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

3.1 TOXICOKINETICS

Studies on toxicokinetics of beryllium in humans are scarce and solely based on indirect measurement of exposure. No available toxicokinetic models have been published that simulate the absorption, distribution, and elimination of beryllium from the human body. Most studies on toxicokinetics of beryllium are in animals.

- Absorption
  - Most beryllium is absorbed via the lungs, particularly among occupationally exposed individuals. Data are not available on the rate and extent of absorption of inhaled beryllium in humans.
  - In animal models, significant absorption of soluble beryllium (e.g., beryllium salts) was observed, compared to insoluble beryllium (e.g., beryllium oxide).
  - Beryllium binds to proteins and nucleic acids within the epidermis, making absorption through intact skin unlikely.
  - No studies were located regarding absorption in humans after oral exposure to beryllium or its compounds. However, human ingestion of beryllium is thought to occur inadvertently via hand-to-face activity following dermal handling of beryllium, or because of mucociliary transport of inhaled beryllium out of the respiratory tract and to the gastrointestinal tract followed by ingestion or by mixed exposure. In animals, beryllium and its compounds are poorly absorbed from the gastrointestinal tract.
- Distribution
  - The lung and respiratory tract are the primary target of inhalation exposure for animals and humans.
  - Absorbed beryllium is distributed throughout the body independent of exposure route. The extent of distribution is dependent on beryllium species, particle size, and solubility.
  - The accumulation of beryllium after absorption through the lung or GI tract is duration specific. Beryllium can accumulate in the liver, lungs, lymph nodes, and bones.
  - Beryllium can be transferred across the placenta.

- Metabolism
  - Beryllium and its compounds are not biotransformed, but soluble beryllium salts are partially converted to less soluble forms in the lung.
- Excretion
  - Beryllium is slowly cleared from the lung; clearance half-lives range from days to years in animals. Pulmonary clearance of beryllium is multiphasic where a fast clearance rate is followed by slower elimination rates.
  - Beryllium can be excreted via breast milk.
  - In animal studies, 99% of beryllium is excreted in feces and <1% is excreted in urine after oral exposure.

3.1.1 Absorption

Limited data are available regarding beryllium absorption via inhalation in humans. Deposition and clearance of beryllium are affected by the form of beryllium, the solubility, size of the inhaled particle, and dose (WHO 2001).

Two case studies of accidental human inhalation exposure suggest potential lung deposition of beryllium. Due to an accidental leakage of beryllium dust in a laboratory, 25 people were exposed to an undetermined concentration for 10–20 hours. The day after exposure, mean serum beryllium levels were 3.5±0.47 ppb beryllium, compared to 1.0 ppb in unexposed controls. Six days later, the mean serum level of the exposed group had decreased to 2.4±0.3 ppb beryllium (Zorn et al. 1986). After accidental exposure of eight male workers to <8 ng Be/m^3 as beryllium chloride for 4–6 hours/day for 10 days, beryllium levels in urine and blood increased 4-fold above normal levels (1 ng Be/g) in urine and blood of unexposed individuals (Stiefel et al. 1980).

Soluble beryllium compounds are absorbed more readily than insoluble beryllium compounds. For example, approximately 20% of the initial lung burden was absorbed following inhalation or intratracheal instillation of soluble beryllium salts. However, absorption was slower and less substantial for similar administration of the less soluble compound, beryllium oxide (Delic 1992; WHO 2001). Studies in guinea-pigs and rats indicate that 40–50% of inhaled soluble beryllium salts are absorbed and retained in the respiratory tract (Delic 1992; HSE 1994; WHO 2001). Soluble beryllium salts can become stored in inflammatory scar tissue, or insoluble precipitates can be formed (Reeves and Vorwald 1967).

Calcination affects absorption of beryllium from the lungs in beagle dogs. More calcined (at 1000°C) BeO (62%), was retained in the lungs 180 days after exposure than BeO calcined at 500°C (17%).

Many studies have demonstrated that the lung is the primary target of inhalation exposure in animals and humans. Beryllium in the atmosphere is mainly particulate matter, and deposition in the lungs depends on particle size (especially aerodynamic diameter), form, and solubility (IRSST 2012). Particle size influences deposition rates in humans and in mice, with mice exhibiting a wider variation in rates than humans. The rate of pulmonary deposition between mice and humans for total particles (Be and BeAl) differed by a factor of 20 with humans having the higher deposition rate based on experimental data from IRRST (2012). For fine particles (Be, BeO, BeAl), the rates of deposition between humans and mice were dependent of the form of beryllium. For beryllium, the deposition rate was 1.2 times higher in humans than in mice and 22.5 times higher in humans for BeAl. IRSST (2012) reports mice with deposition rate five times higher than in humans for BeO (Table 3-1). The lung concentration of fine particulate BeO was significantly higher than the other fine particulate beryllium forms.

Table 3-1. Percentage of Lung Deposition as a Function of MMAD

Chemical form	MMAD (µm)	Rate of pulmonary deposition in mice (%)	Rate of pulmonary deposition in humans (%)
Be-T	4.1	0.5	10
Be-F	1.5	11	15
BeO-F	0.4	40	8
BeAl-T	6.5	0.25	5
BeAl-F	4.4	0.4	9

Source: IRSST 2012
MMAD = Mass median aerodynamic diameter; T = Total particulate matter; F = Fine particulate matter; Rates determined using data from Raabe et al., 1988 and IRSST, 2006

Genotoxic actions in metal toxicity often are a result of the ion form. Strupp (2011a) compared the dissolution behavior of beryllium metal and beryllium chloride (a soluble beryllium compound) in an ion formation test. The conditions were designed to simulate inhaled beryllium metal in the human lung (Strupp 2011a). Beryllium chloride dissolved immediately up to the limit of solubility in the normal lung medium and ~90% in the lysosomal fluid with the lower pH, and the amount dissolved did not significantly increase over 28 days. In contrast, beryllium metal increasingly dissolved over time in both fluids, but remained lower than the amount of beryllium chloride that dissolved. The data indicate that dissolution kinetics of the soluble forms and the metal are largely different (Strupp 2011b).

Stefaniak et al. (2012) investigated solubilization of 17 beryllium-containing materials (ore, hydroxide, metal, oxide, alloys, and process intermediates) using artificial human airway epithelial lining fluid. Beryllium-containing particles deposited in the respiratory tract dissolved into the artificial lung epithelial lining fluid and created ions that can be absorbed in the lung and interact with immune cells resulting in

sensitization. The highest releases, based on mass, of ionic beryllium were observed from the beryl ore particles (from 3.88% for the smaller size particle samples to 11.78% for the larger size particles) in 7 days, while release from the other beryllium sources was < 1%. Dissolution half-times ranged from 30 days (reduction furnace material) to 74,000 days (hydroxide). The rapid clearance of beryllium ions leads to an increased release in the respiratory tract via dissolution in airway lining fluid (Stefaniak et al. 2012).

In rats exposed to an aerosol of beryllium sulfate (Reeves and Vorwald 1967) accumulation of beryllium was found in either the lungs or tracheobronchial lymph nodes. Half of the initial pulmonary load was cleared quickly; the rest was retained in the lungs for longer periods and may have been incorporated into pulmonary cell nuclei. Beryllium accumulation in tracheobronchial lymph nodes was greater in males than in females. The authors suggest that body weight only partially contributed to the difference. Males exhibited a greater enlargement of the lymph nodes than females. According to the authors, this indicates that there were sex differences associated with beryllium exposure where males were more sensitive than females (Reeves and Vorwald 1967).

Beryllium is retained and accumulated in the lungs upon repeated exposure. Pulmonary accumulation of beryllium after repeated chemical exposure could lead to beryllium sensitization, and potentially the development of CBD (Benson et al. 2000). For example, C3H/HeJ mice exposed to beryllium metal for several months resulted in beryllium lung burdens of generally >20 μg/lung with the development of lesions that had Be-containing macrophages, granulomatous pneumonia, lymphocytic interstitial aggregates, and mononuclear interstitial infiltrates (Finch et al. 1998; Nikula et al. 1997). Rodent vs. human responses differ. Humans develop fibrosis and heart enlargement with CBD, and rodents do not.

Studies suggest that beryllium is unlikely to be systemically absorbed through intact skin because beryllium binds to proteins and nucleic acids of the epidermis, leading to poor diffusion. Beryllium has been demonstrated to bind to alkaline phosphatase and nucleic acids in guinea pig epidermis *in vitro* (Belman 1969). This binding could account for the inefficient transfer of beryllium from the epidermis to the blood. Skin exposure to soluble beryllium salts can lead to beryllium sensitization in humans (Curtis 1951).

Skin ulceration in workers exposed to beryllium occurred when skin was accidentally cut or abraded (Williams et al. 1987). Beryllium exposure on injured skin may allow a larger fraction of the applied dose to be absorbed into the body, compared to intact skin. Ivannikov observed a significant absorption of beryllium into systemic circulation during a 24-hour exposure to $BeCl_2$ applied directly to the skin of live animals with three types of wounds: 7.8 - 11.4% for abrasions (superficial skin trauma), 18.3 - 22.9% for cuts (skin and superficial muscle trauma), and 34 - 38.8% for penetrating wounds (deep muscle trauma)

(Ivannikov et al. 1982). In situations where both skin injury and the level of dermal exposure to beryllium-containing dust are high, dermal exposure to beryllium may contribute to systemic exposure (Deubner et al. 2001b).

The beryllium occupational air standard is intended to protect workers from high inhalation exposures. However, beryllium is also slightly absorbable through the skin, particularly if the skin is broken. Skin absorption of beryllium may create skin ulcers and damaged skin can increase the amount of beryllium absorbed as described further below (Kreiss et al. 2007).

CBD cases at a copper-beryllium alloy finishing facility were more likely to have reported ulcers or small craters in the skin, compared to employees without CBD (IRSST 2012; Schuler et al. 2005), suggesting a possible role of skin contact in sensitization, especially following exposure to fine particles. In an *in vitro* study, Tinkle et al. (2003) observed that fine particles (0.5- and 1.0-μm) penetrated the stratum corneum, epidermis, and dermis of human skin, thereby initiating an immune response when beryllium particles were <1 μm in diameter with the skin in motion. Topical application of beryllium salts and beryllium oxide to C3H mice generated beryllium-specific cutaneous sensitization (Tinkle et al. 2003). These examples suggest that skin contact with poorly soluble beryllium oxide particles can cause beryllium sensitization in animal and humans.

Deubner et al. (2001c) calculated the expected amount of workplace beryllium exposure through intact and damaged skin, and then compared this to the expected dose from inhalation of workplace air (Kent et al. 2001), or to the OSHA exposure limit (2 μg/m^3) established at that time (Table 3-2). These calculations assume a dermal loading rate of beryllium on skin of 0.43 μg/cm^2, based on the studies of loading on skin after workers performed cleaning duties (Sanderson 1999), multiplied by a factor of 10 to approximate the workplace concentrations, and the very low absorption rate of 0.001 percent.

Table 3-2. Hypothetical Calculation of Workday Beryllium Doses: Dermal Versus Inhalation

	Dermal (μg/workday)		Inhalation (μg/workday)
	Undamaged skin	Damaged skin	
Lower limit	0.0036	0.0318	0.116
Maximum value	1.68	5.36	1.63

Source: Deubner et al. 2001c

No studies were located regarding absorption in humans after oral exposure to beryllium or its compounds. Accidental ingestion of beryllium can be assumed from hand-to-face activity following dermal loading of beryllium or as a result of mucociliary transport of inhaled beryllium out of the

respiratory tract and to the gastrointestinal tract, with the former being more likely (Table 3-3). Actual loading and ingestion of beryllium in the workplace is not known, thus several assumptions are incorporated into calculations to approximate the assumed exposures.

Table 3-3. Hypothetical Calculation of Workday Beryllium Doses: Ingestion versus Inhalation

	Ingestion (μg/workday)					Inhalation (μg/workday)
	Hand to mouth	Tracheobronchial-mucociliary-aided ingestion	Head airways ingestion	Total dose via ingestion	Alternative ingestion scenario[a]	
Lower limit	0.0330	0.0003	0.0032	0.0365	10	0.116
Maximum value	4.02	0.0041	0.0865	4.11	20	1.63

Source: Deubner et al. 2001*c*

Beryllium and its compounds are poorly absorbed from the gastrointestinal tract in animals. The amount absorbed depends on the dose and solubility of the compounds and is limited by the formation of insoluble colloidal phosphate in the intestine (IRSST 2012).

Less than 1% of radiolabeled beryllium in mice, rats, monkeys and dogs was absorbed from the gut after oral dosage. The lower large intestine received the greatest amount of radiation from ingestion of the radionuclide. The amount of Be^7 in the urine indicates the degree of absorption. In rats, mice, dogs, and monkeys, intestinal absorption of beryllium varies, but in general, beryllium was poorly absorbed. The urinary output of rats, mice, dogs, and monkeys was 0.11, 0.24, 0.38, and 3.71% of the total dose, respectively, with most of the radiolabel excreted in the feces (104, 98, 107, and 108%, respectively) (Furchner et al. 1973). Urinary excretion was 0.9 and 0.2% beryllium when administered to male Sprague-Dawley rats at 0.019 and 0.19 mg beryllium/kg/day via drinking water for 24 weeks, while 60 – 90% appeared in the feces. Oral absorption of beryllium and its compounds may be reduced by the formation of beryllium phosphate precipitates in the alkaline environment of the intestine (Reeves 1965). Rats exposed to 31 mg beryllium/kg/day as beryllium sulfate in drinking water for 2 years excreted very little beryllium via the urine (Morgareidge et al. 1975).

3.1.2 Distribution

Following inhalation exposure, beryllium can be retained and may accumulate in lung tissue upon repeated exposure (Benson et al. 2000). Absorbed beryllium primarily transports and distributes via the bloodstream and has been found to widely distribute to various organs in animals. The size, form, and route of exposure of beryllium affects levels of accumulation among various tissues and organs.

A study of children living in southern Poland measured the concentration of beryllium in the pharyngeal tonsils of 379 children (176 girls, 203 boys) between the ages of 2 and 17 years (median 6.0 years) (Nogaj et al. (2014). The average concentration found in pharyngeal tonsil samples was 16 ng/g with a range of 1 to 58 ng/g. The mean concentration of beryllium was higher ($p<0.05$) in girls than in boys, and concentrations also varied significantly by location.

After absorption of beryllium, short-term accumulation happens in the liver, especially when concentrations are high. In the long term, beryllium distributes to the lymph nodes and bones (IRSST 2012). The half-life of skeletal beryllium has been estimated at 450 days (WHO 2001). A study by Krachler et al. (1999a) provides evidence that beryllium is transferred across the human placenta and excreted via breast milk. Beryllium has been identified in human umbilical cord and maternal blood (IRSST 2012). The levels of beryllium in umbilical cord serum and in colostrum were higher than in maternal serum (Krachler et al. 1999a).

In the past, beryllium concentrations in several human organs have been reported as follows: 0.21 ppm in lungs; 0.08 ppm in brain; 0.07 ppm in both the kidney and spleen; 0.04 ppm in each of liver, muscle, and vertebrae; 0.03 ppm in heart; and 0.02 in bone (Meehan and Smythe 1967). Further details regarding the source of the organs were not provided. In eight males accidentally exposed to <8 ng beryllium/m^3 as beryllium chloride for 4–6 hours/day for 10 days, the beryllium levels in urine and blood increased 4-fold above the levels of <1ng beryllium/g of either blood or urine in unexposed individuals. Beryllium concentration in serum reached a steady state 10 hours after exposure. Since transport and distribution of beryllium occurs in the blood, the authors also evaluated the distribution of beryllium in individual components of the blood and reported that 60-70% of beryllium was bound to two serum proteins: prealbumin and γ-globulin (Stiefel et al. 1980).

Rats exposed to 34.25 μg beryllium/m^3 as an aerosol of beryllium sulfate 7 hours/day, 5 days/week for 72 weeks achieved steady state concentrations in the lungs in 36 weeks of exposure. The beryllium concentration in tracheobronchial lymph nodes peaked between 36 and 52 weeks and decreased thereafter (Reeves and Vorwald 1967). Beryllium concentrations in serum and urine samples of Wistar rats and guinea pigs exposed to 2–40 mg beryllium/m^3 as an aerosol of beryllium nitrate for 16 hours, were up to 36 and 300 ng Be/g, respectively.

Concentrations of beryllium in the blood of guinea pigs increased exponentially and reached a steady-state after 8–12 hours of exposure with a concentration in serum of 10 ng Be/g (Stiefel et al. 1980). In rats and hamsters exposed to an aerosol of beryllium oxide particles, initial alveolar depositions ranged from

12 µg to 160 µg beryllium with a retention half-life of about 6 months. Only the pulmonary lymph nodes accumulated detectable amounts of translocated beryllium oxide (Sanders et al. 1975).

Beryllium tissue burdens were examined in C3H/HeJ male mice exposed to 0 or 250 µg/m^3 fine particle of (<5 µm) beryllium as beryllium metal, beryllium oxide (BeO), or beryllium aluminum (BeAl) via nose-only inhalation chamber for 6 hours/day, 5 days/week for 3 weeks and sacrificed 1 week after exposure termination (Muller et al. 2010; 2011; IRSST 2012). Mass median aerodynamic diameter (MMAD) was measured using a Marple Personal Cascade Impactor with the smallest reading for BeO (0.41±0.03 µm) and the largest for BeAl (4.40±1.64 µm). As compared to controls, significant increases in beryllium concentrations were observed in the spleen, liver, kidney, lung, and blood. Significantly higher levels of beryllium in the liver, kidney, and blood and lower levels in the spleen were found in the BeAl group as compared to the other beryllium groups. No differences in beryllium levels in spleen, liver, kidney, or blood were found between the Be metal and BeO groups.

Pulmonary beryllium concentrations were significantly different in the three beryllium groups; the highest concentration was found in the BeO group and the lowest concentration was found in the BeAl group. The pulmonary concentration in the mice exposed to fine particulates was highest in the BeO group at approximately 25 times more than the BeAl group (~3,500 ng/g) and four times higher than the Be group (~15,000 ng/g) . In the lung, mice were exposed to fine (4.4±1.64 µm) and larger particles of BeAl (MMAD 6.5±1.96 µm), there was an almost 3-fold increase in fine particle vs. larger particle concentrations (IRSST 2012).

Distribution from the lungs to other organs is dependent on the form of beryllium. In beagle dogs exposed to BeO calcined at 500°C, 14% and 8.8% of the initial lung burden was found in the skeleton and tracheobronchial lymph nodes, respectively 64 days post exposure. After 180 days, 16% of the initial lung burden was found in the skeleton, which was comparable to the amount retained in the lungs. The liver also saw increases in beryllium over time. In contrast, the distribution of BeO calcined at 1000°C 64 days post exposure was different than BeO calcined at 500°C. The lungs retained 88% of the initial lung burden, 1.9% was found in the tracheobronchial lymph nodes and 1.5% in the skeleton. At 180 post exposure, 62% of the initial lung burden was still present. The difference in distribution was attributed to the greater solubility of BeO calcined at 500°C (Finch et al. 1990; Finch et al. 1988; Haley et al. 1989).

Wagner et al. (1969) also found that after exposure to beryl ore, concentrations were highest in lungs followed by the skeleton, liver, and kidney. Beryllium sulfate distribution is affected more by solubility and absorption rate than are beryllium oxide or beryllium ores (Wagner et al. 1969). Wagner et al. (1969) and Stokinger et al. (1950) exposed different species, including mice, hamsters, guinea pigs, dogs, cats,

monkeys, goats, rats and rabbits, via inhalation to beryllium (Table 2-1) and found little variation in the beryllium distribution with the highest amounts retained in the lungs or tracheobronchial lymph nodes, followed by femur, spleen, liver, and kidney. Only the pulmonary lymph nodes accumulated detectable amounts of translocated BeO. Seven days post exposure, no detectable beryllium concentrations appeared in the liver, skeleton, or urine of rats or hamsters exposed to BeO (Rhoads and Sanders 1985; Sanders et al. 1975).

No studies were located regarding distribution in humans after oral exposure of beryllium or its compounds. In the case of inhalation, a portion of the inhaled material is transported to the gastrointestinal tract by the mucociliary escalator or by the swallowing of the insoluble material deposited in the upper respiratory tract (Kjellström and Kennedy 1984). Beryllium is thought to be poorly absorbed from the gastrointestinal tract in animals; however, beryllium that is absorbed is distributed to the organs and tissues. Beryllium was found in the liver, large intestine, small intestine, kidneys, lungs, stomach, and spleen in hamsters given beryllium sulfate, beryllium oxide, or beryllium metal in the diet for 3–12 months (Watanabe et al. 1985). Beryllium was retained in the gastrointestinal tract of mice exposed to a radioactive dose of beryllium chloride by gavage. The amount found in the tissues other than intestinal was <0.1%. Three hours after exposure, the accumulation of radioactivity was greatest in the liver followed by the kidney, mesenteric lymph nodes, lungs, blood, and carcass (LeFevre and Joel 1986).

After exposure of male Sprague-Dawley rats to beryllium sulfate at 0.019 and 0.190 mg beryllium/kg/day via drinking water for 24 weeks, beryllium content ranged from 1 to 3 μg, most of which appeared in the bones followed by blood and liver. The pattern of beryllium distribution to rat tissues and organs indicated that as the exposure duration increases, accumulation levels also increase (Reeves 1965).

Other studies indicate that in animals, high levels of beryllium accumulate in bone as a result of oral exposure to the chemical or its compounds. In rats treated by gavage with radioactive beryllium chloride, the greatest accumulation (other than that in the gastrointestinal tract) was detected in the bone, followed by viscera, pelt, and muscle (Furchner et al. 1973). Beryllium accumulation in the bones of rats exposed for 2 years to dietary concentrations of the chemical was proportional to the administered dose (Morgareidge et al. 1975).

No studies were located regarding distribution in humans or animals after dermal exposure to beryllium or its compounds. The lack of data is expected because beryllium is poorly absorbed after dermal exposure. Intraperitoneal injection of beryllium sulfate in rats elicited brain accumulation of beryllium in a dose-dependent manner (Drobyshev et al. 2019).

3.1.3 Excretion

Excretion of absorbed beryllium is generally via urine, whereas unabsorbed ingested beryllium is excreted through the feces (WHO 2001). In eight men accidentally exposed to <8 ng beryllium/m^3 as beryllium chloride 4–6 hours/day for 10 days, urinary levels were four times higher than the average levels of <1.0 ng Be/g in unexposed individuals (Stiefel et al. 1980).

Concentrations of Be measured in the exhaled breath condensate (Be-EBC) and urine of workers exposed occupationally to 0.015 – 0.354 μg m^{-3} cumulative beryllium exposure index (CBEI) in an aluminum production plant indicates inhalation exposure (Hulo et al. 2016). Concentrations of 1.01±0.16 (mean± standard error) ng Be/L in the EBC of exposed subjects after adjustment for smoking status were higher than in controls (0.62±0.08 ng/L). Urinary concentrations of 14.85±3.61 ng g $creatinine^{-1}$ in workers and 15.16±2.88 ng g $creatinine^{-1}$ in controls were not substantially different between groups. Metal concentrations measured in EBC of workers were not correlated with concentrations in their urine (Hulo et al. 2016). Due to an accidental leakage of beryllium dust in a laboratory, 25 people were exposed to an undetermined concentration for 10–20 hours. The mean serum level of the exposed group had dropped to similar levels as the unexposed group two to eight weeks after exposure, suggesting a biological half-life of 2 to 8 weeks (Zorn et al. 1986). The detection of beryllium in the lungs ~30 years after occupational exposure to beryllium powder and dust for ~6 years in a fluorescent light bulb factory indicated that beryllium can be recovered from the lungs of workers with CBD years after exposure has ceased (Verma et al. 2003).

Inhaled beryllium is cleared from the respiratory tract by various mechanisms. More soluble forms of beryllium are cleared by absorption, whereas less soluble or insoluble forms may reside in the lungs for many years after exposure. Clearance mechanisms for less soluble or insoluble forms of beryllium depend on the deposition location in the respiratory tract (Table 3-4).

Table 3-4. Clearance Mechanisms for Less Soluble and Insoluble Forms of Beryllium

Location	Clearance Mechanism
Nasal Passages	Sneezing, mucociliary transport, dissolution
Tracheobronchial region	Coughing, mucociliary transport, phagocytosis, dissolution
Alveolar	Phagocytosis, translocation, dissolution

Sources: OSHA 2015 and Schlesinger et al. 1997

Following deposition, beryllium is slowly cleared from the lung. Clearance half-lives range from days to years in animals. Evidence suggests that clearance may be biphasic with an initial rapid clearance via mucociliary transport from the lungs to the gastrointestinal tract followed by a slower phase involving

translocation to the tracheobronchial lymph nodes, uptake by alveolar macrophages, and solubilization (WHO 2001). The rapid phase half-time ranges from 1 to 60 days and the slow phase half-time is 0.6-2.3 years in rats. The half-time depends on the solubility of the beryllium compound with more soluble forms having a shorter half-time than less soluble forms. After prolonged inhalation exposure to beryllium sulfate, pulmonary clearance half-time was approximately 2 weeks and thereafter the elimination rate diminished rapidly. Clearance of beryllium chloride, a soluble beryllium salt, is faster than that of the oxide. Though soluble beryllium salts also precipitate out in the respiratory tract, their initial clearance is faster than the clearance of insoluble or sparingly soluble forms (Magos 1991; WHO 2001). Hart et al. (1980) exposed guinea pigs for 55 minutes nose-only to 230 μg Be/m^3 as beryllium chloride. Immediately after the end of exposure, 34% of the initial body burden was in the gastrointestinal tract, indicating significant mucociliary clearance during exposure. By 48 h post exposure, 50% of the initial lung burden had been removed by mucociliary clearance or alveolar clearance. However, 34% of the initial body burden was still present at 14 days, primarily in the lungs, indicating that clearance is biphasic (Hart et al. 1980). Most inhaled beryllium metal remains in the lung of experimental animals for an extended period of time (Finch et al. 1990).

Haley et al. (1990) exposed rats for 50 minutes nose-only to a mean concentration of 800 μg/m^3 of beryllium metal. The clearance half-time of beryllium over the period studied was 240 days. Clearance of beryllium from 3 to 171 days post exposure was best described by a single-component negative exponential function (Haley et al. 1990).

Hart et al. (1984) exposed male F344 rats to 447 μg Be/m3 as beryllium oxide heat-treated at 560°C and found rapid clearance of beryllium from the lavageable lung compartment (fluids and free lung cells, half-time < 2 days) but minimal clearance in 21 days from the non-lavageable compartment (lung tissue). Female and male rats exposed to beryllium oxide were able to clear 12% and 21% of the alveolar lung burden within 63 days of exposure respectively. Female and male hamsters cleared 38% and 45% of the beryllium in the alveoli respectively in the same amount of time. The study indicates that male rats are better able to clear beryllium particles from the lungs than female rats. The biological half-life for beryllium oxide in the rat lung was estimated to be 6 months (Sanders et al. 1975). Approximately 95% of the beryllium was excreted through the feces. Stiefel et al. (1980) found that rats and guinea pigs exposed to 2–40 mg beryllium/m^3 as beryllium nitrate for 16 hours had increased concentrations of urinary beryllium (300 ng beryllium/g), compared to normal concentrations (2.1 ng beryllium/g). Clearance half-times have been reported to be 180–260 days in rats (Finch et al. 1990; Strupp 2011b).

In dogs exposed nose-only to 28±9 μg/L as beryllium oxide calcined at 500°C or 1000°C (the solubility of beryllium oxide decreases as the temperature at which it is calcined increases) for sufficient durations to result in low (14-15 μg beryllium/kg) initial lung burdens and high (36–64 μg beryllium/kg) initial lung burdens, there were no differences in whole-body retention with regard to initial lung burdens. Whole-body clearance after exposure to beryllium oxide calcined at 500°C was described by a two-component, negative exponential function. The short-term component accounted for 59% of the initial lung burden and had a half-life of 54 days. Whole-body clearance after exposure to beryllium oxide calcined at 1000°C was described by a single-component negative exponential function with a half-life of 310 days. Clearance from the lung was more rapid and greater amounts were translocated to the liver, blood, and skeleton in the dogs exposed to beryllium calcined at the lower temperature than in dogs exposed to beryllium calcined at the higher temperature. Lung clearance of both was described by a single-component negative exponential function. Lung clearance half-lives were 64 days for 500°C calcined beryllium oxide and 240 days for 1000°C calcined beryllium oxide. Fecal excretion predominated at early times after exposure to either beryllium oxide aerosols and at all time periods for 1000°C calcined beryllium oxide, while urinary excretion predominated at later times. Dogs exposed to beryllium oxide calcined at 500°C excreted a significantly ($p<0.05$) greater total percentage of the initial lung burden of beryllium than dogs exposed to beryllium calcined at higher temperature by 180 days. Thus, beryllium oxide calcined at 500°C was cleared more rapidly than beryllium oxide calcined at 1000°C (Finch et al. 1990). Although clearance of beryllium oxide calcined at the lower temperature was relatively fast during the first few days after exposure due to mucociliary clearance, later clearance may result from slow translocation of tracheobronchial lymph nodes, macrophage clearance from the pulmonary to the tracheal regions, and pulmonary solubilization of beryllium followed by mobilization through blood to liver and bone or excretion in urine (Finch et al. 1990)

Benson et al. (2000) investigated the pulmonary toxicity and clearance of Beryllium/copper (BeCu) alloy (2% Be; 98% Cu) and metal beryllium particles with MMAD of 1–3 μm in female C3H/HeJ mice administered 12.5, 25, and 100 μg BeCu alloy or 2 and 8 μg beryllium metal via intratracheal instillation and reported slow lung clearance of beryllium metal with a half-life of 2 weeks to a year.

Beryllium deposited in the lungs of rats exposed for 30-180 minutes to beryllium oxide fired at 1000°C was cleared biphasically as well. In the first phase, 30% of the total lung burden was cleared; the half-life was 2.5 days. In the second phase, the remaining 70% of the beryllium in the lung was cleared with a half-life of 833 days. The whole-body clearance yielded a single-phase exponential curve with a half-life of 356 days (Rhoads and Sanders 1985). In the Muller et al. (2010b) study discussed in Section 2.14, increased levels of beryllium were found in the mice’s urine after 1, 2, or 3 weeks of exposure and 1 week after

exposure termination. During the exposure period, urinary beryllium levels were much lower in the beryllium oxide group than in the beryllium metal or beryllium aluminum groups. In the beryllium oxide group, beryllium urinary levels were similar after 1, 2, or 3 weeks of exposure, whereas in the beryllium metal and beryllium aluminum groups, the highest levels occurred after 1 week of exposure (Muller et al. 2010b).

No studies were located regarding excretion in humans after oral exposure to beryllium or its compounds. Animals exposed to oral doses of beryllium or its compounds excrete the greatest percentage of the dose via the feces, which indicates that beryllium is poorly absorbed by the gastrointestinal tract. Analysis of the excreta of rats exposed to 0.019 and 0.190 mg beryllium/kg/day as beryllium sulfate in drinking water indicated that 99% of the dose was excreted in the feces and <0.5% was excreted in the urine. The excretion pattern of beryllium in the feces reached steady-state after 9 weeks (Reeves 1965). Similarly, excretion of beryllium occurred mainly via the feces of rats exposed to 0.3, 2.8, and 31 mg beryllium/kg/day as beryllium sulfate in the diet for 2 years. The feces contained 10.7 ppm and the urine 29.7 ppb of the 0.3 mg beryllium/kg/day dose, and a similar pattern was observed with the other doses (Morgareidge et al. 1975). Rats, monkeys, mice, and dogs orally exposed to radioactive beryllium chloride excreted 98% of the dose via the feces. About 50% was excreted within 1/4 day after parenteral dosage (Furchner et al. 1973). No studies were located regarding excretion in humans or animals after dermal exposure to beryllium or its compounds.

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

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models 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 (Clewell and Andersen 1985). 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.

No PBPK modeling studies were located for beryllium.

3.1.5 Animal-to-Human Extrapolations

As reviewed in EPA (1998) and Finch et al. (1996), several animal models of human CBD have been developed, but no models to date mimic all aspects of the human disease. Numerous studies in dogs,

monkeys, and rats have reported granulomatous inflammation in the lungs. However, the lung lesions do not histopathologically resemble CBD in humans; effects are transient or are not consistently associated with beryllium-specific immune responses.

Studies in mice suggest that mice may be an appropriate model (Huang et al. 1992; Nikula et al. 1997). In the model developed by Huang et al. (1992), mice were preimmunized with beryllium sulfate and then administered a single intratracheal dose of beryllium sulfate (Huang et al. 1992). A number of mice displayed symptoms consistent with CBD such as the influx of $CD4^+$ T lymphocytes into the lungs, sensitization of T lymphocytes to beryllium, interstitial inflammation, and granuloma formation. However, these effects were observed at 8 months and were resolved by 10 months. The study by Nikula et al. (1997), in which mice received a 90-minute nose-only exposure to beryllium metal, also found many similarities between effects observed in mice and human CBD. The study authors concluded that this mouse model can be used to study the influence of dose, exposure pattern, and physicochemical form of beryllium on the development of CBD. A comparison of the histologic characteristics of beryllium-induced disease in humans and mice is presented in Table 3-5.

Table 3-5. Histologic Characteristics of Beryllium-induced Disease in Mice and Humans

Histologic findings	Mice	Humans
Interstitial cellular infiltration of macrophages, lymphocytes, and variable numbers of plasma cells	Mild to moderate	Moderate to marked
Granulomas	Present; poorly formed	Variable; absent to poorly formed to well formed
Giant cells	Numbers variable; may be scattered or associated with granulomas	Numbers variable; may be scattered or associated with granulomas
Cholesterol clefts	Numerous and often seen within giant cells	Numerous and often seen within giant cells
Interstitial fibrosis	Present in 75% of cases; minimal to mild	Present in large percentage of cases; minimal to mild in 50% and moderate to marked in 50%
Calcific inclusions	Absent	Present in approximately 55% of cases
Hyalinized nodules	Absent	Present in lung or hilar lymph nodes of 40% of cases
Interstitial compact aggregates of lymphocytes	Present	Not described
Beryllium	Metal evident in H&E sections	Increased tissue levels found by spectrographic and chemical analysis

Source: Nikula et al. 1997

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 beryllium are discussed in Section 5.7, Populations with Potentially High Exposures.

Children are not small adults and have needs and behaviors that may result in higher exposures. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. As children grow their nutritional needs change from breast milk or formula to solid foods. They may eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Specific information on the exposure of children to beryllium is limited. Nadal et al. (2005) found that beryllium concentrations in the hair of children in Tarragona County, Spain were below the limit of detection (0.13 μg/g) in urban areas, near a chemical complex, and near a large oil refinery and two incinerators. A cancer risk assessment of teenagers living in New York City and Los Angeles measured beryllium concentrations in personal samples of air of 0.002 ng/m^3 in New York City and 0.003 ng/m^3 in Los Angeles (Sax et al. 2006). Sax et al. (2006) concluded that EPA models of beryllium concentrations tended to overestimate personal cancer risks for beryllium. An x-ray health survey was conducted in 1948 in the neighborhood surrounding a beryllium manufacturing facility in Lorain, Ohio. In this survey, 2,000 children were examined, and none of the children exhibited signs of chronic berylliosis disease (AEC 1948).

As with adults in the general population, small exposures in children occur from normal ingestion of food and drinking water and inhaling air. These exposures may be higher in areas with naturally high beryllium

soil levels, and near beryllium processing sites, electric power plants, and waste sites containing beryllium. There is suggestive evidence that beryllium is transferred across the placenta and excreted via breast milk.(Krachler et al.1999a). No information on beryllium levels in amniotic fluid, meconium, or neonatal blood was located.

At waste sites, beryllium that is found in excess of natural background levels is most likely to be in soil and presents a special hazard for young children. Hand-to-mouth activity and eating contaminated dirt will result in oral exposure to beryllium. The hazard depends on the form of beryllium present at the waste site. Beryllium in soil at waste sites is almost entirely in the form of insoluble oxides and hydroxides of beryllium which would be expected to be less biologically available than more soluble forms (see Section 5.4.1).

Household products are not likely to contain beryllium except for copper-beryllium wire, which is used in and around the home in electronics or other electrical devices. Products used in crafts, hobbies, or from cottage industries may contain significant amounts of beryllium, so exposure is expected to be higher when undertaking these activities.

The on-going process of development for neonates and breast-fed infants may make them more susceptible to the effects of a contaminant. Mothers exposed to beryllium can pass it to their neonates and breast-fed infants. A study by Krachler et al. (1999a) provides suggestive evidence that beryllium is transferred across the placenta and excreted via breast milk. The levels of beryllium in umbilical cord serum and in colostrum were higher than in maternal serum. The average concentrations of beryllium in the umbilical cords of healthy newborn children were measured for arterial (1.3 μg/L), venous (0.8 μg/L), and mixed (0.6 μg/L) sera (Krachler et al. 1999b). No information on beryllium levels in amniotic fluid, meconium, or neonatal blood was located.

Beryllium exposure to children from parents' work clothes, skin, hair, tools, or other objects from the workplace is possible if the parent uses beryllium at work. In a report to Congress by NIOSH, several historical cases of home contamination by beryllium were reported (NIOSH 1995). Workers who do not change their work clothes at the end of the workday can increase the probability of home contamination with beryllium.

Data on the toxicity of beryllium in children is limited. Dietary studies with beryllium carbonate have found beryllium rickets in young rats (Guyatt et al. 1933; Jacobson 1933; Kay and Skill 1934). The potential of beryllium to induce developmental effects has not been adequately investigated. A chronic study did not find developmental effects (gross and skeletal malformations, fetal survival, and fetal body weights were examined) in dogs exposed to beryllium sulfate in the diet (Morgareidge et al. 1976).

However, intratracheal and intravenous exposure studies have found increases in fetal/neonatal mortality, internal abnormalities, and behavioral abnormalities in rat and mouse offspring (Mathur et al. 1987; Tsujii and Hoshishima 1979).

A fecal test for an infant who was clinically diagnosed with Bartter syndrome (kidney disorder causing imbalance of some ions and related molecules in the body) revealed elevated levels of beryllium and other heavy metals. The mother was occupationally exposed to beryllium-copper alloy through lead soldering for 14 years. This case study suggests beryllium exposure may be transferred to the infant *in-utero* or through lactation (Crinnion and Tran 2010). This observation is supported by findings from Sharma et al. (2002) who observed beryllium crossed the placental barrier and accumulated in the offspring of pregnant rats exposed to a single dose of beryllium as 50 mg/kg beryllium nitrate. The results of a study by Krachler et al. (1999a) suggest that beryllium is transferred across the placenta and via maternal milk. Beryllium levels in the umbilical cord sera and in colostrum were higher than maternal sera levels (Krachler et al. 1999a).

No human or animal data were located that examined possible age-related differences in the toxicokinetics of beryllium. There are no data on the toxicokinetic properties of beryllium in children or immature animals.

Subsequent sections of this chapter (Sections 3.3 and 3.4) discuss the available information on biomarkers of beryllium exposure and effect, as well as interactions between beryllium and other chemicals. The available information is from adults and mature animals; no child-specific information was identified. It is likely that this information will also be applicable to children.

A susceptible population will exhibit a different or enhanced response to beryllium than will most persons exposed to the same level of beryllium in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of beryllium, or compromised function of organs affected by beryllium. Populations who are at greater risk due to their unusually high exposure to beryllium are discussed in Section 5.7.

There are strong data to suggest that a genetic susceptibility factor may predispose certain individuals to development of CBD. CBD is a hypersensitivity granulomatosis characterized by beryllium hypersensitivity and mediated by CD4+ T cells (Rossman 2001). However, not all individuals with beryllium hypersensitivity will develop CBD. Genetic differences in the MHC seem to determine whether an individual is able to present beryllium to a T cell and mount a proliferative response (Maier 2002).

A case control study by Maier et al. (1999) was designed to assess whether polymorphisms in the angiotensin converting enzyme were associated with CBD and the disease severity. No statistically significant associations between angiotensin converting enzyme genotype and CBD were found in the comparisons of individuals with CBD to beryllium-exposed controls or non-beryllium exposed controls (Maier et al. 1999).

Animal data support the human data that genetically determined cellular immune mechanisms may be involved in CBD, as indicated by studies in different strains of guinea pigs and mice. Intratracheal instillation of beryllium oxide (calcined at 560°C) resulted in the development of granulomatous lung disease in outbred Hartley and in strain 2 guinea pigs, but not in strain 13 guinea pigs (Barna et al. 1981; 1984). Granulomatous lung disease also was produced in the F_1 offspring of mated strain 2 and strain 13 guinea pigs, but the severity was milder in the hybrid strain than in the strain 2 guinea pigs (Barna et al. 1984). In addition, when guinea pigs were exposed intradermally or intratracheally to beryllium oxide and challenged by dermally applied beryllium sulfate, the strain 2 and the F_1 guinea pigs showed positive skin tests for delayed-type hypersensitivity, while strain 13 guinea pigs did not. Granulomatous lung disease was also induced in strain A/J ($H\text{-}2_a$ haplotype) mice, but not in BALB/c ($H\text{-}2_d$ haplotype) or C57BL/6 ($H\text{-}2_b$ haplotype) mice, after intratracheal instillation of beryllium sulfate, suggesting that genetic differences at the H-2 major histocompatibility gene complex may account for the differential responses to beryllium sulfate in mice (Huang et al. 1992). These results suggest that genetically determined factors may make some humans more susceptible to CBD.

Other animal data indicates that females may be more susceptible than males to the effects of beryllium. Female rats exposed to 0.034 mg beryllium/m^3 as beryllium sulfate for 72 weeks had higher mortality rates and more severe weight loss than males (Reeves et al. 1967). Female rats exposed to 131 mg beryllium/kg/day as beryllium sulfate in the diet for 2 years developed transient glucosuria while renal effects were not observed in males (Morgareidge et al. 1975).

Translocation of beryllium from bone to liver eventually causes a systemic disease characterized by weight loss and liver necrosis (Clary et al. 1972). This study exposed guinea pigs and mice to radioactive beryllium oxide intratracheally after hormone biosynthesis was inhibited by metyrapone injection. The results indicated that altered adrenal hormone synthesis shifted beryllium concentrations from bone to liver, causing weight loss. A combination of adrenal dysfunction and compromised liver function could exacerbate beryllium disease. Therefore, people with lowered adrenal and/or liver functionality may be unusually susceptible to the effects of beryllium.

A human leukocyte antigen (HLA) class II marker has been strongly associated with CBD (Lombardi et al. 2001). Studies conducted by Fontenot et al. (2000) and Lombardi et al. (2001) suggest that the HLA–DP allele, in particular alleles with HLA–DP containing Glu at DP69, are involved in the presentation of beryllium to CD^{4+} T cells, which are involved in the pathogenesis of CBD (Fontenot et al. 2000; Lombardi et al. 2001). Richeldi et al. (1993) found a higher frequency of allelic variants of the HLA–DP gene coding for a glutamate in position 69 of HLA–DPB1 chain (HLA–DPB1 Glu^{69}) among individuals with CBD than in beryllium-exposed individuals without the disease (Richeldi et al. 1993). The HLA–DPB1 Glu^{69} DNA marker was found in 5 of 6 beryllium workers with CBD, 0 of 2 beryllium-sensitized individuals without disease and 36 of 119 (30%) unsensitized beryllium-exposed individuals (Richeldi et al. 1997).

Further analysis by Wang et al. (1999) found the Glu^{69} marker on the HLA–DPDBP1 gene was not very predictive of CBD (predictive value of 0.36). However, the presence of the relatively rare HLA–DP allele, HLA–DPnon*0201 DPB1 Glu^{69}, had a predictive value of 0.57. Wang et al. (1999) also found a higher percentage of homozygous Glu^{69} carriers in the CBD group as compared to controls. The study authors estimated that carriers of the Glu^{69}/Glu^{69} markers or non-*0201Glu^{69} allele accounted for 85% of the CBD cases and only 16% of unaffected beryllium-exposed individuals. Additional studies by this group found that most beryllium-sensitized individuals without CBD also carried rare HLA–DPnon*0201 Glu^{69} DPB1 alleles (Wang et al. 2001).

It is likely that CBD is a multigenetic disease with a number of genetic factors contributing to the development of an immune response to beryllium. Rossman et al. (2002) also suggested that HLA–DPB1-E69 is a marker for susceptibility to hypersensitivity and not just a progression marker for CBD. The study also found that HLA amino acid epitopes on HLA–DPDRB1 and -DQB1, in concert with or independently of HLA–DPB1-E69, may be associated with progression to CBD (Rossman et al. 2002). Stubbs et al. (1996) also found allelic differences in the -DR (D-related)) isotype of class II HLAs. The HLA–DPDRB1 alleles associated with beryllium sensitization were *0103, *09, *1302, *0403, and *0302 (Stubbs et al. 1996).

Newer studies have shown that 68– 93% of beryllium-sensitized workers and 84–92% of workers with CBD carried the HLA–DPB Glu69 allele, compared to 36–48% in beryllium workers without beryllium sensitization or CBD (Amicosante et al. 2005; McCanlies et al. 2004; Rosenman et al. 2011; Saltini et al. 2001; Sato et al. 2007b; Van Dyke et al. 2011a, 2011b). A 6-fold increase in the risk of beryllium sensitization or CBD was found in beryllium workers positive for HLA–DPDBP1 Glu69 (OR 6.06; 95% CI 1.96–18.7) (Van Dyke et al. 2011a). Separating beryllium sensitization from CBD, Sato et al. (2007b) calculated ORs of 8.2 (95% CI 4.2–15.9) and 11.9 (95% CI 5.5–23.5), respectively.

Rosenman et al. (2011) found higher prevalence of HLA–DPB Glu69 homozygotes and heterozygotes among subjects with beryllium sensitization or CBD as compared to beryllium-exposed non-sensitized subjects (Table 3-6); however, there were no differences between the workers with CBD or beryllium sensitization. Van Dyke et al. (2011b) showed that subjects with beryllium sensitization or CBD were more likely to be HLA–DPB1 Glu69 homozygotes (25.7 and 19.7%, respectively) than non-sensitized subjects (4.3%). The study also found differences in the HLA–DPB1 Glu69 alleles; subjects with beryllium sensitization had a significantly higher frequency of *0201 (51.4%) and *0601 (12.9%) alleles than non-sensitized subjects (26.3 and 1.2%, respectively); a higher frequency of *0601 alleles was also found in subjects with CBD (18.0%). Logistic regression modeling showed that carriage of a single *02 allele, a single non-*02 allele, or a *02 and a non-*02 allele was significant predictors of beryllium sensitization or CBD; the ORs are summarized in Table 3-6. Similarly, Rosenman et al. (2011) found a significantly higher distribution of non-HLA–DPB1*0201 alleles in CBD subjects; 10 alleles were more frequently found (*0202, *0301, *0601, *0901, *1001, *1101, *1401, *1601, *1701, and *7101). Silveria et al. (2012) demonstrated that beryllium-sensitized or CBD subjects were more likely to carry non-HLA–DPB1*02 alleles than *02 alleles (Silveira et al. 2012).

Table 3-6. Risk of BeS and CBD by HLA–DPB1 Glu69 Genotype in Beryllium Workers

Genotype	Odds ratio (95% CI)
BeS	
Homozygote	3.54 (1.29–9.51)
Heterozygote	3.28 (1.63–6.64)
CBD	
Homozygote	2.90 (1.16–7.14)
Heterozygote	6.88 (3.53–13.55)

BeS = beryllium sensitization; CBD = chronic beryllium disease; CI = confidence interval; Glu69 = glutamic acid at position 69

Source: Rosenman et al. 2011

Two studies conducted by Van Dyke (2011a, 2011b) examined the relationship between beryllium exposure carriage of the HLA–DPB1 Glu69 genotype and beryllium sensitization or CBD and found that both exposure and E69 genotype contribute to the development of CBD. The OR for beryllium sensitization and CBD (combined) among HLA–DPB1 Glu69 carriers with beryllium exposure >0.1 $\mu g/m^3$ was 24.1 (95% CI 4.77–122) (Van Dyke 2011a). The top half of Table 3-7 shows the significant predictors (type of allele) for beryllium sensitization based on multiple logistic regression (Van Dyke et al. 2011b).

The predictors of CBD using a similar multiple logistic regression model are shown in the bottom half of Table 3-7 (Van Dyke et al. 2011b). Among workers with a lifetime weighted average beryllium exposure of 2 μg/m^3, the ORs for CBD were increased from 5-fold for E69-negative genotypes to more than 100-fold for the E69 homozygotes (Van Dyke et al. 2011b). Among workers with a lifetime weighted average beryllium exposure of 2 μg/m^3, the ORs (95% CI) for CBD were 4.91 (1.46–16.56) for HLA–DPB1 Glu69 genotype, 17.01 (3.80–76.17) for single *02 allele, 58.77 (13.43–257.2) for single non-*02 Glu69 allele, and 110.7 (19.87–619.3) for Glu69 copy number with one *02 allele plus one non-*02 GluE69 allele (Glu69 homozygote) (Van Dyke et al. 2011b).

Table 3-7. Risk of BeS and CBD by HLA–DPB1 Glu69 Genotype in Former and Current Beryllium Workers

Genotype	Odds ratio (95% CI)
Beryllium sensitization	
Single *02 allele	12.01 (4.28–33.71)
Single non-*02 Glu69 allele	29.54 (10.33–84.53)
Glu69 copy number with one *02 allele plus one non-*02 Glu69 allele	55.68 (14.8–209.40)
CBD	
Single *02 allele	3.46 (1.42–8.43)
Single non-*02 Glu69 allele	11.97 (5.12–28.00)
Glu69 copy number with one *02 allele plus one non-*02 Glu69 allele	22.54 (7.00–72.62)

BeS = beryllium sensitization; CBD = chronic beryllium disease

Source: Van Dyke et al. 2011b

To date, 43 HLA-DPb1 alleles that code for Glu69 (E69) have been described. E69 alleloforms of MHC class II antigen-presenting proteins with the greatest negative surface charge convey the highest risk of CBD, however, irrespective of allele, they convey equal risk of beryllium sensitization. The same alleles that cause the greatest risk of CBD are also thought to be important for the progression from beryllium sensitization to CBD (Snyder et al. 2008).

The HLA–DPB1^{E69} allele is a susceptibility marker that has been suggested as useful for pre-employment screening. HLA–DPB1^{E69} allele has been shown to be associated with CBD and beryllium sensitization in at least three sufficiently-sized, well characterized study populations (Maier et al. 2003; Rossman et al. 2002) and several smaller studies, and essentially all of the studies agree (as reviewed in Weston 2011). Inheritance of HLA–DP DPB1^{E69} (HLA–DP beta 1E69) carries an increased risk of 2- to 30-fold in beryllium exposed workers (Weston et al. 2005). However, positive predictive value ranged only 8.3 – 14.3% for carriers with an assumed disease frequency of 5%. For high risk subgroups with disease frequencies of 15%, the range of positive predictive values was found to span between 25 – 43%. Allelic/carrier frequencies were found to be 0.21/0.33, 0.24/0.40, 0.27/0.47, and 0.38/0.59 for

Caucasians, African-Americans, Hispanics, and Chinese, respectively, indicating Chinese could be at highest risk of developing CBD (Weston et al. 2002).

Although HLA-DPB1-Glu69 is associated with the development of CBD, it cannot fully explain susceptibility. About 15% of CBD patients do not possess a Glu69-containing HLA-DP allele, suggesting that other MHC class II alleles may be involved in disease susceptibility. In CBD patients without a Glu69-containing HLA-DP allele, an increased frequency of HLA-DR13 alleles has been described, and these alleles possess a glutamic acid at position 71 of the β-chain (Bill et al. 2005). Studies have also examined the frequencies of other HLA genotypes among individuals who were DPB1 Glu69 negative; 100% were positive for DRB E71, compared to 19.2% in controls. The HLADPB1 Glu69 and HLA–DPDRB Glu71 genotypes accounted for 100% of the beryllium-sensitized and CBD subjects compared to 50.3% in the beryllium exposed non-sensitized workers. Sato et al. (2007b) found that 70% of the CBD subjects who were HLA–DPB1 Glu69 negative were HLA–DPDRB1*13 carriers; the prevalence in the non-sensitized workers was 15.7%, and no association was found for beryllium sensitization. Another study found that HLA–DPDRP Phe47 was significantly associated with beryllium sensitization /CBD among HLA–DPB1 Glu69 negative subjects; 95% of the HLA–DPB1 Glu69 negative subjects were positive for HLA–DPDRP Phe47 (Amicosante et al. 2005).

There are other genes that may be involved in regulating the immune and inflammatory response in the pathogenesis of this disease. Several studies have examined the association of polymorphisms with CBD or beryllium sensitization. Functional gene polymorphisms of the TNF-α and transforming growth factor (TGF) β1 genes are suspected to modify the course of granulomatous disorders.

High TGF-β1 protein production has been associated with several diseases including pulmonary sarcoidosis. TGF-β1, a multifunctional cytokine involved in mediating the fibrotic/Th1 response, has several genetic variants which might predispose individuals to these lung diseases. Genotype GG produces more TGF-β1, which inhibits cytokine production and IL-2 dependent T cell activation, potentially limiting the inflammatory response. A single nucleotide polymorphism (SNP) in the TFNA gene promotor, -308 (A for G), may increase the release of TNF-α from Be stimulated BAL cells of CBD patients. Both TGF-β1 (codon 25) polymorphisms and TNFA (-308) were analyzed in patients with CBD. Both TGF-β1 polymorphism and TFNA (-308) genotype frequencies from United States CBD patients differed significantly from those of European and Israeli patients/controls (Gaede et al. 2005).

The genotype profile of the CBD positive sub-cohort of European and Israeli patients was 62.5% CC/GC compared to 13.8% in healthy controls ($P<0.001$). This pattern was not observed in the U.S. cohort.

However, for TNFA2 allele, the U.S. CBD positive cohort had increased frequencies of the TFNA SNP (28.20% vs. 8.96% in healthy controls; P<0.005) (Gaede et al. 2005).

Increase in TGF-β1 (codon 25) genotype (CC or GC) frequency associated with a low TGF-β1 protein release suggests that they are involved in the pathogenesis of CBD. This indicates that multiple genes can determine susceptibility for the same immunopathological reaction and disease (Gaede et al. 2005). For example, the TNF-α response is independent of the activation of beryllium-specific HLA-DP restricted T-cells (Amicosante et al. 2002).

Gene polymorphisms associated with the control of the immune response, such as TNF-α and TGF-β1, act in synergism with a specific immune response gene such as HLA-DPB1-Glu69 or other HLA-class II polymorphisms; driving the immune response to beryllium and in the development CBD (Gaede et al. 2005). Using DNA from sarcoidosis cases/controls, TGF-β1-variants were analyzed by sequence-specific primer PCR. Specific TGF-β genotypes and haplotypes are associated with a more severe pulmonary phenotype in both sarcoidosis and CBD, although not affecting disease susceptibility per se. The -509C and codon 10T were significantly associated with disease severity indicators in both CBD and sarcoidosis (Jonth et al. 2007).

TNF- α may also play a central role in the determination of susceptibility to beryllium hypersensitivity (Dotti et al. 2004). Examining TNF-α polymorphisms, Saltini et al. (2001) found a significantly higher frequency of the TNF-α-308*02 allele among subjects with beryllium sensitization or CBD, as compared to controls; however, there were no significant differences between the frequencies in CBD or beryllium sensitized subjects (Saltini et al. 2001). In contrast, McCanlies et al. (2007) did not find significant associations between TNF-α-308*02 or TNF-α-238*02 and beryllium sensitization or CBD. Similarly, Sato et al. (2007a) did not find any significant differences in the frequencies of several TNF-α promoter polymorphisms in subjects with beryllium sensitization, CBD, or the combined groups.

Relationships between CBD disease severity and TNF-α promoter polymorphisms have been reported (Maier et al. 2001; Sato et al. 2007a). TNF-α expression was not upregulated for THP-1 macrophages upon beryllium stimulation in comparison to the untreated control cells. Alveolar macrophages (THP-1 monocytes into macrophages) could have some level of tolerance to beryllium and this may explain why most Be-exposed individuals remain healthy throughout life (Ding et al. 2009).

Studies by Sato et al. (2010), Bekris et al. (2006), and McCanlies et al. (2010) looked for associations between polymorphisms of genes coding for CC chemokine receptor 5 (CCR5), glutamate cysteine ligase (GCL, rate-limiting enzyme for glutathione synthesis), and several interleukins (IL-1A, IL-1B, IL-1RN,

IL-2, IL-9, and IL-9R). No significant differences in the frequency of CCR5 polymorphisms were found between subjects with beryllium sensitization, CBD, or controls (subjects exposed to beryllium but not sensitized), suggesting that CCR5 or GCL polymorphisms do not increase susceptibility (Sato et al. 2010). However, polymorphisms were found to be associated with the progression of CBD. Greater declines in lung function were found among CBD subjects who were homozygous for specific CCR5 polymorphisms or carried specific CCR5 alleles. When analyzed in a combined group of beryllium sensitized and CBD subjects, associations were found between BAL lymphocyte percentages and specific CCR5 polymorphisms.

Bekris et al. (2006) found differences in GCL polymorphisms between CBD subjects and beryllium sensitized subjects or controls (exposed to beryllium but not sensitized). However, no differences in GCL polymorphisms were found between the controls and beryllium sensitized subjects (Bekris et al. 2006). In the McCanlies et al. (2010) study, the frequency of three IL-1A single nucleotide polymorphisms were significantly different in subjects with CBD compared to those with beryllium sensitization or non-sensitized subjects (Table 3-8). The authors controlled for HLA-DPBI^{Glu69} status in the analyses, as it is well documented that this can also impact sensitivity to CBD. In Table 3-8, various changes were observed depending on the substitution that was observed due to the specific polymorphism. It demonstrates that specific changes in the IL-1A gene can lead to greater susceptibility to developing CBD.

Table 3-8. Adjusted[a] OR and 95% CI for Significant IL-1A SNPs for Different Genetic Models

SNP/Genetic Model	CBD vs Non-sensitized OR (95% CI)	BeS vs Non-sensitized OR (95% CI)	CBD vs BeS OR (95% CI)
IL-1A-1142			
Additive			
AC vs AA	2.23 (1.27-3.93)[b]	0.68 (0.36-1.27)	3.04 (1.34-6.91)[b]
CC vs AC	0.76 (0.34-1.69)	0.76 (0.24-2.41)	1.04 (0.26-4.11)
Dominant			
CC, AC vs AA	2.03 (1.18-3.48)[b]	0.63 (0.35-1.15)	3.02 (1.36-6.70)[b]
Recessive			
CC vs AC, AA	1.05 (0.48-2.28)	0.64 (0.22-1.85)	1.49 (0.40-5.57)
IL-1A-3769			
Additive			
AG vs GG	1.75 (1.03-2.96)[b]	0.70 (0.39-1.24)	2.29 (1.08-4.85)[b]
AA vs AG	0.95 (0.43-2.12)	0.84 (0.26-2.66)	1.15 (0.31-4.33)
Dominant			
AA, AG vs GG	1.73 (1.45-2.88)[b]	0.65 (0.37-1.13)	2.51 (1.21-5.19)[b]
Recessive			
AA vs AG, GG	1.36 (0.64-2.88)	0.74 (0.26-2.17)	1.86 (0.53-6.50)

Table 3-8. Adjusted[a] OR and 95% CI for Significant IL-1A SNPs for Different Genetic Models

SNP/Genetic Model	CBD vs Non-sensitized OR (95% CI)	BeS vs Non-sensitized OR (95% CI)	CBD vs BeS OR (95% CI)
IL-1A-4697			
Additive			
CT vs TT	1.91 (1.13-3.23)[b]	0.69 (0.38-1.20)	2.56 (1.21-5.41)[b]
CC vs CT	0.82 (0.37-1.81)	0.73 (0.23-2.29)	1.18 (0.31-4.39)
Dominant			
CC, CT vs TT	1.72 (1.04-2.85)[b]	0.62 (0.36-1.08)	2.56 (1.24-5.29)[b]
Recessive			
CC vs CT, TT	1.04 (0.48-2.25)	0.63 (0.22-1.82)	1.51 (0.42-5.43)

[a]All ORs adjusted for plant and HLA-DPBI^{Glu69}
[b]Significant ORs

A = adenine; BeS = beryllium sensitization; C = cytosine; CBD = chronic beryllium disease; CI = confidence interval; G = guanine; IL = interleukin; OR = odds ratios; SNP = single nucleotide polymorphisms; T = thymine

Source: McCanlies et al. 2010

Alveolar macrophages from patients with CBD and beryllium sensitization demonstrated significantly greater cell surface CD16 (encoded by the FCGR3A gene). The V158F polymorphism (with significantly higher frequencies of the 158V allele and 158VV homozygotes) of the FCGR3A gene is associated with CBD compared to beryllium sensitized subjects and controls and may impact lung function in CBD. FCGR3A 158VV homozygous genotypes could contribute to the accelerated lung function decline in CBD. The FCGR3A polymorphisms may serve as a risk factor of CBD (Liu et al. 2019). It is possible that this polymorphism could be related to other adverse effects, but further research is needed.

Poorly soluble beryllium materials undergo dissolution in artificial sweat, suggesting that skin exposure is a biologically plausible pathway for development of sensitization. Skin surface acidity, which is regulated by sweat chemistry and bacterial hydrolysis of sebum lipids, varies by anatomical region and may be an exposure-modifying factor for beryllium particle dissolution (Stefaniak et al. 2010).

Beryllium is used in dental alloys where prolonged exposure may cause some toxic effects. Haberman et al. (1998) examined the use of beryllium dental materials which may cause allergic contact dermatitis in some patients. Prolonged exposure to beryllium in dental alloy causes symptoms consistent with gingivitis, oral lichen planus, leukoplakia, aphthous ulcers, and pemphigus (Haberman et al. 1998).

Although, beryllium is poorly absorbed after ingestion, gastro-intestinal conditions might result in increased absorption. For example, Magos (1991) reported that about 20% of the dose is absorbed from the acidic stomach. The majority of the dose is precipitated as in insoluble form in the gut (Magos 1991).

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 1989).

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 beryllium 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 1989). 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 Beryllium 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 1989). 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 Beryllium 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

An alternative approach to assessing beryllium exposure is biological monitoring. There are several tests for measuring beryllium in excreta (e.g., saliva, urine) or other biological tissue (e.g., hair or blood), or measuring a biological effect (e.g., enzyme induction or inhibition) (Frame et al. 1974; Foreman et al. 1970; IARC 1980; Martinsen and Thomassen 1986; Xiao-Quan et al. 1989). Biological monitoring has

the advantage of measuring the total absorbed dose from all routes of exposure, and it is becoming more common and accepted as the understanding of the pharmacokinetic process and feasibility of monitoring improves. Biomonitoring for assessing worker exposure to beryllium would provide an integrated assessment of total worker exposure, and an indication of exposure over time. (Deubner et al. 2001b).

Beryllium levels in urine were analyzed in eight laboratory workers and compared to the levels of beryllium in the laboratory atmosphere for 30 days after an accidental leakage of beryllium chloride. The urinary levels appear to be directly proportional to atmospheric levels at 8 ng/m^3 (Zorn et al. 1986). These are the only available data that associate airborne beryllium levels with urinary levels in humans. The latest NHANES data for beryllium show beryllium levels below the limit of detection for the entire population for years 1999-2010 (Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019).

Biomarkers of oral or dermal exposure to beryllium were not located, probably because very little beryllium is absorbed after exposure by these routes. Reduction in inhalation exposure to beryllium has not resulted in a comparable reduction in the occurrence of beryllium sensitization or CBD, perhaps due to dermal exposure to beryllium particles. Skin exposure may be sufficient to cause an immune response (e.g., beryllium sensitization), while inhalation exposure may be necessary for manifestation of CBD. Biological monitoring of beryllium in bodily fluids could be used to assess for the contribution of both the inhalation and dermal routes to total beryllium exposure. Early attempts at biological monitoring found that beryllium levels in urine indicated whether exposure to beryllium occurred but did not correlate to exposure level or severity of disease (Klemperer et al. 1951; DeNardi et al. 1953; Stoeckle et al. 1969). More recent analytical chemistry methods have greatly improved feasibility of biological monitoring for beryllium (Apostoli and Schaller 2001; Wegner et al. 2000; Saribal 2019; Goullé et al. 2005; Caldwell et al. 2005; Devoy et al. 2013). Apostoli and Schaller (2001) used inductively coupled plasma-mass spectrometry (ICP-MS) to demonstrate that levels of airborne beryllium correlated with levels of urinary beryllium among metallurgical workers. These successes hold promise for future development of approaches to assess and minimize dermal and inhalation exposures to beryllium. (Day et al. 2006a).

Biopsy tissue has been analyzed to determine beryllium concentrations in the body (Nadal et al. 2019). Lung tissue of two employees of a beryllium extraction and processing plant, where beryllium concentrations exceeded the April 2018 and earlier recommended standards of 2 μg beryllium/m^3 for an 8-hour day and 25 μg beryllium/m^3 for a 30-minute maximum level, contained 0.18 and 0.65 μg beryllium/g dry weight compared to the normal level of 0.02 μg beryllium/g (Kanarek et al. 1973). New lower standards (effective May 11, 2018) are provided in Chapter 7. The subject with the higher beryllium level did not have lung lesions; however, the subject with the lower beryllium level had granulomas. Thus, beryllium

levels in lung biopsies indicate the exposure to beryllium but may not confirm the presence of CBD. Therefore, further testing is required to confirm that these granulomas are a direct result of beryllium exposure, as CBD manifests identically to sarcoidosis, and is often misdiagnosed (Cullinan et al. 2017; Mayer et al. 2014; Chen et al. 2019) . Though one can determine the beryllium concentration in the lung using lung biopsy, it as an invasive procedure and does not provide information on how recently the exposure occurred because beryllium form, solubility, and particle size influence the amount of time the different types are present in the lung.

For occupational health monitoring, the internal dose of beryllium received by the lungs is more relevant than urinary or atmospheric beryllium. Beryllium in the exhaled breath condensate (EBC), was shown to be a good marker of occupational exposure in an aluminum (Al) production plant (Hulo et al. 2016). Hulo et al. (2016) measured the concentrations of beryllium in EBC (Be-EBC) and urine of controls and workers recently exposed to beryllium occupationally and calculated a cumulative beryllium exposure index (CBEI). Concentrations of Be-EBC (1.01±0.16 (mean + standard error) and 0.62±0.08 ng L^{-1} in exposed vs. controls) were significantly higher in exposed subjects after adjustment for smoking status, but urinary concentrations (14.85±3.61 (mean + standard error) and 15.16±2.88 ng g $creatinine^{-1}$ in exposed vs. controls) were not significantly different between groups. Concentrations of Be-EBC and Al-EBC of exposed subjects were highly correlated ($r = 0.85$; $p < 0.0001$). Concentrations of Be-EBC were significantly correlated with CBEI, but, not with urinary concentration. Due to its relationship with CBEI, but not with urinary concentrations of beryllium, Be-EBC could be used as a marker of occupational exposure and provide additional toxicokinetic information in occupational health studies (Hulo et al. 2016).

The contribution of skin exposure to beryllium causing beryllium sensitization has been recognized for over 60 years (Curtis 1951; Day et al. 2006b; Stefaniak et al. 2010). Studies suggest that hair follicles could be a marker of exposure to relatively insoluble particles <1 mm in diameter (Tan et al. 1996; Tinkle et al. 2003).

Drolet-Vives et al. (2009) evaluated whether hair and bone beryllium levels could be used as biomarkers of beryllium exposure. In the study, groups of C3H/HeJ mice were nose-only exposed to filtered air (n=7) or 250 μg/m^3 beryllium metal with a fine (MMAD 1.5 μm; n=40) or large (MMAD 4.1 μm; n=35) particle size for 6 hours/day, 5 days/week for 3 weeks. Beryllium levels in washed hair were significantly higher in groups of beryllium-exposed mice sacrificed 1 week after exposure as compared to controls. A significantly higher beryllium hair level was found in the mice exposed to fine beryllium and sacrificed 3 weeks post exposure as compared to those sacrificed 1 week after exposure. Beryllium exposure also significantly increased bone beryllium levels; the levels in the mice exposed to fine beryllium were

significantly higher than in the large particle beryllium group. Beryllium levels in the bone of mice killed 3 weeks after termination of exposure to fine beryllium metal was significantly higher than in those killed after 1 week. A report of beryllium accumulation in hair and bones of mice exposed to contaminated dusts suggested the potential use of hair and bone as biomarkers of beryllium exposure (Drolet-Vives et al. 2009).

The amount of beryllium found in the lungs could be used to differentiate CBD from sarcoidosis and controls. In a study by Verma et al. (2003), CBD cases had a much higher average beryllium level in the lungs than both sarcoidosis cases and controls. However, occupational history is an equally important factor in the differentiation between CBD and sarcoidosis (Verma et al. 2003).

3.3.2 Biomarkers of Effect

ABD affects most regions of the respiratory tract; some reported symptoms include nasopharyngitis, shortness of breath, labored breathing, and chemical pneumonitis.

Beryllium sensitization does not typically present with signs or symptoms however consistent abnormal or borderline results for blood and/or lung BeLPT results are a biomarker of effect. Likewise subclinical CBD does not present with clinical signs but BeS individuals do have histopathological evidence in the lung. Clinical CBD is an effect biomarker when BeS individuals have histopathological evidence in the lung with respiratory symptoms, changes on chest radiographs, or altered pulmonary physiology.

The lung is the most sensitive target organ of beryllium exposure. Long-term beryllium exposure often results in reduced lung function. This decrease has been measured by spirometry, such as forced expiratory volume in one second, maximum breathing capacity, maximum mid-expiratory flow, and vital capacity (Andrews et al. 1969; Kriebel et al. 1988a, 1988b). Blood gases such as carbon dioxide tension, oxygen tension, alveolar oxygen tension, alveolar carbon dioxide tension, and carbon monoxide diffusion capacity have also been analyzed.

Silveria et al. (2017) observed disease phenotypes associated with baseline patient characteristics, suggesting that CBD is a heterogeneous disease with variable severity. Lung physiology tests, including pulmonary function tests (PFT) plus data from exercising twice gave unique and meaningful information towards the characterization of CBD and sarcoidosis. These tests may be used in future studies to define mechanisms and risk factors for CBD severity (Silveira et al. 2017).

Radiographic examinations revealed opacities in the lung following chronic exposure to beryllium (Kanarek et al. 1973). X-rays have been used to determine three stages of chronic beryllium poisoning: a

fine diffuse granularity in the lungs, followed by a diffuse reticular pattern, followed by the appearance of distinct nodules. However, x-ray results cannot distinguish between CBD and sarcoidosis.

A patch test using soluble beryllium salts was evaluated in 32 patients with known CBD (Curtis 1959) and 18 other lung disease patients. The patch test was positive in all 32 CBD patients and negative in 16 of 18 patients with other lung diseases, indicating that the patch test may be useful in the diagnosis of CBD. However, the patch test using soluble beryllium compounds may be sensitizing and may exacerbate the condition in patients with CBD (Stoeckle et al. 1969; Tepper and VanOrdstrand 1972) (Cotes et al. 1983; Epstein 1983). Therefore, this method is not recommended as a diagnostic tool.

Analysis of secretions and cells of the lower respiratory tract obtained using outpatient bronchoscopy with bronchoalveolar lavage and transbronchial biopsy is useful for detecting granulomatous lung disease sub-clinically (Kreiss et al. 2007). However, it alone cannot distinguish CBD from sarcoidosis (Mayer et al. 2014). The presence of beryllium in the bronchoalveolar fluid, aids the CBD diagnosis.

CBD develops sequentially. Beryllium-exposed workers may develop immunologic beryllium sensitization without CBD. Some with beryllium sensitization develop CBD. Initially, CBD is sub-clinical (sCBD), meaning it is mild and largely asymptomatic. Many individuals with sCBD will progress to advanced CBD. Beryllium sensitization precedes CBD and develops after as little as 9 weeks of beryllium exposure (Harber and Su 2014; Maier 2001). Since CBD is caused by an immune reaction to beryllium, assessments of beryllium hypersensitivity, most often the BeLPT, are used in both medical surveillance and the diagnosis of beryllium sensitization and CBD, even at the sub-clinical stage (Maier 2001). The BeLPT measures cell proliferation via thymidine incorporation in cultured cells in the presence or absence of beryllium salts.

The standards for classifying individuals with beryllium sensitization and CBD have evolved with time. At one-point, patch tests were used to diagnose beryllium sensitization. Then an abnormal BeLPT without evidence of lung disease indicated beryllium sensitization. Now, beryllium sensitization is operationally defined by having two or more positive BeLPTs. At least two abnormal beryllium sensitization tests with evidence of a granulomatous inflammatory response in the lung is considered diagnostic of CBD (Schuler et al. 2012). The determinants of progression from beryllium sensitization to CBD are uncertain, however higher exposures and the presence of a genetic variant in the HLA–DP β-chain appear to increase the risk (Balmes et al. 2014; Rogliani et al. 2004).

Both peripheral blood cells and bronchioalveolar lavage cells can be used in the BeLPT. In early studies, abnormal blood BeLPT results were found in 100% of the subjects with CBD (Williams and Williams 1983; Williams and Williams 1982). Normal results were found in all individuals who were suspected of

having CBD, and abnormal results in approximately 2% of the healthy beryllium workers (Williams and Williams 1983). Despite these results, the blood BeLPT test was not widely used because it was very difficult to perform and not very reproducible. Refinement of the test methodology resulted in a more reproducible test that could be used as a screening tool (Newman 1996a).

A number of large-scale screening studies have utilized blood BeLPT for identifying beryllium sensitization in workers (Kreiss et al. 1993, 1996, 1997; Newman et al. 2001; Stange et al. 2001). In these studies, the majority (53–86%) of the workers with consistently abnormal blood BeLPT results were also diagnosed as having CBD. Using the blood BeLPT to screen workers for beryllium sensitization and CBD is not foolproof. Kreiss et al. (1993, 1996) found a small percentage of workers with CBD and normal or inconsistent blood BeLPT results. Deubner et al. (2001d) assessed the predictive value of the blood BeLPT as a screening tool for CBD. The incidences of CBD among workers with a single unconfirmed abnormal blood BeLPT result was 7/19 (37%), Forty-five percent of the workers had CBD and confirmed abnormal blood BeLPT results (34/75), and 49% of workers with first-time double abnormal blood BeLPT results (both laboratories in agreement) had CBD (17/35) (Deubner et al. 2001d). One limitation of the blood BeLPT is high inter- and intra-laboratory variability. Using split blood samples, Stange et al. (1996b) found an 85–96% agreement rate among three laboratories; however, the agreement rate was only 21–33% for positive blood BeLPT results. Similarly, Deubner et al. (2001d) found poor to moderate agreement in blood BeLPT results among three laboratories.

Stange et al. (2004) examined the sensitivity and specificity of the BeLPT test using data from >25,000 BeLPT tests from 12,194 workers employed at 18 DOE sites (national laboratories, production, and support sites); most of the workers were employed at the Rocky Flats Environmental Technology Site. At 17 of the sites, workers were exposed to beryllium or beryllium oxide; at the last site, workers were exposed to beryllium-copper alloy. 458 subjects with no known beryllium exposure were also tested. A false positive result was defined as an abnormal test result that could not be confirmed by additional BeLPT retests conducted within 2 months of the original sample. Data from the subjects with no known beryllium exposure were used to calculate the false positive rate.

The diagnosis of BeS based on blood BeLPT results alone may have been problematic in older studies. False positive rates in four laboratories conducting the BeLPT ranged from 0.00 to 3.35%, with an average false positive rate of 1.09% (Stange et al. 2004). False negative results were assessed among workers with two or more abnormal results and were defined as a normal result occurring within 2 years of the initial abnormal result. Overall, the false negative rate was 31.7% when only normal and abnormal results were considered and 27.7% when borderline-abnormal rates were considered abnormal (Stange et al. 2004). Methodological and diagnostic criteria have improved the reliability of the BeLPT throughout

the years. Splitting samples, defining the amount of proliferation needed to be considered abnormal or borderline, increasing the number of indices with proliferative responses, and repeat sampling have increased the sensitivity and specificity of the BeLPT. With these changes, the BeLPT has sensitivity estimated at 88% with a 96% specificity (Balmes et al. 2014). It is recommended that any probable BeS diagnosis that has solely used blood lymphocyte proliferation be followed up with a BAL BeLPT as many will rely on the BAL results to either support or reverse a BeS diagnosis.

Inter-laboratory agreement of abnormal results from the four test laboratories ranged from 26.2 to 61.8%; there was a greater agreement (36.6–64.7%) when only sensitized cases were considered. The intra-laboratory agreement of abnormal results ranged from 80.4 to 91.9%. Test sensitivity, the probability that a patient with CBD will have an abnormal BeLPT result, was 68.3%. Test specificity, the proportion of normal tests in all patients who do not have CBD, was 96.9%.

The study also evaluated the predictability of the BeLPT and found that 25.3% of the participants with one abnormal BeLPT result and 38.9% of the participants diagnosed with beryllium sensitization were diagnosed with CBD (Stange et al. 2004). Using the data from the Stange et al. (2004) study, Middleton et al. (2006) examined two algorithms used for BeLPT testing. In the first algorithm, one laboratory analyzed the initial blood sample and two laboratories analyzed a split sample for confirmation of abnormal or borderline tests. Using this algorithm, the test sensitivity was 65.7%; the specificity was estimated to be 99.9%. In the second algorithm, split samples sent to different laboratories were used for the initial and confirmation (abnormal and borderline results) tests. The test sensitivity was 86.0% using the second algorithm, and the test specificity was 99.8% (Middleton et al. 2006).

Middleton et al. (2008) examined the sensitivity and specificity of three beryllium sensitization criteria using the Stange et al. (2004) data. The three criteria were: (1) one abnormal BeLPT result; (2) one abnormal and one borderline (or abnormal) BeLPT result; or (3) two abnormal BeLPT results. The sensitivities of the three criteria were similar: 68.2, 65.7, and 61.2%, respectively (the respective specificities were 98.89, 99.92, and 99.98%). The positive predictive value (the likelihood that a person who meets the criteria is truly sensitized to beryllium) varies with the beryllium sensitization prevalence in the test population. If the prevalence in the test population is low (1%), then the positive predictive values for three criteria were 38.3, 89.3, and 96.8%; thus, the first criteria only correctly predicted beryllium sensitization for 38.3% of the subjects with one abnormal test result. At a 10% prevalence of beryllium sensitization, the positive predictive values were 87.2, 98.9, and 99.7% (Middleton et al. 2008).

In a subsequent analysis, Middleton et al. (2011) estimated the predictability of several combinations of results when three BeLPT tests were administered (single test in the first round and split samples in the

second round); this analysis also used the Stange et al. (2004) data set. The positive predictive values at 1–10% population beryllium sensitivity prevalence rates were >99% when three abnormal or two abnormal and one borderline test results were identified. When three borderline results were found, the positive predictive values were 83.7 and 98.3% at 1 and 10% population prevalence, respectively. When the results were one abnormal, one borderline, and one normal, the positive predictive values were 55.7 and 93.2% for 1 and 10% prevalence, respectively (Middleton et al. 2011).

Common interpretation strategies aim to limit false positives to 5%. A continuous variable is used in the BeLPT test and is called the stimulation index. The stimulation index is considered positive if it is greater than a criterion value, which is chosen to limit false positives to 5%. Nevertheless, beryllium-exposed workers in screening programs have the test performed many times, often on an annual basis. The test is imperfect, having both false positives and false negatives. Sensitization is operationally defined by having two or more positive BeLPTs, and the likelihood of having at least two positive tests increases as the number of tests increases. Simple measures such as sensitivity/specificity are less applicable when screening testing is applied multiple times (Harber and Su 2014). When prevalence is less than 50%, repeat testing will ultimately reduce accuracy. This adverse effect is increasingly important with more testing cycles, lower prevalence, and lower specificity.

Skin patch testing in three individuals with CBD resulted in strongly positive reactions to beryllium sulfate application, presenting extensive granulomatous inflammation in the skin. T cell clones in skin overlapped with those in BAL in all patients tested. Analysis of peripheral blood T cells before and after patch testing demonstrate T cell influx and mobilization into the blood during granulomatous inflammation (Fontenot et al. 2002).

Nonspecific immunologic findings in CBD include an increase in serum gamma-globulin levels (Resnick et al. 1970). The existence of specific antibodies to beryllium have been reported (Clarke 1991). While the results of peripheral blood BeLPTs have been variable in patients with CBD (Kreiss et al. 1989; Newman et al. 1989; Saltini et al. 1989; Stokes and Rossman 1991; Williams and Williams 1983), the results of lung BeLPTs have been consistently positive (Rossman et al. 1988; Saltini et al. 1989).

Martin et al. (2011) investigated whether a cytokine-based assay of $CD4^+$ T cells would be a better predictor of beryllium sensitization than the BeLPT test. The investigators used an enzyme-linked immune spot (ELISspot) analysis to measure IFN-γ secreting $CD4^+$ T cells. In a study of former beryllium workers, similar rates of sensitization were found using BeLPT (8.1%) and an IFN-γ ELIS spot response test (10%); however, among current workers, the BeLPT identified 1.3% sensitized workers compared to 9.9% identified using the IFN-γ ELISspot response test. The investigators suggested that the difference in

the test results among the current workers was due to the poor proliferation of beryllium-specific $CD4^+$ T cells after antigen exposure with no alteration on the cell's ability to secrete TH1-type cytokines such as IFN-γ. The IFN-γ ELISspot response test had a sensitivity of 85% and a specificity of 100%. The study also demonstrated that the IFN-γ ELISspot response test could also be used to differentiate between beryllium sensitization and CBD. More than 93% of the beryllium-sensitized subjects had less than 10 spot-forming units (SPU) and subjects with >40 SFUs had an 81% probability of progressing to CBD (Martin et al. 2011).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Most studies involving chemical interactions were performed to assess whether a substance could ameliorate beryllium toxicity. Mortality rates were lower if rats exposed to 2.59 mg beryllium/m^3 as beryllium sulfate were injected daily with ferric ammonium citrate beginning 4 days prior to beryllium exposure (Lindenschmidt et al. 1986; Sendelbach and Witschi 1987a). The protective action of iron on beryllium toxicity may be related to the ability of iron to increase ferritin synthesis, making more ferritin available to bind with beryllium (Lindenschmidt et al. 1986). Ferritin chelates with beryllium to protect against the inhibition of phosphoglucomutase (Joshi et al. 1984).

Ingested soluble beryllium compounds may interact with phosphate to form insoluble beryllium phosphate particles that are sequestered in Kupffer cells of the liver (Tepper and VanOrdstrand 1972). Diffusion of beryllium from the deposited particulates may cause damage to these cells and necrosis of the liver.

Intravenous injection of rats or mice with the ammonium salt of aurine tricarboxylic acid increased the survival of both species that were injected intravenously with lethal doses of beryllium sulfate (White et al. 1951). The protective effect was observed when the aurine tricarboxylic acid was administered from 1 hour before to 8 hours after injection of beryllium sulfate. Aurine tricarboxylic acid provides a protective effect that was attributed to its ability to complex with the beryllium ion, thereby reducing the amount of beryllium ion available to induce tissue injury.

Co-exposure of Chinese hamster ovary cells to beryllium sulfate and x-rays resulted in an increased rate of chromatid-type exchanges compared to the rates resulting from exposure to beryllium sulfate or x-rays alone (Brooks et al. 1989). The increase was multiplicative rather than additive. Experiments on cell cycle kinetics suggested that the multiplicative interaction occurs only in cells in the S and G_2 stages.

BeSO4 and beryllium chloride were weak mutagens by themselves, but a strong comutagen when used in conjunction with 1-methyl-3-nitro-1-nitrosoguanidine (MNNG). BeSO4 significantly enhanced the

mutagenicity of MNNG up to 3.5-fold over MNNG alone in a forward mutant detection system developed in *Escherichia coli* (Taylor-McCabe et al. 2006).

Benson et al. (2000) investigated the pulmonary toxicity and clearance of Beryllium/ copper (BeCu) alloy (2% Be; 98% Cu) and beryllium metal in female C3H/HeJ mice by administering 12.5, 25, and 100 μg BeCu alloy or 2 and 8 μg beryllium metal via intratracheal instillation. Slow lung clearance of the beryllium component (half-time of 2 weeks to a year) indicated that beryllium accumulates in the lung upon repeated exposure, while the Cu component rapidly clears the lung. The presence of Cu does not affect pulmonary beryllium clearance when comparing dose groups of 2 μg beryllium as a component of the 100 μg BeCu and beryllium alone (Benson et al. 2000).

Maternal and developmental beryllium-induced toxicity in rats altered metabolic indices. Treatment of Tiron (4,5-dihydroxybenzene 1,3-disulfonic acid disodium salt) restored metabolic indices largely to normal in both mothers and fetuses. Tiron may be effective in restoring altered biochemical parameters due to the available binding sites and the stability constant of the metal–chelator complex formed. Two molecules of Tiron may form a stable complex by substituting their hydrogen atoms and binding to beryllium with its oxygen atom (Sharma et al. 2000; 2002). Beryllium concentration in liver and kidney of adult female rats after intraperitoneal administration of 1 mg/kg beryllium nitrate for 3 weeks decreased following the therapy of 471 mg/kg chelating agent, Tiron (Shukla et al. 1998).

A chelating agent, 2,3-dimercapto-1-propanesulfonic acid (50 mg/kg), administered to male rats depleted beryllium from the liver, spleen and kidneys, but resulted in the redistribution of beryllium to the blood (Flora et al. 1995). Tiferron has been shown to have an antioxidant effect mobilizing beryllium ions from different tissues and recovering beryllium-induced systemic toxicity in combination with α-tocopherol and piperine respectively; however, combination of tiferron and piperine presented more pronounced therapeutic potential (Nirala et al. 2007). Likewise, crocin “saffron” protects against beryllium chloride toxicity in rats through diminution of oxidative stress and enhancing gene expression of antioxidant enzymes (El-Beshbishy et al. 2012).