CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- Submicron-size particles of a substance such as cobalt can be almost completely absorbed through the respiratory tract, whereas larger particles may be moved after deposition in the respiratory tract by mucociliary clearance and swallowed. Inhaled cobalt absorption ranges from 52 to 78%. The fraction of ingested cobalt that is absorbed from the gastrointestinal tract depends on an individual's nutritional status, the cobalt dose, and the type of cobalt. The ingested cobalt absorption rates range from 5 to 97%. Absorption rates vary widely among humans. Cobalt may also be absorbed through the skin; absorption through intact skin was <1%, while absorption through abraded skin was almost 80%.

- Cobalt is primarily distributed to the serum, whole blood, liver, kidneys, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, and pancreas.

- Cobalt is not subject to metabolism by enzymatic pathway but tends to get distributed between organ systems and excreted via urine and feces.

- Cobalt is excreted primarily in urine and feces regardless of the route of exposure. The elimination of cobalt is often represented as a multi-compartmental model with compartments having half-lives of several hours to a week. Values for cobalt have been calculated based on urinary excretion of either stable cobalt or its radioactive isotopes, ^{57}Co and ^{60}Co.

3.1.1 Absorption

In general, regional deposition of cobalt in the lungs depends on both biological and physical characteristics such as particulate size, breathing patterns, and airstream velocity. Deposition of particulates >2.5 µm occurs in the upper portion of the airway, whereas particulates <2.5 µm are deposited in the lower portion of the respiratory tract (James et al. 1994). Absorption of deposited cobalt is dependent on its solubility and location within the lung. Physiologically insoluble cobalt particles are cleared by phagocytosis and/or mucociliary transport and have a low systemic absorption (Bailey and Roy 1994; Kreyling 1990). More soluble forms of cobalt are absorbed into the bloodstream through the alveolar or bronchial walls. Particles located in the alveolar region undergo phagocytosis or dissolution and are subsequently absorbed (Kreyling 1990). Particle dissolution rates in lung fluids, n secretions, or in macrophages, as well as cobalt's biochemical reactions and binding to tissue components, affect the rate of absorption (Bailey and Roy 1994; Kreyling 1990).

There are limited data available for either humans or animals regarding cobalt absorption following inhalation exposure. In a small study of four individuals utilizing radiolabeled (^{57}Co) cobalt oxide with

geometric mean diameters of 0.8 and 1.7 µm, the average fractional deposition of the smaller particles was 52% and the average fractional deposition of the 1.7 µm particles was 78% (Foster et al. 1989). Urinary cobalt levels measured in workers can be an indicator of cobalt lung absorption. Lison et al. (1994) found that in workers exposed via inhalation to more soluble forms of cobalt, there was an increase in cobalt in the urine at the end of their shift, possibly indicating rapid absorption from the lungs. However, urine measurements following exposure to cobalt oxides, a less-soluble form, were lower than the amount in urine for the more soluble forms, which may be an indication of a lower absorption rate from the lungs (Lison et al. 1994). Similarly, Christensen and Poulsen (1994) found higher levels of cobalt in the blood and urine of pottery plate painters when the painters used a soluble pigment compared to levels when they used a less-soluble pigment.

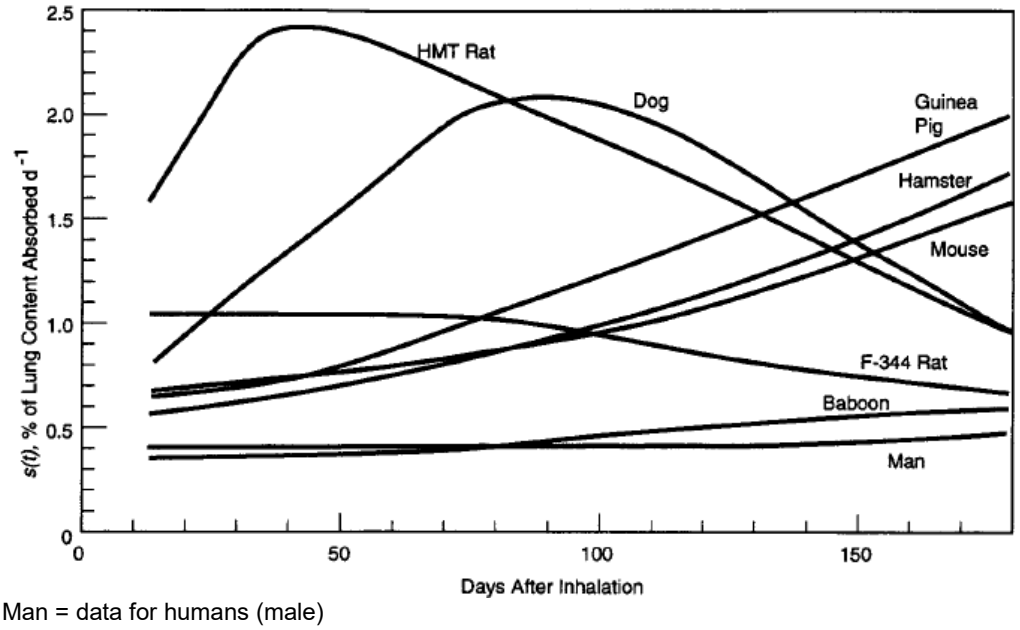
NTP (2014) exposed female rats and mice for 2 weeks, 3 months, or 2 years to cobalt metal particulate aerosol via inhalation. Median diameters of the cobalt particles were measured at regular intervals during the study and were maintained at 1.86–1.92 µm for the 2-week inhalation study, 1.4–2.0 µm for the 3-month study, and 1.5–2.0 µm for the 2-year inhalation study. Lung deposition rates were calculated. For both rats and mice, lung deposition rates generally increased with exposure. The 2-week study used doses of 2.5, 5, 10, and 20 mg/m^3. The corresponding lung deposition rates were 1.46, 2.48, 3.12, and 8.91 µg Co/day for rats and 0.57, 1.25, 1.87, and 2.34 µg Co/day for mice. For the 3-month and 2-year studies, lung deposition rates were calculated for the following doses: 1.25, 2.5, and 5 mg/m^3. The corresponding deposition rates for rats were 1.45, 2.13, and 5.6 µg Co/day for the compartment with higher absorption rates and 0.018, 0.08, and 0.29 µg Co/day for the compartment with lower absorption rates. The corresponding deposition rates for mice were 0.87, 1.84, and 1.18 µg Co/day for the fast compartment and 0.027, 0.075, and 0.22 µg Co/day for the slow compartment (NTP 2014).

In Syrian golden hamsters exposed to cobalt oxide, absorption was reported to be approximately 27% of inhaled cobalt oxide (particle size, 1–2.5 µm), with 60% recovered in the gastrointestinal tract, which could reflect mucociliary transport and swallowing of particles (Wehner and Craig 1972). Collier et al. (1991) calculated translocation rates from lungs to blood in rats exposed to ^{57}Co-labelled cobalt tetraoxide over the duration of the study and reported that translocation rates increased with time from 0.5–1% per day initially to 1.5–4.0% 150 days postexposure, with the youngest rats exhibiting the highest rates.

Absorption rates from the lungs into the blood were measured in two interspecies studies using ^{57}Co-labelled cobalt tetraoxide. Bailey et al. (1989) measured the rate of absorption of ^{57}Co-labelled cobalt tetraoxide at two different particle sizes (0.8 and 1.7 µm) in humans, baboons, dogs, hamsters,

guinea pigs, mice, and three strains of rats (Sprague-Dawley, F344, and HMT). Mice were only exposed to the 0.8 µm size particles. The fraction of cobalt translocated for the 0.8 µm particles was twice that of the 1.7 µm particles for all species except mice (Bailey et al. 1989). Dogs, baboons, and HMT rats showed the greatest differences in translocation rates. To further investigate the differences in translocation, a second study was conducted in which the three species were exposed via inhalation to a form of ^{57}Co-labelled cobalt tetraoxide that was denser and had a smaller specific surface area than the particles in the first study. The particle size used was 0.9 µm (Kreyling et al. 1991). Initial translocation rates ranged from 0.001%/day in baboons to 0.007%/day in rats. Kreyling et al. (1991) reported that the rate-determining process for translocation to blood is the intracellular particle dissolution in the macrophage, as transfer of the dissociated material to blood is fast. The translocation rates varied widely across species ranging from 0.004 to 0.0015%/day for the smaller particles and 0.002 to 0.006%/day for the larger particles (Bailey and Roy 1994; Bailey et al. 1989). Results are summarized in Figures 3-1, 3-2, and 3-3.

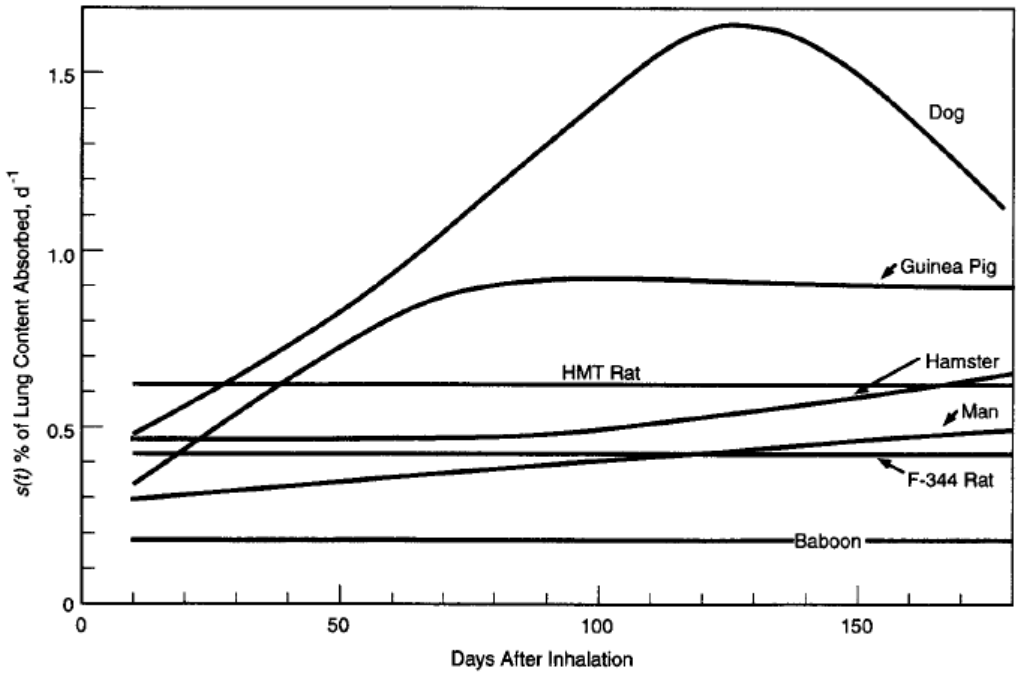
Figure 3-1. Modeling Indicates Particle Size is Crucial for Absorption of Cobalt from Lungs to Blood after Inhalation Exposure: 0.8 µm Porous Cobalt Tetraoxide



Man = data for humans (male)

Source: Bailey and Roy (1994)

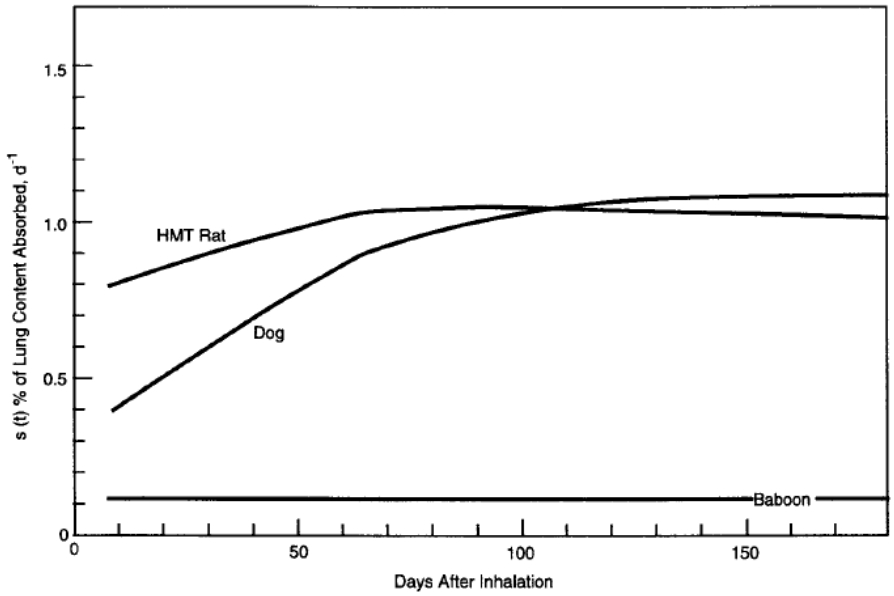
Figure 3-2. Modeling Indicates Particle Size is Crucial for Absorption of Cobalt from Lungs to Blood after Inhalation Exposure: 1.7 µm Porous Cobalt Tetraoxide



Man = data for humans (male)

Source: Bailey and Roy (1994)

Figure 3-3. Modeling Indicates Particle Size is Crucial for Absorption of Cobalt from Lungs to Blood after Inhalation Exposure: 0.9 µm Solid Cobalt Tetraoxide



Source: Bailey and Roy (1994)

Absorption following oral exposure to cobalt in humans varies and is dependent on individual nutritional status, cobalt dose, and type of cobalt. Studies in humans have reported a large interindividual variability for absorption rates. The reported absorption rates range from 5 to 97% (Harp and Scoular 1952; Smith et al. 1972; Sorbie et al. 1971; Valberg et al. 1969). More recent estimates of absorption indicate that the absorption rates range from 10 to 25% for soluble forms of cobalt administered as a solid and from 20 to 45% for soluble forms of cobalt administered as a liquid (Tvermoes et al. 2015).

Christensen et al. (1993) measured the absorption of both soluble cobalt chloride and insoluble cobalt tetraoxide in 12 male and 11 female volunteers. Based on urinary excretion of cobalt, uptake of cobalt chloride was greater than the uptake of the insoluble cobalt tetraoxide. Values for non-radiolabeled cobalt have been calculated based on urinary excretion of cobalt. Both overnight fasting and iron deficiency resulted in increased cobalt absorption (Smith et al. 1972; Sorbie et al. 1971; Valberg et al. 1969). Amino acids and sulfhydryl groups that bind with cobalt ions might reduce absorption (Paustenbach et al. 2013). Serum ferritin levels were strongly inversely correlated with blood cobalt levels in Norwegian women (Meltzer et al. 2010). Barany et al. (2005) also reported an inverse relationship between iron levels and cobalt in both adolescent girls and 15-year-old boys. This result was not observed in 17-year-old boys, most likely due to a better iron status. Adolescent boys had lower blood cobalt levels overall compared to adolescent girls. Lower levels of both ferritin and total iron resulted in higher levels of cobalt in the blood. Higher activity levels in males also resulted in higher cobalt levels in the blood due to decreased iron levels (Tvermoes et al. 2014).

Cobalt and iron share a common absorptive pathway in the intestines, although cobalt absorption can take place without ferritin (Reuber et al. 1994; Schade et al. 1970; Thomson et al. 1971). The duodenum and proximal jejunum are the primary sites for cobalt ion absorption, where absorption is mediated by the (DMT1) (Danzeisen et al. 2020a; Knopfel et al. 2005). Since cobalt and iron share similar characteristics, it is thought that both may compete for uptake by DMT1. DMT1 is involved in transporting iron into the duodenum and is upregulated by iron deficiency or by increased demand for iron (Garrick et al. 2006; Meltzer et al. 2010; Paustenbach et al. 2013). Another protein, Nramp1, may also facilitate uptake of cobalt, iron, and manganese (Forbes and Gros 2003).

Studies of gastrointestinal cobalt absorption in humans have shown differences in absorption rates based on sex, with females generally having higher absorption rates likely due to higher rates of iron deficiency in women (Looker et al. 1997).

Christensen et al. (1993) reported higher levels of blood and urinary cobalt in the female volunteers compared to the male volunteers following oral administration of cobalt. After 31 days of cobalt supplementation, blood levels of cobalt were 2 times higher in females than in males (Finley et al. 2013). Tvermoes et al. (2014) extended the cobalt supplementation to 90 days and reported that the male volunteers had lower blood levels than females. The total amount of cobalt in an adult as vitamin B_{12} via ingestion is about 0.25 mg, of which 50–90% is contained in the liver (IARC 1991).

Absorption studies in rats have reported differences in absorption based on solubility, with 13–34% of the more soluble forms of cobalt being absorbed compared to 1–3% of insoluble forms being absorbed (Ayala-Fierro et al. 1999; Bailey et al. 1989; Barnaby et al. 1968; Collier et al. 1989; Hollins and McCullough 1971; Kirchgessner et al. 1994; Patrick et al. 1989; Schade et al. 1970; Taylor 1962). Ayala-Fierro et al. (1999) also reported an absorptive half-life of 0.9 hours for orally administered cobalt chloride in male Fisher rats. Water-soluble cobalt forms exhibit greater absorption than non-water-soluble forms (Deka et al. 1981; Firriolo et al. 1999; Inaba et al. 1980; Kinoshita and Fujita 1972; Kreyling et al. 1986). Absorption was not affected by particle size of cobalt administered to baboons, guinea pigs, HMT rats, F344 rats, hamsters, or CBA/H mice (Bailey et al. 1989).

Danzeisen et al. (2020a) estimated the bioavailability of cobalt compounds in rats following oral exposure based on substance elution in gastric and intestinal fluids. As reported in human studies, sex differences in bioavailability were reported for cobalt chloride, with slightly higher estimated bioavailability in females (12%) than males (7%). These predictions are lower than estimates in humans (20–45%; Tvermoes et al. 2014), potentially due to differences in study design, including a single bolus administration in the rat study compared to lower doses over a 3-month period in the human study (Danzeisen et al. 2020a). The estimated bioavailabilities of cobalt sulfide and cobalt tetraoxide in rats, relative to cobalt chloride, were <0.1%, suggesting that these compounds are not well absorbed (Danzeisen et al. 2020a).

Administration of cobalt chloride (labeled with radioactive ^{58}Co) and complexed with histidine, lysine, glycylglycine, ethylenediaminetetraacetic acid (EDTA), casein, or glycine resulted in decreased gastrointestinal absorption of cobalt in rats, whereas significantly greater absorption occurred when radiolabeled cobalt chloride was administered in cows' milk. Cobalt (II) glycine complex was absorbed in greater quantities than a cobalt (III) glycine complex (Taylor 1962). Taylor (1962) performed this study to better elucidate the mechanism by which absorption occurs in the gastrointestinal tract.

Similar to humans, iron deficiency led to increased absorption of cobalt in rats, whereas simultaneous administration of cobalt and iron reduced the amount of cobalt absorbed (Reuber et al. 1994; Schade et al. 1970). Increasing oral doses of cobalt resulted in decreased fractional absorption (Houk et al. 1946; Kirchgessner et al. 1994; Taylor 1962). In young rats and guinea pigs (≤60 days old), reported absorption is 3–15-fold greater than in adult animals (200 days of age) (Naylor and Harrison 1995).

Species differences in estimated absorption following oral exposure are reported for more soluble cobalt compounds. Absorption of soluble cobalt compounds is greater in rats (13–34%) than in dairy cows (1–2%) and guinea pigs (4–5%) (Ayala-Fierro et al. 1999; Barnaby et al. 1968; Hollins and McCullough 1971; Kirchgessner et al. 1994; Naylor and Harrison 1995; Schade et al. 1970; Taylor 1962; Van Bruwaene et al. 1984).

Studies evaluating absorption through dermal exposure are limited. In an assessment of hard metal workers, Klasson et al. (2017) reported a significant correlation with cobalt on the skin and uptake into blood and noted that dermal exposure may contribute as much to uptake as inhalation exposure. A doubling of cobalt levels on skin resulted in a 3–14% increase in blood cobalt levels (Klasson et al. 2017). Kettelarij et al. (2018b) also reported a positive association between cobalt levels in the urine of hard metal workers and measured dermal (index finger) exposure and collected both before and after their work shift. For each doubling of pre- and post-shift dermal levels of cobalt, the median urinary cobalt levels increased by 70 and 32%, respectively. The stronger association between pre-shift dermal levels and urinary cobalt levels, compared to post-shift dermal levels, may reflect ongoing absorption from the previous day's exposure despite end-of-shift hand cleaning, possibly due to unremoved residue. Kettelarij et al. (2018b) collected urine samples over the course of 24 hours (4–11 samples per worker) and collected removable skin contamination sample (via skin wipes) pre- and post-shift, compared to the study by Klasson et al. (2017), which collected urine samples pre- and post-shift and collected samples of skin exposure once. In an experiment to measure absorption through the skin, four subjects held their right hands for 90 minutes in a box filled with either freshly mixed powder (5–15% cobalt) or waste dry powder. Both conditions resulted in an increase of urinary cobalt by an order of magnitude postexposure and continued for 48–60 hours (Scansetti et al. 1994); unlike Kettelarji et al. (2018b), end-of-shift hand washing was not addressed.

Data on absorption of cobalt through human skin are available from a limited number of *ex vitro* studies. Using cobalt powder applied in synthetic sweat, the reported steady-state percutaneous permeation through excised human abdominal skin was 0.0123±0.0054 μg/cm^2/hour, with a lag time of

1.55±0.71 hours with much of the cobalt remaining in the skin (Leggett 2008). Another study evaluated skin permeation and distribution of cobalt chloride hexahydrate dissolved in ammonium formate buffer solution in full thickness human skin samples (Hagvall et al. 2021). While cobalt accumulated primarily in the stratum corneum (which is ~20 µm thick), with peak intensity at 10 µm beneath the surface of the skin, it did penetrate into the epidermis to a depth of 60 µm.

Animal studies suggest that dermal absorption of cobalt depends on whether the skin is intact or damaged. Absorption through intact skin is comparatively low, while absorption through damaged skin is much higher (Inaba and Suzuki-Yasumoto 1979; Lacy et al. 1996). Inaba and Suzuki-Yasumoto (1979) examined the absorption of $2.2x10^{-5}$ mg ^{60}Co/kg as cobalt chloride in 1.4 N HCl through 1 cm^2 of intact or abraded skin of guinea pigs. Absorption measured 3 hours postexposure through intact skin was <1%, while absorption through abraded skin was almost 80%. A study in hamsters also reported a low amount of absorption of cobalt through unabraded skin (Lacy et al. 1996).

3.1.2 Distribution

As a component of vitamin B_{12}, cobalt is an essential element and is found throughout the body. Cobalt is distributed to the serum, whole blood, liver, kidneys, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, and pancreas (Collecchi et al. 1986; Forbes et al. 1954; Hewitt 1988; Ishihara et al. 1987; Muramatsu and Parr 1988; Teraoka 1981; Yamagata et al. 1962; Yukawa et al. 1980). The total body content of cobalt is estimated at 1.1–1.5 mg (ICRP 1979; Yamagata et al. 1962), with approximately 0.11 mg in the liver (ICRP 1979). Approximately 85% of the total cobalt body burden in adults is in the form of the vitamin B_{12} organometallic complex (Paustenbach et al. 2013). The amount of cobalt available to partition into, and accumulate in, tissues is dependent on the concentration of free cobalt ions in serum. At serum cobalt concentrations up to 3,000 µg/L, it is estimated that 8.3–8.5% exists as free cobalt ions; the rest is bound to serum proteins, primarily albumin (Paustenbach et al. 2013). Two protein carriers, albumin and α_2-macroglobulin, bind to cobalt in the blood and serum (Paustenbach et al. 2013). Cobalt (II) ions can bind to lipoproteins and haptoglobin, and cobalt (III) has been reported to bind to transferrin, resulting in a decrease in iron transferrin binding (Paustenbach et al. 2013). The transport/binding mechanisms for cobalt ions in blood and tissues may involve competitive interactions with receptor binding affecting feedback mechanisms that involve other divalent cations like iron and calcium. The transport binding mechanisms are not well understood (Paustenbach et al. 2013).

Autopsy results from workers exposed to cobalt via inhalation found increased cobalt levels in tissues. Significant increases in cobalt in the lung were found in copper smelter and metal workers and coal miners occupationally exposed to cobalt (Gerhardsson et al. 1984; Hewitt 1988; Hillerdal and Hartung 1983; Teraoka 1981). Gerhardsson et al. (1984) reported a median lung concentration in deceased smelter workers of 15 ppb, which is twice that of the control group; however, there were no significant differences in cobalt levels in the kidneys or liver. Hewitt (1988) reported a median lung concentration of cobalt of 0.007 µg/g wet tissue in Swedish smelter workers. In an airplane painter, increased cobalt levels were also found in the lymph nodes (0.76 ppm), lung (1.4 ppm), liver (0.46 ppm), spleen (0.45 ppm), and kidneys (0.35 ppm) (Teraoka 1981). In a mother-infant study from the African Copperbelt mining region, in which populations have high environmental exposure to cobalt, a high degree of placental transfer of cobalt from mother to infant was observed, with higher concentrations in umbilical cord blood, compared to maternal cord blood (Kayembe-Kitenge et al. 2023). The levels in paired maternal-cord blood samples were highly correlated, as were maternal-placental and placental-cord samples.

The tissue distribution of cobalt in animals and humans are similar. In dogs exposed to either ^{60}Co-labelled cobalt oxide or ^{60}Co-labelled cobalt tetraoxide via inhalation and following translocation from the lung, the highest cobalt concentrations were recorded in the liver, kidney, and skeleton, with ^{60}Co-labelled cobalt oxide having higher concentrations than ^{60}Co-labelled cobalt tetraoxide (Barnes et al. 1976). Cobalt oxide is more soluble than ^{60}Co-labelled cobalt tetraoxide; only 10% of the initial lung burden remained in the lung, compared to 85% for ^{60}Co-labelled cobalt tetraoxide, 8 days after inhalation exposure (Barnes et al. 1976). Brune et al. (1980) exposed rats to cobalt particles via inhalation for 8 hours/day for up to 107 days; cobalt levels accumulated in the lungs and were almost 500 times that of controls. Dust particles remaining in the lungs were primarily found in the macrophages. After the lungs, the kidneys and liver had the next highest cobalt levels (Brune et al. 1980). Tissue distribution in rats following exposure to ^{57}Co-labelled cobalt tetraoxide was found mainly in the thoracic tissues 182 days postexposure. Seven days postexposure, the majority of the extrathoracic cobalt tissue distribution was found in the gastrointestinal tract, pelt, and carcass (Collier et al. 1991). Both Patrick et al. (1989) and Talbot and Morgan (1989) reported similar results in rats and mice, respectively, with most of the cobalt remaining in the lungs and very little distributing to other organs. In dogs exposed to various forms of cobalt oxides, the lungs retained much of the cobalt followed by the bones, muscle, and skin; the stomach, liver, and kidneys contained less cobalt (Kreyling et al. 1986).

In Syrian golden hamsters, the carcass (23%) and gastrointestinal tract (60%) had the most cobalt 24 hours postexposure to Cobalt oxide (Wehner and Craig 1972). In swine, the kidney cortex and spleen had higher cobalt levels than controls (Kerfoot 1974).

NTP (2014) reported the following distribution order for cobalt tissue concentrations (cobalt as µg Co/g tissue) in F344/N rats (in decreasing order): lung, liver, kidney, femur, heart, serum, and blood. The tissue cobalt burden (µg Co/tissue) distribution was similar except that the liver accumulated more cobalt than lung, and the heart accumulated more cobalt than the femur. In general, both the order for tissue concentrations and burdens were similar in mice.

There are limited data regarding distribution of cobalt following oral exposure in humans. However, the available studies show that cobalt is distributed by serum and blood (Finley et al. 2013; Tvermoes et al. 2014). Following oral administration of cobalt in volunteers for 31 days, Finley et al. (2013) reported that the rate of uptake for serum cobalt levels was 1.3 µg/L for every 1.0 µg/L of cobalt in whole blood. Tvermoes et al. (2014) also reported higher serum cobalt levels than whole-blood cobalt levels from a 90-day dosing study in volunteers. Both Finley et al. (2013) and Tvermoes et al. (2014) reported that women had higher concentrations in blood and serum than men. Steady-state concentrations of cobalt in whole blood and red blood cells were reached within 14–24 days following a 31-day supplementation with cobalt (Finley et al. 2013). Steady-state conditions were achieved after 20 days in men and 35 days in women following a 90-day supplementation of cobalt (Tvermoes et al. 2014). The time course data of cobalt levels in blood and serum suggest that cobalt may be sequestered in red blood cells, resulting in slower clearance (Finley et al. 2013; Tvermoes et al. 2014). Protein-bound cobalt comprised 95% of the total serum cobalt during dosing. Kargar et al. (2013) reported that approximately 96% of serum cobalt was bound to large molecular proteins in a 90-day study of volunteers who ingested approximately 1 mg cobalt/day of a dietary cobalt supplement. The study authors also reported an increase in percent of cobalt bound from 95 to 99% during the post-dosing time frame. The study authors suggested that the increase in the fraction of bound cobalt was due to the movement of bound cobalt from extravascular to intravascular space because of the depletion of cobalt in the intravascular space by excretion and red blood cell uptake (Kargar et al. 2013).

Studies in animals show that cobalt is found primarily in the liver, with smaller amounts in the kidneys, heart, stomach, and intestines following oral exposure of cobalt that resulted in gastrointestinal absorption (Ayala-Fierro et al. 1999; Greenberg et al. 1943; Persson et al. 1992; Simesen 1939; Thomas et al. 1976). In a study examining the distribution of orally administered radiolabeled cobalt ($^{60}CoCl_2$) in rats, Barnaby

et al. (1968) reported that after 1 day, the liver contained the highest level of cobalt (4% of the radioactivity administered) with <1% in all other organs. However, after 132 days, the highest levels of cobalt were reported in the muscle and skeleton, both <1%, and the amount of cobalt in the liver had dropped to 0.016% (Barnaby et al. 1968). Following a single oral dose of cobalt naphthenate, the highest levels were found in the liver, followed by the kidney and heart; negligible amounts were found in the spleen and testes (Firriolo et al. 1999). Pehrsson et al. (1991) also reported a 30-fold increase in cobalt levels in the myocardium of rats orally exposed to cobalt in the diet. Bourg et al. (1985) reported higher cobalt levels in the blood, brain, and testis of rats exposed to cobalt in the diet. Following a 90-day exposure to 7.44 mg Co/kg/day as cobalt chloride, distribution of cobalt in the tissues was primarily to the liver and kidney (Danzeisen et al. 2020a). Additional distribution was observed to the following organs, in descending order: adrenal gland, pancreas, bone plus bone marrow, ovary, uterus, prostate, brain, lungs, and testes. As expected (since cobalt is a component of vitamin B_{12}), low levels of cobalt were observed in most tissues in sham controls.

Szakmary et al. (2001) reported a dose-dependent increase in cobalt levels in fetal blood and amniotic fluid following oral exposure to cobalt in pregnant rats. Clyne et al. (1988) measured the amount of cobalt in the myocardium, soleus muscle, and serum in rats orally administered cobalt sulfate in the diet for 8 weeks. Cobalt levels in the myocardium, soleus muscle, and serum were higher in the exposed group compared to controls. While the highest cobalt levels were in the myocardium, followed by the soleus muscle, and then serum, cobalt levels were 100-fold higher than controls in serum, 30-fold higher than controls in the myocardium, and 26-fold higher than controls in the soleus muscle.

Skalny et al. (2021) administered cobalt chloride in drinking water to pregnant mice from 3 days prior to gestation through lactation. At weaning (day 25), the offspring were removed and exposed to cobalt chloride in drinking water until postnatal day 30. Cobalt tissue concentrations were measured on days 18, 25, and 30 in kidneys, liver, spleen, skeletal muscle, and serum. Cobalt distribution in those tissues showed time- and dose-dependent increases. Serum levels were 140-, 194-, and 300-fold higher than controls on days 18, 25, and 30, respectively. Skeletal muscle levels followed the same pattern, increasing with age. However, for the liver, kidney, and spleen, the maximal difference between exposed mice and controls occurred on day 25.

Gluhcheva et al. (2014) showed that cobalt passes through breastmilk and that distribution in mice is dependent upon lifestage. In the study, mouse dams were exposed via drinking water to cobalt chloride hexahydrate at 19 or 31 mg Co/kg/day for 2 or 3 days prior to parturition and throughout the lactation

period, and pups continued exposure through 90 days of age. Pups were sacrificed at intervals between PNDs 18 and 90. Cobalt accumulation in the liver and kidneys was greater in younger animals, compared to older animals, potentially due to immature enzyme systems. Cobalt distribution to the spleen was comparable across timepoints.

No studies were identified regarding distribution in humans or animals after dermal exposure to cobalt.

Smith et al. (1972) administered intravenous cobalt, as ^{60}Co, to 23 men and one woman and performed whole-body scans up to 1,018 days postexposure. The results indicated that 10–30% of the cobalt was found in the liver. Jansen et al. (1996) administered ^{55}Co-labelled cobalt chloride intravenously to two healthy adult human males. The scans showed that 50% of the cobalt accumulated in the liver, while 40% was found in the bladder.

Similar results have been reported in animals. Houeto et al. (2018) administered cobalt chloride intraperitoneally daily for 3 weeks. At the end of exposure, the tissues with the highest cobalt concentrations were the liver and kidney. Two hours after intravenous injection of ^{57}Co-labelled cobalt chloride in rats, cobalt was found in the liver (22.8% of the dose), kidneys (10.2%), and intestines (3.16%) (Gregus and Klaassen 1986). Similar results (29% liver, 10% kidneys, 4.6% intestines) were found following intracardiac injection of cobalt nitrate in rats (Patrick et al. 1989) or intravenous injection of ^{55}Co-labelled cobalt chloride in rats (Jansen et al. 1996). After intravenous injection of ^{60}Co-labelled cobalt chloride in rats, the greatest concentrations were found in the liver and kidney; however, 100 days after injection, the highest concentrations were found in the spleen, heart, and bone (Thomas et al. 1976). Barnaby et al. (1968) reported similar results following intraperitoneal injection of ^{60}Co-labelled cobalt chloride in rats. Following intramuscular injection of cobalt mesoporphyrin in rats, the liver and blood had the highest cobalt concentrations, followed by the kidney, lung, spleen, adrenal glands, and heart, at 7 days post-injection and later (Feng et al. 1998). Four weeks after subcutaneous administration of cobalt protoporphyrin, the highest concentrations of cobalt occurred in the kidney, followed by spleen, liver, lung, thymus, and gonads (Rosenberg 1993).

3.1.3 Metabolism

Metabolism of cobalt consists of formation of complexes with a variety of protein and nonprotein ligands. Cobalt is not subject to direct metabolism by enzymatic pathways but tends to predominantly get distributed in organ systems as discussed in Section 3.1.2 or to be excreted as detailed in Section 3.1.4

(Andre et al. 1989; Bailey et al. 1989; Collier et al. 1989; Patrick et al. 1989; Talbot and Morgan 1989; Van Bruwaene et al. 1984).

3.1.4 Excretion

In excretion following inhalation exposure, the mucociliary escalator is the main clearance mechanism for insoluble particles deposited in the conducting zone (trachea and primary bronchi), whereas soluble particles are cleared by diffusional and pinocytotic processes from this region. In the alveolar region, insoluble particles are removed by phagocytosis and transport to the mucociliary escalator. Soluble forms in the alveolar region are cleared by diffusional and pinocytotic processes (Oberdörster 1993).

Following exposure of humans to physiologically insoluble cobalt compounds (e.g., cobalt metal, cobalt tetraoxide), clearance from the body appears to follow three-phase kinetics. The first phase, mucociliary clearance of particles deposited in the tracheobronchial region, has a half-time of 2–44 hours (Apostoli et al. 1998; Mosconi et al. 1994). The second phase, which involves a macrophage mediated clearance from the lungs, has a half-time of 10–78 days (Beleznay and Osvay 1994; Mosconi et al. 1994). The third clearance phase, representing long-term clearance from the lungs, has a half-time on the order of years (Bailey et al. 1989; Beleznay and Osvay 1994; Mosconi et al. 1994; Newton and Rundo 1971).

Following a controlled aerosol exposure in humans, about 40% of the initial lung burden of inhaled ^{57}Co-labelled cobalt oxide was retained for a period of 6 months after exposure (Foster et al. 1989). Six months after exposure, a cumulative elimination of 33% of the initial lung burden was found in the urine and 28% was found in the feces (Foster et al. 1989). The ratio of peak absorption rate to average mechanical clearance rate was about 5 to 1. The peak translocation and average mechanical clearance of cobalt from the lungs for different species are reported in Table 3-1. Humans, baboons, and dogs had the lowest mechanical clearance rates among the different species, and humans and baboons had the lowest translocation rates for 0.8-µm particles. Cobalt elimination is affected by time (e.g., urinary excretion increases with time) and particle size, with more cobalt mechanically removed via the mucociliary escalator when the aerosol consists of larger particles (Bailey et al. 1989; Foster et al. 1989).

Table 3-1. Peak Translocation and Average Mechanical Clearance Rates (%) After Inhalation of Cobalt Oxide for 180 Days[a]

	Percent of lung content cleared per day for 180 days				
	Translocation at peak				Average mechanical
Species (strain)	0.8 µm	Peak day	1.7 µm	Peak day	clearance (%)[b]
Human	0.45	180	0.5	180	0.1
Baboon	0.6	180	0.2	c	0.1
Beagle dog	2.1	85	1.7	180	0.03
Guinea pig	2.1	180	1	75	0.3
Rat (HMT)	2.4	40	0.6	c	0.9
Rat (F344)	1.1	10	0.4	c	1
Hamster	1.8	180	0.7	180	0.8
Mouse	1.7	180	No data	No data	1.05

[a]Cobalt-57 was used as a tracer.
[b]Clearance rates were virtually identical in both particle size groups.
[c]Constant value over 180 days.

Source: Bailey et al. (1989)

Elimination half-lives in rats and mice exposed for 2 weeks to cobalt metal were 9–11 days in blood (rats), 4–7 days in blood (mice), approximately 3 days in serum (rats), 3–4 days in serum (mice), 4–6 days in lungs (rats), and 6–7 days in lungs (mice). In rats exposed for 3 months to cobalt, the pulmonary clearance followed a two-phase elimination. The first, a rapid phase, had a half-life of 2–3 days and the second phase, a slow phase, had a half-life between 19 and 23 days. For 2-year exposures in rats, dose-dependent rapid clearance phase half-lives were between 1.5 and 2.9 days, and the slow clearance phase half-lives were between 83 and 789 days for respective doses of 1.25 and either 2.5 or 5 mg/m^3, indicating that steady state was achieved for the two highest doses. Between 95 and 99% of the cobalt was eliminated in the rapid phase, with 1–5% eliminated in the slow phase. For mice exposed to cobalt for 2 weeks, the half-lives decreased as the dose increased. Like rats exposed for 3 months, the pulmonary clearance exhibited a two-phase elimination. For mice exposed for 2 years to 1.25, 2.5, and 5 mg/m^3, the rapid phase half-lives were 1.2, 1.1, and 5.2 days, respectively, indicating a slightly longer half-life in animals exposed at the highest dose. The total slow phase lung cobalt clearances ranged from 3.1 to 17.6%, while the total rapid phase lung cobalt clearances ranged from 96.9 to 82.4% with increasing exposure concentration (NTP 2014).

Following inhalation exposure, the rate of urinary excretion appears to correlate with the rate of translocation of cobalt from the lungs to the blood and the rate of fecal clearance appears to correlate with the rate of mechanical clearance of cobalt from the lungs to the gastrointestinal tract (Andre et al. 1989;

Bailey et al. 1989; Collier et al. 1989; Kerfoot 1974; Kreyling et al. 1986, 1989; Palmes et al. 1959; Patrick et al. 1989; Talbot and Morgan 1989). The solubility of cobalt affects the rate of clearance in animals, with moderately soluble forms, such as cobalt oxide, clearing faster than insoluble forms, such as cobalt tetraoxide (Barnes et al. 1976; Kreyling et al. 1984).

Urinary excretion was the primary route of cobalt elimination after a single inhalation exposure (Palmes et al. 1959) or after 3 months of exposure (Kerfoot 1974; Palmes et al. 1959) in rats and swine. In several species of animals, most of the inhaled cobalt oxide (labeled with ^{57}Co) following a single exposure was cleared from the lungs by 6 months after exposure (Table 3-2) (Andre et al. 1989; Bailey et al. 1989; Collier et al. 1989; Kreyling et al. 1989; Patrick et al. 1989; Talbot and Morgan 1989).

Table 3-2. Initial (Day 3) Lung Deposits of Cobalt Oxide and Summary of Lung Retention at 90 and 180 Days[a]

	Mean initial ^{57}Co activity in lung L(3)[b] (kBq)		Lung retention L(90)[c]/L(3) (%)		Lung retention L(180)[d]/L(3) (%)	
Species (strain)	0.8 µm	1.7 µm	0.8 µm	1.7 µm	0.8 µm	1.7 µm
Human	53	42	64	75	45	56
Baboon	2,100	1,700	55	55	26	37
Beagle dog	1,150	1,450	27	45	5.5	12
Guinea pig (Harwell)	8.4	1.4	49	46	8.3	15
Rat (HMT, 1985)	10.8	4.7	5.2	20	1.3	8
Rat (HMT, 1986)	3.2	0.7	5.3	18	1.2	9.2
Rat (F344, SPF)	8.8	4.4	14	25	4.7	9.2
Rat (Sprague-Dawley)	0.9	0.1	8	39	1	15
Syrian hamster	4	1.2	21	35	3.4	12
Mouse (CBA/H)	1.8	No data	15	No data	2.8	No data

[a]Cobalt-57 was used as a tracer.
[b]Lung deposits at Day 3.
[c]Lung deposits at Day 90.
[d]Lung deposits at Day 180.

Source: Bailey et al. (1989)

Excretion of unabsorbed cobalt following oral exposure in humans is primarily through the feces, whereas absorbed cobalt is primarily excreted in the urine with a small amount excreted in the feces. Sorbie et al. (1971) reported that within 24 hours of oral administration of radioactive ^{57}Co- or ^{60}Co-labelled cobalt chloride, 18% (9–23%) of the administered dose was excreted via the urine in volunteers with normal iron levels. The amount of cobalt excreted in volunteers with iron deficiency increased to 31% (23–42%).

Valberg et al. (1969) also reported similar differences in excretion between iron-sufficient and iron-deficient volunteers. Paley et al. (1958) reported an approximately 10-fold difference in cobalt levels in the urine and feces, with the feces having the higher amount. Postdosing distribution showed a different pattern, with serum cobalt concentrations falling faster than whole-blood cobalt levels and with whole-blood cobalt levels exceeding the serum cobalt levels (Finley et al. 2013). Finley et al. (2013) reported a 66% decrease in serum cobalt levels and a 52% decrease in blood cobalt levels 1-week postdosing. Elimination of cobalt in blood and serum follows a two-phase exponential decay curve, with an initial rapid phase followed by a slower second phase. The fast phase elimination half-life was 3 days, with 61% of cobalt concentration at the end of dosing found in the whole blood, while 77% was found in the serum. For the slow phase, the half-life was 16 days for serum and 39 days for whole blood, with 23% of the cobalt found in serum and 39% found in whole blood (Finley et al. 2013).

Blood cobalt levels 1 and 2 weeks post oral dosing decreased by 63 and 69%, respectively, in healthy male volunteers who received 0.4 mg cobalt/day for 15 days, indicating that much of the cobalt was rapidly eliminated from the blood postdosing (Tvermoes et al. 2013).

Tvermoes et al. (2014) reported that elimination of cobalt from whole blood and serum followed a two-phase exponential decay curve, with a fast initial phase followed by slower second phase, following ingestion of 1 mg/day cobalt for 90 days (Table 3-3). Elimination from red blood cells was linear with time and correlated with the red blood cell life span of 120 days (Tvermoes et al. 2014). Serum cobalt concentrations were correlated with urine cobalt concentrations for both men and women; however, women retained more cobalt than men. Renal clearance differences between men and women likely reflect the different glomerular filtration rates between men (120 mL/minute) and women (99 mL/minute) and the differences in urine production volume between men (2,900 mL) and women (1,800 mL). The ratios of urine to serum cobalt concentrations for men and women throughout dosing were 3.4 and 3.3, respectively. Urinary excretion of cobalt appears to be mediated by a saturable reabsorption process (Tvermoes et al. 2014).

Table 3-3. Retention of Cobalt (Cobalt Chloride) in Whole Blood and Serum in Humans after Oral Dosing

	First phase			Second phase		
	Fraction eliminated[a]	Elimination rate constant (per day)	Half-life (days)	Fraction eliminated	Elimination rate constant (per day)	Half-life (days)
Whole blood	0.52	0.62	2.8	0.48	0.020	36
Serum	0.76	0.58	3.08	0.24	0.037	22

[a]Over a period of 22–36 days.

Source: Tvermoes et al. (2014)

Animal studies demonstrate that soluble cobalt is excreted through the urine and insoluble cobalt is excreted through the feces. Table 3-4 provides the cumulative urinary and fecal elimination in several species following oral administration of cobalt tetraoxide (with a ^{57}Co tracer) (Bailey et al. 1989). No significant differences in elimination of cobalt tetraoxide were found among several species of animals, and >96% was quickly eliminated in the feces (Andre et al. 1989; Bailey et al. 1989; Collier et al. 1989; Patrick et al. 1989; Talbot and Morgan 1989). For the more soluble cobalt (II) chloride, reported fecal elimination levels ranged from 70 to 83% of the administered dose for rats, with urinary excretion accounting for most of the remainder of the dose (Ayala-Fierro et al. 1999; Barnaby et al. 1968; Hollins and McCullough 1971). In lactating dairy cows, about 97% of an oral dose of cobalt chloride was recovered in the feces by day 70 postexposure, while the urine and milk contained 0.26 and 0.012% of the dose, respectively (Van Bruwaene et al. 1984). Following a single exposure in beagle dogs, 90% of the more insoluble cobalt tetraoxide was eliminated in the feces and 5% in the urine, whereas 70% of the more soluble cobalt nitrate was excreted in the feces and 25% was excreted in the urine (Kreyling et al. 1986).

Table 3-4. Summary of Retention and Excretion After Intragastric Administration of Cobalt Tetraoxide Particles (Mean Percentage of Recovered Activity at 7 Days Post Administration)[a]

	Cumulative fecal excretion (%)		Whole body retention (%)		Cumulative urinary excretion (%)		Absorption(%)	
Species (strain)	0.8 µm	1.7 µm	0.8 µm	1.7 µm	0.8 µm	1.7 µm	0.8 µm	1.7 µm
Baboon	97.8	98.4	0.12	0.2	2	1.4	2.6	1.9
Guinea pig	98.7	97.6	0.16	0.66	1.1	1.9	1.3	2.3
Rat (HMT)	96.3	99.4	0.09	0.02	2.8	0.6	3.9	1
Rat (F344)	99.6	99.7	0.04	0.03	0.4	0.3	0.4	0.3

Table 3-4. Summary of Retention and Excretion After Intragastric Administration of Cobalt Tetraoxide Particles (Mean Percentage of Recovered Activity at 7 Days Post Administration)[a]

	Cumulative fecal excretion (%)		Whole body retention (%)		Cumulative urinary excretion (%)		Absorption(%)	
Species (strain)	0.8 μm	1.7 μm	0.8 μm	1.7 μm	0.8 μm	1.7 μm	0.8 μm	1.7 μm
Hamster	96	96.3	0.5	0.18	3.5	3.5	5.1	5.1
Mouse (CBA/H)	99.1	No data	0.3	No data	0.6	No data	0.8	No data

[a]Cobalt-57 was used as a tracer.

Source: Bailey et al. (1989)

Following oral exposure, iron-deficient rats eliminated less of a given dose in the feces than normal rats, while co-administration of iron compounds resulted in an increased fecal excretion of cobalt compounds (Reuber et al. 1994).

Danzeisen et al. (2020a, 2020b) reported plasma toxicokinetic parameters and fecal and urinary excretion kinetics for different cobalt compounds administered by gavage. The results are presented in Tables 3-5 and 3-6. Values for the maximum plasma concentration (C_{max}), half-life ($t_{1/2}$), and elimination constant (K_{el}) were comparable for all three substances (at administered doses), except that C_{max} for cobalt tetraoxide was twice as high in male as in female rats. Large differences in estimated oral bioavailability (AUC) due to substance solubility were expected and confirmed. The predominant excretion pathway was via fecal excretion, which decreased with solubility (>80% for cobalt chloride hexahydrate; >95% for cobalt tetraoxide), with some urinary excretion. Excretion levels were highest on day 1 following exposure, with levels on subsequent days at least an order of magnitude lower (Table 3-6).

Table 3-5. Pharmacokinetic Parameters for Orally Administered Cobalt in Rats

Test item	Dose level (mg Co/kg)	C_{max} (mg/L)	$t_{1/2}$ (hours)	K_{el} (1/hour)	$AUC_{0-t\ last}$/cobalt dose [(hour mg/L)/(mg/kg)]
Male rats					
Cobalt chloride hexahydrate	2.48	2.51	14.2	0.0489	20
Cobalt tetraoxide	214	2.08	17.3	0.0402	0.18
Cobalt sulfide	194	2.01	16.8	0.0413	0.1

Table 3-5. Pharmacokinetic Parameters for Orally Administered Cobalt in Rats

Test item	Dose level (mg Co/kg)	C_{max} (mg/L)	$t_{1/2}$ (hours)	K_{el} (1/hour)	$AUC_{0–t\ last}$/cobalt dose [(hour mg/L)/(mg/kg)]
Female rats					
Cobalt chloride hexahydrate	2.48	2.61	13.7	0.0508	13.7
Cobalt tetraoxide	214	1.10	16.1	0.043	0.12
Cobalt sulfide	194	2.01	14.9	0.0464	0.1

$AUC_{0-t\ last}$ = area under the curve from time (t) zero to t last; C_{max} = maximum plasma concentration; K_{el} = elimination constant; $t_{1/2}$ = half-life

Source: Danzeisen et al. (2020a)

Table 3-6. Cobalt Levels in Urine and Feces in Rats Following Oral Exposure to Cobalt

Substance	Dose level (mg Co/kg)	Cobalt concentration in urine (µg Co/L)			Cobalt concentration in feces (µg Co/g)		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Male rats							
Cobalt chloride hexahydrate	2.48	9,791	834	239	41.11	3.91	1.69
Cobalt tetraoxide	220	6,344	379	307	5,502.63	258.5	23.33
Female rats							
Cobalt chloride hexahydrate	2.48	4,974	411	247	47.55	2.77	0.35
Cobalt tetraoxide	220	5,158	572	172	5,335.27	233.6	6.59

Source: Danzeisen et al. (2020b)

Urinary excretion of cobalt increased from a pre-exposure level of 18.1 nmol (1.07 µg) to 38.5 nmol (2.27 µg) 24 hours after exposure in five subjects who were dermally exposed for 1 hour by keeping their hands in a solution containing 1,600 mg Co/L. The maximum amount of cobalt excreted occurred 4–6 hours after exposure in two subjects; in two other subjects, the urinary excretion rate increased monotonically up to 24 hours following exposure. No increase in urinary cobalt was reported for one subject (Leggett 2008).

Lacy et al. (1996) reported that much of the absorbed dose of cobalt chloride was excreted in urine 48 hours after a single dermal exposure in Syrian hamsters. No other studies were identified regarding excretion in animals after dermal exposure to cobalt.

In humans with metal-on-metal hip replacements, urinary cobalt levels were 3-fold higher than concentrations in plasma. Cobalt clearance increased from 1.3 mL/minute in the preoperative group to 3.7 mL/minute in the follow-up group; with increasing daily output, the renal clearance in the postoperative group increased from 1.9 to 7.1 mL/minute (Daniel et al. 2010). Smith et al. (1972) reported that 24 hours after intravenous administration of ^{60}Co-labelled cobalt chloride, 22% of the administered dose was excreted in the urine, 1.8% of the administered dose was excreted in the feces, and >90% was removed from plasma within 30 minutes. The urinary to fecal ratio was 6.7:1. Retention times for two of the subjects followed over the course of 1,000 days showed the following half-times and corresponding percentages leaving the body: 0.5 days (44%); 6 days (32%); 60 days (13%); and 800 days (11%). The liver retained an average of 20% of the total body burden from a few days post administration through 1,000 days post injection (Smith et al. 1972).

Paley et al. (1958) reported that 56–73% of the dose was excreted in urine 48 hours after intravenous administration and Kent and McCance (1941) reported that 57% was excreted in 2 weeks. The average urinary excretion of ^{57}Co in 13 healthy human subjects (9 males and 4 females) during the first 24 hours after intravenous injection of cobalt glycinate was 34%, with no gender differences reported. Following intravenous injection of ^{57}Co glycinate, the urinary to fecal excretion ratio of 6:1 was measured in one subject over the course of 3 days (Leggett 2008).

Following intravenous injection of cobalt nitrate in various species of animals, more than half was excreted within the first day and approximately 80% was excreted in the urine within 21 days (Table 3-7) (Andre et al. 1989; Bailey et al. 1989; Collier et al. 1989; Patrick et al. 1989; Talbot and Morgan 1989). Other investigators have also found that urine is the primary route of cobalt excretion following intravenous administration, with approximately 5–30% excreted in the feces (Ayala-Fierro et al. 1999; Barnaby et al. 1968; Gregus and Klaassen 1986; Kreyling et al. 1986; Onkelinx 1976; Thomas et al. 1976). Excretion of cobalt (2–7% of the injected dose) in the bile was also reported in dogs and rats (Cikrt and Tichy 1981; Gregus and Klaassen 1986; Sheline et al. 1946). Urinary excretion following intraperitoneal injection is the major route of elimination, with fecal excretion accounting for much of the remaining dose (Barnaby et al. 1968; Hollins and McCullough 1971; Talbot and Morgan 1989). However, longer-term clearance may be more balanced between urinary and fecal excretion (Hollins and McCullough 1971). Urinary excretion was also the predominant route following subcutaneous injection of cobalt chloride and cobalt nitrate, and both were excreted rapidly from the body (Rosenberg 1993; Talbot and Morgan 1989).

Table 3-7. Summary of Retention and Excretion of Cobalt Following Injection of Cobalt Nitrate Solution (Mean Percent Recovery)[a]

	Whole-body retention on day			Cumulative urinary excretion on day			Cumulative fecal excretion on day		
Species (strain)	1	7	21	1	7	21	1	7	21
Baboon	–	–	–	57	74	80	5	17	20
Beagle dog	–	–	–	71	86	87	3.4	4.4	4.9
Guinea pig	34	8	3.5	64	82	85	2.2	10	12
Rat (HMT)	18	4.2	1.9	64	72	74	18	24	24
Rat (F344)	–	–	2.9	–	–	80	–	–	18
Hamster	27	4.3	1.9	55	68	69	17	28	29
Mouse	23	2.9	1.1	59	71	72	18	26	27

[a]Cobalt-57 was used as a tracer.

Source: Bailey et al. (1989)

The chemical form of the cobalt compound may affect its rate of elimination. Subcutaneous injection of cobalt protoporphyrin, a substance where the cobalt atom is chelated within the porphyrin ring, resulted in a slower clearance from plasma ($t_{½}$ of 3 days) in rats than cobalt chloride, where >95% was measured in plasma 30 minutes after injection. Approximately 20% of the cobalt from cobalt protoporphyrin remained in plasma 14 days after injection (Rosenberg 1993). Intramuscular injection of cobalt mesoporphyrin resulted primarily in fecal excretion, with high systemic retention (Feng et al. 1998).

Nishimura et al. (1978) intravenously injected ^{60}Co-labelled cobalt chloride and ^{58}Co-labelled cobalt-cyanocobalamin into rats. After 21 days post administration of ^{60}Co-labelled cobalt chloride, the liver and kidney contained 26.4 and 13.1% of the body burden, respectively, with most of the isotope activity excreted in the urine. Comparatively, the body burden of ^{58}Co-labelled cobalt-cyanocobalamin in the kidneys was 43.8%, with 12% in the liver. Most of the isotope activity was in the feces. Cumulative excretion over 9 days was different for the two forms of cobalt. Cumulative excretion rates for cobalt chloride were 80 and 9% of the dose for urine and feces, respectively. Cumulative excretion rates for cobalt cyanocobalamin were 5 and 14% for urine and feces, respectively (Nishimura et al. 1978).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

The International Commission on Radiological Protection (ICRP) developed two models to evaluate the kinetics of cobalt: a Human Respiratory Tract Model (HRTM) (Bailey et al. 2007; ICRP 1994, 1995), and a systemic model (ICRP 2016; Legget 2008). The HRTM simulates the deposition, clearance, and absorption of inhaled particulates. The systemic model simulates the distribution and excretion of cobalt absorbed from the respiratory tract or gastrointestinal tract.

The ICRP (2016) has published HRTM parameter values for absorption of cobalt compounds (Table 3-8). The HRTM assumes that absorption into blood occurs at equivalent rates in all parts of the respiratory tract, except in the anterior nasal passages, where no absorption occurs. Absorption is simulated as a two-stage process that begins with particle dissolution, followed by transfer of dissolved material into blood. Dissolution is simulated as a biphasic process with a rapid phase, fraction f_{rapid} dissolving at rate k_{rapid} (day^{-1}), and a slow phase, fraction, f_{slow} ($1-f_{rapid}$) dissolving at rate k_{slow} (day^{-1}). A fraction of the dissolved material is bound (f_{bound}) and is transferred to blood at rate (k_{bound}). The unbound fraction, $1-f_{bound}$, is transferred instantaneously to blood. In the absence of specific estimates for absorption kinetics, compounds are classified into absorption types F (fast), M (medium), and S (slow).

Table 3-8. ICRP (2016) Absorption Parameter Values for Cobalt

Type[a]	Rapid fraction	Slow fraction	Rapid rate (day^{-1})	Slow rate (day^{-1})	Bound fraction	Bound rate (day^{-1})	GI tract absorption fraction[b]
Inhaled cobalt							
Fast (F)	1.0	0	1	NA	0.03	0.002	0.1
Medium (M)	0.2	0.8	1	0.005	0.03	0.002	0.02
Slow (S)	0.01	0.99	1	$1x10^{-4}$	0.03	0.002	0.001
Ingested cobalt							
Insoluble oxides							0.05
All other cobalt compounds							0.1

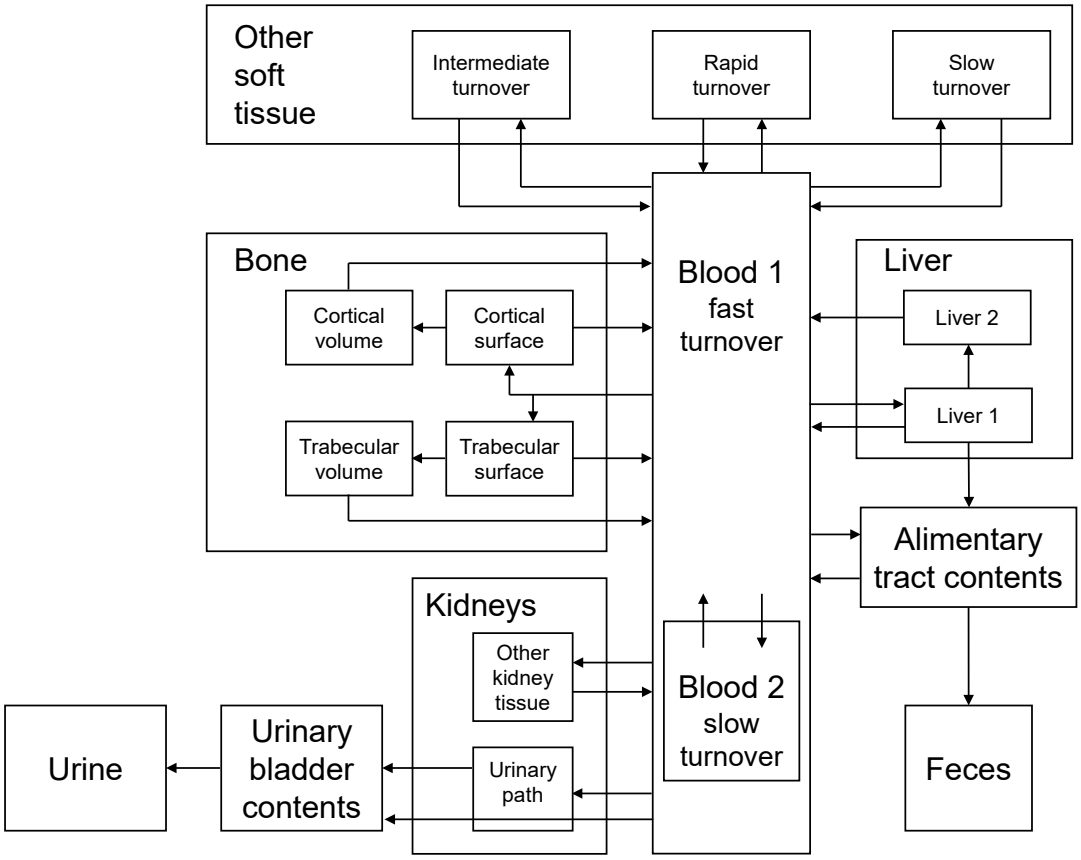
[a]Type F: cobalt chloride, cobalt nitrate; Type M: default for other cobalt compounds in the absence of specific estimates for absorption kinetics; and Type S: cobalt oxide.
[b]Cobalt cleared from the respiratory tract to the GI tract.

GI = gastrointestinal; HRTM = Human Respiratory Tract Model; ICRP = International Commission for Radiological Protection

The ICRP systemic model (Legget 2008; ICRP 2016;) includes compartments representing blood (central distributing compartment), bladder, bone, gastrointestinal tract, kidney, liver, and other soft tissues (Figure 3-4). The blood compartment includes fast and slow turn-over subcompartments. Transfers of cobalt between blood and tissues occurs to and from the fast turn-over compartment. Other tissue compartments also have subcompartments representing different kinetic pools. Transfers between compartments are governed by first-order rate coefficients (day^{-1}). Absorption from the gastrointestinal tract into the fast turn-over compartment of blood is simulated with an absorption fraction. ICRP (2016) assigned absorption fractions of 0.05 for ingested cobalt oxides and 0.1 for all other cobalt compounds.

Unice et al. (2012, 2014a, 2014b) modified the Leggett (2008) model in several ways. The Unice et al. (2012) model assigned a central tendency estimate for the gastrointestinal absorption fraction of 0.25, with a minimum of 0.1 and a maximum of 0.35 based on available data for men and women, assumed that cobalt was ingested in a soluble form, and incorporated total blood volume and urinary excretion rates to better calculate cobalt levels in blood and urine. The model output for blood and urine was compared to the results of a Danish study of 23 subjects who ingested a soluble form of cobalt. The model predictions were in concordance with the test population (Unice et al. 2012). In addition, Tvermoes et al. (2013) conducted a study that compared the measured cobalt levels in whole blood of 20 healthy male volunteers who ingested cobalt to the model predictions of whole-blood concentrations. The mean measured values were within 5% of the model's concentration range when bounded by a 15–35% absorption rate (Tvermoes et al. 2013).

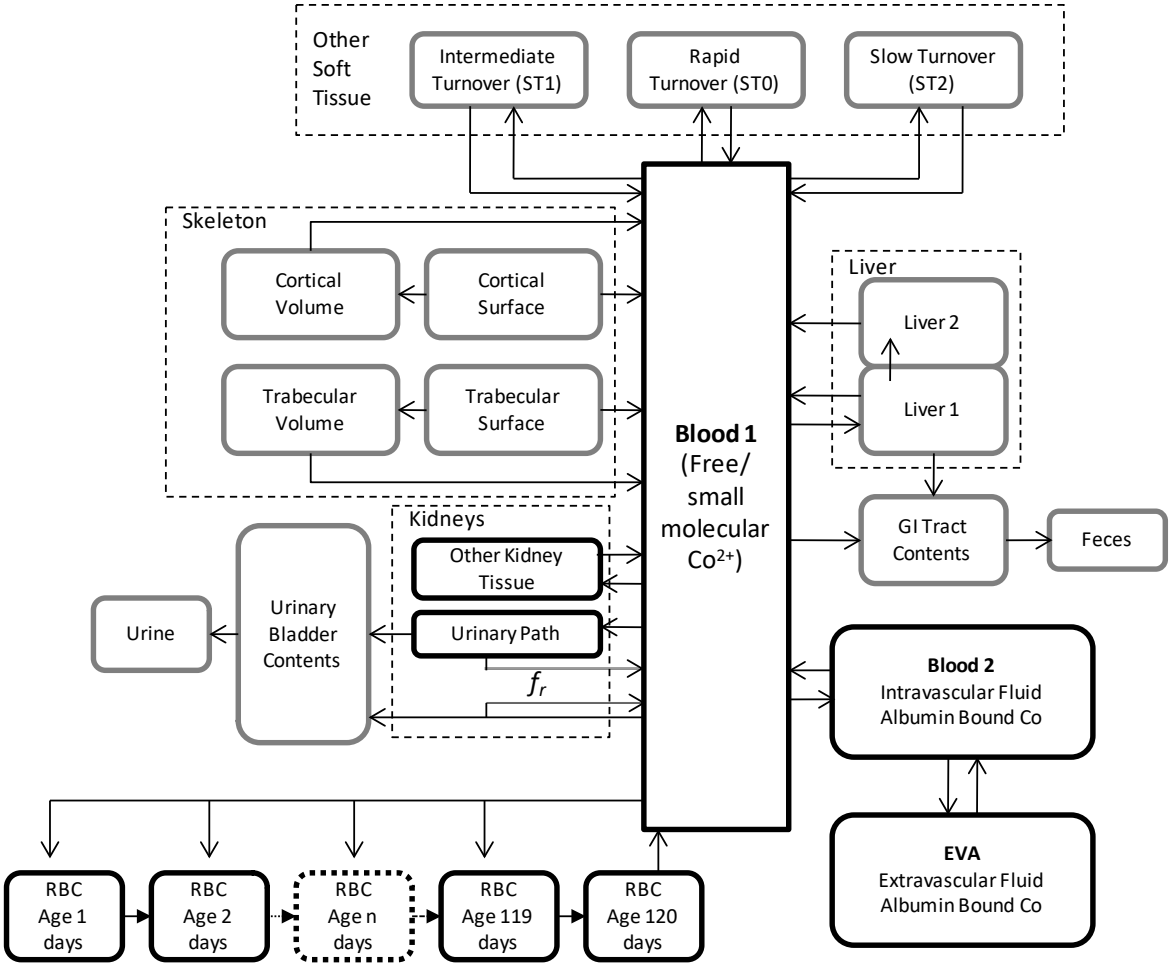
Figure 3-4. Structure of ICRP (2016) Cobalt Systemic Model



Source: ICRP (2016), with permission from The International Commission on Radiological Protection

Unice et al. (2014a, 2014b) updated their model to reflect new toxicokinetic data involving cobalt albumin binding, uptake and storage of cobalt in red blood cells, saturable renal reabsorption of Co^{2+}, and effect of glomerular filtration rates and free Co^{2+} on cobalt excretion. The changes incorporated included increasing the fraction of serum protein bound cobalt from 95% during dosing to 99% postdosing; adding a postdosing linear decrease in cobalt red blood cell concentration; adjusting renal clearance to fit with glomerular filtration rates and free cobalt concentration; and adding compartments to account for serum albumin-bound cobalt, exchange rates between albumin-bound cobalt between intravascular and extravascular fluids, and individual red blood cell compartments representing each day in the lifetime of a red blood cell (120 days). Unice et al. (2014a) compared the model output to several data sets including healthy volunteers, whole-body retention studies, dialysis patients, anephric (with non-functioning kidneys) patients, and a cobalt poisoning incident. The model compared well with all external datasets. Tvermoes et al. (2015) used this model to estimate cobalt concentrations in tissues at varying doses. Figure 3-5 depicts the model design.

Figure 3-5. Structure of Unice et al. (2014b) Cobalt Systemic Model



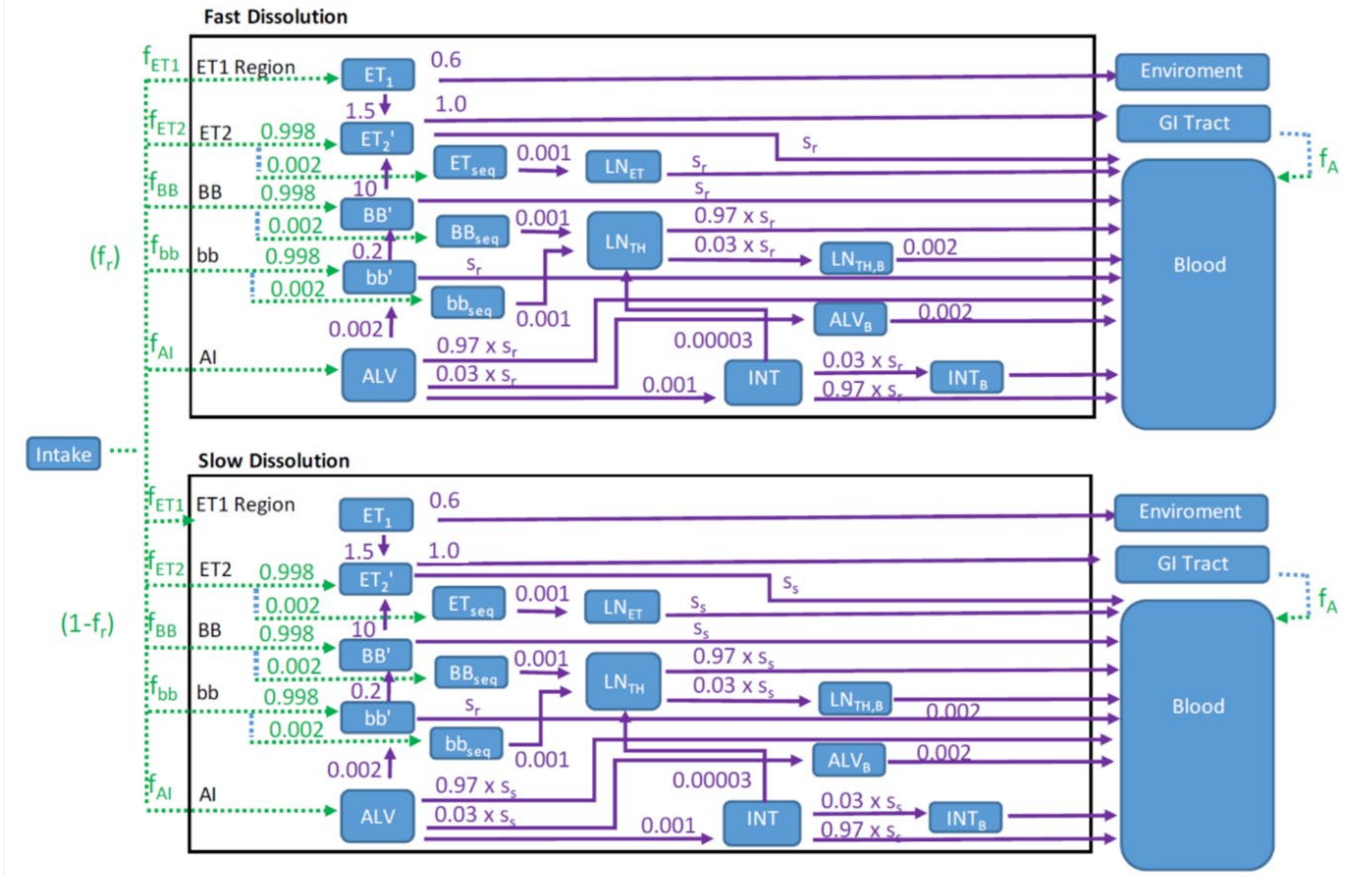
Source: Unice et al. (2014b), with permission from Elsevier

Unice et al. (2020a, 2020b) further updated their cobalt models to include an inhalation pathway based on the ICRP HRTM (Figure 3-6). Their modified ICRP HRTM accounts for particle-size-dependent deposition in the extrathoracic region, both the bronchial and bronchiolar airways, and the alveolar–interstitial region of the lungs. A default particle density of 3 g/cm^3 was used. Other assumptions for most forms of cobalt included moderate respiratory absorption rates (ICRP 'Type M'), with a rapid fraction of 0.2, rapid dissolution half-life of 17 hours, slow dissolution half-life of 137 days, and absorption fraction from the alimentary tract of 0.02. For the oral absorption component of the model, both ingested dust along with the estimated absorption fraction from the alimentary tract (due to particles clearance from the respiratory tract) were utilized. These oral components were used together with the HRTM in Unice et al. (2020a). Modeling of other chemical forms of cobalt (e.g., cobalt oxides) used the following assumptions: slow absorption rates (ICRP 'Type S'), with a rapid fraction of 0.01, rapid dissolution half-life of 17 hours, slow dissolution half-life of 19 years, and absorption fraction from the alimentary tract of 0.001. To account for species differences in regional lung deposition, animal doses were modeled using human equivalent concentrations. The modeled data and measured data showed good agreement, within a factor of two, for blood, liver, testes, and tissue concentrations. When the model was run using occupational inhalation exposure scenarios, the results showed that the systemic body burden was higher for ingestion than for inhalation (Unice et al. 2020a).

3.1.6 Animal-to-Human Extrapolations

Retention and clearance of physiologically insoluble ^{57}Co particles after inhalation varies widely across species, illustrating the potential difficulty of extrapolating the results of animal lung retention experiments to humans even qualitatively (Bailey et al. 1989). Conversely, differences in absorption of physiologically insoluble cobalt oxide following oral exposure do not appear to exist between species (humans were not included in the study) (Bailey et al. 1989). Absorption of soluble cobalt compounds is greater in rats (13–34%) than in dairy cows (1–2%) and guinea pigs (4–5%) following oral exposure (Ayala-Fierro et al. 1999; Bailey et al. 1989; Barnaby et al. 1968; Hollins and McCullough 1971; Kirchgessner et al. 1994; Naylor and Harrison 1995; Schade et al. 1970; Taylor 1962; Van Bruwaene et al. 1984). While PBPK models are available (Section 3.1.5), they are either restricted to kinetic modeling in humans (ICRP models) or model assumptions for cobalt are based on human data (Unice et al. 2020a). Therefore, they are not suitable for animal-to-human dose extrapolations.

Figure 3-6. Updated Model Inhalation



Compartments and regions

ID (1994)	Symbol (2015)	Region or compartment
1,2,3	ALV, AI	Alveoli (ALV), Alveolar-Interstitial (AI)
1,2,3	INT	Interstitium
4,5	bb, bb'	Bronchioles
6	bb_{seq}	Sequestered in bronchioles
7,8	BB, BB'	Bronchi
9	BB_{seq}	Sequestered in bronchi
10	LN_{TH}	Lymph nodes in thoracic region
11	ET_2, ET_2'	Posterior nasal passage, larynx and pharynx
12	ET_{seq}	Sequestered in extrathoracic tissue
13	LN_{ET}	Lymphatic system in extrathoracic region
14	ET_1	Anterior nasal
15	GI	GI tract
16	Environment	environment (e.g. tissue paper)

Source: Unice et al. (2020b), with permission from Elsevier

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to cobalt are discussed in Section 5.7, Populations with Potentially High Exposures.

Age-Related Exposure and Pharmacokinetic Differences. No studies that examined pharmacokinetic differences between adults and children were identified. Animal studies have suggested several differences in pharmacokinetic behavior of cobalt compounds between children and adults. Following inhalation exposure to cobalt tetraoxide, deposition tended to increase with age (Collier et al. 1991). The youngest animals exposed (3 weeks postnatal) had significantly lower fractional retention 182 days postexposure compared to 13-, 21-, and 46-week-old animals. There were no significant differences in fractional retention among the older animals until 281 days postexposure where there were significant differences among all age groups. The study authors attributed this to a faster rate of translocation of cobalt from the lung to the blood, which could enhance subsequent excretion. The youngest animals had a significantly faster translocation rate, which was not further explained by the study authors. There were no significant differences in mechanical clearance rates of ^{57}Co-labelled cobalt tetraoxide in animals of different ages (Collier et al. 1991). Naylor and Harrison (1995) reported that in rats and guinea pigs, fractional absorption of cobalt following oral exposure was highest 1 day after birth, remained elevated in rats, but not guinea pigs, during the suckling stage, and diminished rapidly with time thereafter.

In animal studies where soluble cobalt compounds were intravenously injected, cobalt was shown to cross the placenta and enter the fetus. Twenty-four hours after intravenous injection of cobalt chloride in rats,

0.14% of the dose was found in the fetus, 0.19% was found in the chorioallantoic placenta, and 0.22% was found in the yolk sac (Zylicz et al. 1975). The amount of cobalt crossing the placenta following intravenous injection was greater in later gestational stages, although <1% of the maternal dose reached the fetus (Nishimura et al. 1978; Zylicz and Zabloina 1976; Zylicz et al. 1975). The form of cobalt may also be important relative to bioavailability to the fetus. Nishimura et al. (1978) reported that the fetal uptake of cobalt, following intravenous administration of either cyanocobalamin or cobalt chloride to the mother, was increased for cyanocobalamin (5% of the maternal dose) compared to cobalt chloride (<1% of the maternal dose).

Cobalt is detected in human breast milk at concentrations in the parts per billion (ppb) range in the inorganic form (Byczkowski et al. 1994). Animal studies reported low amounts of cobalt in the breast milk. Milk obtained 70 days postexposure from lactating dairy cows contained 0.012% of the exposure dose (Van Bruwaene et al. 1984). Cobalt given intravenously to mother rats as cyanocobalamin was transferred to offspring via the breast milk (1–2%) (Nishimura et al. 1978).

Health Effects from Exposure to Cobalt. Available data have not clearly defined whether children are at greater risk from exposure to stable cobalt than adults. Data on effects of cobalt in children following inhalation exposures are lacking. Jacobziner and Raybin (1961) reported two cases of children who had accidentally ingested unknown amounts of cobalt chloride. In one case, a 19-month-old male ingested acetylsalicylic acid followed by stomach lavage and was asymptomatic; the following day, he ingested cobalt chloride, developed poisoning symptoms (bluish skin and lips, swollen lips and tongue, restless, then drowsiness), and received stomach lavage, but died approximately 6.5 hours after the ingestion. However, a 3-year-old male who swallowed a mixture of cobalt and chloride from a chemistry set (compounds not specified) showed no symptoms before or after stomach lavage (Jacobziner and Raybin 1961).

Enlarged thyroid glands have been reported in children given cobalt chloride for treatment of anemia. However, the thyroid glands returned to normal size upon cessation of treatment (Chamberlain 1961; Little and Sunico 1958; Sederholm et al. 1968; Washburn and Kaplan 1964). Patch testing of children aged 4–14 years revealed that 13 out of 45 girls and 3 out of 26 boys reacted to cobalt chloride with contact dermatitis (Romaguera and Vilaplana 1998). A review of the literature suggests that the effects of cobalt may not be the same for all humans. Individuals, including children, who do not have functioning kidneys, suffer from sepsis, or have sickle-cell disease could have higher levels of free cobalt in organ tissues due to either a decrease in serum albumin levels or an increase in serum ischemia-modified

albumin, which might result in a stronger response to cobalt at doses that would not adversely affect healthy individuals (Paustenbach et al. 2013). Jin et al. (2018) analyzed the 2003–2012 National Health and Nutrition Examination Survey (NHANES) data for an association between urinary mineral cation concentrations and estimated glomerular filtration rate and reported that a decrease in renal function (decrease in filtration rate) was associated with a decrease in cobalt in urine.

Individuals who are sensitized to cobalt could be at risk for developing cobalt-induced asthma (Shirakawa et al. 1988, 1989). Sensitization to cobalt in hard metal workers causes cobalt-specific increases in serum antibodies (IgE and IgA) resulting in the development of hard metal asthma (Bencko et al. 1983; Shirakawa et al. 1988, 1989). Two studies by Potolicchio et al. (1997, 1999) suggested that the presence of a polymorphism (for glutamate 69 in the β chain) in the HLA-DP gene might increase susceptibility to hard metal lung disease. Following oral exposure, individuals with iron deficiency could also have an increased risk, as both human and animal studies have shown increased absorption of cobalt compounds in iron-deficient animals and humans (Barany et al. 2005; Meltzer et al. 2010; Reuber et al. 1994; Schade et al. 1970; Sorbie et al. 1971; Valberg et al. 1969).

Developmental effects have not been observed in animals exposed only during gestation, even at maternally toxic oral exposure levels (Paternian and Domingo 1988; Seidenberg et al. 1986). However, effects have been reported in animals following oral exposure during both pre- and postnatal developmental periods, including impaired growth and survival as well as systemic effects similar to those observed in adult animals (e.g., hematological effects) (Danzeisen et al. 2020a; Domingo et al. 1985b; Gluhcheva et al. 2020). Since these findings were examined only in a limited number of studies at a limited number of doses and/or were often observed at doses associated with maternal toxicity or at doses comparable to adult toxicity, it is unknown if the developing fetus or infant will be more susceptible to cobalt toxicity compared to an adult.

Genetic polymorphisms may infer differential susceptibility to risk of cancer from exposure to high levels of cobalt (e.g., occupational exposure). Mateuca et al. (2005) evaluated potential associations between polymorphisms associated with reduced capacity for DNA repair and chromosomal abnormalities (micronucleated mononucleates or binucleates), DNA damage, and oxidative DNA damage (urinary 8-OHdG) in workers exposed to cobalt or hard metal (tungsten-cobalt) and in unexposed controls. Genes evaluated included those involved in base-excision (hOGG1, XRCC1) and double-strand break (XRCC3). The analysis showed that the *XRCC3*^{241} and *hOGG*^{1326} variants were associated with increased micronucleated mononucleates in both the exposed workers and the total study population (workers and

controls. The Arg/His or His/His *XRCC*^{1280} variant was associated with increased DNA damage in the total study population.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for cobalt from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to cobalt are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by cobalt are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Levels of cobalt in the blood, feces, and urine can be used to indicate exposure to this chemical. Most of the data for this response come from occupational studies. Non-occupational studies are available; however, although they provide internal dose metrics (e.g., cobalt levels in urine and/or blood), information regarding external exposure levels is typically not available. Typical background levels (geometric mean) of urinary cobalt in humans ranged from 0.32 to 0.42 µg/L and blood cobalt was 0.151 µg/L (Hoet et al. 2013). Elevated levels of cobalt have been measured in the blood after supplementation of inorganic cobalt (Finley et al. 2013; Tvermoes et al. 2013, 2014).

Goldoni et al. (2004) measured cobalt in the exhaled breath of hard metal workers and found cobalt in the exhaled breath at 11.9–741 nanomoles/L, with levels higher at the end of the shift. Conversely, another study reported that exhaled breath concentrations of cobalt were not correlated to workplace air concentrations, which may limit its usefulness as a biomarker (Broding et al. 2009).

Wahlqvist et al. (2020) examined the relationships between concurrent inhalation and dermal exposure to cobalt particulates in the air to blood and urine levels in hard metal workers. Exposure to cobalt in the air was correlated with both cobalt in blood and urine, whereas dermal exposure was correlated with blood but not urine. Cobalt uptake was higher than expected based on low air concentrations, and the study authors suspected that it was from oral co-exposure from eating with unwashed hands and using oral tobacco products while working. Klasson et al. (2017) also reported associations in hard metal workers between blood cobalt levels and measured cobalt levels in the air and on the skin. Kettelarij et al. (2018b) reported a strong association between measured air levels of cobalt and urinary cobalt levels in hard metal workers. Associations were also observed between cobalt deposits on the skin (measured pre- and post-shift) and urinary cobalt levels; however, the association was stronger pre-shift compared to post-shift, potentially due to continued absorption from the previous workday. Kettelarij et al. (2018b) suggested that these data indicate that urine may be a good biomarker of dermal exposure to cobalt. However, based on low solubility of cobalt dust, and a weaker association between urinary cobalt and post-shift skin cobalt levels despite much higher post-shift skin levels, urinary cobalt levels are more likely reflective of inhalation exposure and potential oral exposure (via hand-to-mouth transfer during eating and/or smoking). Hutter et al. (2016) reported urinary cobalt levels of 200 µg/L at an exposure of 1 mg/m^3 cobalt in air based on scant information (147 air samples, 1,166 urine results for 253/1969 workers, during 27 years). The study authors proposed that results indicated oral co-exposure because urinary

cobalt was higher in smokers than nonsmokers, based on exposure via the "dust-hand cigarette-mouth path." Common among these studies was that inhalation exposure dominated the relationship with both blood or urine levels, dermal cobalt was incompletely removed by washing, and the impact of oral co-exposure on dermal uptake assessments was unclear.

Earlier studies reported associations between cobalt exposure and cobalt levels in blood and urine (Alexandersson 1988; Ichikawa et al. 1985; Lison et al. 1994; Nemery et al. 1992; Scansetti et al. 1985). Occupational exposure to 0.1 mg/m^3 cobalt resulted in blood levels of cobalt of 0.57–0.79 μg/dL and urinary levels of 59–78 μg/L (Ichikawa et al. 1985). Timing of biomarker measurement may be important. Apostoli et al. (1994) reported that for workers in hard metal manufacturing, urinary cobalt increases rapidly postexposure, peaking 2–4 hours after the workday ended and decreasing thereafter over time. Correlations between recent worker exposure and cobalt levels in the blood or urine are more consistent for exposure to soluble cobalt compounds than for less-soluble compounds (Lison et al. 1994).

Elevated cobalt levels have also been identified in hair and toenail samples of populations living near a mine tailings repository in Zambia, with mean levels of 0.9 and 1.0 mg/kg, respectively (Nakaona et al. 2020). Levels measured and hair and toenails were positively correlated with each other. According to the study authors, hair and toenail levels were elevated, but they did not correlate with exposures from food or water; levels in the air were not evaluated (Nakaona et al. 2020). Based on available data, it is unclear if either hair or nails are reliable biomarkers of exposure. Elevated cobalt levels were also observed in toenail samples of children living near mine tailings in Western Uganda (2.21 mg/kg), compared to referents living >400 km from the mine (0.49 mg/kg); however, findings were similar between adults living near the mine (0.37 mg/kg) and referents (0.42 mg/kg) (Mwesigye et al. 2016). Mwesigye et al. (2016) noted that presence of dust was prevalent in toenails, even after washing; therefore, toenails may be unreliable biomarkers of exposure via food and water contamination from mine tailings in human populations in which subjects' feet are frequently directly exposed to contaminated soil. Ren et al. (2020) also noted the importance of thorough and efficient washing strategies when using hair samples for metal(loid) exposure analysis to reduce interference for ambient air pollution. Additionally, care should be taken to prevent the washing technique from stripping cobalt from within the hair and toenails. Seasonal patterns have also been observed for metals and other trace elements in toenails collected in women the United States, including cobalt, with peak levels observed in summer months (Wojcik et al. 2024). Increased levels in toenail samples (not correlating to increased environmental levels) may be due to changes in footwear in the summer, use of nail polish in warmer months (which may trap trace elements), and/or seasonal variation in nail growth. Regardless of the mechanism, based

upon these findings, the study authors suggested that studies using toenail concentrations as biomarkers of exposure should correct exposure levels for the season during which the toe clipping were collected.

3.3.2 Biomarkers of Effect

No cobalt-specific biomarkers of effects resulting from cobalt toxicity were identified. Diminished respiratory function and polycythemia are the most notable signs of cobalt toxicity. Diminished respiratory function in humans includes decreased values for the FEV_1 and FVC lung parameters, in particular a decrease in FEV_1/FVC ratio, plus increased cough, dyspnea, and sputum. The FEV_1 and FVC measures correlated with urinary cobalt concentrations (Gennart and Lauwerys 1990; Kusaka et al. 1986a). Elevated levels of CC16, a lung surfactant protein, is a proposed biomarker for identification of early lung damage in workers with dust and chemical exposures due to its correlation with pulmonary inflammation and epithelial damage (Andersson et al. 2020). Elevated serum CC16 levels were associated with increasing cumulative exposure to inhalable cobalt in Swedish hard metal workers; cumulative exposure levels in this cohort were not associated with decrements in lung function (Andersson et al. 2020).

Oral exposure to cobalt caused increases in hemoglobin and hematocrit in humans (defined as polycythemia by the study authors) (Davis and Fields 1958). Increases in hemoglobin and hematocrit were also observed in animal studies after inhalation exposure (NTP 1991, 1998, 2014). Finley et al. (2012b) examined the human and animal toxicology and reported that the blood cobalt levels exceeded 300 μg/L. In another study by the same study authors, polycythemia and reduced iodide uptake were reported (Finley et al. 2012a). Additionally, monitoring of cobalt-specific changes in serum antibodies (IgE and IgA) could indicate that sensitization to cobalt occurred (Bencko et al. 1983; Shirakawa et al. 1988, 1989). More research is needed in this area to identify biomarkers of effect after exposure to cobalt as changes in respiratory function, development of polycythemia, and changes in serum antibodies are not unique to cobalt-induced toxicity.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Animal studies suggest that exposure to cobalt affects metal ion metabolism. Zaksas et al. (2013) administered cobalt chloride to mice and measured the effect of cobalt on several mineral ions in plasma. Cobalt increased the plasma levels of iron, magnesium, aluminum, and silicon, while reducing the amount of boron. Since cobalt binds to plasma transferrin, which also binds iron, the potential exists for cobalt to

affect iron transport or metabolism. Cobalt can adversely affect the metabolism of other essential minerals by competing for binding sites, altering signal transduction, and affecting protein biosynthesis (Zaksas et al. 2013). Skalny et al. (2021) evaluated the effect of cobalt chloride exposure on tissue distribution of metal ions in immature mice (see Section 3.12 for details on the experiment). A time- and dose-dependent effect by cobalt chloride on tissue levels of copper, iron, manganese, and zinc was reported. The study authors suggested that exposure to cobalt alters essential mineral metabolism. Moshtaghie et al. (2004) evaluated the competition between cobalt and iron in binding to human serum transferrin. Iron binding to human serum transferrin was reduced by 20% when cobalt ions were present, and iron uptake was reduced by 30%, indicating competition for binding.

Studies suggest an adverse impact of cobalt ions on calcium ions. Soluble cobalt can block inorganic calcium uptake channels, limiting calcium influx into cells. This effect may be linked to a reduction of steroidogenesis in mouse Leydig cells (Henquin et al. 1983; Moger 1983; Yamatani et al. 1998). Cobalt can alter calcium influx for mice into liver cells following exposure to glucagon (Yamatani et al. 1998) and pancreatic β cells (Henquin and Lambert 1975) and for rats into isolated pancreatic islet cells (Henquin and Lambert 1975). Cobalt might also affect neuromuscular transmission through antagonism with calcium ions (Weakly 1973).

An *in vivo* study designed to determine the effects on ribonucleic acid (RNA) expression patterns using human bronchial epithelial cells exposed to cobalt, lead, and cadmium concurrently reported four specific alterations in RNA expression patterns associated with cell cycle regulation: oxidative stress response; GSH metabolism and steroidogenesis; and xenobiotic metabolism (Glahn et al. 2008).

Radioactive cobalt-57, in combination with bleomycin, is used in cancer treatment as a therapeutic agent (Goodwin and Meares 1976; Hansen et al. 1976; Kapstad 1978, 1979; Li et al. 2018). When used in combination, the anti-tumor effects are amplified. The bleomycin cobalt ion combination acts by binding to and cleaving the DNA in tumor cells (Kakinuma and Orii 1982). However, bleomycin has been associated with adverse effects (hair loss, emesis, weight loss, pneumonitis, fibrosis, and loss of lung diffusion capacity for carbon monoxide) (Li et al. 2018).

Cobalt chelators have been tested in rats to evaluate their mitigation potential in reducing the toxic effects of cobalt (Baker and Czarnecki-Maulden 1987; Domingo et al. 1983; Llobet et al. 1988). In rats previously exposed to cobalt, urinary excretion of cobalt was increased by treatment with GSH and diethylenetriaminepentaacetic acid and fecal excretion of cobalt was increased by treatment with EDTA

and 2,3-dimercaptosuccinic acid. Treatment with N-acetyl-L-cysteine (NAC) increased both urinary and fecal excretion of cobalt. The amino acid, cysteine, also reportedly reduced the toxicity of cobalt in chicks (Baker and Czarnecki-Maulden 1987). British anti-Lewisite bound to hyaluronic acid (BAL-HA) has also been shown to be an effective chelator *in vitro*; this particular chelator was developed to reduce toxicity of cobalt ions in synovial fluid following metal-on-metal joint replacement surgeries (Ude et al. 2023).

An interrelationship between cobalt and nickel sensitization has been reported in individuals exposed to the two metals. The dermatological impact is greater in individuals sensitized to both metals (Rystedt and Fisher 1983; Veien et al. 1987). One animal study using guinea pigs showed some interaction between nickel and cobalt (Wahlberg and Liden 2000). Studies of cultured alveolar type II cells showed a synergistic (greater-than-additive) response with co-exposure to cobalt and nickel chlorides (Cross et al. 2001). Bonefeld et al. (2015) reported that mice dermally exposed to a mixture of nickel and cobalt had increased immune response to both metals in combination than to either metal alone.

Hard metal dusts, consisting of 5–10% cobalt with the balance being tungsten carbide, were considerably more toxic than cobalt or tungsten carbide particles alone (Harding 1950). The increase in toxicity could be the result of the oxidation of cobalt metal to ionic cobalt, which results in increased solubility of cobalt and leads to the generation of active oxygen species (Lasfargues et al. 1995; Lison et al. 1995, 1996).