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TOXICOLOGICAL REVIEW

OF

BERYLLIUM AND COMPOUNDS

(CAS No. 7440-41-7)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

April 1998
Revised May, 2008
(Inhalation cancer assessment and other selected text, as indicated)

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U.S. Environmental Protection Agency
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Note to reviewers: There are no changes in the non-cancer assessment and the existing RfD/RfC values have been retained. New text in the cancer assessment is highlighted, although no changes have been made to the inhalation unit risk (IUR) for cancer and the existing IUR has been retained.

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to beryllium. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of beryllium and compounds.

This Toxicological Review contains a cancer reassessment. The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

Note: In the May 2008 revised draft assessment, there are no changes in the non-cancer assessment and the existing RfD/RfC values have been retained. New text in the cancer assessment is highlighted, although no changes have been made to the inhalation unit risk (IUR) for cancer and the existing IUR has been retained.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations based on the 1998 assessment are provided in Appendix B.

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of beryllium. IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for beryllium has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to*

Carcinogens (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature for the inhalation cancer assessment was reviewed through December 2007. The assessments of the RfD and RfC, completed April 3, 1998, have not been updated.

2. CHEMICAL AND PHYSICAL INFORMATION

The element beryllium (Be) was discovered in 1798 by the French chemist Vauquelin, who prepared the hydroxide of beryllium. The metallic element was first isolated independently in 1828 by Wöhler and Bussy, and the latter named the new element glucinium (Gl) because of the sweet taste of its salts (Bussy, 1828). Today, this name is still used in the French chemical literature. In 1957, Wöhler's name Aberyllium@ was officially recognized by IUPAC (Ballance et al., 1978).

The Chemical Abstracts Service (CAS) names, registry numbers and respective atomic or molecular formulas for pure beryllium or beryllium compounds are listed along with some alloys of beryllium (IARC, 1980):

Beryllium	7440-41-7	Be
Acetic acid, beryllium salt	543-81-7	Be $(C_2H_3O_2)_2$
Hexakis[acetato-0:0]-oxotetraberyllium	19049-40-2	$Be_4O(C_2H_3O_2)_6$
Bis[carbonato-(2-)]dihydroxytriberyllium	66104-24-3	$(BeCO_3)_2.Be(OH)_2$
Beryllium chloride	7787-47-5	$BeCl_2$
Beryllium fluoride	7787-49-7	BeF_2
Beryllium hydroxide	13327-32-7	$Be(OH)_2$
Beryllium oxide	1304-56-9	BeO
Phosphoric acid, beryllium salt (1:1)	13598-15-7	$BeHPO_4$
Phenakite	13598-00-0	Be_2SiO_4
Sulfuric acid, beryllium salt (1:1)	13510-49-1	$BeSO_4$
Silicic acid, beryllium zinc salt	39413-47-3	ND
Bertrandite	12161-82-9	$4BeO.2SiO_2.H_2O$
Beryl	1302-52-9	$3BeO.Al_2O_3.6SiO_2$
Aluminum alloy, Al, Be	12770-50-2	ND
Copper alloy, Cu, Be	11133-98-5	ND
Nickel alloy, Ni, Be	37227-61-5	ND

ND = exact composition unknown or undetermined.

Elemental beryllium has many unique physical properties. It is the lightest of all solid and chemically stable substances, with an unusually high melting point of 1,278EC, low density, and very high specific heat, heat of fusion, sound conductance and strength-to-weight ratio. Beryllium is lighter than aluminum but is more than 40% more rigid than steel.

The chemical properties of beryllium differ considerably from those of the other alkaline earth metals. It has a number of chemical properties similar to aluminum even though the two elements have different oxidation states (Be^{+2}, Al^{+3}) based on their different positions in the periodic table; namely, Groups IIA and IIIA, respectively. The ionic radius of beryllium is only

0.31 angstroms, with a large ionic charge-to-radius ratio of 6.45. Because of this, the most stable beryllium compounds are formed with smaller anions such as fluoride and oxide (Krejci and Scheel, 1966). This high charge-to-radius ratio of bivalent beryllium also accounts for the amphoteric nature (like aluminum, beryllium behaves as an acid in presence of a base and vice versa) of the ion (Basolo, 1956; Cartledge, 1928) and the strong tendency of beryllium compounds to hydrolyze. The degree of hydrolysis is dependent on the nature of the salt [i.e., BeF_2 ~1%; $BeCl_2$ ~4.6%] (Drury et al., 1978). In addition to forming various types of ionic bonds, beryllium has a strong tendency for covalent bond formation. For example, it can form organometallics such as $(CH_3)_2Be$.

Most metallic salts formed from hydrochloric, hydrofluoric and nitric acids are very soluble in water; beryllium is no exception. However, anhydrous beryllium sulfate, beryllium hydroxide, beryllium oxide and beryllium carbonate are for the most part relatively insoluble in water. In hot water, however, anhydrous beryllium sulfate is converted to the tetrahydrate, with a solubility of 425 g/L (Table 2-1). Aqueous solutions of beryllium salts are acidic as a result of the formation of $Be(OH_2)_4^{+2}$, the tetrahydrate. Because of its amphoteric character, beryllium is capable of forming positive ions in dilute acids at a pH <5 and negative ion called beryllates [$(BeO_2)^{=}$] above pH of 8, with insoluble hydroxides and complexes forming between pH 5 and 8 (Drury et al., 1978). Salts of strong bases and weak acids (e.g., beryllium acetate) are capable of hydrolyzing and reacting with water to form insoluble hydroxides. Beryllium is likely to occur in natural waters only in trace quantities (<1 μg/L), since beryllium compounds are relatively insoluble at the pH of natural waters (Hem, 1970). Such is shown in Figure 1, which demonstrates the degradation, fate and transport of beryllium compounds in any neutral environment. In neutral environments the oxides, sulfates, hydroxides and nitrates and the beryllium oxy organic compounds are shown as forming insoluble beryllium compounds and remain in the particulate, rather than the dissolved, species. The fluorides of beryllium are soluble and will remain in the dissolved state (Dairy et al. 1996). Detectable concentrations of beryllium are found in acidified waters. In view of the increased acidification of some natural waters, there is potential for an increased solubility of beryllium salts.

The use of beryllium in alloys is based on a combination of outstanding properties that are conferred on other metals: low density combined with strength, high melting point, resistance to oxidation, and a high modulus of elasticity. These alloys are suitable as lightweight materials that must withstand high acceleration or centrifugal forces. However, beryllium-rich alloys have not played a significant role because of the brittle nature imparted by beryllium to other metals and the low solubility of most elements in solid beryllium. The only alloy with a high beryllium content is lock-alloy, containing 62% beryllium and 38% aluminum.

Table 2-1. Physical and chemical properties of beryllium compounds

Properties	Chemical name						
	Beryllium oxide	**Beryllium sulfate**	**Beryllium hydroxide**	**Beryllium carbonate**	**Beryllium fluoride**	**Beryllium chloride**	**Beryllium nitrate**
Molecular formula	BeO	$BeSO_4$	$Be(OH)_2$	$BeCO_3$ + $Be(OH)_2$	BeF_2	$BeCl_2$	$Be(NO3)_2 \cdot 3 H_2O$
Molecular weight	25.01	105.07	43.03	112.05	47.01	79.93	187.1
CAS registry number	1304-56-9	13510-49-1	13327-32-7	13106-47-3	7787-49-7	7787-47-5	13597-99-4
Specific gravity (20°)	3.01	2.44	1.92	NR	1.986 (25°)	1.899 (25°)	1.557
Boiling point, °C	3900	NR	NR	NR	NR	482.3	142
Melting point, °C	2530+30	decomposes 550-600	NR	NR	555	399.2	60
Vapor pressure, mm Hg	NR	NR	NR	NR	NR	1291°C	NR
Water solubility, mg/L	0.2, 30°C	Insoluble in cold water; converted to tetrahydrate in hot water	Slightly soluble	Insoluble in cold water; decomposes in hot water	Extremely soluble	Very soluble	Very soluble

NR = not reported.

Sources: Windholz et al. (1976); Weast (1977).

Ammonium Tetrafluoroberyllate
(Ammonium Beryllium Fluoride)
$(NH_4)_2BeF_4$! $2[NH_4]^+aq + [BeF_4]^{2-}aq$ *Remains soluble in a neutral environment*
Excess H_2O
pH 7

Beryllium Oxide
$BeO + H_2O$! $Be(OH)_2$ *Forms insoluble beryllium hydroxide in a neutral*
Excess H_2O environment
pH 7

Beryllium Hydroxide
$Be(OH)_2$! No Reaction *Beryllium hydroxide is insoluble in a neutral environment*
Excess H_2O
pH 7

Beryllium Fluoride
$BeF_2 + 2H_2O$! $[BeF_2(H_2O)_2]_{aq}$ and other complexes *Remains soluble in a neutral environment*
Excess H_2O
pH 7

Beryllium Nitrate Trihydrate
$Be(NO_3)_2$ X $3H_2O$ + 2MOH ! $Be(OH)_2$ + $2[M]^+_{aq}$ + $2[NO_3]^-_{aq}$ + $3H_2O$
Base Excess H_2O Beryllium Hydroxide Nitrate Salt in Solution
pH 7
Forms insoluble beryllium hydroxide in a neutral environment

Beryllium Sulfate Tetrahydrate
$BeSO_4$ X $4H_2O$ + 2MOH* ! $Be(OH)_2$ + $2[M]^+_{aq}$ + $[SO_4]^{2-}_{aq}$ + $4H_2O$
Base Excess H_2O Beryllium HydroxideSulfate Salt in Solution
pH 7
*M signifies a cation such as sodium, potassium, calcium, etc.
Forms insoluble beryllium hydroxide in a neutral environment

Beryllium Oxalate Trihydrate
BeC_2O_4 X $3H_2O$ + 2MOH ! $Be(OH)_2$ + $2[M]^+_{aq}$ + $[C_2O_4]^{2-}_{aq}$ + $3H_2O$
Base Excess H_2O Beryllium Hydroxide Oxalate Salt in Solution
pH 7
Forms insoluble beryllium hydroxide in a neutral environment

Beryllium Basic Acetate*
$Be_4O(C_2H_3O_2)_6$ + 6MOH + H_2O ! $4Be(OH)_2$ + $6[M]^+_{aq}$ + $6[C_2H_3O_2]^-_{aq}$
Base Excess H_2O Beryllium Hydroxide Acetate Salt in Solution
pH 7
*Beryllium basic acetate is not a true basic salt; it is a covalent compound.
Forms insoluble beryllium hydroxide in a neutral environment

Source: Hertz et al. (1996)

Figure 1. Precipitation of beryllium compounds in a neutral (pH 6.5-9.5) environment.

3. TOXICOKINETICS

3.1. ABSORPTION

Inhalation is the primary route of uptake of beryllium to occupationally exposed persons; however, no human data are available on the deposition or absorption of inhaled beryllium. With respect to deposition and clearance, particles of beryllium, like other inhaled particles, are governed by important factors such as dose, size and solubility. Particles formed from volatile emissions, as a result of high temperature by either nucleation (where gas molecules come together) or condensation (where gas molecules condense onto an existing particle), tend to be much smaller in size than those produced by mechanical processes in which small but more coarse particles are produced from larger ones. In its *Air Quality Criteria for Particulate Matter* document, the U.S. EPA (1996a) defines air particles according to a bimodal distribution as fine (<1 μm aerodynamic equivalent diameter [d_{ae}]) and coarse (>2.5 μm d_{ae}). Studies concerned with measurement of particulate matter often report such results as PM_{10}, referring to samplers that collect increasing fractions as the particle diameter decreases below 10 μm MMAD (d_{ae}). For dosimetric purposes in the respiratory tract, 50% of particles of this d_{ae} will penetrate beyond the larynx.

Beryllium particles produced from anthropogenic processes (more than 99% of beryllium emitted into the atmosphere is the result of oil or coal combustion for electric power generation) are generally emitted as the oxide; namely, beryllium oxide (BeO) (U.S. EPA, 1987). The inhalation toxicity of insoluble beryllium oxide depends to a great extent on its physical and chemical properties, which can be altered considerably depending on production conditions. It is well known that the toxicity of beryllium oxide is dependent on the particle size, with smaller particles (<10 μm, d_{ae}) able to penetrate beyond the larynx. However, most inhalation studies and occupational exposures involve quite small (<1-2 μm) BeO particles that would penetrate deeply into the lungs. In inhalation studies with beryllium ores, particle sizes are generally much larger, with deposition occurring in several areas throughout the respiratory tract for particles <10 μm (d_{ae}).

Today, toxicologically relevant exposure to beryllium appears to be almost exclusively confined to the occupational settings; however, it should be noted that the similar prevalence of chronic beryllium disease (CBD) in the community compared to workers exposed to much higher levels (100-1,000 $\mu g/m^3$) was attributed to the smaller particle size of beryllium emitted to the outside air compared to beryllium particles inside the plant (Eisenbud et al., 1949; Eisenbud and Lisson, 1983). The temperature at which beryllium oxide is calcined influences its particle size (surface area), solubility, and ultimately its toxicity. Beryllium oxide calcined at 500EC produces a

more toxic oxide than at 1,000EC, which has been attributed to its greater specific surface area compared to the material calcined at 1,000EC (Finch et al., 1988; Haley et al., 1989).

Occupational studies also show compound-specific differences in beryllium toxicity, but are less clear about whether beryllium metal or beryllium oxide is more toxic, and if these differences can be attributed to variability in particle size or solubility differences. Eisenbud and Lisson (1983) found a higher prevalence of CBD in people who work with beryllium metal than in those who worked with beryllium oxide, and Sterner and Eisenbud (1951) found a much higher prevalence of CBD in people who worked with beryllium oxide than in those who worked with other beryllium compounds. By contrast, Cullen et al. (1987) found a greater frequency of CBD in workers presumably exposed to beryllium oxide fumes compared to the beryllium metal, but the small particle size of the fume compared to the beryllium metal dust may have contributed to the higher toxicity in this study.

Natural and anthropogenic emissions of beryllium to the atmosphere are depicted in Table 3-1 (U.S. EPA, 1987a). Atmospheric beryllium oxide returns to earth through wet and dry deposition. Beryllium, once deposited on land as the oxide, remains bound to the soil within the environmental pH range of 4-8 and does not dissolve in water, thus preventing release to ground water. In addition, beryllium is believed not to biomagnify to any extent within food chains. Beryllium generally enters the water as beryllium oxide and slowly hydrolyzes to beryllium hydroxide [$Be(OH)_2$], which is insoluble in water. BeO and $Be(OH)_2$ are almost impervious to attack from dilute acids and alkalis. However, such particles are solubilized by a fluoride source or sources of extremely strong acid acids (pH <0) and strong bases (pH >14). Such solubility in strong acids and bases once again demonstrates the amphoteric nature of this metal as the hydroxide. The estimated average concentration of beryllium in any fresh surface water is 1 μg/L or 1 ppb.

Table 3-1. Natural and anthropogenic emissions of beryllium to the atmosphere

Emission source	Total U.S. production[a] (10^6 tons/year)	Emission factor (g/ton)	Emissions (ton/year)
Natural:			
Windblown dust	8.2	0.6	5.0
Volcanic particles	0.41	0.6	0.2
Total			5.2
Anthropogenic:			
Coal combustion	640	0.28	180
Fuel oil	148	0.048	7.1
Beryllium ore processing	0.008	37.5[b]	0.3
Total			187.4

[a]Units of metric tons.
[b]Production of beryllium ore expressed in equivalent tons of beryl; the emissions factor of 37.5 is hypothetical.

3.1.1. Respiratory Absorption

There are no human data on the deposition and absorption of inhaled beryllium. In animals, beryllium deposited in the lung is cleared slowly, with clearance half-times of days to years, depending on the beryllium compound and, in the case of processed beryllium oxides, on the processing temperature. Initial clearance from the lung, which includes uptake, is typically biphasic, and occurs rapidly via the mucociliary escalator, followed by slower clearance via translocation to the tracheobronchial lymph nodes, alveolar macrophage clearance to the tracheal region, and solubilization of beryllium. The initial rapid and slow phase clearance of particles in the tracheobronchial tree leads to half-times of ~1-60 days and 0.6-2.3 years in rats, respectively. Thus, the amount of beryllium in the lung at any time after exposure depends on the amount deposited and the rate of clearance. In human beings, the residence time for beryllium in the lung may be several years, since appreciable amounts of beryllium have been found in the lung many years after exposure was stopped. Lung clearance of beryllium from the alveoli is more rapid in hamsters than in rats and, in both species, is greater in males than females (Sanders et al., 1975). Clearance is also affected by whether a soluble beryllium compound is capable of ionizing in the lung. Non-ionized soluble forms of beryllium, such as citrate, are cleared from the lungs in about 1-4 days; however, the ionized soluble forms precipitate in lung tissue and simulate particulate matter in behavior. Beryllium excretion occurs primarily in the feces, with a larger percentage in the urine at longer postexposure times, as more beryllium is solubilized and systemically distributed.

As discussed in further detail in Section 4.1, the presence of beryllium in the lung is one criterion used to diagnose CBD. In a study of 20 subjects with suspected CBD, the beryllium concentration in lung tissue ranged from 8-1,925 μg/g, with an average of 282 μg/g dried tissue (Schepers, 1962). Hasan and Kazemi (1974) defined elevated levels of beryllium in the lung as

>0.02 μg/g dry weight of lung. Beryllium lung burden is not predictive of CBD. Stiefel et al. (1980) found that the average beryllium concentration in the blood of 20 people without occupational exposure was 0.9 ng/g.

Several authors have investigated the pulmonary and whole-body clearance of beryllium compounds following inhalation exposure. The more soluble compounds, such as beryllium sulfate and beryllium oxide calcined (heated as part of its preparation) at 500EC, are cleared more rapidly than less soluble compounds, such as beryllium oxide, calcined at 1,000EC. The influence of calcining temperature of beryllium oxide on the compound's solubility, toxicity and clearance is discussed in more detail in Section 4.4. In an experiment with beagle dogs exposed for 5-42 minutes to beryllium oxide calcined at 500 or 1,000EC, Finch et al. (1990) found that pulmonary clearance of both forms from 4 days through one year after exposure was described by a single component exponential function. The half-time for pulmonary clearance was 64 days for beryllium oxide calcined at 500EC and 240 days for beryllium oxide calcined at 1,000EC. Whole-body clearance was biphasic for the low-temperature calcined material, with 59% of the initial lung burden cleared with a half-life of 54 days and the half-life for the long-term component being more than 1,000 days. The long-term component was attributed to beryllium that dissolved from particles and bound to extrapulmonary compartments such as the skeleton and liver. Whole-body clearance of beryllium oxide calcined at 1,000EC could be described by a single-component exponential function with a half-life of 310 days. After an interval of 2.5 years, these dogs received a second acute exposure (<1 hour) to beryllium oxide calcined at 500EC, and the whole-body clearance time was similar to that seen for 500EC BeO in the initial exposure (Haley et al., 1992).

Rhoads and Sanders (1985) observed biphasic lung clearance in rats exposed for 30-180 minutes to beryllium oxide fired at 1,000EC. The first component accounted for 30% of the initial lung burden and had a half-life of 2.5 days. The second component had a half-life of 833 days. They found that whole-body clearance was uniphasic, with a half-life of 356 days. Sanders et al. (1975) reported an alveolar retention half-life for beryllium oxide of approximately six months in rats and hamsters exposed to beryllium oxide calcined at 1,000EC. Hart et al. (1984) exposed male F344 rats to 447 μg Be/m^3 as beryllium oxide heat-treated at 560EC 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 nonlavageable compartment (lung tissue).

The accumulation of beryllium in the lung was measured in male and female Sprague-Dawley rats exposed to 34.25 μg Be/m^3 as beryllium sulfate for 7 hours/day, 5 days/week for up to 72 weeks, with three of each sex sacrificed monthly during exposure (Reeves and Vorwald, 1967).

The lung burden tended to plateau in both sexes after about 36 weeks. This plateau was attributed to the attainment of an equilibrium between deposition and clearance for this soluble salt. The concentration of beryllium in the tracheobronchial lymph nodes peaked at about 44 weeks, with markedly higher levels in males. This was interpreted as better lymphatic clearance of the lungs in males. Over half of the beryllium in the lungs was still present at four weeks after the end of exposure. The study authors suggested that soluble beryllium salts become sequestered in inflammatory scar tissue, or that insoluble precipitates are formed.

Clearance of beryllium chloride, a soluble beryllium salt, is faster than that of the oxide. 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 hours 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. In dogs exposed to 191 μg Be/m^3 as beryllium fluoride for 6 hours/day, 5 days/week for various exposure durations, the rate of increase of the beryllium level in lungs and pulmonary lymph nodes increased with duration of exposure, suggesting decreased clearance (Stokinger et al., 1953). In the lung, beryllium accumulation was 0.013, 0.089 and 0.062 μg/g lung/day for 47, 87 and 207 days of exposure, respectively. A continuous increase in the rate of beryllium accumulation was observed in the pulmonary lymph nodes.

There is evidence that the initial lung burden does not affect the pulmonary clearance of beryllium but does affect the clearance of other particles. No significant differences in beryllium lung clearance half-times (250-380 days) were noted among male F344/N rats receiving inhalation exposure to beryllium metal aerosol resulting in initial lung burdens of 1.8, 10 or 100 μg (Finch et al., 1994). Exposure was to 4.7-150 mg/m^3 for 14-30 minutes. (Additional animals received an initial lung burden of 0.32 μg, but clearance could not be calculated for this dose.) However, there was a dose-related decrease in the clearance of a radioactive tracer particle. The reason for a dose-related effect on the tracer particle, but no dose-related effect on clearance of beryllium itself, is unclear. However, the study authors suggest that the beryllium particles might be sequestered at the sites of inflammation and thus shielded from normal clearance mechanisms. Sanders et al. (1975) found that clearance of a radioactive tracer was decreased by 40% for at least 60 days postexposure in female rats receiving an initial alveolar deposition of 30 μg beryllium as beryllium oxide calcined at 1,000EC (exposure level and duration not reported).

3.1.2. Gastrointestinal Absorption

Gastrointestinal absorption can occur by both the inhalation and oral (diet, drinking) routes of exposure. In the case of inhalation, a portion of the inhaled material is transported to the GI tract by the mucociliary escalator or by the swallowing of the insoluble material deposited in the upper respiratory tract (Kjellstrom and Kennedy, 1984). Unlike inhalation, where a significant part of the inhaled dose is incorporated into the skeleton (ultimate site of beryllium storage, half-life of 450 days), oral administration results in <1% absorption and storage (as reviewed by U.S. EPA, 1991a). Most of the beryllium taken up by the oral route passes through the gastrointestinal tract unabsorbed and is eliminated in the feces.

3.1.3. Dermal Absorption

Dermal absorption, like oral absorption, contributes only very small amounts to the total body burden of beryllium-exposed persons; however, because of the skin effects elicited by beryllium compounds, this route is of some significance. As most beryllium salts do not remain soluble at physiological pH, there is no ready systemic diffusion following local skin contact since beryllium is bound by epidermal (alkaline phosphatase and nucleic acids) constituents.

3.2. DISTRIBUTION

In animals, beryllium is cleared from the lung and distributed primarily in the skeleton, with additional deposition in tracheobronchial lymph nodes. After a single 5- to 42-minute exposure of beagle dogs to beryllium oxide calcined at 500EC, 14% and 16% of the initial lung burden was found in the skeleton at 64 and 180 days postexposure, respectively (Finch et al., 1990). At 180 days, comparable levels were in the lung and skeleton. The amount of beryllium in the tracheobronchial lymph nodes peaked at 8.8% of the initial lung burden at 64 days postexposure, and the amount of beryllium found in the liver increased with time. By contrast, for the material calcined at 1,000EC, 88%, 1.9% and 1.5% of the initial lung burden was found in the lungs, tracheobronchial lymph nodes and skeleton, respectively, at 64 days postexposure. Soluble beryllium appears to cross the placenta, based on findings in mice injected intravenously with approximately 0.1 mg/kg radiolabeled beryllium chloride (Bencko et al., 1979).

In dogs exposed by inhalation to beryllium fluoride, beryllium sulfate or beryllium oxide, Stokinger et al. (1953) found beryllium primarily in the lung, pulmonary lymph nodes, skeleton and liver. The more soluble compounds (the fluoride and sulfate) had a larger percentage of the total body burden in the skeleton and liver, indicating greater systemic distribution. Rats sacrificed three

weeks after receiving a single intratracheal instillation of radiolabeled beryllium oxide calcined at 1,000EC had beryllium primarily in the lung, with much smaller amounts in the liver, kidney, femur and heart (Clary et al., 1975). Sanders et al. (1975) found that deposition in tracheobronchial lymph nodes increased with time after inhalation exposure to beryllium oxide calcined at 1,000EC.

3.3. METABOLISM

Beryllium and its compounds are not biotransformed. However, soluble beryllium salts may be converted to less soluble forms in the lung, while insoluble forms of beryllium ionized by myeloperoxidases as it is being engulfed by phagocytes (ATSDR, 1993; Leonard & Lauwerys, 1987; Lansdown, 1995).

3.4. ELIMINATION

Excretion of unabsorbed beryllium is primarily via the fecal route shortly after exposure (inhalation or intratracheal) through mucociliary clearance from the respiratory tract and ingestion of swallowed beryllium (Hart et al., 1980; Finch et al., 1990). Urinary excretion becomes more important at later time points, especially for the more soluble beryllium compounds, as absorbed beryllium is removed from the body. Beryllium oxide calcined at 1,000EC is less soluble, and fecal excretion dominated at all time periods in dogs through one year after exposure (Finch et al., 1990). At 32 days after an acute inhalation exposure of beagle dogs, fecal excretion accounted for 59% of total excretion of beryllium oxide calcined at 500EC, and 68% for beryllium oxide calcined at 1,000EC (Finch et al., 1990). By 180 days postexposure, fecal excretion accounted for 47% of total excretion of the low-fired material and 54% of total excretion for the high-fired material. In guinea pigs exposed to 230 μg Be/m^3 as beryllium chloride for 55 minutes, Hart et al. (1980) described a 40% reduction of beryllium body burden within 48 hours, primarily by fecal excretion (90%). Rhoads and Sanders (1985) reported that virtually all excreted beryllium was in the feces, following a single inhalation exposure of rats to beryllium oxide calcined at 1,000EC.

Andre et al. (1987) measured excretion of beryllium metal powder and of hot-pressed (at 1,000EC) beryllium metal after intratracheal instillation in *Papio papio* baboons and rats. Urinary excretion showed a clear relationship to the amount of beryllium instilled. Mean daily excretion of beryllium metal was 4.6×10^{-6} % of the administered dose in baboons and 3.1×10^{-6} % in rats. Hot-pressed beryllium was more soluble, with a daily excretion of 13.8×10^{-6}% of the dose in rats.

Urinary excretion of beryllium following occupational exposure correlates qualitatively with the degree of exposure but does not correlate with the severity of CBD (Klemperer et al., 1951). The

average daily excretion of beryllium in a group of former beryllium workers ranged from 1.2-8.3 μg/L with an average of 4.0 μg/L. Stiefel et al. (1980) reported 2 μg/L beryllium in the urine of smokers who used unfiltered cigarettes; no information was provided on urinary levels in nonsmokers without occupational exposure. The average urinary beryllium level in dental technicians exposed to beryllium was 0.37 μg/L, while the average in the general population in an area with a high density of metallurgical manufacturing industries was 0.24 μg/L (Apostoli et al., 1989).

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Physiologically based toxicokinetic models have been developed to assess environmental exposure levels for other metals, such as cadmium and lead. However, no toxicokinetic models have been developed for beryllium in either human or animal species.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS AND CLINICAL CONTROLS

4.1.1. Noncancer Effects

4.1.1.1. *Acute Beryllium Disease*

Acute beryllium disease is defined as beryllium-induced pulmonary disease with less than a year's duration (Sprince and Kazemi, 1980). Acute beryllium lung disease is likely to be due to direct toxicity, unlike the immune mechanism of chronic beryllium disease described below.

4.1.1.2. *Chronic Beryllium Disease*

Chronic beryllium disease (CBD), formerly known as Aberylliosis@ or Achronic berylliosis,@ is an inflammatory lung disease that results from inhalation exposure to beryllium. It is characterized by the formation of granulomas (pathologic clusters of immune cells) with varying degrees of interstitial fibrosis and involves a beryllium-specific immune response. A particularly important part of the diagnosis of CBD is to distinguish it from sarcoidosis, a granulomatous lung disease of unknown cause. Varying definitions of CBD do exist. The Beryllium Case Registry lists the following criteria for diagnosing CBD:

1) Establishment of significant beryllium exposure based on sound epidemiologic history
2) Objective evidence of lower respiratory tract disease and clinical course consistent with beryllium disease
3) Chest X-ray films with radiological evidence of interstitial fibronodular disease
4) Evidence of restrictive or obstructive defect with diminished carbon monoxide diffusing capacity (DL_{CO}) by physiologic studies of lung function

5a) Pathologic changes consistent with beryllium disease on examination of lung tissue

5b) Presence of beryllium in lung tissue or thoracic lymph nodes.

More recent studies typically use the following criteria based on the availability of more advanced diagnostic tools that provide higher sensitivity and specificity than earlier methods (Newman et al., 1989):

1) History of beryllium exposure
2) Histopathological evidence of noncaseating granulomas or mononuclear cell infiltrates in the absence of infection
3) Positive blood or bronchoalveolar lavage (BAL) lymphocyte transformation test

A key aspect of the identification of CBD is the demonstration of beryllium sensitization in the beryllium lymphocyte transformation test (BeLT, also known as the LTT, BeLPT) (reviewed by Newman, 1996). In this test, lymphocytes obtained from either BAL fluid or from peripheral blood are cultured *in vitro* and then exposed to soluble beryllium sulfate to stimulate lymphocyte proliferation. The observation of beryllium-specific proliferation indicates beryllium sensitization. Early versions of the test had high variability, but the use of tritiated thymidine to identify proliferating cells has led to a more reliable test (Mroz et al., 1991; Rossman et al., 1988). Tests utilizing peripheral blood have now been found to be as sensitive as the BAL assay, although larger abnormal responses are generally observed in the BAL assay (Kreiss et al., 1993a; Pappas and Newman, 1993). False negative results can occur with the BAL BeLT in cigarette smokers who have marked excess of alveolar macrophages in lavage fluid (Kreiss et al., 1993a). The BeLT has also been used in animal studies to identify those species with a beryllium-specific immune response (see Section 4.4). As described below, the BeLT test can detect beryllium sensitization and has a higher predictive value in CBD screening than clinical exam, spirometry, or chest radiography.

Evaluation of the exposure-response to beryllium has been made more difficult because CBD is an immune disease and only a small percentage of the population appears to be susceptible. Nonetheless, exposure-response relationships are evident. Several studies have observed CBD in people chronically exposed in plants that are generally in compliance with the beryllium permissible exposure limit of 2 $\mu g/m^3$.

The most complete investigation of community cases of CBD was conducted by Eisenbud et al. (1949), who evaluated exposure related to 11 cases of CBD based on radiographic and pathologic examination. Radiological screening of 10,000 residents was conducted, with questionable cases undergoing clinical evaluation. CBD was diagnosed based on radiological and clinical findings and on a consensus of specialists. One case was exposed to beryllium dust on worker clothes and will not be discussed further. Of the other cases, five lived within 0.25 miles of a beryllium production plant, and all lived within 0.75 miles of the plant. A follow-up to this study reported three additional cases at less than 0.75 miles from the plant but no additional cases of CBD at greater than 0.75 miles (Sterner and Eisenbud, 1951). Measurements downwind from the plant found that the beryllium concentration at 0.75 miles was about 0.045 $\mu g/m^3$, and continuous sampling stations found that the average concentration at about 700 feet from the plant (the furthest distance within the affected area)

was 0.05 μg/m^3 (range 0-0.46 μg/m^3). The emitted beryllium was primarily as beryllium oxide, although beryllium fluoride and beryl (beryllium ore) were also present. The study authors also calculated an estimated exposure, based on emissions levels, stack heights and wind speed data. These estimates were generally in good agreement with the downwind data. Based on these calculations, the authors estimated that the average exposure levels at 0.75 miles from the plant during the period of exposure monitoring were 0.004-0.02 μg/m^3. Averaging this value to 0.01 μg/m^3 and noting that both plant production and emissions were about 10-fold higher in earlier years, the authors estimated that the concentration at 0.75 miles was 0.01-0.1 μg/m^3. However, the only population data available are within 0.25 miles of the Lorain plant. Eisenbud and Lisson (1983) were quite certain that a population of approximately 500 people was exposed to levels of 0.1 μg/m^3. Beyond 0.25 miles, estimates of exposure are very uncertain. The similar prevalence of CBD in the community compared to workers exposed to much higher levels (up to 100 μg/m^3) was attributed to the smaller particle size of beryllium emitted to the outside air compared to beryllium particles inside the plant (as discussed in Eisenbud and Lisson, 1983). Thus, this study establishes a NOAEL(HEC) of 0.01-0.1 μg/m^3 for the development of CBD in a population exposed to beryllium in ambient air.

Kanarek et al. (1973) measured beryllium exposure levels and respiratory effects in 214 of 245 full-time workers who were employed for 1-14 years at a beryllium extraction and processing plant. Because most operations occurred only during a small fraction of the day, the study authors considered peak air concentrations more important than TWAs, and reported only the former value. Measured beryllium concentrations ranged from 0.31-1,310 μg/m^3, with the lower levels corresponding to times that the operations were not occurring. For some processes, the lower range was as high as 7 μg/m^3. They identified 31 workers with radiographic findings consistent with interstitial disease, 20 workers with significant hypoxemia (decreased arterial oxygen tension, Pa_{02}), and 11 workers with both symptoms. Two cases of CBD were identified, but screening for CBD was incomplete because biopsies were conducted on only these two subjects. A follow-up study was conducted in 1974, after exposure levels had been markedly reduced, with peak beryllium concentrations of 15 μg/m^3 and <2 μg/m^3 for the two worst processes (Sprince et al., 1978). Hypoxemia was significantly decreased in the 13 hypoxemic workers who were available for follow-up, and 9 of the workers with initial evidence of interstitial lung disease had normal chest radiographs. This study suggests that early clinical signs of CBD can be reversed by reduced exposure to beryllium. However, because neither beryllium sensitization nor CBD were shown in the workers exposed between 1971 and 1974, the strength of this conclusion is weakened.

Cotes et al. (1983) evaluated beryllium exposure and its effects in 130 of the 146 men who had worked at a beryllium manufacturing plant for at least six months. Exposure was measured as

area samples, and the geometric mean concentration for each sample site and year was estimated by eye after plotting the data on logarithmic graph paper. Mean exposure for different job processes was 0.029-0.72 μg Be/m^3 as beryllium oxide in 1952, and 0.022-0.21 μg Be/m^3 in 1960. Four definite clinical cases of CBD, one highly probable case of CBD and two cases of radiographic abnormality were identified. The definition of CBD was not reported, but two of the definite cases were described as having typical radiographic and lung function changes but no overt clinical symptoms. Two other cases of Asubacute@ beryllium disease were identified in follow-up studies, one of whom developed CBD after a beryllium patch test. The probable cases had small radiographic opacities with no other explanation, and one had a somewhat reduced carbon monoxide diffusing capacity (DL_{CO}). The two definite cases identified in the main study worked entirely in the slip-casting bay, where the geometric mean beryllium concentration was 0.036 μg/m^3 in 1952 and 0.18 μg/m^3 in 1960. Their exposure duration was about 6 years. The overall average exposure levels were not reported. However, based on the reported months of exposure and cumulative exposure level, the average exposure can be estimated as 0.1 μg/m^3 for the two definite cases and 0.05-0.16 μg/m^3 for the cases of radiographic changes only. There was no evidence of an association between CBD and brief high exposures. Seventeen men at the plant recalled brief periods of high exposure and two developed acute beryllium disease, but none of these men developed CBD. This study is limited by the poor description of the definition used for CBD, but it identifies a LOAEL of 0.1 μg/m^3, corresponding to a LOAEL (HEC) of 0.036 μg/m^3.

In a three-year prospective study of beryllium mine and mill workers, Rom et al. (1983) found that beryllium sensitization, based on blastogenic lymphocyte transformation (LT), was reversible when exposure levels decreased. In the initial assessment of 197 workers, there were 15.9% (13/82) positives (LTs) , based on the peripheral blood BeLT; 8.2% (5/61) were positive in the follow-up of 1982. Of 11 of the 13 workers who were positive (LTs) initially and were tested in the follow-up study, 8 had lost their sensitivity at the second test. In the baseline year, one-third of the beryllium monitoring samples exceeded 2 μg/m^3, and the mean exposure level was 7.18 μg/m^3. By contrast, only 11% of the samples exceeded 2 μg/m^3 in the following 3 years, and the average was 0.25, 0.40 and 0.99 μg/m^3 in successive years. Only qualitative individual exposure levels were reported. Most of the sensitized individuals had higher exposure levels, but cases of sensitization were also reported in people with low exposure. A positive BeLT result was not associated with decreased respiratory function. None of the study participants developed CBD. Although the study authors presented some data on reproducibility of results, their assay does not appear to be as sensitive or reproducible as later versions of this assay (e.g., Mroz et al., 1991), and the apparent reversibility may have been due to false positives in the initial assay or false negatives in the repeat assay. Acute chemical pneumonitis resulting from high-level beryllium exposure has also been reported. Acute beryllium lung disease is likely to be due to direct toxicity, unlike the immune

mechanism of CBD. As of 1977, there were 887 cases in the beryllium case registry. Of these, 631 cases were classified as chronic, 212 as acute, and 44 as acute developing to CBD. Thus, early cases of CBD sometimes also had acute beryllium disease. Due to the markedly decreased levels of occupational exposure to beryllium, acute chemical pneumonitis is now quite rare. Only one acute case was added to the registry in 1972-75, but there was about one case of CBD per month during the same period (Sprince and Kazemi, 1980).

Cullen et al. (1987) reported five likely cases of CBD (using the beryllium case registry definition of CBD) in workers who were presumably exposed to beryllium oxide fumes at a precious metals refinery for 4-8 years before the development of symptoms. Time-weighted average personal air samples for beryllium ranged from 0.22 42.3 μg/m^3 throughout the plant, and 10% of the samples were >2.0 μg/m3. However, four of the cases worked predominantly in the furnace area, where beryllium exposure was measured at 0.52 0.44 μg/m^3 (maximum measurement 1.7 μg/m^3). No additional cases were found in the screening of current workers, but a fifth was identified after the screen. This subject worked as a crusher, where exposure was to beryllium metal dust at 2.7-7.2 μg/m^3. The CBD cases had the classic signs of CBD, including hilar adenopathy visible radiologically, noncaseating granuloma and pulmonary fibrosis in biopsy samples, and decreased DL_{CO}. Symptoms progressed even after the removal from exposure. Beryllium sensitization was shown *in vitro* with BAL lymphocytes. Three of the cases were considered to have CBD, while diagnosis of two (both in the furnace area) was complicated by confounding factors. One had a history of hilar enlargement and the other had schistosomiasis and no BAL stimulation data. The study authors also analyzed beryllium exposure levels by job classification and screened 45 of 70 current workers for CBD using interviews and analysis of spirometry data and chest radiographs from routine testing. No in vitro screening for beryllium sensitization was conducted on the general worker population. Noting that the prevalence of CBD was highest at a task with a lower exposure levels, the study authors suggested that the beryllium oxide fumes to which workers were exposed in the furnace area were more toxic than the beryllium metal dust to which workers were exposed at other tasks. The study authors considered alternative explanations for the development of disease following low-level exposure to be unlikely. Although sampling efficiency was less than 100% for particles <0.8 microns, these small particles were not considered to contribute significantly to the overall mass. However, such small particles may be even more toxic because of their large surface area per unit mass. There was concomitant exposure to arsenic at 0.82 0.26 μg/m^3, cadmium at 38.927.2 μg/m^3, lead at 20.315.2 μg/m^3, and nickel at 91.967.6 μg/m^3, but these levels were all within acceptable exposure limits. Although the study authors note that there have been no significant changes in work practices during the past 20 years, it is possible that the small number of retrospective air samples collected in a two-week period may not accurately reflect past and present

exposure conditions. The LOAEL in this study was 0.52 $\mu g/m^3$, with a LOAEL (HEC) after adjusting for occupational exposure of 0.19 $\mu g/m^3$.

In a cross-sectional study of 297 white male workers at a beryllium extraction facility, Kriebel et al. (1988a) analyzed the following pulmonary function parameters: forced vital capacity (FVC); forced expiratory volume in 1 second (FEV_1); maximum mid-expiratory flow (MMEF); and alveolar-arterial oxygen gradient ($AaDO_2$). Exposure data using area samplers and/or personal sampling were available from 1947 and later. Individual exposure was estimated on the basis of reported job titles and exposure at each job during different periods (Kriebel et al., 1988b). Exposure decreased dramatically during the period of assessment; average exposures at dirty jobs were above 25 $\mu g/m^3$ prior to the 1960s. The median cumulative exposure was 65 $\mu g/m^3$-year, and the median of the mean lifetime exposures was 4.3 $\mu g/m^3$. Both exposures to beryllium fumes and to beryllium or beryllium oxide dust occurred. Correlations between pulmonary function parameters and exposure were determined, but no attempt was made to separately identify workers with CBD. A significant ($p<0.05$) correlation was observed between decreased FVC and FEV_1 and exposure for $\geq$21 years. There was also a significant correlation between cumulative exposure and increased (worse) $AaDO_2$ values, but not with other pulmonary function parameters. Mild radiological abnormalities were seen in some workers. The absence of more severe findings can probably be attributed to the healthy worker effect. Labor turnover in the plant was very high during the years when exposure was high, and the sensitive workers had probably left because of acute beryllium disease or CBD. Because workers were not grouped by exposure level, the data are insufficient to determine levels at which such effects are seen. In addition, because those with CBD were not separately identified, the magnitude of the exposure-response relationship was probably decreased.

Kreiss et al. (1989) used the peripheral blood BeLT to screen an occupationally exposed population for CBD and found that 6/51 (11.8%) of the currently exposed workers were sensitized. One of the sensitized workers had an equivocal BeLT result and did not have CBD, based on the lack of granulomas on transbronchial lung biopsy. Historically, exposure at this plant was to beryllium oxide dust and fumes, but exposure at the time of the study was only to beryllium oxide dust. In a screen of 505 workers at a plant that had manufactured ceramics from beryllium oxide (beryllia) from 1958 through 1975, the prevalence of CBD among various exposed subgroups ranged from 2.9 to 15.8% (Kreiss et al., 1993b). Two cases of CBD that were identified on the basis of chest radiographs had normal or inconsistently abnormal blood BeLT results. No sensitized cases who had not yet developed CBD were identified, perhaps because of the long period since first exposure (23.7 years on average). The latency for CBD ranges from a few months to more than 20 years (Kreiss et al., 1993a). There is at present no clear relationship between exposure duration and the development of the disease. Cases have developed following exposures as short as a few months

(Kreiss et al., 1996). Studies suggest that early stages of CBD can be reversed (Rom et al., 1983; Sprince et al., 1978), although these studies are weakened by methodological limitations, as described in the following paragraphs.

Although subjects identified based on BeLT test results may not have overt symptoms of CBD, many do exhibit functional impairment. Pappas and Newman (1993) measured spirometry, lung volumes, arterial blood gases, DL_{CO}, and exercise physiology in a group of 21 workers identified using the blood BeLT ("surveillance-identified") and 15 workers identified based on symptoms or radiographic abnormalities ("clinically identified"). Exercise physiology was the most sensitive test, with 52% of the surveillance-identified subjects exhibiting abnormal pulmonary physiology at maximum exercise; 93% of the clinically identified subjects showed similar abnormalities. Present and former smokers were included in the groups. DL_{CO} was a less sensitive measure. The study authors noted that most of the subjects exhibited a rise in the ratio of dead space to tidal volume (VD/VT), even though most subjects had a normal recruitment of VT. They suggested that this indicates that a pulmonary vascular abnormality occurs early in CBD. As support, they noted that granulomas and fibrosis developing early in the course of the disease are often located in the interstitium in a perivascular distribution.

The clinical severity of CBD, measured by pulmonary function, exercise physiology and degree of radiographic abnormalities, is reflected by the stimulation index in the BAL BeLT, the BAL white cell count, and the BAL differential cell count (Newman et al., 1994a). Interestingly, the blood BeLT results did not correlate with severity of CBD. Eight subjects without CBD were beryllium-sensitized as demonstrated by abnormal results in the blood test but normal BAL BeLT results and no evidence of granulomas. These results are consistent with other data (Kreiss et al., 1993a) showing that sensitization precedes inflammatory infiltration of the lung.

The development of fiber optic bronchoscopy and transbronchial biopsy methods has allowed the identification of subclinical cases of CBD. Newman et al. (1989) evaluated respiratory symptoms and physical examination results in 12 cases of newly-identified CBD based on the following: (1) a history of beryllium exposure; (2) histopathological evidence of noncaseating granulomas or mononuclear cell infiltrates; and (3) a positive blood or BAL BeLT. Eight of the cases were exposed to beryllium oxide dust or beryllium fumes (exposure duration of 1-25 years). The other four were exposed primarily to beryllium oxide dust (exposure duration of 0.1-5 years, none with current exposure). Only five sought medical attention for respiratory symptoms and none had systemic symptoms associated with CBD, although at least one had mild respiratory symptoms that were observed in a detailed physical examination. Five of the subjects also had no increase over normal in interstitial markings on chest radiography. Lung volumes and flow rates were abnormal in

only 4/12 cases, and oxygen exchange during exercise was abnormal in only 3/9. Based on these findings, the authors suggested that CBD be classified into the following stages: (1) sensitization; (2) subclinical beryllium disease (sensitized subjects with histopathological evidence, but no clinical signs); and (3) beryllium lung disease [same as (2), but with respiratory symptoms, changes on chest radiographs, or altered pulmonary physiology].

Varying, and generally low, prevalences of CBD have been observed in occupationally exposed populations, even when exposure was as high as 100 $\mu g/m^3$ (Sterner and Eisenbud, 1951). However, the BeLT has allowed the identification of an exposure-response relationship for beryllium sensitization. Kreiss et al. (1993a) used the BeLT test to evaluate a stratified random sample of nuclear weapons workers (n=895), but the author did not report exposure levels. Subjects with beryllium sensitization underwent further clinical testing, including a lung biopsy and a BAL BeLT test. Of 18 sensitized subjects, 12 had CBD and 3 others developed CBD within two years. The sensitization rate correlated with participant-reported exposure level and ranged from 1.5% in the no-exposure group (some of whom had suspected exposures) to 3.4% in the consistent exposure group; machinists (a job title with consistent exposure) had an even higher sensitization rate (4.7%). Longer-term longitudinal studies are necessary to determine whether all sensitized subjects eventually develop CBD. The beryllium-sensitized machinists had a longer time since first exposure than the nonsensitized machinists, suggesting that earlier exposures were higher or that the latency period was not sufficient for all cases of sensitization to have developed in the latter group. Beryllium sensitization, and then CBD, was also detected in a secretary, indicating that a transient, possibly high exposure to beryllium can cause sensitization. Beryllium sensitization was observed to progress to CBD even in the absence of continued exposure, suggesting that abnormal BeLT test findings are predictive of future development of CBD.

Stange et al. (1996) assessed beryllium sensitization and CBD in 1,885 current employees and 2,512 former employees at the Rocky Flats Environmental Technology site. Beryllium concentrations in the main beryllium production building were measured from 1970 to 1988 using fixed airhead samplers and from 1984 to 1987 using personal air monitoring devices. The mean beryllium concentrations from fixed airhead samplers and personal monitoring devices were 0.16 $\mu g/m^3$ (95% confidence interval of 0.10-0.22 $\mu g/m^3$) and 1.04 $\mu g/m^3$ (95% confidence interval of 0.79-1.29 $\mu g/m^3$), respectively. Beryllium sensitization (positive blood BeLT results from two different laboratories or positive results in two consecutive blood BeLTs) was diagnosed in 22 current employees (1.2%) and 47 former employees (1.9%). CBD was diagnosed in 6 (0.3%) and 22 (0.9%) current and former employees, respectively. The combined incidence of CBD and beryllium sensitization was 1.49% and 2.75% among the current and former employees (2.21% for both groups combined). Current and former employees with negative BeLT results or unconfirmed

positive results were retested 3 years later, along with employees not participating in the previous screening and employees with abnormal X-rays; employees with no definitive diagnosis of CBD were offered a BeLT and chest X-ray one year after the initial screening. The 3- and 1-year retesting resulted in a beryllium sensitization and CBD incidence of 9/518 (1.7%) and 1/518 (0.2%), respectively. The total incidence of beryllium sensitization and CBD among the current and former employees (includes cases from initial and follow-up screenings) was 107/4,397 (2.43%). A total of 29 cases of CBD were diagnosed among the current and former employees at the Rocky Flats site: 17 cases had evidence of granulomas on biopsy, seven had no evidence of granulomas, and biopsies were not performed for five cases. Thus, this study identified a LOAEL of 1.04 $\mu g/m^3$ for beryllium sensitization and CBD; the LOAEL (HEC) after adjusting for occupational exposure is 0.37 $\mu g/m^3$.

Kreiss et al. (1996) conducted a cross-sectional study of 136/139 of then-current beryllium workers in a plant that made beryllia ceramics from beryllium oxide powder. An additional 15 workers who had been exposed to beryllium at other jobs were excluded from exposure calculations because their earlier exposure was not known. Because the plant opened in 1980, high-quality industrial hygiene measurements were available for almost the entirety of the exposure period. Measurements from 1981 and later were reviewed and included area samples, process breathing-zone samples, and personal lapel samples (the last year only). Quarterly daily-weighted average (DWA) exposures were calculated using a formula based on all of these measurements for each job title. However, general area and breathing zone samples were not recorded for machining processes until the last quarter of 1985, soon after machining production was transferred to that plant, even though a limited amount of machining had been conducted since 1982. Although total beryllium exposure was generally well characterized for most of the affected workers, two of the seven beryllium-sensitized machinists started machining prior to the systematic environmental monitoring. Since exposure levels generally declined with time, exposure estimates for these two subjects may have been underestimated. The median breathing zone measurement of beryllium for machining was 0.6 $\mu g/m^3$, and 0.3 $\mu g/m^3$ for other processes. The frequency of excursions to higher exposure levels decreased with time, with the percentage of machining breathing zone measurements above 5 $\mu g/m^3$ falling from 7.7% during early sampling years to 2.1% during later sampling years.

Beryllium lymphocyte transformation tests were performed by two different laboratories on blood samples collected from 136 employees. Positive results from one or both laboratories were confirmed by analyzing a subsequent blood sample. Of 136 tested employees, 5 had consistently abnormal blood BeLT results from the two laboratories and were diagnosed with CBD based on observation of granulomas in lung biopsy samples. An additional two employees had abnormal blood results from one of the two laboratories and had no granulomas in lung biopsy samples. Both employees developed abnormal blood results in other laboratory tests within 2 years. One of these

two employees also developed symptoms of CBD. The other employee declined clinical follow up. An additional case of CBD was found during the study in an employee hired in 1991, who had a nonhealing granulomatous response to a beryllium-contaminated skin wound. This subject had a confirmed abnormal blood test and after several additional months developed lung granulomas. Only one CBD case had an abnormal chest x-ray (defined as mall opacity profusion of 1/0 or greater). An additional 11 former employees had CBD, for a total prevalence of 19/709. Beryllium-sensitized cases were similar to nonsensitized ones in terms of age, ethnic background and smoking status, but did have significantly fewer pack-years of smoking. There was also no significant difference in percent exposed to beryllium dust or mist in an accident or unusual incident, or those in areas with a posted high air count. Of the eight sensitized workers, seven had worked in machining at some point, while one had never worked in a production job. The beryllium sensitization rate was 14.3% among the machinists, compared to 1.2% among all other employees. The individual average beryllium exposures for the six CBD cases and two sensitized cases among current employees ranged from 0.2 1.1 μg/m3, and the cumulative exposure ranged from 92.6 1,945 $\mu g/m^3$ days. The median of estimated average beryllium exposure for the sensitized cases was about 0.55 $\mu g/m^3$. The sensitized cases without disease did not have lower exposures than the CBD cases. Machinists may have been more susceptible than other groups because of their higher overall exposure, or because the particles produced during machining were primarily respirable in size, while other exposures were to particles larger than the respirable range. Other characteristics of the machining exposure, such as the particle morphology and surface properties, or adjuvants in machining fluids may also have affected sensitization. The study authors noted that median breathing zone levels tended to be lower than the DWAs derived from these levels, because much of the day was typically spent in high-exposure tasks. This study identified a lowest-observed-adverse-effect level (LOAEL) of 0.55 $\mu g/m^3$, and a LOAEL Human Equivalent Concentration (LOAEL[HEC]), adjusted for an occupational exposure (5 days/7 days, 10 m^3 per 8 hour workday/ 20 m^3 per day), of 0.20 $\mu g/m^3$.

Few data are available on the particle characteristics of beryllium under occupational exposure conditions. However, Hoover et al. (1990) found that 5.7% of the particles released during sawing of beryllium metal had aerodynamic diameters smaller than 25 μm but larger than 5 μm, and 0.3% were smaller than 5 μm. For milling of beryllium metal, 12-28% of the particles had aerodynamic diameters between 5 and 25 μm, and 4-9% were smaller than 5 μm, depending on the milling depth. More than 99% of the particles generated from operations conducted with beryllium alloys were larger than 25 μm.

CBD has been reported in people not occupationally exposed to beryllium, including people living in communities near beryllium plants (Chesner, 1950; Dattoli et al., 1964; Lieben and Metzner, 1959) and in families of beryllium workers who wore contaminated clothing at home.

These cases have been markedly reduced by better industrial hygiene, including mandatory work clothes exchange, but nonoccupational CBD has still been reported following low-level episodic exposure of family members (Newman and Kreiss, 1992).

A number of studies have characterized the signs and symptoms of CBD. Initial symptoms of early cases of CBD typically include dyspnea, cough, fatigue, weight loss and chest pain (Aronchik, 1992; Hasan and Kazemi, 1974; Kriebel et al., 1988b; Meyer, 1994; Sterner and Eisenbud, 1951; Williams, 1993). Other symptoms included bibasilar crackles, clubbing of the fingers and skin lesions, heart failure, and an enlarged liver or spleen. Prominent diagnostic findings are diminished vital capacity, diffuse infiltrates and hilar adenopathy visible radiographically. Fibrosis occurs at late stages in the disease. Granulomatous inflammation has also been reported in extrapulmonary sites, such as extrapulmonary lymph nodes, skin, liver, spleen, kidney, bone, myocardium, central nervous system, and skeletal muscle. As noted above, clinical, radiographic, and traditional spirometric signs of CBD are less sensitive than histologic findings and immunologic screens using the BeLT. Computed tomography (CT) can identify some CBD cases missed by chest radiography, but even CT missed 25% of histologically-confirmed cases (Newman et al., 1994b).

CBD has resulted in death, especially prior to the implementation of more rigid controls in 1949, when exposure was much higher than it is now. In a cohort mortality study of 689 patients with CBD who were included in the case registry, there was a high rate of deaths due to pneumoconiosis, primarily CBD (Standardized Mortality Ratio [SMR] = 34.23, 95% confidence interval of 29.1-40.0, 158 deaths) (Steenland and Ward, 1991). Similar results (SMR=1640, $p<0.05$, 52 deaths) were observed in an earlier analysis of deaths in the beryllium case registry due to nonneoplastic respiratory disease (Infante et al., 1980). Deaths have also been reported in community cases of CBD, including a 10-year-old girl (Lieben and Williams, 1969). Some of these cases have been confirmed based on histological evidence of CBD and evidence of beryllium in the lungs.

4.1.2. Cancer Effects

A series of epidemiology studies has investigated the carcinogenic potential of beryllium exposure among beryllium processing workers and patients enrolled in the Beryllium Case Registry (BCR). Some of these studies have found positive, statistically significant, associations between beryllium inhalation exposure and lung cancer (Table 4-1). Prior to 1947, beryllium concentrations greater than 1,000 $\mu g/m^3$ were not uncommon. Due to concerns about CBD, the Atomic Energy Commission (AEC) and subsequently OSHA established a permissible exposure limit of 2 $\mu g/m^3$.

Bayliss (1971)

The U.S. Public Health Service study of approximately 8,000 past and current workers employed in beryllium plants revealed a slightly elevated risk of lung cancer among male workers employed between 1942 and 1967 (standard mortality ratio [SMR][1] = 1.07; 95% CI = 0.75–1.47) (Bayliss, 1971). Some of the study limitations included elimination of 2,000 workers due to incomplete data, lack of analysis of data according to length of time since initial employment, and the combination of populations from several plants into one cohort.

Mancuso (1980, 1979)

Mancuso (1980) extended an historic cohort study (Mancuso, 1979), following 3,685 workers employed in Ohio and Pennsylvania beryllium production facilities between 1937 and 1948. The author compared the beryllium workers to the U.S. white male population (Mancuso, 1979) and 5,929 workers employed at a viscose rayon plant from 1938–1948 (Mancuso, 1980). Lung cancer mortality was followed in both groups through 1976. A statistically significant increase in lung cancer mortality was observed in the beryllium cohort compared with the entire rayon cohort (SMR = 1.40; 95% CI = 1.12–1.74) (Table 4-1). An elevated risk of lung cancer was found to be most pronounced among long-term workers. When the cohorts were stratified by duration of employment, the number of cancer deaths among beryllium workers employed for greater than 49 months was double that expected based on viscose rayon workers (SMR = 2.22; 95% CI = 1.26–3.62).

Limitations of this study included potential confounding by cigarette smoking, no exposure assessment, no latency analysis (other than duration of employment), and lack of clarity in the description of the analytic methods. The use of rayon workers as a control group may have helped control for variables such as smoking and socioeconomic status. However, if the rayon workers also had an increased risk of lung cancer mortality because of exposure to other lung carcinogens, the SMRs would be attenuated.

[1]The ratio of the number of deaths observed in the study group or population to the number that would be expected if the study population had the same specific rates as the standard population (Last, 1995).

Table 4-1. Summary of epidemiologic studies assessing the relationship between beryllium exposure and lung cancer

Reference	Cohort or plant location (cohort size)	Period of employment	Termination of follow-up	Comparison population	SMR or odds ratio	95% CI	Observed lung cancer deaths
Bayliss (1971)	USPHS (multiple plants) (n ~ 8,000)	1942–1967	1967	U.S. population	SMR = 1.07	0.75–1.47	37
Mancuso (1979)	Lorain, OH, & Reading, PA (n = 3,685)	1937–1948	1974	U.S. white male population	Overall Lorain SMR = 1.97[a] Reading SMR = 1.37 ≥15 years since hire Lorain SMR = 2.23[a] Reading SMR = 1.63[a]	 1.32–2.90 1.00–1.85 1.43–3.32 1.16–2.24	 25 40 22 36
Mancuso (1980)	Lorain, OH, & Reading, PA (n = 3,685)	1937–1948	1976	Viscose rayon workers	Overall SMR = 1.40[a] Duration of employment: ≤12 months SMR = 1.38[a] 13–48 months SMR = 1.06 ≥49 months SMR = 2.22[a]	1.12–1.74 1.04–1.80 0.60–1.73 1.26–3.62	80 52 14 14
Wagoner et al. (1980)	Reading, PA (n = 3,055)	1942–1967	1975	U.S. white male population	Overall SMR = 1.37[a] Interval since hire <15 years SMR = 0.95 15–24 years SMR = 1.28 ≥25 years SMR = 1.85[a]	1.01–1.81 0.46–1.75 0.78–1.98 1.16–2.81	47 9 18 20
Infante et al. (1980)	BCR (n = 421)	Entry into registry 1952–1975	1975	U.S. white male population	Overall SMR = 2.12 By respiratory illness group Acute SMR = 3.14[a] Chronic SMR = 0.72	0.93–4.19 1.27–6.53 0.04–3.58	7 6 1
Steenland and Ward (1991)	BCR (n = 689)	Entry into registry 1952–1980	1988	U.S. population	Overall SMR = 2.00[a] Time since 1st exposure ≤20 years SMR = 1.95	1.33–2.89 0.94–3.59	28 10

Table 4-1. Summary of epidemiologic studies assessing the relationship between beryllium exposure and lung cancer

Reference	Cohort or plant location (cohort size)	Period of employment	Termination of follow-up	Comparison population	SMR or odds ratio	95% CI	Observed lung cancer deaths
					>20 years SMR = 2.03[a]	1.20–3.21	18
Ward et al. (1992)	Seven beryllium plants (n = 9,225)	1940–1969	1988	U.S. male population	Overall SMR = 1.26[a] Latency	1.12–1.42	280
					≤15 years SMR = 0.89	0.60–1.28	27
					15–30 years SMR = 1.20[a]	1.00–1.43	119
					>30 years SMR = 1.46[a]	1.22–1.71	134
Levy et al. (2007)	Reanalysis of Ward et al. (1992) (n = 9,225)	1940–1969	1988	U.S. veterans[b]	Overall SMR = 1.04[c]	0.92–1.17	280
Sanderson et al. (2001a)	Reading, PA (n = 242 lung cancer cases)	1940–1969	1992	U.S. male population (SMR)	Overall SMR = 1.22[a]	1.03–1.43	142
				Workers at same plant as controls (OR)	Average exposure >20µg/m^3 Odds ratio (exposure unlagged) = 2.23 Odds ratio (exposure lagged 10 years) = 4.17[a] Odds ratio (exposure lagged 20 years) = 2.19[a] Maximum exposure >20µg/m^3 Odds ratio (exposure unlagged) = 2.22 Odds ratio (exposure lagged 10 years) = 4.58[a] Odds ratio (exposure lagged 20 years) = 2.34[a]		

[a]p value < 0.05.
[b]U.S. decennial census age-specific population data for Lorain, OH, and Reading, PA.
[c]Estimates categorized by latency were not reported.

Note: BCR = Beryllium Case Registry; SMR = standard mortality ratio; USPHS = U.S. Public Health Service; CI = confidence interval.

Objective information about exposures was limited to occasional air measurements or educated guesses because there were few worker safety measures. Limited resources prevented the acquisition of personal information about smoking or other behaviors on the scale required for a study of a rare outcome such as lung cancer. These methods had inherent limitations and required further, more rigorous research for the findings to be convincing. The consistently positive results seen in these early beryllium research studies stimulated more refined research studies that attempted to overcome early limitations.

Wagoner et al. *(1980)*

A cohort mortality study of 3,055 white males employed between 1942 and 1967 at the beryllium extraction, processing, and fabrication facility in Reading, Pennsylvania, followed workers from plant start-up through 1975 (Wagoner et al., 1980). The reference group for this cohort was the U.S. white male population. Since U.S. statistics were not available for the period 1968–1975, SMRs for this period were based on the 1965–1967 period. No retrospective exposure assessment was attempted, but the authors stratified the results by interval since hire (a proxy for latency), date of employment (a proxy for potential improvements in industrial hygiene), and duration of employment. This study revealed a statistically significant increase in lung cancer mortality (SMR = 1.37; 95% CI = 1.01–1.81). When deaths from lung cancer were stratified by interval since onset of employment, the SMR was highest among workers with a more than 25-year latency (Table 4-1). There was also a statistically significant increase in the number of lung cancer deaths among workers hired before 1950, 31 observed versus 16.73 expected (SMR = 1.85; 95% CI = 1.28–2.60).

Vital statistics from an earlier period were used. U.S. EPA (1987a) adjusted the lung cancer SMRs from this study for the problems of using non-concurrent vital statistics and for lack of information on smoking. This analysis, based on information submitted to EPA at that time, increased expected lung cancer death rates by 11% to account for the underestimation that may have occurred from using older vital statistics (nationwide lung cancer rates were increasing) and by 4.1% to account for the potential for differences in smoking habits between the beryllium cohort and the U.S. population. Although the SMRs for latency of 25 years remained elevated after this adjustment, they were no longer statistically significant (SMR = 1.36) (U.S. EPA, 1987a). Despite the limitations, the above results indicated that an association between beryllium and lung cancer could not be ruled out. Subsequent studies of the beryllium workers further investigated this relationship by using more sophisticated epidemiologic methods.

Infante et al. *(1980)*

Infante et al. (1980) examined the possible relationship between beryllium exposure and lung cancer in a cohort mortality study of 421 white males entered into the Beryllium Case Registry (BCR) with the diagnosis of beryllium disease between July 1952 and December 1975. The cohort did not include subjects who were deceased at the time of BCR entry. No information on occupations was provided in the report, but IARC (1993), in its review of this study, mentioned that the majority of individuals in the BCR worked in beryllium extraction and smelting, metal production, and fluorescent tube production, and a small number were not exposed occupationally but lived near the plants. Cause-specific mortality data from the U.S. population for the period of 1965–1967 (matched for race, sex, age, and calendar time period) were used for comparisons. An increase in the number of observed cancer deaths (SMR = 1.53; 95% CI = 0.95–2.35) and the number of lung cancer deaths grouped as cancer of the trachea, bronchus, and lung was noted (SMR = 2.12; 95% CI = 0.93–4.19). The small number of observed deaths may have contributed to the lack of statistically significant associations (Table 4-1).

Cancer mortalities were segregated by diagnosis of either acute beryllium-related respiratory illness (n = 223) or chronic beryllium-related diseases (n = 198). Significant increases in deaths from lung cancer were observed in the acute beryllium illness group (SMR = 3.14; 95% CI = 1.27–6.53) (Table 4-1). Five of these six lung cancer deaths were observed among workers with >15-year latencies (SMR = 3.21; 95% CI = 1.17–7.10). Of the 198 workers with chronic respiratory disease, only one lung cancer death was observed. The authors noted that this observation may be due to the high case-fatality rate for nonneoplastic respiratory disease in the workers with chronic beryllium illnesses. Significant increases in deaths from nonneoplastic respiratory disease were observed in the group with acute beryllium illness (SMR = 10.31; 95% CI = 5.23–18.38) and in the chronic beryllium illness group (SMR = 64.62; 95% CI = 47.16–86.51), based on 10 and 42 observed deaths, respectively. The lung cancer mortality rates were not adjusted for cigarette smoking because smoking habit information was not obtained from the cohort. The authors (Infante et al., 1980) noted that it was highly unlikely that workers with acute beryllium illnesses had smoking habits of sufficient magnitude to account for the excessive lung cancer risk observed in that group.

Steenland and Ward (1991)

Steenland and Ward (1991) conducted an analysis similar to that of Infante et al. (1980), extending the period of entry into the BCR by five years and following cases until 1988. The authors analyzed incidence of lung cancer mortality in a cohort of patients with beryllium disease who had been entered into the BCR. The study cohort consisted of 689 patients listed in the BCR with acute and chronic beryllium disease, which was thought to include most of the known cases of beryllium disease in the U.S. The authors developed SMRs from U.S. population cancer mortality

rates. The results were stratified by time since first exposure, duration of exposure, gender, type of industry involved in exposure, and acute versus chronic beryllium disease (individuals with acute beryllium disease are presumed to have had a higher dose of beryllium according to the authors). Information on the smoking habits of 32% of the cohort in 1965 was compared to U.S. smoking statistics to determine the effect that smoking differences might have had on lung cancer SMRs among the subjects.

There was a statistically significant twofold excess of lung cancer death in the beryllium disease cohort compared to the U.S. population (SMR = 2.00; 95% CI = 1.33–2.89) (Table 4-1). The excess risk appeared to be somewhat higher among patients with the acute form of beryllium disease (Table 4-2). Women had a higher risk of lung cancer than men, with SMRs being 4.04 (95% CI = 1.47–8.81) and 1.76 (95% CI = 1.02–2.67), respectively. Latency and length of exposure did not affect the SMRs. The authors suggest that patients in the cohort smoked less than the general public in 1965, perhaps as a result of their respiratory disease. Steenland and Ward (1991) calculated that, without beryllium exposure, the SMR for lung cancer due to smoking would have been less than 1.0 compared to smoking prevalence in the U.S. population.

Table 4-2. SMRs for lung cancer deaths among persons enrolled in the Beryllium Case Registry

	Observed lung cancer deaths	SMR	95% CI
Overall lung cancer SMR	28	2.00[a]	1.33–2.89
Acute beryllium disease	17[b]	2.32[a]	1.35–3.72
Chronic beryllium disease	10[b]	1.57	0.75–2.89
Males	22	1.76[a]	1.02–2.67
Females	6	4.04[a]	1.47–8.81
Exposure ≤4 years	17	2.01[a]	1.11–3.23
Exposure >4 years	11	1.98	0.99–3.55
≤20 years since 1st exposure	10	1.95	0.94–3.59
>20 years since 1st exposure	18	2.03[a]	1.20–3.21

[a]$p < 0.05$; SMR = standard mortality ratio.
[b]The authors state that the sums may not add up to the total number of cause-specific deaths because disease type was unknown for 2% of the cohort.

Source: Steenland and Ward (1991).

A major strength of the study was that the entire cohort had definite beryllium exposure, based on the criteria for enrollment into the BCR. The fact that most of the excess number of lung

cancer deaths occurred among acute, as opposed to chronic, beryllium disease cases may be due to chance as the confidence intervals of the acute and chronic groups overlap. It is also possible that high case fatality of CBD may not allow for the development of lung cancer. Additionally, higher levels of exposure among acute cases may be needed for the development of lung cancer. In other words, intensity of exposure rather than cumulative exposure over a long period of time might be influential in the development of disease.

MacMahon (1994) suggested that there may have been preferential enrollment into the BCR by individuals with beryllium disease already diagnosed with lung cancer. However, Steenland and Ward (1991) state that only five individuals were known to have cancer when they entered the registry and none of these individuals had lung cancer. MacMahon (1994) pointed out that the smoking information was obtained after the occurrence of beryllium respiratory disease. The true prevalence of smoking before onset of disease was thus unknown, and the authors' estimate of a baseline risk ratio of less than 1.0 for lung cancer may have been inaccurate. Finally, the study may be subject to recall bias since smoking history may have been gathered around the time the individual was diagnosed with respiratory disease.

Ward et al. (1992)

Ward et al. (1992) conducted a retrospective cohort study of 9,225 men employed for at least 2 days between January 1, 1940, and December 31, 1969, at seven beryllium-processing facilities located in Pennsylvania and Ohio. The cohort was followed through December 31, 1988. The study was sponsored by the National Institute for Occupational Safety and Health (NIOSH) and the National Cancer Institute. The workers at the beryllium-processing facilities were involved in extracting of beryllium hydroxide from beryl ore; producing beryllium oxide, pure beryllium metal, and beryllium copper alloy; and machining beryllium-containing products. The beryllium compounds to which the workers were potentially exposed included beryllium ammonium fluoride mists and fumes, beryllium oxide dusts, beryllium metal, and beryllium copper alloy dusts and fumes. The workers were also exposed to ore dust, silicon dioxide fumes, lead sulfide, copper sulfide, sulfur trioxide, acid fluoride mists, hydrogen fluoride, and ammonium fluoride. Sulfuric acid mist and fume exposure occurred in the Lorain facility (BISAC, 1997). The study did not address the beryllium dose or type of beryllium compound to which workers were specifically exposed. The authors noted that before 1949, when environmental controls were not mandated, air concentrations of beryllium were high, often exceeding 1,000 $\mu g/m^3$.

Expected numbers of lung cancer for the SMR calculation were derived from the U.S. population mortality statistics. County-level mortality-rate statistics were also used to control for geographic variation in mortality rates. The use of the latter reference group limited the analysis to

post-1950 mortality because county-level statistics were not available for the 1940s. Smoking habits of workers in several of the plants were obtained in the 1968 U.S. Public Health Service survey. These were used to estimate the relative risk of lung cancer between the exposed cohort and the U.S. population due to smoking alone.

The authors found a statistically significant excess in risk of lung cancer at the seven facilities combined (SMR 1.26; 95% CI 1.12–1.42) compared with the U.S. population (Table 4-3). Most of the excess risk came from the two oldest plants in Lorain, Ohio, and Reading Pennsylvania, with statistically significant SMRs of 1.69 (95% CI 1.29–2.17) and 1.24 (95% CI 1.03–1.47), respectively (Table 4-3). The county-derived SMRs were of a similar magnitude to the SMRs based on national data. The SMRs were higher among cases with a latency >15 years since job start date in the Lorain and Reading facilities (SMRs = 2.09 and 1.17 for 15–30 years since hire and 1.66 and 1.40 for >30 years since hire date in Lorain and Reading, respectively). When lung cancer mortality was stratified by employment duration, the only statistically significant increase in lung cancer SMRs was among workers employed <1 year over all and in the <1 year and 1–5 year categories in the Lorain facility. A statistically significant increase in lung cancer was noted among workers hired before 1950 (SMR = 1.42; 95% CI = 1.22–1.64). This finding was influenced by mortality in the Lorain plant, which closed in 1948. In the Reading facility (in operation before 1950), an increased lung cancer rate was found ($SMR_{Reading}$ = 1.26; 95% CI = 1.02–1.54) among workers hired before 1950. With the exception of those hired before 1950, no other significant increases in lung cancer deaths were observed when workers were grouped by decade of hire. Nonstatistically significant increases were seen for the 1950s decade at the Reading (SMR = 1.42), Cleveland (SMR = 1.32), Elmore (SMR = 1.42), and Hazelton (SMR = 1.86) facilities.

Comparing the smoking information from the 1965 survey with smoking prevalence in the U.S. population, the authors estimated that smoking could have accounted for an SMR of 1.13 for beryllium. With the smoking adjustment factor, SMRs were estimated to be 1.12 (95% CI = 0.99–1.25), 1.49 (95% CI = 1.14–1.92), and 1.09 (95% CI = 0.91–1.30), for the entire cohort, the Lorain plant, and the Reading plant, respectively. Among the 1,192 workers from the Lorain plant, 98 were identified with beryllium disease through the BCR, the highest proportion of beryllium disease cases among all seven plants (8.2%). Eleven of these cases died of lung cancer (SMR = 3.33; 95% CI = 1.66–5.95). Among the remaining 1,094 Lorain plant workers, 46 lung cancer deaths were observed (SMR = 1.51; 95% CI = 1.11–2.02).

Table 4-3. Lung cancer SMR from a cohort of workers employed at seven beryllium plants

	Sample size	Observed lung cancer deaths	U.S. SMR[a]	County SMR[b]	City SMR[c]
Overall lung cancer SMR	9,225	280	1.26[e]	1.32[e]	–
Plant (start-up year)					
Lorain, OH (1935)	1,192	57	1.69[e]	1.60[e]	1.14
Reading, PA (1935)	3,569	120	1.24[e]	1.42[e]	1.07
Lucky, OH (1950)	405	9	0.82	0.84	–
Cleveland, OH (1937, 1963)[d]	1,593	44	1.08	1.05	–
Elmore, OH (1958)	1,323	15	0.99	1.06	–
Hazleton, PA (1958)	590	13	1.39	1.50	–
Employed at multiple sites	257	13	1.67	–	–
Unknown	296	8	1.33	–	–
Latency (years from job start date)					
<10		10	0.70	–	–
10–15		17	1.05	–	–
15–20		32	1.26	–	–
20–25		36	1.06	–	–
25–30		51	1.29	–	–
>30		134	1.46[e]	–	–
Latency: Lorain, OH (years)					
<15		1	0.38	–	–
15–30		21	2.09[e]	–	–
>30		35	1.66[e]	–	–
Latency: Reading, PA (years)					
<15		9	0.78	–	–
15–30		44	1.17	–	–
>30		67	1.40[e]	–	–
Duration of employment (years)					
<1		152	1.32[e]	–	–
1–5		61	1.19	–	–
5–10		21	1.26	–	–
>10		46	1.19	–	–

[a]The SMR was based on expected numbers of lung cancer from U.S. population mortality statistics.
[b]The SMR was based on expected numbers of lung cancer from county-level mortality statistics.
[c]SMR adjusted for lung cancer rates in Lorain and Reading.
[d]Two plants in Cleveland were combined.
[e]$p < 0.05$.

Source: Ward et al. (1992).

The association between a 30-year latency of first beryllium exposure and lung cancer found in this study was consistent with a prolonged latency period for development of lung cancer and possible exposure to higher levels of beryllium before the OSHA mandates were instituted. The

association with the shorter duration could be influenced by the possibility that workers left the plant early because they experienced beryllium disease or other respiratory disease or because of potentially poor working conditions that could be possibly related to high exposure levels at the time. The major limitations of this study included the lack of exposure information, incomplete ability to correct for smoking, and use of population-based SMRs. The lack of information about beryllium exposure would result in attenuated risk estimates because the cohort contained a mixture of unexposed and exposed workers and the exposed workers were exposed to a variable degree. However, there were indications in the study that higher exposure levels were associated with lung cancer. For example, in two plants that went into operation later than the Lorain and Reading plants, the SMRs for workers who developed lung cancer were elevated among those hired before 1960, when beryllium exposure levels were presumably higher. Eisenbud and Lisson (1983) reported levels as high as 4,700 $\mu g/m^3$ in the Lorain facility in 1950.

The authors stated that neither smoking nor geographic location explained the excess in lung cancer and that occupational exposure to beryllium was the most plausible explanation for the finding.

Levy et al. (2002)

Levy et al. (2002) reanalyzed the data from the Ward et al. (1992) study by using different analysis methods and smoking data from a variety of surveys to compute smoking corrected SMRs (Table 4-4). The reanalysis produced lower SMRs compared with those within Ward et al. (1992), with a statistically significant elevated risk only in the Lorain plant. Levy et al. (2002) disputed the comparison population used by Ward et al. (1992) to develop SMRs, particularly county-based rates based on age-specific census data and cancer deaths provided by the state health departments. The counties around Lorain and Reading tended to be rural, while the beryllium workers predominantly resided in the cities. Levy et al. (2002) argued that background lung cancer rates due to smoking were much higher in Lorain, OH, and Reading, PA, than in the general U.S. population. Deriving SMRs from the Lorain, OH, and Reading, PA, populations reduced the estimates to 1.39 and 1.02, respectively (Table 4-4).

Table 4-4. SMRs and 95% CIs for death from lung cancer, corrected for cigarette smoking by using various control populations

	Unadjusted U.S. SMR[a] (95% CI)	Ward et al. SMR[b] (95% CI)	Wagoner et al. SMR[c] (95% CI)	Levy et al. SMR[d] (95% CI)
Plant (start-up year)				
Lorain, OH (1935)	1.69[e] (1.28–2.19)	1.49[e] (1.13–1.93)	1.31 (0.99–1.70)	1.39[e] (1.05–1.79)
Reading, PA (1935)	1.24[e] (1.01–1.48)	1.10 (0.91–1.31)	0.96 (0.80–1.15)	1.02 (0.84–1.22)
All plants	1.26[e] (1.12–1.42)	1.12 (0.99–1.26)	0.98 (0.87–1.10)	1.04 (0.92–1.17)

[a]Uncorrected U.S. population-based SMR.
[b]Used smoking correction factor from Ward et al. (1992).
[c]Used smoking correction factor from Wagoner et al. (1980).
[d]Used smoking correction factors based on a U.S. veterans' survey (Levy et al., 2002).
[e]$p < 0.05$.

Source: Levy et al. (2002).

Sanderson et al. (2001a, b)

Sanderson et al. (2001a) conducted a nested case-control study, based on 242 lung cancer deaths from a cohort of 3,569 male workers employed at the beryllium processing facility in Reading, Pennsylvania, between January 1, 1940, and December 31, 1969. The cohort in which this case-control study was embedded was followed through 1992 for lung cancer mortality. Each case was age and race matched to five controls. These 710 controls were selected based on the period of incidence of lung cancer in the cases. Beryllium exposures among the controls were limited to the date of the cases' deaths.

In the development of a job exposure matrix for exposure assessment, workers' job histories were updated through 1992 and coded by using a list of jobs and departments developed by a review of company and union records, information from NIOSH, Department of Energy archives, and published literature (Sanderson et al., 2001b). These were linked to a file of quantitative time-specific exposure estimates for each job and department based on the exposure estimates described above. Exposure to beryl ore, beryllium fluoride, beryllium hydroxide, beryllium copper alloy, and beryllium aluminum alloy were analyzed both separately and together. These measures were used to create number of days of exposure (tenure) and cumulative, average, and maximum beryllium exposure estimates for each worker. The measures were presented as unlagged (exposure at any time), lagged 10 years (exposure that occurred 10 years before lung cancer diagnosis), and lagged 20 years (exposure that occurred 20 years before lung cancer diagnosis).

As for exposure documentation, only short-term impinger and high volume filter samples were available prior to 1961. The former represented task or job-specific exposures, the latter were collected in the beryllium alloy production areas where most employees worked. In the 1940s, AEC

set a quarterly DWA of 2 µg/m^3 that was later adopted by ACGIH and OSHA as an 8-hour time weighted average threshold limit value. AEC sampled the general work air, computing daily air average exposures for each job in the plant. AEC reported extensive ventilation and environmental control measures throughout the plant in 1959. Before that, only the mixing and sinter furnace areas had dust control equipment, and these may have had nonfunctioning exhaust systems. Rules about wearing respirator equipment were often ignored. Because many work areas had no dust control, beryllium dust from furnaces contaminated other departments. The company began collecting air measurements regularly in 1971. Only sporadic measurements were found before that time. There were no measurements found between 1961 and 1971. Long-term employees reported that after ventilation controls were installed (1959–1962) exposures began to decrease and that housekeeping practices improved. Before this, employees reported that fumes and suspended aerosol were clearly visible in many areas of the plant (personal communication, Dr. Wayne Sanderson, University of Iowa). Long-term employees and plant archives confirmed that exposures dramatically decreased again after 1971 when the Occupational Safety and Health Act was passed and standards for beryllium were implemented.

Sanderson et al. (2001b) calculated the geometric means of the DWA samples, stratified by work area. The DWA was time weighted by multiplying the average beryllium concentration for each task or area by the time spent by the workers on that task, then dividing that value by the length of the workday. Because the length of time each worker spent at a task was unknown when the samples were collected, the estimated duration of tasks from DWA measurements in later years was used, assuming that the time needed to complete a task remained constant between 1935 and 1971. For the time period between 1960 and 1971 when exposure data were missing, exposure levels were estimated to be midway between the more contaminated period before 1960 and the regulated period after 1970. Job descriptions in the plant's archives indicated that production processes and job duties remained relatively constant. Where there were dramatic changes, the authors adjusted task times. However, some time changes were probably unknown to the authors and individual variability in time taken to complete tasks could not be taken into account.

Sanderson et al. (2001a) reported an overall SMR for lung cancer of 1.22 (95% CI 1.03–1.43). It was also found that lung cancer cases had higher levels of exposure to beryllium than controls when the exposures were lagged 10 or 20 years (Table 4-5). Odds ratios were also calculated by quartile of days of exposure, cumulative exposure, average exposure, and maximum exposure and stratified by unlagged and lagged by 10 and 20 years (Table 4-5). The lowest exposure quartile was the reference category. The odds ratios for the lagged exposures indicated that lung cancer was significantly associated with beryllium compared with the lowest exposure level (Table 4-5). There was more than a doubling of risk of lung cancer in the next-to-lowest

quartiles of each exposure indicator. Among workers with at least 20 years since first exposure, the odds ratio for lung cancer was 2.18 (p value < 0.05) at cumulative beryllium exposure levels of 21–2,195 µg/m^3-day. Among this group, the odds ratio for lung cancer was 1.92 (p value < 0.05) at average beryllium exposure levels of 1.1–19.3 µg/m^3 and 1.95 (p value < 0.05) at maximum beryllium exposure levels of 1.1–23.0 µg/m^3. In the third quartile of exposure, the odds ratios, in some cases, were higher: 1.89 (p value < 0.05), 3.06 (p value < 0.05), and 2.89 (p value < 0.05) for cumulative, average, and maximum exposure, respectively. The odds ratios for each exposure metric in the fourth or highest exposure quartile tailed off and were not statistically significant. The maximum exposure, when lagged for 20 years, is over twofold lower than the maximum exposure when lagged for 10 years (i.e., 13.1 µg/m^3 versus 30.8 µg/m^3 among cases and 6.5 µg/m^3 versus 16.1 µg/m^3 among controls) (Table 4-5).

Table 4-5. Mean exposure and odds ratio estimates between cases and controls

	Geometric mean of exposure indicators (GSD[a])		Odds ratio by quartile[b] of beryllium exposure		
	Cases, n = 142	Controls, n = 710	Quartile 2	Quartile 3	Quartile 4
Tenure (days), 0 lag	202.1 (9.4)	328.0 (9.4)[c]	[66–298 days] 1.09	[299–1,647 days] 0.74	[>1,647 days] 0.54[c]
Lagged 10 years	178.4 (19.7)	133.0 (19.7)	[35–203 days] 1.64	[204–1,195 days] 1.28	[>1,195 days] 0.87
Lagged 20 years	58.4 (40.6)	31.3 (40.6)	[2–93 days] 2.23[c]	[94–698 days] 2.48[c]	[>698 days] 1.61
Cumulative exposure (µg/m^3-day), 0 lag	4606.0 (9.3)	6,328.0 (9.3)	[1,426–5,600 µg/m^3-day] 0.73	[5,601–28,123 µg/m^3-day] 0.85	[>28,123 µg/m^3-day] 0.57[c]
Lagged 10 years	4057.0 (38.9)	2,036 (38.9)[c]	[809–3,970 µg/m^3-day] 1.38	[3,971–20,996 µg/m^3-day] 1.38	[>20,996 µg/m^3-day] 0.92
Lagged 20 years	844.0 (134)	305 (134)[c]	[21–2,195 µg/m^3-day] 2.18[c]	[2,196–12,376 µg/m^3-day] 1.89[c]	[>12,376 µg/m^3-day] 1.89[c]
Average exposure (µg/m^3), 0 lag	22.8 (3.4)	19.3 (3.4)	[11.3–24.9 µg/m^3] 1.61	[25.0–34.0 µg/m^3] 1.75[c]	[>34.0 µg/m^3] 1.27
Lagged 10 years	22.6 (6.6)	12.3 (6.6)[c]	[9.6–23.6 µg/m^3] 2.39[c]	[23.7–32.8 µg/m^3] 2.71[c]	[>2.8 µg/m^3] 1.83[c]
Lagged 20 years	10.2 (11.2)	5.3 (11.9)[c]	[1.1–19.3 µg/m^3] 1.92	[19.4–25.5 µg/m^3] 3.06[c]	[>25.5 µg/m^3] 1.70
Maximum exposure (µg/m^3), 0 lag	32.4 (3.8)	27.1 (3.8)	[17.1–25.0 µg/m^3] 1.82[c]	[25.1–71.5 µg/m^3] 1.08	[>71.5 µg/m^3] 1.14
Lagged 10 years	30.8 (7.6)	16.1 (7.6)[c]	[10.1–25.0 µg/m^3] 3.34[c]	[25.1–70.0 µg/m^3] 2.19[c]	[>70.0 µg/m^3] 1.92[c]
Lagged 20 years	13.1 (13.9)	6.5 (13.9)[c]	[1.1–23.0 µg/m^3] 1.95[c]	[23.1–56.0 µg/m^3] 2.89[c]	[>56.0 µg/m^3] 1.67

[a]GSD = geometric standard deviation.
[b]Quartile 1 was used as the reference group.
[c]$p < 0.05$.

Source: Sanderson et al. (2001a).

Using the log-transformed exposure indicators as continuous predictors, a conditional logistic regression noted that for the 10 and 20 year lagged exposure measures, there was a statistically significant positive association between beryllium exposure and lung cancer (Table 4-6). Continuous exposure-response analysis of the logged variables revealed a significant negative sloping relationship between cancer and unlagged tenure but a positive slope between cancer and tenure lagged 20 years (Table 4-6).

Table 4-6. Conditional logistic regression analysis of logs of continuous exposure variables

Variable	Parameter estimate	Wald statistic
Log tenure (days), 0 lag	−0.096	5.45[a]
Lagged 10 years	0.045	2.51
Lagged 20 years	0.045	4.39[a]
Log cumulative exposure (µg/m^3-days), 0 lag	−0.064	2.38
Lagged 10 years	0.060	5.35[a]
Lagged 20 years	0.041	5.62[a]
Log average exposure (µg/m^3), 0 lag	0.110	2.14
Lagged 10 years	0.184	12.62[a]
Lagged 20 years	0.088	8.35[a]
Log maximum exposure (µg/m^3), 0 lag	0.098	2.06
Lagged 10 years	0.171	12.81[a]
Lagged 20 years	0.085	8.63[a]

[a]$p < 0.05$.

Source: Sanderson et al. (2001a).

In terms of type of beryllium exposure, over 70% of the cases were classified as being "ever exposed" to beryllium oxide or beryllium-copper alloy (Table 4-7). Significant associations between lung cancer and exposure to these beryllium types were observed when a 10 year and 20 year lag analysis was introduced. The author also noted that workers were exposed to other chemicals, such as acid, copper, and fluorides at the worksite; the latter two exposures were found to be associated with increased lung cancer (Table 4-7). It should be noted that all workers were exposed to two or more chemicals while at the plant.

Table 4-7. Odds ratio for "ever exposed" to select types of beryllium and other chemicals

Exposure	No lag		10 Year lag		20 Year lag	
	Cases (%)	Odds ratio	Cases (%)	Odds ratio	Cases (%)	Odds ratio
Be[a] ore	47.9	1.07	47.9	1.27	43.0	1.50[b]
Be oxide	88.7	1.52	88.0	2.35[b]	75.4	1.93[b]
Be-copper alloy	85.2	1.45	84.5	2.11[b]	71.8	1.80[b]
Acid	39.4	1.12	39.4	1.34	31.0	1.28
Copper	56.3	1.29	55.6	1.47[b]	48.6	1.55[b]
Fluorides	76.8	1.18	75.4	1.51[b]	66.2	1.66[b]

[a]Be = beryllium.
[b]$p < 0.05$.

Source: Sanderson et al. (2001a).

No information was available on current smoking status of workers. The only data available on smoking status were gathered from a 1968 U.S. Public Health Service study in which 368 workers of the current cohort had participated. To determine whether smoking was a confounder in the association between beryllium exposure and lung cancer, an internal analysis was performed to determine if an association existed between smoking status and level of exposure. Since the sample size was too small to differentiate between cases and controls, professional status was used as a crude method to adjust for smoking status. Beryllium exposure was higher among nonprofessionals than professionals, with cumulative exposure of 82,607µg/m^3-day and 3,649 µg/m^3-day, respectively. Within the professional and nonprofessional groups, there were no statistically significant differences in exposure levels among current smokers, former smokers, and nonsmokers, indicating that smoking was not a major confounder because it was not related to beryllium exposure. With professional status identified as a potential confounder, 14 professional workers among the 142 cases and 88 professionals among the 710 controls were excluded and the data were reanalyzed. As observed in the original analysis, cumulative, average, and maximum exposures lagged for either 10 or 20 years were higher among the cases than controls (Table 4-8).

Table 4-8. Mean exposure and odds ratio estimates between cases and controls, excluding workers with professional status

	Geometric mean of exposure indicators (GSD[a])		Odds ratio by quartile[b] of beryllium exposure		
	Cases, n = 128	Controls, n = 622	Quartile 2	Quartile 3	Quartile 4
Tenure (days), 0 lag	176.0 (9.9)	277.0 (10.0)[c]	[54–236 days] 0.92	[236–1,356 days] 0.80	[>1,356 days] 0.50[c]
Lagged 10 years	154.0 (19.6)	118.0 (19.9)	[35–155 days] 1.76	[156–950 days] 1.45	[>950 days] 0.84
Lagged 20 years	58.2 (41.1)	28.5 (41.9)[c]	[2–93 days] 2.52c	[75–540 days] 2.49[c]	[>540 days] 1.86
Cumulative exposure (µg/m^3-day), 0 lag	4935.0 (10.1)	7182.0 (10.2)	[1,626–6,055 µg/m^3-day] 0.81	[6,056–35,710 µg/m^3-day] 0.87	[>35,710 µg/m^3-day] 0.52
Lagged 10 years	4228.0 (41.7)	2371 (42.4)	[909–4,595 µg/m^3-day] 1.45	[4,596–26,692 µg/m^3-day] 1.40	[>26,692 µg/m^3-day] 0.91
Lagged 20 years	991.0 (155.6)	347 (159.3)[c]	[26–2,444 µg/m^3-day] 2.10[c]	[2,445–15,400 µg/m^3-day] 2.47[c]	[>15,400 µg/m^3-day] 1.63
Average exposure (µg/m^3), 0 lag	28.0 (2.7)	25.9 (2.7)	[16.8–25.0 µg/m^3] 1.81[c]	[25.1–42.3 µg/m^3] 1.36	[>42.3 µg/m^3] 1.03
Lagged 10 years	27.1 (6.3)	16.3 (6.4)[c]	[14.0–25.0 µg/m^3] 3.63[c]	[25.1–43.3 µg/m^3] 2.47[c]	[>43.3µg/m^3] 1.66
Lagged 20 years	12.3 (12.8)	6.7 (12.9)[c]	[4.1–23.0 µg/m^3] 2.77[c]	[23.1–31.0 µg/m^3] 3.39[c]	[>31.0 µg/m^3] 1.76
Maximum exposure (µg/m^3), 0 lag	39.0 (3.1)	36.3 (3.1)	[23.1–25.1 µg/m^3] 2.30[c]	[25.2–71.5 µg/m^3] 0.99	[>71.5 µg/m^3] 1.00
Lagged 10 years	36.5 (7.2)	21.2 (73)[c]	[21.0–25.1 µg/m^3] 3.09[c]	[25.2–74.8 µg/m^3] 1.44	[>74.8 µg/m^3] 1.84[c]
Lagged 20 years	15.7 (14.9)	8.1 (15.1)[c]	[3.1–23.0 µg/m^3] 2.23[c]	[23.1–60.0 µg/m^3] 3.05[c]	[>60.0 µg/m^3] 1.79

[a]GSD = geometric standard deviation.
[b]Quartile 1 was used as the reference group.
[c]$p < 0.05$.

Source: Sanderson et al. (2001a).

The Sanderson et al. (2001a) study, coupled with the Sanderson et al. (2001b) study, is the first study to provide detailed exposure analyses with the development of a job exposure matrix. Sanderson et al. (2001b) confirmed verbal accounts that beryllium levels in the Reading, Pennsylvania, plant decreased from very high levels in the 1930s and 1940s to relatively low levels beginning in the 1970s. Lung cancer cases were more prevalent among workers who had higher cumulative, average, and maximum exposures 10 and 20 years before death than among controls of the same age. Lagged analyses also revealed positive exposure response trends. The lagged estimates also restrict the analysis to exposures before the current occupational 2 $\mu g/m^3$ exposure limit was established. The use of a control group of workers within the same plant may have decreased the healthy worker effect, with controls having similar socioeconomic and demographic attributes. The authors note that there were other chemicals present in the worksite in the Reading, Pennsylvania, plant and the odds ratios for the relationship between copper and fluorides and lung cancer were statistically significant. These associations are likely to have occurred because exposure to copper and fluorides was highly correlated with beryllium in this group of workers. Professional status was found to be a confounder in examining the relationship between beryllium exposure and lung cancer. After workers with professional status were removed from the study cohort, cases lagged for 10 and 20 years had higher cumulative, average, and maximum exposure levels of beryllium and cases were almost two times as likely to be exposed to higher levels of beryllium as were controls.

Levy et al. (2007) examined the effect of log transformation on the findings from Sanderson et al. (2001a). Using non-transformed exposure metrics, rather than the log-transformed metrics used in the Sanderson et al. (2001a), Levy et al. (2007) reported no elevated odds ratios for any of the exposure variables (cumulative, average, or maximum exposure).

Summary of epidemiology studies

Epidemiology studies investigating the association between beryllium exposure and lung cancer have been based on either workers from one or a set of beryllium manufacturing plants or the BCR. The USPHS study (Bayliss, 1971) was one of the first studies to identify excess lung cancer mortality among beryllium exposed workers. Studies from the BCR reported a twofold increase in lung cancer deaths among people exposed to beryllium (Steenland and Ward, 1991; Infante et al., 1980). This observation was also noted in studies based in one or more beryllium plants, with statistical significance noted with longer latency (Sanderson et al., 2001a; Ward et al., 1992; Mancuso et al., 1980; Wagoner et al., 1980). Earlier studies, such as Wagoner et al. (1980) and Mancuso (1980), used U.S. mortality rates for the period ending in 1967. This methodology may have resulted in an underestimation of expected lung cancer deaths. Risk estimates were primarily in the form of SMRs, and the study cohort/population for most studies included workers from the

Reading, Pennsylvania, beryllium plant. Prior to the studies by Sanderson et al. (2001a, b), duration of employment was an assumed surrogate for cumulative exposure. However, as previously discussed, the duration interval may have included periods of high beryllium exposure rather than being a corollary to cumulative exposure.

The study by Ward et al. (1992) was limited by the assumption used to account for lung cancer deaths due to cigarette smoking; the lack of job history data, without which there can be no objective quantitative exposure assessment; the lack of control for potential exposure to other carcinogens, including co-exposure to sulfuric or hydrofluoric acid mists during employment in the beryllium industry or nonconcurrent exposure to other carcinogens during employment outside of the beryllium industry; and the relatively small increases in lung cancer risks (MacMahon, 1994).

In the reanalysis of the data from Ward et al. (1992), Levy et al. (2002) argued that using locality-specific (or city) cancer mortality rates as the control population would be more appropriate than using the U.S. general population or county rates because the cities had higher background cancer rates. Levy et al. (2002) recalculated the SMRs, using smoking correction factors. This resulted in a lowering of the SMRs previously reported by Ward et al. (1992), but the observed number of lung cancer deaths in one of the beryllium plants was still significantly higher than expected. Earlier studies revealed that there was beryllium exposure in the area around the plants (Sterner and Eisenbud, 1951; Eisenbud et al., 1949). This suggests that the county- and municipality-based SMRs contained individuals not employed by the plants who were beryllium-exposed, which could have resulted in an attenuation of the true associations.

Sanderson et al. (2001a, b) are the most comprehensive studies to date that address the association between occupational exposure to beryllium and lung cancer. Sanderson et al. (2001b) compiled a detailed job-exposure matrix, which not only facilitated the estimation of the level of exposure but also the type of beryllium and other compounds that workers may have been exposed to as well. Using this information, Sanderson et al. (2001b) show that exposures to beryllium in the 1940s and 1950s were up to 500-fold higher than in the 1970s (i.e., 1,000 $\mu g/m^3$ versus 2 $\mu g/m^3$). Using a lagged analysis, Sanderson et al. (2001a) showed that cases were exposed to twice the level of beryllium that controls were exposed to. To address the issue of smoking, Sanderson et al. (2001a) conducted a separate analysis of nonprofessional workers as a surrogate for smoking status and found that both groups (professionals and nonprofessionals) had elevated risks of lung cancer. Earlier criticisms that smoking was not adequately addressed were answered by these investigators—smoking would have to be related to both lung cancer and exposure to be a confounder.

The cohort studies described in this section have consistently found an elevated risk of lung cancer related to exposure to beryllium and its compounds in beryllium plants in the U.S. This consistency could be attributed in part to the reanalysis of the same cohort over time. The studies have progressed from crude observations of excesses in workers in the industry to more sophisticated approaches based on objective exposure estimation and use of comparable controls. The beryllium levels in the U.S. plants often exceeded 1,000 μg/m^3 and were recorded to be as high as 4,700 μg/m^3 before plants came into compliance with the OSHA permissible exposure level (PEL) of 2 μg/m^3 beryllium limits (U.S. DOE, 1999). The use of lagging to take into account latency of lung cancer and exposure to potential high levels of beryllium revealed statistically significant risk ratios of 2–4, indicating that beryllium exposure is associated with more than a doubling of lung cancer risk. Because there was a sharp decline in the level of beryllium exposure from the 1940s to the 1970s and because length of employment may have been reduced due to the onset of acute or chronic respiratory disease, latency as an indicator for high beryllium exposure is of greater relevance than duration in the assessment of potential carcinogenicity of beryllium.

4.2. PRECHRONIC, CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS - ORAL AND INHALATION

4.2.1. Oral Exposure

In a chronic toxicity study by Morgareidge et al. (1977, 1975), groups of Wistar albino rats were fed diets containing 0, 5, 50, or 500 ppm beryllium as beryllium sulfate tetrahydrate. The rats were administered the beryllium-containing diet from four weeks of age through maturation, mating, gestation, and lactation. Fifty male and 50 female offspring were then placed on the same diets as the parents and fed the beryllium-containing diet for 104 weeks. Using estimated TWA body weights of 0.467, 0.478, and 0.448 kg for males in the 5, 50, and 500-ppm groups and 0.294, 0.302 and 0.280 kg for the females, respectively, and U.S. Environmental Protection Agency's (U.S. EPA, 1988) allometric equation of food intake, doses of 0.36, 3.6, and 37 mg/kg-day for males in the 5, 50, and 500 ppm groups and 0.42, 4.2 and 43 mg/kg-day for females in the 5, 50, and 500-ppm groups, respectively, were calculated. Clinical observations, body weight, food consumption, organ weights (liver, kidney, testes, ovaries, thyroid, pituitary, adrenal), gross necropsy and histopathological examination of most tissues and organs (25-26 tissues examined) were used to assess the toxicity and carcinogenicity of beryllium in the offspring; it does not appear that the parental generation rats were examined. Tissues from 20 rats/sex/group in the control and 500-ppm groups were examined microscopically (the study authors did not state whether the animals undergoing histopathological examination were randomly selected), as well as all tissues with gross

abnormalities (all groups) and tissues (excluding bone marrow, eyes, and skin) from animals found dead or sacrificed moribund (all groups).

No overt signs of toxicity were observed, and mortality appeared to be similar in the controls (30/50 males and 28/50 females died) and beryllium groups at 5 ppm (30/50 and 24/50, respectively), 50 ppm (31/50 and 18/50) and 500 ppm (24/50 and 17/50) at the end of the 104 weeks of the study. During the first 40-50 weeks of the study, exposure to beryllium did not appear to affect growth. Slight decreases in growth (body weights of males and females in the 500-ppm group were within 10% of control body weights) were observed in the latter part of the study; however, no statistically significant alterations were observed. Alterations in organ weights were limited to statistically significant ($p<0.05$) increases in relative kidney weight in males exposed to 50 ppm, decreases in relative kidney and adrenal weights in 500-ppm females, and decreases in relative testes weights in 5- and 50-ppm males. Histological examination of the major organs and tissues did not reveal beryllium-related noncarcinogenic alterations. These data suggest that the maximum tolerated dose (MTD) was not reached.

Reticulum cell sarcomas were observed in a number of tissues examined, including the lungs, lymph nodes, spleen, liver, kidneys, and pancreas; the highest incidence was in the lungs. Because lymphomas (reticulum cell sarcoma is a type of lymphoma) are almost always detected grossly, reticulum cell sarcoma incidences were calculated based on the number of tissues grossly examined (all gross diagnoses were confirmed histopathologically) rather than on the number of tissues microscopically examined. In most organs, the incidence of reticulum cell sarcomas was not significantly higher in the beryllium-exposed rats, as compared to controls. In the lung, the incidences of reticulum cell sarcoma were 10/50, 17/50, 16/50, and 12/50 in males and 5/50, 7/50, 7/50, and 5/50 in females exposed to 0, 5, 50, or 500-ppm beryllium, respectively. The incidences of lung reticulum cell sarcomas in the beryllium-exposed rats were not significantly different than in controls. The incidences of reticulum cell sarcoma-bearing rats in the 0, 5, 50, and 500-ppm groups were 12/50, 18/50, 16/50, and 13/50, respectively, for males and 8/50, 11/50, 7/50, and 8/50 for the females; no significant increase in tumor-bearing rats was found. No other treatment-related increases in tumor incidence were observed.

Morgareidge et al. (1976) conducted a long-term feeding study in which groups of 5 male and 5 female beagle dogs (aged 8 -12 months) were fed diets containing 0, 5, 50, or 500-ppm beryllium as beryllium sulfate tetrahydrate for 172 weeks. The basal diet was a commercial dog chow (Purina®) moistened with warm water; the dogs were given access to the food for 1 hour per day. Because of overt signs of toxicity, the 500-ppm group was terminated at 33 weeks. At this time, a group of 5 male and 5 female dogs was added to the study and fed a diet containing 1-ppm

beryllium; duration of exposure for this group was 143 weeks. Using estimated TWA body weights of 13.0, 12.7, 13.8, and 12.3 kg for males in the 1, 5, 50, and 500-ppm groups, respectively, and 10.2, 10.3, 11.2, and 8.6 kg for females, and the reported average food intake of 300 g/day, the 1, 5, 50, and 500-ppm concentrations correspond to doses of 0.023, 0.12, 1.1, and 12.2 mg/kg-day for male dogs and 0.029, 0.15, 1.3, and 17.4 mg/kg-day for females. The following parameters were used to assess toxicity: daily observations, food consumption, body weight, hematology and serum clinical chemistry (blood samples collected after 1, 3, 6, 16, 18, 24, 30, and 36 months of exposure), urinalysis (samples collected after 1, 3, 6, 18, 24, 30, and 36 months of exposure), organ weights (heart, liver, kidney, brain, spleen, pituitary, thyroids, adrenals and gonads), and histopathology of the spleen, thymus, pancreas, lungs, gonads, stomach, small and large intestines, urinary bladder, heart, aorta, muscle, adrenals, thyroids, lymph nodes, salivary glands, gallbladder, liver, kidneys, pituitary, brain, spinal cord, skin, mammary gland, bone marrow, and eyes.

Two moribund animals in the 500-ppm group were sacrificed during week 26; the remaining animals in the 500-ppm group were killed during week 33. Overt signs of toxicity observed in the 500-ppm group included lassitude, weight loss, anorexia, and visibly bloody feces, indicating that the MTD is <500 ppm. Four other animals died during the course of the study or were killed moribund; two dogs died during parturition, and one male and one female dog in the 50-ppm group died. The appearance, behavior, food intake, and body weight gain of the animals in the other beryllium groups did not differ from controls. No beryllium-related hematological, serum chemistry, or urinalysis alterations were observed in the 1, 5, or 50-ppm groups. In the 500-ppm group, a slight anemia (slight decreases in erythrocyte, hemoglobin, and hematocrit; statistical analysis not reported), more apparent in the females than in the males, was observed after three and six months exposure; however, there were no alterations in the bone marrow and none of the animals was seriously affected. The authors' noted that the anemia might have been related to hemorrhaging of the gastrointestinal tract rather than a direct effect of beryllium on the hematological system. No alterations in organ weights were observed. All animals in the 500-ppm group showed fairly extensive erosive (ulcerative) and inflammatory lesions in the gastrointestinal tract. These occurred predominantly in the small intestine, and to a lesser extent in the stomach and large intestine, and were regarded by the authors as treatment-related effects. This conclusion is supported by independent review of the study report; the lesions were not considered related to some other cause such as intestinal worms (Goodman, 1997). All of the animals with stomach or large intestinal lesions also had lesions in the small intestine, except for one animal with stomach lesions only. This animal had stomach lesions that were very localized and not very severe. Lesions in the small intestine (4/5 males and 5/5 females) considered treatment-related include desquamation of the epithelium, edema, fibrin thrombi, acute inflammation, subacute/chronic inflammation, necrosis and thinning/atrophy of the epithelium, and ulceration (Goodman, 1997). High-dose animals also

showed moderate to marked erythroid hypoplasia of the bone marrow, which the authors and an independent reviewer also considered treatment-related (Goodman, 1997). Bile stasis and vasculitis in the liver and acute inflammation in the lymph nodes occurring in these animals are attributed to a likely systemic bacterial invasion through the damaged intestinal mucosa. A generalized low-grade septicemia likely initiated kidney damage.

In the 50-ppm group, one female dog died after 70 weeks of treatment. This animal showed gastrointestinal lesions, but less severe, occurring in the same locations and appearing to be of the same types as those in dogs administered 500 ppm. The authors stated that the death of this animal appeared related to beryllium administration. Other animals in this treatment group survived until study termination and had no remarkable gross or microscopic findings. No neoplasms were observed in the beryllium-exposed dogs. Reproductive endpoints are discussed in Section 4.3.

Groups of 52 male and 52 female Long-Evans rats were maintained on a low-metal diet and given drinking water containing 0 or 5 ppm beryllium as beryllium sulfate (hydration not stated) from weaning to natural death (Schroeder and Mitchener, 1975b). The water also contained 5-ppm chromium III, 50-ppm zinc, 5-ppm copper, 10-ppm manganese, 1-ppm cobalt, and 1-ppm molybdenum. Doses of 0.63 and 0.71 mg/kg-day were calculated for male and female rats, respectively, using estimated TWA body weights of 0.42 and 0.26 kg and U.S. EPA (U.S. EPA, 1988) allometric equation for water consumption. The following parameters were used to assess toxicity: body weights (animals weighed at weekly and monthly intervals for the first year and at 3 month intervals thereafter); blood glucose; cholesterol, and uric acid (blood samples collected from 12 rats/sex after an 18 hour fast); urine protein, pH, and glucose; heart weight; gross pathology; and histopathology of heart, lung, kidney, liver, spleen and tumors. Twenty male and 8 female rats in the beryllium group died at 20 months of age from pneumonia; a similar number of animals in the control group also died from pneumonia.

At 30 days, the male and female rats exposed to beryllium weighed significantly more than the control animals. At 60, 90, 120, and 180 days, the beryllium-exposed male rats weighed significantly less than the controls; no significant alterations in body weight were observed at the other time intervals (150, 360, or 540 days). Because decreases in body weight were generally <10% and not prolonged, these data indicate the doses may have been close to, but did not reach, the MTD (U.S. EPA, 1986a). No significant alterations in mortality or longevity were observed. Glucosuria (females only) and alterations in serum glucose levels were observed in the beryllium-exposed rats. The alterations in serum glucose levels consisted of significantly lower levels in males aged 475 days and higher levels in males and females aged 719 days. It should be noted that the control rats were at least 50 days older than the beryllium-exposed rats when blood samples were

collected, and in the controls, blood glucose levels declined with increasing age. Significantly increased serum cholesterol levels were observed in female rats exposed to beryllium at ages 475 and 719 days. The results of the histological examination were not reported. The alterations in serum glucose, cholesterol levels and urine glucose levels were not considered adverse because the alterations were not large enough to suggest impairment in organ function.

The incidence of gross tumors was 4/26 (15%) and 17/24 (70%) in the male and female control rats and 9/33 (27%) and 14/17 (82%) in the male and female rats exposed to beryllium. The incidences of malignant tumors (tumors were considered malignant if there were multiple tumors in the same animal) were 2/26 (7.7%) and 8/24 (33%) in the male and female controls and 4/33 (12%) and 8/57 (14%) in the male and female beryllium-exposed rats. The incidences of gross or malignant tumors in the control and beryllium-exposed groups were not significantly different. It should be noted that in an unpublished report (Schroeder and Nason, 1976), the incidence of gross tumors in male and female beryllium-exposed rats was 4/25 and 13/20 (control data the same as reported in published paper). In the published paper, the same values were listed as the tumor incidence for tungsten-exposed rats. It is difficult to determine which is the correct tumor incidence data for the beryllium-exposed rats; however, neither set of incidence data is statistically significantly different from controls.

In a lifetime exposure study, groups of 54 male and 54 female Swiss mice were administered 0 or 5 ppm beryllium as beryllium sulfate (hydration not stated) in drinking water from weaning to natural death (Schroeder and Mitchener, 1975a). The mice were fed low-metal diets and the drinking water was supplemented with 50-ppm zinc, 10-ppm manganese, 5-ppm copper, 5-ppm chromium III, 1-ppm cobalt, and 1-ppm molybdenum. The 5-ppm water concentration is equivalent to doses of 1.2 mg Be/kg-day for the male and female mice, using an estimated TWA body weight of 0.042 and 0.035 kg and the U.S. EPA (U.S. EPA, 1988) allometric equation for water consumption. In the beryllium group, statistically significant alterations in body weight were observed; the alterations included heavier male mice at 30 days and lighter female mice at 90 and 120 days. Overall, the decrease in body weight was <10%, indicating that the MTD was not reached. No significant alterations in mortality or survival were observed in the beryllium-exposed mice. No alterations in tumor incidence were observed.

Matsumoto et al. (1991) fed groups of 10 male Wistar rats diets containing 0 or 3% beryllium carbonate for four weeks. The diet contained adequate amounts of calcium. U.S. EPA's (U.S. EPA, 1988) allometric equation for daily food consumption and an estimated TWA body weight of 0.10 kg were used to calculate a dose of 3,700 mg/kg-day beryllium carbonate (480 mg/kg-day beryllium). Body weight and serum calcium, phosphate, protein, alkaline phosphatase and acid

phosphatase levels were the only parameters measured. At four weeks, the rats fed the beryllium diet weighed approximately 18% less than the controls (statistical significance not reported). Serum phosphate concentrations and serum alkaline phosphatase activity were significantly lower in the beryllium-exposed rats. No statistically significant alterations in serum calcium or protein levels or serum acid phosphatase activity were observed.

In addition to the oral study by Matsumoto et al. (1991) using rats fed diets containing beryllium carbonate, older oral studies by Guyatt et al. (1933) and Kay and Skill (1934) have demonstrated rickets in rats fed diets containing up to 3% beryllium carbonate; however, the bone lesions observed were not attributed to any direct effects from beryllium itself, but to the deprivation of phosphate in the intestine by precipitation as beryllium phosphate.

In a series of experiments conducted by Guyatt et al. (1933), young rats were fed a "normal" stock diet containing 0.125-3.0% beryllium carbonate (13-300 mg/kg-day beryllium using a food factor of 0.05 (U.S. EPA, 1986a), and the authors' estimate that the beryllium carbonate used in the study contained 20% beryllium). It appears that the animals were fed this experimental diet for at least 24-28 days; however, no additional information on the exposure protocol was provided. Decreases in body weight gain, decreases in activity, and a "waddling" gait and arched back were observed in the beryllium-exposed rats, and the severity and onset appeared to be dose-related, but it was not stated whether these effects were observed in all groups. X-ray examination revealed rickets in rats fed diets of 0.125% beryllium carbonate and higher; a considerable decrease in bone density and an almost complete lack of calcification of epiphyseal cartilage was observed in the $\geq$1% beryllium carbonate diet groups. Histological examination of the femur and tibia showed evidence of decreases in mineral deposition in the metaphysis and reduced amounts of mineral salts in the trabeculae and cortex of the tibia. Decreases in plasma inorganic phosphorus levels, decreases in acid soluble phosphorus levels in the liver, and decreases in kidney phosphatase levels were observed in the beryllium-exposed rats; no changes in liver inorganic phosphorus levels were observed.

(Kay and Skill, 1934) fed groups of eight albino rats (strain and sex not reported) a basal diet ("Bill's stock diet"), which contained 0 or 0.5% beryllium carbonate for 21-22 days. Using a food factor of 0.05 (U.S. EPA, 1986a) and the estimate of beryllium content of the beryllium carbonate (20%) from Guyatt et al. (1933), a dose of 50 mg/kg-day beryllium was estimated. Eight groups of rats were fed the beryllium carbonate diets; five of the groups also received daily subcutaneous injections of 0.5-25% sodium glycerophosphate[2] and two groups received daily subcutaneous injections of 1 or 10% saline solution. "Excellent skeletal development" was found in the control

[2] Sodium glycerophosphate is used as source of phosphate in the treatment of calcium and phosphate metabolism

group. In the beryllium-exposed group (without glycerophosphate or saline injections), severe rickets and decreased blood inorganic phosphorus, "plasma phosphatase," total erythrocyte phosphorus, liver "ester phosphorus," and kidney phosphatase levels were observed. In the beryllium-exposed rats administered glycerophosphate, the severity of the rickets and the decreases in phosphorus levels were diminished; this was not observed in the beryllium-exposed rats administered saline solution.

To assess the effect of beryllium on body weight, Freundt and Ibrahim (1990) administered 0 or 100 ppm beryllium sulfate tetrahydrate in drinking water to groups of five female Sprague-Dawley rats for 91 days. Using a TWA body weight of 0.28 kg and a water intake of 0.039 L/day (calculated using U.S. EPA's (U.S. EPA, 1988) allometric equation), a dose of 13.9 mg/kg-day beryllium sulfate (0.71 mg/kg-day beryllium) was calculated. The rats were fed a standard diet. The rats were weighed at weekly intervals and food and water consumption was measured weekly. Although the beryllium-exposed rats weighed more than the controls, the difference was not statistically significant. Administration of beryllium in the drinking water also resulted in increases in food intake (approximately 3-5% higher than controls) and water consumption (approximately 5-10% higher than controls).

In a dietary exposure study (Goel et al., 1980), a group of eight male albino rats (strain not specified) were fed a standard diet and given 20 mg beryllium nitrate orally every third day for 2.5 months (40 doses administered). A control group of 4 male rats was fed the standard diet. In the beryllium-exposed rats, a number of histological alterations were observed in the lungs. These included congestion and ruptured ciliated epithelial cells of the respiratory bronchioles, thickened epithelium cells and necrosis in the alveoli, and damage to the arteriole endothelium. The other ingestion studies, as well as the parenteral administration studies, did not report respiratory effects. Although the method was not adequately described, it appears that the beryllium nitrate was placed on the food in a powder form; thus, it is possible that the animals inhaled some of the beryllium.

imbalances.

4.2.2. Inhalation Exposure

Although a number of chronic studies in laboratory animals have been conducted with beryllium compounds, few have been done using modern criteria for high-quality toxicology studies. In addition, whereas several laboratory animal species (such as mice, dogs, and monkeys) respond to beryllium exposure with several features of human CBD, no laboratory animal model fully mimics all features of human CBD. Specifically, the animal models fail to demonstrate a progressive granulomatous pulmonary response with a concomitant beryllium-specific immune response. In addition, no chronic studies are available on non-neoplastic effects of beryllium oxide, the most environmentally relevant form.

Reeves and Vorwald (1967) exposed 150 male and 150 female Sprague-Dawley rats for 7 hours/day, 5 days/week to 34.25 μg Be/m^3 as beryllium sulfate aerosol (average particle size was 0.118 μm, electron microscopy) for up to 72 weeks, with three of each sex sacrificed monthly during exposure. An equal number of control rats were exposed to distilled water aerosol. Lung weights were markedly increased in the exposed rats, and an inflammatory lung response (characterized as a marked accumulation of histiocytic elements and thickened and distorted alveolar septa) was noted, as was accumulation of alveolar macrophages. A proliferative response was also noted, progressing from hyperplasia to alveolar adenocarcinomas in 100% of the exposed rats at 13 months, compared to 0% of the controls. Histopathologic examination was limited to the lungs. The authors noted that 8 male and 4 female rats in the control group and 9 male and 17 female rats in the beryllium group died during the course of the study. The plateau body weight in the beryllium-exposed female rats was approximately 25% less than found in the controls (statistical significance not reported).

Lung granulomas, inflammation, and adenomas were also observed in a group of 127 Sherman rats (males and females combined) exposed to 28 μg/m^3 beryllium as beryllium sulfate aerosol for 8 hours/day, 5.5 days/week for up to 6 months and killed in groups of 5-15 immediately after the end of exposure, or approximately monthly for up to 18 months postexposure (Creedon et al., 1957). There was a lag period for the development of granulomas, with most of these lesions developing several months after the end of exposure.

Vorwald and Reeves (1959) exposed Sherman rats (number and sex not reported) via the inhalation route to aerosols of beryllium sulfate (hydration not stated) at 6 and 54.7 μg Be/m^3 for 6 hours/day, 5 days/week for an unspecified duration (particle size not reported). Animals were sacrificed periodically and examined histopathologically. Initially, inflammation consisted of histiocytes, lymphocytes, and plasma cells scattered throughout the lung parenchyma. Following more prolonged exposures, more focal lesions consisting primarily of histiocytes were observed.

Multinucleated giant cells were also observed. Thickened alveolar walls and fibrotic changes were also observed. Lung tumors, primarily adenomas and squamous cell cancers, were observed in the animals sacrificed after nine months of this exposure regime.

Similar results to those of Vorwald and Reeves (1959) were observed in a study by (Reeves and Deitch, 1971) (as reviewed by (U.S. EPA, 1987)). In this study, groups of 20-25 Charles River CD rats were exposed to 35.66 μg Be/m^3 as beryllium sulfate (hydration not stated) for 35 hours/week; the mean particle size was 0.21 μm (dae). The exposure durations were 800 hours (5 groups), 1,600 hours (2 groups), and 2,400 hours (1 group). No information about a control group is provided. Age at the initiation of exposure appeared to be a more important variable for tumor development than was exposure duration. The lung tumor incidence (19/22, 86%) for young rats exposed for three months was the same as in rats exposed for 18 months (13/15, 86%) but was higher than in older rats exposed for three months (3-10/20-25, 15-40%). Tumors were typically observed after a latency period of nine months. In the beryllium-exposed rats, the epithelial hyperplasia observed at one month progressed to metaplasia at 5-6 months, and anaplasia by 7-8 months.

Stokinger et al. (1953) conducted a chronic study in which groups of 14 dogs, 5 cats, 10 male rabbits, and 120 male rats were exposed to 186 μg Be/m^3 as beryllium fluoride for 6 hours/day, 5 days/week for 207 calendar days, with some intermediate sacrifices. There was no control group for any species. Three of the dogs and 73 rats died during the experiment, with all deaths by day 70 for the dogs and day 19 for the rats. Dogs exhibited significant increases in plasma fibrinogen after 9-17 days of exposure, followed by a second peak at 117 or more days of exposure. Decreases in red blood cell counts and hemoglobin concentration and increases in mean corpuscular volume were observed in rabbits and dogs. Histopathological lesions were observed only in the lungs, and occurred in most animals of all of the tested species. Lesions included an infiltration of large monocytes, polymorphonuclear leukocytes in the alveoli and interstitial infiltration of monocytes and lymphocytes.

Stokinger et al. (1950) exposed rats, dogs, cats, rabbits, guinea pigs, hamsters, monkeys, and goats via inhalation to 40, 430, or 2,000 μg Be/m^3 as beryllium sulfate for 6 hours/day, 5 days/week for 100, 95, or 51 days, respectively (particle size not reported). No animals died following exposure to the low concentration. Following exposure to 430 μg Be/m^3, 23/47 rats, 1/5 cats, 2/24 rabbits, and 2/34 guinea pigs died, but all dogs, monkeys, and goats survived. Mortality was higher in animals exposed to 2,000 μg Be/m^3. Signs of toxicity included weight loss and anemia. All histopathological lesions were confined to the lungs and included the following influx of cells: interstitial and intraalveolar infiltration of monocytes, polymorphonuclear leukocytes, lymphocytes,

and plasma cells. Macrophages containing cellular debris were observed within the alveoli. The exposure levels at which histopathological lesions were observed were not specified for each species; therefore, no NOAEL or LOAEL could be assigned.

In a study to test the carcinogenicity of beryllium ores, Wagner et al. (1969) exposed groups of 12 male squirrel monkeys (*Saimiri sciureus*), 60 male CR CD rats, 30 male Greenacres Controlled Flora (GA) rats, and 48 male Golden Syrian hamsters to 0 or 15 mg/m^3 bertrandite or beryl for 6 hours/day, 5 days/week for 17 months (rats and hamsters) or 23 months (monkeys). The test atmospheres generated from the bertrandite ore ($Be_4Si_2O_7[OH]_2$; 1.4% beryllium) and beryl ore ($Be_3Al_2Si_6O_{18}$; 4.14% beryllium) contained 210 and 620 μg Be/m^3, respectively, and the geometric mean diameters of the particles were 0.27 μm (geometric standard deviation of 2.4) and 0.64 μm (geometric standard deviation of 2.5). Both ores contained very high silicon dioxide[3] levels (63.9% by weight). Exposed and control monkeys, rats, and hamsters were serially sacrificed upon completion of six and 12 months of exposure; rats and hamsters at the 17th month, and monkeys at the 23rd month. Five control rats and five rats from the 12th and 17th month exposure groups were sacrificed in order to determine the free-silica content of the lung tissue. At exposure termination, beryllium concentrations in the lungs were 18.0 and 83 μg/g fresh tissue in the bertrandite- and beryl-exposed rats, 14.1 and 77.4 μg/g fresh tissue in the bertrandite- and beryl-exposed hamsters, and 33 and 280 μg/g fresh tissue in the bertrandite- and beryl-exposed monkeys. Free silica (SiO_2) levels in the rat lungs were 30 - 100 times higher in the beryllium ore-exposed rats than in the controls. Increased mortality was observed in the monkeys (11%), rats (13%), and hamsters (25%) exposed to either bertrandite or beryl ore, with the highest mortality rates in the bertrandite ore-exposed animals (no further details provided). No significant alterations in body weight gain were observed in the monkeys or hamsters.

In the rats, decreased body weight gains (terminal body weights were 15% lower compared to controls) were observed beginning after six months of exposure, and from 12 months to exposure termination at 17 months. In the beryl-exposed rats, small foci of squamous metaplasia or tiny epidermoid tumors were observed in the lungs of 5/11 rats killed after 12 months of exposure. At exposure termination, lung tumors were observed in 18/19 rats (18 had bronchiolar alveolar cell tumors, 7 had adenomas, 9 had adenocarcinomas, and 4 had epidermoid tumors). Additional alterations in the lungs included loose collections of foamy macrophages and cell breakdown products, lymphocyte infiltrates around the bronchi, and polymorphonuclear leukocytes and lymphocytes present in most of the bronchiolar-alveolar cell tumors. In the bertrandite-exposed rats, granulomatous lesions composed of several large, tightly packed, dust-laden macrophages were

[3]The International Agency for Research on Cancer (IARC) classifies crystalline silica in the form of quartz or cristobalite from occupational sources as a human carcinogen (IARC 1997).

observed in all rats exposed for 6, 12, or 17 months. No tumors were observed. Neoplastic or granulomatous pulmonary lesions were not observed in the control rats. In the beryl- and bertrandite-exposed monkeys, the histological alterations consisted of aggregates of dust-laden macrophages, lymphocytes and plasma cells near respiratory bronchioles and small blood vessels. No tumors were found. In the bertrandite-exposed hamsters, granulomatous lesions consisting of tightly packed, dust-laden macrophages were observed after six months, and the number did not increase after 17 months. These alterations were not observed in the beryl-exposed or control hamsters. A typical proliferation and lesions, which were considered bronchiolar alveolar cell tumors except for their size, were observed in the hamsters after 12 months of exposure to beryl or bertrandite. After 17 months of exposure, these lesions became larger and more adenomatous in the beryl-exposed hamsters. It should be noted that silicosis was not observed in any of the animals exposed to the beryllium ores that contained a large amount of free silica. No significant gross or histologic alterations were observed in the thymus, spleen, liver, or kidneys of the beryllium-exposed rats, hamsters and monkeys.

In a monkey carcinogenicity study (Vorwald, 1968), a group of 7 male and 9 female rhesus monkeys (*Macaca mulatta*) (aged 18 months) were exposed to 35 μg Be/m^3 beryllium sulfate mist 6 hours/day, 5 days/week (particle size not reported). The author notes that the "exposure was interrupted, often for considerable periods of time, in order to maintain the best possible overall well-being of the animal, to prevent a threatening acute beryllium pneumonitis, and to favor survival to old age or at least long enough for the inhaled beryllium to exert its maximal chronic effects in terms of epithelial proliferation, metaplasia, and cancer." The exposure schedule was presented in a figure, but it was difficult to determine the exposure protocol from this figure. The longest exposure was for 4,070 hours. Most of the exposure was during the first 4.5 years of the study with an approximate 6-month exposure 2.5 years later. Four animals died within the first two months of the study; the cause of death was acute chemical pneumonitis. Lung cancer was observed in 8 of the 12 remaining animals. The first tumor was observed in a monkey 8 years of age exposed for 3,241 hours. The tumors were described as a gross mass located in either the hilar area or more peripheral portions of the lung, or as small and large tumors scattered irregularly throughout the pulmonary tissue.

A single-exposure inhalation study of beryllium metal in F344/N rats resulted in a 64% incidence of lung carcinomas over the lifetime of the animals (Nickell-Brady et al., 1994). Groups of 30 males and 30 females were administered a single, nose-only exposure to a beryllium metal aerosol (mass median aerodynamic diameter (MMAD) = 1.4 μm, geometric standard deviation (GSD) = 1.9) at 500 mg/m^3 for 8 minutes, 410 mg/m^3 for 30 minutes, 830 mg/m^3 for 48 minutes, or 980 mg/m^3 for 39 minutes. Control rats were exposed to filtered air alone. Mean lung burdens

resulting from these exposures were 40, 110, 360, and 430 μg of beryllium, respectively. Tumors became apparent by 14 months after exposure, and the incidence (apparently for all groups combined) was 64% over the lifetime of the rats. Multiple tumors were frequently found; the majority were adenocarcinomas, and some were >1 cm. The tumors were analyzed for gene mutations (see Section 4.4.3.).

Hall et al. (1950) performed seven experiments in which a total of 133 animals, representing six species, were exposed to beryllium oxide dust. The six species included mongrel cats and dogs, a mixed English strain of guinea pigs, New Zealand hares, albino rats (Wistar derived), and rhesus macaques. All the animals were kept in the chamber 6 hours daily, 5 days per week during the exposure period. Preceding each experiment, the animals were conditioned in a dust-free chamber 6 hours daily, 5 days each week for 2-3 weeks. A summary of each study is described below.

In the first set of experiments, Hall et al. (1950) exposed 10 female guinea pigs, 3 male rabbits, and 20 young adult female rats to Grade I beryllium oxide (88 mg/m^3; 10 exposure days) (Table 4-9). All animals were killed following the last exposure. In exposed guinea pigs, histopathological changes in the lungs were noted and characterized as a minimal phagocytic response provoked by inhaled particulate foreign matter. Lung, liver, kidney, and spleen histopathology of exposed animals was consistent with controls. Exposed animals exhibited minimal changes in bodyweight. No differences were observed between exposed and control animals' activity or alertness. Nausea or vomiting was not observed with exposed or control.

Lung, liver, kidney, and spleen histopathology of exposed male rabbits revealed no evidence of injury that might be attributed to effects of beryllium. The sole evidence of toxicity found in male rabbits was a progressive decrease in the mean red blood cell count, from 5.4 to 4.1 million per cubic millimeter. Leukocyte counts in exposed animals remained consistent with controls. Minimal changes in bodyweight were observed in exposed animals. The exposed rabbits were exhibited normal activity and alertness, and the condition of their coats remained good. No significant changes were found in the blood non-protein nitrogen, serum proteins, or urinary proteins. Anorexia, nausea, or vomiting was not observed. Microscopic evaluation of 20 tissues, including lung, liver, kidney, and spleen, of the young female rats revealed no evidence of injury that might be attributed to effects of beryllium. There was no detectable increase of rales among exposed rats, and no treatment related changes were found in the blood elements (i.e., erythrocyte and leukocyte counts). As with the exposed guinea pigs and rabbits, the exposed rats exhibited minimal changes in bodyweight and remained normally active and alert with groomed coats. Anorexia, nausea, or vomiting was not observed.

In a second set of experiments, Hall et al. (1950) exposed 3 male rabbits and 10 young adult male rats to Grade II beryllium oxide (83 mg/m^3; 60 exposure days) (Table 4-9). All animals were killed following the last exposure. In male rabbits, steady weight gain was observed during the duration of exposures, and no external signs of intoxication were observed. Histopathology of the lungs from exposed animals was consistent with controls. Toxicological effects of beryllium were observed with changes in the peripheral blood that resembled the progressive development of macrocytic anemia. Beginning after about 14 days exposure, the average red cell count decreased from 6.0 ± 0.15 to 4.9 ± 0.17 million per cubic millimeter. The maximal decrease, observed after 35 days exposure, exceeded 1.2 x 10^6 corpuscles per cubic millimeter. Concomitantly with the decrease in red cell count, the average mean corpuscular volume, based on the hematocrit value and the cell count, rose from 68.5 ± 4.5 to 85.6 ± 4.4 cubic microns. There was a tendency toward development of hypochromia, indicated by transient decreases in the average mean corpuscular hemoglobin concentration. No changes were observed between exposed rats and controls for all parameters tested (i.e., bodyweight, external signs of intoxication, and histopathology of lungs).

In a third set of experiments, Hall et al. (1950) exposed 10 male guinea pigs, 3 male rabbits, and 20 male rats to Grade III beryllium oxide (87 mg/m^3; 10 exposure days) (Table 4-9). Guinea pigs, rabbits, and 15/20 male rats were killed following the last exposure. Five male rats were kept alive for continued observation. In exposed male guinea pigs, the only evidence of toxicity was a slight phagocytic infiltration in the lungs. No changes in bodyweight were observed between exposed and control animals.

The only observed toxicological response in exposed male rabbits was phagocytic infiltration of the lungs. No significant changes in the amount of protein excreted in the urine were observed between exposed and control rabbits, and no significant changes in blood non-protein nitrogen, serum protein concentrations, blood cell counts, or bodyweight gain were found. In exposed rats, pulmonary changes were observed by a histological response of phagocytic infiltration in the lungs of all rats killed following the last exposure. No signs of toxicity were evident in blood cell counts of exposed, terminal sacrifice rats; however, three of the 5 hold over rats developed moderate leukocytosis. Of the 5 rats held for observation following the last exposure, one rat died a week later. Two of the remaining 4 holdover rats lost weight and also had increased rales and unkempt coats (time period not stated).

In a fourth set of experiments, Hall et al. (1950) exposed two male cats and two male monkeys to Grade III beryllium oxide (84 mg/m^3; 15 exposure days) (Table 4-9). Following the last exposure, the animals were monitored for at least 1.5 years. In the exposed male cats, anorexia was accompanied by a marked loss of weight. One cat became so weakened and emaciated that it was

killed about 2.5 months after the termination of exposure. Histopathology of the killed cat's lung revealed slight evidence of pulmonary damage. The remaining exposed cat appeared to be in normal health at 1.5 years (time based on publication of study) following the last exposure.

Table 4-9. Summary of study conditions and beryllium oxide properties

Grade of BeO	Purity (% BeO)	Duration of exposure (hrs)[a]	Concentration mg/m^3 (mean ± SD)	MMPD (µm; mean, range)	Firing temperature (°C)	Solubility[b] (mg/L) 48 hrs, 4 hrs
Grade I	99.3	56	88 ± 8.9	0.71, 0.23-1.04	1,350	8, 2
Grade II	100	360	83 ± 22.3	1.13, 0.69-1.70	1,150	--, --
Grade III	99.2	60	87 ± 10.5	1.1, 0.66[c]	1,150	--, --
		90	84 ± 7.8			
Grade IV	99.2	105	86 ± 9.6	1.1, 0.66[d]	1,150	--, --
Grade V	97.0	90	82 ± 10.3	0.59, 0.35-0.86	400	66, 54
	97.0	236	10 ± 2.7	0.47, 0.16-2.60	400	66, 54

MMPD = mass-median particle diameter; SD = standard deviation
[a]Exposures were conducted for 6 hrs/day, 5 days/week.
[b]Values represent solubility in bicarbonate-citrate buffer at pH 7.0.
[c]The distribution of particle sizes was atypical. The listed value represents the mass-median particle diameter for the cumulative percentage of particles by weight for 96% of the mean and 47% for the range.
[d]The listed value represents the mass-median particle diameter for the cumulative percentage of particles by weight for 90% of the mean and 41% of for the range.

Source: Hall et al. (1950).

Similar to the exposed cats, the exposed male monkeys exhibited anorexia that was accompanied by marked loss of weight. One monkey had increasing exertional dyspnea and was eventually killed in a greatly weakened condition about two months after exposure was terminated; however, only slight histologic evidence of pulmonary damage was seen. The remaining monkey appeared to be in normal health at 1.5 years (time based on publication of study) after the last exposure.

In a fifth set of experiments, Hall et al. (1950) exposed two male and two female dogs to Grade IV beryllium oxide (86 mg/m^3; 17.5 exposure days) (Table 4-9). Animals were killed following the last exposure. In the exposed male dogs, a small transient decrease in arterial oxygen tension occurred between 24 and 54 hours (4 and 9 days) exposure in one of the dogs. No significant change was detected in carbon dioxide tension in the blood of either animal. Further, no significant changes in red blood cell counts, or leukocyte counts were noted. Serum protein concentration and albumin-globulin ratio varied only within the normal limits. Consistently normal findings were reported for blood non-protein nitrogen and urinary protein. Histopathological evidence of only slight tissue damage was found in the lungs of one male dog, whereas in the lungs of the other male dog, there was moderately severe inflammation with considerable obliteration of structure due to the presence of areas of atelectasis and emphysema.

One of the female dogs, which was subsequently found to be pregnant, exhibited a sharp transient decrease in arterial oxygen tension reaching a minimal value of 63 mm Hg after 36 hours of exposure (6 days), with return to the normal range (87-97.5 mm Hg) 2 days later. The red blood cell count of the pregnant female dog averaged 6.8 ± 0.33 million per cubic millimeter during the pre-exposure period and decreased to 5.7 ± 0.33 million after 35 hours of exposure. The decrease in red cell count was not accompanied by a corresponding change in the leukocyte count, and there were no significant changes in the blood cell counts of the other female dog. In the nonpregnant female dog, serum protein concentration and the albumin-globulin ratio varied only within normal limits; consistently normal findings were reported for blood non-protein nitrogen and urinary protein.

In a sixth set of experiments, Hall et al. (1950) exposed 23 rats and 2 dogs to Grade IV beryllium oxide (82 mg/m^3; 15 exposure days) (Table 4-9). Rats surviving the exposure regimen were held for observation, whereas both dogs were killed following the last exposure. Ten rats were killed serially during the exposure period: 5 rats after 5 days and 5 after 10 days exposure, seven rats were killed terminally. Eight of 23 rats, 6 of them females, died while being exposed for 15 days to the special low-fired beryllium oxide, 82 mg/m^3, and 5 of the 15 survivors, 2 males and 3 females, died during the first week after the period of exposure (Table 4-10). Death was preceded by obvious signs of respiratory distress, increased rales and loss of weight, most severe in the females.

Table 4-10. Cumulative mortality among rats inhaling beryllium oxide (400°C), 82 mg/m^3, for 15 exposure days[a]

Cumulative hours of exposure	Cumulative exposure (mg/m^3-min × 10^{-5})	Males		Females	
		# deaths/ # exposed	%	# deaths /# exposed	%
60	2.9	0/13	0	1/10	10
66	3.2	1/13	8	5/10	50
78	3.8	2/13	15	6/10	60
90	4.4	6/13	46	9/10	90

[a]Exposures were conducted for 6 hrs/day, 5 days/week.

Source: Hall et al. (1950).

Among the 20 surviving rats, there were 13 males and 7 females. The females lost weight steadily after about the fourth day of exposure, whereas the males merely stopped gaining at about the same time. The difference of response in the two sexes was correlated with an age difference, since the females were nearly full grown at the beginning of the experiment, whereas the males were still actively growing. The initial mean weight of the females was 201 ± 8 grams, corresponding to an average age of about 210 days, whereas the average weight of the males was 196 ± 10 grams, which corresponds to an average age of only about 65 days. Moderate progressive leukocytosis was observed in five rats. Little evidence of tissue response other than phagocytosis of the inhaled dust particles was found in sections of the rats' lungs.

Both exposed dogs exhibited loss of appetite and loss of weight; the latter amounted to 7 and 14 per cent of their respective initial weights. There was a progressive decrease of oxygen tension in the peripheral arterial blood of one dog, a female. The maximal change was observed after nine days (54 hours) exposure and amounted to about 12 mm of Hg. The other dog, a male, exhibited only a transient lowering that never reached significant proportions. Moderate progressive leukocytosis was observed in both dogs. No significant changes were found in the red blood cell counts, hemoglobin concentrations, or hematocrit readings of either dog. Evidence of rather copious intra-alveolar edema was seen in lung sections from one of the dogs. In most areas the exudate was granular rather than homogeneous in appearance. There was moderate phagocytic infiltration. In the terminal bronchi, considerable loss of bronchial epithelium and some early regeneration were observed. Occasionally, a rather definite membrane taking a dense eosin stain was seen lining the alveolar wall. The changes in the lung sections from the other dog in this experiment were similar.

In a final set of experiments, Hall et al. (1950) exposed four female dogs to Grade IV beryllium oxide (10 mg/m^3; 40 exposure days). Three of the dogs exhibited anorexia and loss of weight during exposure; the mean loss of weight amounted to more than one quarter of their initial weight. These animals also had marked reductions in arterial oxygen tension during the exposure.

The greatest change occurred between the fifth and sixth days (30-36 hours) and averaged 21 mm of Hg. The fourth dog failed to show any loss of appetite at any time during the 9-week exposure period and gained steadily in weight. In all four animals, increased effort of breathing was noticed. A consistent trend was found toward lower albumin-globulin ratios due to a progressive decrease in the concentration of the albumin fraction and a concomitant increase of the globulins. Evidence of progressive development of macrocytic anemia was found in all dogs. A decrease of nearly 2 million cells per cubic millimeter in the average red cell count was measured during the 40-day period of exposure, and this was rather closely correlated chronologically with an increase in mean corpuscular volume from an average of 75 to as high as 101 cubic microns. There was little change in average hemoglobin concentration and transient changes in serum proteins, plasma fibrinogen, and alkaline phosphatase. Lung damage of advanced degree was found in sections from one of the 2 dogs that were killed immediately after exposure. There was active bronchial epithelial proliferation, with formation of large adenomatoid nests about the bronchi. Entire low power fields were seen which were composed chiefly of infiltrating cells and in which most of the alveoli were collapsed, only scattered air-containing sacs being present. In many areas, the alveolar walls were thickened; in others, they were thin, and many alveoli were emphysematous. Interstitial inflammation was prominent, but little intra-alveolar edema was present. A lesser degree of damage was observed in lung sections from the other dog, in which there were moderate amounts of bronchial epithelial proliferation and interstitial inflammation. Little alveolar edema was present, but frank vascular congestion was noted in the dog that had eaten well, gained weight steadily, and exhibited the least clinical, chemical, and hematological evidences of intoxication.

A summary of the findings from the series of experiments by Hall et al. (1950) are provided in Table 4-11. Intratracheal instillation studies are discussed in Section 4.4.2.

Table 4-11. Summary of effects of inhaled beryllium oxide dust

Grade of beryllium	Degree of exposure		Species and # of animals (sex)	Criteria of toxicity				
	Hours in chamber	mg/m^3-min × 10^{-5}[a]		Mortality (%)	Weight loss	Histologic (lungs)	Hematologic	Biochemical
Grade I	56	3.0	10 guinea pigs (F) 3 rabbits (M) 20 rats (F)	0	0	Minimal or no damage	Decrease of red cell count in rabbits	None positive for toxicity
Grade II	360	18.0	3 rabbits (M) 10 rats (M)	0	0	No damage	Macrocytic anemia in rabbits	None positive for toxicity
Grade III	60	3.1	10 guinea pigs (M) 3 rabbits (M)	0	0	Phagocytic infiltration	Moderate leukocytosis in 3/5 rats examined	None positive for toxicity
			20 rats (M)	5				
	90	4.5	2 cats (M) 2 monkeys (M)	0	Marked	Slight injury in 1 cat and 1 monkey examined	No change	No change
Grade IV	105	5.4	4 dogs	0	0	Slight to moderate injury in 2 dogs examined	Decrease of red cell count in pregnant dog	Transient hypoxia
Grade V	90	4.4	20 rats (M)	46	Slight	Minimal damage	Leukocytosis in 5 rats examined	No change
			20 rats (F)	90	Marked			
			2 dogs (M & F)	0	Marked	Moderate damage	Leukocytosis in both dogs	Hypoxia in F dog
	236	1.4[b]	4 dogs (F)	0	Marked in ¾	Moderate in 2 dogs examined	Macrocytic anemia in all 4 dogs	Marked transient hypoxia in ¾ dogs; transient changes in serum proteins, plasma fibrinogen, and alkaline phosphatase

[a]Cumulative exposure = (mg beryllium oxide/m^3) × total time exposed (minutes).
[b]Concentration = 10 mg of beryllium oxide/m^3.

Source: Hall et al. (1950).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

There are limited data on the reproductive and developmental toxicity of beryllium compounds following oral exposure. In the chronic dog oral exposure study conducted by Morgareidge et al. (1976) (described in Section 4.2), the male and female dogs exposed to 1, 5, or 50 ppm beryllium sulfate in the diet (0.023, 0.12 and 1.1 mg/kg-day for males and 0.029, 0.15 and 1.3 mg/kg-day for the females) were housed together at the time of the second heat after treatment initiation, allowed to mate and wean (at 6 weeks of age) their pups, which were then returned to community floor pens (with the exception of the first litter, which was killed 5 days after whelping). The number of pregnant females for 0, 1, 5, and 50 ppm were as follows: 3, 2, 5, and 3. Treated females had between 1 and 3 litters; controls had 1-4, each litter by the same sire. First-litter pups surviving to postnatal day 5 were sacrificed for soft tissue gross examination and were stained for evaluation of skeletal malformations. Pups from subsequent litters were grossly examined at weaning. Beryllium did not appear to adversely affect reproductive or developmental endpoints (number of pregnancies, number of pups, number of live pups, pup weight) in the beryllium-exposed dogs. No beryllium-related decreases in post-natal survival (day 7 or weaning) were observed. The authors reported no gross or skeletal abnormalities in the surviving first-litter pups, but data were not shown; stillborn or cannibalized pups dying within the first few postnatal days were not examined.

4.3.2. Inhalation Exposure

Only limited information is available on potential reproductive and developmental effects of beryllium, but there appear to be no effects following exposure via environmentally relevant routes. Savitz et al. (1989) found no association between occupational exposure to beryllium and the risk of stillbirth, preterm delivery, or small-for-gestational age infants in a case-control study using National Natality and National Fetal Mortality Survey data. Analyses were conducted for 2,096 mothers and 3,170 fathers of stillbirths, 363 mothers and 552 fathers of preterm babies, and 218 mothers and 371 fathers of small-for-gestational age babies. For beryllium, analyses were conducted only for paternal exposure, not for maternal exposure. In light of the small population exposed to beryllium, case-control studies have limited sensitivity for reproductive effects.

No animal experiments of the developmental toxicity of inhaled beryllium are available. No standard two-generation reproductive studies have been carried out.

4.3.3. Parenteral Administration

Several studies (as reviewed by (U.S. EPA, 1991b) have tested the reproductive and developmental toxicity of beryllium following intratracheal instillation and intraperitoneal injection. Clary et al. (1975) conducted a continuous breeding experiment in which male and female Sprague-Dawley rats received a single intratracheal administration of 200-µg beryllium as beryllium oxide (calcined at 960°C in the first experiment and at 500°C in the second experiment). Groups of four or five females and two males were placed together for mating. In the first experiment, groups of eight exposed rats and four controls were sacrificed after the first, second, and fourth pregnancies, and at 12 and 15 months. No alterations in average number of pregnancies, numbers of live or dead pups per litter, lactation index, or fetal body weights were observed. In the second experiment, 10 exposed and 10 control rats were sacrificed at 12 months after exposure. There was no adverse effect of beryllium in either experiment. Indeed, significant increases in the number of live pups/female were observed in the dosed groups.

Developmental effects (increased fetal mortality, decreased fetal body weight, internal abnormalities, and delayed neural development) were observed in the offspring of rodents following intratracheal or intraperitoneal administration of beryllium chloride, beryllium oxide, or beryllium sulfate during gestation. Mathur et al. (1987) administered intravenous injections of 0.021 mg/kg Be as beryllium nitrate to mated Sprague Dawley rats (n=5-8/group) ($1/10^{th}$ the LD_{50}) on postcoital day 1, 11, 12, 13, 15, or 17. Rats were laparotomized on gestation days 10 and 20 and then allowed to deliver. All pups died within 2-3 days of birth and all pups in the group injected on postcoital day 11 died *in utero*, but these effects may have been due to the repeated surgeries.

4.4. OTHER STUDIES

4.4.1. Mechanistic Studies

Considerable research has investigated the mechanism of CBD and attempted to identify an appropriate animal model for CBD. An appropriate animal model for CBD is one that forms immune granulomas following the inhalation of beryllium, demonstrates beryllium specificity of the response, and mimics the progressive nature of the human disease. These immune granulomas are distinct from granulomas formed by foreign-body reactions (Haley, 1991). Immune granulomas result from persistent antigenic stimulation, while foreign-body granulomas result from persistent irritation. Histologically, foreign-body granulomas consist predominantly of macrophages and monocytes, and small numbers of lymphocytes. By contrast, immune granulomas are characterized by larger numbers of lymphocytes, primarily T lymphocytes (known as T cells). T cells in

granulomas are primarily antigen-specific T-helper cells, which are recognized by the presence of the CD4 antigen on their cell surfaces. The predominance of T cells in immune granulomas and the responsiveness to beryllium in skin patch tests (reviewed in Kreiss et al., 1994) indicate that the immune response in CBD is primarily cell-mediated.

Numerous studies with laboratory animals have demonstrated that exposure to beryllium often results in chronic granulomatous inflammation of the lung that is often progressive, even after cessation of beryllium exposure (Haley et al., 1990, 1989; Sendelbach et al., 1986). However, not all of these lesions can be attributed to an immune inflammation.

Animal models for CBD.

Beryllium-induced lung lesions in rats are formed by foreign-body reactions, rather than immune mechanisms as observed with humans, and therefore, are not considered an appropriate model for CBD. Male F344 rats exposed to a beryllium metal aerosol at 800 μg/m^3 for 50 minutes (initial lung burden, 625 μg) developed an acute necrotizing, hemorrhagic, exudative pneumonitis and intraalveolar fibrosis, that peaked at day 14 postexposure (Haley et al., 1990). By 31 days postexposure, inflammatory lesions were replaced by minimal interstitial and intravascular fibrosis. After a period of minimal inflammation, an increase occurred and progressed to chronic active inflammation. The chronic lung lesions were characterized by severe alveolar macrophage, alveolar epithelial hyperplasia, and interstitial fibrosis. These granulomatous lesions had only low numbers of lymphocytes, and lymphocyte levels in the BAL were also not elevated.

Chronic inflammation and Type II cell hyperplasia were observed at initial lung burdens (ILB) as low as 1.8 μg (4,700 μg/m^3 beryllium for 30 minutes) with no effect at an ILB of 0.32 μg (8,600 μg/m^3 for 14 minutes) and with an exposure-related severity (Finch et al., 1994). Preliminary experiments also found no evidence of beryllium-induced proliferation of splenic lymphocytes obtained from rats exposed to an initial lung burden of 50 μg beryllium as beryllium metal, and tested in the BeLT at 210 days postexposure (Haley, 1991). Similarly, the lungs of F344 rats exposed for 1 hour to 0.013 μg Be/m^3 as beryllium sulfate aerosol had injury-related cell proliferation, Type II alveolar cell hyperplasia, and infiltrates of interstitial macrophages, but few lymphocytes (Sendelbach et al., 1986). The response was largely resolved by three weeks postexposure.

Hart et al. (1984) found no effect on lymphocyte level in BAL of F344 rats exposed for 1 hour to 447 μg Be/m^3 as beryllium oxide aerosol heat-treated at 560^{o}C, and the resulting inflammatory lesions of the lung consisted of macrophages and polymorphonuclear (PMN) leukocytes, with few lymphocytes. Effects in rats exposed to beryllium oxide calcined at about

1,000°C (1-100 μg Be/m^3 for 30-180 minutes) were milder, with small granulomatous lesions consisting primarily of foamy macrophages (Sanders et al., 1975). The absence of lymphocytes in beryllium-induced lesions in these studies shows that acute beryllium disease occurs in the rat, but the rat is not an appropriate model for CBD, because it does not mount an immune response to inhaled beryllium. F344 rats previously immunized by injection of beryllium sulfate and then exposed 2 weeks later to a single dose of beryllium sulfate *via* intratracheal instillation developed pulmonary granulomas 6 weeks after exposure, but the granulomas were resolving by 12 weeks postexposure (Votto et al., 1987). Total lung tissue exhibited an increase in both T- and B-lymphocytes, and T helper cells were increased in BAL fluid. This system may provide a rat model for CBD, but the study did not show a beryllium-specific immune response.

Mice may be an appropriate model for CBD, although not all aspects of the disease have been replicated in this species. BAL fluid of mice (sex not reported) preimmunized with beryllium sulfate and then administered a single intratracheal dose of beryllium sulfate had increased lymphocytes at 1 to 8 weeks postexposure, primarily because of increased T-helper cells, although Bursa (B) cells were also increased (Huang et al., 1992). Interstitial inflammation and granuloma formation were observed, but these changes only occurred at 8 months postexposure, not earlier, and had resolved by 10 months. This protocol did not produce lesions in BALB/c or C57BL/6J mice, suggesting that genetic differences at the H2 major histocompatibility locus (MHC) could be responsible for differences in sensitivity.

Nikula et al. (1997) exposed female A/J mice and C3H/HeJ mice to a beryllium metal aerosol (nose-only exposure) for 90 minutes, resulting in mean initial lung burdens of 49 and 62.50 μg, respectively. At 28 weeks after exposure, the mice had a marked, multifocal granulomatous pneumonia with mild interstitial fibrosis. The histopathological lesions were similar for both mouse strains. The interstitial aggregates exhibited lymphocyte proliferation and contained elevated numbers of T helper cells; the observed histopathological lesions may have been due to toxic and foreign-body properties of beryllium and an immune response. However, beryllium-specific proliferation of lymphocytes was not observed in the BeLT using lymphocytes from peripheral blood, the spleen, or bronchial lymph nodes. Although these two studies differed in the beryllium compound studied and neither demonstrated a beryllium-specific response, the observed granulomas did have an immune component.

The guinea pig appears to model certain aspects of CBD. An immune granulomatous lung disease was observed in strain 2 guinea pigs that received a single intratracheal injection of 1.8 mg beryllium as beryllium oxide (Barna et al., 1984a). The calcining temperature was not reported, but an earlier study by the same authors used beryllium oxide calcined at 560°C (Barna et al., 1981).

The granulomas contained interstitial infiltrates of lymphocytes and other cells, but fibrosis was not observed. Lesions developed by 6 weeks, with all animals affected by 10 weeks and a marked decrease in the incidence and severity of the lesions by 1.5 years postexposure. Spleen and lymph node cells proliferated in response to beryllium sulfate stimulation in the BeLT, although tests with other metals were not conducted to show beryllium sensitivity. Results from the BeLT with BAL lymphocytes were not informative, because the unstimulated cells incorporated large amounts of tritiated thymidine and were refractory to further stimulation by mitogens (Barna et al., 1984b). However, an increased percentage of T lymphocytes was observed in BAL fluid from treated guinea pigs. By contrast, strain 13 guinea pigs, which differ from strain 2 only at the MHC Ia locus, had no significant increase in granulomatous lung disease compared to controls, and no evidence of beryllium sensitization. These studies show that intratracheal instillation of beryllium oxide can induce in guinea pigs both immune granulomas containing a T lymphocyte component, and a beryllium-specific immune response. However, the effect has not yet been demonstrated under physiological conditions (inhalation exposure), and specificity for beryllium over other metals has not been demonstrated.

The beagle dog appears to model most aspects of human CBD (Haley et al., 1989). Granulomatous lesions and lung lymphocyte responses consistent with those observed in humans with CBD were observed following a single exposure to beryllium oxide aerosol generated from a nebulized solution of beryllium oxide calcined at 500^{o}C or 1,000^{o}C (10 mg/ml). The aerosol was administered to the dogs perinasally for 5-40 minutes to attain initial lung burdens of 17 or 50 µg beryllium oxide/kg body weight. Control dogs were sham-exposed to nebulized distilled water for 30 minutes. Actual doses are presented in Table 4-12.

Table 4-12. Initial lung burdens in dogs evaluated by bronchoalveolar lavage after inhalation of beryllium oxide

Level	Actual dose[a] (µg Beryllium oxide/kg body weight)
500^{o}C	
High dose	42.5 ± 11.0
Low dose	18.4 ± 2.7
1,000^{o}C	
High dose	47.6 ± 17.9
Low dose	18.0 ± 1.7

[a]Values are means ± SD.

Source: Haley et al. (1989).

Pulmonary lesions were characterized by peribronchiolar and perivascular lymphocytic-histiocytic inflammatory cell infiltrates of similar intensity 8 and 32 days after exposure with the suggestion of a peak response at 64 days. Lymphocytes were small and well-differentiated in early lesions but progressed to larger, lymphoblastic cells at later times. Aggregation of lymphocytes with large epithelioid macrophages formed distinct lymphofollicular nodules and/or microgranulomas within the parenchyma. Microgranulomas consisted centrally of large vacuolated and/or epithelioid macrophages surrounded by irregular mantles of lymphocytes. Alveolar macrophages were frequently large and vacuolated with abundant yellow-brown, flocculant to granular, intracytoplasmic material. Areas of the most intense interstitial inflammation were accompanied by moderate to marked interstitial fibrosis, epithelial cell hyperplasia, and air space organization.

The authors reported that the percentages of lung lymphocyte in dogs with high ILBs of 500^{o}C-treated beryllium oxide had a marked increase at 3 months, which declined rapidly through 22 months. Dogs with low ILBs of 500^{o}C heat-treated beryllium oxide also had an increase in percent lymphocytes at 3 months, which returned to control levels. Total lung lymphocyte numbers were likewise elevated in animals exposed to 500^{o}C-treated beryllium oxide with the high ILB group again having the greatest increases at 3 months with a rapid decline thereafter. Only one dog with high ILB 1,000^{o}C-treated beryllium oxide had a variable and mild increase in percentages of lymphocytes. None of these dogs showed an increase in lymphocyte numbers. Dogs with low ILB 1,000^{o}C-treated beryllium oxide also had no identifiable changes in the percentages or numbers of lung lymphocytes.

Beryllium specificity of the immune response was demonstrated by positive results in the BeLT, although there was considerable interindividual variation (Haley et al., 1989). Positive results were observed with BAL lymphocytes only in the group with a high ILB of the material calcined at 500^{o}C, but positive results with peripheral blood lymphocytes were observed at both doses with material calcined at both temperatures. Although there was striking variability in the severity and distribution of lesions, generally dogs exposed to material calcined at 500^{o}C developed more severe lesions. Based on the foregoing data, the author's noted the similarities between their findings and the lymphocytic and histologic responses reported in humans with CBD, which included the following: 1) production of severe granulomatous lung lesions; 2) positive blast transformation of blood and lung lymphocytes; 3) increased numbers of pulmonary lymphocytes, and 4) marked individual variation in sensitivity to beryllium.

In a follow up experiment, control dogs and those exposed to beryllium oxide calcined at 500^{o}C were allowed to rest for 2.5 years, and then re-exposed to filtered air (controls) or beryllium oxide calcined at 500^{o}C for ILB target of 50 μg BeO/kg body weight (Haley et al., 1992). Immune

responses of blood and BAL lymphocytes, and lung lesions in dogs sacrificed 210 days postexposure, were compared with results following the initial exposure. Histologic lesions were characterized by perivascular and interstitial infiltrates of lymphocytes and macrophages with progression to patchy granulomatous pneumonia accompanied by focal septal fibrosis. The severity of lung lesions was comparable under both conditions, suggesting that a 2.5-year interval was sufficient to prevent cumulative pathologic effects.

Conradi et al. (1971) found no exposure-related histological alterations in the lungs of six beagle dogs exposed to a range of 3,300 4,380 μg Be/m^3 as beryllium oxide calcined at 1,400^{o}C for 30 minutes, once per month for three months. Because the dogs were sacrificed two years postexposure, the long time period between exposure and response may have allowed for the reversal of any beryllium-induced changes. Alternatively, the high calcining temperature may have contributed to the low toxicity, continuing the trend observed with beryllium oxide calcined at 500^{o}C and 1,400^{o}C.

Haley et al. (1994) exposed male cynomolgus monkeys (*Macaca fascicularis*) to either beryllium metal or beryllium oxide[4] calcined at 500^{o}C by intrabronchiolar instillation as a saline suspension. Lymphocyte counts in BAL fluid were significantly increased in monkeys exposed to beryllium metal on postexposure days 14 to 90, but only on postexposure day 60 in monkeys exposed to beryllium oxide. The lungs of monkeys exposed to beryllium metal had lesions characterized by interstitial fibrosis, Type II cell hyperplasia, and lymphocyte infiltration; some monkeys exhibited immune granulomas. Similar lesions were observed in monkeys exposed to beryllium oxide, but the incidence and severity were much less. BAL lymphocytes from monkeys exposed to Be metal, but not from monkeys exposed to beryllium oxide, proliferated in response to beryllium sulfate in the BeLT.

In an experiment similar to the one conducted with dogs, Conradi et al. (1971) found no effect in monkeys (*Macaca irus*) exposed *via* whole-body inhalation for three 30 minute monthly exposures to a range of 3,300 - 4,380 μg Be/m^3 as beryllium oxide calcined at 1,400^{o}C. The lack of effect may have been related to the long period (two years) between exposure and sacrifice, or to low toxicity of beryllium oxide calcined at such a high temperature. The data from Haley et al. (1994) show that beryllium can induce immune granulomas and beryllium sensitization in monkeys *via* intrabronchiolar instillation, although this was not shown using a physiologically relevant route.

[4] Mass median aerodynamic diameter (MMAD) was 1.6 micrometers; geometric standard deviation was 1.9.

Genetics of beryllium sensitivity

Evidence from a variety of sources shows that genetic susceptibility plays a role in the development of CBD. Early occupational studies proposed that CBD was an immune reaction with a genetic component, based on the high sensitivity of certain individuals and the lack of CBD in others who were exposed to levels several orders of magnitude higher (Sterner and Eisenbud, 1951). Animal studies support these results. Immune granulomas were observed in strain 2 guinea pigs, but not in strain 13 guinea pigs, which differ from strain 2 only at the MHC Ia locus (Barna et al., 1984a). Similarly, beryllium inhalation caused immune granulomas in A/J mice, but not in BALB/c or C57BL/6J mice, which have different MHC class II genes (Huang et al., 1992). These studies suggest that differences in CBD susceptibility are related to differences at the MHC locus.

MHC class II antigens and functional IL2 receptors are needed in order for BAL CD4+ T cells from patients with CBD to proliferate *in vitro* in response to beryllium stimulation (Saltini et al., 1989). This requirement, known as class II restriction, is typical of the response of CD4+ T cells to soluble antigens, but not to nonspecific mitogens. In other words, the T cells only respond to the antigen (in this case, beryllium or beryllium plus some protein) in association with MHC class II molecules on the surface of the antigen-presenting cell. Granuloma formation has been hypothesized to result from a cytokine amplification loop involving macrophages, lymphocytes, and other factors (Newman, 1996).

Recent studies have identified a genetic marker linked to CBD susceptibility. The MHC class II region includes the HLA-DR, DQ, and DP genes. Richeldi et al. (1993) reported a strong association between the MHC class II allele HLA-DPβ1, which has a glutamate at position 69, and the development of CBD in beryllium-exposed workers. This marker was found in 32/33 of the workers who developed CBD, but in only 14/44 similarly exposed workers without CBD. Stubbs et al. (1996) also found a biased distribution of HLA DPβ1 alleles in beryllium-sensitized subjects, with the glutamine 69 allele present in 86% of the sensitized subjects but in only 48% of exposed, nonsensitized subjects. They also found a biased distribution of the MHC class II HLA DR gene but found no association with specific amino acid changes. Thus, neither of these markers are completely specific for CBD; however, the data do support a strong genetic contribution to CBD susceptibility, and these markers may be useful for screening for sensitive workers. It is also not clear if the association between either allele and CBD is a causal one. Preliminary findings show that the anti-HLA DR antibody blocked beryllium-specific lymphocyte proliferation, while an anti-HLA DP antibody had a minimal effect. It is not yet clear which, if either, of these class II genes interact directly with the beryllium ion, although the antibody inhibition data suggest that the HLA DR gene product may be involved in the presentation of beryllium to T lymphocytes. However, Richeldi et al. (1993) noted that structure-function studies of MHC class II molecules indicate that

the amino acid change associated with CBD may affect an amino acid that plays a critical role in antigen binding. The more common allele of HLA DPβ1 has a positively charged amino acid (lysine) at position 69, while the glutamine 69 variant is negatively charged at this site and could directly interact with the beryllium ion. Nonetheless, the high percentage (~30%) of exposed workers without CBD who had this allele suggests that other factors also contribute to the development of CBD. The beryllium exposure level plays at least some role, since the overall prevalence of CBD in exposed workers is 2-5%, while the prevalence at certain highly exposed tasks is as much as 15% (Kreiss et al., 1996, 1993a).

Relationship between beryllium speciation and toxicity

The toxicity of beryllium compounds is related to the solubility and surface area of the compound. For example, in a subacute inhalation study with female monkeys (*Macaca mulatta*), beryllium fluoride was markedly more toxic than beryllium sulfate, which was somewhat more toxic than beryllium phosphate (Schepers, 1964). Beryllium metal appeared to induce a greater toxic response than beryllium oxide following intrabronchiolar instillation in cynomolgus monkeys, as evidenced by more severe lung lesions, a larger effect on BAL lymphocyte counts, and a positive response in the BeLT with BAL lymphocytes only after treatment with beryllium metal (Haley et al., 1994). Comparable doses were calculated based on comparable levels of the Be^{2+} ion, based on an assumed dissolution rate for Be metal, rather than on comparable levels of instilled beryllium. This form of normalization was chosen in light of data suggesting that the toxicity of beryllium metal results from a thin surface layer of beryllium oxide on the metal particles (Hoover et al., 1989).
Occupational studies also show compound-specific differences in beryllium toxicity, but are less clear about whether beryllium metal or beryllium oxide is more toxic, probably because of variability in particle size. (Eisenbud and Lisson, 1983) found a higher prevalence of CBD in people who worked with beryllium metal than in those who worked with beryllium oxide, and (Sterner and Eisenbud, 1951) found a much higher prevalence of CBD in people who worked with beryllium oxide than in those who worked with other beryllium compounds. By contrast, Cullen et al. (1987) found a greater frequency of CBD in workers exposed to beryllium oxide fumes than those exposed to beryllium metal, but the small particle size of the fume compared to the beryllium metal dust may have contributed to the higher toxicity of the beryllium oxide in this study.

The temperature at which beryllium oxide is calcined influences its solubility, and hence its toxicity. Haley et al. (1989) found more severe lung lesions and a stronger immune response in beagle dogs receiving a single inhalation exposure to beryllium oxide calcined at 500°C than in dogs receiving an equivalent initial lung burden of beryllium oxide calcined at 1,000°C. The higher toxicity of beryllium oxide calcined at 500°C has been attributed to its greater surface area compared to the material calcined at 1,000°C (Finch et al., 1989). These authors found that the *in vitro*

cytotoxicity to Chinese hamster ovary (CHO) cells and cultured lung epithelial cells of 500°C beryllium oxide was greater than that of 1,000°C beryllium oxide, which was greater than that of beryllium metal. However, when toxicity was expressed in terms of particle surface area, the cytotoxicity of all three forms was similar. Finch et al. (1991) obtained similar results in a study comparing the cytotoxicity of beryllium metal particles of various sizes to cultured rat alveolar macrophages, although specific surface area did not entirely predict cytotoxicity. The similar solubilities of beryllium metal particles and beryllium oxide are attributed to a fine layer of beryllium oxide that coats the metal particles (Hoover et al., 1989).

In an *in vitro* study with dog alveolar macrophage cultures, Eidson et al. (1991) found that uptake of beryllium oxide by macrophages was independent of the calcination temperature, but soluble beryllium sulfate was poorly taken up. Intracellular dissolution of the oxide correlated with cytotoxicity, and was higher for the material calcined at 500°C. The authors concluded that beryllium oxide is phagocytized by the macrophages, dissolved in lysosomes, and becomes cytotoxic once sufficiently high dissolved concentrations are achieved. Extracellular soluble beryllium was concluded to be noncytotoxic.

4.4.2. Carcinogenicity Studies—Parenteral and Dermal Administration

A number of studies have examined the carcinogenic potential of beryllium and beryllium compounds in animals following intratracheal, intravenous, intramedullary, and intracutaneous administration. The results of these studies have been extensively reviewed by U.S. EPA (U.S. EPA, 1987, 1991b). Lung tumors have been observed in rats following a single intratracheal instillation of beryllium metal, passivated beryllium metal (99% beryllium, <1% chromium), beryllium-aluminum alloy (62% beryllium), or beryllium hydroxide. Beryllium alloys containing <4% beryllium did not result in increases in lung tumors. Lung tumor incidences of 11-51% were observed in rats following intratracheal instillation of beryllium oxide fired at high, low, and medium temperatures. The types of lung tumors found in animals receiving intratracheal instillations of beryllium included adenocarcinomas, adenomas, squamous cell carcinoma, and malignant lymphoma. Osteosarcomas have been observed in rabbits and possibly in mice following intravenous or intramedullary injection of zinc beryllium silicate, beryllium oxide, beryllium phosphate, or beryllium metal. Tumors have not been observed following: 1) intracutaneous injection of beryllium sulfate; 2) after accidental introduction of beryllium oxide, beryllium phosphate or beryllium-containing fluorescent phosphors into the skin; or 3) following percutaneous administration of beryllium compounds. Granulomatous ulcerations were observed when the beryllium penetrated the epidermal layer of the skin.

4.4.3. Genotoxicity

The genotoxicity of beryllium has been previously reviewed by U.S. EPA (1987), IARC (1993), IPCS (2001), and ATSDR (2002) (see also Gordon and Bowser [2003]; Leonard and Lauwerys [1987]). A summary of the genotoxicity and mutagenicity studies with beryllium and compounds is provided in Table 4-13. Studies reported that beryllium chloride, beryllium nitrate, and beryllium sulfate did not induce gene mutations in the *Salmonella typhimurium* (Ames) assays, with or without metabolic activation.

Beryllium chloride was negative in a variety of nonmammalian studies, including the *Bacillus subtilis* rec assay (Nishioka, 1975), *Escherichia coli* WP2 *uvr* A (Rossman et al., 1984), and the *S. typhimurium* (Ames) test (Kuroda et al., 1991). In addition, beryllium chloride failed to induce SOS repair in *E. coli* (Rossman et al., 1984). However, positive results were reported for *B. subtilis* rec assay (Kuroda et al., 1991), *E. coli* forward mutation assay (Zakour and Glickman, 1984), and the *Photobacterium fischeri* gene mutation test (Ulitzur and Barak, 1988).

Beryllium nitrate was positive in a *B. subtilis* rec assay but negative in the Ames assay (Kuroda et al., 1991; Tso and Fung, 1981).

In the case of beryllium sulfate ($BeSO_4$), the *B. subtilis* rec assay (Kada et al., 1980; Kanematsu et al., 1980) and the *E. coli* rec assay (Dylevoi, 1990) were positive. The *E. coli* WP2 uvr A (Dunkel et al., 1984), Ames (Ashby et al., 1990; Arlauskas et al., 1985; Dunkel et al., 1984; Rosenkranz and Poirier, 1979; Simmon, 1979a), and *Saccharomyces cerevisiae* (Simmon, 1979a) mutagenicity studies were negative. Taylor-McCabe et al. (2006) examined the mutagenicity of $BeSO_4$ and the comutagenicity of beryllium with a known mutagen 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) by using a forward mutant detection system developed in *E. coli*. In this system, $BeSO_4$ was shown to be weakly mutagenic alone and significantly enhanced the mutagenicity of MNNG up to 3.5-fold over MNNG alone. This study also describes the proteins regulated by beryllium in vitro and suggests several potential pathways/mechanisms underlying beryllium-induced genotoxicity.

Positive results have been observed in mammalian cells cultured with beryllium chloride, beryllium sulfate, and beryllium nitrate. For beryllium chloride, positive results were reported for sister chromatid exchange (SCE) (Kuroda et al., 1991) and gene mutations (Miyaki et al., 1979) in Chinese hamster V79 cells. For beryllium nitrate, positive results were also reported for SCE frequencies in Chinese hamster V79 cells (Kuroda et al., 1991).

The ability of beryllium sulfate to cause SCE frequencies, chromosomal aberrations, and morphological transformation among human and animal cell lines was also examined. Larramendy et al. (1981) reported that $BeSO_4$ induced similar SCE frequencies in both human and hamster cells. However, in a study by Anderson (1983) negative SCE results were reported with murine macrophage-like and human lymphocyte cells. Increases in chromosome aberrations were observed in human lymphocytes and Syrian hamster cells (Larramendy et al., 1981) but not in Chinese hamster lung or ovary cells (Ashby et al., 1990; Brooks et al., 1989). It was found that $BeSO_4$ has the ability to induce morphological transformation in cultured BALB/c-3T3 cells and Syrian hamster embryo cells (Keshava et al., 2001; DiPaolo and Casto, 1979). In addition, $BeSO_4$ resulted in genomic instability in BALB/c mouse cells and genetic mutations in Chinese hamster V79 cells (Keshava et al., 2001; Hsie et al., 1979). A DNA repair test with $BeSO_4$ on rat hepatocytes yielded negative results (Williams et al., 1982).

Data on the in vivo genotoxicity of beryllium are limited to two studies. First, beryllium sulfate (1.4 and 2.3 g/kg, 50 and 80% of median lethal dose) administered by gavage did not induce micronuclei in the bone marrow of CBA mice, although a marked depression of erythropoiesis suggestive of bone marrow toxicity was evident 24 hours after dosing (Ashby et al., 1990). Second, F344/N rats that received a single nose-only exposure to beryllium metal and subsequently developed lung tumors were examined for genetic mutations. No mutations were detected in p53 or c-raf-1, and only weak mutations were detected in K-ras in lung carcinomas from F344/N rats given a single nose-only exposure to beryllium metal (Nickell-Brady et al., 1994). The authors concluded that the mechanisms for the development of lung carcinomas from inhaled beryllium in the rat do not involve gene dysfunctions commonly associated with human non-small-cell lung cancer.

In summary, the evidence for beryllium as a direct acting mutagen in bacterial test systems is equivocal. Mutations, SCEs, and chromosomal aberration assays in mammalian test systems have yielded both positive and negative results for beryllium compounds.

Table 4-13. Summary of studies on the direct mutagenicity and genotoxicity of beryllium and beryllium compounds

Compound	Test system	Endpoint	Result (+/− S9)[a]	Reference
		Nonmammalian test systems		
Beryllium chloride	*B. subtilis* rec assay	Gene mutation	ND/−	Nishioka (1975)
	B. subtilis rec assay	Gene mutation	ND/+	Kuroda et al. (1991)
	E. coli	SOS repair	ND/−	Rossman et al. (1984)
	E. coli WP2 *uvr* A	Gene mutation	ND/−	Rossman et al. (1984)
	E. coli	Forward mutation	ND/+	Zakour and Glickman (1984)
	P. fischeri	Gene mutation	ND/+	Ulitzur and Barak (1988)
	S. typhimurium	Gene mutation	−/−	Kuroda et al. (1991)
Beryllium nitrate	*B. subtilis* rec assay	Gene mutation	ND/+	Kuroda et al. (1991)
	S. typhimurium	Gene mutation	ND/−	Kuroda et al. (1991); Tso and Fung (1981)
Beryllium sulfate	*B. subtilis*	Gene mutation	ND/+	Kada et al. (1980); Kanematsu et al. (1980)
	E. coli WP2 *uvr* A	Gene mutation	ND/−	Dunkel et al. (1984)
	E. coli rec assay	Gene mutation	ND/+	Dylevoi (1990)
	S. typhimurium	Gene mutation	−/−	Arlauskas et al. (1985); Ashby et al. (1990); Dunkel et al. (1984); Rosenkranz and Poirier (1979); Simmon (1979a)
	S. cerevisiae	Gene mutation	ND/−	Simmon (1979b)
Beryllium sulfate + MNNG	*E. coli*	Forward mutation	+	Taylor-McCabe (2006)
		Mammalian test systems		
Beryllium metal (single, nose-only exposure)	F344/N rats[b]	Genetic alterations	−	Nickell-Brady et al. (1994)
Beryllium chloride	Chinese hamster V79 cells	Gene mutation	+	Miyaki et al. (1979)
	Chinese hamster V79 cells	SCE	+	Kuroda et al. 1991)
Beryllium nitrate	Chinese hamster V79 cells	SCE	+	Kuroda et al. (1991)
Beryllium sulfate	BALB/c-3T3 cells[c]	Genomic instability	+	Keshava et al. (2001)
	BALB/c-3T3 cells[c]	Morphological transformation	+	Keshava et al. (2001)
	P338D1 macrophage cell line	SCE	−	Andersen (1983)
	Chinese hamster ovary cells	Chromosomal aberration	−	Brooks et al. (1989)
	Chinese hamster lung cells	Chromosomal aberration	−	Ashby et al. (1990)
	Chinese hamster V79 cells	Gene mutation	+	Hsie et al. (1979)
	Syrian hamster cells	Chromosomal aberration	+	Larramendy et al. (1981)
	Syrian hamster cells	SCE	+	Larramendy et al. (1981)

Table 4-13. Summary of studies on the direct mutagenicity and genotoxicity of beryllium and beryllium compounds

Compound	Test system	Endpoint	Result (+/− S9)[a]	Reference
	Syrian hamster embryo cells	Morphological transformation	+	DiPaolo and Casto (1979)
	Rat hepatocytes	DNA repair	−	Williams et al. (1982)
	Human lymphocytes	Chromosomal aberration	+	Larramendy et al. (1981)
	Human lymphocytes	SCE	+	Larramendy et al. (1981)
	Human lymphocytes	SCE	−	Andersen (1983)
Beryllium sulfate (gavage; 80% [2.3 g/kg] and 50% [1.4 g/kg] of median lethal dose)	Male CBA mice	Micronuclei	−	Ashby et al. (1990)

[a]ND = no data.
[b]Lung tumors from the rats were analyzed for genetic alterations.
cBeryllium sulfate transformed cells were injected into nude mice; 100% of mice developed fibrosarcomas.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

4.5.1. Oral Exposure in Animals

There are no reliable data on the oral toxicity of beryllium in humans. The database for animal oral exposure studies is composed of short-term and chronic studies, many of which tested low doses of beryllium and did not find any adverse effects.

Gastrointestinal lesions and bone marrow hypoplasia were observed in male and female dogs fed diets containing 1-17 mg/kg-day and 12-17 mg/kg-day beryllium sulfate, respectively, for approximately three years (Morgareidge et al., 1976). Chronic oral exposure of rats (0.4-43 mg/kg-day) and mice (1.2 mg/kg-day) to beryllium sulfate did not result in any adverse effects (Morgareidge et al., 1977, 1975; Schroeder and Mitchener, 1975a, b).

"Beryllium rickets" have been observed in rats exposed to beryllium carbonate (13-300 mg/kg-day) in the diet for 3 to 4 weeks (Guyatt et al., 1933; Kay and Skill, 1934). It has been suggested that the rickets are the result of decreased absorption of phosphorus through the gastrointestinal tract, rather than a direct effect on bones or alterations in calcium balance. This is supported by the findings of Matsumoto et al. (1991) on rats fed beryllium carbonate (480 mg/kg-day) in the diet. One hypothesis is that, in the gut, the beryllium binds to soluble phosphorus and forms an insoluble beryllium phosphate that cannot be absorbed.

The oral studies in animals suggest that the gastrointestinal and the skeletal systems are target organs for beryllium. In dogs exposed to beryllium sulfate, the gastrointestinal tract is a sensitive target and lesions appear to be induced in the gut at doses less than those for bone marrow hypoplasia. Gastrointestinal effects were not observed in rats or mice exposed to dietary beryllium sulfate, and the gastrointestinal tract was not examined in the beryllium carbonate studies. It is not known if exposure to beryllium compounds other than beryllium carbonate will result in rickets, because the available studies on beryllium sulfate (the only other beryllium compound with available oral toxicity data) did not examine the skeletal system or measure serum phosphate levels. Schroeder and Mitchener (1975a) noted that rickets were not observed in their beryllium-exposed rats, but the criteria used to assess potential rachitic effects were not reported. Morgareidge et al. (1976) did not mention the occurrence of rickets in dogs that were observed daily and who underwent histological examination of the bone.

The potential of beryllium to induce developmental and/or reproductive effects has not been adequately assessed. In the only oral exposure study examining reproductive or developmental

endpoints, beryllium did not affect fertility or pup survival, weight or skeletal formation (Morgareidge et al., 1976). However, only small numbers of animals were evaluated, and visceral examinations of pups, examination of dying pups, or postnatal development were not evaluated. Developmental endpoints may be important to evaluate because, as with other metals, beryllium may cross the placenta and there is the potential for greater gastrointestinal absorption in young animals. There are no multigeneration studies, nor are there studies of male reproductive toxicity.

Beryllium sensitization progressing to CBD is the critical effect in humans exposed by inhalation. Oral exposure studies in animals have not evaluated measures of immune response or dysfunction.

The dog appears to be the species most relevant for extrapolation of dose-effect to humans. Dogs appear to model most aspects of CBD in humans. The dog is typically a better model than the rodent for the absorption kinetics of elements in humans. In addition, the dog appears to be more sensitive to beryllium than rats, showing greater effects at comparable doses.

4.5.2. Inhalation Exposure in Humans and Animals

In humans, the lung is the primary target of inhalation exposure to beryllium. Exposure to levels at or near mean values of 1 $\mu g/m^3$ for an indeterminate period of time may result in the development of a chronic inflammatory lung disease (CBD) characterized by the formation of granulomas (Cotes et al., 1983; Cullen et al., 1987; Kreiss et al., 1996). These granulomas result from an immune reaction, primarily based on cell-mediated immunity. A genetic component to CBD susceptibility has been identified (Richeldi et al., 1993). The toxicity of beryllium compounds increases with increasing solubility (Finch et al., 1988; Haley et al., 1989). Beryllium oxide calcined at 500°C is more soluble, more toxic and has a greater surface area than beryllium calcined at 1,000°C. The toxicity of inhaled aerosolized beryllium metal appears to resemble that of beryllium oxide calcined at 500°C because of a thin layer of oxide on the beryllium metal particles (Hoover et al., 1989).

An animal model of human CBD is defined by the development of immune granulomas, a beryllium-specific immune response, and a disease progression that mimics the human disease. Based on these criteria in single-exposure studies, the beagle dog appears to model several aspects of CBD (Haley et al., 1989). Monkeys (Haley et al., 1994), mice (Huang et al., 1992), and guinea pigs (Barna et al., 1984a), although they have not been studied in as great detail, also appear to develop immune granulomas. Rats form granulomas after inhaling beryllium compounds, but the granulomas do not have an immune component and rats do not mount a beryllium-specific immune

response (Finch et al., 1994; Haley et al., 1990; Hart et al., 1984). Using mice and guinea pigs gives the advantage of being able to use larger numbers of animals in experiments, but of these two species, a beryllium-specific immune response has only been shown in guinea pigs. No exposure-response studies have been published using species that are appropriate models for CBD, and all studies using appropriate models have been conducted only with acute exposures.

4.6. EVALUATION OF CARCINOGENICITY

4.6.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of beryllium via the oral route. There are no epidemiological studies of the effect of ingested beryllium on cancer in humans. Oral exposure studies in rats and mice did not find significant increases in tumor incidences. These studies tested relatively low doses, and no toxic effects were observed at any dose tested. Thus, a maximum tolerated dose (MTD) may not have been achieved.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), beryllium falls along a continuum between "likely to be carcinogenic to humans" and "carcinogenic to humans" by the inhalation route. These guidelines recognize that it may not be possible to select one of the five cancer descriptors in every case. The guidelines state: "Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence. These descriptors and narratives are intended to permit sufficient flexibility to accommodate new scientific understanding and new testing methods as they are developed and accepted by the scientific community and the public. Descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases that are clarified by the full narrative." Given the current inhalation database, beryllium falls along a continuum between "likely to be carcinogenic to humans" and "carcinogenic to humans."

A series of epidemiological studies of beryllium processing workers (Sanderson et al., 2001a; Ward et al., 1992; Mancuso, 1980, 1979; Wagoner, 1980) and of BCR members (Steenland and Ward, 1991; Infante et al., 1980) provides evidence of an association between beryllium inhalation exposure and lung cancer risk. Many of these studies have shown a twofold or greater increase in lung cancer mortality (SMR) among persons who may have been exposed to high levels of beryllium. However, a sound causal association between human exposure and cancer has yet to be elucidated. The human studies are supported by animal studies. Inhalation exposure to beryllium has resulted in increases in lung cancer in rats and monkeys (Nickell-Brady et al., 1994; Reeves and Deitch, 1971; Wagner et al., 1969; Vorwald, 1968; Reeves et al., 1967). Combining findings from

both the human and animal studies supports the notion of beryllium being classified as along a continuum between "likely to be carcinogenic to humans" and "carcinogenic to humans." NIOSH is currently reanalyzing the data for the cohort of beryllium processing workers. Completion of this analysis may provide better insight for the cancer classification of beryllium.

4.6.2. Human, Animal and Other Supporting Evidence

4.6.2.1. *Oral*

There are no epidemiological studies of the effect of ingested beryllium on cancer in humans. Oral exposure studies in rats (Morgareidge et al., 1975, 1977; Schroeder and Mitchener, 1975a) and an oral study in mice (Schroeder and Mitchener, 1975a, b) did not find significant increases in tumor incidences. All three studies tested relatively low doses and no toxic effects were observed at any dose tested. Thus, the MTD was not achieved.

4.6.2.2. *Inhalation*

The human epidemiology of beryllium offers evidence of an association between inhaled beryllium and lung cancer. A series of lung cancer mortality studies has been conducted in a set of beryllium processing plants in the U.S. (Sanderson et al., 2001a; Ward et al., 1992; Mancuso, 1980; Wagoner et al., 1980). Additionally, two studies of lung cancer in patients enrolled in the BCR were conducted (Steenland and Ward, 1991; Infante et al., 1980). All of these studies have found statistically significant increases (overall SMRs ranging from 1.22–2.00) in lung cancer mortality among persons exposed to beryllium (Sanderson et al., 2001a; Ward et al., 1992; Steenland and Ward, 1991; Infante et al., 1980; Wagoner et al., 1980; Mancuso, 1980, 1979).

The study by Sanderson et al. (2001a, b) used objective quantifiable beryllium measurements, accounted for latency in the development of lung cancer, and used a comparable control group rather than population-based SMRs. This study utilized a job exposure matrix to create objective measures of beryllium exposure. The exposure assessment found high beryllium levels were documented in the work environment before 1971, leading to latency or time since first exposure rather than duration of employment as an important measure of levels of exposure. With this application of a 10 year and 20 year lag, the cumulative, average, and maximum level of beryllium exposure (GSD) for cases was double the level estimated for the controls (Table 4-5, refer to GSD). The comparison group used in this study was derived from the pool of workers who had not died of lung cancer, effectively controlling for general health as well as location and socioeconomic status. The authors demonstrated that cigarette smoking was unlikely to be a

confounder because smoking was not related to beryllium exposure in the subset of workers with information on smoking. This study overcame most of the methodological inadequacies of the earlier studies and of occupational studies in general and provided robust, less biased risk estimates that indicated that exposure to levels of beryllium greater than the OSHA PEL of 2 $\mu g/m^3$ (U.S. DOE, 1999) may double or triple the risk of lung cancer (Table 4-1).

Several issues have arisen when the body of evidence was weighed for the association between beryllium exposure and lung cancer. In terms of risk observed, there seems to be a consistent elevated risk among the studies. However, there is potential overlap in the study population among these studies. For example, the study population for several studies included workers at the Reading, Pennsylvania, plant (Sanderson et al., 2001a; Ward et al., 1992; Mancuso 1980, 1979; Wagoner et al., 1980). Thus, there is a possibility that the consistent elevated risk observed may be due to reassessment of the same subset of people. Conversely, this observation could be interpreted as a continuum of evidence in support of an association between beryllium exposure and lung cancer risk.

Comparing risk estimates based on one plant to estimates that combine risk from multiple plants diminishes the ability to recognize variability in lung cancer risk at individual beryllium plants, thus impacting the interpretation of an observed association between beryllium exposure and lung cancer. In other words, comparing the overall SMR from a single plant study, such as Sanderson et al. (2001a), to the multiplant study by Ward et al. (1992) may overlook the individual range in SMR from each plant in the latter study, in turn lowering the confidence in the observed consistent elevated risk.

An internal EPA review of the beryllium data presented by Sanderson et al. (2001a) revealed that cumulative exposure and average exposure may have dissimilar associations with lung cancer risk. With the majority of the workers being exposed to high levels of exposure over a short period of time, an association between average exposure and lung cancer risk is observed, but the data set appears to be inadequate to effectively evaluate chronic long-term exposure to beryllium and lung cancer. NIOSH is currently conducting an updated cohort mortality study, which, in addition to adding 13 years of follow-up on the Reading cohort, will also investigate the discrepancies with cumulative and average exposure associations with lung cancer risk (Dr. Mary Schubauer-Berigan, NIOSH, April 23, 2007, telephone communication). This study will also address the issue of smoking as a potential confounder and the influence of older age of hire on the relationship between beryllium exposure and lung cancer risk.

Among the studies that assess the association between beryllium exposure and lung cancer from the BCR, there is a possibility of bias from the workers' access to health care as a result of

being part of the registry, although data to test this hypothesis are not available. In other words, if workers identified in BCR were more likely to be diagnosed with lung cancer because of their access to health care, then health care access (as a benefit of being in the case registry) will be the determining factor from which cases of lung cancer are identified among the worker population. Thus, the odds of detecting lung cancer cases among BCR workers would be higher if being part of that registry meant that workers had preferential access to health care compared with workers with limited access to health care. Questions about the requirements to be included in the case registry (optional or mandatory) also add to the uncertainty surrounding the studies that utilize the BCR. Lastly, as the level of elevated risk is small (overall SMR ranging from 1.07–2.12), with wide CIs, the level of confidence that the elevated risk observed is most likely due to beryllium exposure is low.

Inhalation exposure to beryllium has resulted in increases in lung cancer in rats and monkeys (Nickell-Brady et al., 1994; Reeves and Deitch, 1971; Wagner et al., 1969; Vorwald, 1968; Reeves et al., 1967). These observations support a possible association noted in the occupational studies. In addition, intravenous and intramedullary injection-induced osteosarcomas in rabbits and possibly in mice have been observed (U.S. EPA, 1991b, 1987). These data are considered sufficient evidence of carcinogenicity to animals.

IARC (1993), NTP (2005), Vainio and Rice (1997), and the U.S. Department of Health and Human Services (ATSDR, 2002b) considered the epidemiological data to be sufficient evidence of the carcinogenicity of beryllium and compounds in humans. IARC (1993) concluded that the issue of adjustments for smoking had been handled adequately. IARC (1993) noted that a limitation of the most recent cohort studies was the absence of discussion of potential exposure to other lung carcinogens, although "there is no evidence that other lung carcinogens were present."

4.6.3. Mode of Action

Experimental studies with animals and human exposures have shown that the lung is the target organ of toxicity following inhalation exposure to beryllium. In animals, adverse outcomes in the pulmonary system include emphysema, pneumonitis, and lung cancer. In humans, acute and chronic beryllium disease and lung cancer are the principal effects observed.

The modes of action for the series of events leading to cancer are unknown. As discussed in Section 4.4.3, evidence of direct mutagenicity is equivocal. Another possibility is that chronic beryllium disease increases one's risk of lung cancer. The following have been identified as potentially relevant to the mode of action for chronic beryllium disease: (1) the beryllium-reactive T-

cell and T-cell antigen receptor utilization (Amicosante and Fontenot, 2006); (2) cytokines in the amplification of the cell-mediated immune response to beryllium (Hong-Geller et al., 2006); and (3) the immunogenetics of beryllium sensitization (Fontenot and Maier, 2005). However, the relevance of these theories to the mode of action for lung cancer in humans is dependent on establishing a correlation between chronic beryllium disease and lung cancer. Another alternative as proposed by epidemiology studies such as Infante et al. (1980) and Sanderson et al. (2001a) is that the development of lung cancer might be the result of an acute, high-intensity exposure. However, as discussed previously, this observation may be a reflection of the type of workers available for sampling within these occupational studies. In other words, as clearly exemplified by Sanderson et al. (2001a), there were more workers who were exposed during a period of high exposure and few who were chronically exposed during the period when exposure levels were lower. A fourth possible mode of action is via an inflammatory route in which an undiagnosed low-grade inflammatory response may result in sustained DNA damage via peroxynitrite formation and eventually the development of lung cancer (Emmendoerffer et al., 2000).

In summary, the carcinogenic mode of action for beryllium is as yet unknown, and no studies have been conducted to confirm any of the theories that can mechanistically link beryllium exposure to lung cancer. Hence, the mode-of-action framework from the *Guidelines for Carcinogen Risk Assessment* has not been applied (U.S. EPA, 2005a).

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

A number of factors may differentially affect the response of children to toxicants such as beryllium and compounds. These factors include diet and physical environment as well as maturation of physiological and biochemical processes. In general, children have higher gastrointestinal absorption and are more susceptible to the effects of metals (for example, lead; Mushak, 1991) than are adults. Also, metals may cross the placenta, affecting the developing fetus. It seems reasonable that these generalizations would apply to beryllium, but there are no substantiating data available.

4.7.2. Possible Gender Differences

The extent to which men differ from women in susceptibility to beryllium metal and beryllium compounds is not known. The SMR for women in the Beryllium Case Registry Cohort study (Steenland and Ward, 1991) was 4.04 and statistically significant compared with an SMR of 1.76 among men in the cohort (also statistically significant). However, this finding was based on only 6 cases of lung cancer in women versus 22 cases among men in the registry. The 95% confidence intervals of the SMRs for men and women overlapped, indicating that there was no statistically significant difference between the SMRs for men and women.

Although some gender differences have been observed in oral animal studies with respect to reticulum cell sarcomas (Morgareidge et al., 1975), body weight alterations in rats and mice, glucosuria, and incidence of gross tumors in controls and exposed rats (Schroeder and Mitchener, 1975a, b), the quality of the studies precludes their significance. Male and female dogs developed the same types of gastrointestinal lesions at the same site following chronic beryllium ingestion (500 ppm as beryllium sulfate tetrahydrate). One of five female dogs showed similar lesions, while no males responded, at the lower dose of 50 ppm (Morgareidge et al., 1976).

Compared to oral exposure conditions, fewer gender differences were observed via inhalation. Reeves et al. (1967) observed differences in plateau body weight between females and males in beryllium-exposed female rats compared to controls.

Data are insufficient to draw definitive conclusions regarding gender differences in response to beryllium exposure.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

There are no human oral exposure studies that could be used to derive an RfD for beryllium.

There are a number of long-term animal studies (Guyatt et al., 1933; Kay and Skill, 1934; Schroeder and Mitchener, 1975a, b; Morgareidge et al., 1975, 1976, 1977) reported for several species (rats, mice, dogs) ingesting beryllium sulfate and/or beryllium carbonate. Morgareidge et al. (1976) is chosen as the principal study and lesions of the small intestine of dogs is the critical effect.

No adverse effects were observed in rats (Morgareidge et al., 1975, 1977) exposed to <500 ppm beryllium sulfate in the diet (≤ 37 - 43 mg/kg-day) or in rats and mice (Schroeder and Mitchener, 1975a, b) exposed to 5 ppm beryllium sulfate in drinking water (0.63 and 0.71 mg/kg day for rats and 1.2 mg/kg-day for mice). These studies are limited by free-standing NOAELs (i.e., highest dose tested is NOAEL), flaws in study design or executions, or inadequate documentation of the study results.

"Beryllium rickets" have been observed in rats exposed to beryllium carbonate (13–300 mg/kg-day) in the diet for 3 to 4 weeks (Guyatt et al., 1933; Kay and Skill, 1934). It has been suggested that the rickets are the result of decreased absorption of phosphorus through the gastrointestinal tract, rather than a direct effect on bones or alterations in calcium balance. This is supported by the findings of Matsumoto et al. (1991) with rats fed beryllium carbonate (480 mg/kg-day) in the diet. One hypothesis is that, in the gut, the beryllium binds to soluble phosphorus and forms an insoluble beryllium phosphate that cannot be absorbed.

Morgareidge et al. (1976) found that dogs fed 500 ppm beryllium as beryllium sulfate tetrahydrate (12 and 17 mg/kg-day for males and females, respectively) developed gastrointestinal lesions, most severely in the small intestine; weight loss, anorexia and lassitude were also observed in these animals. Exposure to 500 ppm was terminated after 33 weeks and the animals were killed in a moribund condition. A 10 fold lower beryllium

concentration (50 ppm; 1.1 and 1.3 mg/kg-day in males and females, respectively) resulted in similar, but less severe, gastrointestinal tract lesions as those seen in one female in the 500 ppm group, dying during week 70. The remaining animals at this dose level survived until study termination at approximately three years and showed no histopathological alterations in the gastrointestinal tract related to treatment. A no-observed-adverse-effect level (NOAEL) of approximately 0.1 mg/kg-day and frank-effect level (FEL) of 12 mg/kg-day for gastrointestinal tract lesions, anorexia, and weight loss in moribund dogs are determined. It is, however, difficult to discern whether the 1.3 mg/kg-day level should also be considered a FEL, because one animal died, or whether it is more appropriately considered a LOAEL, since while one animal was affected after a year of treatment, the other animals at the same level survived two years longer without adverse gastrointestinal pathology. Alternatively, some may think this one animal overly sensitive and discount it. However, the gastrointestinal lesions in this animal were of the same types and occurred in the same region, but with lesser severity, as in the higher dose group.

Therefore, the critical effect is small intestinal lesions in dogs in Morgareidge et al. (1976). A dose of 0.1 mg/kg-day is a NOAEL, 12 mg/kg/day is a FEL, and it is difficult to ascertain the LOAEL dose.

5.1.2. Methods of Analysis—Benchmark Dose

For small intestinal lesions, dose-response information is available for more than one dose level and can be used to determine the BMD_{10}, thereby decreasing the reliance on the one animal at 50 ppm.

For BMD calculations, the dose was converted from ppm beryllium to mg/kg-day using food intake reported by the authors as 300 g/day and the time-weighted average body weight (kg) for each sex/dose group for the study (females: 0, 0.029, 0.15, 1.3, 17.4; males: 0, 0.023, 0.12, 1.1, 12.2 mg/kg-day). The average of the doses (0, 0.026, 0.135, 1.2, 14.8 mg/kg-day) and the combined male and female incidence for small intestinal lesions (0/10, 0/10, 0/10, 1/10, 9/10) were modeled by the exponential polynomial, THRESH, and Weibull models. A 10% change (extra risk) was chosen as the benchmark response. The exponential polynomial model gave a fit to the data (p value for goodness-of-fit = 0.94). The BMD (the lower 95% confidence bound on the concentration from the MLE [maximum likelihood estimate], 10% extra risk) obtained for these data with this model was 0.46 mg/kg-day

(MLE= 1.4 mg/kg-day). For the THRESH model, the BMD10 was 0.47 mg/kg-day (MLE=1.2); the p value for goodness of fit is 1.0. The Weibull model was also applied with similar results (p value for goodness-of-fit= 0.96; MLE= 1.3; BMD_{10}= 0.46 mg/kg-day). The BMD_{10} of 0.46 mg/kg-day is used for all subsequent quantitation in the RfD dose-response assessment.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

A 300-fold uncertainty factor (UF) is applied to the BMD10 for lesions in the small intestines of male and female dogs for derivation of the RfD. This UF is composed of 10-fold each for intra- and interspecies extrapolation and a 3-fold factor for database deficiencies. Although there are several chronic oral animal studies, there is a lack of human toxicity data by the oral route, and reproductive/developmental and immunotoxicologic endpoints have not been adequately assessed in animals. Database gaps include lack of adequate studies for evaluation of reproductive and developmental toxicity (including multigenerational studies, studies on male reproductive toxicity, teratology and postnatal development) owing to the possible crossing of the placenta and greater absorption of beryllium in young animals. In addition, oral studies examining immunologic endpoints, the most sensitive endpoint by the inhalation route, are lacking. Since the principal study is of chronic duration and a benchmark dose is used, there are no uncertainty factors for duration or NOAEL/LOAEL extrapolation. No modifying factor is proposed for this assessment. It should be noted that the RfD is imprecise to perhaps an order of magnitude.

$$RfD = 0.46 \text{ mg/kg-day} \div 300 = 2 \times 10^{-3} \text{ mg/kg-day}$$

5.2. INHALATION REFERENCE CONCENTRATION

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

There is an extensive body of evidence documenting beryllium sensitization and CBD as the most sensitive effect of inhalation exposure to beryllium, and explaining the

unusual exposure-response pattern. The Kreiss et al. (1996) occupational exposure study, which identified a $LOAEL_{(HEC)}$ of 0.20 $\mu g/m^3$, and the Eisenbud et al. (1949) community monitoring study, which identified a $NOAEL_{(HEC)}$ of 0.01 0.1 $\mu g/m^3$, were selected as the co-principal studies. Although the method of identifying CBD cases in the Eisenbud et al. (1949) study was relatively insensitive compared to modern methods, this study has the advantage of being conducted with the general population, rather than a worker population. In addition, because the incidence of CBD was evaluated at different distances from the plant (and hence at different estimated exposure levels), this was the only study that was able to identify a NOAEL for CBD. The $NOAEL_{(HEC)}$ range reflects the uncertainty associated with the estimations of exposure level.

Occupational exposure studies by Cullen et al. (1987) and Cotes et al. (1983) identified low $LOAEL_{(HEC)}$s for CBD. Using the beryllium case registry definition of CBD, Cullen et al. (1987) identified a $LOAEL_{(HEC)}$ of 0.19 $\mu g/m^3$. Although the $LOAEL_{(HEC)}$ identified in this study was similar to that found in Kreiss et al. (1996), the Cullen et al. (1987) study was not selected as a principal study because no historical exposure monitoring data were available and worker exposure levels were estimated using a small amount of monitoring data. Cotes et al. (1983) reported a $LOAEL_{(HEC)}$ of 0.033 $\mu g/m^3$, but the definition of CBD used in this study was not well defined. This study was not selected as a principal study because only two cases of CBD were identified and the exposure concentrations were estimated using area samplers rather than personal and/or breathing zone samplers.

5.2.2. Methods of Analysis—NOAEL/LOAEL

A $NOAEL_{(HEC)}$ of 0.01 0.1 $\mu g/m^3$ was observed based on general population inhalation exposure to beryllium near the Lorain beryllium plant (0.75 miles), using insensitive screening methods (Eisenbud et al., 1949). An occupational study by Kreiss et al. (1996) found a LOAEL of 0.55 $\mu g/m^3$ ($LOAEL_{(HEC)}$ of 0.20 $\mu g/m^3$) using more sensitive screening methods.

A benchmark concentration (BMC) analysis could not be conducted for two reasons. First, neither study provided exposure-response information for more than one exposure level. Second, CBD is a sensitization disease, and the maximum susceptible population appears to be about 16% of the exposed population (Kreiss et al., 1993b). Therefore, a

response level of 10% would correspond to 1.6% (i.e., $BMC_{1.6}$) of the susceptible population developing the disease. No studies have yet been conducted evaluating the exposure-response in the subset of the population that appears to be genetically susceptible to CBD.

Calculation of the HEC for the occupational studies is as follows. The occupational LOAEL value was adjusted for the default occupational ventilation rate and for the intermittent work week schedule using the equation:

$LOAEL_{(HEC)}$ = LOAEL ($\mu g/m^3$) × (10 m^3 per 8-hour work shift)/(20 m^3 per day) × 5 days/7 days

Thus, a LOAEL of 0.55 $\mu g/m^3$ (Kreiss et al., 1996) corresponds to a $LOAEL_{(HEC)}$ of 0.20 $\mu g/m^3$.

5.2.3. RfC Derivation - Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

The available data suggest that only a small percentage of the population (1-5%) appears to be susceptible to CBD (Kreiss et al., 1994). Because individuals developing beryllium sensitization and CBD are the most sensitive subpopulation, an uncertainty factor of 1 was used to account for human variability. An uncertainty factor of 1 was also used to adjust for the less-than-chronic exposure duration of the Kreiss et al. (1996) study; use of this uncertainty factor is supported by the evidence that the occurrence of CBD does not appear be related to exposure duration. Because the screening method used in the Kreiss et al. (1996) study was more sensitive than the methods used in the Eisenbud et al. (1949) study, the RfC was derived from the LOAEL (Kreiss et al., 1996) with an uncertainty factor of 3 to account for the sensitive nature of the subclinical endpoint (beryllium sensitization). A database uncertainty factor of 3 was used to account for the poor quality of exposure monitoring in the co-principal studies and other epidemiology studies that assessed the incidence of beryllium sensitization and CBD among exposed workers and community residents. Although there are no developmental studies or two-generation reproduction studies, a limited continuous breeding study found that beryllium does not cause reproductive or developmental effects following intratracheal administration (Clary et al.,

1975). In addition, systemic distribution of beryllium is less than 1% (U.S. EPA, 1987a), and any systemic effects would be expected to occur at exposure levels much above the very low levels at which CBD is observed.

No modifying factor is proposed for this assessment (MF=1).

$$RfC = 0.2\ \mu g/m^3 \div 10 = 0.02\ \mu g/m^3, \text{ or } 2 \times 10^{-2}\ \mu g/m^3$$

5.3. CANCER ASSESSMENT

5.3.1. Choice of Study/Data—Rationale and Justification

Under the *Guidelines for Carcinogenicity Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of beryllium by the oral route. Derivation of a quantitative cancer risk estimate is therefore precluded. In general, the oral animal studies (Schroeder and Mitchener, 1975a, b; Morgareidge et al., 1977, 1976, 1975) did not find statistically significant increases in tumors upon ingestion of beryllium sulfate.

As discussed in Section 4.6, beryllium, by the inhalation route, falls along a continuum between "likely to be carcinogenic to humans" and "carcinogenic to humans." The classification is based on the interpretation of the body of evidence available, including the combined weight-of-evidence evaluation of data from both human occupational and animal studies and the aforementioned issues surrounding the key epidemiology studies in the assessment. In the 1987 IRIS assessment on beryllium , this chemical was classified as a B2: probable human carcinogen based on the 1986 Guidelines for Carcinogenic Ris Assessment (U.S. EPA, 1987). Later, based on the limited evidence of carcinogenicity in humans exposed to airborne beryllium (lung cancer) and sufficient evidence of carcinogenicity in animals (lung cancer in rats and monkeys inhaling beryllium, lung tumors in rats exposed to beryllium via intratracheal instillation, and osteosarcomas in rabbits and possibly mice receiving intravenous or intramedullary injection), beryllium was reclassified from a B2 (inadequate human data) to a B1 probable human carcinogen (limited human data) using criteria of the same version of the Guidelines for Carcinogen Risk Assessment. In the reassessment of beryllium in 1998 (U.S. EPA, 1998c), inhaled beryllium was characterized as a "likely" carcinogen in humans, using the 1996 proposed Guidelines for Carcinogen Risk

Assessment,

In 1987, as part of the first IRIS beryllium assessment, U.S. EPA (1987) derived a cancer inhalation unit risk (IUR) based on Wagoner et al. (1980), a cohort study of 3,055 employees who worked in a beryllium plant in Reading, Pennsylvania,, between 1942 and 1967. The 1987 IUR derivation used exposure values that NIOSH estimated from occupational monitoring data not specifically related to the Reading plant and its workers.

When EPA updated the IRIS beryllium assessment in 1998, it considered whether Ward et al. (1992), a cohort study of 9,225 workers employed at seven beryllium plants, including the Reading plant, between 1940 and 1969, would support a reassessment of the IUR. Because Ward et al. (1992) lacked adequate exposure information, EPA decided to retain the 1987 IUR until additional analysis of the beryllium worker cohort became available.

Since the 1998 IRIS assessment, a new analysis of workers at the Reading plant has been published. Sanderson et al. (2001a) conducted a nested case-control study of beryllium workers (n = 852) employed in one of the beryllium processing plants included in the Ward et al. (1992) seven-plant cohort mortality study. The plant, in Reading, Pennsylvania, was selected by the investigators because it had both a large number of lung cancer cases and adequate personnel and beryllium exposure records to construct historical exposure estimates. To generate estimates of beryllium exposure, Sanderson et al. (2001b) developed a job-exposure matrix that provided airborne beryllium exposure estimates (based on historic beryllium monitoring data) for every job that lung cancer cases and matched controls may have held during their tenure at the plant. Using these estimates, Sanderson et al. (2001a) then compared beryllium exposures among cases and controls.

Sanderson et al. (2001a) compared cases and controls by quartiles of beryllium exposure (cumulative, average, and maximum), using a categorical or quartile approach. In this analysis, using both 10 and 20 year lagged exposures, the odds ratios appeared to be the highest in the second or third quartiles of exposure (with the first quartiles used as the baseline) and then stayed the same or decreased in the highest exposure quartile. This pattern suggests a nonlinear relationship between increasing beryllium exposure (cumulative, average, and maximum) and the odds ratio. In addition, Sanderson et al. (2001a) employed

conditional logistic regression and log of exposure in a continuous analysis of the data. In this analysis, significantly elevated odds ratios were observed for 10 year and 20 year lagged exposures, primarily for average and maximum but not cumulative exposure metrics.

In evaluating both the categorical approach and the continuous logistic regression analysis conducted by Sanderson et al. (2001a), several issues were identified. In the categorical analysis, the odds ratios declined in the highest exposure quartile for each of the exposure metrics considered (cumulative, average, and maximum exposure). One might remove cases from the highest exposure quartile (along with their matched controls) by using the assumption that the highest quartile may be skewed by outliers of the overall distribution. However, this approach leaves insufficient data to generate a dose-response relationship. In the continuous analysis, using conditional logistic regression and the log of exposure, the resulting dose-response relationship yielded a "supralinear" curve with nearly infinite slope at low dose, which then plateaued at high dose. This shape of the dose-response curve makes derivation of an IUR extremely uncertain. However, in this same analysis, finding a significant relationship between beryllium exposure and lung cancer seems to be dependent on whether exposure metrics are log-transformed or not. Though not reported by Sanderson et al. (2001a), regression analysis without log-transformation of the exposure did not yield a statistically significant relationship between beryllium exposure and lung cancer. The treatment of zero values in the log-transformation may impact the observation of a statistically significant result. This fact adds to the uncertainty of the dose-response relationship, especially given that there is no underlying statistical rationale for log-transforming the data.

As summarized in Section 4.1, other epidemiology studies have evaluated the relationship between beryllium exposure and lung cancer risk, including Ward et al. (1992) and Wagoner et al. (1980). However, as with other studies prior to Sanderson et al. (2001a), no quantification of individual worker beryllium exposures was available or attempted, and duration of employment was typically used as a surrogate. As already indicated, length or duration of exposure may not have been an appropriate surrogate for estimating beryllium exposure because dramatic declines in DWA beryllium exposures have been observed, in some cases more than 100-fold, from the 1930s to the 1970s (Sanderson et al., 2001b).

After determining that the Sanderson et al. (2001a) data could not be modeled to obtain a point of departure, EPA investigated whether the Sanderson et al. (2001a) exposure

estimates and odds ratios could be substituted for the NIOSH exposure estimates and SMRs from Wagoner et al. (1980) in the method used to develop the current IRIS IUR (U.S. EPA, 1998b, 1987). Because associations between the exposure levels and the odds ratios seem to depend on the choice of exposure metric (average, maximum, or cumulative) and because associations were seen only when the data were log transformed, EPA determined that the values were too uncertain to use in the derivation. Additionally, Sanderson et al. (2001a) was designed as a case-control study. All the cases and controls worked at the Reading plant and were exposed to beryllium. Odds ratios were calculated by comparing cancer deaths in the upper three quartiles of exposure to those in the lowest quartile of exposure. Sanderson et al. (2001a) did not provide the data needed to calculate SMRs for the exposed workers compared to unexposed groups, which is an essential input for method used to calculate the current IUR. In addition to the above challenges, given the difficulty in choosing appropriate cut points for categorical analyses, difficulty in replicating conditional logistic regression parameter estimates for continuous exposure metrics (i.e., average and maximum exposures), and the fact that the beryllium-lung cancer relationship in this cohort may be driven by acute, high exposures to beryllium, EPA was unsuccessful in developing an IUR using the data from Sanderson et al. (2001a).

Appendix C provides additional discussion of EPA's attempts to use the data from Sanderson et al. (2001a) to derive an IUR.

Many of the experimental animal studies summarized in Section 4.2 were conducted prior to the implementation of good laboratory practice guidelines and possess inherent shortcomings that make interpretation of these data difficult. Some of these shortcomings include the lack of control groups, outbreaks of non-treatment-related diseases such as pneumonia, and co-exposures to other potential carcinogens. Wagner et al. (1969) was one of the few studies that compared beryllium-exposed animals to controls. However, Wagner et al. (1969) found rats exposed to beryllium ores also had high levels of silicon dioxide (30–100 times higher levels of silicon dioxide than beryllium) in their lungs. Thus, this study was not appropriate for quantifying the risk to the general human population of exposure to beryllium. Nickell-Brady et al. (1994) also utilized controls in a study with F344/N rats, but exposure was via a single, short-term, nose-only exposure to beryllium metal (500 mg/m^3 for 8 minutes, 410 mg/m^3 for 30 minutes, 830 mg/m^3 for 48 minutes, or 980 mg/m^3 for 39 minutes). Tumors became apparent by 14 months following exposure, and the incidence (apparently for all groups combined) was 64% over the lifetime of the rats. However, the

design of this study hinders the ability to derive an inhalation unit risk, which assumes long-term chronic exposure.

In conclusion, as summarized above, the studies that have become available since the 1998 IRIS beryllium assessment (U.S. EPA, 1998b) are inadequate to support a reassessment of the current IUR on IRIS. As mentioned previously, NIOSH is currently conducting an updated cohort mortality study that includes an additional 13 years of follow-up on the Reading cohort as well as two additional beryllium processing facilities (both of which started in the late 1950s) (Dr. Mary Schubauer-Berigan, NIOSH, April 23, 2007, telephone communication). Because this updated study will cover a broader range of years of plant operation and associated beryllium exposure levels, a wider birth cohort distribution with a wider range of beryllium exposures should result. This should provide insight into the relationship between lung cancer and exposure, older age at hire (which leads to a tendency for person-time and events from the earliest birth cohort to accrue in the lowest exposure category), and baseline disease risk that appear to be confounding the ability of Sanderson et al. (2001a) to evaluate the association between beryllium and lung cancer. Future studies should also improve upon smoking estimation methods and further elucidate the potential for modification of beryllium risk ratios by smoking status. It is expected that such studies will provide a more definitive assessment of the carcinogenicity of beryllium.

It is recommended that the existing unit risk based on the Wagoner et al. (1980) study be retained as the basis for a quantitative estimate. There is limited information reported on beryllium exposure levels for the seven beryllium processing facilities that were examined in the cohort mortality studies. Prior to 1950 when exposure levels of $\leq$2 $\mu g/m^3$ were mandated by the Atomic Energy Commission, beryllium levels at the Lorain and Reading facilities (facilities with the highest lung cancer mortality rates) were very high. NIOSH (1972) estimated that the lower-bound estimate of the median exposure concentration exceeded 100 $\mu g/m^3$ and concentrations in excess of 1,000 $\mu g/m^3$ were commonly found (Eisenbud and Lisson, 1983). In 1947 and 1948, beryllium concentrations of 590-43,300 $\mu g/m^3$ were measured at the Lorain facility. Beryllium levels exceeding the Atomic Energy Commission's permissible levels were frequently found after 1950. At the Elmore, OH facility, TWA beryllium levels of 3.8-9.5, 6.8-19.1, and 23.1-54.6 $\mu g/m^3$ were found in 1953, 1956 and 1960, respectively (Zielinski, 1961). Another study of this facility found that in 1960 and 1966, beryllium concentrations ranged from <0.1 to 1,050 $\mu g/m^3$ depending on the production area; the average and median levels for all areas were 60.3 and 28.4 $\mu g/m^3$,

respectively, in 1960 and 18.1 and 11.4 μg/m^3 in 1966 (Cholak et al., 1967). The available exposure data suggest that beryllium processing workers can be exposed to a wide range of beryllium concentrations depending on the facility where they worked, decade they were employed, and the type of work performed. The lack of monitoring data relating cancer risk to beryllium exposure levels or reliable exposure surrogates is reason for concern; however, it does not preclude the use of the human exposure data estimated by NIOSH [range of median exposure levels inside plants (100-1000 μg/m^3)] for quantitative cancer risk estimates.

5.3.2. Dose-Response Data

Dose-response data are inappropriate for oral exposure. Dose-response data for inhalation include the occupational exposure study of a cohort of workers exposed to beryllium at the Reading facility (Wagoner et al., 1980). Lung cancer SMRs were elevated, particularly for workers hired prior to 1950 when exposures to beryllium were very high, and who were followed for at least 25 years (SMR=1.87). U.S. EPA (1987a) further analyzed the data and concluded that the adjusted SMRs, while still elevated, were not statistically significant. The adjustments accounted for differences in smoking habits between the cohort and the U.S. population and for the use of older vital statistics, and eliminated an ineligible cancer death. The adjusted lung cancer deaths for the subcohort followed for at least 25 years ranged from 13.91 to 14.67, in comparison with 20 observed, resulting in SMRs or relative risks of 1.44 to 1.36, respectively.

Beryllium concentration in the workplace (μg/m^3)	Ratio of years of exposure to years at risk (f/L)	Effective dose (μg/m^3)	95% upper-bound estimate of relative risk	Unit risk (μg/m^3)$^{-1}$
100	1.00	21.92	1.98	1.61×10^{-3}
			2.90	1.79×10^{-3}
	0.25	5.48	1.98	6.44×10^{-3}
			2.09	7.16×10^{-3}
1000	1.00	219.18	1.98	1.61×10^{-4}
			2.09	1.79×10^{-4}
	0.25	54.79	1.98	6.44×10^{-4}
			2.09	7.16×10^{-4}

5.3.3. Dose Conversion

Not applicable by the oral route.

With respect to the inhalation route, the effective dose was determined by adjusting for duration of daily (8/24 hours) and annual (240/365) exposure, and the ratio of exposure duration to duration at risk, i.e., f years out of a period of L years at risk (from onset of employment to termination of follow-up). Two values of f/L were used in the calculations, namely, f/L=1 and 0.25. An f/L of 1.0 would avoid overestimating the risk (but could underestimate the risk) if the observation by Reeves and Deitch (1971)—that tumor yield depends not on the length of exposure but on age at exposure—is valid. For a given "effective" dose d and a relative risk R, the carcinogenic potency (q1*) is calculated by the formula B= (R-1) x 0.036/d, where 0.036 is the estimated lung cancer mortality rate in the U.S. population. The risk estimates were based on the data of Wagoner et al. (1980) in which the smoking adjusted, expected lung cancer deaths were found to range from 13.91-14.67, in comparison to 20 observed. Relative risk estimates of 1.36 ($p>0.05$) and 1.44 ($p>0.05$) were derived and the 95% upper confidence limits of these estimates, 1.98 and 2.09, respectively, were used to estimate the lifetime cancer risk (q1*).

5.3.4. Extrapolation Method(s)

Not applicable for the oral route

With respect to inhalation studies in humans, the response is measured in terms of the relative risk of the exposed cohort of individuals as compared with the control group. The mathematical model employed for low-dose extrapolation assumes that for low exposures the lifetime probability of death from cancer, Po, may be represented by the linear equation

$$Po = A + B_H\ x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in units such as $\mu g/m^3$. The factor B_H is the increased probability of cancer associated with each unit increase of x, the agent in air.

5.3.5. Oral Slope Factor and Inhalation Unit Risk

An oral slope factor was not derived.

With regard to the inhalation route of exposure, data from the epidemiological study by Wagoner et al. (1980) and the industrial hygiene reviews by NIOSH (1972) and Eisenbud

and Lisson (1983) have been used to develop a cancer risk estimate associated with exposure to air contaminated with beryllium. Two upper-bound relative risk estimates, 1.98 and 2.09, calculated from the human data ($p<0.05$ for both relative risk values), have been used in the calculations. In recognition of the greater uncertainty associated with the exposure estimation, four different "effective" levels of exposure that reflect various uncertainties, along with two relative risk estimates, have been used in the present calculations. As a result, eight potency estimates have been calculated ranging from 1.6×10^{-4} per ($\mu g/m^3$) to 7.2×10^{-3} per ($\mu g/m^3$), with the geometric mean of the eight estimates being 2.4×10^{-3} per ($\mu g/m^3$). This "unit risk" estimate could be considered an upper-bound estimate of the cancer risk because low-dose linearity is assumed in the extrapolation and the 95% upper-confidence limits (1.98 and 2.09) are used in the calculations.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Beryllium is a light, stable, bivalent metal belonging to the alkaline earth family. It is used in metal alloys, in particular beryllium-copper alloy, and in high-performance products in the metallurgical, aerospace, and nuclear industries. The primary anthropogenic emission source of beryllium is the combustion of coal and fuel oil, which releases beryllium-containing particulates and fly ash. The general population is exposed to beryllium through inhalation of air and consumption of food and drinking water.

There are no human data on the oral toxicity of beryllium. Chronic oral studies in rodents generally have not shown adverse effects of ingested beryllium (Morgareidge et al., 1975, 1977; Schroeder and Mitchener, 1975a, b). However, these studies have had several limitations in design and execution. A long-term (3-year) oral study in dogs indicates the gastrointestinal tract, particularly the small intestine, is the target organ for ingested beryllium (Morgareidge et al., 1976).

Beryllium sensitization and CBD have been observed following occupational exposure and in residents living near a beryllium manufacturing facility (Kreiss et al., 1996; Eisenbud et al., 1949). CBD is a well-characterized granulomatous immune disease that occurs in a susceptible subset of the population. A genetic component of CBD has been identified. This genetic marker identifies most, but not all, of the CBD cases, and a portion of those with the marker do not develop CBD after exposure to beryllium, indicating that other factors also contribute to determining the sensitive subpopulation. Although some studies indicate that early stages of CBD can be reversed, the degree of reversibility and exposure levels that allow reversibility have not been characterized. Although animal models that mimic several aspects of human CBD appear to be available in the dog, monkey, mouse, and guinea pig, an animal model that mimics all aspects of CBD, in particular the progressive nature of the disease, has not been identified.

The potential of beryllium to induce developmental and/or reproductive effects has not been adequately assessed. No effect on fertility or pup survival, body weight, or skeletal

formation was observed in a chronic dog feeding study. However, this study did not conduct visceral examinations of pups or monitor postnatal development. Developmental effects (increased fetal mortality, decreased fetal body weight, internal abnormalities, and delayed neurodevelopment) have been reported in the offspring of rodents following intratracheal or intraperitoneal administration of beryllium. No reproductive effects were observed in rats receiving a single intratracheal instillation of beryllium.

The areas of scientific uncertainty concerning the noncancer hazard assessment for beryllium include examination of immunologic endpoints or sensitive indicators for rickets in chronic oral studies in animals, an animal model that mimics the progressive nature of CBD in humans, and adequate oral developmental and reproductive toxicity studies.

A series of epidemiological studies on beryllium processing workers (Sanderson et al., 2001a; Ward et al., 1992; Mancuso, 1980, 1979; Wagoner, 1980) and on BCR members (Steenland and Ward, 1991; Infante et al., 1980) suggest a relationship between inhalation exposure to beryllium and lung cancer. Scientific uncertainties in the assessment of the human carcinogenicity data include inadequate control for confounders such as smoking and potential occupational exposure to other lung carcinogens. The increased incidence of lung cancers among workers with acute beryllium disease (presumably these workers were exposed to very high concentrations of beryllium), the higher incidence of lung cancers among workers first employed when exposure levels were very high and a consistent finding of lung cancer excess in beryllium processing facilities suggest a relationship between beryllium exposure and an increased risk of lung cancer.

Inhalation exposure or intratracheal administration of beryllium has resulted in lung cancer in rats and monkeys (Nickell-Brady et al., 1994; U.S. EPA, 1991b, 1987; Reeves and Deitch, 1971; Wagner et al., 1969; Vorwald, 1968; Reeves et al., 1967). These observations support the association noted in the occupational studies. In addition, intravenous and intramedullary injection induced osteosarcomas in rabbits and possibly in mice (U.S. EPA, 1991b, 1987). These data are considered sufficient evidence of carcinogenicity to animals.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of beryllium via the oral route. Via the inhalation route, beryllium falls along a continuum between "likely to be carcinogenic to humans" and "carcinogenic to humans."

Studies of beryllium processing workers (Sanderson et al., 2001a; Ward et al., 1992; Mancuso, 1980, 1979; Wagoner, 1980) and of BCR members (Steenland and Ward, 1991; Infante et al., 1980) have shown a twofold increase in lung cancer mortality among persons who may have been exposed to high levels of beryllium. However, a sound causal association between human exposure and cancer has yet to be elucidated. The human studies are supported by animal studies. Inhalation exposure to beryllium has resulted in increases in lung cancer in rats and monkeys (Nickell-Brady et al., 1994; Reeves and Deitch, 1971; Wagner et al., 1969; Vorwald, 1968; Reeves et al., 1967). Combining findings from both the human and animal studies supports the notion of beryllium being classified as between "carcinogenic to humans" and "likely to be carcinogenic to humans."

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

A quantitative estimate of human risk as a result of low-level chronic beryllium oral exposure is based on animal experiments, since no adequate human oral exposure data are available; the gastrointestinal system appears to be the primary target of toxicity in dogs. Quantitative estimates of human risk as a result of low-level chronic beryllium inhalation exposure are based on human data. The lung appears to be the primary target of toxicity and carcinogenicity in human and animal inhalation studies.

The human chronic dose of ingested beryllium considered to be safe (RfD) is 2×10^{-3} mg/kg-day. This is 1/300 of the BMD_{10}, using small intestinal lesions in a long-term dog study as the indicator of adverse effects. The BMD_{10} dose is the 95% lower confidence limit of the dose which produces a 10% incidence of small intestinal lesions. It was calculated using data from the four beryllium dose groups and the control group.

The overall confidence in the RfD assessment is low to medium, derived from medium confidence in the principal study and low to medium confidence in the database. Beryllium was administered by a relevant route (oral) at multiple dose levels for a chronic duration, demonstrated effects at two dose levels, and relatively comprehensive histopathologic evaluations were conducted. However, there were small groups of animals (5/sex/dose), early mortality at the high dose level, no evidence of randomization or control

for potential litter effects, and no measure of immune response or function, the critical endpoint by the inhalation route. Confidence in the database is low to medium because there is only one chronic dog study showing adverse effect levels; other chronic studies in rodents demonstrated NOELs at the highest doses tested. Confidence in this assessment is improved over the earlier version on IRIS due to the inclusion of additional chronic studies in rats and dogs.

6.2.2. Noncancer/Inhalation

The human chronic air concentration (RfC) considered to be safe is 2×10^{-2} μg/m^3. This concentration is 1/10 of the adjusted adverse effect level for beryllium sensitization and CBD in workers (Kreiss et al., 1996).

The overall confidence in the RfC assessment is medium. The RfC is based on an occupational inhalation study performed with a moderate to large group size (136 subjects screened) in which sensitive measures were used to identify the affected population (Kreiss et al., 1996). No NOAEL was identified in this study, but a NOAEL slightly below the LOAEL(HEC) was suggested in a study using less sensitive methods of diagnosing CBD in a population exposed to high levels of beryllium in ambient air (Eisenbud et al., 1949). The poor quality of the exposure monitoring in the co-principal studies decreases the confidence in the principal studies to medium. The confidence in the database is also medium. A common limitation in the database is the lack of adequate exposure monitoring in the epidemiology studies and some uncertainty regarding the mechanism (and beryllium exposure levels) associated with the progression to CBD in beryllium sensitized individuals. Several human and animal studies are currently being conducted which may provide additional information on the mechanisms of action and data which would be useful for dose-response assessment. Although no inhalation developmental or multigenerational reproductive studies were available for beryllium, no reproductive effects were observed in an intratracheal reproduction study in animals at exposure levels above those causing CBD (Clary et al., 1975). In addition, the unusually low level at which CBD occurs, together with the low systemic distribution of inhaled beryllium, mean that any developmental effects would occur at levels much higher than those causing CBD. Reflecting the medium confidence in the principal studies and database, confidence in the RfC is medium.

6.2.3. Cancer—Oral and Inhalation

In 1987 as part of the first IRIS beryllium assessment, EPA derived a cancer IUR based on Wagoner et al. (1980), a cohort study of 3,055 employees who worked in a beryllium plant in Reading, Pennsylvania, between 1942 and 1967 (U.S. EPA, 1987). The 1987 IUR derivation used exposure values that NIOSH estimated from occupational monitoring data not specifically related to the Reading plant and its workers. When EPA updated the IRIS beryllium assessment in 1998, it considered whether Ward et al. (1992), a cohort study of 9,225 workers employed at seven beryllium plants, including the Reading plant, between 1940 and 1969, would support a reassessment of the IUR. Because Ward et al. (1992) lacked adequate exposure information, EPA decided to retain the 1987 IUR until additional analysis of the beryllium worker cohort became available.

Since the 1998 IRIS assessment, a new analysis of workers at the Reading plant has been published. Sanderson et al. (2001a) conducted a nested case-control study of beryllium workers (n = 852) employed in one of the beryllium processing plants included in the Ward et al. (1992) seven-plant cohort mortality study. EPA encountered several problems in attempting to derive an IUR for beryllium based on the data currently available from the Sanderson et al. (2001a) cohort. These problems included difficulty in choosing appropriate cut points for categorical analyses, difficulty in replicating conditional logistic regression parameter estimates for continuous exposure metrics (i.e., average and maximum exposures), and the fact that the beryllium-lung cancer relationship in this cohort may be driven by acute, high exposures to beryllium. In addition, the case-control study design did not allow estimation of SMRs in the form needed for application of methodology used to derive the current IUR on IRIS.

For these reasons, EPA has determined that the data in Sanderson et al. (2001a) are inadequate to support a reassessment of the IUR currently on IRIS, and the IUR of 2.4×10^{-3} per $\mu g/m^3$ is retained.

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APPENDIX A. SUMMARY OF AND RESPONSE TO EXTERNAL PEER REVIEW COMMENTS

The Toxicological Review for Beryllium and all individual beryllium assessments have undergone both internal peer review performed by scientists within EPA or other Federal agencies and a more formal external peer review performed by scientists chosen by EPA in accordance with U.S. EPA (1994a). Comments made by the internal peer reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. In addition, the external peer reviewers met to discuss the individual beryllium assessments; issues raised at this meeting are also discussed below. A summary of comments made by the external reviewers and EPA's response to these comments follows. The nine external peer reviewers (see Contributors and Reviewers) recommended that this document and the accompanying assessments be accepted with revisions.

The external peer reviewers offered editorial comments and many minor but valuable suggestions; these have been incorporated into the text to the extent feasible. Substantive scientific comments are addressed below. Several reviewers provided citations and/or copies of papers they would like to see added to the Toxicological Review; studies that supported the hazard identification and dose-response assessments have been incorporated into the document.

(1) Comments on General Questions

Question 1. Are there other studies that should be included as additional or supporting studies for the RfD? (This assumes the RfD is based on the Morgareidge et al. dog study [1976]).

Comments: Three reviewers thought the studies cited for the RfD and oral toxicity of beryllium were appropriate. One reviewer thought the document could also draw upon the larger metal literature for general information relating to the biokinetics/bioavailability of beryllium. No important papers were discovered in independent literature searches conducted by one reviewer.

Response to Comments: The revised *Toxicological Review* bases the RfD on the Morgareidge et al. (1976) chronic feeding study in dogs. Chronic studies in mice and rats serve as the supporting studies. When appropriate, reference is made to the larger body of information on metals.

Question 2. Are the uncertainty and modifying factors appropriate for the RfD? (Comments by reviewers refer to the RfD based on a benchmark dose approach for the gastrointestinal lesions in the Morgareidge et al. [1976] dog study.)

Comments: Five reviewers agreed that a range of 100 to 300 for the uncertainty factor seemed prudent or reasonable. While these reviewers agreed that 10-fold factors for intraspecies and interspecies extrapolation were needed, there was less agreement as to whether the database uncertainty factor (UF) should be 1 or 3. The reviewers agreed that a UF for database deficiencies should be reviewed by EPA. Other reviewers felt it was outside their expertise to comment.

Response to Comments: There is a 300-fold UF applied to the benchmark dose for small intestinal lesions in male and female dogs. This UF is composed of 10-fold each for intra- and interspecies extrapolation and a 3-fold factor for database deficiencies. Database gaps include lack of adequate studies for assessment reproductive and developmental toxicity (including multigenerational studies and studies on male reproductive toxicity, teratology and postnatal development) owing to beryllium's possible crossing of the placenta and greater absorption in young animals. In addition, oral studies examining immunologic endpoints, the most sensitive endpoint by the inhalation route, are lacking.

Question 3. Is the confidence statement appropriate for the RfD? (Comments by reviewers refer to the RfD based on a benchmark dose approach for the gastrointestinal lesions in the Morgareidge et al. [1976] dog study.)

Comments: Three reviewers agreed that confidence of low to medium for an RfD based on the Morgareidge et al. (1976) dog study seem reasonable.

Response to Comments: Confidence statements for the RfD reflect the above comments.

Question 4. Was the RfC based on the most appropriate critical effect and study (studies)?

Comments: The peer reviewers felt that beryllium sensitization and progression to CBD were the most appropriate critical effects and recommended using Kreiss et al. (1996) and Eisenbud et al. (1949) as co-principal studies. The reviewers felt that the beryllium air concentrations measured retrospectively over a 2-week period in Cullen et al. (1987) may not be representative of exposures over the previous 20 years, and suggested that this study should not be used as a principal study. One reviewer felt that the critical effect should be described

as Asubclinical beryllium lung disease (SBLD)@ rather than CBD. In addition, the document should include a discussion of SBLD, in particular that individuals with SBLD appear to be at risk for developing symptoms of CBD, the transition from SBLD to CBD does not require additional beryllium exposure, and not all individuals with SBLD will develop CBD.

Response to Comments: The critical effect for the RfC was changed from beryllium sensitization to beryllium sensitization and progression to CBD based on the LOAEL identified in the Kreiss et al. (1996) study and the NOAEL from the Eisenbud et al. (1949) study. The Cullen et al. (1987) study was used as a supporting study rather than a principal study. A discussion of the subclinical aspects of CBD and the potential progression to overt CBD (with or without additional beryllium exposure) are discussed in the document.

Question 5. Are there other data that should be considered in developing the uncertainty factors or modifying factors for the RfC?

Comments: Three reviewers recommended adding an additional uncertainty factor to account for uncertainties in the database, in particular the poor quality of exposure monitoring data. Two reviewers recommended using a 3 uncertainty factor and one reviewer recommended a total uncertainty factor of greater than 3 but less than 10. At the peer review panel meeting, one reviewer voted to increase the uncertainty factor to 10 (although he did not discuss the uncertainty factor in his written comments). Three reviewers were comfortable with a total uncertainty factor of 3. Two reviewers did not comment on the uncertainty factor.

Response to Comments: U.S. EPA agreed with the recommendation of the majority of the peer reviewers and increased the total uncertainty factor to 10; 3 for use of a LOAEL and 3 for database limitations.

Question 6. Does the Confidence Statement for the RfC present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects to humans, and the comprehensiveness of the database, and does the statement make sufficiently apparent all the underlying assumptions and limitations of the RfC assessment?

Comments: Three reviewers agreed with the confidence limits (medium for principal studies, high for database, and medium-to-high for the RfC). Three reviewers recommended changing the confidence in the database to medium and noting that there are several ongoing human and animal studies. Three reviewers did not comment on the confidence statement.

Response to Comments: In response to the peer review comments, the confidence in the database was changed to medium; the confidence in the RfC was changed to medium to reflect the medium confidence in both the principal studies and the database.

Question 7. For the cancer assessment, are the tumors biologically significant and relevant to human health?

Comments: The lung cancer SMRs in the human occupational studies and BCR entrant studies were felt to be relevant by three reviewers. One reviewer felt that the reticulum cell sarcomas seen in the rat study are of dubious significance to humans and that these tumors should be examined as a combined incidence across tissues rather than on a tissue-by-tissue basis; this point also was raised by one reviewer and in the peer review. One reviewer had reservations regarding the human studies, as expressed in comments on the specific question regarding changing the weight-of-evidence classification for carcinogenicity. The remaining reviewers did not comment.

Response to Comments: The reticulum cell sarcoma data have been further analyzed as suggested and found to be not significant statistically; this information has been added to the document. Further discussion regarding the strengths and limitations of the human data has been added to the document.

Question 8. Does the cancer weight-of-evidence statement present a clear rationale?

Comments: Two reviewers said yes. One reviewer commented that the IRIS summary of the animal data focused on the oral data, but that the inhalation animal data should also be included as they provide support for the carcinogenicity assessment. The other reviewers did not respond to this question.

Response to Comments: The inhalation carcinogenicity studies in animals have been incorporated into the summary sheet.

(2) Comments on Chemical-Specific Questions

Question 1. Is there a minimum database for derivation of an RfD? Do you think the present IRIS RfD (Schroeder and Mitchener, 1975a, b) meets minimum database requirements? Do you agree with the Anot verifiable@ status recommended in the draft document reviewed by the panel? Should the Morgareidge et al. (1975, 1977) rat or dog (1976) studies be used as the bases of the RfD? What is the most appropriate critical effect and study/studies?

Comments: The majority of the reviewers thought the database was sufficient to establish an RfD as per EPA guidelines. There are four chronic studies available in three species (dogs, rats, mice) by a relevant route of exposure. Although each study has difficulties and alone would be considered deficient by contemporary toxicology standards, collectively they establish a range of doses that is unlikely to evoke noncancer toxicity. Although the Morgareidge dog study (1977) was not published as a peer review paper and did not measure immune response or function (an endpoint important by the inhalation route), the reviewers felt it was a properly conducted, multiple-dose chronic oral study with complete histopathology that showed effect levels. The dog study is preferred over the rat studies for several reasons: the rat is typically a poorer model than the dog for the absorption kinetics of elements in humans, and the dog study used lower Be doses/kg body weight than the rat studies and showed a dose-response for an adverse effect. However, the reviewers suggested that the Agency: review the Morgareidge dog study to possibly establish a BMD-based RfD based on GI tract lesions, and have a veterinary pathologist review the pathology data (specifically gastrointestinal lesions). Since there is an effect in the dog study, the issue of a free-standing NOAEL is moot, although when faced with only NOAELs from several studies (or even a series of NOAELs from just one study), the Agency has determined RfDs. The Schroeder and Mitchener studies (1975a, b) used doses that were too low to establish an effect level. The Morgareidge rat study was considered inconclusive because not all tissues or animals were analyzed.

One reviewer further suggested that various dose-response curves (quantal linear, Weibull, gamma multi-hit, multistage) can be fitted to the gastrointestinal data and recommended EPA consider the quantal linear model for the gastrointestinal lesion data set (stomach, small GI or large GI).

One reviewer thought the Anot verifiable@ status is reasonable as the cited studies are weak and of questionable use in Aunequivocally establishing@ a NOAEL and LOAEL. The Morgareidge et al. (1976) dog study appears useful for RfD derivation, although the death of one animal in the 50 ppm group, while the other animals appeared to be minimally affected, is of some concern.

Response to Comments: The RfD in the *Toxicological Review* is derived from small intestinal lesions in male and female dogs in the Morgareidge et al. (1976) chronic feeding study.

A board-certified veterinary pathologist reviewed the Morgareidge et al. (1976) study report (but not the slides, which are unavailable) and concluded, A...given that the GI tract lesions occurred in both the small and large intestines, occurred in most of the high-dose animals, both male and female, and occurred in animals without roundworms, it appears that the GI lesions are related to beryllium treatment rather than some other cause.@ Further, the lesions that appeared to be related to treatment occurred predominantly in the small intestine and were erosive and inflammatory in nature. Treatment-related small intestinal lesions grouped together for determination of incidence data were: desquamation of the epithelium, edema, fibrin thrombi, acute inflammation, subacute/chronic inflammation, necrosis , thinning/atrophy of the epithelium and ulceration. The lesions in the one female in the 50 ppm treatment group that was killed earlier in the study (week 70) appeared to be of the same types as those found in dogs in the 500 ppm group, suggesting to the pathologist that the lesions in this dog were treatment-related.

A BMD approach using the exponential polynomial model was applied to these data to derive the 95% lower confidence limit on dose producing a 10% response (extra risk), or the BMD10, of 0.46 (MLE= 1.4; goodness-of-fit= 0.13) mg/kg-day using the mean doses for male and females and the combined incidence for small intestinal lesions . The BMD approach was chosen over the NOAEL/LOAEL approach because it utilizes all of the dose-response information and decreases reliance on the response of the one animal in the 50 ppm dose. The Weibull model, using the same inputs, determined a BMD10 of 0.46 (MLE=1.3; goodness of fit= 0.08) mg/kg/day. Other models were also run on these data with similar results (THRESH, BMD10=0.47, MLE=1.2, goodness-of-fit = 8.7E-18).

Question 2. Is it appropriate to base the RfD on soluble beryllium salts, even though beryllium oxide appears to be the most environmentally relevant form of beryllium? Since beryllium precipitates in the gut as the insoluble phosphate and is not well absorbed (<1%), is it appropriate to use various parenteral routes to mimic the oral route of ingestion? If so, are the studies of sufficient quality to be used in risk assessment?

Comments: The reviewers agreed that it seemed reasonable to base the RfD on animal studies using soluble beryllium salts, particularly in the absence of compelling information otherwise. By analogy to other chemical elements, the chemical form of the inorganic salt most likely will modify the bioavailability, but the different salts are less likely to have different mechanisms of action and toxicities. Statements of beryllium solubility and that beryllium precipitates in the gut as insoluble phosphate may not be an adequate generalization for predicting biokinetics in humans. However, whatever the chemical form it is accurate to say that beryllium is very poorly absorbed through the gastrointestinal tract. Parenteral administration can be useful in studying the disposition of beryllium in the body, but its usefulness in dose-response assessment is likely limited.

One reviewer noted that it is probably correct to say that oxidized forms of beryllium are the most environmentally relevant, but the document should present information to substantiate this. However, the reviewer was not aware of studies for beryllium in water that would indicate the most common form in water is the oxide.

Response to Comments: The statement about beryllium precipitating in the gut as the insoluble phosphate is not well documented and has been deleted as a generalization.

Chapter 3 states that beryllium from anthropogenic sources is generally emitted as the oxide and cites the U.S. EPA's Health Assessment Document for Beryllium (1987). A citation for ATSDR's Toxicological Profile for Beryllium (1993) has also been added..

Question 3. Should the RfDs/RfCs be presented as a point estimate or as a range?

Comments: RfD (Comments by reviewers refer to the RfD based on a benchmark dose approach for the gastrointestinal lesions in the Morgareidge et al. [1976] dog study.): While one reviewer thought a range was appropriate, while another preferred a point estimate with a statement that the RfD is imprecise to perhaps an order of magnitude (and that an RfD, based on animal data, is less precise than an RfD based on human data). Another reviewer did not have a strong opinion regarding a point estimate or range as being more appropriate, but thought the approach for beryllium should reflect that there is weak and /or uncertain information in the oral database because the studies all have questions and sources of uncertainty associated with them. Based on discussions at the meeting, one reviewer noted that a range for the RfD would arise from use of the range of uncertainty factors (100 to 300). (See also General Question 3).

RfC: Two reviewers preferred a point estimate and one of these reviewers preferred a statement that the RfC is imprecise to perhaps an order of magnitude. Two reviewers noted that there was some uncertainty associated with the exposure monitoring in the Kreiss et al. study (1996), and questioned whether it was appropriate to use a single exposure level; one reviewer thought it was appropriate to express the RfC as a range of the Eisenbud et al. (1949) and Kreiss et al. (1996) exposure levels.

Response to Comments: The RfD and RfC are presented as a point estimate with the statement that they are imprecise, perhaps to an order of magnitude. This approach is consistent with current EPA policy for the RfD/RfC.

Question 4. Does the following statement accurately reflect current knowledge Aalthough a number of chronic studies in laboratories have been conducted with beryllium compounds, few have been done using modern toxicological methods and none of those in animals that are appropriate models for CBD@?

Comments: Five of the reviewers noted that an animal model that mimics all aspects of human CBD has not been identified. However, the reviewers noted that there are several adequate animal models that mimic certain aspects of CBD. The other three reviewers did not comment on the statement.

Response to Comments: In response to the comments made by the peer reviewers, the statement regarding animal models was modified. The revised statement notes that there are several animal models that adequately mimic certain aspects of human CBD. However, a laboratory animal model that mimics all features of human CBD, in particular the progressive nature of the disease, has not been identified.

Question 5. Based on more recent epidemiological follow-up studies of beryllium processing workers (Ward et al., 1992) and entrants on the BCR (Steenland and Ward, 1991), is there sufficient support for changing the weight-of-evidence classification for carcinogenicity from a B2 to a B1 carcinogen and maintaining the present quantitative inhalation carcinogenic assessment?

Comments: Two reviewers agreed that the data supported a change to B1 (probable human carcinogen), but one reviewer expressed a concern that this characterization differs from that of IARC (1993), which considered the human data sufficient, whereas EPA concluded it was limited. One reviewer expressed reservations about changing the characterization to B1, based on the reviewer's intensive review of the earlier Wagoner et al. (1980) study and impressions

of the review panel's discussion of the Ward et al. (1992) study. One reviewer said B, but lacked experience to differentiate between B1 and B2. Reviewer #7 thought that beryllium should be classified in Group A. Three reviewers declined to comment. Reviewer #1 said the data do not clearly demonstrate carcinogenicity from inhalation exposure (hence not Group A) and that the choice of B1 rather than B2 may depend on analyses suggested during the peer review meeting (i.e., of the possible confounding by acid mists). One reviewer suggested that further analysis of the acid mist issue might bring EPA,s assessment into agreement with IARC,s, or could be used to explain the differences between the assessments.

In addition, one reviewer and the peer review panel commented that evidence for carcinogenicity by the oral route was inadequate. The peer review panel concluded that no oral risk estimate should be derived.

The majority of reviewers did not address the issue of whether to maintain the present quantitative inhalation carcinogenic assessment. One reviewer said that a quantitatively derived unit risk should not be calculated with the existing data because they are not sufficient. The reviewer recommended that quantitation be deferred until the NIOSH study with its better exposure estimates is available. One reviewer suggested that all the exposure information from the NIOSH criteria document be used with the Ward et al. (1992) data to calculate a unit risk. The peer review panel also suggested that the exposure range estimates by NIOSH that were used with the Wagoner et al. (1980) data for quantitative cancer assessment could be used with the Ward et al. (1992) data to estimate a unit risk. One reviewer pointed out that the dose conversion for the Wagoner et al. (1980) incorporates some unstated assumptions concerning the appropriate dose metric with regard to cumulative lifetime exposure that may not be appropriate and that the rationale for the dose conversion should be added.

Response to Comments: Further analysis of the issues regarding potential confounding by exposure to acid mists did not clearly implicate acid mists, nor did it completely resolve the issue (see response to comments on the subsequent question regarding sulfuric and hydrofluoric acid mists). Reflecting this and other limitations in the human data, the Agency concludes that the appropriate classification is B1 rather than A. The change from B2 to B1 is appropriate, because the increased incidences of lung cancers among workers with acute beryllium disease (and therefore assumed to be exposed to very high concentrations of beryllium), the higher incidences of lung cancers among workers first employed when exposure levels were very high, a consistent finding of lung cancer excesses in six of seven beryllium processing facilities, and the occurrence of the highest risks for lung cancer in plants where the risk for nonmalignant respiratory disease is the highest are indicative of a causal

relationship between beryllium exposure and an increased risk of lung cancer. A discussion regarding the difference between IARC's and EPA's conclusions regarding the adequacy of the human data has been added.

The Agency agrees that the data are inadequate for the assessment of carcinogenicity by the oral route and that no oral risk estimate should be derived. The oral toxicological review document and IRIS summary sheet have been revised slightly to clarify this conclusion.

Use of additional exposure information from the NIOSH criteria document with the data of Ward et al. (1992) is problematic because of the lack of specific job history data in the study that would link workers with the job- and work-area specific exposure data in the NIOSH document. Similarly, use of the NIOSH exposure range estimates with the Ward et al. (1992) data to estimate a new unit risk study does not overcome a major limitation common to both the Wagoner et al. (1980) and Ward et al. (1992) studies—the lack of personal monitoring data or detailed job history data from which exposure could be fully assessed. NIOSH, however, has recently completed a lung cancer study in a large cohort of beryllium processing workers. This study developed an exposure matrix and calculated airborne beryllium exposure concentrations, and may therefore provide the best basis for a quantitative cancer estimate. The study is currently in peer review, and will be evaluated as the basis for a new quantitative estimate when available. Until that time, the current inhalation estimate will be retained. The explanation of the dose conversion for the unit risk has been revised so that it is consistent with the original explanation (U.S. EPA, 1987).

Question 6. Given that IARC (1992) has designated sulfuric acid mist a human carcinogen, is there reason to think that the elevated SMRs for lung cancer at the Lorain and Reading beryllium processing facilities were due to sulfuric and hydrofluoric acid mist, respectively, rather than to beryllium?

Comments: Three reviewers agreed that there is reason to suspect exposure to these acid mists as a potential confounder. Two reviewers said that these mists were not the principal culprits. Reviewer #3 did not answer the question because of lack of information. Three reviewers did not address the question. The majority of reviewers, and the peer review panel as a whole, suggested that additional analysis of this issue be undertaken, including an investigation of what levels of sulfuric acid mist are associated with increased cancer risk, the SMRs, and the tumor types as compared with the beryllium data.

Response to Comments: Investigation of this issue revealed that exposure to sulfuric acid mists has not been strongly associated with lung cancer, but rather with laryngeal cancer (IARC, 1992; Sathiakumar et al., 1997). Limitations in the evidence for an association between exposure to sulfuric acid mist and lung cancer include poor or no quantitation of exposure, possible confounding by other occupational exposures and smoking, and low SMRs. The majority of lung cancer SMRs in the studies that reported a positive association between exposure to sulfuric acid mists and lung cancer were in the range of 1.18 to 1.39. The studies of lung cancer in workers exposed occupationally to beryllium and/or sulfuric acid or other acid mists do not, for the most part, categorize the type of cancer. Thus, the data are insufficient to determine whether different types of lung cancer may be associated with beryllium exposure versus sulfuric acid exposure. There are no carcinogenicity studies of sulfuric acid in animals. Information regarding the potential carcinogenicity of hydrofluoric acid was not available. IARC (1992) considered hydrofluoric acid to be a weak inorganic acid and did not assess it in the monograph on strong inorganic acids. A more detailed discussion of these findings has been added to the toxicological review document, and a brief discussion has been added to the IRIS summary. The results of this investigation do not change the Agency's conclusions regarding the cancer weight-of-evidence classification for beryllium.

Question 7. Based on the overall evidence from *in vivo* and *in vitro* studies, can one say unequivocally that beryllium is not a genotoxic carcinogen?

Comments: One reviewer said yes, while four other reviewers said no but indicated that beryllium is probably acting by a nongenotoxic mechanism, and four reviewers felt this question was outside their area of expertise.

Response to Comments: The document is consistent with the reviewers conclusions.

Question 8. Is it appropriate to base the cancer assessment on soluble beryllium salts, even though beryllium oxide appears to be the most environmentally relevant form of beryllium?

Comments: One reviewer noted that the qualitative and quantitative cancer assessments were based on human occupational studies and animal studies involving exposure to a variety of beryllium compounds and the metal, and that the evidence suggests that the various forms appear to be carcinogenic. One reviewer stated that lung cancer has been observed in animals dosed with soluble salts by the respiratory route. The other reviews did not specifically address this issue with regard to cancer assessment.

Response to Comments: The occupational studies involved exposure to various soluble and insoluble forms of beryllium, as did the positive animal studies (inhalation, intratracheal, intravenous and intramedullary). Thus, carcinogenicity does not appear to be a property exclusive to the soluble salts.

APPENDIX B. BENCHMARK DOSE FOR RfD

(1) Computational Models- Quantal Data for Small Intestine Lesions in Male and Female Dogs (Morgareidge et al., 1976)

The polynomial mean response regression model (THRESH, I.C.F. Kaiser, 1990), the exponential polynomial model, and the Weibull model were used to fit data by the maximum likelihood method. The following are the forms of the three equations used.

THRESH $P(d)= 1-\exp[-q1(d-d_0)^1-...-qk(d-d_0)^k]$

Exponential
Polynomial $P(d)= 1-\exp[-q1(d)^1-...-q(d)^k]$

Weibull $P(d)= 1-\exp[-\alpha (d)^\beta]$

where:

d= dose
d_0= threshold
P(d)= probability of a response (health effect) at dose d
q1...qk, d_0 α, β, k= estimated parameters

For data input to THRESH and polynomial exponential models, the degree of the polynomial k= 2, gave the best representation of the data, and the response type was extra [P(d)-P(0)]/1-P(0). For the THRESH model, a threshold was estimated.

(2) Data

Group	Dose (mg/kg-day)	# Response/# animals
1	0	0/10
2	0.026	0/10
3	0.135	0/10
4	1.2	1/10
5	14.8	9/10

Doses are average of male and female doses. Incidence is combined for males and females.

(3) Model Fit

Model fit was judged by the *p*-values associated with the χ^2 goodness-of-fit generated by the models.

(4) Results

Model	BMD_{10} (mg/kg/day)	MLE (mg/kg/day)	Estimated parameters	*p*-Value	χ^2 Goodness-of -fit	Degrees of freedom
Exponential polynomial	0.46	1.4	$q1 = 6.9 \times 10^{-2}$ $q2 = 5.9 \times 10^{-3}$	0.94	0.13	2
Thresh	0.47	1.2	$q1 = 9.4 \times 10^{-2}$ $q2 = 4.3 \times 10^{-3}$ $d_0 = 1.4 \times 10^{-1}$	1.0	8.7×10^{-18}	1
Weibull	0.46	1.3	$\alpha = 0$ $\beta = 7.3 \times 10^{-2}$	0.96	0.08	2

(5) Discussion

There was good correlation among the three models for the BMD_{10}. The BMD_{10} of 0.46 mg/kg-day (rounded to 0.5 mg/kg-day) is used for further quantitation of the RfD.

APPENDIX C. ANALYSIS OF DATA FROM SANDERSON ET AL. (2001a)

Although ultimately unsuccessful, several different approaches were attempted by EPA in order to derive an IUR for beryllium from Sanderson et al. (2001a). These approaches and the problems the Sanderson et al. (2001a) data presented in applying these approaches are discussed below.

As discussed in Section 5.3.1, an attempt to apply the methodology used to derive the IUR in the current IRIS assessment of beryllium (U.S. EPA, 1987a) to the Sanderson et al. (2001a) study was unsuccessful because of study design issues and uncertainties in the relationship between lung cancer and beryllium exposure levels.

A methodology originally described by van Wijngaarden and Hertz-Picciotto (2004), which estimates carcinogenic potency (i.e., IUR) through use of categorical exposure-response data from epidemiologic studies, was considered. This methodology, based on linear weighted least squares regression, requires that the inverse variance of the rate ratio estimates from each exposure category be employed as weights in deriving a potency estimate. The variance of each rate ratio estimate is derived from the confidence interval for the rate ratio in each exposure category. In Sanderson et al. (2001a), odds ratios (i.e., rate ratio estimates) were reported for each exposure quartile, but the corresponding confidence intervals were not provided. Therefore, EPA obtained the original data from the study authors in order to generate these CIs.

EPA was successful in obtaining the original data from NIOSH that were used in the analysis conducted by Sanderson et al. (2001a). However, the accompanying documentation regarding each variable in the data set and how it was created was limited, and EPA was unable to duplicate exposure quartiles for the three beryllium exposure metrics (i.e., average, cumulative, and maximum exposures) employed by Sanderson et al. (2001a) in their categorical analysis. Although the discrepancies in the exposure quartiles were small, they raised questions about the validity of any subsequent quantitative categorical analysis that EPA might perform. In addition, when trying to recreate the exposure quartiles of Sanderson et al. (2001a), a high frequency of cases was found to exist right at the cut point between the first and second exposure quartiles rendering any subsequent quantitative analysis based on a categorical approach potentially sensitive to whether these cases were classified in the first (i.e., referent) or second exposure quartile.

The methodology of van Wijngaarden and Hertz-Picciotto (2004) recommends converting cumulative exposure metrics, usually expressed in units of concentration × time (e.g., $\mu g/m^3$-day), to units of mass (e.g., mg) over a working lifetime. In attempting this conversion with the Sanderson et

al. (2001a) data, EPA found that a high proportion of cases in the cohort of Sanderson et al. (2001a) was exposed to very high levels of beryllium for short time periods (e.g., 7,000 µg/m^3 for 1 day). This observation is consistent with the fact noted by Sanderson et al. (2001a) that half of the cases in the cohort were employed at the beryllium processing facility for <5 months. These facts suggest that any potential association between beryllium exposure and lung cancer risk observed in this cohort may be driven by acute, high exposure to beryllium rather than by cumulative exposure.

In the Sanderson et al. (2001a) categorical analysis, an increase in the odds ratios with increasing beryllium exposure was not observed across the exposure quartiles. More specifically, the pattern that was generally seen was that the odds ratios estimated for the second and third exposure quartiles were significantly elevated over the odds ratios in the lowest (or referent) exposure quartile, but the odds ratios in the highest exposure quartiles decreased relative to those in the second and third exposure quartiles. This result indicates the absence of a linear dose-response relationship between beryllium exposure and lung cancer risk, especially at high levels of beryllium exposure, and underscores the importance of accurately classifying cases and controls across the exposure quartiles when conducting a categorical analysis. As did Sanderson et al. (2001a), EPA also attempted to analyze these data treating beryllium exposure as a continuous variable. This approach presented an additional set of problems, as further discussed below.

Sanderson et al. (2001a) conducted their continuous exposure-response analysis by using conditional logistic regression employing the log of three beryllium exposure metrics (i.e., cumulative, average, and maximum exposures). The premise behind log transforming exposure was to help "linearize" the dose-response curve observed in the categorical analysis and to obviate the need for and influence of the specific cut points used in the categorical analysis. Sanderson et al. (2001a) found some significant positive associations (i.e., parameter estimates significantly greater than 0) by using the log-transformed exposure variables, primarily when exposure metrics were lagged. Thus, the analysis of the log-transformed exposure variables confirmed the observations from the categorical analysis and suggested that the dose-response relationship between beryllium exposure and lung cancer risk may even be "supralinear" at low doses (i.e., exhibit a slope much steeper than 1). EPA attempted to replicate this continuous analysis of Sanderson et al. (2001a) but was only successful in duplicating the logistic regression parameter estimates when using the log of the cumulative exposure metric (i.e., µg/m^3-day).

Finally, when lung cancer risk is being evaluated in any epidemiologic study, smoking as a potential confounder always has to be considered because of the strong association between smoking and lung cancer. Only a relatively crude adjustment for smoking was employed in the categorical analysis of Sanderson et al. (2001a) (i.e., "professional status" was used as a stratification variable),

while, in their continuous analysis, no adjustment was made for smoking. In a letter to the editor regarding a recently published reanalysis of Sanderson et al. (2001a), Schubauer-Berigan et al. (2007) from NIOSH stated, "Birth year is a potentially important confounder of the beryllium-lung cancer association because background lung cancer risk due to smoking was expected to be lower for workers born before 1900 (25% of the case-control group) than for workers born later." Because of this potential confounding, Schubauer-Berigan et al. (2007) reference another continuous analysis of the Sanderson et al. (2001a) data conducted by NIOSH (but not yet published) adjusted by birth year. In this analysis, NIOSH found that cumulative beryllium exposure was not associated with lung cancer risk at any lag but the elevated odds ratios previously seen with average and maximum exposure metrics were still present. This newest continuous analysis, adjusted for smoking, provides additional evidence that, in the Sanderson et al. (2001a) cohort, short-duration, high-intensity beryllium exposure, rather than the cumulative exposure measure, appears to be associated with lung cancer risk.

In summary, EPA encountered several problems in attempting to derive an IUR for beryllium based on the data currently available from the Sanderson et al. (2001a) cohort. These problems included difficulty in choosing appropriate cut points for categorical analyses, difficulty in replicating conditional logistic regression parameter estimates for continuous exposure metrics (i.e., average and maximum exposures), and the fact that the beryllium-lung cancer relationship in this cohort may be driven by acute, high exposures to beryllium.

In conclusion, based on the uncertainties and problems raised above, EPA has determined that the data in Sanderson et al. (2001a) are inadequate to support a reassessment of the IUR currently on IRIS.