missing values = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
   background = 0
   intercept = 3.0285
   slope = 1

Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s) -background   -slope
   have been estimated at a boundary point, or have been specified by the user,
   and do not appear in the correlation matrix )

   intercept
   intercept  1

Parameter Estimates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>intercept</td>
<td>2.97347</td>
<td>0.157916</td>
</tr>
<tr>
<td>slope</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table
### Model Comparison

<table>
<thead>
<tr>
<th>Model</th>
<th>Log(likelihood)</th>
<th>Deviance</th>
<th>Test DF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>-41.8291</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitted model</td>
<td>-41.9887</td>
<td>0.319256</td>
<td>2</td>
<td>0.8525</td>
</tr>
<tr>
<td>Reduced model</td>
<td>-54.9105</td>
<td>26.1628</td>
<td>2</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

AIC: 85.9774

### Goodness of Fit

<table>
<thead>
<tr>
<th>Dose</th>
<th>Est._Prob.</th>
<th>Expected</th>
<th>Observed</th>
<th>Size</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>0.0000</td>
<td>0.000</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>0.0095</td>
<td>0.0462</td>
<td>2.263</td>
<td>3</td>
<td>49</td>
<td>0.5015</td>
</tr>
<tr>
<td>0.0320</td>
<td>0.3197</td>
<td>15.985</td>
<td>15</td>
<td>50</td>
<td>-0.2987</td>
</tr>
</tbody>
</table>

Chi-square = 0.34  DF = 2  p-value = 0.8434

### Benchmark Dose Computation

- Specified effect = 0.1
- Risk Type = Extra risk
- Confidence level = 0.95
- BMD = 0.0141927
- BMDL = 0.0109984
Probit Model with 0.95 Confidence Level

Fraction Affected vs. Dose

BMDL BMD

09:26 09/10 2005
Male mouse – A/B adenoma or carcinoma:

All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest $p$-value and lowest AIC)
Best estimate of BMDL = 0.015 mg/m$^3$

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>AIC</th>
<th>BMD mg/m$^3$</th>
<th>BMDL mg/m$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma (power $\geq 1$)</td>
<td>0.944</td>
<td>251.10</td>
<td>0.033</td>
<td>0.0215</td>
</tr>
<tr>
<td>logistic</td>
<td>0.759</td>
<td>251.54</td>
<td>0.048</td>
<td>0.0359</td>
</tr>
<tr>
<td>log logistic (slope $\geq 1$)</td>
<td>0.999</td>
<td>250.99</td>
<td>0.026</td>
<td>$\textbf{0.0150}$</td>
</tr>
<tr>
<td>2 degree polynomial (pos betas)</td>
<td>0.944</td>
<td>251.10</td>
<td>0.033</td>
<td>0.0215</td>
</tr>
<tr>
<td>1 degree polynomial (pos betas)</td>
<td>0.944</td>
<td>251.10</td>
<td>0.033</td>
<td>0.0215</td>
</tr>
<tr>
<td>probit</td>
<td>0.775</td>
<td>251.50</td>
<td>0.046</td>
<td>0.0349</td>
</tr>
<tr>
<td>log probit (slope $\geq 1$)</td>
<td>0.594</td>
<td>252.03</td>
<td>0.059</td>
<td>0.0397</td>
</tr>
<tr>
<td>quantal linear</td>
<td>0.944</td>
<td>251.10</td>
<td>0.033</td>
<td>0.0215</td>
</tr>
<tr>
<td>quantal quadratic</td>
<td>0.412</td>
<td>252.76</td>
<td>0.080</td>
<td>0.0633</td>
</tr>
<tr>
<td>weibull (power $\geq 1$)</td>
<td>0.944</td>
<td>251.10</td>
<td>0.033</td>
<td>0.0215</td>
</tr>
</tbody>
</table>

Output from BMD v1.3.2 is shown below:

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\ROJECTS\COBALT\BMDS\MOMALU\MOMALULOLOGLOG.(D)
Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOMALU\MOMALULOLOGLOYLOG.plt
Fri Sep 09 16:57:38 2005

BMDS MODEL RUN

The form of the probability function is

\[
P[\text{response}] = \frac{\text{background}+(1-\text{background})}{1+\exp(-\text{intercept}-\text{slope}\times \log(\text{dose}))}
\]

Dependent variable = INMM
Independent variable = ECMM
Slope parameter is restricted as slope $\geq 1$

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008
User has chosen the log transformed model

Default Initial Parameter Values
background = 0.22
intercept = 1.47367
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -slope
    have been estimated at a boundary point, or have been specified by the user,
    and do not appear in the correlation matrix )

<table>
<thead>
<tr>
<th></th>
<th>background</th>
<th>intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>1</td>
<td>-0.62</td>
</tr>
<tr>
<td>intercept</td>
<td>-0.62</td>
<td>1</td>
</tr>
</tbody>
</table>

Parameter Estimates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>0.22179</td>
<td>0.0478621</td>
</tr>
<tr>
<td>intercept</td>
<td>1.45848</td>
<td>0.375385</td>
</tr>
<tr>
<td>slope</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

<table>
<thead>
<tr>
<th>Model</th>
<th>Log(likelihood)</th>
<th>Deviance</th>
<th>Test DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>-123.493</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitted model</td>
<td>-123.494</td>
<td>0.00271986</td>
<td>2</td>
<td>0.9986</td>
</tr>
<tr>
<td>Reduced model</td>
<td>-130.684</td>
<td>14.3818</td>
<td>3</td>
<td>0.002429</td>
</tr>
</tbody>
</table>
**AIC:** 250.988

### Goodness of Fit

<table>
<thead>
<tr>
<th>Dose</th>
<th>Est. Prob.</th>
<th>Expected</th>
<th>Observed</th>
<th>Size</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>0.2218</td>
<td>11.090</td>
<td>11</td>
<td>50</td>
<td>-0.03047</td>
</tr>
<tr>
<td>0.0180</td>
<td>0.2777</td>
<td>13.884</td>
<td>14</td>
<td>50</td>
<td>0.03649</td>
</tr>
<tr>
<td>0.0590</td>
<td>0.3793</td>
<td>18.963</td>
<td>19</td>
<td>50</td>
<td>0.0109</td>
</tr>
<tr>
<td>0.1800</td>
<td>0.5613</td>
<td>28.065</td>
<td>28</td>
<td>50</td>
<td>-0.0185</td>
</tr>
</tbody>
</table>

Chi-square = 0.00  DF = 2  p-value = 0.9986

### Benchmark Dose Computation

- **Specified effect** = 0.1
- **Risk Type** = Extra risk
- **Confidence level** = 0.95
- **BMD** = 0.0258434
- **BMDL** = 0.0149697
Log-Logistic Model with 0.95 Confidence Level

Fraction Affected vs. Dose

BMDL and BMD values are indicated on the graph.
Male mouse – A/B adenoma or carcinoma:

All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.023 mg/m$^3$

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>AIC</th>
<th>BMD</th>
<th>BMDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma (power ≥1)</td>
<td>0.571</td>
<td>196.12</td>
<td>0.0455</td>
<td>0.0296</td>
</tr>
<tr>
<td>logistic</td>
<td>0.273</td>
<td>197.61</td>
<td>0.0735</td>
<td>0.0562</td>
</tr>
<tr>
<td>log logistic (slope ≥1)</td>
<td>0.700</td>
<td>195.72</td>
<td>0.0384</td>
<td>0.0231</td>
</tr>
<tr>
<td>2 degree polynomial (pos betas)</td>
<td>0.571</td>
<td>196.12</td>
<td>0.0455</td>
<td>0.0296</td>
</tr>
<tr>
<td>1 degree polynomial (pos betas)</td>
<td>0.571</td>
<td>196.12</td>
<td>0.0455</td>
<td>0.0296</td>
</tr>
<tr>
<td>probit</td>
<td>0.300</td>
<td>197.42</td>
<td>0.0697</td>
<td>0.0528</td>
</tr>
<tr>
<td>log probit (slope ≥1)</td>
<td>0.167</td>
<td>198.57</td>
<td>0.0768</td>
<td>0.0524</td>
</tr>
<tr>
<td>quantal linear</td>
<td>0.571</td>
<td>196.12</td>
<td>0.0455</td>
<td>0.0296</td>
</tr>
<tr>
<td>quantal quadratic</td>
<td>0.117</td>
<td>199.26</td>
<td>0.0959</td>
<td>0.0739</td>
</tr>
<tr>
<td>weibull (power ≥1)</td>
<td>0.571</td>
<td>196.12</td>
<td>0.0455</td>
<td>0.0296</td>
</tr>
</tbody>
</table>

Output from BMD v1.3.2 is shown below:

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.(D)
Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.plt
Fri Sep 09 17:05:08 2005

BMDS MODEL RUN

The form of the probability function is

$$P[\text{response}] = \text{background} + (1 - \text{background})/[1 + \exp(-\text{intercept-slope}*\text{Log(dose)})]$$

Dependent variable = INMF
Independent variable = ECMF
Slope parameter is restricted as slope ≥ 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008
User has chosen the log transformed model

Default Initial Parameter Values
background = 0.08
intercept = 1.17812
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix )

background    intercept
background            1         -0.6
intercept         -0.6            1

Parameter Estimates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>0.0920048</td>
<td>0.035283</td>
</tr>
<tr>
<td>intercept</td>
<td>1.06119</td>
<td>0.354864</td>
</tr>
<tr>
<td>slope</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

<table>
<thead>
<tr>
<th>Model</th>
<th>Log(likelihood)</th>
<th>Deviance</th>
<th>Test DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>-95.5104</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitted model</td>
<td>-95.8619</td>
<td>0.702985</td>
<td>2</td>
<td>0.7036</td>
</tr>
<tr>
<td>Reduced model</td>
<td>-102.791</td>
<td>14.5619</td>
<td>3</td>
<td>0.002232</td>
</tr>
</tbody>
</table>
AIC: 195.724

Goodness of Fit

<table>
<thead>
<tr>
<th>Dose</th>
<th>Est. Prob</th>
<th>Expected</th>
<th>Observed</th>
<th>Size</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>0.0920</td>
<td>4.600</td>
<td>4</td>
<td>50</td>
<td>-0.2937</td>
</tr>
<tr>
<td>0.0170</td>
<td>0.1345</td>
<td>6.726</td>
<td>7</td>
<td>50</td>
<td>0.1135</td>
</tr>
<tr>
<td>0.0580</td>
<td>0.2223</td>
<td>11.117</td>
<td>13</td>
<td>50</td>
<td>0.6403</td>
</tr>
<tr>
<td>0.1700</td>
<td>0.3911</td>
<td>19.556</td>
<td>18</td>
<td>50</td>
<td>-0.451</td>
</tr>
</tbody>
</table>

Chi-square = 0.71  DF = 2  p-value = 0.7003

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.0384492
BMDL = 0.0231
Log-Logistic Model with 0.95 Confidence Level

Fraction Affected vs. dose

BMDL, BMD

09:36 09/10 2005