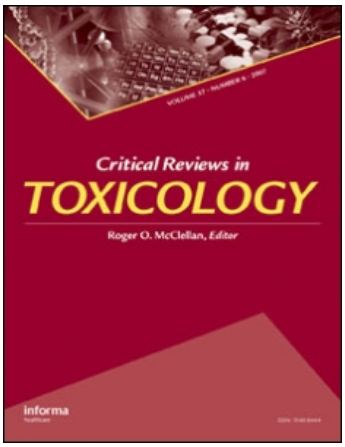

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D. M. Hollins [a]; M. A. McKinley [a]; C. Williams [b]; A. Wiman [b]; D. Fillos [a]; P. S. Chapman [a]; A. K. Madl [a]
[a] ChemRisk Inc., San Francisco, California [b] ChemRisk Inc., Tallahassee, Florida, USA

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REVIEW ARTICLE

Beryllium and lung cancer: A weight of evidence evaluation of the toxicological and epidemiological literature

D. M. Hollins[1], M. A. McKinley[1], C. Williams[2], A. Wiman[2], D. Fillos[1], P. S. Chapman[1], and A. K. Madl[1]

[1]*ChemRisk Inc., San Francisco, California, and* [2]*ChemRisk Inc., Tallahassee, Florida, USA*

Abstract

The potential carcinogenicity of beryllium has been a topic of study since the mid-1940s. Since then, numerous scientific and regulatory bodies have assigned beryllium to various categories with respect to its carcinogenicity. Past epidemiologic and animal studies, however, have been marked with notable methodological shortcomings. Because it has been about 16 yr since IARC evaluated beryllium and approximately 50 relevant papers on the topic have been published since that time, we conducted a weight-of-evidence analysis of the historical as well as recent animal and human literature. We also assessed whether recently published studies improved upon methodological shortcomings or shed light upon uncertainties in prior studies. Thirty-three animal studies, principally designed to evaluate the cancer hazard or related mechanisms, and seventeen epidemiologic studies were considered in this assessment. Based on this analysis, the evidence for carcinogenicity of beryllium is not as clear as suggested by previous evaluations, because of the inadequacy of the available smoking history information, the lack of well-characterized historical occupational exposures and shortcomings in the animal studies. We concluded that the increase in potential risk of lung cancer was observed among those exposed to very high levels of beryllium and that beryllium's carcinogenic potential in humans at exposure levels that exist in modern industrial settings should be considered either inadequate or marginally suggestive.

Keywords: *Beryllium; epidemiology; IARC; lung cancer; toxicology*

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Address for Correspondence: A. K. Madl, ChemRisk Inc., 25 Jessie Street, Suite 1800, San Francisco, CA 94105, USA. E_mail: amadl@chemrisk.com

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1. Introduction

Beryllium is a ubiquitous, naturally occurring element present in numerous environmental media, household products, foodstuffs, and drinking water (WHO 2001; ATSDR 2002). Because of its unique combination of thermal, chemical, nuclear, and mechanical properties, beryllium is an essential material in numerous industries, including aerospace, medical, electrical, energy, and automotive (IARC 1993; U.S. EPA 1998). Beryl ore is extracted, processed, and converted into different forms used in these industries: metal, ranging from 100% beryllium through low-beryllium-content alloys, and ceramics. Beryllium-containing particles are generated during processing and manufacturing activities such as melting and casting, machining, molding, grinding, and cutting of beryllium materials. Changes in production processes, ventilation controls, work practice controls, and production levels have resulted in generally decreasing airborne beryllium concentrations since levels in excess of 1000 $\mu g/m^3$ in some operations in the 1940s (Rossman et al. 1991; Kolanz 2001).

Inhalation exposure to beryllium can result in acute and chronic forms of disease (Eisenbud and Lisson 1983; ATSDR 2002). In the 1940s, cases of acute beryllium disease in the United States that occurred following exposure to beryllium oxide and salts prompted researchers to explore the inhalation hazards of beryllium in experimental animal studies (Eisenbud 1982; Borak 2006). In 1946, scientists reported that intravenous administration of certain beryllium silicate powders to rabbits resulted in the development of osteogenic sarcoma (bone cancer) (Gardener and Heslington 1946). Subsequently, numerous animal studies were conducted between the late 1940s and 1970s wherein soluble and insoluble forms of beryllium were administered by several different exposure routes. Unfortunately, much of this research involved questionable methodologies even by the research standards at that time (Rossman et al. 1991).

The first epidemiologic study to evaluate the possible relationship between beryllium exposure and lung cancer was published in 1969 (Mancuso and El-Attar 1969). Since that time, nearly 20 epidemiological studies have evaluated beryllium's potential carcinogenicity, and has been a topic of evaluation by various organizations, including the International Agency for Research on Cancer (IARC), the U.S. Environmental Protection Agency (U.S. EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), the American Conference of Governmental Industrial Hygienists (ACGIH), the World Health Organization (WHO), and the National Toxicology Program (NTP), as well as health agencies of several countries. As has been noted by others, the quality of the epidemiology literature varies considerably with respect to sample size, reference population, exposure metric, and adjustment for smoking (U.S. EPA 1987; MacMahon 1994; Deubner, Lockey et al. 2001; Levy et al. 2002; Deubner et al. 2007; Garabrant 2007; Levy et al. 2007). Moreover, as has been noted in past evaluations by IARC (1993) and U.S. EPA (1998), the animal literature is characterized by notable shortcomings with respect to assessing dose-response and temporality, demonstrating consistency, and showing statistical significance of tumor incidence in beryllium-treated animals.

Since the last evaluation conducted by the IARC Working Group in 1993, several mechanistic studies and five epidemiological assessments of historical beryllium cohorts and only one new in vivo animal study, have been conducted and published. In light of these recently

published studies, the newly published U.S. EPA criteria for the classification of carcinogens, and the IARC decision to reevaluate the listed chemicals under their carcinogenicity classification schemes, we have conducted a weight-of-evidence analysis of the potential carcinogenicity of beryllium. The purposes of this review are to revisit the historical animal and human literature, to assess whether recently published studies improved upon prior methodological shortcomings, and to shed light upon the potential causal relationship between beryllium and lung cancer. The following sections present and review (1) the experimental animal literature, (2) the epidemiology literature, and (3) the key findings and limitations of this literature. As a part of this analysis, standard criteria for causality (i.e., Hill's "criteria") were considered in evaluating the supporting evidence for classifying beryllium as a lung carcinogen.

2. Background

2.1 Agency evaluations of beryllium carcinogenicity

The potential for carcinogenicity of beryllium and beryllium compounds was considered by IARC Working Groups in 1972, 1980, 1987, and 1993, and additionally by the U.S. EPA in 1987, 1991, 1998 and 2008 (IARC 1980, 1987, 1993, 2006; U.S. EPA 1987, 1991, 1998, 2008). Most recently, this topic has been reviewed by the ACGIH, the German Research Foundation (DFG), and the United Kingdom's Health and Safety Executive (HSE) (Greim 2005; HSE 2006; ACGIH 2008). Each is worthy of discussion so that the evolution of views on beryllium's carcinogenicity can be understood.

In IARC's first evaluation in 1972, after assessing four epidemiology studies and one publication of case reports conducted between 1967 and 1970, the Working Group concluded that these studies have "not provided evidence" of the existence of a relationship between exposure to beryllium compounds and the occurrence of cancer in humans (IARC 1972, p. 25). IARC noted several limitations of these studies, including lack of information on workers' occupational histories and personal habits, and limited lengths of follow-up. IARC also noted that evidence for the carcinogenicity of beryllium, beryllium salts, or beryl existed for three animal species, including lung tumors in rats following inhalation exposure (Schepers et al. 1957; Reeves et al. 1967; Wagner et al. 1969), tumors in monkeys following intrabronchial implantation (Vorwald 1966) or inhalation exposure, and bone tumors in rabbits following intravenous administration of beryllium metal and beryllium phosphate (Gardener and Heslington 1946; Cloudman et al. 1949; Barnes 1950; Barnes and Denz 1950; Dutra and Largent 1950; Hoagland et al. 1950; Araki et al. 1954; Janes et al. 1954; Kelly et al. 1961; Schepers 1961; Komitowski 1968; IARC 1972).

Noting that new data had become available, a second IARC Working Group (1980) reevaluated beryllium. Four additional cohort studies were reviewed: Mancuso (1979 and 1980), Infante et al. (1980), and Wagoner et al. (1980). This Working Group noted that although the previously reviewed epidemiological studies did not provide evidence of a relationship between beryllium exposure and human cancer, the four subsequent studies of the same population all showed a small excess risk of lung cancer in cohort members occupationally exposed to beryllium. With regard to the animal studies, the IARC Working Group concluded that there was "sufficient evidence" that beryllium metal and several beryllium compounds were carcinogenic based on three experimental animal species, and that the epidemiological evidence suggesting that beryllium exposure may lead to increased risk for lung cancer in humans was "limited." After assessing both the animal and human data, the Working Group concluded that beryllium's carcinogenicity in humans should be considered "suspect" (IARC 1980, p. 190).

In 1987, IARC published a supplemental update of the beryllium monograph, although no new epidemiology studies were available at the time of this third review (IARC 1987). An additional toxicological study was added to the assessment, in which inhalation exposures to various beryllium compounds were found to produce lung tumors in rats (Litvinov et al. 1983). This Working Group again cited "limited" evidence of carcinogenicity in humans and "sufficient" evidence of carcinogenicity to animals, and therefore classified beryllium and beryllium-containing compounds as a Group 2A carcinogen (IARC 1987, p. 87).

In 1993, a fourth Working Group reviewed the previously evaluated epidemiology literature and added two recently published cohort studies and three case-control studies to their analysis (Hinds et al. 1985; Carpenter et al. 1988; Steenland and Ward 1991; Feingold et al. 1992; Ward et al. 1992). This Working Group stated that smoking had been adequately addressed and that the recent cohort studies were limited by the absence of discussion regarding exposures to other lung carcinogens; however, it was noted that evidence of exposures to other carcinogens was not apparent. In addition, the Working Group noted that lung tumors were produced in rats via inhalation and intratracheal instillation, and that osteosarcoma was observed in rabbits after injection with various beryllium compounds. The Working Group concluded that there was sufficient evidence in both humans and experimental animals of the carcinogenicity of beryllium and beryllium-containing compounds, and thus classified as a Group 1 carcinogen (IARC 1993).

In 1998, the U.S. EPA performed a toxicological review of beryllium and beryllium compounds with regards to lung cancer, reviewing most of the studies included in the previous four IARC assessments (U.S. EPA 1998). In the preceding 1987 and 1991 U.S. EPA assessments, epidemiology studies conducted prior to 1987 were considered to be insufficient in assessing the carcinogenic potential of beryllium in humans. Contrary to IARC's conclusion about the adequacy of controlling for confounders in the most recent epidemiology studies, the U.S. EPA stated that "the issues of incomplete smoking data and exposure to other potential lung carcinogens are not completely resolvable with the data currently available, and therefore concludes

that the evidence of carcinogenicity of beryllium and compounds is limited in humans" (IARC 1993; U.S. EPA 1998, p. 53). The U.S. EPA concluded that there was sufficient evidence for carcinogenicity in animals, based on lung tumors and osteosarcoma observed in rats and rabbits following various routes of exposure to beryllium. Thus, after placing weight on the animal studies, using the 1986 Guidelines for Carcinogen Risk Assessment, the U.S. EPA reclassified beryllium from a B2 carcinogen (inadequate human data) to a B1 (limited human data). Using the 1996 proposed Guidelines for Carcinogen Risk Assessment, however, the U.S. EPA concluded that inhaled beryllium would be characterized as "likely" carcinogenic in humans (U.S. EPA 1998, p. 53). In light of recent studies, the U.S. EPA has updated the cancer assessment in the draft Toxicological Review of Beryllium and Compounds[1] (U.S. EPA 2008). An external peer review of the draft 2008 updated cancer assessment has recently occurred, in which peer reviewers were charged to comment on the weight of evidence to classify beryllium "along a continuum between *likely to be carcinogenic to humans* and *carcinogenic to humans*" (U.S. EPA 2005; 2008, p. 80; Peer Review Summary Report 2008). In addition to other available studies, the primary studies that both IARC and U.S. EPA relied upon when classifying this metal are discussed in greater detail in later sections of this paper.

A number of additional agencies have evaluated beryllium's carcinogenic potential, both in the United States and internationally. There is general agreement among these organizations that beryllium should be classified as a potential or known human carcinogen. In the United States, the National Toxicology Program (NTP) has categorized beryllium as "known to be a human carcinogen" based on both animal and human data (NTP 2005). The National Institute for Occupational Safety and Health (NIOSH) considers beryllium compounds to be potential occupational carcinogens (NIOSH 2005). The European Commission (EC) currently has beryllium listed as a Category 2 carcinogen (ESIS 2008). Additionally, the German Research Foundation (DFG) has classified beryllium and its organic compounds as a Category 1 carcinogen, primarily based on the results of the Sanderson et al. (2001b) study. In the German evaluation, it was noted that "the data as a whole indicate that the carcinogenic effects described occurred mainly at high concentrations, which, as a rule, are no longer to be expected in workplaces today" (Greim 2005, p. 152).

The ACGIH similarly has listed beryllium compounds as an A1—Confirmed Human Carcinogen, primarily based on the observations in the Ward et al. (1992) study and to be consistent with the 1993 IARC evaluation (ACGIH 1996, 2001). In the 2001 documentation of the Threshold Limit Value (TLV), ACGIH cited that the carcinogenic potency of beryllium was "quite low" and in 2008 the "controversy over interpretation of the epidemiologic evaluations of the carcinogenicity of beryllium" was noted[2] (ACGIH 2001, p. 5; 2008, p. 11). The classification is based upon the evidence that "high exposure among workers before the 1950s was associated" with increased risk of lung cancer (ACGIH 2008, p. 11); the majority of these persons had suffered from acute beryllium disease (ABD), which has not been reported in nearly 30 yr in the United States (Eisenbud and Lisson 1983; ATSDR 2002).

Potency of beryllium was also recently evaluated by the Health and Safety Executive (HSE) of the United Kingdom. Based on both animal and human data, beryllium has been assigned a provisional potency estimate of Level A due to the "relatively short exposure periods [that] have been associated with increased lung cancer risk (mean tenure of occupation among lung cancer cases in epidemiological study was < 1 year; lung tumors in rats after 1 hour/day, 4 months exposure to beryllium oxide dust)" seen in some studies (HSE 2006, p. 6).

Further, the ATSDR published a review and assessment of the available animal and human literature in 2002 (ATSDR 2002). In a summary of animal studies whereby beryllium was administered via inhalation, the ATSDR cited that many of these studies "have been criticized because of poor documentation, being conducted at single dose levels, or failure to include controls" and concluded that "collectively, the animal data indicate that beryllium is carcinogenic in animals"(ATSDR 2002, p. 73). Regarding the human literature, the ATSDR believed that the recent studies by Ward et al. (1992) and Sanderson et al. (2001b) "provide strong data on the carcinogenic potential of beryllium in humans," but limitations with the existing cancer database, such as "poor exposure characterization, relatively low excess cancer risk and the lack of discussion of exposure to other lung carcinogens," are also noted (ATSDR 2002, p. 72).

In order to elucidate the relationship between beryllium and lung cancer further, the body of literature reviewed by the agencies just described as well as the recently published studies are critically evaluated in this analysis, with particular attention to whether the historical literature supports the current classifications of beryllium carcinogenicity.

3. Evaluation of the literature

3.1 Studies of cancer in animals

We identified 149 toxicology studies that examined the effects of chronic exposure to various beryllium compounds. Many of these studies were not of sufficient duration to explore a possible association between beryllium exposure and tumor formation (carcinogenicity), and/or examined endpoints other than carcinogenicity, and/or

[1] The U.S. EPA announced the release of the updated cancer assessment in the Toxicological Review of Beryllium and notice of the External Peer Review Panel Meeting in the May 12, 2008, *Federal Register* notice. The draft document that was available for external peer review and the Peer Review Summary Report were reviewed in this analysis (U.S. EPA 2008; Peer Review Summary Report 2008).

[2] The 2008 ACGIH draft documentation for beryllium was reviewed for this publication. The ACGIH has adopted the draft documentation and it will be available March 13, 2009. (http://www.acgih.org/resources/press/TLV2009list.htm).

were conducted in species other than mammals (62 studies); some were in vitro studies that were not relevant to the mechanism of beryllium carcinogenicity (39 studies), and some were articles with insufficient information, including non-English language abstracts/journal articles (15 studies). Because of these factors, such studies were not considered in our analysis. The remaining 33 studies that were principally designed for the observation of tumor formation from beryllium treatment or supporting mechanistic data were considered in this assessment. These studies are grouped and summarized according to the route of administration (Table 1). Other than the mechanistic studies described in this article, no new animal cancer bioassays have been reported since IARC's 1993 Working Group evaluation.

In our review of the animal data, we placed the greatest emphasis on chronic inhalation studies involving beryllium metal and beryllium metal alloys, since inhalation is the primary occupational route of exposure to beryllium, and these particular forms of beryllium (pure metal and the alloys) are the most commonly used in industrial applications. It is important to note, however, that most of the studies reviewed herein examined soluble beryllium compounds, which are rarely used in industrial settings and are not utilized in any consumer applications. Because early cases of acute beryllium disease in the United States occurred following exposure to soluble beryllium salts, a large number of animal studies were designed to include exposure to these compounds.

When considering the quality of the animal studies, we evaluated various aspects of the study design and methodology based on criteria described by the IARC Working Group (IARC 2006). As outlined in the IARC Preamble, qualitative aspects of the toxicological study were assessed, including experimental conditions, consistency of results, range of neoplastic responses, and any potential modifying factors. Further, the qualitative aspects of the studies were evaluated, including route and duration of exposure, dosing regimen, and the characteristics of the animals being studied. Regarding long-term animal experiments, the number of animals in each treatment group, the number and types of tumors observed, and length of survival were considered. Lastly, we considered whether the statistical methods and techniques employed to analyze the data relied upon generally accepted methods and were transparently presented. Other factors that we evaluated in both human and animal studies included kinetic factors, acute or chronic toxic effects, genetic effects, and structure–activity patterns (IARC 1993).

3.1.1 Inhalation studies

Eight chronic animal inhalation studies involving beryllium compounds were identified (Dutra et al. 1951; Schepers et al. 1957; Schepers 1961; Vorwald 1966; Reeves et al. 1967; Wagner et al. 1969; Litvinov et al. 1984). The most recent study (Finch et al. 1996) is a comprehensive review of beryllium metal inhalation studies conducted at the Inhalation Toxicology Research Institute (ITRI) and published (usually in the Institute's annual reports) between 1990 and 1994. The experimental design of many of the ITRI studies involved co-exposures of beryllium and 239PuO2 but the focus of the studies reviewed herein are those for which animals (rats and mice) were exposed to beryllium metal only. In addition, some of the ITRI studies were designed to examine DNA mutations and not tumor formation; this work is reviewed in this paper separately (Nickell-Brady et al. 1994; see below).

A number of ITRI studies exposed F344 or F344/N rats to a single dose of beryllium metal by inhalation at concentrations of up to 1,200 mg/m^3 to produce initial lung burdens ranging from 0.32 to 450 μg/g lung tissue beryllium. At the lowest initial lung burdens (0.32 to 17 μg), only chronic inflammation, fibrosis, and Type II cell hyperplasia were observed at 8 to 365 days post exposure. In the range of 17 to 50 μg beryllium initial lung burden, crude tumor incidence (the combined incidence of benign and malignant tumors) was generally around 50%–60% (2% incidence in controls). At higher initial lung burdens (50 to 450 μg beryllium), crude tumor incidence was in the range of 81% to 93%. The most common type of tumor observed was papillary adenocarcinoma; also observed were tubular carcinoma, squamous cell carcinoma, bronchioloalveolar carcinoma, adenosquamous carcinoma, and solid adenocarcinoma.

Similar studies (single exposures to beryllium metal at concentrations up to 1,000 mg/m^3) were conducted in mice. In lung tumor-resistant mice (C3H or C3H/HeJ strains) exposed to beryllium to produce an initial lung burden of 60−70 μg, no tumors were reported but some histopathological changes (enlarged bronchial lymph nodes, free particles, and particle-laden macrophages, hyperplasia of alveolar macrophages, neutrophilic alveolitis, and interstitial mononuclear infiltrates) were observed. At an initial lung burden of 60 μg in lung tumor-sensitive mice (A/J strain), the incidence of pulmonary adenomas was 30% and that for adenocarcinomas was 46%, although the corresponding tumor incidence in control animals was 26% and 37%, respectively. It was concluded in these studies that there was no difference in incidence, multiplicity, or latency of pulmonary neoplasia in beryllium exposed C3H/HeJ mice compared to controls, whereas the incidence and multiplicity of pulmonary neoplasia were slightly increased in beryllium exposed A/J mice. Although statistical analyses of tumor incidence rates in beryllium-exposed compared to controls were not reported, it was noted that the results of two strains of mice sharply contrasted those observed with F344/N rats.

It is noteworthy that the majority of beryllium inhalation studies were conducted 40 yr ago, long before rigorous standardized bioassay criteria were established. With the exception of the ITRI studies, none of the studies would meet current expectations regarding design, statistical power, histopathology, or quality control. These studies were conducted in a variety of species (rabbits, rats, monkeys, hamsters, and guinea pigs), using many beryllium forms. Tumor types reported were osteosarcoma and adenocarcinoma

Table 1. Summary of animal studies evaluated with respect to beryllium carcinogenicity.

Study	Be Form	Species	Tumor Type	Increased Tumor in Exposed[a]	Sufficient Controls	Sufficient Sample Size	Temporality	Dose-Response Tested	Dose-Response Found	High Mortality
Inhalation										
Dutra et al. 1951	Be oxide	Rabbits	Osteosarcoma	N	N	N	N	Y	N	Y
Schepers et al. 1957	Be sulfate tetrahydrate	Rats	Adenoma/adenocarcinoma	Y	Y	Y	N	N	N	N
Schepers 1961	Multiple	Multiple	Not specified	N	N	Not reported	N	N	N	N
Reeves et al. 1967	Be sulfate tetrahydrate	Rats	Adenocarcinoma	Y	Y	Y	N	N	N	N
Vorwald 1966	Be sulfate	Monkeys	Anaplastic pulmonary tumor	N	N	Y	N	N	N	N
Wagner et al. 1969	Bertrandite and beryl ores	Rats	Adenoma/adenocarcinoma	Y	Y	Y	Y	N	N	N
		Hamsters	Bronchiolar/alveolar cell tumor	Y	Y	Y	Y	N	N	N
		Monkeys	Aggregates of dust-laden macrophages	N[b]	Y	Y	Y	N	N	N
Litvinov et al. 1984	Be oxide	Rats	Malignant epithelial lung tumor	N	Y	Not reported	N	Y	Y	Y
Finch et al. 1996	Be metal	Rats	Adenoma/adenocarcinoma	Y	Y	Y	Y	Y	Y	N
	Be metal	C3H mice	None	N[b]	Y	Y	Y	Y	Y	N
	Be metal	A/J Mice	Adenoma/adenocarcinoma	Y[c]	Y	Y	Y	Y	Y	N
Intratracheal Instillation										
Schepers 1961	Multiple	Multiple	Not specified	N	N	Not reported	N	N	N	N
Groth et al. 1980	Be metal and alloys	Rats	Adenoma/adenocarcinoma	Y	Y	Y	Y	Y	Y	Y
Ishinishi et al. 1980	Be hydroxide	Rats	Squamous cell carcinoma/adenoma/ adenocarcinoma	N	Y	N	N	N	N	N
Litvinov et al. 1983	Be oxide	Rats	Malignant epithelial lung tumor	N	Y	Not reported	N	Y	Y	N
Intrabronchial Intubation/Bronchmural Injection										
Vorwald 1966[d]	Be oxide	Monkeys	Bronchogenic tumor	N	N	Y	N	Not reported	Not reported	N
Intraosseal Injection										
Yamaguchi 1963	Be oxide	Rabbits	Chondroma/osteoma/ chondrosarcoma/ osteochondrosarcoma	N	N	Y	N	N	N	N
Tapp 1966	Zinc Be silicate	Rabbits	Osteogenic sarcoma	N	N	N	N	N	N	Y
Tapp 1969	Multiple	Rabbits	Osteogenic sarcoma	N	N	N	N	N	N	N
Komitowski 1974	Be oxide	Rabbits	Osteogenic sarcoma w/lung metastases	N	N	Not reported	N	N	N	N
Matsuura 1974	Multiple	Rabbits	Osteosarcoma	N	N	N	N	N	N	N
Mazabraud 1975	Zinc Be silicate	Rabbits	Osteogenic sarcoma	N	N	Y	N	N	N	N
Hiruma 1991	Be oxide	Rabbits	Osteosarcoma	N	N	N	N	N	N	N
Intravenous Injection										
Gardner and Heslington 1946	Zinc Be silicate/Be oxide	Rabbits	Osteosarcoma	N	N	Not reported	N	N	N	N

Table 1. Continued on next page

Table 1. Continued

Study	Be Form	Species	Tumor Type	Increased Tumor in Exposed[a]	Sufficient Controls	Sufficient Sample Size	Temporality	Dose-Response Tested	Dose-Response Found	High Mortality
Cloudman et al. 1949	Zinc Be silicate/Be oxide	Rabbits/mice	Malignant bone tumor	N	Y	Not reported	N	N	N	N
Barnes 1950	Be metal and alloys	Rabbits	Bone sarcomata	N	N	Y	N	N	N	N
Barnes and Denz 1950	Zinc Be silicate/Be silicate	Rabbits	Osteosarcoma	N	Y	N	N	N	N	Y
Dutra and Largent 1950	Be oxide	Rabbits	Osteosarcoma	N	Y	Not reported	N	N	N	N
Hoagland et al. 1950	Zinc Be silicate/Be oxide	Rabbits	Osteogenic sarcoma	N	N	N	N	N	N	N
Araki et al. 1954	Be oxide	Rabbits	Osteosarcoma	N	N	N	N	N	N	N
Janes et al. 1954	Zinc Be silicate	Rabbits	Osteogenic sarcoma	N	N	N	N	N	N	N
Kelly et al. 1961	Zinc Be silicate	Rabbits	Osteogenic sarcoma	N	N	N	N	N	N	N
Schepers 1961	Multiple	Multiple	Not specified	N	N	Not reported	N	N	N	N
Komitowski 1968	Be oxide	Rabbits	Osteosarcoma	N	N	Not reported	N	N	N	N
Fodor 1977	Be oxide	Rabbits	Sarcoma	N	N	Y	N	N	N	Y
Intraperitoneal Injection										
Schepers 1961	Multiple	Guinea pigs/ rabbits	Not specified	N	N	Not reported	N	N	N	N
Nesnow et al. 1985	Be sulfate	Mice	Skin papilloma	N	N	Y	N	Y	Y	N
Ashby et al. 1990	Be sulfate tetrahydrate	Mice	Lung tumor	N	Y	N	N	Y	Y	N
Intracardial Injection										
Schepers 1961	Be phosphate	Guinea pigs	None	N	N	Not reported	N	N	N	N
Subcutaneous Injection										
Schepers 1961	Be phosphate	Pigs	None	N	N	Not reported	N	N	N	N
Oral										
Schroeder and Mitchener 1975	Be sulfate	Rats	Not specified	Y	Y	Y	N	N	N	N
Morgareidge et al. 1976	Be sulfate tetrahydrate	Beagle dogs	Ulcerative/inflammatory lesions of the gastrointestinal tract	N[e]	Y	Y	Y	Y	Y	N

[a] Y (for "yes") is indicated if an increase in tumor, if it occurred, in a study that was conducted using sufficient controls and sufficient sample size. A statistically significant increase was reported only in Groth et al. (1980). The significance of other increases in tumor incidence was either not reported or was not measured.
[b] No tumors were reported but accumulations of particle-laden macrophages were noted in the histopathology analysis.
[c] The incidence of pulmonary adenoma in treated animals was 30% and that for adenocarcinoma was 46%. The corresponding tumor incidence in control animals was 26% and 37%, respectively.
[d] No control animals were tested. Use of more than one test dose was not described.
[e] No tumors were reported but ulcerative/inflammatory lesions only observed in 500 ppm treatment group. This treatment group terminated at 33 weeks (in a 172-week study) because of overt toxicity. Authors indicated that 500 ppm exceeded the maximum tolerated dose.

in mostly rabbits and rats, respectively (Schepers et al. 1957; Vorwald 1966; Reeves et al. 1967; Wagner et al. 1969; Litvinov et al. 1984). However, not all studies reported tumor type. For example, Schepers (1961) simply noted a positive carcinogenic response in rats exposed to magnesium beryllium silicate, beryllium fluoride, beryllium phosphate, and beryllium sulfate, and in monkeys exposed to beryllium phosphate. Of these eight studies, four focused on insoluble beryllium forms (Dutra et al. 1951; Wagner et al. 1969; Litvinov et al. 1984; Finch et al. 1996), while the remaining studies evaluated beryllium salts or ores.

Collectively, the four studies that exposed animals to insoluble beryllium are remarkable in that the tumorigenic response varied widely between species despite the common route of exposure—i.e., osteosarcoma in rabbits with beryllium oxide (Dutra et al. 1951), epithelial lung tumors in rats with beryllium oxide (Litvinov et al. 1984), and a variety of benign and malignant changes in lung tissue of rats and hamsters with beryllium-containing ores (Wagner et al. 1969; Finch et al. 1996). The relevance of the beryllium forms (insoluble) and the lack of consistency with respect to tumor type and target organ significantly reduce the power of these studies to be reliable predictors of the tumorigenic response in humans. Similarly, studies in primates are generally considered to be excellent predictors of the possible carcinogenicity of substances in humans; however, the three inhalation studies describing beryllium exposure in monkeys do not report a consistent tumor response (unspecified tumors in Schepers 1961; anaplastic pulmonary tumors in Vorwald 1966; aggregates of dust-laden macrophages in Wagner et al. 1969). Despite the report of tumors in many of these inhalation studies, study design shortcomings and incomplete reporting limit interpretation of their results. For example, several studies failed to include a control group of animals (Dutra et al. 1951; Schepers 1961; Vorwald 1966). Incomplete reporting (e.g., exposure frequency, exposure duration, number of exposed animals, tumor type) was characteristic of several studies (Schepers 1961; Vorwald 1966; Litvinov et al. 1984). High mortality in treated animals was reported in two studies (Dutra et al. 1951; Litvinov et al. 1984). Excessive exposure (greater than 300 times the current occupational exposure limit) and use of rats with a high incidence of spontaneous lung disease were reported in one study (Wagner et al. 1969). Further, only three studies employed more than one exposure concentration (Dutra et al. 1951; Litvinov et al. 1984; Finch et al. 1996) yet no dose-response relationship was observed in one of these (Dutra et al. 1951). In summary, while the animal data suggest that chronic inhalation exposures to different forms of beryllium can lead to the appearance of tumors in different organs in more than one species, the utility of these data for assessing carcinogenic potential in animals, and further in humans, is limited because of shortcomings in study design, lack of detail in the description of methods and results, and utilization of beryllium forms not commonly encountered in the workplace (i.e., soluble beryllium).

3.1.2 Intratracheal instillation studies

Four studies reported on the effects of beryllium compounds administered as a single dose to animals using intratracheal instillation of low solubility beryllium forms (Schepers 1961; Groth et al. 1980; Ishinishi et al. 1980; Litvinov et al. 1983). While intratracheal instillation may have some advantages relative to inhalation studies (more accurate delivery of a known amount of material, less cost), the obvious disadvantage is the lack of relevancy to actual human exposures. Specifically, intratracheal instillation bypasses the normal scrubbing mechanisms of the nasal turbinate, and therefore particles administered in this manner are delivered to a region of the lungs that may not otherwise be accessible. In addition, bolus application results in unavoidably high local concentrations of the delivered material in the lower lobes of the lung (Vorwald 1966). Caution should therefore be taken when interpreting patterns of particle translocation and retention and pulmonary histopathology, since particle clumping, local inflammation, and irregular particle retention may be a reflection of the administration method, and not of the inherent nature of the administered materials (Madl and Pinkerton 2008).

Intratracheal instillation has produced different tumors in different studies. For example, adenomas and adenocarcinomas have been observed in rats exposed to beryllium metal, passivated beryllium, and beryllium oxide (Groth et al. 1980); a squamous-cell carcinoma, an adenocarcinoma, and adenomas were reported in rats exposed to beryllium hydroxide (Ishinishi et al. 1980), and malignant epithelial lung tumors were reported in rats exposed to high-temperature- and low-temperature-fired beryllium oxide (Litvinov et al. 1983). In Schepers (1961), none of the treatments (with beryllium metal and zinc magnesium beryllium silicate) resulted in tumors in guinea pigs or rats. Of the four intratracheal instillation studies, two studies exposed animals to multiple concentrations of beryllium to allow for assessing tumor incidence dose-response (dose response was reported in both studies) (Groth et al. 1980; Litvinov et al. 1983). In addition, the incidence of adenoma or adenocarcinoma was statistically significant only in Groth et al. (1980). However, high mortality was observed in the exposed groups: 40% at the low dose and 54% at the high dose of beryllium metal, and 43% at the low dose and 26% at the high dose of passivated beryllium metal.

Of note is the fact that some tumor types (i.e., adenoma, adenocarcinoma and malignant epithelial lung tumors—were reported in both the inhalation studies and these intratracheal instillation studies. In particular, studies by Litvinov et al. (1983; 1984) reported epithelial lung tumors in rats exposed to beryllium oxide by inhalation and via intratracheal instillation. Consistency in response between inhalation and intratracheal exposures gives added weight to relevance and importance of the tumor types reported in these studies.

General shortcomings of the majority of the intratracheal studies included incomplete reporting (e.g., number of

exposed animals), small group sizes, and use of only a single dose.

3.1.3 Intrabronchial intubation/bronchomural injection studies

A single study exposed 20 Rhesus monkeys to beryllium oxide and reported a bronchogenic tumor 4.5 yr after treatment; two additional tumors (described as highly anaplastic with adenomatous and epidermoid patterns) were detected in the following year (Vorwald 1966). Even though monkeys are often the most relevant model for assessing the carcinogenic potential of chemicals in humans, this study suffers from a number of methodological weaknesses: (1) No control animals were used, (2) little to no information was provided concerning the dose of beryllium oxide used or the number or frequency of dosing (if more than once), and (3) information concerning specific tumor types was not provided. Because it is not possible to determine whether more than one dose was employed in the study, the study results are not useful for assessing a dose response relationship. It should also be noted that the intention of the study was not to assess the carcinogenicity of beryllium, but to demonstrate that the monkey in general was a plausible model for assessing carcinogenicity. Animals were exposed not only to beryllium, but to cigarette smoke and other "exposures concurrently that were expected to be co-carcinogenic" (OSHA 1977). Thus, the results of this study are of limited usefulness to characterize the carcinogenic potential of beryllium alone.

3.1.4 Intraosseal injection studies

Seven studies examined the possible carcinogenicity of various beryllium compounds in animals treated via injection or implantation into bone (Yamaguchi 1963; Tapp 1966, 1969; Komitowski 1974; Matsuura 1974; Mazabraud 1975; Hiruma 1991). All of these studies were conducted in rabbits. Tumors observed included a chondroma, osteoma, chondrosarcoma, and osteochondrosarcoma following exposure to beryllium oxides and silicates (Yamaguchi 1963; Tapp 1966, 1969; Komitowski 1974; Matsuura 1974; Mazabraud 1975; Hiruma 1991).

Even though osteosarcomas were consistently observed in these studies, study limitations are common and, as discussed later, osteosarcoma is a tumor type that is only seen with rabbits after exposure to beryllium. In each study, either no control animals were used at all, or the controls consisted of injections of substances into other bones of treated animals (Yamaguchi 1963; Tapp 1966, 1969; Komitowski 1974; Matsuura 1974; Mazabraud 1975; Hiruma 1991). Incomplete reporting (e.g., the total number of injections per animal; dosing regimen) was a characteristic of some studies (Yamaguchi 1963; Komitowski 1974; Matsuura 1974). High (>30%) mortality was reported in treated animals in one study (Tapp 1966); small group sizes were used in four studies (Tapp 1966, 1969; Matsuura 1974; Hiruma 1991). Only single doses were tested in these seven intraosseal injection studies (Yamaguchi 1963; Tapp 1966, 1969; Komitowski 1974; Matsuura 1974; Mazabraud 1975; Hiruma 1991). These studies are of limited usefulness in the sense that one might expect tumors in bones injected even with inert substances, simply as a consequence of a reaction to substances that cause chronic irritation. Such studies may be useful for addressing kinetic or mechanistic questions even though they have little bearing on assessing carcinogenic potential as a result of workplace exposures. The consistency of the bone tumor response in rabbits is noteworthy nonetheless. The strength of this species-specific response is reinforced by the observation of osteosarcoma formation in rabbits exposed to beryllium via inhalation (Dutra et al. 1951).

3.1.5 Intravenous injection studies

Some of the earliest studies of beryllium in experimental animals were those involving intravenous injection (Gardener and Heslington 1946; Cloudman et al. 1949; Barnes 1950; Barnes and Denz 1950; Dutra and Largent 1950; Hoagland et al. 1950; Araki et al. 1954; Janes et al. 1954; Kelly et al. 1961; Schepers 1961; Komitowski 1968; Fodor 1977). Rabbits were the principal species used in these studies, but some studies were also conducted with mice, rats, and dogs; beryllium compounds used in these studies included zinc beryllium silicate, beryllium oxide, beryllium metal, beryllium silicate, beryllium phosphate, beryllium hydroxide, beryllium sulfate, and zinc magnesium beryllium silicate; and tumor types reported were principally osteosarcomas (Table 1).

Although tumors were observed in a number of intravenous injection studies, the lack of an appropriate control group was characteristic of a number of studies, such that it was not possible to determine if tumors reported in these studies were increased compared to controls. Similar to the studies in which rabbits were exposed to beryllium via inhalation and intraosseal implantation, intravenous injection studies consistently demonstrated osteosarcoma in rabbits, which may be a further indication of the potential susceptibility of the rabbit to beryllium as a tumor-causing agent.

Limitations of these studies included incomplete reporting of the number of treated animals and/or amount and number of treatments, no control animals, single-dose only testing, high mortality in treated animals, and small group sizes (Table 1).

3.1.6 Intraperitoneal, intracardial, subcutaneous, and oral studies

Several studies were conducted by administering beryllium compounds via intraperitoneal, intracardial, and subcutaneous injection or via drinking water or food (Schepers 1961; Schroeder and Mitchener 1975; Morgareidge et al. 1976; Nesnow et al. 1985; Ashby et al. 1990).[3] These studies were either negative for carcinogenicity (Schepers 1961;

[3] Although not published as a peer-reviewed paper, Morgareidge (1976) was reviewed by the U.S. EPA for the 2001 Toxicology Review of Beryllium in support of summary information on the Integrated Risk Information System (IRIS) (U.S. EPA 1998).

Morgareidge et al. 1976), or if tumors were observed, the incidence was not statistically significant (Schroeder and Mitchener 1975; Nesnow et al. 1985; Ashby et al. 1990). With the exception of Morgareidge et al. (1976), these studies generally suffer from many of the same design, execution, and reporting flaws of the studies summarized to this point, and thus interpretation of study results is likewise problematic.

3.1.7 Consideration of species-specific responses

Osteosarcoma in rabbits was observed when animals were exposed intravenously to different beryllium compounds. Curiously, Dutra et al. (1951) also reported one osteosarcoma in rabbits exposed to beryllium via inhalation. Intravenous injection exposes many of an animal's organs to beryllium, including bone; whether there is bone-specific uptake or sequestration mechanisms (similar to lead) particular to rabbits has not been demonstrated. However, in rats receiving a single intravenous injection of radiolabeled beryllium sulfate, circulating beryllium was found almost exclusively in the plasma (Vacher and Stoner 1968). Two fractions of plasma beryllium were identified. The smaller fraction was bound to plasma organic acids. In the larger fraction, the beryllium sulfate was converted to beryllium phosphate, which formed aggregates associated with plasma globulins. The facts that beryllium phosphate forms preferentially upon intravenous injection and that phosphate distributes preferentially to bone upon uptake may explain why intravenous injection of beryllium forms osteosarcoma in rabbits (HSDB 1997). This is also supported by the observation that beryllium oxide granules accumulate in skeletal marrow after intravenous administration of beryllium oxide to rabbits (Fodor 1977).

That inhalation exposure may serve as a route for osteosarcoma development in rabbits may be explained by Dutra et al. (1951), who noted that in experimental animals exposed to dusts of beryllium oxide, lung macrophages travel via pulmonary lymphatics and mediastinal lymph nodes to the blood and provide a ready route by which beryllium distributes systematically and ultimately to bone. Dutra et al. (1951) further stated that beryllium particles may pass directly through the walls of alveolar capillaries, from which it would be distributed in the blood and to other organs of the body. Beryllium is widely distributed to the organs of animals as a result of pulmonary absorption. Immediately after rats were exposed to radioactive beryllium salts (beryllium sulfate and beryllium chloride) for 3 h, the majority of total body radioactivity in tissues was in the lungs (60%) and skeleton (13.5%) (Zorn et al. 1977). Given these mechanistic characteristics of injected beryllium as described in rats, it might be expected that this species would develop osteosarcoma when treated in this manner. However, the available data do not support this hypothesis, suggesting that rabbits have some extraordinary susceptibility to the development of osteosarcoma.

The development of osteosarcoma has been noted by other researchers as a common occurrence in rabbits. Kondo et al. (2007) reported a case of a forelimb osteosarcoma in a male cross-breed rabbit, and several additional studies of spontaneous osteosarcoma formation in domestic and laboratory rabbits have been reported in the literature (Salm and Field 1965; Weisbroth and Hurvits 1969; Amand et al. 1973; Walberg 1981; Hoover et al. 1986; Renfrew and Rest 2001; Mazzullo et al. 2004). Such findings raise the possibility that osteosarcoma in beryllium-exposed rabbits (either via bone implantation or inhalation) may not be the direct result of the exposure but rather may be the manifestation of spontaneous bone tumor formation in this species. In support of this, Renfrew et al. (2001) published a report of a rabbit that presented with a mass from which tissue samples were taken at three different stages of the disease process and diagnosed sequentially as an osteogenic sarcoma, a fibroblastic tumor with rudimentary osteoid formation, and, finally, as a fibrosarcoma. The final diagnosis, however, was a fibroblastic osteosarcoma. The authors concluded that without the first two tissue samples, an incorrect diagnosis would have been reached, and incorrect diagnoses could indicate that osteosarcoma in rabbits may be underrepresented in the literature.

Several studies provided evidence of beryllium carcinogenicity in rats via inhalation and intratracheal instillation, but not by other routes of exposure. Principal tumor types were lung adenoma and adenocarcinoma. Groth et al. (1980) was the only study that reported statistically significant increases in tumors compared to controls. In all other inhalation/intratracheal studies in rats in which tumors were reported, the statistical significance of tumor incidence was either not assessed or not reported (Schepers et al. 1957; Schepers 1961; Reeves et al. 1967; Wagner et al. 1969; Ishinishi et al. 1980; Litvinov et al. 1983, 1984; Nickell-Brady et al. 1994), although in these studies, where discernable, tumors were generally present in greater numbers in treated animals compared to controls. Relative differences in cancer defense mechanisms, however, may make rats a poor model for human cancer concerns. Humans have evolved many types of defenses that collectively ensure that they are orders of magnitude more resistant to spontaneous tumors than rats (Ames and Gold 1990). In addition, rats appear to be susceptible to tumors when fibrosis develops; this progression from fibrosis to tumor formation is often associated with doses that overwhelm clearance mechanisms and are unrealistic compared to human exposure conditions (Hext 1994; Mauderly et al. 1994; Mauderly 1996; ILSI 2000).

A report by Sivulka (2006) of historical control data for respiratory lesions in rodents supports the view that rats may be poor human models for beryllium carcinogenicity assessment. The authors examined 24 chronic inhalation studies conducted by the National Toxicology Program between 1990 and 2000 and found that chronic active inflammation in historical control animals ranged from 0 to 54% in male rats and from 0 to 62% in female rats. If other effects, such as granulomatous inflammation, suppurative inflammation, hemorrhage, and fibrosis, are taken into

account, the rat's predisposition to spontaneous inflammatory effects becomes more pronounced. As a consequence, Sivulka speculates that these lesions could be exacerbated when rats are exposed to any particulate aerosol, regardless of chemical composition. In addition, Turnton and Hooson (1998) have shown that fibrotic reactions occur readily in rats but not in humans, for whom pulmonary fibrosis is mainly a sequel to extensive lung damage. Nikula et al. (1997) reported markedly less epithelial responses to inhaled particles in nonhuman primates than in rats.

3.1.8 Mechanistic studies of beryllium carcinogenicity

Numerous test methods have been developed over the years to examine mechanisms of chemical carcinogenesis. The Organization for Economic Cooperation and Development (OECD) lists more than 15 different standardized protocols for assessing the potential for genetic toxicity, including both in vitro and in vivo methods, in its Series on Testing and Assessment (OECD 1997). Many of these methods, such as bacterial mutation tests, mammalian chromosome aberration tests, sister chromatid exchange tests, and DNA damage, repair, and unscheduled synthesis tests, have been employed to elucidate possible mechanisms of beryllium carcinogenesis. In addition, standard protocols for identifying specific mutations in cancer genes (proto-oncogenes) and disregulation of tumor suppressor genes have existed for more than two decades, and have been used in mechanistic studies of beryllium. Table 2 provides a summary of available studies and reviews designed to examine possible mechanisms of beryllium carcinogenesis.

A number of reviews have discussed the mutagenicity and genotoxicity of various forms for beryllium (Leonard and Lauwerys 1987; Kuroda et al. 1991; Gordon and Bowser 2003). Short-term in vitro tests with soluble beryllium compounds (e.g., beryllium chloride and beryllium sulfate) have demonstrated infidelity of DNA synthesis and gene mutations in bacteria and mammalian cells (Leonard and Lauwerys 1987). Neither beryllium chloride nor beryllium nitrate was mutagenic in *Salmonella*, but they did produce

Table 2. Summary of beryllium genotoxicity and mutagenicity studies.

Study	Review?	Be Form	Test	Result[a]	Standard Test?[b]
In vitro studies					
Léonard and Lauwerys 1987	Y	Be chloride/Be sulfate	DNA synthesis infidelity	+/+	N
			DNA synthesis enzyme inhibition	+/+	N
			Forward gene mutations	+/+	Y
			Cell transformation	+/+	Y
			Nucleoprotein binding	+/+	N
			Nucleic acid binding	+/+	N
Kuroda et al. 1991	Y	Be chloride/ Be nitrate/	Salmonella mutagenicity assay	−/−/+	Y
		Be oxide	Sister chromatid exchange	+/+/−	Y
Joseph et al. 2001	N	Be sulfate	Cancer gene mutations/activation	+	N
			DNA synthesis, repair, and recombination genes	+ (inhibition)	N
Lavastre et al. 2002	N	Be sulfate	Superoxide (ROS) production	−	N
			Chemotaxis	−	N
			Apoptosis	−	N
Gordon and Bowser 2003	Y	Multiple	Non-mammalian mutagenicity tests	+ (weak)	Y
			Mammalian mutagenicity tests	+ (weak)	Y
			Mammalian chromosomal aberrations	+ and −	Y
			Mammalian transformation assays	+	Y
			Cancer gene activation	+ and −	N
Rana 2008	Y	Not specified	Mammalian cell transformation	+	Y
			Superoxide (ROS) production	+	N
			Apoptosis	+	N
In vivo studies					
Haley et al. 1990	N	Be metal	Histologic changes in bronchiolar lavage fluid	+	N
Nickel-Brady et al. 1994	N	Be metal	Cancer gene mutations/activation	−	N
Keshava et al. 2001	N	Be sulfate	Cancer gene mutations/activation	+	N
			Genomic instability	+	N
Gordon and Bowser 2003	Y		Cancer gene mutations/activation	+ and −	N

[a] Results key:
+ = positive result.
− = negative result.
+/+/etc. = results if more than one beryllium form was used in a study.
+ (weak) = weakly positive result.
+ (inhibition) = positive result for inhibition.
+ and − = some positive and some negative results, depending on beryllium form used (for review studies).
[b] Based on OEDC guidelines for Testing and Assessment.

a significant increase in sister chromatid exchange in V79 cells compared to controls (Kuroda et al. 1991).

A recent review by Gordon and Bowser (2003) noted that mutation and chromosomal aberration assays with beryllium have produced contradictory results—bacterial mutagenicity tests are generally negative, but tests with beryllium in mammalian cells have produced mutations, chromosomal aberrations, and cell transformation (Gordon and Bowser 2003). The analysis concluded that results in genotoxicity assays are highly dependent on the chemical form of beryllium used in the assay, and results from tests using forms encountered in the workplace (e.g., airborne particles of beryllium metal, alloys, or ceramics) are absent from the literature. There is a report in the review by Kuroda et al. (1991), however, of genotoxicity of beryllium oxide (an occupationally relevant form of beryllium) in bacteria, but not in mammalian cells (see Table 2).

Nickell-Brady et al. (1994) used in vivo methods to examine the molecular mechanisms underlying the carcinogenicity of beryllium metal. The study attempted to relate genetic changes in adenocarcinoma in rats to changes observed in human lung tumors, particularly human small-cell lung tumors. This analysis related genetic changes in rat adenocarcinoma to non-small cell lung cancer in humans is based on the assumption by the authors that non-small cell lung cancer is the type usually associated with epidemiology studies of beryllium-exposed workers. Non-small cell lung tumors, or, more correctly, non-smal cell lung carcinoma (NSCLC), has three main subtypes: squamous-cell lung carcinoma, large-cell lung carcinoma, and adenocarcinoma. Since adenocarcinoma was the histological cell type observed in rats in their study, the authors attempted to relate these findings in these tumors to the corresponding tumor type in humans (adenocarcinoma being a subtype of NSCLC).

More specifically, gene dysfunctions including mutations, amplifications, rearrangements, and overexpression of proto-oncogenes and their protein products such as K-*ras* and c-*raf* have been found in human respiratory-tract cancers (Pfeifer et al. 1989; Gazdar 1990). Inactivation of tumor suppressor genes, such as *p53,* has also been detected in human lung cancer (Chiba et al. 1990). With these specific genetic targets in mind, Nickell-Brady et al. (1994) examined potential changes involving these loci in F344/N rats receiving a single, nose-only exposure to beryllium metal (up to 980 mg/m^3). The authors reported in their study that (1) no K-*ras* codon 12, 13, or 61 mutations were detected by direct sequencing techniques in all of 24 beryllium-induced lung adenocarcinomas in the rats and K-*ras* codon 12 GGT-GTT transversions were detected in only two of 12 adenocarcinomas; (2) no mutant *p53* nuclear immunoreactivity was observed in any Be-induced tumor, nor were mutations detected in *p53* by direct sequencing; and (3) no rearrangement of the *c-raf-1* proto-oncogene was detected by Southern blot analysis. The study concluded that some gene mutations commonly associated with human non-small-cell lung cancer may be found as beryllium associated in rat adenocarcinoma, but these mutations are not obligatory nor necessarily causative in rats.

More recent studies have provided indirect evidence that cancer gene alterations and other genetic mechanisms may be responsible at least in part for cell transformation and tumor formation in animal models using beryllium. One study showed that cancer-related genes such as R-*ras*, c-*myc*, and c-*fos* were expressed at a rate two to five times greater in cells developed from tumors grown in nude mice injected subcutaneously with BALB/c-3T3 cells morphologically transformed with beryllium sulfate compared to control (i.e., non-transformed BALB/c-3T3) cells. It was additionally noted that expression of genes involved in DNA synthesis, repair, and recombination was downregulated in the tumor cells compared with the control cells (Joseph et al. 2001). It is noteworthy that this experimental method, although not necessarily a standard genotoxicity assay, has the advantages of an in vitro short-term test, with the additional benefit of studying the sequential processes that occur during tumor formation in the nude mouse assay. These results suggest that gene alterations, as well as changes in control of DNA replication and repair, may be acting in concert in beryllium carcinogenesis. Another recent study demonstrated amplification of the cancer genes K-*ras* and c-*jun*, but not the *p53* tumor suppressor gene, in BALB/c-3T3 (mammalian) cells treated with beryllium sulfate (Keshava et al. 2001). No change in protein products of these cancer genes was observed, suggesting a discrepancy between genetic changes and the products of these genes that may carry out cell transformation. In addition, genetic instability, defined as band shifts and changes in banding intensity using DNA fingerprinting techniques, was indicated in beryllium-treated BALB/c-3T3 cells, but the role of this instability in cell transformation is unclear.

These recent studies, although demonstrating the activation of specific oncogenes possibly related to beryllium, failed to directly evaluate genetic changes that might be found in tumors (pathologically similar to those found in humans) of animals treated in vivo with beryllium. This fact, combined with the general lack of evidence of remarkable alterations in human lung cancer genes from the earlier study of Nickell-Brady et al. (1994), raises questions about the role of proto-oncogenes and tumor suppressor genes in the development of beryllium induced tumors.

Other studies suggest epigenetic mechanisms for beryllium carcinogenicity. For example, it has been shown in rats that beryllium metal induces inflammatory lesions in the lung that progress to interstitial and intra-alveolar fibrosis and alveolar macrophage and epithelial hyperplasia (Haley et al. 1990). The deposition of beryllium particles in the lung thus elicits a chronic inflammatory response involving the recruitment of neutrophils and macrophages with cytokine release and oxygen radical production, leading to cellular damage and cell proliferation. Another recent review suggests that beryllium sulfate stimulates the formation of reactive oxygen species (ROS) and that ROS may be responsible for beryllium-induced macrophage apoptosis,

inflammation, and malignancy (Rana 2008). In contrast, Lavastre et al. (2002) suggested that beryllium does not cause ROS-mediated apoptosis in human neutrophils treated with beryllium sulfate. Recently, ROS was also not implicated in tumors from nude mice injected subcutaneously with BALB/c-3T3 cells morphologically transformed with beryllium sulfate (Joseph et al. 2001). ROS may or may not be dependent on the beryllium form used in the test system, in contrast to evidence in studies of beryllium mutagenicity and genotoxicity, where these attributes clearly are dependent on chemical form.

In summary, beryllium mutagenicity and genotoxicity appear to be highly dependent on the chemical form of beryllium (e.g., metal, alloy, or salt) and the test system used. Unfortunately, very few studies have examined occupationally relevant beryllium forms such as beryllium metal or beryllium alloys. Data from studies seeking to elucidate possible epigenetic mechanisms of carcinogenesis such as chronic inflammation and the possible involvement of ROS likewise are conflicting and incomplete. The available in vitro and in vivo studies do not allow for any clear determination of the mechanism of beryllium carcinogenicity, or at least fail to point to a predominant mechanism.

3.1.9 Animal studies and implications for the assessment of beryllium carcinogenicity

From the 33 chronic animal studies reviewed, beryllium exposure did not cause tumors via several routes of exposure in several animal models. No evidence for carcinogenicity was indicated in studies in which beryllium compounds were administered via the intraperitoneal, intracardial, subcutaneous, or oral (i.e., drinking water) routes, with the exception of the study by Ashby et al. (1990) in which the occurrence of tumors was not statistically significant. Animals for which there was no or extremely limited evidence of carcinogenicity included monkeys, pigs, dogs, hamsters, guinea pigs, and mice. Rabbits and rats exposed to beryllium compounds via inhalation, intratracheal instillation, implantation, and intravenous injection provided the only evidence of beryllium carcinogenicity in animals, although study design shortcomings limit the conclusions that can be drawn from these studies.

Of the 33 studies reviewed herein, only seven were conducted using reasonably sound methods, reflected by study designs that included sufficient controls and sample sizes, and complete reporting (Schepers et al. 1957; Reeves et al. 1967; Wagner et al. 1969; Schroeder and Mitchener 1975; Morgareidge et al. 1976; Groth et al. 1980; Finch et al. 1996). In five of these six studies, dose response was either not tested or not found (Schepers et al. 1957; Reeves et al. 1967; Wagner et al. 1969; Schroeder and Mitchener 1975; Morgareidge et al. 1976). In these five studies, the statistical significance of any increase in tumor incidence compared to controls was either absent or not reported. Only Groth et al. (1980) observed statistically significant increases in tumors (i.e., adenomas and adenocarcinomas) compared to controls. However, in this study, high mortality was reported in the treated animals, only female animals were used, and, in one subset of experiments, only one dose was used. Studies conducted more recently (early 1990s) by ITRI suggest carcinogenicity in rats and in lung tumor-sensitive mice (but not in other mice); however, rat lung tumors were produced in animals exposed to very high (up to 1,200 mg/m^3) beryllium concentrations, resulting in high (up to 450 μg/g lung) initial beryllium lung burdens. Thus, taken as a whole, these 33 studies offer little to resolve the issue of whether carcinogenic responses to beryllium in experimental animals support the designation of beryllium as carcinogenic to humans.

Although adenocarcinoma in rats and osterosarcoma in rabbits are common outcomes reported in many of the chronic animal studies and may suggest a carcinogenic potential of beryllium in animals, such a correlation is significantly undermined by questions regarding the propensity of certain animal models or dosing regimens to elicit tumorigenic responses irrespective of the material administered. Additionally, the vast majority of studies reviewed for beryllium, including the studies that reported a positive tumor response in rats, suffered from incomplete reporting and serious methodological flaws. In addition, several studies did not report the age, strain, sex, or numbers of animals used in the study. Very few of the studies employed an adequate control group or any control group at all. Many studies reported high mortality in exposed animals; in some instances mortality was greater than 50%. Many failed to use sufficient numbers of animals in either the exposed or control groups. Most were not designed to assess dose response, the temporal relationship between exposure and tumor development, or the statistical significance of the results. Shortcomings of some studies may be due to the era in which they were conducted (i.e., in the 1940s and 1950s before the general advent of Good Laboratory Practices). Whatever the reason for these reporting and design flaws, interpreting results in studies both positive and negative for carcinogenicity is difficult such that a clear determination concerning beryllium's carcinogenicity in animals may not be possible without additional animal studies utilizing current standardized chronic toxicity testing methods. Notwithstanding the methodological weaknesses observed with the historical animal literature, more recent investigations do not present any additional insights as to possible mechanisms (e.g., mutations, ROS) for tumor development in animals.

3.2 Studies of cancer in humans

In light of the shortcomings in the various animal studies, it was recognized that significant weight needs to be placed on the epidemiologic literature when deciding how to classify the carcinogenic hazard of beryllium for occupationally exposed cohorts. These studies can be divided into four groups: (1) studies of workers in the manufacturing setting (eight studies), (2) studies that evaluate registrants of the beryllium case registry (BCR) (two studies), (3) reanalyses of the data of workers in the manufacturing setting (three studies), and (4) case-control studies of cancer patients (four studies). We have given greater weight to studies in the first three groups

with particular attention given to the various methods used in the reanalyses to address confounding in earlier studies.

When considering the quality of the human cancer studies, we evaluated aspects of the study design and methodology based on criteria set forth by the IARC Working Group. As outlined in the IARC Preamble, the study population, disease, and exposure should be clearly outlined and exposures should be ascertained independent of disease status (IARC 2006). Second, the study should properly address potential confounding by variables and the most appropriate comparisons should be made between the control and exposed populations (e.g., using local disease rates rather than national rates, if applicable). Third, the study should clearly report the data on which the conclusions are derived, such as observed and expected cases in cohort studies. Further, issues dealing with temporality need to be addressed, such as latency or duration of employment. Fourth, statistical methods employed should be clearly defined and the means for adjustment of confounding should be addressed. Then, after the quality of the study has been evaluated, the criteria for causality are evaluated on the strength of association (i.e., size of relative risk), consistency of results across study designs using different epidemiological approaches, and mode of action on target organs. If inconsistent results are observed among studies, reasons for this are investigated and those studies deemed of higher quality are given more weight. In addition, the Working Group does note that a dose-response relationship is strong evidence for causality; however, the lack of dose response is not evidence against a causal relationship (IARC 1993).

3.2.1 Historical background on beryllium plants and cohort sources

Seven beryllium production facilities in the United States have been in operation since the mid-1930s. The Brush Beryllium Company employed workers at five plants in Ohio (Lorain, Luckey, Elmore, Perkins, and St. Clair), and the Kawecki Berylco Company employed workers at two plants in Pennsylvania (Reading and Hazelton). The Perkins and St. Clair plants are collectively referred to as "Cleveland" in some epidemiology studies. These plants have been involved in various phases of extraction of beryllium hydroxide from beryl ore, ore refining, beryllium processing, including the production of beryllium oxide, pure beryllium metal, and beryllium copper alloy, and machining of beryllium-containing materials. Each plant was involved in aspects of these major beryllium processes during various time periods, ranging from the mid 1930s until the late 1970s (Ward et al. 1992). While all seven plants were integral in major beryllium production, the two oldest plants, Lorain and Reading, have been studied extensively because of reported increased rates of lung cancer; 70% of the observed lung cancer cases and more than 50% of the person-years at risk (PYAR) were reported at these two plants.

The Lorain plant was in operation from 1936 until 1948 when it was destroyed by a fire. During this period of large-scale beryllium production, Lorain was the only commercial beryllium plant that used a sulfuric acid-dependent process. While Lorain was operational, the occupational health risks associated with airborne sulfuric acid were not fully appreciated and processes involving these chemicals were not ventilated properly (BISAC 1997). Sulfuric acid and other strong inorganic acids have been classified by IARC as carcinogenic to humans, although the U.S. EPA has determined that the evidence for carcinogenicity in humans is limited (IARC 1992; U.S. EPA 1998). Beryllium exposure standards were mandated by Atomic Energy Commission (AEC) contracts, and control measures were introduced throughout the United States in 1949, markedly reducing exposures in beryllium facilities and beryllium disease incidence in AEC-associated facilities (IARC 1980; Ward et al. 1992; Sanderson et al. 2001a). While acute cases of beryllium disease were generally associated with airborne concentrations of beryllium salts in excess of 100 $\mu g/m^3$, beryllium exposures were reported to frequently exceed 1000 $\mu g/m^3$ at Lorain, as compared to today's standard of 2 $\mu g/m^3$ (Laskin et al. 1950; Eisenbud 1982; Eisenbud and Lisson 1983; OSHA 1989; Rossman et al. 1991; Borak 2006).

The Reading plant opened in 1935 and primarily produced copper beryllium alloy products in addition to beryllium aluminum, nickel and chromium alloys (Sanderson et al. 2001a). During the beryl ore extraction process, this facility used a fluoride process, and many other facilities used either fluoride or sulfate processes. The implementation of local exhaust ventilation in 1959 and cessation of beryl ore extraction in 1964 were some of the many changes undertaken at the Reading facility (Ward et al. 1992; BISAC 1997). Information on the operations of the five other plants, however, is limited, and is not presented here.

Controversies regarding the interpretation of carcinogenesis bioassay studies in the late 1940s and the continued appearance of beryllium-related respiratory illnesses eventually led to numerous field studies of beryllium workers aimed at resolving the ambiguities regarding beryllium toxicity (Rossman et al. 1991). The newly formed AEC, the largest consumer of beryllium in the late 1940s, appointed a committee to review a proposed occupational exposure limit (OEL) for beryllium. As a corollary to recommending an OEL, the committee formed a beryllium case registry (BCR) at Columbia University to collect data on reported chronic beryllium disease cases. The registry was transferred to the Massachusetts General Hospital in 1952, to the U.S. Public Health Service in the late 1960s, and ultimately to NIOSH (Rossman et al. 1991). The main goals of the BCR were to collect medical information and exposure data from patients with beryllium disease, to study the course of the diseases, and to establish criteria for diagnosis of beryllium-related illness (Sprince 1986). Thus, the aggregation of clinical data from multiple collaborating physicians was an important step in moving from case summaries and anecdotal reports to a standardized system of reporting. Rossman et al. (1991) noted, however, that the registry has been heavily criticized as being deficient in acquiring sufficient data on individual exposures, on populations at risk, and in maintaining follow-up of questionable cases.

Numerous studies have been conducted on workers at one or more of the seven beryllium-producing plants in Ohio and Pennsylvania. These studies evaluate overlapping subsets of the same cohort of workers, and differ primarily in the methodologies used to evaluate latency, confounding by smoking, or exposure-response relationships. These studies are discussed next in chronological order and grouped by cohort definition: plant population-based versus BCR population-based study. Following the description of these studies, four case-control studies conducted in populations outside the manufacturing facility environment are discussed. If a risk estimate (e.g., odds ratio) or confidence limit was not presented in the original publication, the risk estimate and the 95% Fisher exact confidence interval were calculated when possible based on the available data using OpenEpi software (Dean et al. 2008). Further, p values are reported when the confidence limits were not presented and sufficient information to calculate them was not provided. The epidemiologic studies included in this analysis are summarized in Table 3.

3.2.2 Epidemiologic plant-based studies

Mancuso studies (1969, 1970, 1979, 1980): The earliest investigations of the health effects of beryllium on workers at the Lorain and Reading plants were performed by Mancuso and colleagues during the 1960s and 1970s. The initial study reported deaths in beryllium workers at two plants and compared the mortality experience with that of workers in the rubber industry (Mancuso and El-Attar 1969). No risk estimates were presented, nonexposed workers were included in the analysis, and information on exposure classification was lacking (ATSDR 2002). The authors concluded that the beryllium-exposed cohort showed a "slightly higher" rate of lung cancer for each of the two plants when compared to the cohort of rubber workers (Mancuso and El-Attar 1969, p. 428). The authors noted the uniqueness of the beryllium workers tenure of employment, and reported that almost 78% had worked in the plants of study for less than 2 yr, a significant limitation that is also apparent in subsequent studies.

In the 1970 update, virtually all of the lung cancers were observed in workers who had been employed less than 18 mo and in those with prior respiratory illness (Mancuso 1970). Therefore, the authors cited that "factors inherent among the short-term employees were contributing to the higher rates" of lung cancer for the workers of 5 quarters or less (Mancuso 1970, p. 270). The authors further concluded that the lack of a dose-response relationship "lessen[s] to some extent the association of beryllium as a carcinogenic agent" (Mancuso 1970, p. 270). ATSDR (2002) noted that based on the small number of deaths in each exposure category and the limitations of the 1969 analysis, conclusions could not be drawn from this study.

In a subsequent analysis in 1979, the cohort was limited to workers employed at Lorain and Reading (Mancuso 1979). Standardized mortality ratios (SMRs) were calculated using expected deaths based on 5-yr mortality rates for the general U.S. white male population. The authors reported that published vital statistics were not available for the period 1968–1975, and thus applied U.S. national mortality rates for 1965–1967 to expected lung cancer deaths for the period 1968–1975. The extrapolation of the mortality rates from 1965–1967 has been criticized as underestimating the expected lung cancer deaths by approximately 10%.[4] The SMR for lung cancer was 2.00 (SMR = 1.8, 95% CI 1.2–2.7, using Saracci's adjustment) among workers at the Lorain plant and was 1.37 (SMR = 1.25, 95% CI 0.9–1.7, using Saracci's adjustment) among workers at the Reading plant. The analysis by interval since first employment and for those workers who were followed for more than 15 yr since first employment resulted in an SMR for Lorain and Reading (using Saracci's adjustment) of 2.0 (95% CI 1.3–3.1) and 1.5 (95% CI 1.0–2.1), respectively. Of the 65 combined bronchogenic cancers observed, about 90% occurred in workers with duration of employment less than 5 yr.

A reanalysis of the 1979 study used as a comparison group viscose rayon industry workers employed at one company during comparable time periods (Mancuso 1980). Eighty lung cancer deaths were observed at Lorain and Reading. The SMR for lung cancer was 1.4 ($p < .01$). Using an expected number of deaths based on viscose rayon workers employed in a single department, a lung cancer SMR of 1.58 ($p < .01$) was also reported. The IARC Working Group noted that "using the latter reference cohort may introduce selection bias since the mortality experience of workers who never change departments while employed in the industry may differ from that of the total workforce of the industry, for non-occupational reasons" (IARC 1993, p. 67). An analysis for effect of latency was not conducted.

The Mancuso studies are significant for the unbiased identification of beryllium workers through the use of U.S. Social Security Administration records; however, limitations noted by IARC include the lack of analysis of risk by job title or exposure category (IARC 1993). Also, the U.S. EPA 1998 document criticized these studies for issues such as the inadequacy of age adjustment and lack of discussion about confounding by smoking. It was noted by U.S. EPA that NIOSH re-analyzed the Mancuso data and found "serious problems" with the analysis (U.S. EPA 1987, pp. 7–50). U.S. EPA concluded that "it would appear that the study is at best only suggestive of an increased risk of lung cancer due to exposure to beryllium" (U.S. EPA 1987, pp. 7–51). Although not necessarily informative for drawing conclusions regarding beryllium carcinogenicity, the study was informative in generating hypotheses and in identifying methodological limitations that could be addressed in future analyses.

[4] The U.S. EPA noted that this methodology likely resulted in an underestimation of expected lung cancer deaths, and a subsequent overestimation of the SMR (U.S. EPA 1998). A correction factor of 10% (referred to as the Saracci adjustment by IARC), has been utilized to correct for differences in the mortality experience of the U.S. population during these two time frames; however, it has also been reported that the expected lung cancer deaths were underestimated by as much as 11% (Saracci 1985; IARC 1993; MacMahon 1994).

Table 3. Summary of epidemiology studies evaluated with respect to beryllium carcinogenicity[a].

Reference	Study design	Population Source	Time period of exposure	Population size	Exposure metric	Latency (yr)	Follow-up	Ascertainment		Adjustment	
								Exposure[b]	Death[c]	Smoking	Other
Mancuso and El-Attar (1969)	Cohort	Lorain, Reading Plants	1937-1948	3,685	Year of first employment	–	1966	SSR	DC, BCR	N	N
Mancuso (1970)	Update of Mancuso and El-Attar (1969)	Lorain, Reading Plants	1937-1948	3,685	Duration of employment (divided into quarters)	–	1967	SSR	DC, BCR	N	N
Bayliss et al. (1971)[d]	Cohort	NA[d]	1942-1967	6,818	Duration of employment Onset of employment Type of industry employment	–	1967	Company personnel files	DC	N	N
Mancuso (1979)	Update of Mancuso (1969, 1970)	Lorain, Reading Plants	1942-1948	3,266	Duration of employment	15	1974 (Lorain) 1975 (Reading)	SSR	DC, BCR	N	N
Mancuso (1980)	Update of Mancuso (1969, 1970, 1979)	Lorain, Reading Plants	1937-1948	3,685	Duration of employment	–	1976	SSR	DC, BCR	N	N
Infante et al. (1980)	Cohort	NA[e]	NA[e]	421	Time since onset of exposure	15	1975	BCR	DC	N	N
Wagoner et al. (1980)	Cohort	Reading Plant	1942-1968	3,055	Duration of employment Onset of employment	15-24 >25	1976	Company personnel files	DC	N	N
Hinds et al. (1985)	Case-control	Lung cancer cases in Hawaii (1979-1982)	NA	261 cases 444 controls	None Low Medium High[f]	–	NA	Interview, JEM	Registry and Hospital Records	Y (pack-years)	Y (age, ethnicity)
Carpenter et al. (1988)	Case-control	CNS cancers in Oak Ridge, TN	1943-1977	89 cases 356 controls	Duration of employment Duration of exposure Ever exposed/un exposed Exposure rank[g]	10	NA	Database for DOE employees[h]	DC	N	Y (duration of employment, SES, radiation)
Steenland and Ward (1991)	Update of Infante (1980)	NA[i]	NA[i]	689	Duration of exposure Time since first exposure	20	1988	BCR	DC	N	N
Feingold et al. (1992)[j]	Case-control	Childhood cancers in Denver, CO (1976-1983)	One year prior to birth	252 cases 222 controls	Ever/Never Exposure Rank[k]	–	NA	Interview of parents, JEM	Registry and Hospital Records	Y[l]	Y (father's education)
Ward et al. (1992)	Cohort	Lorain, Reading, Luckey, Perkins, St. Clair, Elmore and Hazelton Plants	1940-1969	9,225	Duration of Employment	<10 10–15 15–20 20–25 25–30 >30	1988	Company personnel files, SSR	DC	Y (correction factor)	Y (geographic variation)
Sanderson et al. (2001)	Case-control	Reading Plant	1940-1969	142 cases 710 controls	Duration (days) Cumulative exposure Average exposure Maximum exposure	10 20	1992	Company personnel files	DC	Y (exclusion of professionals)	N

Table 3. Continued on next page

Table 3. Continued.

Reference	Study design	Population Source	Time period of exposure	Population size	Exposure metric	Latency (yr)	Follow-up	Ascertainment		Adjustment	
								Exposure[b]	Death[c]	Smoking	Other
Levy et al. (2002)	Reanalysis of Ward (1992)	Lorain, Reading, Luckey, Perkins, St. Clair, Elmore and Hazelton Plants	1940-1969	9,225	Duration of employment	-	1988	Company personnel files	DC	Y (correction factor)	Y (geographic variation)
Brown et al. (2004)	Case-control	Rocky Flats Plant, CO	1952–1989	180 cases 720 controls	Cumulative exposure[m]	5 10 15	1996	Company personnel files	DC	Y (pack-years)	NA
Levy et al. (2007)	Reanalysis of Sanderson (2001)	Reading Plant	1940-1969	142 cases 710 controls[n]	Duration (days) Cumulative exposure Average exposure Maximum exposure	10	1992	Company personnel files	DC	N	N
Schubauer-Berigan et al. (2007)	Reanalysis of Sanderson (2001)	Reading Plant	1940-1969	142 cases 710 controls	Cumulative exposure Average exposure	10 20	1992	Company personnel files	DC	N	Y (birth cohort)

[a] Lung cancer was the primary endpoint for all studies except for Carpenter et al. (1988) which evaluated central nervous system cancers.
[b] Ascertainment of occupation/exposure: SSR = Social Security records, BCR = Beryllium Case Registry, JEM = Job-exposure matrix.
[c] Ascertainment of death: DC = Death certificates, BCR = Beryllium Case Registry.
[d] Bayliss et al. (1971) was a presentation at the ACGIH conference in 1971. The 6,818 study subjects were former employees of Brush Wellman and Kawecki Berylco; plant locations not specified.
[e] The study subjects in Infante et al. (1980) were members of the beryllium case registry (BCR), enrolled while alive between 1952 and 1975. The time of employment in the beryllium industry and the locations were not presented.
[f] Exposure in Hinds et al. (1985) was determined based on reported occupation. Occupation was coded and linked to a job-exposure matrix identifying various agent exposures at 4 levels.
[g] In Carpenter et al. (1988), each job title/department was given a rank for potential exposure to each of the 26 chemicals evaluated from 0 (none likely) to 3 (high potential for exposure to the specified chemical).
[h] Department of Energy.
[i] Steenland and Ward (1991) was an extension of Infante et al. (1980). BCR members enrolled while alive between 1952 and 1980 were included. The time of employment in the beryllium industry and the locations are not presented.
[j] Feingold et al. (1992) was a study of parental occupation and childhood cancer.
[k] Estimated level of exposure in Feingold et al. (1992) was ranked as high, medium, low, or unknown.
[l] While maternal smoking was evaluated in Feingold et al. (1992), it was found not to be a confounder when adjusting for father's education.
[m] The cumulative exposures for beryllium, analyzed as continuous and design variables, were not presented in Brown et al. (2004).
[n] The Levy et al. (2007) study re-evaluated the initial cases and matched controls in Sanderson et al. (2001), in addition to performing an analysis for the 142 cases and 200 controls matched within 3 years of age.

Bayliss et al. (1971): One of the earliest investigators of the health effects of beryllium, David Bayliss, a NIOSH scientist, evaluated former male workers at the Brush Wellman and Kawecki Berylco facilities and found no statistical difference between observed and expected lung cancer cases based on duration of employment and date of first employment (Bayliss et al. 1971). Of the 36 observed male lung cancer deaths, two-thirds were among workers who started prior to 1947, although Bayliss did not specify in which plants these workers may have worked. It was noted that "office workers tended to show slightly more lung cancer than did production workers" and that production workers exhibited higher mortality rates from respiratory disease than office workers; however, the analysis was not controlled for smoking and an analysis by latency was not performed (Bayliss et al. 1971, p. 97). No statistically significant excess risks were reported when considering length of employment or date of first employment. In fact, there were no significant excess risks of any cancers in this cohort, which Bayliss indicated might be attributable to the healthy worker effect.

Wagoner et al. (1980): In 1980, Wagoner et al. published an update of the Bayliss study evaluating workers from the Reading plant (Wagoner et al. 1980). Statistically significant increases in risk were reported for those with latency of greater than 25 yr since onset of employment and for the short-term workers. Analysis by latency showed that workers with 25 yr or more since start of employment had a lung cancer SMR of 1.85 (95% CI 1.13–2.86). The number of cases was small, and only 3 of the 20 lung cancers observed worked at the plant for greater than 5 yr. The reported lung cancer SMR for those who worked less than 5 yr was 1.4 (95% CI 1.00–1.91). Expected lung cancer deaths for the 1968 to 1975 period were calculated using 1965–1967 national lung cancer death rates for white males. IARC noted that because of this extrapolation, the Saracci (1985) adjustment is appropriate to apply. This resulted in an overall SMR of 1.25 (95% CI 0.9–1.7).

This publication was criticized both by the senior author (David Bayliss) and by the U.S. EPA Health Assessment Document in 1987 for underestimating the expected lung cancer rates (which, when corrected using Saracci's adjustment, eliminates the statistically significant excess risk reported), lack of adjustment for confounding by smoking, lack of adjustment for latency, and the inclusion of one case who was interviewed but never worked at the plant (Bayliss 1980; U.S. EPA 1987; ATSDR 2002). The U.S. EPA presented a re-analysis of this data controlling for these confounders, and concluded that there was no significant difference between observed and expected cases (U.S. EPA 1987). They cited that the risk of lung cancer in the beryllium workers was "exaggerate[d]" and that the authors "underemphasized" the shortcomings of the study (U.S. EPA 1987, p. 7–39).

Ward et al., 1992 and the Reanalysis (Levy et al., 2002): A retrospective cohort mortality study was performed by researchers at NIOSH, evaluating workers employed at seven beryllium processing facilities (Ward et al. 1992). This analysis greatly improved upon methodologies previously used because of the longer follow-up period, indirect control for smoking, and latency analysis. The lung cancer SMR for the total cohort, not adjusted for smoking, was 1.26 (95% CI 1.12–1.42) (Figure 1). Adjusting for smoking,[5] the SMR of lung cancer decreased to 1.12 (95% CI 0.99–1.25). Unadjusted SMRs were also reported for the individual plants; the only significantly elevated SMRs were for Lorain (SMR = 1.69, 95% CI 1.28–2.19) and Reading (SMR = 1.24, 95% CI 1.03–1.48) (Figure 1). After adjusting for smoking, the SMR decreased for both the Lorain plant (SMR = 1.49, 95% CI 1.13–1.93) and Reading (SMR = 1.09, 95% CI 0.91–1.30).[6] The authors conclude that "neither smoking nor geographic location fully explains the increased lung cancer risk," a finding contrary to the 2002 reanalysis (Ward et al. 1992). According to data presented in Ward et al. (1992), there is evidence of increased

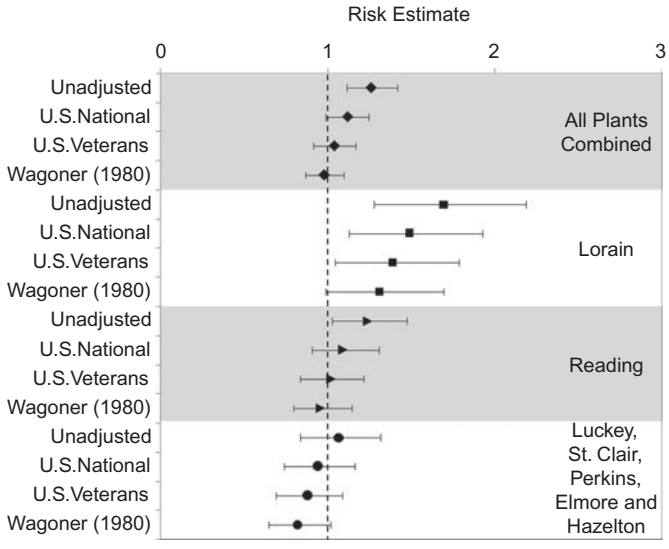

Figure 1. Unadjusted and smoking-adjusted risk estimates for lung cancer at U.S. beryllium production plants. Unadjusted and U.S. smoking-adjusted estimates presented in Ward et al. (1992). Risk estimates adjusted using U.S. Veterans smoking rates and the Wagoner smoking correction factor presented in Levy et al. (2002).

[5] The effect of smoking was analyzed using a procedure described by Axelson and Steenland (1988) based on information from a 1968 U.S. Public Health Survey of the beryllium workers. A smoking correction factor of 1.13 was calculated to account for differences in smoking rates between the cohort and the U.S. population. The SMR is divided by the smoking correction factor to take into account the smoking habits of the cohort.

[6] Ward noted that smoking data were collected during a 1968 Public Health Service medical survey of four beryllium facilities (Reading, Hazelton, Elmore, and St. Clair) and that 94% of the lung cancer cases occurred among workers hired in the 1940s and 1950s. Thus, the validity of the adjustment for smoking depends on the assumption that the difference in smoking habits between the cohort and U.S. population was the same in the 1940s and 1950s as in the late 1960s, and that the distribution of smoking at the four facilities adequately represented the distribution of smoking at all plants. At the four facilities, 1466 subjects contributed data on smoking; this comprised 16% of the total cohort (9225). Levy et al. (2002) noted that these data did not adequately represent smoking habits of the entire cohort and most importantly did not characterize smoking habits at the Lorain plant, the only plant that showed significantly elevated lung cancer rates in subsequent analyses after adjustment for this confounder.

smoking-related illnesses in this cohort (SMR = 1.21, 95% CI 1.06–1.38 for nonmalignant diseases of the respiratory system for the total cohort). To address geographic variation in lung cancer rates, SMRs derived from county lung cancer rates were compared to those derived from U.S. rates. Ward concluded that lung cancer SMRs based on local county mortality rates differed only slightly from those based on U.S. rates.

In an analysis of employment duration, there was no observed trend of increased risk corresponding to duration of employment. The only reported significant increased risk was among workers employed for less than one year, at a latency of greater than 25 yr from employment for the total cohort. The latency analysis showed significantly elevated SMRs for the Lorain plant at 15–30 yr (2.09, $p < .01$) and > 30 yr latency (1.66, $p < .05$), and for Reading at > 30 yr latency (1.40, $p < .05$) (Figure 2). For the total cohort, significant increased risk with increasing latency was observed for > 30 yr (SMR = 1.46, 95% CI 1.22–1.72).[7] Decade of hire, specifically before 1950, was one of the strongest correlates of lung cancer mortality risk in the total cohort (SMR = 1.42, 95% CI 1.22–1.65), and was noted to be heavily influenced by workers at the Lorain plant. For workers hired during the 1960s, the SMR for lung cancer was significantly less than one (SMR = 0.62, 95% CI 0.37–0.98).

In a reanalysis of the data presented in Ward et al. (1992), Levy et al. (2002) focused primarily on the issues of smoking and geographic variation in lung cancer rates in an attempt to further clarify their potential confounding effects. In an effort to provide a comparable referent population, Ward compared county-specific SMRs of lung cancer to SMRs based on the U.S. population and reported that the differences were negligible. However, using county-specific rates that incorporate large rural areas where lung cancer rates are generally lower may potentially underestimate the expected deaths, resulting in higher SMRs, and the majority of workers at Lorain and Reading were residents of the city where the plant was located (i.e., 89% and 68%, respectively) (Levy et al. 2002). To address this, the residency rates at the Lorain and Reading plants were applied proportionally to the city and county lung cancer death rates to create a combined city/county rate.[8] Based on U.S. rates, the lung cancer SMR presented in Ward et al. (1992) for Lorain was 1.69 (95% CI 1.28–2.19) and decreased to 1.14 (95% CI 0.86–1.48) using combined city/county rates. For Reading, the SMR for lung cancer based on U.S. rates was 1.24 (95% CI 1.03–1.48) and decreased to 1.07 (95% CI 0.89–1.28) using combined city/county rates. Using these mortality rates, the rates of lung cancer in these two plants were not higher than the rates of lung cancer in the areas in which the workers lived, potentially reflecting higher air pollution levels in these two industrialized areas (Levy et al. 2002).

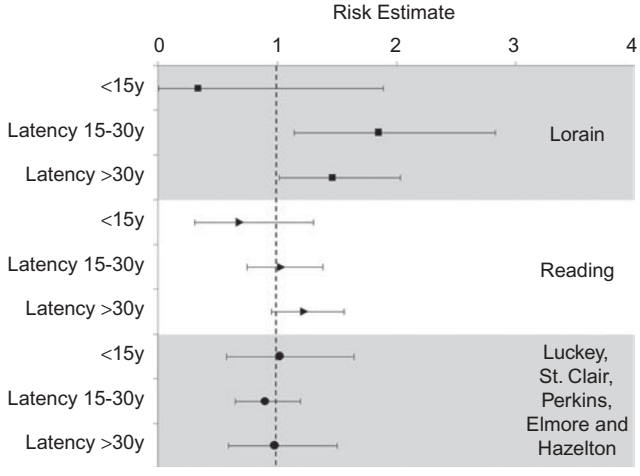

Figure 2. Smoking adjusted latency risk estimates by plant using Ward et al. (1992) data. Smoking adjusted estimates obtained by using correction factor of 1.13. SMRs and 95% confidence intervals (Fisher exact) were calculated using OpenEpi.

In addition to adjusting for geographic variation in lung cancer mortality, the authors compare alternatives to the smoking correction factor used in the Ward analysis. As presented in Ward et al. (1992), the available smoking data indicate that smoking rates among the workers were higher than in the U.S. population, and as a result, the use of the correction factor based on the 1968 survey of beryllium workers and U.S. smoking data taken in 1965 and 1970 potentially underestimates confounding by smoking. In Levy et al. (2002), the authors evaluated three alternative correction factors (applied to the U.S. unadjusted rates): (1) 1.13, the 1968 estimate used in Ward et al. (1992); (2) 1.29, calculated from Mancuso (1980) and referred to as Wagoner-ACS; and (3) 1.21, derived from a major study of smoking and lung cancer in U.S. veterans (Kahn 1966; Levy et al. 2002). The adjusted-SMRs using the U.S. veterans correction factor and Wagoner factor are presented in comparison with the unadjusted and U.S. population-adjusted SMRs that were presented in Ward, for the total cohort, Lorain, Reading, and remaining plants in Figure 1. Compared to the unadjusted risk estimate, these correction factors reduce the SMRs to approximately one for Reading ($SMR_{Ward} = 1.10$, 95% CI 0.91–1.31; $SMR_{Wagoner} = 0.96$, 95% CI 0.80–1.15; $SMR_{U.S.\ Veterans} = 1.02$, 95% CI 0.84–1.22). For Lorain, the SMRs also decreased using the various smoking correction factors ($SMR_{Ward} = 1.49$, 95% CI 1.13–1.93; $SMR_{Wagoner} = 1.31$, 95% CI 0.99–1.70; $SMR_{U.S.\ Veterans} = 1.39$, 95% CI 1.05–1.79). In addition, Levy et al. (2002) calculated attributable risk indicating that 85% (237 of 280) of lung cancer deaths among the cohort may be attributable to smoking.

Overall, it is important to note that the methods used to adjust for smoking in both the Ward and Levy studies are indirect methods, and, as such, residual confounding is likely to exist. As the authors point out, "workers hired prior to 1960 (the group in which 93% of all lung cancer cases

[7] Statistically significant increased risk was reported for latency of 15–30 yr, but when recalculating the risk estimate to obtain confidence intervals, we found that the confidence interval for the reported risk included 1 (reported SMR 1.20, calculated 95% CI 0.99–1.44, $p = .057$).

[8] Based on personal communication with Dr. Neil Roth.

occurred) were not well represented in the survey" (Levy et al. 2002, p. 1012). Levy et al. (2002) also noted that there were potential errors in the calculation of the smoking correction factor used in Ward et al. (1992) due to inconsistencies in the collection of data of the surveys upon which this factor is based. Unfortunately, the original data are unavailable to investigate this further.

Sanderson et al., 2001 and the Reanalyses (Levy et al. 2007 and Schubauer-Berigan et al. 2008): Sanderson et al. (2001b) provided an additional 4 yr of follow-up of the Reading cohort studied in Ward et al. (1992). This study made available, for the first time, actual exposure estimates based on air sampling for specific jobs. A job-exposure matrix was used to assess the relationship between beryllium exposure intensity and mortality from lung cancer, which allowed for a more thorough evaluation of exposure-response relationship (Sanderson et al. 2001a). Two reanalyses of the data were also performed (Levy et al. 2007; Schubauer-Berigan et al. 2008).

Beryllium plant records were used to construct historical exposure estimates in a job-exposure matrix that was published separately (Sanderson et al. 2001a). In addition to employment duration (measured in days), the authors also evaluated cumulative, average, and maximum exposure. A crude adjustment for smoking was performed by excluding "professionals" who were reported to have lower smoking rates than the worker population. Exposure metrics in this analysis were transformed to their natural logarithms. For those workers who had no exposure within the disease latency period, values of zero were assigned a value of 0.1. Conditional logistic regression was used to estimate exposure odds ratios (ORs) by quartiles of exposure and continuous exposure to evaluate a dose-response relationship between beryllium exposure and lung cancer. In total, 142 cases and 710 controls were evaluated (i.e., 5 controls per case selected using risk set sampling). In order to discount exposures that may not have contributed to causing lung cancer, the authors lagged the 3 exposure matrices by 10 and 20 yr. Lagging is often used in occupational epidemiology studies to evaluate disease induction and latency periods. Since information on disease induction is often unknown, lagging exposures to include those that occur during the most relevant time period is useful (Checkoway et al. 1990).

For the Reading facility, the risk estimate for lung cancer was 1.22 (95% CI 1.03–1.43). In the analysis of quartiles of exposure, with respect to all workers and the analysis that excluded professionals (surrogate control for smoking), elevated ORs were not observed with increasing exposure. Significantly increased ORs for tenure were reported in the second and third quartiles in the 20-yr lagged analysis (2.23 and 2.48, $p < .01$); however, there was a significant negative association for the highest cumulative exposure group (fourth quartile) compared to the reference group in the unlagged analysis (OR = 0.54, $p < .05$). Likewise, for cumulative exposure, a significant negative association was reported (OR = 0.57, $p < .05$) for the highest exposure group for the unlagged analysis, and significant increased risks in the 20-yr lagged analysis were reported for the second and third quartiles compared to the reference (2.18, $p < .01$, and 1.89, $p < .05$). With regard to average and maximum exposure, significant increased risks are again reported in the second and third quartiles in the 10- and 20-yr lagged analysis compared to the reference group, with a reduction in risk in the highest exposure group. The 20-yr lagged results are illustrated in Figure 3.

In the continuous analysis, a significant positive association was seen with the log of tenure lagged 20 yr. Significant positive associations were also seen with the log of cumulative, average, and maximum exposures lagged 10 and 20 yr. No association was observed for unlagged exposure using the maximum, average, or cumulative metric, and a significant negative association was seen with the log of the unlagged tenure. The regression coefficients are summarized in Table 4.

Overall the authors concluded that "confounding by professional status (as a surrogate for smoking) did not occur," and that the increased lung cancer among workers with higher lagged exposures provided evidence of the carcinogenicity of beryllium (Sanderson et al. 2001b, p. 138). However, it has been noted that several methodological issues likely affected the results, such as the reliability of data used to construct the historical exposures, lack of proper smoking adjustment, and major differences noted between cases and controls that affect the results when lagging is performed (Deubner, Lockey et al. 2001; Levy et al. 2002; Schubauer-Berigan et al. 2007; Schubauer-Berigan et al. 2008). Therefore, the conclusions drawn by Sanderson et al. (2001b) must be reviewed in the context of the two reanalyses that followed.

The reliability of the exposure estimates used in the job-exposure matrix was discussed in Levy et al. (2002). After reviewing the historical data, Levy and his colleagues noted that few measurements from the 1970s were available, whereas the bulk of person-years at risk of exposure occurred prior to this time. The authors also concluded that "possibly serious overestimation of exposures owing to the fact that most of the measurements reflect the presence of nonrespirable particles" may have occurred (Levy et al. 2002, p. 1011).

Levy and his colleagues reported that the control selection methods used by Sanderson et al. (2001b) resulted in imbalances between the cases and controls, leading to a greater percentage of controls that had no exposure during the latency period and thus were assigned a value of 0.1 compared to cases (Levy et al. 2007). In other words, the controls were more likely to have exposure outside of the latency period of the matching case because age at first employment and age at employment termination for controls were several years greater than that of the matching case. This disparity reportedly resulted in 10-yr and 20-yr lagged regression coefficients that were elevated. Levy et al. (2007) reported that when using the nontransformed exposure metrics for lag of 10 yr and assigning a value of zero to subjects having no exposure during the latency period, all

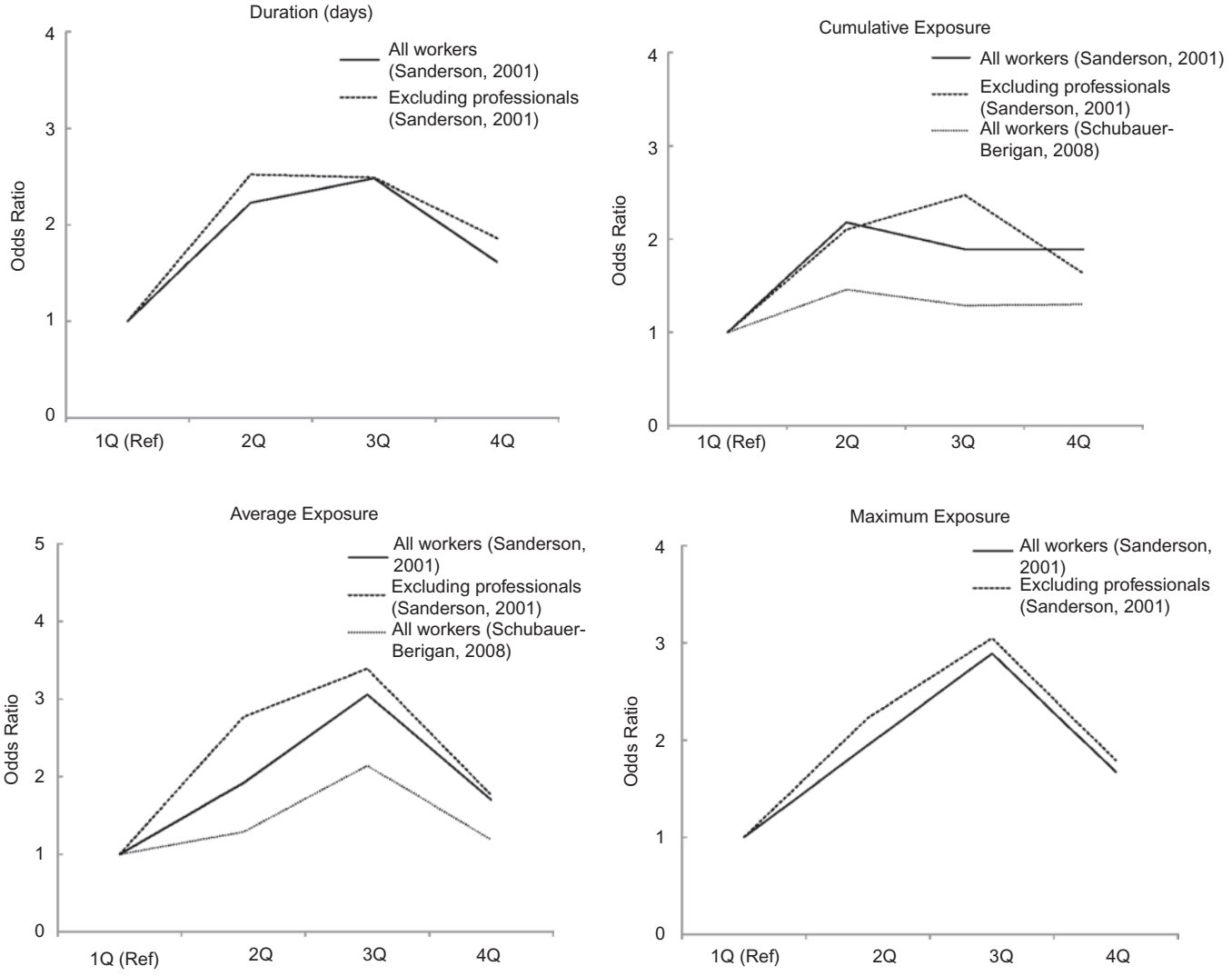

Figure 3. Results of categorical analysis of exposure metrics for 20-yr lagging and effect of controlling for year of birth (Sanderson et al., 2001; Schubauer-Berigan et al., 2008). Quartiles of exposure metrics compared to reference category (1st quartile). Schubauer-Berigan odds ratios are controlled for year of birth (categories < 1899, 1900–1910, 1911–1920, > 1921).

regression coefficients were very close to zero, indicating no relationship between beryllium exposure and lung cancer. In the second part of the analysis, Levy et al. (2007) addressed the age disparity between cases and controls (on average controls were 9.7 yr older at age of death) in Sanderson et al. (2001b) by selecting a subset of controls and matching them more closely to the cases (within 3 yr). In doing so, the significantly increased risks observed in the Sanderson analysis for 10-yr lagged cumulative, average, and maximum exposure were eliminated (Table 4).

Deubner et al. (2007) further assessed the impact of correlations between time-related covariates in the nested case-control study design employed in Sanderson et al. (2001b), and the possible spurious confounding generated. Although the limitations of their empirical evaluation were noted, the possibility of generating bias from certain study design methodologies, including matching and exposure lagging, was demonstrated. Garabrant (2007) also commented on the importance of performing empirical evaluations or simulations to assess study design as a source of confounding. Specifically, he noted that "correlations among time-related variables may produce apparent associations between outcome variables and covariates that have no meaningful relationship to outcome" (Garabrant 2007, p. 941).

The rates of lung cancer vary significantly for U.S. males born during the late 1800s and early 1900s, as seen in Table 1 of Schubauer-Berigan et al. (2008). This "birth cohort effect" is likely related to differences in smoking patterns for workers born before 1900, as well as the tendency for workers hired during World War II to have been older at time of hire. In a critique of the Sanderson study, Deubner et al. (2001) noted that cases were hired at a younger age than controls because of bias in the selecting cases and controls as a result of matching methods (i.e., risk-set sampling or incidence density sampling) (Deubner, Lockey et al. 2001). The Schubauer-Berigan et al. (2008) reanalysis evaluated whether uncontrolled confounding resulted in the observed differences between cases and controls in age at hire, and

Table 4. Results of continuous analyses of exposure metrics presented in Sanderson et al. (2001), Levy et al. (2007), and Schubauer-Berigan et al. (2008).

	Sanderson et al. (2001)[a]			Levy et al. (2007)[b]		Schubauer-Berigan et al. (2008)[c]		
Exposure Metric	Regression coefficient	OR	p value	OR	95% Cl	Regression coefficient	OR	p value
Duration (days)								
0 lag	−**0.096**	**0.91**	**0.020**	–	–	–	–	–
10 y lag	0.045	1.05	0.113	0.91	(0.82–1.02)	–	–	–
20 y lag	**0.045**	**1.05**	**0.036**	–	–	–	–	–
Cumulative exposure (ug/m^3 days)								
0 lag	−0.064	0.94	0.123	–	–	−0.055	0.95	0.181
10 y lag	**0.060**	**1.06**	**0.021**	0.99	(0.91–1.08)	0.038	1.04	0.192
20 y lag	0.041	1.04	0.018	–	–	0.012	1.01	0.585
Average exposure (ug/m^3)								
0 lag	0.110	1.12	0.143	–	–	0.119	1.13	0.107
10 y lag	**0.184**	**1.20**	**0.0004**	1.11	(0.95–1.31)	**0.159**	**1.17**	**0.0041**
20 y lag	**0.088**	**1.09**	**0.0039**	–	–	0.048	1.05	0.207
Maximum exposure (ug/m^3 days)								
0 lag	0.098	1.10	0.151	–	–	–	–	–
10 y lag	**0.171**	**1.19**	**0.0003**	1.06	(0.92–1.22)	–	–	–
20 y lag	**0.085**	**1.09**	**0.0033**	–	–	–	–	–

[a] Reported logs of continuous exposure variables.
[b] Reanalysis of Sanderson, et al. (2001) using nontransformed exposure metric, limited to controls within 3 years of age of matching case as a surrogate control for birth cohort.
[c] Reanalysis of Sanderson, et al. (2001) adjusting for birth year (<1900, 1900–1910, 1911–1920, >1920) to account for known differences in smoking rates.
Bolded values are statistically significant at $p < 0.05$.

the effect of adjusting for birth year on lung cancer risk in the Sanderson et al. case control study (Sanderson et al. 2001b; Sanderson 2001c; Schubauer-Berigan et al. 2007; Schubauer-Berigan et al. 2008).

Schubauer-Berigan et al. (2007) replicated the categorical and continuous analyses performed in the original study for the cumulative and average exposure metrics controlling for birth cohort for unlagged, 10-yr and 20-yr latency periods. When controlling for birth cohort using birth cohort quartiles (<1899, 1900–1910, 1911–1920, and >1920) decreased lung cancer risk estimates associated with log of cumulative exposure are observed. Decreased risk estimates are also observed when evaluating the log of the continuous cumulative exposure estimate. However, for log of average exposure, unlagged and 10- and 20-yr lagged analyses show significantly increased risk among the second and third quartiles of exposure, but not for the highest level of exposure, and the increases are nonlinear. The risk estimate for log of continuous average exposure is also significantly elevated when adjusting for birth cohort in the 10-yr lagged analysis, but not for the 20-yr lagged analysis (Table 4). Schubauer-Berigan et al. (2008) concluded that average but not cumulative exposure was related to lung cancer risk after adjustment for birth cohort, specifically for 10-yr lag. In addition, they concluded that the differences in age at hire among cases and controls were due to the fact that birth years are earlier for controls than for cases. It is therefore appropriate to control for this variable since there is a much lower baseline risk for lung cancer for men born prior to 1900.

The conclusions of Levy et al. (2007) and Schubauer-Berigan et al. (2008) confirm that when using the unlagged analysis, there is no observed relationship between beryllium and lung cancer for any of the exposure metrics; however, a weak association was observed for lung cancer and average beryllium exposure, at a lagging of 10 yr (Schubauer-Berigan et al. 2008). With lagging, the results of Schubauer-Berigan et al. (2008) are consistent with those of Levy (2007), showing no observed relationship between cumulative exposure or duration of employment and lung cancer.

3.2.3 Beryllium case registry studies

Infante et al. (1980): Infante et al. (1980) analyzed the mortality experience of white males who entered the BCR while alive. They were then classified into two mutually exclusive categories: those diagnosed with ABD and those with a diagnosis of chronic beryllium disease (CBD). Because of the use of U.S. national mortality rates for 1965–1967 to calculate expected lung cancer deaths for the period 1968–1975, the IARC reported that Saracci's adjustment factor of 10% is appropriate for considering the results of Infante et al. (1980) (IARC 1993).

Seven lung cancer deaths were observed and 3.30 were expected (SMR 2.12, not statistically significant). IARC reported that the recalculated expected number of lung cancer deaths was 3.63, resulting in an adjusted SMR of 1.93 (95% CI 0.8–4.0) (IARC 1993). For those classified in the registry with an ABD diagnosis, Infante et al. (1980) reported a statistically significant unadjusted lung cancer SMR of 3.14. IARC (using Saracci's adjustment) calculated a lung cancer SMR of 2.86 (95% CI 1.0–6.2). One subject diagnosed with CBD died of lung cancer (1.52 expected). For this case, the authors originally reported a non-statistically significant lung cancer SMR of 0.72, while IARC's Working Group (using Saracci's adjustment) reported a lung cancer SMR of 0.66 (95% CI 0.1–3.7). Six of the seven lung cancer deaths were observed in workers who were involved in beryllium

extraction and smelting, and five of the seven had exposure to beryllium for less than one year.

Infante et al. (1980) did not control for potential confounding by smoking. However, the authors noted that it was unlikely that the excess lung cancer risk observed among workers with acute beryllium illnesses was due to smoking. The investigators indicated that the lack of consistency between excess lung cancer risk in those with ABD and CBD resulted from the small expected number of lung cancer deaths, particularly among workers with chronic lung disease and the relatively short follow-up time for those workers who entered the registry after 1965 (≤ 10 yr). Small sample sizes in the exposure groups of interest possibly limited the ability to detect a statistically significant difference, if an association truly existed. Additionally, IARC (1993) noted that although no information on occupation was provided in the report, 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.

Steenland and Ward (1991): Steenland and Ward (1991) conducted an extended analysis of the Infante et al. (1980) BCR investigation, by including females and nonwhite males and adding 13 yr of follow-up. U.S. national mortality rates were available for all years, thus eliminating the need for Saracci's adjustment to the reported lung cancer risks. The SMR for lung cancer was 2.00 (95% CI 1.33–2.89). Steenland and Ward (1991) reported that the lung cancer SMR varied little by time since first exposure or by duration of exposure. The SMR for ≤ 20 yr since first exposure was 1.95 (95% CI 0.94–3.59) and the SMR for > 20 yr since first exposure was 2.03 (95% CI 1.20–3.21). For exposure duration of ≤ 4 yr the SMR was 2.01 (95% CI 1.11–3.23) and for exposure duration of > 4 yr the SMR was 1.98 (95% CI 0.99–3.55). The authors noted, however, a possible explanation for this observation was that the registry likely contained inaccuracies in duration of exposure.

Of the 689 subjects included in the analysis, 237 subjects were reported as being diagnosed with ABD (17 of these cases died of lung cancer), and 439 subjects were diagnosed with CBD with clinical symptoms (10 of these cases died of lung cancer). The lung cancer SMR was 2.32 (95% CI 1.35–3.72) for those diagnosed with ABD, while the lung cancer SMR was 1.57 (95% CI 0.75–2.89) for those diagnosed with CBD. Eisenbud (1993) described this greater risk of lung cancer for those with ABD as being entirely related to the experience of the Lorain plant. For example, in reviewing the employment records, he discovered that all 17 lung cancer cases had work histories involving the Lorain facility. He also reported that 145 of the 235 patients diagnosed with ABD in the registry (the Sanderson study identified 237) were employed at the Lorain facility, and that no lung cancer cases were found in the remaining 90 subjects, who were employed at beryllium facilities other than Lorain. This observation is noteworthy, as ABD cases are generally associated with concentrations of airborne soluble beryllium salts in excess of 100 μg/m^3, and exposures at the Lorain facility were reported as high as 4700 μg/m^3 (Laskin et al. 1950; Eisenbud 1982). As noted earlier, the Lorain facility had potential confounding exposures, possibly accounting for the difference in lung cancer disease incidence in these two groups. Steenland and Ward (1991) further cite that the occurrence of lung cancer in ABD and CBD cases was not all that different and may have occurred by chance.

The effect of smoking on the incidence of lung cancer was not controlled for in this analysis. The authors reported that information on smoking habits as of 1965 was available for 223 (32%) of registry members and was compared with smoking habits of the U.S. population of similar demographics as of 1965. It was observed that the cohort had fewer current male smokers (26%) than did the U.S. age-adjusted population (39%) in 1965, and that current smokers in the cohort smoked fewer cigarettes than current smokers in the overall U.S. population in 1965. Steenland and Ward, however, concluded that if smoking habits of the 32% of subjects were representative of the entire cohort, then the excess lung cancer risk in this population would unlikely be attributable to smoking. However, the U.S. EPA noted that other studies, such as Ward et al. (1992) and Wagoner et al. (1980), found that failing to correct for cigarette smoking in regards to lung cancer deaths resulted in an underestimation of expected deaths, and a subsequent overestimation of the SMR (U.S. EPA 1998). This contradicts Steenland and Ward (1991), who indicated that not correcting for differences in smoking habits between the cohort and U.S. population may overestimate the expected deaths, since smoking rates in the study population were actually less than the general population, and therefore resulted in an underestimated SMR.

3.2.4 Additional human studies evaluating carcinogenicity of beryllium

In addition to the cohort studies discussed earlier, the IARC Working Group cited three case-control studies in their evaluation of the human carcinogenicity of beryllium. Hinds et al. (1985) reviewed data from newly diagnosed male lung cancer cases in Hawaii. A job-exposure matrix, constructed from lists of occupation codes, was linked to each case, and each chemical agent of interest was classified into three exposure levels (low, medium, and high). The authors found an excess cancer risk at the low and high levels of beryllium exposure. A nested case-control study of central nervous system (CNS) cancers was conducted at two nuclear facilities in Oak Ridge, TN, and exposures to 26 chemicals of interest were ranked by potential for exposure (Carpenter et al. 1988). Although the authors did not find appreciable excess risk of CNS cancers associated with occupational exposures to any of the researched chemicals, a weak association between beryllium and these cancers was noted. However, the results were not statistically significant. Further, Feingold et al. (1992) reported results from a parental occupation and childhood cancer case-control study. Exposure to beryllium or

its compounds was not associated with excess risk of all cancers in children. Although not reviewed by the most recent IARC evaluation, Brown et al. (2004) investigated exposures to radiation, asbestos, hexavalent chromium, nickel, and beryllium and lung cancer mortality in workers employed at the Rocky Flats Plant in Colorado. Data from a job-exposure matrix was used to estimate annual exposures to these chemicals. No associations between lung cancer mortality and cumulative exposures to these chemicals were identified.

3.2.5 Epidemiologic studies and implications for the assessment of beryllium carcinogenicity

Based on our review of the relevant epidemiology literature regarding the potential association between beryllium and lung cancer, we identified and evaluated 17 publications. The U.S. EPA and others have criticized the studies conducted prior to 1987 as likely being influenced by confounding factors (e.g., smoking) and methodological limitations (U.S. EPA 1998). While useful in generating hypotheses, contributing to the research of this issue, and identifying additional areas of research, the results of these studies are undermined by confounding factors and provide limited insight into further elucidating the disease effect association. The U.S. EPA, in addition to others, further noted that the studies conducted by Steenland and Ward (1991), Ward et al. (1992), and Sanderson et al. (2001b) are more complete analyses. The reanalyses conducted by Levy and Schubauer-Berigan have further addressed the issues of confounding with earlier studies (Levy et al. 2007; Schubauer-Berigan et al. 2008). Modestly elevated risk estimates were observed in the studies included in this analysis and cited by IARC. However, these investigations lacked the data to control for confounding by smoking and they contained uncertainties regarding study methodologies, further limiting the interpretation of the significance of these slightly elevated risks (BISAC 1997; MacMahon 1994; ATSDR 2002; Garabrant 2007; Deubner et al. 2009).

Reanalyses of the data used by Ward have provided analytic enhancements by controlling for confounders, using more complete databases, incorporating a longer follow-up, and conducting more appropriate statistical analyses (Levy et al. 2002; Deubner et al. 2007; Levy et al. 2007; Schubauer-Berigan et al. 2008). These studies, however, do not provide independent evidence as they largely utilize overlapping cohorts from the same study population. Other issues related to confounding of the disease relationship by smoking, exposures to sulfuric acid and other strong inorganic acids, the lack of an association between risk of lung cancer and duration of exposure, marginal lung cancer risk estimates, and exceedingly high historical exposures experienced at the Lorain and Reading plants suggest that the evidence supporting carcinogenic potential in humans for beryllium is either inadequate or marginally suggestive. At worst, beryllium appears to have low potency for producing lung cancer at high exposure concentrations.

3.3 Short-term worker effect

It has been postulated that increased cancer risk can be attributed to characteristics or factors inherent among short-term employees. In the beryllium manufacturing cohort, numerous studies have shown elevated and often statistically significantly elevated SMRs for those employed for less than 1, 4, or 5 yr, depending on the study (Mancuso and El-Attar 1969; Mancuso 1970; 1979; 1980; Wagoner et al. 1980; Steenland and Ward 1991). Although information on individual personal habits of the beryllium workers was not available, other researchers have investigated this issue in other populations with short-term employees.

Stewart et al. (1990), for example, evaluated estimates of exposure and mortality experience by job type in a formaldehyde plant, and found that short-term workers (≤ 1 yr in the job) were more likely to be in jobs with higher levels of exposure to particulates than were long-term workers (> 1 yr). Short-term workers had greater risks of dying from diseases of the circulatory system, arteriosclerotic heart disease, emphysema, digestive-tract diseases, cirrhosis of the liver, motor vehicle accidents, suicide, and cancer of the stomach, colon, lung, prostate, or brain than long-term workers. The authors suggest that these observed increased risks are consistent with the influence of socioeconomic and lifestyle factors, particularly tobacco and alcohol use (Stewart et al. 1990). Similarly, Lamm et al. (1988) stated that including short-term workers may magnify, rather than dilute, the estimates of risk in studies. They found lung cancer mortality to be significantly increased for short-term workers, but not for long-term workers, and differences in smoking habits or other unknown factors between the two groups were suggested as possible explanations (Lamm et al. 1988).

Doll and Peto (1985) further suggest that short-term workers have unusual, often important confounding characteristics such as atypical smoking habits, previous occupational exposures, or other "lifestyle" differences compared with the general population. Further studies have suggested that unskilled jobs, particularly in the dusty trades, attract shifting populations with similar atypical lifestyles (Browne 1986). In 1999, Kolstad and Olsen reported that workers with two or more pre-employment hospitalizations related to alcohol abuse or violence had rate ratios of 2.30 (95% CI 1.74–3.06) and 1.86 (95% CI 1.35–2.56) for short-term and early termination of employment, respectively. They concluded that an unhealthy lifestyle was a determinant of short-term employment (Kolstad and Olsen 1999). Similarly, short-term workers were found to have higher mortality rates from virtually all causes, not just occupational ones, compared to long-term employees. These findings were attributed to individual risk factors, such as high levels of tobacco and alcohol consumption among short-term workers. Similar findings were also reported in a study of male workers at chromate pigment factories and in a review of cement workers (Davies 1984; Ohlson and Hogstedt 1985). Extrapolating findings of mortality rates and the influence of lifestyle habits and employment choices for typical short-

term workers from other cohorts to those observed with the beryllium industry suggests that a number of factors could contribute to the excess lung cancer mortality among beryllium workers employed for less than 5 yr.

3.4 Effect of limited smoking data

Smoking is one of the most investigated risk factors in epidemiologic research. Proper adjustment for smoking history is critical when studying the effects of exposures on diseases strongly linked to smoking, with lung cancer studies heavily impacted by this confounder (Blair et al. 1988; Brown and Kessler 1988). There is a lack of consensus, however, on how to adequately account for smoking. Some argue that the accuracy of self-reported smoking status is severely limited and cite the need to secure better estimates of smoking exposure, while others note that the lack of adjustment for smoking introduces only limited bias in occupational studies on lung cancer (Gillies et al. 1982; Vesey et al. 1982; Leffondre et al. 2002; Richiardi et al. 2005). As reported by Jarvis et al. (1987), "self-reported smoking rates are likely to give a substantial underestimate of the true prevalence of smoking," leading to an underestimation of the effects of smoking on the disease process (Jarvis et al. 1987, p. 1437). Poor correlations between reported smoking status and biochemical markers of smoking suggest that misclassification can occur when smoking status is limited to self-reports (Ohlin et al. 1976; Jarvis et al. 1987; 2008; Stram et al. 2002; Sillett et al. 1978; Jarvis et al. 1987; Vesey et al. 1982).

Because some misclassification of smoking information is likely, some residual confounding by smoking is inevitable (Greenland 1980; Greenland and Robins 1985; Stram et al. 2002; Rothman et al. 2008). The problem of residual confounding may be amplified if the only smoking information available is a dichotomous variable such as "ever" versus "never" smoked or if detailed smoking histories are not available (i.e., smoking duration, intensity, time since cessation) (Leffondre et al. 2002). The lack of detailed information on smoking habits, which is often unavailable in historical epidemiology investigations, significantly limits the adjustment for confounding when attempting to associate increases in lung cancer risk to a specific industrial chemical, as is the case in the beryllium cohort. The issue of residual confounding is also problematic, as it may appear to the reader that confounding by smoking has been fully controlled, when in fact it may not have been. As Rothman (2008) states, "if the confounding is strong and the exposure-disease relation is weak or zero, misclassification of the confounder can produce extremely misleading results, even if the misclassification is independent and nondifferential," as is the case with beryllium, smoking, and lung cancer (Rothman et al. 2008, p. 145). Potential error in self-reported smoking status or exposure classification may underestimate the risk from smoking, and may overestimate the effect of the other potentially related exposures, such as an industrial chemical. Based on our review, residual confounding, misclassification, and bias with regard to smoking in the epidemiologic studies cannot be ruled out with reasonable confidence in the various studies of beryllium carcinogenicity.

There are several approaches in occupational epidemiology studies to quantitatively assess the potential confounding by smoking when cigarette smoking data are either lacking or incomplete. These indirect methods include, among others, (1) estimating the smoking prevalence for the entire occupational cohort and (2) evaluating the mortality pattern of smoking related diseases that are not expected to be associated with the occupational exposure of interest (Checkoway et al. 2004). As discussed later, it is desirable for the data from these two sources to generate quantitatively consistent results. Ward et al. (1992) reported a higher prevalence of smoking in the occupational cohort, in comparison to the U.S population, reporting an overall smoking adjustment factor for the cohort of 1.13 (based on a 1968 medical survey conducted at four of the seven facilities). However, an SMR of 1.34 for emphysema was reported (Ward et al. 1992). These two findings are not internally consistent and it does not appear that there is a distribution of smoking in the beryllium cohort that can generate both the magnitude of SMR for emphysema (1.34) and the magnitude of the 1.13 smoking adjustment. The two main reasons to discount the SMR for emphysema and to preclude this argument would be if beryllium causes emphysema, or if there were errors in the diagnosis. However, this does not appear to be the case as emphysema has been previously shown to be associated with either smoking or a deficiency in proteinase inhibitors and there likely would not be a differential misclassification due to errors in diagnosis (Stulbarg 1996).

Given this inconsistency, one is left with choosing between two options to control for smoking: the first option resulting in the conclusion that beryllium is associated with an SMR of 1.12 for the total cohort, as presented in Ward et al. (1992), and the second option resulting in a conclusion that lung cancer may not be associated with beryllium exposure. It is no less appropriate to use a smoking-related disease to indirectly control for smoking than it would be to use limited data from a smoking survey that may or may not have collected a random representative sample. Further, while Ward et al. (1992) reported that there is greater smoking in the subsample of cohort members for which smoking data exists, Steenland and Ward (1991) report less smoking among the registrants of the BCR in comparison to the general population, potentially due to underreporting of smoking habits. Again, the inconsistencies cited in the two analyses between the smoking habits in these overlapping cohorts further support the previous arguments posed by the U.S. EPA and others that there is insufficient evidence to exclude confounding by smoking in these analyses.

4. Assessment of evidence for causality

As a part of this analysis, the postulates for causality presented by Bradford Hill were considered in evaluating the supporting evidence for the association between beryllium and lung cancer. Originally adapted from a 1964 report on

smoking and health, the various postulates of Bradford Hill have been included in many discussions of causality, including the IARC Working Groups (Hill 1965; IARC 2006). Although not intended to be applied rigorously to the determination of causation, these postulates are meant to serve as guidelines in assessing whether or not a causal relationship truly exists. Several of these concepts are discussed in terms of the weight of evidence for beryllium exposure and carcinogenicity.

4.1 Strength of association

Virtually all of the studies included in this analysis reported lung cancer risk estimates < 2.0, many of which fall below 1.5. As cautioned by Hill (1965), in general, strong associations are unlikely to be explained by bias or confounding, while weak associations may be due to subtle, unrecognized errors. These errors may potentially lead to faulty or misleading outcomes. Thus, the strength of association between exposure and disease is best determined by the risk estimates derived from the most reliable available studies. Based on our review, we found the Ward et al. (1992) analysis and Levy et al. (2002) reanalysis to present the most reliable risk estimates for the entire cohort. Unadjusted lung cancer SMRs were reported as 1.69 (95% CI 1.28–2.19) and 1.24 (95% CI 1.03–1.48), for Lorain and Reading, respectively. After adjusting the expected values using a smoking correction factor based on the U.S. population, the SMRs decrease to 1.49 (95% CI 1.13–1.93) and 1.09 (0.91–1.31), respectively (Ward et al. 1992). As demonstrated in the Levy reanalysis, depending upon the smoking correction factor used, the SMRs decreased to nonsignificance with the exception of workers at Lorain, a unique plant with documented high exposures during the 1940s (Laskin et al. 1950; Ward et al. 1992; Eisenbud 1993; Levy et al. 2002). Because misclassification is likely to occur even with more accurate smoking information, some residual confounding is inevitable (Rothman et al. 2008). The evidence presented in this analysis suggests a weak association between beryllium exposure, which cannot be considered out of context of the limitations of the smoking data and other potential confounders.

4.2 Consistency

Consistent study findings among different populations in different places, circumstances, and times, and among different study designs can support a causal relationship between exposure and disease. However, consistent results across the epidemiology literature do not exist in the context of the carcinogenicity of beryllium. It is important to acknowledge that the human studies relied upon in support of the classification of beryllium have been conducted using data from the same cohort of workers; therefore, consistency among the results can be misleading. Moreover, depending on the approaches used to analyze the data, the findings of the studies are not consistent. For example, because smoking is a known confounder in lung cancer studies and accurate smoking data do not exist for the entire cohort, different methodologies have been used to address potential confounding by smoking: (1) smoking correction factors used to adjust the risk estimates (Wagoner et al. 1980; Ward et al. 1992; Levy et al. 2002), (2) exclusion of a subset of the cohort (Sanderson et al. 2001b), and (3) adjustment for birth cohort (Schubauer-Berigan et al. 2008). Consistent results using these methods are not evident. Because of the disparities in age-specific lung cancer rates across birth cohorts between the late 1800s and 1930, and the tendency to hire older workers during the 1940s, it has been shown that adjusting for birth cohort is important (Schubauer-Berigan et al. 2008). In light of the limited smoking data for this cohort upon which the smoking correction factors are based, control for birth cohort seems to be the most valid technique to control for this confounding effect.

Likewise, consistency is not apparent when correction is made using combined city/county lung cancer rates compared to county or national lung cancer rates as seen in Levy et al. (2002). Further, consistency is not apparent among the latent periods in which an increased risk of lung cancer is associated (Ward et al. 1992; Sanderson et al. 2001b; Schubauer-Berigan et al. 2008). Finally, using a different methodology for matching cases to controls resulted in different conclusions, as seen in the Levy reanalysis of the Sanderson case-control study (Sanderson et al. 2001b; Levy et al. 2007). The observed association between lung cancer and beryllium exposure in Sanderson et al. (2001b) was shown to be related to case-control differences in date of birth and age at hire, and not to beryllium exposure (Deubner et al. 2007; Levy et al. 2007).

Consistency implies that a similar exposure–disease relationship is demonstrated across studies using different methodological approaches. This is not evident in the published literature reviewed for this analysis. Rather, what has been consistently demonstrated throughout these studies is that when adjusting for confounders, or by using local lung cancer rates for comparison, despite the limitations of the methods used, the risk estimates decreased and generally were no longer statistically significant.

4.3 Temporality

Evidence that the exposure occurred prior to the disease, or temporality, must be exhibited to determine that a causal association exists. Since lung carcinogens require a latency period, it has been suggested that performing a lagged analysis is necessary to evaluate the association between beryllium exposure and lung cancer (Sanderson et al. 2001b). The beryllium manufacturing cohort studies have employed a similar approach by evaluating time since first employment and date of death. In all studies, the estimate of exposure, whether from Social Security Administration records, personnel files, or the BCR, demonstrated that work in a beryllium plant preceded death (ascertained by death certificates). It is important to note that lung cancer has been associated with a variety of causes, including several occupational exposures, genetic susceptibility, and smoking (U.S. Surgeon General 1985).

Ascertaining information from death certificates can result in misclassification, given that they do not provide the date of diagnosis, frequently lack information on the type of lung cancer, and provide limited and inconsistent data on occupational history. As seen in Ward et al. (1992), 50% of the cohort worked for less than 1 yr; at Lorain and Reading, 85% and 54%, respectively worked for less than 1 yr. Information regarding occupations outside of the beryllium plants has not been evaluated or presented. Consequently, while there is sufficient evidence to determine a temporal relationship between occupational beryllium exposure and death from lung cancer, the question of whether occupational exposure to beryllium or other confounding exposures resulted in the disease has not been definitively addressed.

4.4 Biological gradient

An important characteristic of both the animal and human evidence is the apparent lack of a biological gradient (i.e., increasing exposure levels to beryllium do not appear to lead to greater incidence of the disease, based on the available data). Rigorous effort has been made to assess a biological gradient in the human studies using employment duration, average exposure, maximum exposure, and cumulative exposure as metrics, and no convincing gradient has been identified (Sanderson et al. 2001b; Levy et al. 2002; Schubauer-Berigan et al. 2008). A consistent dose-response relationship was not demonstrated using any metric in the unlagged, 10-yr lagged, and 20-yr lagged analyses (Sanderson et al. 2001b). Further, as stated earlier, short-term workers may be more likely to be employed in jobs with higher levels of exposure and may also be at increased risk of certain diseases consistent with the influence of socioeconomic and lifestyle factors (Stewart et al. 1990), and numerous studies evaluated here showed increased risk primarily in workers of short duration (Mancuso 1980; Wagoner et al. 1980; Ward et al. 1992; Sanderson et al. 2001b). If the beryllium exposure–lung cancer relationship in humans exhibited a biological gradient, we would expect to see a similar increased risk in long-term workers and in those with higher cumulative exposures; yet we do not. However, several factors that may explain, in part, the reason that we do not see an increased risk in those with the highest exposures have been presented by Stayner et al. (2003). These include (1) bias introduced by the healthy worker survivor effect, (2) a depletion of susceptible persons in the population at high exposure levels, (3) a natural limit on the relative risk for diseases with a high background rate, (4) misclassification of exposures, (5) the influence of other risk factors that vary by the level of exposure, and (6) the saturation of key enzyme systems or other processes involved in the development of disease (Stayner et al. 2003). In the beryllium manufacturing studies, the reliability of the exposure data and small size of groups with longer employment duration and higher exposures do contribute significant uncertainty (Sanderson et al. 2001b; Levy et al. 2002).

Moreover, it was shown that lagging exposures to account for latency, as was done in Sanderson et al. (2001b), exaggerated the difference in exposure between cases and controls because of the censoring of exposure information (Garabrant 2007; Levy et al. 2007). The Levy reanalysis (2007) demonstrated that there was very little difference in exposure between cases and controls, particularly lagged 20 yr. This observation was not discussed in the context of the Sanderson et al. (2001b) study, which concluded that increased lung cancer risk in the greater lagged exposures "provide further evidence" that beryllium is a carcinogen (Sanderson et al. 2001b, p. 133).

It has been shown that there is an increased incidence of lung cancers in subjects diagnosed with ABD when evaluating workers at Lorain, who were exposed to extremely high airborne beryllium concentrations in the 1940s (Steenland and Ward 1991; Eisenbud 1993). It is possible, if not likely, that beryllium may be acting as a "high-dose" carcinogen; i.e., high doses of beryllium may cause tumors, while lower doses are completely without effect on tumor formation. There are countless examples of high-dose carcinogens as reported in various animal studies, which, ultimately, are of limited significance when predicting the human risk at much lower doses. In fact, about half of the chemicals tested in both rats and mice have been found to be carcinogens in chronic rodent bioassays at the high doses administered (Ames and Gold 1990). Further, the ATSDR noted that "[i]t is possible that the beryllium disease process (particularly the acute disease) contributes to the development of lung cancer" (ATSDR 2002, p. 71). As noted by the U.S. EPA and others, the risks predicted by models generally are based on high-dose studies, which may have little to no ability to characterize human risk and were never intended to quantitatively estimate human health risks at low exposures (Paustenbach 1989).

In the chronic rodent bioassays, it is postulated that high doses can cause a high rate of cell killing and a corresponding stimulation of cell division (mitogenesis), which may promote mutagenesis and ultimately, carcinogenesis (Ames and Gold 1990). As this relates to the beryllium epidemiology studies, ABD cases experienced very high exposures to airborne beryllium at peak concentrations upwards of 4700 $\mu g/m^3$, which represent a unique environment that does not exist in any industrial environment today (Laskin et al. 1950; Eisenbud 1993).

4.5 Plausibility

The biological plausibility of beryllium carcinogenicity in humans is difficult to determine based on these observational studies. Concerning the experimental animal studies, biological plausibility is conflicting, and there is incomplete evidence concerning the mechanisms of beryllium carcinogenicity as demonstrated in various in vitro and in vivo tests. Several studies in rats and mice indicate that gene mutations commonly associated with human lung cancer have been present, but were ultimately postulated not to be the root cause of carcinogenic transformation (Joseph et al.

2001; Keshava et al. 2001). Mutagenicity and genotoxicity studies indicate beryllium may cause genetic changes under certain conditions (e.g., with soluble beryllium forms) and not under others (e.g., in bacterial assays). Results from mutagenicity and genotoxicity tests with beryllium forms commonly encountered in the work place are generally lacking in the literature. In vivo studies examining possible epigenetic mechanisms of carcinogenesis have shown that beryllium metal may induce chronic lung inflammation with cytokine release and oxygen radical production, leading to cellular damage and cell proliferation; however, there are conflicting reports in animal studies about whether ROS are involved in beryllium carcinogenesis, and whether ROS is dependent on the chemical form of beryllium. Further, bone tumor formation in rabbits exposed to beryllium may not be specific to beryllium, but could be a general response to chronic inflammation/irritation and/or a response occurring in aging animals. Studies at present prevent any clear determination of the mechanism of beryllium carcinogenicity, or at least fail to point to any one predominant mechanism.

5. Conclusion

We conducted a weight-of-evidence assessment of both the historical and recently published literature to evaluate the potential carcinogenicity of beryllium. Based on this analysis, it is our view that the evidence for carcinogenicity of beryllium is not as clear as has been implied by past evaluations. Much of the inadequacy of the data, as they relate to the animal literature, is attributed to methodological weaknesses and responses that lack sufficient scientific strength to be considered reliable or informative (e.g., small sample sizes, inadequate or no control group, and high mortality in exposed animals), lack of dose-response comparisons, possible unique animal species susceptibility (e.g., osteosarcomas in rabbits), exceedingly high doses, and questionably irrelevant routes of exposure (e.g., intraosseal and intravenous), as well as to a lack of a mechanistic basis for tumor development. Further, toxicology studies specific to occupationally relevant forms of beryllium do not show consistent correlations between beryllium exposure and cancer in animals. In addition, tumor responses were not consistent across animal species or beryllium administered via several routes.

In relation to the available epidemiology literature, limited data result in incomplete exposure information and limited control for smoking as a confounder. As such, caution must be applied when evaluating the potential causal association sometimes reported in these studies. There are two primary reasons for this view. First, exposures to beryllium have been poorly defined by the majority of the epidemiology studies. Steenland and Ward (1991) suggest that the excess lung cancer observed among the BCR registrants with ABD (acute disease is assumed to be associated with high exposure to soluble beryllium compounds) supports the argument that beryllium exposure explains, at least partly, the lung cancer excess, and is indicative of a dose-response relationship. Although the relationship between high beryllium exposures and ABD and lung cancer incidence may be suggestive, the existing confounding (e.g., smoking) of the analysis of workers at the Lorain facility, where all of these workers were employed, coupled with the very weak association (RR < 2), weakens the strength of this argument (Eisenbud 1993). Additionally, based on the IARC Working Group's evaluation criteria, such classifications of exposure should be addressed independently of disease status (e.g., ABD).

Further, the strength of associations or relative risk estimates are low, generally < 1.5, and after controlling for smoking or other confounders, most risk estimates are no longer significant, with the exception of one historical plant cohort (Lorain) in which smoking and other chemical exposures have not been well characterized (Ward et al. 1992; Sanderson et al. 2001a; Levy et al. 2002; Levy et al. 2007; Schubauer-Berigan et al. 2008). Because some misclassification of relevant smoking information is likely, some residual confounding by smoking is inevitable (Greenland 1980; Greenland and Robins, 1985; Stram et al. 2002). Additionally, results of different study methodologies vary greatly between studies, upon comparing risks from log transformed versus untransformed exposure data and various matching techniques (Deubner, Goodman et al. 2001; Deubner, Lowney et al. 2001; Deubner and Kent 2007). Thus, there is a lack of consistency across studies that utilize different statistical and epidemiologic approaches. The effect of smoking is nearly impossible to separate from any effects of beryllium, particularly at such low risk estimates where there is a lack of well-characterized smoking history. It is likely that no additional improvements can be made with the analysis of the Lorain cohort without additional data on smoking and the exposure history of these workers. However, issues surrounding residual confounding from smoking and study design shortcomings, such as control matching, are likely to be improved upon through additional cohort follow-up of other beryllium manufacturing plants.

While recent evaluations have addressed methodological limitations apparent in earlier cohort studies (e.g., more appropriate reference population, correction for smoking), risk estimates of a possible association between beryllium exposure and lung cancer in humans cannot be considered out of context of the limitations that still exist in the literature (e.g., other occupational exposures, confounding by birth year, discrepancies in lagging periods, appropriate matching). In addition, because of the lack of well-characterized historical occupational exposures, the lack of an observed dose-response relationship in the animal and human studies, and lack of consistent findings of an increased risk of lung cancer, we find that the evidence is weak for an association between beryllium exposure and lung cancer, using the Hill postulates for causality. These findings are comparable to more historic analyses of the epidemiology literature of beryllium and lung cancer using the same criteria for

disease causation (Monson 1990). In the context of a weight-of-evidence analysis of the historical and recent animal and human literature, we therefore conclude that the evidence for beryllium's carcinogenic potential in humans should be considered either inadequate or marginally suggestive in modern industrial settings.

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References

ACGIH. 1996. Documentation of threshold limit values for beryllium and compounds. Cincinnati, OH: American Conference of Governmental Industrial Hygenists.

ACGIH. 2001. Documentation of threshold limit values for beryllium and compounds. Cincinnati, OH: American Conference of Governmental Industrial Hygenists.

ACGIH. 2008. Draft Documentation of threshold limit values for beryllium and compounds. Cincinnati, OH: American Conference of Governmental Industrial Hygenists.

Amand, W. B., W. H. Riser, and D. N. Biery. 1973. Spontaneous osteosarcoma with widespread meastasis in a belted dutch rabbit. *J Am Anim Hosp Assoc* 9:577–581.

Ames, B. N., and L. S. Gold. 1990. Chemical carcinogenesis: too many rodent carcinogens. *Proc Natl Acad Sci USA* 87(19):7772–7776.

Araki, M., S. Okada, and M. Fujita. 1954. Beryllium. Experimental studies on beryllium-induced malignant tumours of rabbits (Jpn.). *Gann* 45:449–451.

Ashby, J., M. Ishidate, Jr., G. D. Stoner, M. A. Morgan, F. Ratpan, and R. D. Callander. 1990. Studies on the genotoxicity of beryllium sulphate in vitro and in vivo. *Mutat Res* 240(3):217–225.

ATSDR. 2002. Toxicological profile for beryllium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Axelson, O., and K. Steenland. 1988. Indirect mthods of assessing the effects of tobacco use in occupational studies. *Am J Ind Med* 13:105–118.

Barnes, J. M. 1950. Experimental production of malignant tumours by beryllium. *Lancet* 255:463.

Barnes, J. M., and F. A. Denz. 1950. Beryllium bone sarcomata in rabbits. *Br J Cancer* 4(2):212–222.

Bayliss, D. L. 1980. Letter to William H. Foege, M.D., Director, Center for Disease Control. Atlanta, GA.

Bayliss, D. L., W. S. Lainhart, L. J. Crally, R. Ligo, H. Ayer, and F. Hunter. 1971. Mortality patterns in a group of former beryllium workers. Paper read at thirty-third annual meeting of the American Conference of Governmental Industrial Hygienists, May 24–28, 1971, at Toronto, Canada.

BISAC (Beryllium Industry Scientific Advisory Committee). 1997. Is beryllium carcinogenic in humans? *J Occup Environ Med* 39(3):205–208.

Blair, A., K. Steenland, C. Shy, M. O'Berg, W. Halperin, and T. Thomas. 1988. Control of smoking in occupational epidemiologic studies: methods and needs. *Am J Ind Med* 13(1):3–4.

Borak, J. 2006. The beryllium occupational exposure limit: Historical origin and current inadequacy *J Occup Environ Med* 48:109–116.

Brown, C. C., and L. G. Kessler. 1988. Projections of lung cancer mortality in the United States: 1985-2025. *J Natl Cancer Inst* 80(1):43–51.

Brown, S. C., M. F. Schonbeck, D. McClure, A. E. Baron, W. C. Navidi, T. Byers, and A. J. Ruttenber. 2004. Lung cancer and internal lung doses among plutonium workers at the Rocky Flats Plant: A case-control study. *Am J Epidemiol* 160(2):163–172.

Browne, K. 1986. Is asbestos or asbestosis the cause of the increased risk of lung cancer in asbestos workers? *Br J Ind Med* 43:145–149.

Carpenter, A. V., W. D. Flanders, E. L. Frome, W. G. Tankersley, and S. A. Fry. 1988. Chemical exposures and central nervous system cancers: A case-control study among workers at two nuclear facilities. *Am J Ind Med* 13(3):351–362.

Checkoway, H., N. Pearce, and D. Kriebel. 2004. *Research Methods in Occupational Epidemiology*. 2nd ed. Vol. 34, *Monographs in Epidemiology and Biostatistics*. New York: Oxford University Press.

Checkoway, H., N. Pearce, J. L. Hickey, and J. M. Dement. 1990. Latency analysis in occupational epidemiology. *Arch Environ Health* 45(2): 95–100.

Chiba, I., T. Takahashi, M. M. Nau, D. D'Amico, D. T. Curiel, T. Mitsudomi, D. L. Buchhagen, D. Carbone, S. Piantadosi, H. Koga, et al. 1990. Mutations in the p53 gene are frequent in primary, resected non-small cell lung cancer. Lung Cancer Study Group. *Oncogene* 5(10):1603–10.

Cloudman, A. M., D. Vining, S. Barkulis, and J. J. Nickson. 1949. Bone changes observed following intravenous injection of beryllium. *Am J Pathol* 25:810–811.

Davies, J. M. 1984. Lung cancer mortality among workers making lead chromate and zinc chromate pigments at three English factories. *Br J Ind Med* 41:158–169.

Dean, A. G., K. M. Sullivan, and M. M. Soe. 2008. *OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.2.1*. Accessed 2008/11/08, www.OpenEpi.com.

Deubner, D. C., M. Goodman, and J. Iannuzzi. 2001. Variability, predictive value, and uses of the beryllium blood lymphocyte proliferation test (BLPT): Preliminary analysis of the ongoing workforce survey. *Appl Occup Environ Hyg* 16(5):521–526.

Deubner, D. C., J. L. Lockey, P. Kotin, M. B. Powers, F. Miller, A. E. Rogers, and D. Trichopoulos. 2001. Re: Lung cancer case-control study of beryllium workers. Sanderson WT, Ward EM, Steenland K, Petersen MR. Am. J. Ind. Med. 2001. 39:133-144. *Am J Ind Med* 40(3):284–288.

Deubner, D. C., Y. W. Lowney, D. J. Paustenbach, and J. Warmerdam. 2001. Contribution of incidental exposure pathways to total beryllium exposures. *Appl Occup Environ Hyg* 16(5):568–578.

Deubner, D. C., H. D. Roth, and P. S. Levy. 2007. Empirical evaluation of complex epidemiologic study designs: Workplace exposure and cancer. *J Occup Environ Med* 49(9):953–959.

Deubner, D., and M. Kent. 2007. Keeping beryllium workers safe: An enhanced preventive model. *J Occup Environ Hyg* 4(3):D23–30.

Deubner D.C., and H.D. Roth. 2009. Rejoinder: Progress in understanding the relationship between beryllium exposure and lung cancer. Epidemiol 20. epub.

Doll, R., and J. Peto. 1985. Asbestos: Effects on health of exposure to asbestos: Health and Safety Commision.

Dutra, F. R., and E. J. Largent. 1950. Osteosarcoma induced by beryllium oxide. *Am J Pathol* 26(2):197–209.

Dutra, F. R., E. J. Largent, and J. L. Roth. 1951. Osteogenic sarcoma after inhalation of beryllium oxide. *AMA Arch Pathol* 51(5):473–479.

Eisenbud, M. 1993. Re: Lung cancer incidence among patients with berylllium disease. *J Natl Cancer Inst* 85:1697–1699.

Eisenbud, M. 1982. Origins of the standards for control of beryllium disease (1947–1949). *Environ Res* 27:79–88.

Eisenbud, M., and J. Lisson. 1983. Epidemiological aspects of beryllium-induced nonmalignant lung disease: A 30-year update. *J Occup Med* 25:196–202.

ESIS. 2008. Data sheet: Result for EC#: 231-150-7, European Inventory of Existing Commercial Chemical Substances. European Chemical Substances Information System.

Feingold, L., D. A. Savits, and E. M. John. 1992. Use of a job-exposure matrix to evaluate parental occupation and childhood cancer. *Cancer Causes Control* 3:161–169.

Finch, G.L., M.D. Hoover, F.F. Hahn, K.J. Nikula, S.A. Belinsky, P.J. Haley, and W.C. Griffith. 1996. Animal models of beryllium-induced lung disease. Environ Health Perspect 104(Suppl 5):973-979.

Fodor, I. 1977. Histogenesis of beryllium-induced bone tumours. *Acta Morphol Acad Sci Hung* 25(2–3):99–105.

Garabrant, D. H. 2007. Case-control study design: Spurious associations between exposure and outcome. *J Occup Environ Med* 49(9):941–942.

Gardener, L. U., and H. F. Heslington. 1946. Osteosarcoma from intravenous beryllium compounds in rabbits. *Fed Proc* 5:221.

Gazdar, A. F. 1990. Cell biology and molecular biology of small cell and non-small cell lung cancer. *Curr Opin Oncol* 2(2):321–327.

Gillies, P. A., B. Wilcox, C. Coates, F. Kristmundsdotir, and D. J. Reid. 1982. Use of objective measurement in the validation of self-reported smoking in children aged 10 and 11 years: Saliva thiocyanate. *J Epid and Commun Health* 36:205–208.

Gordon, T., and D. Bowser. 2003. Beryllium: Genotoxicity and carcinogenicity. *Mutat Res* 533(1–2):99–105.

Greenland, S. 1980. The effect of misclassification in the presence of covariates. *Am J Epidemiol* 112:564–569.

Greenland, S., and J. M. Robins. 1985. Confounding and misclassification. *Am J Epidemiol* 122:495–506.

Greim, H, ed. 2005. *Beryllium and its compounds*. Vol. 21, *Occupational toxicants*. Weinheim, Germany: Wiley-VCH.

Groth, D. H., C. Kommineni, and G. R. Mackay. 1980. Carcinogenicity of beryllium hydroxide and alloys. *Environ Res* 21(1):63–84.

Haley, P. J., G. L. Finch, M. D. Hoover, and R. G. Cuddihy. 1990. The acute toxicity of inhaled beryllium metal in rats. *Fundam Appl Toxicol* 15(4):767–778.

Hext, P. M. 1994. Current perspectives on particulate induced pulmonary tumours. *Hum Exp Toxicol* 13:700–715.

Hill, A. B. 1965. The environment and disease: Association or causation? *Proc R Soc Med* 58:295–300.

Hinds, M. W., L. N. Kolonel, and J. Lee. 1985. Application of a job-exposure matrix to a case-control study of lung cancer. *J Natl Cancer Inst* 75(2):193–197.

Hiruma, T. 1991. Rabbit osteosarcoma induced by hydroxypropylcellulose mixed beryllium oxide pellet—Comparison between implantations into bone marrow cavity and into fracture callus of the femur (Jpn.). *Nippon Seikeigeka Gakkai Zasshi* 65(9):775–786.

Hoagland, M. B., R. S. Grier, and M. B. Hood. 1950. Beryllium and growth. I. Beryllium-induced osteogenic sarcomata. *Cancer Res* 10(10): 629–635.

Hoover, J. P., D. B. Paulsen, C. W. Qualls, and R. J. Bahr. 1986. Osteogenic sarcoma with subcutaneous involvement in a rabbit. *J Am Anim Hosp Assoc* 9:1156–1158.

HSDB. 1997. Beryllium, elemental: Hazardous Substances Data Bank, National Institutes of Health. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~rreHS4:1. Accessed September 17, 2008.

HSE. 2006. Beryllium metal and beryllium compounds. In *Disease reduction programme—Cancer project: Profiling of occupational carcinogens*. Health and Safety Executive (HSE) Working Group on Action to Control Chemicals (WATCH), United Kingdom.

IARC. 1972. IARC monographs on the evaluation of carcinogenic risks of chemicals to man, Vol. 1: Some inorganic substances, chlorinated hydrocarbons, aromatic amines, *N*-nitroso compounds and natural products. Lyon, France: International Agency for Research on Cancer, World Health Organization.

IARC. 1980. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 23: Some metals and metallic compounds. Lyon, France: International Agency for Research on Cancer, World Health Organization.

IARC. 1987. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Suppl. 7: Overall evaluations of carcinogenicity: An updating of IARC monographs Volumes 1 to 42. Lyon, France: International Agency for Research on Cancer, World Health Organization.

IARC. 1992. IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 54: Occupational exposures to mists and vapours from strong inorganic acids, and other industrial chemicals. Lyon, France: International Agency for Research on Cancer, World Health Organization.

IARC. 1993. IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 58: Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Lyon, France: International Agency for Research on Cancer, World Health Organization.

IARC. 2006. IARC monographs on the evaluation of carcinogenic risks to humans: Preamble. Lyon, France: International Agency for Research on Cancer, World Health Organization.

ILSI. 2000. The relevance of the rat lung response to particle overload for human risk assessment: A workshop consensus report. International Life Sciences Institute (ILSI) Risk Science Institute Workshop Participants. *Inhal Toxicol* 12:1–17.

Infante, P. F., J. K. Wagoner, and N. L. Sprince. 1980. Mortality patterns from lung cancer and nonneoplastic respiratory disease among white males in the beryllium case registry. *Environ Res* 21(1):35–43.

Ishinishi, N., M. Mizunoe, T. Inamasu, and A. Hisanaga. 1980. Experimental study on carcinogenicity of beryllium oxide and arsenic trioxide to the lung of rats by an intratracheal instillation (author's transl, Jpn.). *Fukuoka Igaku Zasshi* 71(1):19–26.

Janes, J. M., G. M. Higgins, and J. F. Herrick. 1954. Beryllium-induced osteogenic sarcoma in rabbits. *J Bone Joint Surg Br* 36-B(4):543–552.

Jarvis, M. J., H. Tunstall-Pedoe, C. Feyerabend, C. J. Vesey, and Y. Saloojee. 1987. Comparison of tests used to distinguish smokers from nonsmokers. *Am J Public Health* 77:1435–1438.

Joseph, P., T. Muchnok, and T. Ong. 2001. Gene expression profile in BALB/c-3T3 cells transformed with beryllium sulfate. *Mol Carcinogen* 32(1):28–35.

Kahn, H. A. 1966. The Dorn study of smoking and mortality among U.S. veterans: Report on eight and one-half years of observation. *Natl Cancer Inst Monograph* 19:1–125.

Kelly, P. J., J. M. Janes, and L. F. Peterson. 1961. The effect of beryllium on bone. A morphological study of the progressive changes observed in rabbit bone. *J Bone Joint Surg Am* 43-A:829–844.

Keshava, N., G. Zhou, M. Spruill, M. Ensell, and T. M. Ong. 2001. Carcinogenic potential and genomic instability of beryllium sulphate in BALB/c-3T3 cells. *Mol Cell Biochem* 222(1–2):69–76.

Kolanz, M. E. 2001. Introduction to beryllium: Uses, regulatory history, and disease. *Appl Occup Environ Hyg* 16(5):559–567.

Kolstad, H. A., and J. Olsen. 1999. Why do short term workers have high mortality? *Am J Epidemiol* 149(4):347–352.

Komitowski, D. 1968. Experimental beryllium-induced bone tumours as a model of osteogenic sarcoma (Pol.). *Chir Narzad Ruchu Ortop Pols* 33:237–242.

Komitowski, D. 1974. Beryllium induced bone sarcomas (author's transl, Ger.). *Verh Dtsch Ges Pathol* 58:438–440.

Kondo, H., M. Ishikawa, H. Maeda, M. Onuma, M. Masuda, H. Shibuya, H. Koie, and T. Sato. 2007. Spontaneous osteosarcoma in a rabbit (*Orcytolagus cuniculus*). *Vet Pathol* 44:691–694.

Kuroda, K., G. Endo, A. Okamoto, Y. S. Yoo, and S. Horiguchi. 1991. Genotoxicity of beryllium, gallium and antimony in short-term assays. *Mutat Res* 264(4):163–170.

Lamm, S. H., M. S. Levine, J. A. Starr, and S. L. Tirey. 1988. Analysis of excess lung cancer risk in short-term employees. *Am J Epidemiol* 127(6):1202–1209.

Laskin, S., R. A. N. Turner, and H. E. Stockinger, eds. 1950. *An analysis of dust and fume hazards in a beryllium plant*. Edited by A. Vorwald. Vol. Leroy U. Gardner Memorial: *Pneuonoconiosis*. New York: Hoeber.

Lavastre, V., C. J. Roberge, M. Pelletier, M. Gauthier, and D. Girard. 2002. Toxaphene, but not beryllium, induces human neutrophil chemotaxis and apoptosis via reactive oxygen species (ROS): Involvement of caspases and ROS in the degradation of cytoskeletal proteins. *Clin Immunol* 104(1):40–48.

Leffondre, K., M. Abrahamowicz, J. Siemiatycki, and B. Rachet. 2002. Modeling smoking history: A comparison of different approaches. *Am J Epidemiol* 156:813–823.

Leonard, A., and R. Lauwerys. 1987. Mutagenicity, carcinogenicity and teratogenicity of beryllium. *Mutat Res* 186(1):35–42.

Levy, P. S., H. D. Roth, and D. C. Deubner. 2007. Exposure to beryllium and occurrence of lung cancer: A reexamination of findings from a nested case-control study. *J Occup Environ Med* 49(1):96–101.

Levy, P. S., H. D. Roth, P. M. T. Hwang, and T. E. Powers. 2002. Beryllium and lung cancer: A reanalysis of a NIOSH cohort mortality study. *Inhal Toxicol* 14:1003–1015.

Litvinov, N. N., V. A. Popov, T. V. Vorozheikina, V. F. Kazenashev, and P. F. Bugryshev. 1983. Blastomogenic activities of various beryllium compounds (Russ.). *Eksp Onkol* 5:23–26.

Litvinov, N. N., V. A. Popov, T. V. Vorozheikina, V. F. Kazenashev, and P. F. Bugryshev. 1984. Data for more precise determination of the maximum allowable concentration of beryllium in the air of the workplace (Russ.). *Gig Tr Prof Zabol* 1:34–37.

MacMahon, B. 1994. The epidemiological evidence on the carcinogenicity of beryllium in humans. *J Occup Med* 36(1):15–26.

Madl, A. K., and K. E. Pinkerton. 2008. *Health effects of inhaled engineered nanoscale materials*. Edited by V. H. Grassian: *Nanoscience and nanotechnology: Environmental and health impacts*. Hoboken, NJ: John Wiley & Sons.

Mancuso, T. F. 1970. Relation of duration of employment and prior respiratory illness to respiratory cancer among beryllium workers. *Environ Res* 3(3):251–275.

Mancuso, T. F. 1980. Mortality study of beryllium industry workers' occupational lung cancer. *Environ Res* 21(1):48–55.

Mancuso, T. F. 1979. Occupational lung cancer among beryllium workers. Paper read at Dusts and Disease, Society for Occupational and Environmental Health Conference, at Park Forest, IL.

Mancuso, T. F., and A. A. El-Attar. 1969. Epidemiological study of the beryllium industry. Cohort methodology and mortality studies. *J Occup Med* 11(8):422–434.

Matsuura, K. 1974. Experimental studies on the production of osteosarcoma by beryllium compounds, and the effects of irradiation (Jpn.). *Jpn J Orthop Assoc* 48:403–418.

Mauderly, J. L. 1996. Lung overload: The dilemma and opportunities for resolution. *Inhal Toxicol* 8:1–28.

Mauderly, J. L., M. B. Snipes, E. B. Barr, S. A. Belinsky, J. A. Bond, A. L. Brooks, I. Y. Chang, Y. S. Cheng, N. A. Gillett, W. C. Griffith, et al. 1994. Pulmonary

toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats. Part 1: Neoplastic and nonneoplastic lung lesions. *Res Rep Health Effects Inst* 68:1–75; discussion 77–97.

Mazabraud, A. 1975. [Experimental production of bone sarcomas in the rabbit by a single local injection of beryllium]. *Bull Cancer* 62(1):49–58.

Mazzullo, G., M. Russo, P. P. Niiutta, and G. De Vico. 2004. Osteosrcoma with multiple metastases and subcutaneous involvement in a rabbit (*Oryctolagus ciniculus*). *Vet Clin Path* 33(2):102–104.

Monson, R. 1990. Case studies in occupational epidemiology. In *Occupational Epidemiology*, 2nd ed. Boca Raton, FL: CRC Press.

Morgareidge, K., G. E. Cox, and M. A. Gallo. 1976. Chronic feeding studies with beryllium in dogs. Food and Drug Research Laboratories, Inc. Submitted to the Aluminum Company of America, Alcan Research & Development, Ltd., Kawecki-Berylco Industries, Inc., and Brush-Wellman, Inc.

Nesnow, S., L. L. Triplett, and T. J. Slaga. 1985. Studies on the tumor initiating, tumor promoting, and tumor co-initiating properties of respiratory carcinogens. *Carcinogenesis* 8:257–277.

Nickell-Brady, C., F. F. Hahn, G. L. Finch, and S. A. Belinsky. 1994. Analysis of K-ras, p53 and c-raf-1 mutations in beryllium-induced rat lung tumors. *Carcinogenesis* 15(2):257–262.

Nikula, K. J., D. S. Swafford, M. D. Hoover, M. D. Tohulka, and G. L. Finch. 1997. Chronic granulomatous pneumonia and lymphocytic responses induced by inhaled beryllium metal in A/J and C3H/HeJ mice. *Toxicol Pathol* 25(1):2–12.

NIOSH. 2005. Pocket guide to chemical hazards, beryllium and beryllium compounds, DHEW (NIOSH) Publication No. 2005-151. Washington, DC: National Institute for Occupational Safety and Health.

NTP. 2005. Report on carcinogens, eleventh Edition: Beryllium and beryllium compounds: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Washington, DC.

OECD. 1997. Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development (OECD). Available at http://titania.sourceoecd.org/vl=3043871/cl=19/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm.

Ohlson, C. G., and C. Hogstedt. 1985. Lung cancer among asbestos cement workers. A Swedish cohort study and review. *Br J Ind Med* 42:397–402.

OSHA. 1977. Informal standard for exposure to beryllium. Transcript of proceedings, August 31, 1977. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration.

OSHA. 1989. OSHA Permissible Exposure Limits. 29 CFR 1910.1000, Table Z-1. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration.

Paustenbach, D. J. 1989. Important recent advances in the practice of health risk assessment: implications for the 1990s. *Regul Toxicol Pharmacol* 10(3):204–243.

Peer Review Summary Report. 2008. Consolidated Comments from the External Peer Review Meeting on the Toxicological Review of Beryllium and Compounds (CAS No. 7440-41-7), Prepared for the U.S. EPA: Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=189634.

Pfeifer, A. M., G. E. Mark, 3rd, L. Malan-Shibley, S. Graziano, P. Amstad, and C. C. Harris. 1989. Cooperation of c-raf-1 and c-myc protooncogenes in the neoplastic transformation of simian virus 40 large tumor antigen-immortalized human bronchial epithelial cells. *Proc Natl Acad Sci USA* 86(24):10075–10079.

Rana, S. V. 2008. Metals and apoptosis: Recent developments. *J Trace Elem Med Biol* 22(4):262–284.

Reeves, A. L., D. Deitch, and A. J. Vorwald. 1967. Beryllium carcinogenesis. I. Inhalation exposure of rats to beryllium sulfate aerosol. *Cancer Res* 27(3):439–445.

Renfrew, H., and J. R. Rest. 2001. Extraskeletal fibroblastic osteosarcoma in a rabbit (*Oryctolagus cuniculus*). *J Small Anim Pract* 42:456–458.

Richiardi, L., F. Forastiere, P. Boffetta, L. Simonato, and F. Merletti. 2005. Effect of different approaches to treatment of smoking as a potential confounder in a case-control study on occupational exposures. *Occup Environ Med* 62:101–104.

Rossman, M. D., O. P. Preuss, and M. B. Powers, eds. 1991. *Beryllium: Biomedical and environmental aspects*. Baltimore, MD: Williams & Wilkins.

Rothman, K. J., S. Greenland, and T. L. Lash. 2008. *Modern epidemiology*, 3rd ed. Philadelphia, PA: Lippincott, Williams & Wilkins.

Salm, R., and J. Field. 1965. Osteosarcoma in a rabbit. *J Pathol Bacteriol* 89:400–402.

Sanderson, W. T. 2001c. Re: Response to criticisms of "Lung Cancer Case-Control Study of Beryllim Workers." *Am J Ind Med* 40:286–288.

Sanderson, W. T., E. M. Ward, K. Steenland, and M. R. Petersen. 2001b. Lung cancer case-control study of beryllium workers. *Am J Ind Med* 39(2):133–144.

Sanderson, W. T., M. R. Peterson, and E. M. Ward. 2001a. Estimating historical exposures of workers in a beryllium manufacturing plant. *Am J Ind Med* 39:145–157.

Saracci, R. 1985. Interpretation of negative epidemiological evidence for carcinogenicity. *IARC Sci Publ* 65.

Schepers, G. W. 1961. Neoplasia experimentally induced by beryllium compounds. *Prog Exp Tumor Res* 2:203–244.

Schepers, G. W., F. T. Creedon, A. B. Delahant, and T. M. Durkan. 1957. The biological action of inhaled beryllium sulfate; a preliminary chronic toxicity study on rats. *AMA Arch Ind Health* 15(1):32–58.

Schroeder, H. A., and M. Mitchener. 1975. Life-term studies in rats: Effects of aluminum, barium, beryllium, and tungsten. *J Nutr* 105(4):421–427.

Schubauer-Berigan, M. K., J. A. Deddens, and M. R. Petersen. 2007. Re: exposure to beryllium and occurrence of lung cancer: A reexamination of findings from a nested case-control study. *J Occup Environ Med* 49(7):708–709; author reply 709–711.

Schubauer-Berigan, M. K., J. A. Deddens, K. Steenland, W. T. Sanderson, and M. R. Petersen. 2008. Adjustment for temporal confounders in a reanalysis of a case-control study of beryllium and lung cancer. *Occup Environ Med* 65(6):379–383.

Sivulka, D. J. 2006. Comparison of non neoplastic respiratory responses in animals after inhalation of water soluble and insoluble metal compounds: White paper available from Nickel Producers Environmental Research Association NiPERA, Toronto, Canada.

Sprince, N. L. 1986. Beryllium disease. In: *Occupational Respiratory Diseases*. Edited by J. Merchant, B. Boehlecke and G. Taylor. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health.

Stayner, L., K. Steenland, M. Dosemeci, and I. Hertz-Picciotto. 2003. Attenuation of exposure-response curves in occupational cohort studies at high exposure levels. *Scand J Work Environ Health* 29(4):317–324.

Steenland, K., and E. Ward. 1991. Lung cancer incidence among patients with beryllium disease: A cohort mortality study. *J Natl Cancer Inst* 83(19):1380–1385.

Stewart, P. A., C. Schairer, and A. Blair. 1990. Comparison of jobs, exposures, and mortality risks or short-term and long-term workers. *J Occup Med* 32(8):703–708.

Stram, D. O., M. Huberman, and A. H. Hu. 2002. Is residual confounding a reasonable explanation for the apparent protective effects of beta-carotene found in epidemiologic studies of lung cancer in smokers? *Am J Epidemiol* 155:622–628.

Stulbarg, M. S., and L. Simmerman. 1996. Chronic obstructive pulmonary disease. In *Occupational and Environmental Respiratory Diseases*. Edited by P. Harber, M. Scheneker and J. Balmer. St. Louis, MO: Mosby.

Tapp, E. 1966. Beryllium induced sarcomas of the rabbit tibia. *Br J Cancer* 20(4):778–783.

Tapp, E. 1969. Osteogenic sarcoma in rabbits. Following subperiosteal implantation of beryllium. *Arch Pathol* 88(1):89–95.

Turton, J., and J. Hooson. 1998. *Target organ pathology: A basic text*. London: Taylor & Francis.

U.S. EPA. 1987. Health assessment document for beryllium. EPA/600/8-84 026F. Research Triangle Park, NC: U.S. Environmental Protection Agency.

U.S. EPA. 1991. Drinking water criteria document for beryllium. NTIS PB92-173301. Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office for the Office of Drinking Water, U.S. Environmental Protection Agency.

U.S. EPA. 1998. Toxicological review of beryllium and compounds (CAS No. 7440-41-7), in supprt of summary information on the Integrated Risk Information System (IRIS). EPA/635/R-98/008. Washington, DC: U.S. Environmental Protection Agency.

U.S. EPA. 2005. Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. Washington, DC: U.S. Environmental Protection Agency.

U.S. EPA. 2008. IRIS toxicological review of beryllium (External review draft). EPA/635/R-08/009A. Washington, DC: U.S. Environmental Protection Agency.

U.S. Surgeon General. 1985. The health consequences of smoking. Cancer and chronic lung disease in the workplace. Rockville, MD: U.S. Department of Health and Human Services, Public Health Service.

Vacher, J., and H. B. Stoner. 1968. The transport of beryllium in rat blood. *Biochem Pharmacol* 17(1):93–107.

Vesey, C. J., Y. Saloojee, P. V. Cole, and M. A. H. Russell. 1982. Blood carboxyhaemoglobin, plasma thiocyanage, and cigarette consumption: Implications for epidemiological studies in smokers. *Br J Med* 284:1516–1518.

Vorwald, A. J. 1966. The induction of experimental pulmonary cancer in the primate. Paper read at Proc 9th Int Cancer Congress, Tokyo, Japan.

Wagner, W. D., D. H. Groth, J. L. Holtz, G. E. Madden, and H. E. Stokinger. 1969. Comparative chronic inhalation toxicity of beryllium ores, bertrandite and beryl, with production of pulmonary tumors by beryl. *Toxicol Appl Pharmacol* 15:10–29.

Wagoner, J. K., P. F. Infante, and D. L. Bayliss. 1980. Beryllium: An etiologic agent in the induction of lung cancer, nonneoplastic respiratory disease, and heart disease among industrially exposed workers. *Environ Res* 21(1):15–34.

Walberg, J. A. 1981. Osteogenic sarcoma with metastasis in a rabbit (*Oryctolagus cuniculus*). *Lab Anim Sci* 31(4):407–408.

Ward, E., A. Okun, A. Ruder, M. Fingerhut, and K. Steenland. 1992. A mortality study of workers at seven beryllium processing plants. *Am J Ind Med* 22(6):885–904.

Weisbroth, S. H., and A. Hurvits. 1969. Spontaneous osteogenic sarcoma in *Oryctolagus cuniculus* with elevated serum alkaline phosphatase. *Lab Anim Care* 19(2):263–265.

WHO. 2001. Beryllium and beryllium compounds. Geneva: Concise International Chemical Assessment Document 32, World Health Organization.

Yamaguchi, S. 1963. Study of beryllium-induced oseogenic sarcoma (Jpn.). *Nagasaki Iggakai Zasshi* 38:127–138.

Zorn, H., T. Stiefel, and H. Diem. 1977. Importance of beryllium and its compunds in occupational medicine. Part 2 (Ger.). Zbl. Arbeitsmed 27:83–88.