DISSERTATION

CONTRIBUTION OF EXPOSURE AND GENETICS TO THE DEVELOPMENT OF BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MICHAEL V. VAN DYKE ENTITLED CONTRIBUTION OF EXPOSURE AND GENETICS TO THE DEVELOPMENT OF BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EXPOSURE, GENETICS, AND CHRONIC BERYLLIUM DISEASE

Beryllium is a low-density metal with unique properties used in a number of industries including automotive, electronics, communications, medical, defense, and aerospace. Workers exposed to aerosols generated by the fabrication of beryllium-containing materials are at risk for developing beryllium sensitization (BeS) and chronic beryllium disease (CBD). While several studies have documented that higher exposures are associated with higher rates of BeS and CBD, there have been consistent difficulties in defining the nature of the exposure-response relationship. In addition, several studies have identified at least one genetic host factor, a glutamic acid at position 69 (E69) of the HLA-DPB1 gene, that increases individual susceptibility to BeS and CBD. The relationship between beryllium exposure and carriage of the E69 genotype has not been well studied. This dissertation research was designed to evaluate the relationship between beryllium exposure and E69 in the risk of BeS and CBD.

In Chapter 2, the combined risk of BeS and CBD was evaluated as a function of beryllium exposure and carriage of any E69 genotype in a case-control study of current and former workers from a U.S. nuclear weapons production facility, the Y-12 National Security Complex (Oak Ridge, TN). Study participants included 35 individuals with BeS, 19 with CBD, and 127 controls with potential beryllium exposure. For this study, beryllium exposures were assessed through a combination of worker interviews and "expert" industrial hygiene assessment. After removing the confounding effect of

potential beryllium exposure at another facility, multivariate models showed a seven-fold (OR 7.41, 95% CI: 2.31-23.75) increased odds for BeS and CBD among HLA-DPB1 E69 carriers and a five-fold (OR 5.13, 95% CI: 1.59 to 16.57) increased odds for those exposed over the median cumulative beryllium exposure (1.8 $\mu g/m^3$-years). Those with both risk factors had additive odds for BeS/CBD (OR 38.0, 95% CI: 6.02 – 240). The study demonstrated that HLA-DPB1 E69 carriage and high exposure to beryllium appeared to be additive risk factors for the development of BeS and CBD. In addition, from this study, it appeared that the magnitude of risk associated with either elevated beryllium exposure or carriage of E69 was similar.

In Chapter 3, the risk of BeS and CBD was evaluated separately as a function of beryllium exposure and specific E69 genotype in a case-control study of former workers from a decommissioned U.S. nuclear weapons production facility, Rocky Flats Environmental Technology Site (RFETS, Arvada, CO). Study participants included 70 individuals with BeS, 61 with CBD, and 255 controls with potential beryllium exposure. For this study, beryllium exposures were assessed through a combination of worker interviews and assessment of task exposures based on facility-specific and industry-wide industrial hygiene exposure measurements. This study showed that different HLA-DPB1 E69 alleles or more than one copy of an E69 allele may confer differential risk of BeS and CBD. Carriage of a single, more common HLA-DPB1 *02 allele conferred a 12-fold increased odds for BeS (OR: 12.01, 95% CI: 4.28-33.71) and a three-fold increased odds for CBD (OR: 3.46, 95% CI: 1.42-8.43). A single copy of a rarer non-*02 E69 allele conferred a 30-fold increased odds for BeS (OR: 29.54, 95% CI: 10.33-84.53) and a nearly 12-fold increased odds for CBD (OR: 11.97 95% CI: 5.12-28.00), and two E69

allele copies conferred a 55-fold increased odds for BeS (OR: 55.68, 95% CI: 14.80-209.40) and a 22-fold increased odds for CBD (OR: 22.54, 95% CI: 7.00-72.62). Lifetime weighted beryllium exposure conferred an approximate two-fold increased odds of CBD (OR: 2.22, 95% CI: 1.21-4.07) regardless of E69 genotype again suggesting an additive relationship between E69 and exposure. Beryllium exposure was not a significant predictor of BeS.

The study in Chapter 4 compared three different, but related, retrospective exposure assessment methods applied to the participants of the case-control study in Chapter 3. Beryllium exposures for each participant were assessed using three different methods: 1) a traditional job exposure matrix (JEM method) that assigned beryllium exposures at the job title level based on interviews with a few workers in each job title and assessment of available industrial hygiene exposure measurements for this job title; 2) individual worker interviews evaluating the tasks each worker performed followed by "expert" assessment of task exposures by two industrial hygienists based solely on professional judgment (IH rating method) as was used in Chapter 2 of this dissertation, and; 3) individual worker interviews as described in #2 followed by extensive analyses of historical facility-specific and industry-wide data to assign exposures to tasks (IH data method), as was used in Chapter 3 of this dissertation. Results from this study suggested that a method of task exposure assessment relying solely on the professional judgment of industrial hygienists (IH rating method) performed similarly to a method involving extensive analyses of historical industrial hygiene measurements (IH data method) in terms of rank order assessment of average task exposure. Participant exposure assignments using all three of the methods were significant predictors of increased CBD

risk with odds ratios ranging from 1.51 (95% CI: 1.03-2.22) for the JEM method to 2.50 (95% CI: 1.47-4.26) for the IH data method, both in terms of each unit increase in lifetime-weighted average exposure. Exposure misclassification likely attenuated the odds ratio point estimates for the odds of CBD by approximately 5% using the IH rating method and approximately 40% using the JEM method.

Taken together, the three studies confirm the importance of both beryllium exposure and E69 genotype in the risk of CBD suggesting an additive relationship between the two. Furthermore, it appears that BeS and CBD risk is differentially distributed among E69 genotypes with carriers of rarer non-*02 E69 alleles at higher risk. These studies also provide additional evidence on the importance of extremely low beryllium exposures in the risk of BeS even after adjusting for genetic susceptibility. Finally, the studies provide evidence to validate a more efficient exposure assessment method based on task exposures assessed using "expert" industrial hygiene assessment rather than resource-intensive compilation and analysis of thousands of exposure measurements.

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DEDICATION

To Marcie for showing me the path to take, Sara for making sure I kept going forward and Mira for showing me a new path.

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LIST OF UNITS

µg	Micrograms
m^3	Cubic meters
µg/m^3	Micrograms per cubic meter
µg/m^3-years	Micrograms per cubic meter years
µm	Micrometers
s	Seconds
° C	Degrees Celsius
bp	Base pairs
mM	Millimolar

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AM	Arithmetic mean
Be	Beryllium
BeLPT	Beryllium lymphocyte proliferation test
BeS	Beryllium sensitization
CBD	Chronic beryllium disease
DPB1	DP beta one
DOE	U.S. Department of Energy
DWA	Daily weighted average
E69	Glutamic acid substitution at position 69
HLA	Human leukocyte antigen
FAH	Fixed airhead samples
GM	Geometric mean
GSD	Geometric standard deviation
IH	Industrial hygiene or industrial hygienist
IL	Interleukin
INF-γ	Interferon gamma
JEM	Job exposure matrix
K-25	K-25 Gaseous Diffusion Plant (Oak Ridge, TN)
LOD	Limit of detection
LTWA	Lifetime-weighted average
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibility complex
MMAD	Mass median aerodynamic diameter
NNSA	U.S. National Nuclear Safety Administration
OSHA	U.S. Occupational Safety and Health Administration
PCR	Polymerase chain reaction
PCR-SSP	Polymerase chain reaction sequence specific primer
PEL	Permissible Exposure Limit
RFETS	Rocky Flats Environmental Technology Site (Arvada, CO)
TLV	Threshold Limit Value
TCR	Receptor T-cells
TNF-α	Tumor necrosis factor alpha
TWA	Time weighted average
Y-12	Y-12 National Security Complex (Oak Ridge, TN)
X-10	Oak Ridge National Laboratories (Oak Ridge, TN)

CHAPTER 1

INTRODUCTION, GOALS, AND BACKGROUND

Introduction

Workers exposed to aerosols generated by the production or fabrication of beryllium-containing materials are at risk for beryllium sensitization (BeS) and chronic beryllium disease (CBD). The risk of BeS/CBD varies by workplace and by exposure level. However, prevalences of up to 15% for BeS and 8% for CBD have been documented in previous studies.[1, 2] With conservative estimates of the exposed U.S. workforce up to 134,000, and increasing use of beryllium in non-traditional industries, chronic beryllium disease represents a significant, occupational lung disease.[3]

Exposure to beryllium causes an immune reaction or BeS in susceptible individuals. This immune reaction is observed as T-Cell proliferation in beryllium stimulated peripheral blood and is measured using the beryllium lymphocyte proliferation test (BeLPT).[2, 4, 5] In some individuals, the immune system is unable to regulate the response to beryllium resulting in T-Cell activation and recruitment of macrophages to encapsulate beryllium particles in the lung.[6] This results in granuloma formation and pulmonary impairment due to the reduction in alveolar surface area available for gas exchange.

Exposure and genetic factors appear to play a role both in the ability of an individual to become sensitized and in the progression from BeS to CBD. While it is clear that higher exposures lead to higher rates of BeS/CBD, the exposure-response relationship does not appear to be linear.[7-9] Some explanations proposed for this lack of a linear exposure-response relationship have included exposure to different physical or chemical forms of beryllium[10] (i.e., beryllium metal versus beryllium oxide), exposure to smaller particle sizes more likely to reach the alveolar region of the lung[11-14], and the failure to account for dermal exposure to beryllium[15-17]. Most epidemiological studies of beryllium disease have been cross sectional in design, and many have suffered from exposure misclassification and lack of power to detect small differences. However, the epidemiological evidence clearly shows the presence of a sub-population of individuals who develop BeS at extremely low exposures.[7, 8, 18] This observation has led to research to define the genetic characteristics of this sensitive sub-population.

Genetic studies have shown significant increased risk of BeS/CBD associated with a particular group of alleles coding for glutamic acid at position 69 (E69) of the human leukocyte antigen class II DP beta one (HLA DPB1) region on chromosome six.[19-28] This region is part of the human major histocompatibility complex (MHC). As the HLA Class II group of genes have been shown to encode cell-surface proteins for presentation of cell processed antigens to T-Cells[29], it is likely this is a functional genetic variant important in the mechanism of BeS/CBD. Some functionality has been confirmed in that proliferation of T-Cells in response to beryllium stimulation can be blocked in vitro by the addition of antibodies to HLA-DP.[30] Molecular biology research has identified that HLA-DPB1 encodes the beta chain of the antigen binding groove and

substitutions at position 69 affect the electrostatic potential and likely the ability to bind the beryllium antigen.[31, 32] However, since the U.S. population carrier frequency of HLA DPB1 E69 is somewhere between 30% and 40%[33] and the prevalence of BeS/CBD in exposed populations is only 1% to 15%[1, 18], it is clear that there are additional factors, likely genetic and exposure related that play a role in the pathogenesis of BeS/CBD. In the only study to evaluate the combination of exposure and genetics, the effects were found to be at least additive.[21] This study involved only six cases of CBD and 121 controls identifying relatively high – albeit, imprecise - odds ratios of 10.1 (95% CI: 1.1-93.7) for high exposure and 11.8 (95% CI: 1.3-108.8) for E69.[21]

Goals of dissertation research

Previous studies have lacked the power or exposure assessment to investigate the individual contribution of genetic and exposure effects, as well as, the interaction of these effects, in the development of BeS/CBD. A better understanding of the differences in exposure-response for BeS/CBD among genetically susceptible individuals is critical for worker protection. The overall goal of this dissertation research was to identify the contribution of exposure and genetic effects in the development of BeS/CBD to better understand the pathogenesis of the disease and provide important information to policy makers considering a new beryllium exposure standard.

Specific aims

1. Examine genetic and exposure effects in relation to BeS/CBD via a case-control study with participants enrolled from a nuclear weapons production facility that

has not been previously studied (Y-12 National Security Complex, Oak Ridge, TN). Due to limited industrial hygiene data, exposure assessment for this cohort was completed using a method combining individual interviews and "expert assessment" by industrial hygienists. This study tested two main hypotheses: 1) genetic variant, specifically, HLA DPB1 E69, and beryllium exposure contribute individually to the development of BeS/CBD; and 2) the average exposure resulting in BeS/CBD is lower for HLA DPB1 E69 carriers compared to non-carriers. This was the first gene-environment study on BeS/CBD to combine HLA genotyping with semi-quantitative exposure assignments.

2. Examine additional genetic and exposure factors, including gene-exposure interaction, effect of multiple DPB1 E69 encoding alleles, and the effect of carriage of rarer non-*02 DPB1 E69 encoding alleles in BeS/CBD via case-control analysis of a larger cohort (Rocky Flats Environmental Technology Site, Arvada, CO) that provides more statistical power and more complete industrial hygiene data for exposure assessment. For this study, exposure assessment was completed using two methods: 1) a method combining individual interviews and industrial hygiene exposure data; and 2) individual interviews and "expert assessment" by industrial hygienists as in the study in specific aim 1. This larger study tested three main hypotheses: 1) there is an additive gene-exposure interaction between exposure and HLA DPB1 E69 status; 2) additional copies of E69 encoding alleles confer increased risk of BeS/CBD; and 3) carriers of rarer non-*02 alleles are at increased risk of BeS/CBD compared to those with the more common *02 alleles.

3. Compare the two exposure assessment methods used in specific aim two. Comparison of the two methods will allow results from the study in specific aim one to be compared to results using a more conventional exposure assessment method based on industrial hygiene data. This aim will test the hypothesis that a method using a combination of individual interviews and expert assessment by industrial hygienists will provide relative exposure assignments comparable to those produced using individual interviews and industrial hygiene exposure data.

Background and significance

Beryllium use and number exposed

Beryllium is a low-density metal with unique properties that make it desirable for use in a number of industries including automotive, electronics, communications, medical, defense, and aerospace. Beryllium is three times lighter than aluminum and has a specific stiffness six times that of steel. The metal conducts heat well and is dimensionally stable over a wide range of temperatures. In addition, it is transparent to X-rays and reflects neutrons. Beryllium has three commercially important forms: metal, oxide or ceramic, and alloy.[34] The world consumption in 2005 was 226 metric tons with a projected consumption of 529 metric tons in 2010.[35]

The number of workers potentially exposed to beryllium is unknown. In the early 1970s, NIOSH estimated a total of 21,233 U.S. workers exposed to beryllium based on a survey of 4,645 facilities in 66 different two digit SIC codes.[36] In the 1980s, this estimate was updated to 800,000 potentially exposed.[37] A more recent study using OSHA sampling data, estimated as many as 134,000 potentially exposed workers in U.S.

government and private industry.[3] However, this estimate likely underestimates the number exposed by not including downstream industries using beryllium products or facilities-related personnel who may have incidental exposure. A study of construction trades workers in the U.S. Department of Energy complex estimated that up to 230,000 construction trades workers alone are potentially exposed to beryllium.[38] In the European Union, it has been estimated that over 67,000 individuals are occupationally exposed to beryllium.[39] Numbers exposed in most other parts of the world have not been clearly defined.

History of beryllium disease

Beryllium has been known for many years to cause two distinct types of pulmonary disease: acute beryllium disease (ABD) and chronic beryllium disease (CBD). The early literature on beryllium cites the development of an acute chemical pneumonitis after fairly high exposures to beryllium.[40, 41] Eisenbud and Lisson[42] observed that nearly all workers exposed to concentrations exceeding 1 mg/m^3 developed ABD while none exposed to less than 0.1 mg/m^3 developed ABD, suggesting a clear exposure-response for ABD. Exposure controls implemented in 1949 by the U.S. Atomic Energy Commission, including a 25 µg/m^3 30-minute exposure limit, are credited with virtually eliminating ABD with the 15 cases reported after 1950 attributed to accidental exposures.[42]

CBD was first described among workers at a fluorescent lamp manufacturing facility in Salem, MA and was attributed to use of beryllium containing phosphors.[43] This report described 17 beryllium exposed workers with common symptoms including

severe dyspnea and weight loss. The report concluded that beryllium caused a delayed onset chemical pneumonitis. In the 1940s and 1950s, additional cases were observed in workers from the primary beryllium industry as well as spouses of workers and residents from communities near beryllium producing facilities.[44-47] It was also noted that about 15% of those diagnosed with ABD eventually developed CBD.[48] The first suggestion that CBD might be an immune mediated disease was published in 1951 by Sterner and Eisenbud.[47] The suggestion was based on several pieces of corroborating evidence: (1) the presence of severe cases of CBD with seemingly low exposures; (2) the lack of correlation between tissue levels of beryllium and the severity of disease; (3) an observed exposure-response at low airborne beryllium levels, but not at high levels; (4) the frequent occurrence of symptoms many years after exposure; and (5) the inability to produce similar effects in laboratory animals. Conclusive evidence for the granulomatous nature of beryllium disease was lacking until a detailed pathological review of 124 cases from the beryllium registry revealed clear granulomatous inflammation.[49] Evidence of a delayed hypersensitivity response to beryllium was demonstrated by Curtis in 1951 through positive patch tests using 2% beryllium sulfate on 13/13 beryllium workers with observed beryllium-related dermatitis.[50]

Based on early observations of CBD in beryllium production and fluorescent light manufacturing, Eisenbud and Machle developed an occupational exposure limit for beryllium. This limit was not based on any specific epidemiological evidence but instead, was extrapolated from existing standards for heavy metals and adjusted for the low atomic mass of beryllium. The standard was set by taking the average exposure limit for heavy metals of 100 $\mu g/m^3$, dividing it by 20 to account for the approximate 20-fold

difference in atomic mass between heavy metals and beryllium, and dividing the result by 2.5 as an "uncertainty" factor. The resulting limit was an eight-hour average exposure of 2 μg/m^3.[51-53] This level was eventually adopted as the OSHA standard in 1971, and remains unchanged to the present day.[54] The apparent success of this standard in reducing the incidence of CBD was outlined by Eisenbud in 1983 who reported that there were only 18 cases of CBD known to have been first exposed since 1962 and none with a first exposure beyond 1972.[42]

As the cases of CBD appeared to be on the decline, important developments were being made in the immunology of CBD. In 1970, Hanifin et al. demonstrated *in vitro* proliferation of peripheral blood lymphocytes when blood cells from patch test positive beryllium exposed workers were stimulated with beryllium oxide or beryllium sulfate.[55] Subsequent studies verified this cell mediated immune response in both blood and lung cells from bronchoalveolar lavage.[56-59] The use of this peripheral blood lymphocyte proliferation as a diagnostic tool started in the 1980s.[58, 60, 61] In the late 1980s, investigators at National Jewish Health modernized this proliferation test by using a radiolabeled DNA precursor, tritiated thymidine, as a marker of proliferation which enabled better reproducibility and easier detection of proliferation.[2, 5] This test became the modern screening test known as the beryllium lymphocyte proliferation test (BeLPT). The BeLPT along with developments in bronchoalveolar lavage for determination of lymphocyte proliferation in lung cells and the ability to less invasively collect transbronchial biopsy specimens for pathological identification of granulomas using a bronchoscope enabled more definitive diagnosis of asymptomatic beryllium workers. Modern diagnosis of beryllium-related health effects classifies an individual with a

repeatable abnormal BeLPT and no evidence of granulomas on biopsy as beryllium sensitized (BeS). It is currently believed that BeS is a pre-cursor to CBD, with the only longitudinal study suggesting that the risk for BeS patients developing CBD is approximately 6 to 8% per year.[62] CBD is diagnosed as evidence of beryllium exposure and classically, granulomas on biopsy. These new diagnostic tools have allowed the detection of BeS and CBD before symptoms developed and have proven that CBD has continued to occur in modern industry.

Beryllium epidemiology in the BeLPT era

The use of the BeLPT enabled the study of many different beryllium exposed workforces, with the clear conclusion that the original beryllium standard of 2 $\mu g/m^3$ has not eliminated beryllium disease in modern industry. As beryllium epidemiology has progressed with different methods depending on the particular industry, it is best presented as a progression of mounting evidence within each of the industries.

Primary beryllium industry

In the early 1990s, Kreiss et al.[9] performed a cross-sectional study at the primary facility where beryllium metal, alloys, and oxide products are produced in the U.S. This study involved full participation of 627 of the 655 current workers at the facility, excluding the five known cases of CBD. The prevalence of BeS was 6.9% (43/627) while the prevalence of CBD was 4.6% (29/632). High-risk processes were identified including ceramic fabrication and beryllium metal production (pebble plant and vacuum melting). Interestingly, two cases of CBD had worked only in purchasing and accounting. There

were no differences in cumulative beryllium exposure between cases and non-cases. As a result of this lack of exposure-response relationship, extensive particle size selective sampling was performed at this facility to determine whether the relationship might be obscured by using total mass sampling. Kent et al.[12] found significant associations between the mass of particles less than 10 µm and less than 3.5 µm on area samples from work areas with elevated risk of CBD and BeS. Significant associations were also observed for the calculated number and surface area concentration of particles less than 10 µm with work areas with high prevalence of CBD.

Another cross-sectional study at a separate beryllium production facility was completed in 2001.[1] This plant operated from 1957 to 1978 and the best estimate of cumulative employment puts the exposed population at 1,351. A total of 577 former employees completed the BeLPT screening in the study which identified 84 (14.5%) individuals with BeS including 44 (7.6%) individuals with CBD (or probable CBD). There were few findings of exposure-response relationships in this cohort with no differences in cumulative, mean, or peak exposures between CBD cases and normal individuals. Those with BeS were, in fact, identified as having consistently lower cumulative and mean exposures.

Beryllium Ceramics Industry

Kreiss et al.[7] conducted a cross-sectional study at the Tucson beryllium ceramics production facility in 1992 to examine the BeS and CBD rates and exposure response relationships. The activities of workers at this plant included powder pressing, extruding, and machining of fired and unfired ceramic materials. This study involved the entire

current workforce (n=139). Of the 136 employees with BeLPTs, eight (5.9%) were eventually identified as BeS of which six (4.4%) were diagnosed with CBD. Seven of the cases, including five CBD cases and both BeS cases, were involved in production activities and had performed machining operations. The remaining CBD case had worked only in the administrative area of the plant. The study identified a BeS prevalence of 14.3% for employees who had ever been a machinist compared to 1.2% among other employees. Elevated BeS risk was not identified in employees ever performing dry pressing or other forming operations which were considered the dustiest operations at the plant.

Exposure assignments at this facility were produced using a combination of standardized employee interviews, company-provided job histories, and air sampling results. The primary air sampling data available were daily weighted average (DWA) measurements, which are calculated 8-hour average concentrations based on time studies and multiple short, high volume, task-based air samples. There were also a limited number of full-shift personal sampling measurements which showed a correlation of approximately 0.65 with the DWA concentrations. Comparisons between cases and non-cases showed no difference in duration, cumulative or average beryllium exposure. However, examination of exposure differences between machining and other operations revealed that, for the period in which most BeS cases had their initial exposure, machining had significantly higher percentages of personal samples >0.1 $\mu g/m^3$, 1.0 $\mu g/m^3$, and 2 $\mu g/m^3$ compared to other processes in the plant. Calculated DWAs were also significantly higher for machining processes than for other processes. The most important finding of this study was the suggestion that exposures resulting in a 14.3 odds

ratio for BeS among beryllium machinists were mostly below the 2.0 μg/m^3 OSHA standard.[7] Follow-up on this cohort in 2002 revealed additional disease burden with a 17.6% cumulative incidence for BeS and 12.5% for CBD.[63]

Henneberger et al.[64] extended the work of Kreiss et al.[7] at the Tucson ceramics facility in 1998 with a cross-sectional study of all current workers not previously diagnosed with CBD. During the period between this study and the previous Kreiss et al.[7] study, the company had worked to reduce machining exposures by enclosing machines and installing additional ventilation. All current employees as of January of 1998, not previously diagnosed with CBD, were eligible to participate. Of those eligible, 151 of 167 employees participated. Of those participating, roughly half (n=74) were short-term workers hired after the initial 1992 study with all but one of the remaining 77 having participated in the 1992 study. Overall, 9.9% (15/151) of the workers were diagnosed as BeS including 10.4% (8/77) of long-term workers and 9.5% (7/74) short-term workers. Over half (8/15) of those diagnosed with BeS were found to have CBD including 7 long-term workers and 1 short-term worker. Lapping, machining, forming, and packaging were identified as processes with statistically higher prevalences of BeS. Interestingly, risk for BeS in machining operations appeared to be elevated only for long-term workers suggesting that process improvements since the 1992 study may have successfully reduced the risk for short-term workers. Non-significant trends were noted for increases in BeS prevalence with higher peak exposures for long-term workers and mean, cumulative, and peak exposures for short-term workers. The study also identified BeS in workers employed fewer than two years at the facility with mean exposures less than 0.1 μg/m^3, cumulative exposures less than 0.1 μg-year/m^3, and peak exposures less

than 0.4 μg/m^3. Most importantly, this study highlights that company efforts to reduce exposures were not successfully preventing BeS.

Based on the results of the 1998 Henneberger et al. study[64] the management of the beryllium ceramics facility embarked on a full-scale preventative program.[65] This program included numerous ventilation changes and enclosures to reduce airborne beryllium exposures, and extensive new personal protective equipment requirements including the requirement of respirators, long sleeves and gloves in production areas. In addition, administrative changes were implemented to reduce the spread of beryllium contamination to non-production areas. Most of these changes were completed by January of 2000 when medical surveillance of new workers began using the BeLPT. Of the 126 employees hired after January of 2000, 97 were eligible for inclusion in the study having worked for more than three months at the facility and completed two rounds of BeLPT screening. Of the 97, four were identified as BeS by the 2004 cut-off date for study inclusion. Three of the four employees had their first abnormal BeLPT "at hire" or the baseline time point and were not considered in the study's estimated 2004 prevalence of 1.0% (1/97). Including workers with positive baseline BeLPTs would increase the estimated prevalence to 4.1% (4/97). The comparison presented by the authors between the 1.0% prevalence among the workers hired after implementation of the preventative program and the revised prevalence estimate of 8.7% among the workers hired between 1993 and 1998 indicates the prevalence in the earlier cohort is 8.4 (95% CI, 1.04-68.49) times greater than that in the 2000-2004 cohort. This reported reduction in BeS occurred with little change in typical airborne beryllium exposures in production areas (0.20 μg/m^3 vs. 0.18 μg/m^3). However, it is important to note the additional requirement of respiratory

protection for the 2000-2004 workers, which would reduce their actual exposures by approximately a factor of 10. The results of the study suggest a full-scale beryllium control program, including respiratory and skin protection likely reduces the incidence of BeS. However, equally important is that the risk for BeS does not appear to be eliminated by the implementation of such a comprehensive program.

Kreiss et al.[66] performed a cross-sectional screening of a more diverse facility with specific departments where beryllium ceramics work was performed. BeLPT screening and chest radiographs on 505 current and former employees of this facility identified nine new cases of CBD. Screening was performed from 1989 to 1990 and the vast majority of beryllium operations were eliminated by 1976. Thus, due to latency, it is not surprising that all seven individuals identified as BeS were diagnosed with CBD. Prevalences of CBD ranged from 1.8% for all participants to 16% for employees who had worked in dusty dry pressing processes. Multivariate analysis identified odds ratios of 11.4 (95% CI: 2.3-55.5) among those ever working in dry pressing, 8.9 (95% CI: 1.8-42.8) among those ever working in process development, and increasing risk per year working with beryllium (OR: 2.2 at 10 year average). Interestingly, one case of CBD was identified in a worker whose hire date was more than seven years after beryllium operations had been terminated, but had performed dry sweeping in legacy beryllium areas.

Beryllium fabrication: nuclear weapons

One of the largest facilities fabricating beryllium parts was the Rocky Flats nuclear weapons plant located in Arvada, CO and later re-named Rocky Flats

Environmental Technology Site (RFETS). In the first coordinated study at the Rocky Flats plant, Kreiss et al.[2] conducted a cross-sectional study to evaluate the use of the BeLPT in the exposed workforce. The plant medical staff identified 58 workers with current beryllium exposure of which 51 participated in the study. At the time of the study, these 58 workers were considered the only workers at the plant to have significant exposure to beryllium with job titles including production machinist (41.2%), experimental operator (27.5%), experimental machinist (41.2%), engineer (5.9%), tool crib attendant (3.9%), and supervisor (3.9%). Although not specifically mentioned in the paper, a personal communication with one of the authors indicated that all of these workers were assigned to either building 444 or building 865 which were later identified as the two main beryllium production buildings. Among these 51 workers, six (11.8%) were identified as having an abnormal BeLPT, and of these six, four of the five who were clinically evaluated were diagnosed with CBD (7.8%). Compared to the normal workers, those with BeS were significantly older and a longer time period had elapsed since their first exposure to beryllium. This report was important in that it identified a rate of beryllium disease among exposed workers that was nearly twice the rate identified in previous reports. In addition, this study was one of the first to clearly document the importance of screening workers using BeLPT to identify early beryllium disease.

Kreiss et al.[8] performed a second cross-sectional study in the late 1980s at Rocky Flats to determine CBD/BeS prevalence and risk factors among all employees. For this study, the entire currently employed workforce (those previously identified as BeS/CBD were excluded) was given a brief survey regarding beryllium exposure. Of the 3,305 employees who returned the questionnaire, a stratified random sample of 1,247 were

selected to participate in a medical screening program. The stratification was based on answers to questions suggesting different levels of beryllium exposure. The strata included all workers classified as beryllium workers, 10% with no reported exposure, 10% with minimal reported exposure, and 40% with definite exposure. Participation included 863 of the stratified random selection and an additional 32 participants who were undergoing clinical evaluation for a total of 895. Medical screening revealed 18 (2.0%) cases of BeS of which 12 (1.3% of total) had CBD on initial evaluation with an additional 3 individuals who developed CBD over the course of the study for a final CBD rate of 1.7%.

A significant increased prevalence of BeS was identified in machinists (4.7%). Exposure duration was not significantly different between BeS machinists and non-BeS machinists, though BeS machinists reported a significantly longer interval since the first beryllium exposure. For non-machinists, there were no significant differences in the cumulative years in beryllium exposed jobs between BeS individuals and other participants. There did appear to be a consistent pattern of increasing prevalence of BeS by self-reported exposure: no exposure (1.5%), minimal exposure (1.1%), intermittent exposure (2.6%), and consistent exposure (3.4%). Specific tasks identified with increased prevalence of BeS included sawing beryllium (4.7%) and band sawing beryllium (6.0%). Based on multivariate logistic regression analysis, the only significant predictors of BeS were reported to be “overexposure” to beryllium and exposure to beryllium before 1970.[8] This study is important in revising the estimated prevalence of BeS at RFETS of 11.8% [2] exclusively among higher exposed workers at the facility. In addition, the study corroborates the risk of BeS for those in low exposure jobs suggesting an increased role

of host factors and likely genetics. However, the study is limited in identification of high risk jobs and processes due to the low number of cases identified and the resulting problem of small subgroups for analysis.[8]

Stange et al.[67] expanded on the work of Kreiss et al.[8] at the Rocky Flats facility with a much larger cohort of employees. The cohort in this study was obtained through the voluntary plant medical screening program or Beryllium Health Surveillance Program (BHSP) for the time period June 1, 1991 to March 31, 1995. Participation in this program was actively offered to all current and former RFETS employees. In this surveillance program, a total of 4,397 current and former workers were tested with the BeLPT. The initial testing identified 97 new cases of BeS of which 28 had CBD. One or three year serial surveillance of the remaining cohort identified an additional 10 cases of BeS of which one had CBD. Overall, the BeS prevalence at the end of the study (1995) was identified as 2.43% and the CBD prevalence of 0.65%. While the authors did not attempt to identify high risk jobs or processes, the analysis suggests that both BeS and CBD occurred at a wide variety of exposures and in jobs not previously considered high risk such as administrative staff and security guards. The occurrence of cases in buildings and jobs with exposures likely below 2.0 $\mu g/m^3$ continued to suggest that the current OSHA permissible exposure limit was not adequate.

Stange et al. further expanded their work including workers participating in the surveillance program through December 31, 1997, as well as an analysis of exposure factors.[68] This new study included a total of 5,173 current and former workers with verifiable work histories. Initial testing, which overlapped with the previous study [69], identified 172 cases of BeS of which 74 had CBD. Three-year serial BeLPT testing

identified an additional 63 cases of BeS of which 7 had CBD. The overall rate of BeS and CBD was 4.5% including both testing scenarios. For CBD, the estimate was 1.6% which is likely low due to the less than 100% participation in medical evaluation procedures by those identified as BeS. Significant univariate odds ratios indicating increased risk of BeS were identified for individuals who had worked in health physics (2.9, 95% CI: 1.1-7.4), as a beryllium machinist (3.0, 95% CI: 2.0-4.8), and in the construction trades (2.4, 95% CI: 1.5-4.0). A protective odds ratio of 0.3 (95% CI: 0.1-0.8) was associated with ever having worked in security services. The risk of ever having worked in particular buildings was also analyzed with significant increased risk associated with ever working in buildings 334 (1.8, 95% CI: 1.2-2.5), 444 (1.5, 95% CI: 1.2-2.0), 776 (1.3, 95% CI: 1.0-1.7), and 865 (1.5, 95% CI: 1.1-2.1). In multivariate logistic models, work as a beryllium machinist, work in the construction trades, work in health physics, time since hire, and work in building 865 were significant predictors of excess risk. While this report suggested a process-related, exposure-response relationship among beryllium exposed workers, it also identified cases among employees thought to be only minimally exposed. More importantly, there were six cases of BeS and one case of CBD among employees hired since 1990 which is important since stringent controls limiting beryllium exposure to 0.5 $\mu g/m^3$ were implemented at that time.[68]

Sackett et al.[70] examined the prevalences of BeS and CBD among Rocky Flats workers hired after the facility had terminated production in 1989. For this report, the population consisted of 2,381 workers undergoing medical surveillance between October 1998 and August 2002. Hire dates were available for 2,342 participants. The cohort included 1,152 considered production workers as they were hired prior to 1990 and 1,190

considered cleanup workers as they were hired after 1990. A total of 13 workers from the production era were identified as BeS of which two had confirmed CBD. From the cleanup era, a total of seven workers were identified as BeS of which one had confirmed CBD. Eleven of the workers identified with BeS did not complete a full clinical evaluation for CBD. These numbers translate to a BeS prevalence of 1.2% from the production era and 0.6% for the cleanup era, a difference which is not statistically significant. This report provides more evidence on the occurrence of BeS in exposure situations believed to be well controlled. In addition, the report provides one of the few examples where self-reports of accidental beryllium exposure prove to be a significant risk factor (OR 3.6, 95% CI: 1.01-12.68).

Viet et al. [71] conducted a case-control study at the Rocky Flats nuclear weapons facility to evaluate the risk of CBD and BeS as it related to various exposure levels. Seventy-four cases of BeS, 50 CBD cases, and 124 age and smoking status matched controls were enrolled in the study. In order to estimate exposures to study participants, the authors constructed a job exposure matrix (JEM). The only exposure information available for the majority of the relevant time period consisted of fixed airhead samples (FAH) rather than personal samples. These FAH samples were only collected in a single building on the Rocky Flats Complex, building 444 which was the beryllium machine shop. However, exposure at RFETS was not limited to work in building 444; there were several other buildings with beryllium exposure. In order to estimate exposures to participants working in other buildings, a relative weighting scheme was established by study personnel and verified by plant industrial hygienists. This weighting scheme relied on establishing building area factors (BAF) and job factors (JF) relative to the exposure

of a machinist in building 444. These exposure factors were used as multipliers to establish cumulative exposures for all participants using yearly averages from FAH samples in building 444 as the base exposure concentration. For example, a lab technician in building 123 had a building exposure factor of 1 and a job exposure factor of 2, as compared to a machinist in building 444 with a building exposure factor of 10 and a job exposure factor of 10. Cumulative exposures were calculated by summing the yearly FAH mean concentration times the BAF times the JF over the entire work history of a participant. This sum was divided by 100 (10 x 10) to rescale the estimate to μg-years/m^3. A mean exposure was calculated by dividing the cumulative exposure by the number of years worked at the facility.

Based on analyses of the study data, years of employment, cumulative exposure, and mean exposure were all greater for CBD cases as compared to matched controls and compared to BeS cases. These same factors were consistently in the direction of longer duration and higher exposure for BeS cases as compared to controls, but not statistically significant. Multiple logistic regression analysis indicated an odds ratio for cumulative exposure of 6.9 (95% CI: 2.3 – 20.6) for each tenfold increase in log-transformed cumulative exposure and, with an alternative model, an odds ratio of 7.2 (95% CI: 2.2-23.5) for each tenfold increase in log-transformed average exposure. Neither cumulative exposure nor mean exposure were identified as significant predictors of BeS odds.[71]

Additional analysis dividing the CBD cases and controls into five increasing exposure categories showed an increasing proportion of CBD cases with increasing exposure, with the exception of the lowest exposure category (0 to 0.03 μg-years/m^3) suggesting an exposure-response relationship for CBD. A similar trend was not observed

for BeS. There is a lengthy analysis on the implications of this study in terms of establishing an occupational exposure limit for beryllium. After making several assumptions including the differences between FAH samples and personal samples, the authors came to the conclusion that, based on their logistic regression model, approximately 1 in 200 individuals exposed at 2 $\mu g/m^3$ would be expected to get CBD.[71]

This study was the first to clearly show an exposure-response relationship for CBD. However, it appears that their model may not account for a large proportion of cases observed in the lowest exposure category. Thus, the model and exposure-response relationship may not account for an extremely sensitive subpopulation; or alternatively, their JEM may not adequately characterize exposures for this subset. In addition, the study failed to show any significant exposure-response relationship for BeS. Finally, the study provides compelling evidence indicating a higher than traditionally accepted risk (1 in 1,000) of CBD among workers exposed at levels equivalent to OSHA's current permissible exposure limit.[71]

Beryllium fabrication: private industry

Newman et al.[72] began medical surveillance at a beryllium machining facility in the Southeast U.S. in 1995. Initial testing on the 235 current and newly hired employees between 1995 and 1997 identified 15 (6.4%) cases of BeS of which 9 of the 12 completing medical evaluation were found to have CBD. Biannual testing identified an additional 6 BeS individuals of which 5 had CBD. This testing resulting in a point BeS prevalence of 9.4%. This study identified BeS in four (6.7%) of the newly hired employees. All four of these individuals had worked at the facility for less than three

months when tested. Three of the four underwent clinical evaluation and two were found to have CBD. This study was the first to identify CBD cases with this short latency and with fairly low exposures (median < 0.3 μg/m^3). In addition, the study confirmed that serial surveillance of exposed workforces will continue to identify new cases of BeS and CBD.

Kelleher et al.[14] conducted a case-control study at the facility reported by Newman et al. [72]. The cohort for the study consisted of all 235 workers who had undergone serial medical surveillance as reported by Newman et al.[72]. The authors constructed a job exposure matrix for all cases and controls using a combination of work history data and exposure measurements. The exposure measurements consisted of 100 personal impactor samples, four samples per job title, collected in two phases between 1996 and 1999. The impactor measurements were supplemented with historical and current industrial hygiene measurements provided by the facility. While the exposure assessment appears somewhat limited in that there were relatively few measurements before 1995, analyses in the paper suggest that exposures prior to this period were likely similar. The only statistically significant exposure risk factor identified was ever working as a machinist with an estimated univariate odds ratio of 4.4 (95% CI: 1.1-17.6). Other exposure measures, including total exposure, exposure to particles greater than 6 μm, and exposure to particles less than 1 μm, suggest higher exposures (both mean and median) among cases as compared to controls. Unfortunately, it appears that significance of the exposure factors was limited by the low power of the study. Suggestive evidence of higher exposures among cases in addition to the reported 60% of cases with average exposures greater than 0.2 μg/m^3 suggested an exposure-response relationship that may

be confounded by other factors such as particle size and exposure misclassification. Interestingly, no cases were identified at exposures less than 0.02 μg/m^3. This observation may suggest an exposure threshold to prevent sensitization.

Madl et al.[73] reanalyzed the data from Kelleher et al.[14] to identify exposure response relationships and the potential for using the data to establish an occupational exposure limit. For this analysis, the authors used a total of five different methods to reconstruct exposures for all cases listed in the study by Kelleher et al.[14] along with seven additional cases from the facility. The exposure reconstruction methods included using the time-weighted average exposure from the highest year exposed and the lifetime weighted average exposure using different grouping strategies to account for years with sparse or missing data. The authors used a 95th percentile exposure as well as a mean and median. The authors argued that the use of an upper bound exposure and the highest year of exposure rather than a mean exposure is more appropriate for an immune mediated disease and for identifying an appropriate occupational exposure limit. This analysis suggested that all cases of BeS and CBD had 95th percentile exposures that exceeded 0.2 μg/m^3. In terms of lifetime weighted average exposures, the new analysis resulted in increased estimates of exposure for 11 cases and decreased estimates for 9 cases as compared to those reported by Kelleher et al.[14]. The data also suggested higher exposures for CBD cases as compared to BeS cases.

Martyny et al.[13] performed particle size selective air sampling at the beryllium facility in the Southeast U.S. to identify potential particle size effects that might explain the high prevalence of CBD identified among individuals machining beryllium. Cascade impactor sampling on five machining processes indicated that more than 50% of the mass

of particles generated by machining beryllium were less than 10 μm. The typical mass median aerodynamic diameters (MMADs) observed from the five machining processes ranged from 0.6 μm to 3.1 μm, with lathe and deburring having the smallest MMAD. These exposures result in total respiratory depositions ranging from 46% to 62% of the measured exposure. While the size of the exposed cohort limits the power to detect associations between specific operations and disease prevalence, it is possible the predominance of small particles generated from these machining operations may explain the high risk of beryllium machining operations.

Beryllium alloy production and fabrication

While cases of BeS and CBD at 2% beryllium-copper facilities were reported by Balkissoon and Newman[74], as well as by Yoshida et al.[75], full scale epidemiological studies were lacking until Shuler et al. conducted a cross-sectional epidemiological study at a copper-beryllium alloy facility.[76] This study involved 153 of the 185 current employees at the facility and involved a work history interview to describe start and end dates of work in specific processes and time spent performing non-routine high exposure tasks, such as clean-up and shutdown maintenance. Beryllium air sampling results were analyzed for each process including data from 1969 to 2000, with 815 high volume task based samples for years before 1995 and 650 personal samples for years beyond 1995.[76]

Between 10 and 18 cases were identified at the facility depending on the diagnosis criteria including six with CBD (3.9%), 12 with BeS (7.8%), and one with BeS who refused clinical evaluation. In evaluating risk factors for BeS/CBD, the authors chose to exclude eight BeS cases due to lack of confirmatory BeLPT results between two

different laboratories. BeS cases were found to have fewer years since first reported beryllium exposure (Mean=1 yr) as compared to non-sensitized workers. CBD cases were more likely to have reported skin ulcers or small craters in the skin than non-sensitized workers. However, other self-reported skin problems were not more prevalent in either BeS or CBD cases. Workers reporting high exposure incidents without respiratory protection were more likely to be BeS. There were no differences in participation in non-routine activities likely to be associated with high beryllium exposures, such as spill clean-up and shut-down maintenance among non-sensitized, BeS, or CBD individuals.

The study did identify work processes with higher prevalences of CBD and BeS including rod and wire production and more specifically point and chamfer, wire annealing and pickling, and wire drawing. In contrast, work in strip metal production was not associated with higher prevalences of BeS or CBD. Exposures at this copper-beryllium alloy facility were considered to be fairly well controlled with a median plant-wide exposure of 0.02 $\mu g/m^3$ for breathing zone samples and 0.09 $\mu g/m^3$ for general area samples between 1969 and 2000. In fact, 99% of all samples were below 2.0 $\mu g/m^3$ and 93% were below 0.2 $\mu g/m^3$. Exposures in rod and wire production were much higher than those in strip metal production, with medians of 0.06 $\mu g/m^3$ and 0.02 $\mu g/m^3$ respectively. In addition, there were significant differences in the upper tails of the exposure distributions for rod and wire production processes compared to strip metal production processes with upper tolerance limits of 0.68 $\mu g/m^3$ compared to 0.10 $\mu g/m^3$. Exposures for wire annealing and pickling were clearly higher than for any other process with a median of 0.12 $\mu g/m^3$ and an upper tolerance limit of 2.32 $\mu g/m^3$.[76]

This study was surprising in that BeS/CBD prevalences for the copper beryllium facility were similar to disease prevalences observed in facilities with much higher documented exposures. Also, the increased prevalence of BeS among employees with less than one year of beryllium exposure suggested that cumulative exposure may not be a very effective metric in predicting BeS risk. The study clearly demonstrates increased BeS/CBD risk from exposure to processes with upper tolerance limits (UTLs) exceeding 0.2 $\mu g/m^3$, and possibly lower risks from processes where the upper tolerance limits are maintained below 0.2 $\mu g/m^3$. [76]

Stanton et al. [18] examined the prevalence of BeS and CBD in copper beryllium distribution centers. Among 88 employees from three distribution facilities, one was identified with CBD for an overall 1% prevalence. Job specific median air sampling for workers at these facilities ranged from 0.01 to 0.07 $\mu g/m^3$. However, there were several processes where UTL exposures exceeded 0.2 $\mu g/m^3$.

Summary of BeS/CBD epidemiology findings

While there has been much difficultly establishing a clear exposure-response relationship for CBD/BeS, epidemiological studies have consistently identified clear "high risk" exposure surrogates. The most consistent findings are those of particular high risk processes within an industry. These processes have often been identified as those producing smaller particles and sometimes described as having higher exposures than other processes within the plant. CBD/BeS has consistently been identified in a small percentage of those considered to be at low risk including administrative personnel and security guards. BeS/CBD appears to have a variable latency with known cases

presenting as early as three months after first exposure and as late as 20+ years after last exposure. There is a clear literature documenting cases of BeS/CBD at exposures less than the current OSHA Permissible Exposure Level (2 μg/m^3). With one exception[71], cumulative exposures to beryllium have not been predictive of disease risk. Relative exposure assignments involving job specific factors, building specific factors, or self-reported exposure have been more frequently associated with disease risk. The lack of an observed exposure-response may be explained by one of the following alternative explanations: 1) the total mass concentration is not relevant to BeS/CBD development; 2) past exposure assessment strategies have produced exposure misclassification that attenuates the true exposure-response relationship; or 3) there is more than one "true" exposure-response relationship for the population and past studies have been unable to stratify the population into the appropriate sub-groups.

Beryllium health effects and genetics

With the lack of clear exposure-response effects identified in the many epidemiological studies and the identification of BeS/CBD cases at very low apparent exposures, attention shifted to the identification of host factors that may influence the effects of beryllium exposure. Based on *in vitro* immunology studies using cells from patients with CBD, Saltini et al. concluded that T-cells are major histocompatibility complex (MHC) class II restricted, meaning that T-cells only proliferate in the presence of MHC class II molecules on the surface of antigen presenting cells.[77] It has been understood for some time that the MHC class II region of the genome is important in presentation of antigens for T-Cell recognition.[78] The MHC region of the human

genome is the most gene-dense and polymorphic region. The MHC region resides on chromosome 6p21.31 with the class II region residing at the centromeric end. The class II region codes for HLA-DP, HLA-DQ, and HLA-DR molecules which are heterodimeric proteins consisting of α and β chains with the α1 and β1 regions of these chains forming the peptide binding region or "groove" important in antigen presentation. As these HLA molecules had been associated with susceptibility to autoimmune disorders in other studies [(79)] and similar metal/MHC complexes had been shown to elicit T-Cell reactivity with other metals such as nickel and colbalt[(80)], researchers hypothesized that HLA molecules may also be important in the development of BeS/CBD. The working hypothesis was that some yet unknown beryllium "moiety", be it a hapten, antigen, or peptide complex, binds to one or more of these HLA molecules on a yet unknown antigen presenting cell allowing it to interact with receptor T-cells (TCR) initiating T-Cell proliferation and cell-mediated inflammatory processes.

Cross-sectional allele and genotype frequency studies

To investigate this hypothesis, Richeldi et al.[(20)] typed the genes of the MHC class II region (HLA-DR, -DQ, and –DP) from the DNA of 33 cases of CBD and 44 beryllium exposed controls. After preliminary evidence did not show strong associations for the HLA-DR and HLA-DQ genes, the researchers focused on the HLA-DP genes finding that 97% of CBD cases had at least one copy of an HLA-DPB1 encoding allele with a glutamic acid substitution at position 69 (E69) of the β chain as compared to just 30% of the controls. This was the seminal evidence suggesting that this E69 substitution conferred increased susceptibility to CBD. Results of this study have since been verified

by many others (Table I-I) showing similar differences in the proportion of E69 encoding HLA-DPB1 alleles between cases of BeS/CBD and controls. Overall, the studies have found HLA-DPB1 E69 prevalences from 30% to 47% among controls and from 73% to 97% among CBD cases with the differences highly significant for all the populations studied. In terms of BeS, HLA-DPB1 E69 prevalences ranged from 39% to 90% and were significantly different from controls in all but one study.

Table I-I – Frequency of HLA-DPB1 E69 alleles by study and diagnosis

Study	Controls		BeS		CBD	
	N	% E69	N	% E69	N	% E69
Richeldi et al. 1993[20]	44	30%	n/a	n/a	33	97%
Richeldi et al. 1997[21]	121	30%	n/a	n/a	6	83%
Wang et al. 1999[24]	34	45%	n/a	n/a	20	95%
Saltini et al. 2001[19]	93	40%	23	39%	22	73%
Wang et al. 2001[23]	163	38%	25	88%	n/a	n/a
Rossman et al. 2002[25]	82	47%	30	90%	25	84%
Maier et al. 2003[26]	125	38%	50	85%	104	86%
McCanlies et al. 2004[27]	727	33%	64	68%	90	82%

HLA-DPB1 allele specific risk

Other results from these genetic studies suggested differential risk of BeS/CBD even among the different HLA-DPB1 E69 alleles and between E69 homozygotes and heterozygotes. The HLA-DPB1 gene is very polymorphic with approximately 100 different alleles. Further, there are approximately 34 allele variants that encode for E69. Wang et al.[24] first suggested the importance of the rarer non-*0201 HLA-DPB1 E69 alleles in conferring increased risk of CBD. Studying 20 CBD cases and 75 beryllium exposed controls, Wang et al.[24] found that the CBD predictive value of having any E69 allele was 0.35 compared to 0.57 for having at least one copy of a non-*0201 E69 allele,

and 0.85 for having two copies of E69 alleles. Thus, it appears that the risk of CBD is increased for non-*0201 E69 alleles and for additional E69 allele copies. These results were later confirmed to apply to BeS as well as CBD in a study comparing 25 BeS cases to 163 controls which identified an odds ratio of 7.33 (95% CI: 2.95-18.17) for non-*0201 E69 alleles and 9.98 (95% CI: 2.78-35.84) for E69 homozygotes.[23]

The importance of the non-*0201 alleles was confirmed in a study involving 94 CBD cases, 48 BeS cases, and 115 controls which showed increased prevalence of both any E69 allele (BeS:85%, CBD:86%) and non-*0201 E69 alleles (BeS:56%, CBD:63%) compared to controls (any E69: 38%, non-*0201: 14%).[26] In this study, while the odds ratios for any E69 allele (CBD: 10.0, 95% CI:5.0-20.2, BeS: 9.5, 95% CI: 3.9-22.9) were similar to the odds ratios for non-*0201 alleles (CBD: 12.2, 95% CI: 6.1-24.4, BeS: 9.3, 95% CI:4.2 -20.6), the fact that over 65% of the cases of CBD and BeS were carriers of the rarer non-*0201 allele confirms its importance.[26] This study also confirmed the importance of homozygosity, especially for CBD, with an odds ratio of 19.4 (95% CI: 4.4-84.5) for CBD and 8.8 (95% CI: 2.0-39.5) among E69 homozygotes.[26] Another study of similar size (90 CBD, 64 BeS, and 727 controls) showed a similar increased prevalence for E69 homozygotes with odds ratios of 24.3 (95% CI: 10.8-54.6) for CBD and 6.4 (95% CI: 2.1-19.7) for BeS as compared to E69 heterozygotes with odds ratios of 9.4 (95% CI: 5.4-16.6) for CBD and 3.3 (95% CI: 1.8-5.9) for BeS.[27]

Functional significance of HLA-DPB1 E69

With the discovery of the increased susceptibility to BeS and CBD among carriers of the HLA-DPB1 E69 variant, several studies were initiated to determine whether this

variant is just a “marker” of susceptibility to beryllium-related health effects, or whether it is functionally significant. Benchtop immunology studies quickly identified its functional significance, as antibodies to HLA-DPB1 can block the proliferative response to beryllium stimulated T-Cells and reduce cytokine production (TNF-α, INF-γ) which are a class of inflammatory markers important in granulomatous lung disease.[30, 81, 82] It has also been identified that among CBD patients, the E69 marker is clinically important with increased gas exchange abnormalities among those with the E69 marker and significantly reduced forced vital capacity among E69 homozygotes.[26] Molecular modeling of the HLA-DP β chain adds support to the hypothesis that E69 is involved in the antigen binding process predicting the location of E69 in an influential position within the antigen binding groove.[30]

Molecular modeling has also led to new hypotheses about the risks conferred by the *0201 alleles as compared to the non-*0201 alleles. Snyder et al.[32] generated three-dimensional models of HLA-DP proteins encoded by the *0201 and a subset of the non-*0201 alleles. From these models, the electrostatic potential of the antigen binding groove encoded by each of the alleles was calculated. These calculations identified substitutions by charged amino acid residues such as aspartic acid (D), glutamic acid (E), arginine (R), and lysine (K) at positions β55, β56, β69, β84, and β85 affect the electrostatic potential at the antigen or peptide binding groove. It was identified that substitutions in non-*0201 E69 alleles increased the electrostatic potential of the encoded β-chain as compared to *0201 alleles. Analysis of pooled epidemiological data from previous studies, including allele specific typing and case status for 67 CBD cases, 78 BeS cases, and 338 controls using the electrostatic potential on the β-chain encoded by

the allele as a continuous covariate, identified a significant log-linear relationship between the magnitude of the electrostatic charge and the log odds of CBD and BeS with correlation coefficients of -0.85 and -0.67 respectively for CBD and BeS. This very intriguing finding suggests that the risk of CBD/BeS is inversely proportional to the charge on the β-chain of the HLA-DP molecule which greatly strengthens the functional significance of the HLA-DPB1 epidemiological findings.

Snyder et al. 2007[31] further extended the hypotheses regarding the effects of electrostatic charge and allele specific risk. Based on the previous study, researchers identified that the charge on most of the E69 encoding alleles ranged from -7 to -9 with most of the non-*0201 E69 alleles at -9.[32] In extending the hypothesis of increased BeS/CBD risk with decreasing charge, researchers surmised that E69 alleles with a -9 charge confer increased risk of CBD and an increased risk of progressing to CBD among those with BeS as compared to those E69 alleles with a -7 charge. In contrast, the researchers surmised that risk of BeS was approximately equal for E69 alleles with either a -7 or -9 charge. To evaluate these hypotheses, the researchers analyzed allele specific typing data and case status on 84 cases of CBD, 72 cases of BeS, and 698 controls. It was identified that individuals with a -9 charge on their E69 allele had a nearly 3 fold increased risk of developing CBD or progressing to CBD if previously diagnosed with BeS as compared to those with a -7 charge on their E69 allele. In contrast, there was no difference in risk for developing BeS comparing the -9 charged E69 alleles to the -7 charged alleles. By adding the charges for an individual's two alleles, the study also identified a trend showing that increased numbers of HLA molecules encoded with a high negative charge results in increased CBD risk, thereby confirming the higher CBD risk

identified in E69 homozygotes and further suggesting additional increased risk from two copies of high negative charge encoding E69 alleles.

Summary of genetic findings

Evidence from genetic studies have allowed the identification of at least a portion (if not the majority) of the BeS/CBD susceptible sub-population. It is clear that carriers of the HLA-DPB1 E69 variant are at higher risk of both BeS and CBD when exposed to beryllium. This variant appears to be functional in the antigen presentation phase of the immune response to beryllium. It also appears that specific non-*0201 E69 encoding alleles confer increased risk compared to the more common *0201 alleles. This increased risk may be explained by the higher negative electrostatic potential imparted to the antigen binding groove by amino acid substitutions encoded by these E69 alleles. Increasing numbers of E69 containing HLA-DP molecules in E69 homozygotes appears to further increase BeS/CBD risk. However, on the basis of these studies, between 3% and 27% of CBD cases do not have the HLA-DPB1 E69 variant which clearly indicates that there are other important factors, both exposure and host, to be studied. Recently, much of this work has focused on HLA-DR, HLA-DQ, and gene polymorphisms associated with pulmonary inflammation such as TNF-308A. However, these studies are beyond the scope of this dissertation.

Gene-exposure interaction

With evidence suggesting that less than 20% of exposed individuals get CBD even at high modern day exposure levels and conversely suggesting that a smaller

number of individuals seem to be at risk for CBD even at extremely low exposures, it is clear that both exposure and host factors play a role in CBD pathogenesis. Only one study to date has evaluated the combined effect of exposure and genetics in CBD. Richeldi et al.[21] studied a population of 127 workers from the Tucson beryllium ceramics facility including six CBD cases and two BeS cases. As a surrogate for exposure, the researchers separated the cohort into those who had ever performed machining operations (exposures estimated at approximately 0.9 $\mu g/m^3$) and those who had never performed machining operations. The prevalence of CBD among HLA-DPB1 E69 negative individuals was 1/86 (1.2%) compared to 5/41 (12.2%) among those with at least one E69 encoding allele. Comparing case prevalence to exposure, the researchers found the prevalence among those who had ever been a machinist was 5/47 (10.6%) compared to 1/80 among those never assigned to machining. Of the six CBD cases, only one did not have a history of machining exposures. This individual was HLA-DPB1 E69 positive. In multivariate analysis, significant odds ratios were identified for both high exposure (OR 10.1, 95% CI: 1.1-93.7) and for carriers of the HLA-DPB1 E69 variant (OR 11.8, 95% CI: 1.3-108.8). This seminal study in gene-environment interactions has important implications in that it suggests a similar magnitude of increased CBD risk from either high exposure or genetic susceptibility. Unfortunately, the study with only six cases did not have sufficient power to evaluate the combined effect of genetics and exposure. In addition, the study has limitations due to the small population size and the lack of more detailed exposure assessment for the non-machinists.

References

1. **Rosenman, K., V. Hertzberg, C. Rice, M.J. Reilly, J. Aronchick, J.E. Parker et al.:** Chronic beryllium disease and sensitization at a beryllium processing facility. *Environmental Health Perspectives* 113: 1366-1372 (2005).

2. **Kreiss, K., L.S. Newman, M.M. Mroz, and P.A. Campbell:** Screening blood test identifies subclinical beryllium disease. *Journal of Occupational Medicine* 31: 603-608 (1989).

3. **Henneberger, P.K., S.K. Goe, W.E. Miller, B. Doney, and D.W. Groce:** Industries in the United States with airborne beryllium exposure and estimates of the number of current workers potentially exposed. *J Occup Environ Hyg* 1: 648-659 (2004).

4. **Kreiss, K., L.S. Newman, and M. Mroz:** Blood testing for chronic beryllium disease. *Journal of Occupational Medicine* 33: 1188-1189 (1991).

5. **Mroz, M.M., K. Kreiss, D.C. Lezotte, P.A. Campbell, and L.S. Newman:** Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *Journal of Allergy and Clinical Immunology* 88: 54-60 (1991).

6. **Maier, L.A., and L.S. Newman:** Beryllium Disease. In *Environmental and Occupational Medicine*, W.N. Rom (ed.), pp. 1021-1035. Philadelphia, PA: Lippincott-Raven, 1998.

7. **Kreiss, K., M.M. Mroz, L.S. Newman, J. Martyny, and B. Zhen:** Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. *American Journal of Industrial Medicine* 30: 16-25 (1996).

8. **Kreiss, K., M.M. Mroz, B. Zhen, J.W. Martyny, and L.S. Newman:** Epidemiology of beryllium sensitization and disease in nuclear workers. *American Review of Respiratory Disease* 148: 985-991 (1993).

9. **Kreiss, K., M.M. Mroz, B. Zhen, H. Wiedemann, and B. Barna:** Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occupational and Environmental Medicine* 54: 605-612 (1997).

10. **Stefaniak, A.B., M.D. Hoover, G.A. Day, R.M. Dickerson, E.J. Peterson, M.S. Kent et al.:** Characterization of physicochemical properties of beryllium aerosols associated with prevalence of chronic beryllium disease. *J Environ Monit* 6: 523-532 (2004).

11. **McCawley, M.A., M.S. Kent, and M.T. Berakis:** Ultrafine beryllium number concentration as a possible metric for chronic beryllium disease risk. *Applied Occupational and Environmental Hygiene* 16: 631-638 (2001).

12. **Kent, M.S., T.G. Robins, and A.K. Madl:** Is total mass or mass of alveolar-deposited airborne particles of beryllium a better predictor of the prevalence of disease? A preliminary study of a beryllium processing facility. *Applied Occupational and Environmental Hygiene* 16: 539-558 (2001).

13. **Martyny, J.W., M.D. Hoover, M.M. Mroz, K. Ellis, L.A. Maier, K.L. Sheff et al.:** Aerosols generated during beryllium machining. *Journal of Occupational and Environmental Medicine* 42: 8-18 (2000).

14. **Kelleher, P.C., J.W. Martyny, M.M. Mroz, L.A. Maier, A.J. Ruttenber, D.A. Young et al.:** Beryllium particulate exposure and disease relations in a beryllium machining plant. *Journal of Occupational and Environmental Medicine* 43: 238-249 (2001).

15. **Tinkle, S.S., J.M. Antonini, B.A. Rich, J.R. Roberts, R. Salmen, K. DePree et al.:** Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental Health Perspectives* 111: 1202-1208 (2003).

16. **Day, G.A., A. Dufresne, A.B. Stefaniak, C.R. Schuler, M.L. Stanton, W.E. Miller et al.:** Exposure pathway assessment at a copper-beryllium alloy facility. *Annals of Occupational Hygiene* 51: 67-80 (2007).

17. **Day, G.A., A.B. Stefaniak, A. Weston, and S.S. Tinkle:** Beryllium exposure: dermal and immunological considerations. *International Archives of Occupational and Environmental Health* 79: 161-164 (2006).

18. **Stanton, M.L., P.K. Henneberger, M.S. Kent, D.C. Deubner, K. Kreiss, and C.R. Schuler:** Sensitization and chronic beryllium disease among workers in copper-beryllium distribution centers. *Journal of Occupational and Environmental Medicine* 48: 204-211 (2006).

19. **Saltini, C., L. Richeldi, M. Losi, M. Amicosante, C. Voorter, E. van den Berg-Loonen et al.:** Major histocompatibility locus genetic markers of beryllium sensitization and disease. *European Respiratory Journal* 18: 677-684 (2001).

20. **Richeldi, L., R. Sorrentino, and C. Saltini:** HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 262: 242-244 (1993).

21. **Richeldi, L., K. Kreiss, M.M. Mroz, B. Zhen, P. Tartoni, and C. Saltini:** Interaction of genetic and exposure factors in the prevalence of berylliosis. *American Journal of Industrial Medicine* 32: 337-340 (1997).

22. **Stubbs, J., E. Argyris, C.W. Lee, D. Monos, and M.D. Rossman:** Genetic markers in beryllium hypersensitivity. *Chest* 109: 45S (1996).

23. **Wang, Z., G.M. Farris, L.S. Newman, Y. Shou, L.A. Maier, H.N. Smith et al.:** Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology* 165: 27-38 (2001).

24. **Wang, Z., P.S. White, M. Petrovic, O.L. Tatum, L.S. Newman, L.A. Maier et al.:** Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. *Journal of Immunology* 163: 1647-1653 (1999).

25. **Rossman, M.D., J. Stubbs, C.W. Lee, E. Argyris, E. Magira, and D. Monos:** Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *American Journal of Respiratory and Critical Care Medicine* 165: 788-794 (2002).

26. **Maier, L.A., D.S. McGrath, H. Sato, P. Lympany, K. Welsh, R. Du Bois et al.:** Influence of MHC CLASS II in susceptibility to beryllium sensitization and chronic beryllium disease. *Journal of Immunology* 171: 6910-6918 (2003).

27. **McCanlies, E.C., J.S. Ensey, C.R. Schuler, K. Kreiss, and A. Weston:** The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *American Journal of Industrial Medicine* 46: 95-103 (2004).

28. **Gaede, K.I., M. Amicosante, M. Schurmann, E. Fireman, C. Saltini, and J. Muller-Quernheim:** Function associated transforming growth factor-beta gene polymorphism in chronic beryllium disease. *Journal of Molecular Medicine* 83: 397-405 (2005).

29. **Apple, R.J., and H. Erlich:** HLA class II genes: structure and diversity. In *HLA and MHC: genes, molecules and function*, M.J. Browning and A.J. McMichael (eds.). Oxford, United Kingdom: BIOS Scientific Publishers Ltd., 1996.

30. **Fontenot, A.P., M. Torres, W.H. Marshall, L.S. Newman, and B.L. Kotzin:** Beryllium presentation to CD4+ T cells underlies disease-susceptibility HLA-DP alleles in chronic beryllium disease. *Proceedings of the National Academy of Sciences of the United States of America* 97: 12717-12722 (2000).

31. **Snyder, J.A., E. Demchuk, E.C. McCanlies, C.R. Schuler, K. Kreiss, M.E. Andrew et al.:** Impact of negatively charged patches on the surface of MHC class II antigen-presenting proteins on risk of chronic beryllium disease. *J R Soc Interface* 5: 749-758 (2008).

32. **Snyder, J.A., A. Weston, S.S. Tinkle, and E. Demchuk:** Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. *Environmental Health Perspectives* 111: 1827-1834 (2003).

33. **McCanlies, E.C., K. Kreiss, M. Andrew, and A. Weston:** HLA-DPB1 and chronic beryllium disease: a HuGE review. *American Journal of Epidemiology* 157: 388-398 (2003).

34. **Kolanz, M.E.:** Introduction to beryllium: uses, regulatory history, and disease. *Applied Occupational and Environmental Hygiene* 16: 559-567 (2001).

35. **IBC:** "International Beryllium Company: Creating a vertically integrated global beryllium company", 2008.

36. **NIOSH:** "Survey Analysis and Supplemental Tables." 1977.

37. **Cullen, M.R., M.G. Cherniack, and J.R. Kominsky:** Chronic berylium disease in the United States. *Seminars in Respiratory Medicine* 7: 203-209 (1986).

38. **Welch, L., K. Ringen, E. Bingham, J. Dement, T. Takaro, W. McGowan et al.:** Screening for beryllium disease among construction trade workers at Department of Energy nuclear sites. *American Journal of Industrial Medicine* 46: 207-218 (2004).

39. **Kauppinen, T., J. Toikkanen, D. Pedersen, R. Young, W. Ahrens, P. Boffetta et al.:** Occupational exposure to carcinogens in the European Union. *Occupational and Environmental Medicine* 57: 10-18 (2000).

40. **Van Ordstrand, H.S., R. Hughes, and M.G. Carmody:** Chemical pneumonia in workers extracting beryllium oxide. *Cleveland Clinic Quarterly* 10: 10 (1943).

41. **Weber, H.H., and W.E. Englehardt:** Investigation of dusts arising out of beryllium extraction. *Zentr Gewerbehyg Unfallverhut* 10: 41 (1933).

42. **Eisenbud, M., and J. Lisson:** Epidemiological aspects of beryllium-induced nonmalignant lung disease: a 30-year update. *Journal of Occupational Medicine* 25: 196-202 (1983).

43. **Hardy, H.L., and I.R. Tabershaw:** Delayed chemical pneumonitis in workers exposed to beryllium compounds. *J. Industr. Hyg. Toxicol.* 28: 197-211 (1946).

44. **Eisenbud, M., R.C. Wanta, C. Dustan, L.T. Steadman, W.B. Harris, and B.S. Wolf:** Non-occupational berylliosis. *J. Industr. Hyg. Toxicol.* 31: 281-294 (1949).

45. **Lieben, J., and F. Metzner:** Epidemiological findings associated with beryllium extraction. *American Industrial Hygiene Association Journal* 20: 494-499 (1959).

46. **Shilen, J., F. Koppenhaver, F. Cleland, L. Lutz, and V.M. Vought:** Beryllium extraction, reduction, and alloy fabrication. *IMS. Industrial Medicine and Surgery* 23: 291-299 (1954).

47. **Sterner, J.H., and M. Eisenbud:** Epidemiology of beryllium intoxication. *Arch Industr Hyg Occup Med* 4: 123-151 (1951).

48. **Tepper, L.B., H. Hardy, and R.I. Chamberlin:** *Toxicity of Beryllium Compounds*. Amsterdam, Netherlands: Elsevier Publishing Co., 1961.

49. **Freiman, D.G., and H.L. Hardy:** Beryllium disease. The relation of pulmonary pathology to clinical course and prognosis based on a study of 130 cases from the U.S. beryllium case registry. *Human Pathology* 1: 25-44 (1970).

50. **Curtis, D.:** Cutaneous hypersensitivity due to beryllium: a study of thirteen cases. *AMA Archs Dermatol Syph.* 64: 470-482 (1951).

51. **Eisenbud, M.:** Origins of the standards for control of beryllium disease (1947-1949). *Environmental Research* 27: 79-88 (1982).

52. **Eisenbud, M.:** The standard for control of chronic beryllium disease. *Applied Occupational and Environmental Hygiene* 13: 25-31 (1998).

53. **Machle, W., E. Beyer, and F. Gregorius:** Berylliosis. *Occupational Medicine* 5: 671-683 (1948).

54. **OSHA:** "OSHA Preambles (29 CFR 1910.1000). Air Contaminants." 1971.

55. **Hanifin, J.M., W.L. Epstein, and M.J. Cline:** In vitro studies on granulomatous hypersensitivity to beryllium. *Journal of Investigative Dermatology* 55: 284-288 (1970).

56. **Van Ganse, W.F., J. Oleffe, W. Van Hove, and C. Groetenbriel:** Lymphocyte transformation in chronic pulmonary berylliosis. *Lancet* 1: 1023 (1972).

57. **Deodhar, S.D., B. Barna, and H.S. Van Ordstrand:** A study of the immunologic aspects of chronic berylliosis. *Chest* 63: 309-313 (1973).

58. **Williams, W.R., and W.J. Williams:** Comparison of lymphocyte transformation and macrophage migration inhibition tests in the detection of beryllium hypersensitivity. *Journal of Clinical Pathology* 35: 684-687 (1982).

59. **Rossman, M.D., J.A. Kern, J.A. Elias, M.R. Cullen, P.E. Epstein, O.P. Preuss et al.:** Proliferative response of bronchoalveolar lymphocytes to beryllium. A test for chronic beryllium disease. *Annals of Internal Medicine* 108: 687-693 (1988).

60. **Williams, W.R., and W.J. Williams:** Development of beryllium lymphocyte transformation tests in chronic beryllium disease. *International Archives of Allergy and Applied Immunology* 67: 175-180 (1982).

61. **Jones-Williams, W., and W.R. Williams:** Value of the beryllium lymphocyte transformation tests in chronic beryllium disease and potentially exposed workers. *Thorax* 38: 41-44 (1983).

62. **Newman, L.S., M.M. Mroz, R. Balkissoon, and L.A. Maier:** Beryllium sensitization progresses to chronic beryllium disease: a longitudinal study of disease risk. *American Journal of Respiratory and Critical Care Medicine* 171: 54-60 (2005).

63. **Kitt, M.M., P.K. Henneberger, D. Deubner, C.R. Schuler, E. McCanlies, and K. Kreiss:** Cumulative incidence of chronic beryllium disease in a ceramics factory cohort. In 131st Annual Meeting of the American Public Health Association. San Francisco, CA, 2003.

64. **Henneberger, P.K., D. Cumro, D.D. Deubner, M.S. Kent, M. McCawley, and K. Kreiss:** Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. *International Archives of Occupational and Environmental Health* 74: 167-176 (2001).

65. **Cummings, K.J., D.C. Deubner, G.A. Day, P.K. Henneberger, M.M. Kitt, M.S. Kent et al.:** Enhanced preventive programme at a beryllium oxide ceramics facility reduces beryllium sensitisation among new workers. *Occupational and Environmental Medicine* 64: 134-140 (2007).

66. **Kreiss, K., S. Wasserman, M.M. Mroz, and L.S. Newman:** Beryllium disease screening in the ceramics industry. Blood lymphocyte test performance and exposure-disease relations. *Journal of Occupational Medicine* 35: 267-274 (1993).

67. **Stange, A.W., D.E. Hilmas, and F.J. Furman:** Possible health risks from low level exposure to beryllium. *Toxicology* 111: 213-224 (1996).

68. **Stange, A.W., D.E. Hilmas, F.J. Furman, and T.R. Gatliffe:** Beryllium sensitization and chronic beryllium disease at a former nuclear weapons facility. *Applied Occupational and Environmental Hygiene* 16: 405-417 (2001).

69. **Stange, A.W., F.J. Furman, and D.E. Hilmas:** Rocky Flats Beryllium Health Surveillance. *Environmental Health Perspectives* 104S: 981-986 (1996).

70. **Sackett, H.M., L.A. Maier, L.J. Silveira, M.M. Mroz, L.G. Ogden, J.R. Murphy et al.:** Beryllium medical surveillance at a former nuclear weapons facility

during cleanup operations. *Journal of Occupational and Environmental Medicine* 46: 953-961 (2004).

71. **Viet, S.M., J. Torma-Krajewski, and J. Rogers:** Chronic beryllium disease and beryllium sensitization at Rocky Flats: a case-control study. *American Industrial Hygiene Association Journal* 61: 244-254 (2000).

72. **Newman, L.S., M.M. Mroz, L.A. Maier, E.M. Daniloff, and R. Balkissoon:** Efficacy of serial medical surveillance for chronic beryllium disease in a beryllium machining plant. *Journal of Occupational and Environmental Medicine* 43: 231-237 (2001).

73. **Madl, A.K., K. Unice, J.L. Brown, M.E. Kolanz, and M.S. Kent:** Exposure-response analysis for beryllium sensitization and chronic beryllium disease among workers in a beryllium metal machining plant. *J Occup Environ Hyg* 4: 448-466 (2007).

74. **Balkissoon, R.C., and L.S. Newman:** Beryllium copper alloy (2%) causes chronic beryllium disease. *Journal of Occupational and Environmental Medicine* 41: 304-308 (1999).

75. **Yoshida, T., S. Shima, K. Nagaoka, H. Taniwaki, A. Wada, H. Kurita et al.:** A study on the beryllium lymphocyte transformation test and the beryllium levels in working environment. *Industrial Health* 35: 374-379 (1997).

76. **Schuler, C.R., M.S. Kent, D.C. Deubner, M.T. Berakis, M. McCawley, P.K. Henneberger et al.:** Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. *American Journal of Industrial Medicine* 47: 195-205 (2005).

77. **Saltini, C., K. Winestock, M. Kirby, P. Pinkston, and R.G. Crystal:** Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. *New England Journal of Medicine* 320: 1103-1109 (1989).

78. **Roitt, I.:** *Essential Immunology*. Boston: Blackwell Scientific Publications, 1994.

79. **Strominger, J.L.:** Biology of the human histocompatibility leukocyte antigen (HLA) system and a hypothesis regarding the generation of autoimmune diseases. *Journal of Clinical Investigation* 77: 1411-1415 (1986).

80. **Lawrence, D.A., and M.J. McCabe, Jr.:** Immunomodulation by metals. *Int Immunopharmacol* 2: 293-302 (2002).

81. **Sawyer, R.T., C.E. Parsons, A.P. Fontenot, L.A. Maier, M.M. Gillespie, E.B. Gottschall et al.:** Beryllium-induced tumor necrosis factor-alpha production by CD4+ T cells is mediated by HLA-DP. *American Journal of Respiratory Cell and Molecular Biology* 31: 122-130 (2004).

82. **Fontenot, A.P., S.J. Canavera, L. Gharavi, L.S. Newman, and B.L. Kotzin:** Target organ localization of memory CD4(+) T cells in patients with chronic beryllium disease. *Journal of Clinical Investigation* 110: 1473-1482 (2002).

CHAPTER 3

EXPOSURE AND GENETICS IN BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE: A CASE-CONTROL STUDY AT ROCKY FLATS ENVIRONMENTAL TECHNOLOGY SITE

Abstract

The development of beryllium sensitization (BeS) and chronic beryllium disease (CBD) are determined by at least one well-studied host genetic factor, a glutamic acid residue at position 69 (E69) of the HLA-DPB1 gene, as well as exposure to beryllium. However, the nature of the relationship between exposure and carriage of the E69 genotype has not been well studied. The goal of this study was to define the relationship between beryllium exposure and E69 for CBD and BeS.

Former workers (n=386) from a decommissioned U.S. nuclear weapons production facility, Rocky Flats Environmental Technology Site (RFETS, Arvada, CO) were enrolled into a case-control study including 70 with BeS, 61 with CBD, and 255 controls. HLA-DPB1 genotypes were determined by PCR-SSP. Beryllium exposures were reconstructed using a task-based exposure matrix developed from worker interviews and historical beryllium exposure measurements.

CBD cases had significantly higher cumulative exposures (median=1.46 $\mu g/m^3$-years) and lifetime weighted average exposures (median=0.07 $\mu g/m^3$) than either BeS cases (median=0.11 $\mu g/m^3$-years, p=0.001 and 0.01 $\mu g/m^3$, p=0.001) or controls

(median=0.39 μg/m^3-years, p=0.018 and 0.03 μg/m^3, p =0.008). Conversely, BeS cases had lower cumulative exposures (median=0.11 μg/m^3-years) than controls (median=0.39 μg/m^3-years, p=0.034) and a trend toward lower lifetime weighted average exposure (median=0.01 μg/m^3 vs. 0.03 μg/m^3, p=0.064). The E69+ genotype frequency was significantly higher for both BeS cases (92.9%, $p < 0.001$) and CBD cases (83.6%, $p < 0.001$) as compared to controls (38.0%). Analyses that jointly considered E69 genotype and Be exposure showed that carriage of any HLA-DPB1 E69 variant conferred about an eight-fold increased odds for CBD while each unit increase in lifetime weighted average Be exposure increased odds approximately two-fold. Compared to HLA-DPB1 E69 negative genotypes, carriage of a single HLA-DPB1 *02 allele conferred a 12-fold increased odds for BeS (OR: 12.01, 95% CI: 4.28-33.71) and a three-fold increased odds for CBD (OR: 3.46, 95% CI: 1.42-8.43). A single copy of a rarer non-*02 E69 allele conferred a 30-fold increased odds for BeS (OR: 29.54, 95% CI: 10.33-84.53) and a nearly 12-fold increased odds for CBD (OR: 11.97 95% CI: 5.12-28.00) and two E69 allele copies conferred a 55-fold increased odds for BeS (OR: 55.68, 95% CI: 14.80-209.40) and a 22-fold increased odds for CBD (OR: 22.54, 95% CI: 7.00-72.62).

HLA-DPB1 E69 carriage and Be exposure each contribute individually and together contribute additively to the odds of CBD. In contrast, while HLA-DBB1 E69 was identified as a significant predictor of BeS, exposure measures were not significant. The increased odds for both CBD and BeS conferred by carriage of HLA-DPB1 E69 alleles appears to be differentially distributed by genotype with carriers of rarer HLA-DPB1 non-*02 E69 alleles and HLA-DPB1 E69 homozygotes at higher odds than those with the more common HLA-DPB1 *02 genotypes.

Introduction

Beryllium is a low-density metal with unique properties including high specific stiffness, high thermal conductivity, corrosion resistance, dimensional stability, and the ability to reflect neutrons. As a result of these properties, nearly all U.S. nuclear weapons have utilized components fabricated from beryllium. In manufacturing nuclear weapons, workers at U.S. and international facilities have cast, machined, polished, pressed, extruded, welded, and plated beryllium components. A subset of workers exposed to aerosols generated by the fabrication of beryllium containing materials at nuclear weapons sites and other manufacturing facilities have developed beryllium sensitization (BeS) and chronic beryllium disease (CBD). BeS is the demonstration of a cell mediated immune response to beryllium. Specifically, beryllium stimulates T-cell proliferation which occurs via presentation of an unknown beryllium antigen by Human Leukocyte Antigen (HLA) class II molecules on antigen presenting cells (APCs) and is demonstrated clinically with the beryllium lymphocyte proliferation test (BeLPT).[1-4] In some individuals, BeS progresses to CBD, a progressive lung disease characterized by non-caseating granulomas.[5, 6] Progression from BeS to CBD is characterized by a Th1 mediated immune response with accumulation of Be-specific T cells and production of IFN-γ, IL-2 and TNF-α in the lung.[7-10] This immune response results in granulomatous inflammation, and eventually fibrosis.

While the prevalence of BeS and CBD appears to vary depending on the workforce studied and other beryllium exposure characteristics, prevalences of up to 15% for BeS and 8% for CBD have been reported in previous studies of beryllium exposed workforces.[2, 11] The Rocky Flats facility or the Rocky Flats Environmental Technology

Site (RFETS) is one of the largest and best studied beryllium-exposed workforces, located approximately 20 miles west of Denver, CO. Since the late 1950s, workers at this former nuclear weapons facility performed a number of beryllium fabrication operations on a production scale including casting, machining, and welding. A cross-sectional study at RFETS in the late 1980s, limited to employees identified as having significant beryllium exposure (beryllium machinists), identified prevalences of 11.8% for BeS and 7.8% for CBD among the 51 workers studied.[2] Later studies expanding the study population identified estimated prevalences ranging from 2.0% to 4.5% for BeS and 0.65% to 1.3% for CBD.[12-14] These later studies also identified specific risk factors for BeS including machining beryllium [12, 14], work in construction trades [14], and work in specific buildings at RFETS[14]. In general, at RFETS and other facilities, higher beryllium exposures have been associated with higher rates of BeS and CBD. However, the nature of the exposure-response relationship does not appear to be linear with reports of both BeS and CBD consistently documented in individuals with low exposure jobs, such as administrative personnel, security guards, and warehouse workers[12, 15, 16]. The strongest evidence for an exposure-response relationship was identified in a matched case-control study conducted at RFETS in the early 1990s which showed a nearly seven-fold increased odds for CBD for each ten-fold increase in log transformed cumulative beryllium exposure.[17] However, neither cumulative nor mean beryllium exposure were identified as significant predictors for BeS odds in this study.[17] Differences in exposure characteristics have been proposed for the lack of a linear exposure-response identified in most other studies, including different physical or chemical forms of beryllium[18], exposure to smaller particle sizes [19-22], and the failure to account for dermal exposure to

beryllium[23-25]. Recent studies suggest that host factors strongly contribute to the risk of BeS and CBD among workers exposed to beryllium.

In a seminal paper, Richeldi et al.[26] demonstrated that HLA-DPB1 alleles with a glutamatic acid at position 69 of the β chain (E69) were overrepresented in CBD cases (97%, n=33) compared to beryllium exposed controls (30%, n=44). More recent studies, including from our group, have verified these results showing increased frequencies of E69 in cases of not only CBD (61-97%), but also BeS (39-90%), compared to controls (30-47%).[27-34] It has been suggested that differential risk of BeS and CBD is conferred by different E69 containing alleles possibly due to alterations in the encoded antigen binding groove for beryllium produced by different amino acid substitutions. Specifically, the less common or rarer non-*02 E69 alleles have been associated with increased odds for CBD and BeS as compared to the *02 E69 alleles.[28, 31, 34] Copy number of E69 encoding alleles may also be important with E69 homozygotes demonstrating greater odds of BeS and CBD as compared to heterozygotes.[28, 31, 32, 34] Immunology studies have verified the functional significance of E69 to the beryllium immune response as antibodies to HLA-DP block beryllium stimulated proliferation and Th1 cytokine production and mutant fibroblasts without the E69 cannot stimulate proliferation or cytokine from T cells.[35-37] Molecular modeling of the HLA-DP β chain has demonstrated that E69 is located in the antigen binding groove allowing it to directly interact with a putative beryllium antigen.[35] Computational modeling has also suggested that the risk of BeS and CBD may be inversely proportional to the electrostatic potential of the β-chain of the HLP-DP molecule with most of the non-*02 E69 alleles having a

greater negative charge and increased risk of BeS and CBD as compared to *02 E69 alleles.[38, 39]

Studies evaluating the combination of genetic and exposure factors on the odds of BeS and CBD are limited. In one study, work as a machinist (OR 10.1, 95% CI: 1.1-93.7), a surrogate for high beryllium exposure, and carriage of HLA-DPB1 E69 (OR 11.8, 95% CI: 1.3-108.8) were each individually associated with increased odds of CBD in a population of 127 workers in the primary beryllium industry, but based on only six CBD cases.[27] The larger study presented in Chapter 2 of this dissertation evaluated nuclear workers (n=181) including 35 with BeS and 19 with CBD, and also demonstrated increased odds of BeS and CBD associated with E69 carriage (OR 7.41, 95% CI: 2.31-23.75) and for those with reconstructed exposures above the median (OR 5.13, 95% CI: 1.59-16.57). In this larger study, the combination of E69 carriage and high exposure resulted in a additive odds (OR 38.0, 95% CI: 6.02 – 240). Neither of these studies used industrial hygiene measurements to define exposure, nor did they have sufficient power to evaluate the effects of E69 copy number or the odds of specific E69 alleles.

Based on the above studies, we hypothesized that HLA-DPB1 E69 homozygosity and carriage of non-*02 E69 alleles both in combination with higher exposure to beryllium would confer increased odds of BeS and CBD. To address this hypothesis, we undertook a case-control study of a large population of nuclear weapons production workers who were involved in fabrication of beryllium components. In addition to addressing HLA-DPB1 E69 status (including copy number and specific E69 alleles), in this study, we used industrial hygiene exposure data and worker interviews to reconstruct beryllium exposures to address the gene-exposure relationship for BeS and CBD in the

largest single workforce cohort of BeS and CBD cases. The aims of the study were to: 1) verify the additive odds for BeS and CBD conferred by E69 and beryllium exposure; 2) define whether E69 homozygotes and carriage of specific E69 alleles impact the odds of BeS and CBD after adjusting for beryllium exposure, and; 3) define beryllium exposure levels important in the risk of BeS and CBD with implications for worker protection and standard setting.

Methods

Worksite description

The current investigation was conducted among workers from Rocky Flats Environmental Technology Site (RFETS). This facility opened in 1951 for the express purpose of processing and machining plutonium, uranium, and other materials into a detonator or "trigger" for nuclear weapons. A change in weapons design in the late 1950's resulted in the wide-scale use of beryllium in components manufactured at RFETS.[40] Beryllium components were cast, pressed, rolled, machined, treated (plated, coated, etc.), and joined on a production scale at the facility. In addition, research and development operations at RFETS included extensive metallurgical testing, as well as the development of new methods for forming, joining, and machining beryllium. The best estimates indicated that approximately 15,063 production workers and 3,250 construction workers were employed at RFETS during the life of the plant.[41] At least 7,820 of these workers were part of a serial medical surveillance program which included testing with the BeLPT and resulted in at least 117 individuals diagnosed with CBD and 184 with

BeS.[42] In 1993, the site's mission changed to cleanup and closure with the official decommissioning and closure of the facility in 2005.

Study recruitment and design

All workers ever working at RFETS and participating in beryllium medical surveillance were eligible to participate in the study. These workers were recruited using three methods: 1) mailings to all workers potentially exposed to radiation or beryllium informing them of their eligibility for participation in free medical screening at National Jewish Health (NJH); 2) presentations at the RFETS beryllium support group which includes some of the cases of BeS and CBD; 3) clinical recruitment of cases of BeS and CBD through the NJH Occupational Medicine clinic.

Cases were defined as BeS if the individual had two or more abnormal blood BeLPT results and/or an abnormal bronchoalveolar lavage (BAL) BeLPT. Cases of BeS had undergone a medical evaluation and had no evidence of CBD (confirmed BeS) or had not undergone a complete medical evaluation to exclude CBD (unconfirmed BeS). CBD cases were required to have evidence of BeS along with either: 1) pathological evidence of granulomatous inflammation on biopsy; or 2) both an abnormal BAL BeLPT and greater than 15% lymphocytes in BAL fluid. Individuals who had worked at the RFETS facility and had at least two normal BeLPTs with at least one in the last five years, along with no abnormal blood BeLPTs were classified as controls.

A case-control study was undertaken with controls frequency matched approximately two to one to cases based on gender, race, and decade of hire at RFETS to allow for similar exposure potential between cases and controls. The study protocol was

reviewed and approved by the National Jewish Health Institutional Review Board. Written informed consent was obtained from each study participant. Following consent administration, all subjects underwent a blood draw to obtain DNA, signed a HIPAA release to obtain medical surveillance data, and were interviewed using a standardized work history/exposure questionnaire.

DNA extraction and HLA-DPB1 genotyping

Genomic DNA was extracted from peripheral blood and DNA was extracted using the Wizard Genomic Purification kit (Promega, Madison, WI). Genotyping was performed using sequence specific primer-polymerase chain reaction (SSP-PCR) for HLA-DPB1 as described by Gilchrist[43]. Briefly, combinations of forward and reverse allele specific primers were used to genotype each DNA strand, cis and trans, allowing determination of alleles for HLA-DPB1. The HLA-DPB1 typing plates were designed to detect all alleles found at greater than or equal to 1% in Caucasian populations.

Exposure questionnaire

A work history/exposure questionnaire was developed using information from focus groups of RFETS workers. This questionnaire was administered by one of four trained interviewers, with industrial hygiene or exposure assessment experience, using an interview script and work history for each participant to establish start and end dates of each work assignment. Participants were asked to verify the start and end dates for each job assignment (i.e., machinist, chemical operator, electrician, etc.) along with an estimate of the average number of hours worked per week in the job. Calendar time and

hours worked were used to establish an exposure time. The specific tasks (i.e., lathe, grind, plating, cleaning, etc.) performed for each job assignment were also recorded. Participants were asked to categorize each task in into one of seven exposure categories listed in order of decreasing qualitative exposure: 1) directly altering a beryllium part; 2) contact with beryllium waste materials; 3) contact with finished and cleaned beryllium parts; 4) work within 5 feet of a beryllium operation with no direct beryllium contact; 5) work in the same room as a beryllium operation with no direct beryllium contact; 6) work in the same building as a beryllium operation with no direct beryllium contact; or 7) no known beryllium exposure. For each of the tasks, the participant provided a percentage of time spent performing the task and a percentage of time spent performing the task with beryllium.

Task exposure estimates

From the exposure questionnaires, a total of 50 unique combinations of exposure category and task were identified (Table III-I) consisting of 27 with direct beryllium exposure involving direct work with a beryllium part or with beryllium waste materials and 23 with indirect beryllium exposure where there was no direct work with beryllium or beryllium waste materials, but instead proximity to a beryllium operation. Based on published information[17, 44, 45] and personal knowledge about beryllium production at RFETS, the 50 combinations were separated into one to three time periods of similar exposure based on installed controls and plant practices as shown in Table III-I. For each of the unique combinations of exposure type, task, and time period that involved direct

exposure to beryllium, the data sources listed below were searched for industrial hygiene data on the specific task or closely related tasks:

1. **RFETS historical data** consisted of approximately 1,800 samples compiled by researchers in the late 1990's constructing a multiple chemical job exposure matrix[46] in addition to a database of over 1,100 beryllium samples compiled from primary sources for this study. The data spans the years 1954 to 1996 and represents most of the major tasks at Rocky Flats. All of the data prior to 1984 was derived from high-volume, short-term air samples. Post-1984 data were derived from full-shift breathing zone samples of mostly machining tasks with a few shorter term samples of casting tasks.
2. **RFETS machining operations pre- and post-control sampling report[44]** described an analysis of 695 personal breathing zone samples collected from machinists in the primary beryllium machine shop at RFETS. The purpose of this report was to quantify average exposures for RFETS machinists before and after the installation of an upgraded low-volume, high-velocity ventilation system in 1986.
3. **RFETS cleanup era data** consisted of beryllium sampling data from the clean-up, demolition, and decontamination of the facility's two major beryllium related buildings (444 and 865). These data consisted of over 8,000 personal samples spanning the years 1999 to 2008 and provided unparalleled data on exposures for maintenance and cleaning tasks using modern control measures. These data were provided to study investigators after removal of all personally identifiable information by colleagues at Oak Ridge Associated Universities.

4. **Published RFETS summary data** were available from studies by Barnard et al.[45] and Viet et al.[17] including a summary analysis of a random sample of over 12,000 of the 500,000 high-volume, fixed airhead samples collected between 1960 and 1988 in the main RFETS beryllium machine shop. These data likely provide the best estimate of exposure for workers indirectly exposed to beryllium from proximity to beryllium machining tasks.
5. **Other Department of Energy site data** consisted of beryllium sampling data from the Y-12 facility in Oak Ridge, TN where similar beryllium tasks were conducted. This limited dataset contains approximately 1,800 personal breathing zone samples on a limited number of tasks and provides relevant beryllium exposure data for metallurgy, laboratory analysis, inspection, and plasma spray tasks.
6. **International data from another atomic weapons facility** were available for a facility in Cardiff, Wales which performed similar operations to RFETS from a study by Johnson et al.[47]. These data consisted of yearly summaries of 217,000 personal beryllium samples by job task. While many of the job tasks at the Cardiff facility used different controls than similar job tasks at RFETS during the early years, the data were useful for establishing exposure estimates for specific tasks including casting after 1986, beryllium inspection, laboratory analysis, and maintenance.
7. **Beryllium precision machine shop data** consisted of beryllium a sampling dataset from a facility the Southeast U.S. spanning the years 1980 to 2008 with more than 6,340 samples of machining, inspection, deburring, administrative, and

maintenance tasks that were compiled by the authors. Many of the job tasks at this facility were similar to those performed at RFETS. In fact, this facility fabricated some of the same beryllium components as were manufactured at RFETS. Analyses of portions of this dataset have been published by Kelleher et al.[22] and Madl et al.[48].

8. **Other relevant beryllium facility data** were available through published literature and government documents. Exposure data from facilities machining beryllium copper were available from public comments to the OSHA Docket for pending legislation[49]. Data on billet cutting in the primary beryllium industry were also available through the OSHA Docket[50].

An arithmetic mean of the available exposure measurements was calculated for each combination of exposure type, task, and time period using the measurements from available data sources that were considered closest in time period and task composition based on the judgment of the authors. The source of the data used for each task is listed in Table III-I. The arithmetic mean was calculated using one of three methods depending on the data available: 1) If less than six measurements were available or the data were determined to follow a normal distribution based on the D'Agostino-Pearson omnibus normality test, a simple average was calculated. 2) With six or more lognormally distributed measurements, the minimum variance unbiased estimate of the arithmetic mean was calculated using methods outlined in Gilbert [51] when no non-detectable values were present in the dataset, or using a maximum likelihood estimation method as described by Finkelstein and Verma [52] when there were non-detectable values in the dataset. 3) When only median values from summary data were available, an arithmetic

mean was calculated using the relationships outlined in Strom and Stansbury [53] assuming the data followed a lognormal distribution and that the geometric standard deviation was 3 which is within the range described by Wambach [54] for frequently monitored high hazard agents. The arithmetic mean was chosen as an appropriate summary measure for each exposure type, task, and time period combination to allow the calculation of cumulative and lifetime weighted average concentrations.[55]

As very little relevant data were available for indirect beryllium exposure tasks, we used a conservative method for assigning average exposures to these tasks. Reported indirect exposures within 5 ft. of a beryllium task were assigned an average of 50% of the task, those in the same room 10% of the task, and those in the same building 1% of the task. While there are limited data to validate this method, Barnard et al.[45] identified that personal breathing zone samples were on average six to seven times greater than fixed airhead monitors from the same area and time period. This suggests that using a 50% reduction for exposures within 5 feet of direct beryllium operations is likely conservative. Approximately 12% of the indirect exposure tasks reported in the exposure questionnaires could not be linked to specific direct exposure tasks. For these situations, an average estimated from other indirect exposure tasks weighted by the amount of time the entire cohort spent in these tasks was used.

Participant exposure assessment

To summarize participants' varying beryllium exposure work histories, cumulative and lifetime weighted mean beryllium exposure were calculated in units of $\mu g/m^3$-years or $\mu g/m^3$, respectively. First, job specific exposure estimates were

determined for each individual by multiplying the exposure estimate for the combination of exposure category, task, and time period in $\mu g/m^3$ by the percent of time worked with beryllium in that time period, the percent of time performing the task, the number of years spent in the job, and the average number of work hours per week divided by 40 hours. Cumulative exposure was calculated by summing all of the job specific exposures for an individual. Lifetime weighted mean exposure was calculated by dividing the cumulative exposure by the total number of years worked. For each participant, beryllium exposure and work time were included over a work history until the date of employment termination at RFETS or until the date of BeS or CBD diagnosis for the cases, whichever came first. The maximum task-based exposure in $\mu g/m^3$ for any exposure time period was used as a surrogate of short-term high exposure regardless of exposure time. Other exposure metrics used in our analyses were determined directly from the exposure questionnaires including the highest reported exposure category (e.g., directly altering beryllium part, contact with beryllium waste materials, etc.), the year of first beryllium exposure, work with beryllium oxide or as a beryllium machinist, and the percent of an individual's work time at RFETS spent directly or indirectly exposed to beryllium.

Statistical analysis

We used SAS v. 9.1 (SAS Institute, Inc., Cary, NC) for statistical analyses. Univariate tests of association between categorical variables were performed using χ^2 and Fisher's exact tests. P-values from categorical analyses were corrected for multiple comparisons using the Bonferroni method. Due to skewed distributions, continuous variables were compared across the three groups (controls, BeS, and CBD) using the Kruskal-Wallis test

followed by pair-wise comparisons using the Mann-Whitney test when significant ($p \leq 0.05$). Unconditional logistic regression was used to model disease state as a function of multiple predictors, including gene and environment variables. Two strategies for inclusion of genetic variables in logistic regression models were used: 1) carriage of any E69 allele, and 2) an allele specific risk model. The allele specific risk model used a set of classification variables coded as: 1) carriage of only E69 negative alleles; 2) carriage of a single copy of an *02 allele along with an E69 negative allele; 3) carriage of single E69 positive non-*02 allele along with an E69 negative allele; or 4) carriage of two E69 allele copies, one *02 allele and one E69 positive non-*02 allele. For this model, the first variable (E69-) was modeled as the reference. Also in this model, E69 homozygotes with two copies of either *02 alleles (n=10) or E69 positive non-*02 (n=3) were excluded from the analysis due to insufficient numbers to classify these genotypes in separate categories. Cumulative and lifetime weighted mean exposure variables were included in logistic regression models as continuous covariates both to reduce the occurrence of sparse classification cells and to increase power. A purposeful model building strategy[56] was used wherein all independent variables with univariate p-values less than 0.25 were evaluated in multivariate models which included one genetic variable specifying E69 status or genotype and one continuous exposure variable. A significance level of 0.05 was required for a variable to remain in the model. First-order interactions with significance levels at or below 0.1 were included in the final model. All demographic variables were tested in the final model for confounding and included in the model when their presence resulted in a at least a 10% change in any of the estimated regression coefficients. Based on significant differences in exposure between CBD and BeS cases,

as well as between CBD cases and controls, CBD and BeS were modeled as separate outcomes. We included the following E69 genetic variables in our final logistic regression models: 1) carriage of only E69 negative alleles; 2) carriage of a single copy of an *02 allele along with an E69 negative allele; 3) carriage of single E69 positive non-*02 allele along with an E69 negative allele; or 4) carriage of two E69 allele copies, one *02 allele and one E69 positive non-*02 allele. E69 homozygotes with two copies of either *02 alleles or E69 positive non-*02 alleles were excluded from the analysis. Candidate exposure variables for logistic regression models included lifetime weighted average exposure, cumulative exposure, maximum task-based exposure, highest reported exposure category, year of first beryllium exposure, work with beryllium oxide or as a beryllium machinist, and the percent of work time spent directly or indirectly exposed to beryllium.

Results

Study population

A total of 399 individuals were enrolled in this study. Thirteen subjects were excluded for the following reasons: five because they did not meet our criteria for diagnosis of CBD or BeS (two with only one abnormal BeLPT, one diagnosed with sarcoidosis without abnormal BeLPTs, two with insufficient medical information to provide an accurate diagnosis), four with either a diagnosis of BeS or CBD before their hire date at RFETS or with long-term beryllium exposure at a facility other than RFETS, and four whose DNA was unavailable for genotyping. The final cohort consisted of 386 former RFETS workers including 255 controls with potential beryllium exposure, 61

subjects with CBD, 53 subjects with confirmed BeS, and 17 individuals who were classified as unconfirmed BeS due to two abnormal BeLPTs, but who had not undergone a bronchoscopy to rule out CBD.

Demographic characteristics

As shown in Table III-II, there were no significant differences in age, year of hire at RFETS, and the number of years worked at RFETS among the three groups. The cohort was predominately male (88.3%) consistent with the traditionally male dominated workforce at RFETS. However, there were proportionately more female cases of BeS (n=15, 21.4%) compared to controls (n=25, 9.8%, p=0.013) and CBD cases (n=5, 8.2%, p=0.050). While participants were predominately Caucasian (97.7%) and non-Hispanic (93.8%), there was a significantly higher proportion of African-American CBD cases (n=4, 6.6%) compared to controls (n=3, 1.2%, p=0.028). Hispanics were over-represented among BeS cases (n=8, 11.4%) compared to controls (n=11, 4.3%, p=0.039). A trend for increased smoking in BeS cases was apparent, with BeS cases more likely to be current smokers (n=8, 11.4%) compared to controls (n=13, 5.1%, p=0.094) and CBD cases (n=2, 3.3%, p=0.104). BeS cases also worked fewer years at the facility (median=12.6) compared to controls (median=15.0, p=0.020). Among those classified as BeS, there were no differences in age, gender, race, ethnicity, smoking status, year of hire at RFETS, or years spent working at RFETS between those with confirmed BeS and those with unconfirmed BeS (data not shown).

Exposure characteristics

Qualitative exposure characteristics

Qualitative self-reported exposure characteristics are shown in Table III-III comparing cases and controls. Overall, 86.3% of the cohort reported direct or indirect exposure to beryllium and 16.9% of the typical participants' time was spent working in jobs with direct or indirect beryllium exposure. Only 12.4% of the participants had ever worked as a beryllium machinist. CBD cases spent a greater percentage of their time directly exposed to beryllium (median=4.3%) as compared to BeS cases (median=0%, p=0.009) and while the comparison was not significant compared to controls, a trend was apparent (median=1.3%, p=0.061). In addition, CBD cases were more likely to report direct exposure to beryllium (68.9%) as compared to BeS cases (45.7%, p=0.026). Interestingly, BeS cases were less likely than controls to report direct exposure to beryllium (45.7% vs. 62.3%, p=0.039), and there was a trend suggesting BeS cases were more likely to report "no known exposure to beryllium" compared to controls (22.9% vs. 12.5%, p=0.111). Together, these data suggest that CBD cases had spent a greater percentage of their work time directly exposed to beryllium than controls or BeS cases, while BeS cases were less likely to have self-reported direct beryllium exposure than controls.

Reconstructed exposures

Reconstructed beryllium exposures comparing cases and controls are shown in Table III-IV. CBD cases had significantly higher cumulative exposures (median=1.46 $\mu g/m^3$-years) and lifetime weighted average exposures (median=0.072 $\mu g/m^3$) than either

BeS cases (median=0.11 μg/m^3-years, p=0.001 and 0.009 μg/m^3, p=0.001) or controls (median=0.39 μg/m^3-years, p=0.018 and 0.03 μg/m^3, p =0.008). Conversely, BeS cases had lower cumulative exposures (median=0.11 μg/m^3-years) than controls (median=0.39 μg/m^3-years, p=0.034) and a trend toward lower lifetime weighted average exposure (median=0.01 μg/m^3 vs. 0.03 μg/m^3, p=0.064). There were no significant differences in any exposure characteristics between confirmed BeS cases and those classified as unconfirmed BeS (data not shown). Separating the lifetime weighted exposures into quartiles (≤ 0.001 μg/m^3, > 0.001 to ≤ 0.03 μg/m^3, > 0.03 to ≤ 0.17 μg/m^3, and > 0.17 μg/m^3) demonstrated that CBD cases had a higher percentage of subjects with exposures over 0.17 μg/m^3 (41.0%) compared to controls (22.3%, p=0.016). In addition, a greater frequency of CBD cases (32.8%) tended to work in the highest tasks based exposures compared to controls (20.0%, p=0.120). Interestingly, BeS cases were more likely to have maximum task based exposures less than 0.02 μg/m^3 (35.7% compared to 19.2% of controls, p=0.017, and 13.1% of CBD cases, p=0.013).

Genotype characteristics

As shown in Table III-Vb, both BeS cases (92.9%, p < 0.001) and CBD cases (83.6%, p < 0.001) were more likely to carry an E69 compared to controls (38.0%). Cases were also more likely to carry two copies of E69 compared to controls (25.7% of BeS cases and 19.7% of CBD cases vs. 4.3% of controls with p < 0.001 and p=0.02 respectively). After correcting for multiple comparisons, the only significant allele specific differences noted in Table III-Va were that BeS cases were more likely to be carriers of the *0201 and *0601 alleles and CBD cases were more likely to be carriers of

the *0601 allele compared to controls. When combined (Table III-Vb), the rarer non-*02 E69 alleles were present at greater frequency in both BeS cases (55.7%, vs. 14.1% of controls, $p < 0.001$) and CBD cases (60.7% vs. controls, $p < 0.001$). The differences were not nearly as significant when BeS cases with the more common *02 genotype were compared to controls (BeS 52.9% vs. 26.7% of controls, $p=0.005$). Interestingly, the frequency of the *02 genotype in CBD cases (39.3%) did not differ significantly from controls. No significant differences were noted in any genotype frequency between BeS and CBD cases or between confirmed and unconfirmed cases of BeS.

Multivariate analyses

Increasing lifetime weighted exposure and E69 were associated with increased odds of CBD in our logistic regression models. In contrast, while E69 was highly predictive of BeS odds in logistic regression models, beryllium exposure metrics including those representing cumulative, average, and short-term high exposure were not associated with BeS odds. Demographic variables including race, ethnicity, gender, age, and year of hire were not significant predictors or confounders in the odds of BeS or CBD.

The significant multivariate predictors of BeS derived from logistic regression are presented in Table III-VI. The model showed point estimates of increasing odds of BeS with carriage of a single *02 allele (OR: 12.01, 95% CI: 4.28-33.71), carriage of a single rarer non-*02 allele (OR: 29.54, 95% CI: 10.33-84.53), and E69 copy number with one *02 allele plus one non-*02 E69 allele (OR: 55.68, 95% CI: 14.8-209.40). In addition, increased odds of BeS was associated with having worked fewer than five years at the

facility (OR: 2.83, 95% CI: 1.31-6.13). If a more simplistic model is used, adjusting for time spent working at the facility, the carriage of any E69 allele increases the odds of BeS 22-fold (OR: 21.89, 95% CI: 8.43-56.80) (data not shown). Beryllium exposure covariates including lifetime weighted average exposure, cumulative exposure, and or short term maximum task-based exposure were not significant predictors of BeS. Race, ethnicity, gender, smoking status, and first-order interactions also did not contribute significantly nor confound the relationship between BeS and other variables in the model.

Using a similar logistic regression model, multivariate predictors of CBD are shown in Table III-VIIa and Figure 3-1. Of note, carriage of a single *02 allele (OR: 3.46, 95% CI: 1.42-8.43), carriage of a single rarer non-*02 E69 allele (OR: 11.97, 95% CI: 5.12-28.00), and E69 copy number with one *02 allele plus one non-*02 E69 allele (OR: 22.54 95% CI: 7.00-72.62) were associated with increased odds of CBD. In addition, each unit increase in lifetime weighted average beryllium exposure (OR: 2.22, 95% CI: 1.21-4.07) was associated with increased odds of CBD. In a simplified model, carriage of any E69 was associated with a nearly eight-fold increased CBD odds (OR: 7.61, 95% CI: 3.66-15.84) when adjusting for lifetime weighted average exposure (OR: 2.27, 95% CI: 1.26-4.09, data not shown in tables). In an alternate model, cumulative beryllium exposure was also a significant predictor of CBD showing a small (< 10%) increase in CBD odds per unit increase in cumulative exposure (OR: 1.04, 95% CI: 1.00-1.07, data not shown). As lifetime weighted average and cumulative exposure were highly correlated (r=0.91), only a single exposure risk factor could be included in the model. In the lifetime weighted average exposure model, neither excluding an exposure outlier (10.7 $\mu g/m^3$) nor excluding all those exposed above a lifetime weighted average of

2 μg/m^3 impacted the genetic regression coefficients or odds ratios significantly, although the latter did impact the lifetime weighted average estimate, more than doubling the regression coefficient. As with BeS, race, ethnicity, gender, smoking status, and first-order interactions were not significant predictors of CBD nor confounders of other variables and CBD odds.

CBD odds ratio estimates were determined by genetic factor and exposure level to estimate how these varied with increasing exposure. The additive relationship between exposure and genetics is illustrated in Table III-VIIb and Figure 3-1. Figure 3-1 demonstrates the output of the logistic regression model from Table III-VIIa and illustrates that the baseline probability of CBD varies by specific E69 allele or E69 copy number and increases with increasing exposure. Considering the current OSHA Permissible Exposure Level (2.0 μg/m^3), point estimates of CBD odds range from five-fold increase for E69 negative genotypes to a more than 100-fold increase for E69 homozygotes. Table III-VIIb also illustrates that compliance with a reduced exposure level, such as 0.1 μg/m^3, could potentially reduce the odds of CBD nearly five-fold across all levels of E69 status.

Discussion

In the largest case-control study of beryllium exposed workers to date, we evaluated the relationship between quantitative beryllium exposure estimates in combination with HLA DPB1 E69 genotype in risk of BeS and CBD. We noted increased exposure associated with CBD as compared to controls which was evident whether considering self-reported exposure assessments or quantitative exposure reconstructions.

However, no exposure-response relationship was apparent for BeS, even with inclusion of genetic risk factors. For both CBD and BeS, E69 conferred increased odds in the absence and presence of exposure variables as has been shown previously in the study in Chapter 2 of this dissertation as well as other studies.[26-32, 34] We found that odds of BeS and CBD appear to be greater among carriers of the rarer non-*02 HLA-DPB1 E69 alleles, and among HLA-DPB1 E69 homozygotes even after adjusting for beryllium exposure. Most importantly, we found evidence supporting an additive relationship between exposure and genetic susceptibility via E69 in the odds of CBD and provided evidence suggesting an exposure-response for CBD and lack thereof for BeS after adjusting for E69 genetic risk factors.

The finding of an exposure-response relationship for CBD and the additive relationship between exposure and genetics has implications for standard setting in the workplace, at a time when OSHA is reconsidering revising the currently out-of-date beryllium exposure standard. In this study, odds of CBD were associated with higher lifetime weighted average and cumulative exposures, whereas increasing exposure was not a risk factor for BeS. This confirms the previous work by Viet et al.[17] at the RFETS facility showing significant relationships for both cumulative and mean exposures and CBD, but not BeS. Also, confirming previous reports [12, 57, 58], we identified CBD cases at very low apparent exposures with three CBD cases reporting no known beryllium exposure and an additional five reporting never having worked in areas or tasks where reconstructed exposures exceeded 0.02 $\mu g/m^3$. Thus, this study, while clearly showing higher prevalence of CBD at higher exposure levels, fails to demonstrate a threshold for the development of CBD. In contrast, for BeS, there was no evidence of either an

exposure threshold or an exposure-response relationship as evidenced by the tendency for BeS cases to be overrepresented in the lowest lifetime weighted average exposure quartile and with over a third of BeS cases never having worked in areas or processes where reconstructed exposures exceeded 0.02 $\mu g/m^3$. This frequent occurrence of BeS among workers with only minimal known exposure combined with evidence of an exposure-response relationship for CBD has important implications for worker protection both in terms of medical surveillance and removal from exposure. The findings suggest even minimally exposed workers should be screened using the BeLPT to detect BeS and facilitate early removal from exposure and possible prevention of progression to CBD.

This study also confirms the individual contributions of exposure and genetics (E69 status) to the development of CBD. In our current study, carriage of any E69 conferred about an eight-fold increased odds of CBD and each unit increase in lifetime weighted average beryllium exposure increased CBD odds approximately two-fold. This eight-fold increased odds for carriage of any HLA-DPB1 E69 variant is similar to the seven-fold increased odds identified in our previous study at another nuclear weapons plant in Chapter 2 of this dissertation and is within the confidence limits of the 12-fold increased odds from the initial gene-environment study of workers in the primary beryllium industry[27]. In comparing the risks from genetics and exposure, our current findings suggest that, in terms of CBD odds, carriage of any single E69 allele even in extremely low exposures incurs similar odds as exposure to an average beryllium concentration of 4 $\mu g/m^3$ for those without an E69 allele. This eight-fold increased odds for any E69 appears to be differentially distributed when considering E69 genotype, with carriers of only a single copy of the more common *02 alleles only at a three-fold

increased odds, those with the rarer non-*02 genotypes at a nearly 12-fold increased odds, and those with two E69 allele copies at more than 20-fold increased odds. Among those exposed at an average of 0.2 $\mu g/m^3$, which is 10% of the current OSHA Permissible Exposure Level and the current Department of Energy action level, our model suggests those without an E69 allele would be at an approximately 20% increased odds of CBD, those with a single *02 allele would be at a four-fold increased odds of CBD, those with a rarer non-*02 allele would be at a 14-fold increased odds of CBD, and E69 homozygotes would be at a more than 25-fold increased odds of CBD. This additive relationship between HLA-DPB1 E69 status and exposure is also similar to that identified in our previous study in Chapter 2 of this dissertation.

In terms of policy development, exposure reduction has the potential to provide a greater public health benefit than pre-employment genetic testing. As has been presented before[59, 60], the low prevalence of BeS and CBD among those exposed and the high carrier frequency of E69 combine to produce an unacceptable positive predictive value for using the E69 marker to determine eligibility for employment in the beryllium industry. Results from this study continue to support this assertion. From this study, considering the greatest genetic risk factors, non-*02 E69 genotype or E69 homozygosity, for the odds of CBD with an odds ratio of approximately 12 for rare genotype and 22 for homozygosity, and assuming a generous CBD prevalence rate of 5%, a non-*02 genotype frequency of 15%, and a 4% frequency of E69 homozygotes, the positive predictive value of genetic testing is only 23% for non-*02 genotype and only 59% for E69 homozygotes. This low positive predictive value implies that for every 100 individuals denied employment due to this genetic trait, the majority of them would not

have developed CBD which represents unacceptable workplace discrimination. Exposure reduction, on the other hand, reduces the odds for all exposed, regardless of E69 status, and might reduce the progression from BeS to CBD. Based on our models, a reduction in exposure from the current OSHA limit of 2 $\mu g/m^3$ to a new limit of 0.1 $\mu g/m^3$ would result in a nearly five-fold reduction in the odds of CBD for all genetic types.

The models from our case-control study can be extrapolated to project the probability of CBD for workers at RFETS given the facility prevalence of CBD of 1.7% identified in a stratified sample by Kreiss et al.[12], and assuming the population characteristics of the participants in this study are representative of all workers at the site. The proportion of participants with CBD in our model is 19% which is approximately a factor of 10 higher than the facility prevalence. The probability of CBD predicted by our model at a lifetime weighted average exposure of 0.2 $\mu g/m^3$ is 6% for those with E69 negative genotypes, 18% for those with a single *02 allele, 44% for those with a single rarer non-*02 E69 allele, and 58% for E69 homozygotes. Assuming the genotype frequencies of the workers at the entire site are similar to the controls in this study (see Table III-Vb, 62% for E69 negative, 22% for *02 genotypes, 11% for non-*02 E69 genotypes, and 4% for homozygotes), a weighted average probability of CBD can be calculated ([0.62 x 0.06] + [0.22 x 0.18] + [0.11 x 0.44] + [0.04 x 0.58] = 0.15) suggesting across the study population the probability of CBD at 0.2 $\mu g/m^3$ is 15%. This probability can be extrapolated using the factor of 10 to account for the difference in the proportion of CBD cases between the case-control study and the facility as a whole indicating the odds of CBD resulting from a 0.2 $\mu g/m^3$ lifetime weighted exposure is 1.5%. From an occupational exposure limit point of view, this suggests strict compliance

with an exposure limit of approximately 0.8 μg/m^3 (assuming a lognormal distribution and a geometric standard deviation of three) using an upper tolerance limit approach as described by Mulhausen et al.[55] would result in a CBD prevalence of about 1.5% in an exposed population. This estimate is much higher than the 1 in 200 (0.5%) odds of CBD at an occupational exposure limit of 2 μg/m^3 suggested by Viet et al.[17]. Using the same extrapolation process, a lifetime weighted average exposure of 0.02 μg/m^3 and associated 0.08 μg/m^3 occupational exposure limit would reduce the CBD prevalence in an exposed population to approximately 1.3% due to the relatively flat exposure response at the lower end.

Our multivariate model for BeS suggested increased odds for those working fewer than five years at RFETS. This apparent reduced odds was consistent with that identified in our previous study in Chapter 2 of this dissertation showing decreasing odds of CBD and BeS combined with increasing work years. While there have been reports of BeS occurring within a short period of time after first exposure [22, 61, 62] and others have reported similar protective effects in multivariate models [63], this study would likely not detect early BeS as most of the cases were first screened many years after first exposure to beryllium. Only 20% of BeS cases in this study were diagnosed as current workers. These cases were diagnosed on average 17 years after starting work at the facility with all diagnosed more than six years after starting work at the facility. It is more likely this increased odds for short-term workers is an artifact of study design as our study did not include frequency matching for the number of years worked. It is also possible that this effect was a result of the increased participation by long term workers in the control

group. However, this finding suggests that workers exposed for only a short time are at risk of BeS.

Limitations

Exposure misclassification could have impacted our results. However, one of the strengths of the study is its detailed exposure reconstruction in which the use of individual interviews accounts for the large variation of work composition within a single job classification. This attention to exposure at the individual level was likely one of the reasons that this study was able to identify an exposure-response relationship for CBD where others using grouping strategies at the job classification level have failed. In this study, cumulative exposures may have been overestimated for cases as exposures accrued until the date of BeS or CBD diagnosis which was likely much later than the date of disease development. The use of reported time percentages to calculate average and cumulative exposures likely resulted in lower exposure estimates than would have been assigned using methods relying on grouping strategies at the job classification level. The use of industrial hygiene data from other time periods and facilities in the development of the task exposures likely resulted in misclassification on an absolute $\mu g/m^3$ scale, but less misclassification on a relative scale for comparing study participants. In assigning exposure estimates to tasks rather than individuals, the misclassification on both the absolute and relative scale should have been non-differential. In spite of these potential misclassifications, we did find exposure response relationships for CBD in our multivariate models.

Conclusions

This is the largest study to evaluate E69, a well known marker of susceptibility for BeS/CBD, along with exposure in the risk of beryllium related health effects. In this study, E69 carriage and beryllium exposure each contributed individually to the odds of CBD. In contrast, E69 genetic risk factors were highly significant in the prediction of BeS, but reconstructed exposures and exposure surrogates did not contribute to BeS risk. Our results also suggest that increased odds for both CBD and BeS conferred by carriage of E69 alleles appear to be differentially distributed by genotype with carriers of rarer non-*02 E69 alleles and E69 homozygotes at higher odds than those with the more common *02 genotypes. The study demonstrates higher prevalence of CBD at higher exposure levels suggesting an exposure-response relationship, but fails to define a threshold below which disease is not apparent. Future studies will be needed to address interactions with other genes in the HLA region and the effects of exposure on CBD severity as higher exposures may be more important with increasing CBD related impairment. Regardless, this study supports efforts at exposure reduction in the workplace aimed at the most susceptible population, those with the E69 genetic variant. It also supports increased medical surveillance for early detection of BeS and removal from exposure to reduce the risk of progression from BeS to CBD.

Table III-I – Task exposures by time period

Description	Time Period One[a]	Task Exposure Time Period One[b] (µg/m3)	Time Period Two[a]	Task Exposure Time Period Two[b] (µg/m3)	Time Period Three[a]	Task Exposure Time Period Three[b] (µg/m3)	Exposure Data Sources[c]
Assembly/Inspection							
General assembly work with Be parts	'52-'05	0.13					7
Hand polishing or etching Be parts	'52-'85	1.0	'86-'05	0.14			7
Brazing/Welding Be parts	'52-'85	1.32	'86-'05	0.7			1
Inspection or handling of Be parts	'52-'85	0.71	'86-'05	0.15			6
Work within 5 feet of Be inspection operations[d]	'52-'85	0.36	'86-'05	0.075			6[e]
Work in same room as Be inspection operations[d]	'52-'85	0.07	'86-'05	0.015			6[e]
Work in same building as Be inspection operations[d]	'52-'85	0.007	'86-'05	0.0015			6[e]
Machining							
Cutting Be with a band saw	'52-'05	1.78					8
Machining Be parts (mill, lathe, bore)	'52-'74	2.56	'75-'85	1.19	'86-'05	0.052	2
Hand grinding of Be parts	'52-'05	0.56					1
Machine grinding Be parts	'52-'74	3.16	'75-'05	0.56			7
Machining BeCu parts	'52-'05	0.09					8
Work within 5 feet of a Be machining operation[d]	'52-'74	1.28	'75-'85	0.6	'86-'05	0.026	2[e]
Work in same room as a Be machining operation[d]	'52-'74	0.35	'75-'85	0.16	'86-'05	0.007	4
Work in same building as a Be machining operation[d]	'52-'74	0.035	'75-'85	0.016	'86-'05	0.0007	4[e]
Foundry							
Be casting and mold breakout (old foundry,444)	'52-'85	73.0	'86-'05	2.0			1, 6
Be casting and mold breakout (new foundry, 865)	'52-'05	2.0					6
Work within 5 feet of Be casting (old foundry, 444)[d]	'52-'85	36.0	'86-'05	1.0			1[e], 6[e]
Work within 5 feet of Be casting (new foundry, 865)[d]	'52-'05	1					6[e]
Work in same room as Be casting (old foundry, 444)[d]	'52-'85	7.3	'86-'05	0.1			1[e], 6[e]
Work in same room as Be casting (new foundry, 865)[d]	'52-'05	0.2					6[e]

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period.
[b]Specifies the arithmetic mean of the exposure for the task and time period combination
[c]Data sources used to establish exposure estimates for each time period and task combination – see methods section for numbers.
[d]For these tasks, there was only indirect exposure to beryllium.
[e]Specifies source of base data, actual exposure estimates were extrapolated based on the method outlined in the methods section.

Table III-I (continued)– Task exposures by time period

Description	Time Period One[a]	Task Exposure Time Period One[b] (µg/m3)	Time Period Two[a]	Task Exposure Time Period Two[b] (µg/m3)	Time Period Three[a]	Task Exposure Time Period Three[b] (µg/m3)	Exposure Data Sources[c]
Forming							
Hot pressing of Be parts	’52-‘05	1.03					1
Rolling Be parts (sheet rolling)	’52-‘05	0.18					1
Cutting Be using a shear	’52-‘05	1.28					1
Annealing/Heat treating Be parts	’52-‘05	0.2					1, 8
Work within 5 feet of Be rolling/pressing[d]	’52-‘05	0.3					1[e]
Work in same room as Be rolling/pressing[d]	’52-‘05	0.06					1[e]
Work in same building as a Be rolling/pressing[d]	’52-‘05	0.006					1[e]
Laboratory							
Metallurgical testing of Be parts	’52-‘05	0.16					5
Laboratory analysis of Be samples	’52-‘85	0.26	’86-‘05	0.13			6
Work within 5 feet of Be laboratory operation[d]	’52-‘05	0.08					5[e]
Work in same room as a Be laboratory operation[d]	’52-‘05	0.016					5[e]
Work in same building as Be laboratory operation[d]	’52-‘05	0.002					5[e]
Treating/Finishing							
Plating/Chemical milling/Etching beryllium parts	’52-‘05	0.32					7
Operating metal spray/plasma machine with Be	’52-‘05	0.52					5
Grit blasting or sand blasting Be parts	’52-‘05	0.3					1
Work within 5 feet of Be plating/chem... Milling[d]	’52-‘05	0.16					7[e]
Work in same room as a Be plating/chem... Milling[d]	’52-‘05	0.03					7[e]
Work in same building as a Be plating/chem.. Milling[d]	’52-‘05	0.003					7[e]

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period.

[b]Specifies the arithmetic mean of the exposure for the task and time period combination

[c]Data sources used to establish exposure estimates for each time period and task combination – see methods section for numbers.

[d]For these tasks, there was only indirect exposure to beryllium.

[e]Specifies source of base data, actual exposure estimates were extrapolated based on the method outlined in the methods section.

Table III-I (continued)– Task exposures by time period

Description	Time Period One[a]	Task Exposure Time Period One[b] (µg/m3)	Time Period Two[a]	Task Exposure Time Period Two[b] (µg/m3)	Time Period Three[a]	Task Exposure Time Period Three[b] (µg/m3)	Exposure Data Sources[c]
Maintenance and D&D							
Cleaning Be contaminated machines/surfaces	’52-‘85	4.5	‘86-‘94	2.25	‘95-‘05	0.05	1
Maintenance on Be contaminated machines/equipment	’52-‘85	1.0	‘86-‘94	0.18	‘95-‘05	0.04	7, 6, 3
Filter replacement/testing on Be contaminated systems	‘52-‘05	23.9					1
Work in same building as a Maint/D&D operation[d]	’52-‘85	0.045	‘86-‘94	0.023	‘95-‘05	0.0005	3[e]
Waste							
Washing Be contaminated laundry	‘52-‘05	0.3					1
Collecting Be waste materials (chip collecting)	’52-‘85	23.9	’86-‘05	3.3			1, 8
Crushing Be parts/shapes	’52-‘85	36.4	’86-‘05	3.3			1
Be waste packaging/re-packaging	’52-‘85	0.6	’86-‘05	0.31			3
Miscellaneous							
Oversight within 5 feet of unspecified Be activities[d]	’52-‘74	0.93	‘75-‘85	0.42	‘86-‘05	0.06	Wt Avg[f]
Oversight in same room as unspecified Be activities[d]	’52-‘74	0.18	‘75-‘85	0.075	’86-‘05	0.015	Wt Avg[f]
Oversight in same bldg as unspecified Be activities[d]	’52-‘74	0.026	‘75-‘85	0.012	‘86-‘05	0.001	Wt Avg[f]

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period.

[b]Specifies the arithmetic mean of the exposure for the task and time period combination

[c]Data sources used to establish exposure estimates for each time period and task combination – see methods section for numbers.

[d]For these tasks, there was only indirect exposure to beryllium.

[e]Specifies source of base data from numbering in methods section, actual exposure estimates were extrapolated based on the method outlined in the methods section.

[f]Weighted averages of other equivalent operations as outlined in the methods section

Table III-II – Comparison of demographic characteristics among CBD, BeS, and controls

	Total (N=386)	**Controls (N=255)**	**BeS (N=70)**	**CBD (N=61)**	**P-Value**
Median age (range)[a]	67 (41 – 89)	67 (41 – 89)	65 (45 – 84)	65 (49 – 86)	
Gender, n (%)[b]					
Male	341 (88.3%)	230 (90.2%)	55 (78.6%)	56 (91.8%)	
Female	45 (11.7%)	25 (9.8%)[c]	15 (21.4%)[c,e]	5 (8.2%)[e]	0.013[c], 0.050[e]
Race, n (%)[b]					
Caucasian	377 (97.7%)	252 (98.8%)	68 (97.1%)	57 (93.4%)	
African American	9 (2.3%)	3 (1.2%)[d]	2 (2.9%)	4 (6.6%)[d]	0.028[d]
Ethnicity, n (%)[b]					
Hispanic	24 (6.2%)	11 (4.3%)[c]	8 (11.4%)[c]	5 (8.2%)	0.039[c]
Non-Hispanic	362 (93.8%)	244 (95.7%)	62 (88.6%)	56 (91.8%)	
Smoking status, n (%)[b]					
Current	23 (6.0%)	13 (5.1%)[c]	8 (11.4%)[c,e]	2 (3.3%)[e]	0.094[c], 0.104[e]
Former	203 (52.6%)	135 (52.9%)	34 (48.6%)	34 (55.7%)	
Never	160 (41.4%)	107 (42.0%)	28 (40.0%)	25 (41.0%)	
Median year of hire (range)[a]	1969 (1952 - 1998)	1968 (1952 – 1993)	1972 (1952 – 1998)	1969 (1952 – 1990)	
Median years at facility (range)[a]	15.0 (0.2 – 40.7)	15.8 (0.2 – 40.7)[c]	12.6 (0.5 – 33.7)[c]	16.8 (1.0 – 40.0)	0.020[c]

[a]Compared using Kruskal-Wallis test followed by pairwise Mann-Whitney tests when significant
[b]Compared using χ^2 or Fisher's exact method
[c]Comparison between BeS and Controls.
[d]Comparison between CBD and Controls.
[e]Comparison between BeS and CBD.

Table III-III – Comparison of reported exposure characteristics among CBD, BeS, and controls

	Total (N=386)	**Controls (N=255)**	**BeS (N=70)**	**CBD (N=61)**	**P-Value**
Any reported exposure to Be, n (%)[a]	333 (86.3%)	223 (87.4%)[c]	54 (77.1%)[c,e]	56 (91.8%)[e]	0.111[c], 0.093[e]
Median year of 1st Be exposure, (range)[b]	1970 (1952-1996)	1969 (1952-1971)	1976 (1952-1996)	1969 (1953-1990)	
Highest reported Be exposure, n (%)[a]					
Any direct Be exposure	233 (60.4%)	159 (62.3%)[c]	32 (45.7%)[c,e]	42 (68.9%)[e]	0.039[c], 0.026[e]
a. Directly alter Be part	112 (29.0%)	72 (28.2%)	15 (21.4%)[e]	25 (41.0%)[e]	0.066[e]
b. Contact with Be waste materials	90 (23.3%)	63 (24.7%)	13 (18.6%)	14 (23.0%)	
c. Contact with finished Be part	31 (8.0%)	24 (9.4%)	4 (5.7%)	3 (4.9%)	
Any indirect Be exposure	100 (25.9%)	64 (25.1%)	22 (31.4%)	14 (22.9%)	
d. Work within 5 ft. of Be operation	25 (6.5%)	18 (7.1%)	2 (2.9%)	5 (8.2%)	
e. Work in same room as Be operation	17 (4.4%)	12 (4.7%)	4 (5.7%)	1 (1.6%)	
f. Work in same bldg as Be operation	58 (15.0%)	34 (13.3%)[c]	16 (22.9%)[c]	8 (13.1%)	0.183[c]
No known exposure to Be	53 (13.7%)	32 (12.5%)[c]	16 (22.9%)[c,e]	5 (8.2%)[e]	0.111[c], 0.093[e]
Median percent of work time exposed to Be (range)[b]					
Directly (categories a – c above)	1.3% (0-100%)	1.4%(0-95.0%)[c,d]	0% (0-100%)[c,e]	4.3% (0-100%)[d,e]	0.061[d], 0.097[c], 0.009[e]
Indirectly (categories d – f above)	5.2% (0-100%)	6.1% (0-100%)	3.1% (0-100%)	10.0% (0-100%)	
Directly or indirectly	16.9% (0-100%)	15.9% (0-100%)	10.6% (0-100%)	33.6% (0-100%)	
Ever exposed to Be oxide, n (%)[a]	22 (5.7%)	13 (5.1%)	5 (7.1%)	4 (6.6%)	
Ever worked as a Be machinist, n (%)[a]	48 (12.4%)	33 (12.9%)	6 (8.6%)	9 (14.7%)	

[a]Compared using χ^2 or Fisher's exact method, p-values Bonferroni corrected (n=3)
[b]Compared using Kruskal-Wallis test followed by pairwise Mann-Whitney tests when significant
[c]Comparison between BeS and Controls
[d]Comparison between CBD and Controls
[e]Comparison between BeS and CBD

Table III-IV – Comparison of reconstructed exposure characteristics among CBD, BeS, and controls

	Total (N=386)	**Controls (N=255)**	**BeS (N=70)**	**CBD (N=61)**	**P-Value**
Median cumulative Be exposure in $\mu g/m^3$-years (Mean)[b]	0.35 (3.68)	0.39 (2.43)[c,d]	0.11 (2.96)[c,e]	1.46 (9.71)[d,e]	0.034[c], 0.018[d], 0.001[e]
Median lifetime weighted average Be exposure in $\mu g/m^3$ (Mean)[b]	0.03 (0.24)	0.03 (0.15)[c,d]	0.01 (0.25)[c,e]	0.07 (0.64)[d,e]	0.064[c], 0.008[d], 0.001[e]
Lifetime weighted average exposure quartiles, n (%)[a]					
≤ 0.001 $\mu g/m^3$	91 (23.6%)	57 (22.3%)[c]	24 (34.3%)[c,e]	10 (16.4%)[e]	0.132[c], 0.081[e]
> 0.001 to ≤ 0.03 $\mu g/m^3$	109 (28.2%)	74 (29.0%)	21 (30.0%)	14 (22.9%)	
> 0.03 to ≤ 0.17 $\mu g/m^3$	89 (23.1%)	67 (26.3%)[c]	10 (14.3%)[c]	12 (19.7%)	0.117[c]
> 0.17 $\mu g/m^3$	97 (25.1%)	57 (22.3%)[d]	15 (24.4%)[e]	25 (41.0%)[d,e]	0.016[d], 0.066[e]
Maximum task-based exposure, n (%)[a]					
< 0.02 $\mu g/m^3$	82 (21.2%)	49 (19.2%)[c]	25 (35.7%)[c,e]	8 (13.1%)[e]	0.017[c], 0.013[e]
≥ 0.02 and < 0.05 $\mu g/m^3$	30 (7.8%)	17 (6.7%)	7 (10.0%)	6 (9.8%)	
≥ 0.05 and < 0.10 $\mu g/m^3$	7 (1.8%)	5 (2.0%)	2 (2.86%)	0 (0%)	
≥ 0.10 and < 0.20 $\mu g/m^3$	27 (7.0%)	18 (7.1%)	6 (8.6%)	3 (4.9%)	
≥ 0.20 and < 0.50 $\mu g/m^3$	24 (6.2%)	16 (6.3%)	4 (5.7%)	4 (6.6%)	
≥ 0.50 and < 1.0 $\mu g/m^3$	29 (7.5%)	24 (9.4%)	2 (2.9%)	3 (4.9%)	
≥ 1.0 and < 2.0 $\mu g/m^3$	100 (25.9%)	75 (29.4%)[c]	8 (11.4%)[c,e]	17 (27.9%)[e]	0.005[c], 0.075[e]
≥ 2.0 $\mu g/m^3$	87 (22.5%)	51 (20.0%)[d]	16 (22.9%)	20 (32.8%)[d]	0.120[d]

[a]Compared using χ^2 or Fisher's exact method, p-values Bonferroni corrected (n=3)
[b]Compared using Kruskal-Wallis test followed by pairwise Mann-Whitney tests when significant
[c]Comparison between BeS and Controls
[d]Comparison between CBD and Controls
[e]Comparison between BeS and CBD

Table III-Va – Comparison of HLA-DPB1 genotype frequency among CBD, BeS, and controls

HLA-DPB1 Genotype	Controls (N=255)	BeS (N=70)	CBD (N=61)	P-Value[a]
E69 Genotypes				
a. *0201	67 (26.3%)[b]	36 (51.4%)[b]	21 (34.4%)	0.011[b]
b. *0202	1 (0.4%)	2 (2.9%)	3 (4.9%)	
c. *0601	3 (1.2%)[b,c]	9 (12.9%)[b]	11 (18.0%)[c]	0.007[b], < 0.001[c]
d. *0801	0 (0)	1 (1.4%)	0 (0)	
e. *0901	2 (0.8%)	4 (5.7%)	4 (6.6%)	
f. *1001	11 (4.3%)	9 (14.8%)	9 (12.9%)	
g. *1301	14 (5.5%)	5 (8.2%)	7 (10.0%)	
h. *1601	1 (0.4%)	2 (3.3%)	2 (2.9%)	
i. *1701	4 (1.6%)[b,c]	7 (11.5%)[b]	7 (10.0%)[c]	0.226[b], 0.113[c]
j. *1901	1 (0.4%)	0 (0%)	0 (0%)	
Non-E69 Genotypes				
k. *0101	20 (7.8%)	7 (10.0%)	5 (8.2%)	
l. *0301	37 (14.5%)	6 (8.6%)	6 (9.8%)	
m. *0401	187 (73.3%)[b,c]	35 (50.0%)[b]	26 (42.6%)[c]	0.037[b], 0.001[c]
n. *0402	62 (24.3%)[b]	2 (2.9%)[b]	9 (14.8%)	0.001[b]
o. *0501	6 (2.3%)	1 (1.4%)	3 (4.9%)	
p. *1101	8 (3.1%)	1 (1.4%)	2 (3.3%)	
q. *1401	10 (3.9%)	2 (2.9%)	1 (1.6%)	
r. *1501	1 (0.4%)	1 (1.4%)	0 (0%)	
s. *2001	3 (1.2%)	0 (0%)	0 (0%)	
t. *2301	2 (0.8%)	0 (0%)	0 (0%)	

[a]Compared using χ^2 or Fisher's exact method, p-values Bonferroni corrected (n=87)
[b]Comparison of BeS cases to controls
[c]Comparison of CBD cases to controls

Table III-Vb – Comparison of grouped HLA-DPB1 E69 genotype frequency among CBD, BeS, and controls

HLA-DPB1 Genotype	Controls (N=255)	BeS (N=70)	CBD (N=61)	P-Value[a]
Grouped E69 Genotypes				
Any E69 allele	97 (38.0%)[b,c]	65 (92.9%)[b]	51 (83.6%)[c]	< 0.001[b,c]
Any *02	68 (26.7%)[b]	37 (52.9%)[b]	24 (39.3%)	0.007[b]
Single *02 with non-E69	57 (22.3%)	21 (30.0%)	13 (21.3%)	
Any Non-*02	36 (14.1%)[b,c]	39 (55.7%)[b]	37 (60.7%)[c]	< 0.001[b,c]
Single E69+ Non*02 with non-E69	29 (11.4%)[b,c]	26 (37.1%)[b]	26 (42.6%)[c]	< 0.001[b], < 0.001[c]
Any Two E69+ copies	11 (4.3%)[b,c]	18 (25.7%)[b]	12 (19.7%)[c]	< 0.001[b], 0.020[c]
Two E69+ copies (*02 alleles)	4 (1.6%)	5 (7.1%)	1 (1.6%)	
Two E69+ copies (*02 + non-*02)	7 (2.75%)[b,c]	11 (15.7%)[b]	10 (16.4%)[c]	0.017[b], 0.020[c]
Two E69+ copies (Non-*02 alleles)	0 (0%)	2 (2.9%)	1 (1.6%)	

[a]Compared using χ^2 or Fisher's exact method, p-values Bonferroni corrected (n=87)
[b]Comparison of BeS cases to controls
[c]Comparison of CBD cases to controls

Table III-VI – Multivariate logistic regression model for BeS considering HLA-DPB1 E69 genotype

Independent Variables	**Regression Coefficient**	**Standard Error**	**P value**	**OR (95% CI)**
Intercept	-3.72	0.48	< 0.001	
HLA-DPB1 E69 genotype				
HLA-DPB1 $E69^{-}$	Ref			
Single HLA-DPB1*02 allele (with E69- allele)	2.48	0.53	< 0.001	12.01 (4.28-33.71)
Single HLA-DPB1 $E69^{+}$ non *02 allele (with E69- allele)	3.39	0.54	< 0.001	29.54 (10.33-84.53)
E69 homozygote (*02 + non-*02 E69+)	4.01	0.68	< 0.001	55.68 (14.80-209.40)
Worked less than 5 years at RFETS	1.04	0.39	0.008	2.83 (1.31-6.13)

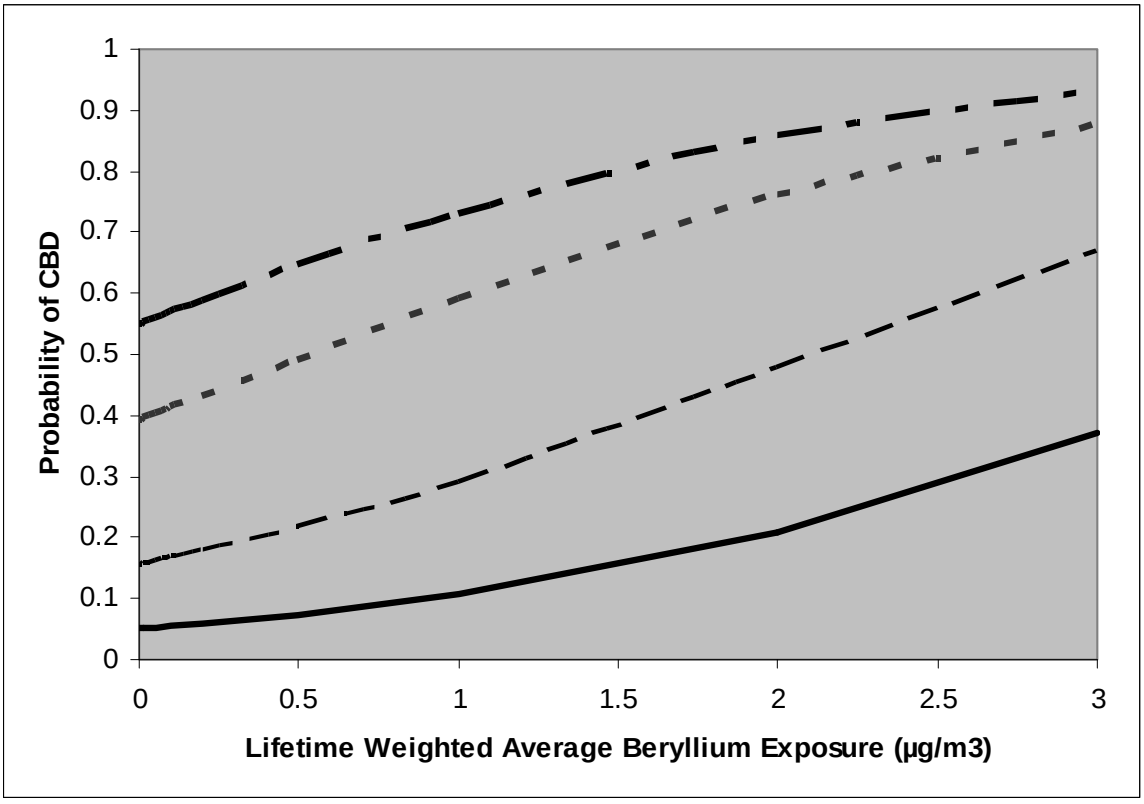
Table III-VIIa– Multivariate logistic regression model for CBD considering HLA-DPB1 E69 genotype and exposure

Independent Variables	**Regression Coefficient**	**Standard Error**	**P value**	**OR (95% CI)**
Intercept	-2.92	0.34	< 0.001	
HLA-DPB1 $E69^-$ genotype				
HLA-DPB1 $E69^-$	Ref			
Single HLA-DPB1*02 allele (with E69- allele)	1.24	0.45	0.006	3.46 (1.42-8.43)
Single HLA-DPB1 $E69^+$ non *02 allele (with E69- allele)	2.48	0.43	< 0.001	11.97 (5.12-28.00)
E69 homozygote (*02 + non-*02 E69+)	3.11	0.60	< 0.001	22.54 (7.00-72.62)
Per unit increase in lifetime weighted average Be exposure ($\mu g/m^3$)	0.80	0.31	0.010	2.22 (1.21-4.07)

Table III-VIIb – Odds ratio estimates by beryllium exposure and HLA DPB1 E69 genotype for odds of CBD

Lifetime weighted average beryllium exposure ($\mu g/m^3$)	**HLA-DPB1 $E69^-$ genotype**	**Single *02 (with E69- allele)**	**Single $E69^+$ non-*02 (with E69- allele)**	**E69 homozygote (*02 + non-*02 E69+)**
0.02 $\mu g/m^3$	1.02 (1.00-1.03)	3.52 (1.45-8.57)	12.16 (5.20-28.45)	22.90 (7.11-73.83)
0.05 $\mu g/m^3$	1.04 (1.01-1.07)	3.61 (1.48-8.77)	12.45 (5.32-29.14)	23.46 (7.27-75.69)
0.10 $\mu g/m^3$	1.08 (1.02-1.15)	3.75 (1.54 -9.14)	12.96 (5.53-30.37)	24.41 (7.55-78.96)
0.20 $\mu g/m^3$	1.18 (1.04-1.32)	4.06 (1.66-9.95)	14.03 (5.95-33.07)	26.43 (8.11-86.11)
0.50 $\mu g/m^3$	1.49 (1.10-2.02)	5.16 (2.02-13.16)	17.82 (7.25-43.81)	33.56 (9.90-113.76)
1.0 $\mu g/m^3$	2.22 (1.21-4.07)	7.68 (2.63-22.43)	26.52 (9.38-75.02)	49.95 (13.07-190.88)
2.0 $\mu g/m^3$	4.91 (1.46-16.56)	17.01 (3.80-76.17)	58.77 (13.43-257.2)	110.7 (19.78-619.3)

Figure 3-1 – Predicted probability of CBD by E69 genotype and lifetime weighted average beryllium exposure



Legend

HLA-DPB1 E69- genotype

Single HLA-DPB1 *02 allele

Single HLA-DPB1 E69+ non-*02 allele

E69 homozygote (*02 + non-*02 E69+)

References

1. **Kreiss, K., L.S. Newman, and M. Mroz:** Blood testing for chronic beryllium disease. *Journal of Occupational Medicine* 33: 1188-1189 (1991).

2. **Kreiss, K., L.S. Newman, M.M. Mroz, and P.A. Campbell:** Screening blood test identifies subclinical beryllium disease. *Journal of Occupational Medicine* 31: 603-608 (1989).

3. **Mroz, M.M., K. Kreiss, D.C. Lezotte, P.A. Campbell, and L.S. Newman:** Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *Journal of Allergy and Clinical Immunology* 88: 54-60 (1991).

4. **Saltini, C., K. Winestock, M. Kirby, P. Pinkston, and R.G. Crystal:** Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. *New England Journal of Medicine* 320: 1103-1109 (1989).

5. **Mroz, M.M., L.A. Maier, M. Strand, L. Silviera, and L.S. Newman:** Beryllium lymphocyte proliferation test surveillance identifies clinically significant beryllium disease. *American Journal of Industrial Medicine* 52: 762-773 (2009).

6. **Newman, L.S., M.M. Mroz, R. Balkissoon, and L.A. Maier:** Beryllium sensitization progresses to chronic beryllium disease: a longitudinal study of disease risk. *American Journal of Respiratory and Critical Care Medicine* 171: 54-60 (2005).

7. **Pott, G.B., B.E. Palmer, A.K. Sullivan, L. Silviera, L.A. Maier, L.S. Newman et al.:** Frequency of beryllium-specific, TH1-type cytokine-expressing CD4+ T cells in patients with beryllium-induced disease. *Journal of Allergy and Clinical Immunology* 115: 1036-1042 (2005).

8. **Tinkle, S.S., L.A. Kittle, B.A. Schumacher, and L.S. Newman:** Beryllium induces IL-2 and IFN-gamma in berylliosis. *Journal of Immunology* 158: 518-526 (1997).

9. **Tinkle, S.S., and L.S. Newman:** Beryllium-stimulated release of tumor necrosis factor-alpha, interleukin-6, and their soluble receptors in chronic beryllium disease. *American Journal of Respiratory and Critical Care Medicine* 156: 1884-1891 (1997).

10. **Maier, L.A., R.T. Sawyer, R.A. Bauer, L.A. Kittle, P. Lympany, D. McGrath et al.:** High beryllium-stimulated TNF-alpha is associated with the -308 TNF-alpha promoter polymorphism and with clinical severity in chronic beryllium disease. *American Journal of Respiratory and Critical Care Medicine* 164: 1192-1199 (2001).

11. **Rosenman, K., V. Hertzberg, C. Rice, M.J. Reilly, J. Aronchick, J.E. Parker et al.:** Chronic beryllium disease and sensitization at a beryllium processing facility. *Environmental Health Perspectives* 113: 1366-1372 (2005).

12. **Kreiss, K., M.M. Mroz, B. Zhen, J.W. Martyny, and L.S. Newman:** Epidemiology of beryllium sensitization and disease in nuclear workers. *American Review of Respiratory Disease* 148: 985-991 (1993).

13. **Stange, A.W., D.E. Hilmas, and F.J. Furman:** Possible health risks from low level exposure to beryllium. *Toxicology* 111: 213-224 (1996).

14. **Stange, A.W., D.E. Hilmas, F.J. Furman, and T.R. Gatliffe:** Beryllium sensitization and chronic beryllium disease at a former nuclear weapons facility. *Applied Occupational and Environmental Hygiene* 16: 405-417 (2001).

15. **Stanton, M.L., P.K. Henneberger, M.S. Kent, D.C. Deubner, K. Kreiss, and C.R. Schuler:** Sensitization and chronic beryllium disease among workers in copper-beryllium distribution centers. *Journal of Occupational and Environmental Medicine* 48: 204-211 (2006).

16. **Kreiss, K., M.M. Mroz, L.S. Newman, J. Martyny, and B. Zhen:** Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. *American Journal of Industrial Medicine* 30: 16-25 (1996).

17. **Viet, S.M., J. Torma-Krajewski, and J. Rogers:** Chronic beryllium disease and beryllium sensitization at Rocky Flats: a case-control study. *American Industrial Hygiene Association Journal* 61: 244-254 (2000).

18. **Stefaniak, A.B., M.D. Hoover, G.A. Day, R.M. Dickerson, E.J. Peterson, M.S. Kent et al.:** Characterization of physicochemical properties of beryllium aerosols associated with prevalence of chronic beryllium disease. *J Environ Monit* 6: 523-532 (2004).

19. **McCawley, M.A., M.S. Kent, and M.T. Berakis:** Ultrafine beryllium number concentration as a possible metric for chronic beryllium disease risk. *Applied Occupational and Environmental Hygiene* 16: 631-638 (2001).

20. **Kent, M.S., T.G. Robins, and A.K. Madl:** Is total mass or mass of alveolar-deposited airborne particles of beryllium a better predictor of the prevalence of disease? A preliminary study of a beryllium processing facility. *Applied Occupational and Environmental Hygiene* 16: 539-558 (2001).

21. **Martyny, J.W., M.D. Hoover, M.M. Mroz, K. Ellis, L.A. Maier, K.L. Sheff et al.:** Aerosols generated during beryllium machining. *Journal of Occupational and Environmental Medicine* 42: 8-18 (2000).

22. **Kelleher, P.C., J.W. Martyny, M.M. Mroz, L.A. Maier, A.J. Ruttenber, D.A. Young et al.:** Beryllium particulate exposure and disease relations in a beryllium machining plant. *Journal of Occupational and Environmental Medicine* 43: 238-249 (2001).

23. **Tinkle, S.S., J.M. Antonini, B.A. Rich, J.R. Roberts, R. Salmen, K. DePree et al.:** Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental Health Perspectives* 111: 1202-1208 (2003).

24. **Day, G.A., A. Dufresne, A.B. Stefaniak, C.R. Schuler, M.L. Stanton, W.E. Miller et al.:** Exposure pathway assessment at a copper-beryllium alloy facility. *Annals of Occupational Hygiene* 51: 67-80 (2007).

25. **Day, G.A., A.B. Stefaniak, A. Weston, and S.S. Tinkle:** Beryllium exposure: dermal and immunological considerations. *International Archives of Occupational and Environmental Health* 79: 161-164 (2006).

26. **Richeldi, L., R. Sorrentino, and C. Saltini:** HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 262: 242-244 (1993).

27. **Richeldi, L., K. Kreiss, M.M. Mroz, B. Zhen, P. Tartoni, and C. Saltini:** Interaction of genetic and exposure factors in the prevalence of berylliosis. *American Journal of Industrial Medicine* 32: 337-340 (1997).

28. **Wang, Z., P.S. White, M. Petrovic, O.L. Tatum, L.S. Newman, L.A. Maier et al.:** Differential susceptibilities to chronic beryllium disease contributed by

different Glu69 HLA-DPB1 and -DPA1 alleles. *Journal of Immunology* 163: 1647-1653 (1999).

29. **Saltini, C., L. Richeldi, M. Losi, M. Amicosante, C. Voorter, E. van den Berg-Loonen et al.:** Major histocompatibility locus genetic markers of beryllium sensitization and disease. *European Respiratory Journal* 18: 677-684 (2001).

30. **Rossman, M.D., J. Stubbs, C.W. Lee, E. Argyris, E. Magira, and D. Monos:** Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *American Journal of Respiratory and Critical Care Medicine* 165: 788-794 (2002).

31. **Maier, L.A., D.S. McGrath, H. Sato, P. Lympany, K. Welsh, R. Du Bois et al.:** Influence of MHC CLASS II in susceptibility to beryllium sensitization and chronic beryllium disease. *Journal of Immunology* 171: 6910-6918 (2003).

32. **McCanlies, E.C., J.S. Ensey, C.R. Schuler, K. Kreiss, and A. Weston:** The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *American Journal of Industrial Medicine* 46: 95-103 (2004).

33. **Gaede, K.I., M. Amicosante, M. Schurmann, E. Fireman, C. Saltini, and J. Muller-Quernheim:** Function associated transforming growth factor-beta gene polymorphism in chronic beryllium disease. *Journal of Molecular Medicine* 83: 397-405 (2005).

34. **Wang, Z., G.M. Farris, L.S. Newman, Y. Shou, L.A. Maier, H.N. Smith et al.:** Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology* 165: 27-38 (2001).

35. **Fontenot, A.P., M. Torres, W.H. Marshall, L.S. Newman, and B.L. Kotzin:** Beryllium presentation to CD4+ T cells underlies disease-susceptibility HLA-DP alleles in chronic beryllium disease. *Proceedings of the National Academy of Sciences of the United States of America* 97: 12717-12722 (2000).

36. **Sawyer, R.T., C.E. Parsons, A.P. Fontenot, L.A. Maier, M.M. Gillespie, E.B. Gottschall et al.:** Beryllium-induced tumor necrosis factor-alpha production by CD4+ T cells is mediated by HLA-DP. *American Journal of Respiratory Cell and Molecular Biology* 31: 122-130 (2004).

37. **Fontenot, A.P., S.J. Canavera, L. Gharavi, L.S. Newman, and B.L. Kotzin:** Target organ localization of memory CD4(+) T cells in patients with chronic beryllium disease. *Journal of Clinical Investigation* 110: 1473-1482 (2002).

38. **Snyder, J.A., E. Demchuk, E.C. McCanlies, C.R. Schuler, K. Kreiss, M.E. Andrew et al.:** Impact of negatively charged patches on the surface of MHC class II antigen-presenting proteins on risk of chronic beryllium disease. *J R Soc Interface* 5: 749-758 (2008).

39. **Snyder, J.A., A. Weston, S.S. Tinkle, and E. Demchuk:** Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. *Environmental Health Perspectives* 111: 1827-1834 (2003).

40. **Chemrisk:** "Reconstruction of Historical Rocky Flats Operations and Identification of Release Points, Project Task 3 & 4 for Phase I, Final Draft Report, prepared for Colorado Department of Public Health and Environment". Alameda, CA, 1992.

41. **U.S. Department of Energy, O.o.H., Safety and Security:** "The Department of Energy Former Worker Medical Surveillance Program". Washington, D.C., 2008.

42. **Stange, A.W., F.J. Furman, and D.E. Hilmas:** The beryllium lymphocyte proliferation test: Relevant issues in beryllium health surveillance. *American Journal of Industrial Medicine* 46: 453-462 (2004).

43. **Gilchrist, F.C., M. Bunce, P.A. Lympany, K.I. Welsh, and R.M. du Bois:** Comprehensive HLA-DP typing using polymerase chain reaction with sequence-specific primers and 95 sequence-specific primer mixes. *Tissue Antigens* 51: 51-61 (1998).

44. **Richen, M.J.:** Surprises during beryllium exposure and operation assessment at Rocky Flats 1984-1986. In U.S. Department of Energy Occupational Exposure Assessment and Chronic Beryllium Disease Prevention Program Implementation Combined Workshop. Northglenn, Colorado, 1997.

45. **Barnard, A.E., J. Torma-Krajewski, and S.M. Viet:** Retrospective beryllium exposure assessment at the Rocky Flats Environmental Technology Site. *American Industrial Hygiene Association Journal* 57: 804-808 (1996).

46. **Ruttenber, A.J., M. Schonbeck, J. McCrea, D. McClure, and J. Martyny:** Improving estimates of exposures for epidemiologic studies of plutonium workers. *Occupational Medicine* 16: 239-258 (2001).

47. **Johnson, J.S., K. Foote, M. McClean, and G. Cogbill:** Beryllium Exposure Control Program at the Cardiff Atomic Weapons Establishment in the United Kingdom. *Applied Occupational and Environmental Hygiene* 16: 619-630 (2001).

48. **Madl, A.K., K. Unice, J.L. Brown, M.E. Kolanz, and M.S. Kent:** Exposure-response analysis for beryllium sensitization and chronic beryllium disease among workers in a beryllium metal machining plant. *J Occup Environ Hyg* 4: 448-466 (2007).

49. **OSHA:** "H005C-6-9-5-8. U.S. Department of Labor, Occupational Safety and Health Administration. Beryllium Docket No. H005C. Exhibit No. 6-9-5-8. Docket Title: Attachment 2.7 Facilities Machining Copper Beryllium. Comments received in response to Federal Register of November 26, 2002." 2002.

50. **OSHA:** "H005C-6-9-5-3. U.S. Department of Labor, Occupational Safety and Health Administration. Beryllium Docket No. H005C. Exhibit No. 6-9-5-3. Docket Title: Attachment 2.1 Primary Beryllium Manufacturing and Processing Facility. Comments received in response to Federal Register of November 26, 2002." 2002.

51. **Gilbert, R.O.:** *Statistical Methods for Environmental Pollution Monitoring*. New York: Van Nostrand Reinhold, 1987.

52. **Finkelstein, M.M., and D.K. Verma:** Exposure estimation in the presence of nondetectable values: another look. *Aihaj* 62: 195-198 (2001).

53. **Strom, D.J., and P.S. Stansbury:** Determining parameters of lognormal distributions from minimal information. *Aihaj* 61: 877-880 (2000).

54. **Wambach, P.F.:** Variation in exposure levels for high hazard frequently monitored agents. *American Industrial Hygiene Association Journal* 63: 424-429 (2002).

55. **Mulhausen, J.R., and J. Damiano:** Quantitative exposure data: Interpretation, decision making, and statistical tools. In *A Strategy for Assessing and Managing*

Occupational Exposures, pp. 117-150. Fairfax, VA: AIHA Press, American Industrial Hygiene Association, 1998.

56. **Hosmer, D.W., and S. Lemeshow:** *Applied Logistic Regression*. New York: John Wiley & Sons, Inc., 2000.

57. **Stange, A.W., F.J. Furman, and D.E. Hilmas:** Rocky Flats Beryllium Health Surveillance. *Environmental Health Perspectives* 104S: 981-986 (1996).

58. **Welch, L., K. Ringen, E. Bingham, J. Dement, T. Takaro, W. McGowan et al.:** Screening for beryllium disease among construction trade workers at Department of Energy nuclear sites. *American Journal of Industrial Medicine* 46: 207-218 (2004).

59. **Silver, K., and R.R. Sharp:** Ethical considerations in testing workers for the -Glu69 marker of genetic susceptibility to chronic beryllium disease. *Journal of Occupational and Environmental Medicine* 48: 434-443 (2006).

60. **Weston, A., J. Ensey, K. Kreiss, C. Keshava, and E. McCanlies:** Racial differences in prevalence of a supratypic HLA-genetic marker immaterial to pre-employment testing for susceptibility to chronic beryllium disease. *American Journal of Industrial Medicine* 41: 457-465 (2002).

61. **Cummings, K.J., D.C. Deubner, G.A. Day, P.K. Henneberger, M.M. Kitt, M.S. Kent et al.:** Enhanced preventive programme at a beryllium oxide ceramics facility reduces beryllium sensitisation among new workers. *Occupational and Environmental Medicine* 64: 134-140 (2007).

62. **Henneberger, P.K., D. Cumro, D.D. Deubner, M.S. Kent, M. McCawley, and K. Kreiss:** Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. *International Archives of Occupational and Environmental Health* 74: 167-176 (2001).

63. **Kreiss, K., M.M. Mroz, B. Zhen, H. Wiedemann, and B. Barna:** Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occupational and Environmental Medicine* 54: 605-612 (1997).

CHAPTER 4

COMPARISON OF THREE METHODS OF RETROSPECTIVE EXPOSURE ASSESSMENT IN A CASE-CONTROL STUDY OF BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE

Abstract

This report provides a comparison of three different, but related, retrospective exposure assessment methods applied to the participants of a case-control study evaluating the risk of beryllium sensitization (BeS) and chronic beryllium disease (CBD) in the nuclear weapons industry.

Workers (n=386) from a former U.S. nuclear weapons production facility, Rocky Flats Environmental Technology Site (RFETS, Arvada, CO), were enrolled in a case-control study including 70 individuals with BeS and 61 with CBD. Beryllium exposures for each participant were assessed using three different methods: 1) a traditional job exposure matrix (JEM method) that assigned beryllium exposures at the job title level based on interviews with three workers in each job title and assessment of available industrial hygiene exposure measurements; 2) individual worker interviews evaluating the tasks each worker performed followed by "expert" assessment of task exposures by two industrial hygienists (IHs) based solely on professional judgment based on their experience in the beryllium industry (IH rating method), and; 3) individual worker interviews as described in #2 followed by extensive analyses of historical facility-specific

and industry-wide data to assign exposures to tasks (IH data method). Task and participant exposures produced using these three methods were compared in terms of specificity, sensitivity, correlation, and absolute agreement. In addition, odds ratios from logistic regression analyses on the odds of CBD by exposure level were compared.

Linear regression of task exposures from the IH rating and IH data methods indicated that IHs' expert assessment of the average beryllium concentrations for each task accounted for approximately 65% of the variability in the task exposures assigned after extensive analyses of historical IH measurements. Compared to the IH data method, the JEM method had high specificity (0.94) but low sensitivity (0.27) considering a binary exposed/unexposed outcome. Participant exposure assignments using all three of the methods were significant predictors of increased CBD odds with odds ratios ranging from 1.51 (95% CI: 1.03-2.22) for each unit increase in lifetime-weighted average (LTWA) exposure for the JEM method to 2.50 (95% CI: 1.47-4.26) for each unit increase in LTWA exposure for the IH data method.

A method of task exposure assessment relying solely on the professional judgment of IHs performed similarly to a method involving extensive analyses of historical IH measurements in terms of rank order assessment of average task exposure. Use of any of the three exposure assessment methods resulted in the identification of increasing odds of CBD with increasing LTWA exposure. Exposure misclassification likely attenuated the odds ratio point estimates for the odds of CBD by approximately 5% using the "expert" assessment method and approximately 40% using the JEM method.

Introduction

Retrospective exposure assessment is one of the most problematic elements of occupational case-control studies requiring intensive effort and resources to establish relatively valid and reliable estimates of past exposure. As the focus of occupational case-control studies is to identify and compare disease risk factors in the most efficient manner possible, rarely does the opportunity arise to formally compare the performance of exposure estimates obtained using different methods. This report provides a comparison of three different, but related, retrospective exposure assessment methods applied to the participants of a case-control study evaluating the risk of beryllium sensitization (BeS) and chronic beryllium disease (CBD) in the nuclear weapons industry.

Beryllium is a low-density metal with unique properties that make it highly desirable for use in a number of industries including automotive, electronics, communications, medical, defense, and aerospace. Workers exposed to aerosols generated by the fabrication of beryllium-containing materials are at risk for developing BeS and CBD. BeS is a Type IV, delayed hypersensitivity reaction, which can be observed as T-cell proliferation in beryllium-stimulated peripheral blood using the beryllium lymphocyte proliferation test (BeLPT).[1-3] In some individuals, BeS progresses to CBD, a lung disease characterized by non-caseating granulomas and interstitial infiltrates often leading to fibrosis.

The risk of BeS and CBD appears to vary by workplace and by exposure level. Prevalences of up to 15% for BeS and 8% for CBD have been reported in cross-sectional studies of beryllium-exposed workforces.[2, 4] While these studies have clearly documented that higher exposures are associated with higher prevalences of BeS and

CBD, there have been consistent difficulties in defining the nature of the exposure-response relationship.[5-7] Due to this previously ill-defined exposure-response relationship, we have recently completed a case-control study (Chapter 3) at a nuclear weapons manufacturing facility to further evaluate the exposure-response relationship and the influence of specific genetic factors in BeS and CBD.

As part of this case-control study, we evaluated subjects' beryllium exposures using three different exposure assessment methods. The first method, termed the JEM method, involved using a previously created facility specific job exposure matrix (JEM) which assigned average beryllium exposures by year based on the job title and the specified work location (building) of the participant. From the average yearly exposures and the total time spent working at the facility, a lifetime weighted average (LTWA) exposure could be assigned to each subject. The second and third methods involved individual interviews with each subject to determine the tasks that they performed and the amount of time spent on each task. From these interviews, descriptions of subjects' tasks were compiled into a list of beryllium exposure tasks. For the second exposure assessment method, termed the IH rating method, two industrial hygienists (IHs) each with over five years experience in beryllium industries were asked to estimate the average exposure in $\mu g/m^3$ for each of the beryllium exposure tasks using only their professional knowledge of beryllium operations. For the third method, termed the IH data method, researchers compiled an extensive database of beryllium exposure measurements for each task from facility-specific historical data and other available industry-wide data. Using this database, researchers calculated time-period specific average exposures for each of the beryllium exposure tasks. Using the exposures assigned to each task by either

the IH rating method or the IH data method, LTWA beryllium exposures were assigned to each study participant using the amount of time the individual spent performing the task and the total time the individual spent working at the facility.

There were several similarities and differences between the exposure assessment methods. All three methods used the same work history rosters as the foundation for establishing changes in job title and work assignment location over time. This work history roster remained unmodified in the JEM method and was modified based on subject interviews in the other two methods. In addition, the JEM and IH data methods relied on some of the same exposure data to establish task exposures. However, expanded beryllium exposure datasets, including those from other nuclear weapons facilities and those from private industry, were also used to determine task exposures for the IH data method. Key differences between the methods included: 1) the JEM method assigned the same average yearly exposure to all workers in a job classification while the IH rating and IH data methods assigned LTWA exposures independently to each individual subject; 2) the IH rating method used IH expert judgment to assign task-based exposures while both the JEM and IH data methods used actual IH exposure measurements; 3) the JEM and IH data methods accounted for differences in task-based exposure due to engineering control or work practice modifications over time at the facility by assigning multiple, time-specific exposures for each task while the IH rating method relied on a single index of exposure for each task; 4) the JEM method consisted of work histories and exposures covering the only the years 1952 to 1996 while the other two methods covered the entire work histories of all the participants up to 2006.

In spite of the differences in the exposure assessment methods, we hypothesized that use of any of the three methods would result in similar estimates of increased odds of CBD with increasing exposure. To address this hypothesis, we analyzed the data from a case-control study of a nuclear weapons production facility where beryllium components were fabricated using separate estimates of LTWA beryllium exposure calculated for each participant from each of the three exposure assessment methods. The aims of the study were to: 1) compare task exposure estimates produced using the IH rating and IH data methods to assess the performance of IH "experts" in estimating beryllium exposures in the absence of exposure data; 2) compare subjects' assigned LTWA exposures between all three methods in terms of sensitivity, specificity, and validity using the IH data method as a "gold" standard; 3) compare the CBD exposure-response profiles produced using LTWA exposures from each of the three methods to assess any important differences in risk at specific exposure levels.

Methods

Worksite description

The current investigation was conducted among workers from Rocky Flats Environmental Technology Site (RFETS). This facility opened in 1951 for the express purpose of processing and machining plutonium, uranium, and other materials into a detonator or "trigger" for nuclear weapons. Starting in the late 1950s and continuing until approximately 1990, workers at this facility cast, pressed, rolled, machined, treated (plated, coated, etc.), and joined beryllium materials on a production scale at the facility. As a very large and diverse facility, including production, maintenance, and research

operations involving many different materials, not all RFETs employees were exposed to beryllium and those who were often worked only a small portion of their career with or around beryllium. In 1993, the site's mission changed to cleanup and closure with the official decommissioning and closure of the facility in 2005. The best estimates indicated that approximately 15,063 production workers and 3,250 construction workers were employed at RFETS during the life of the plant.[8] At least 7,820 of these workers were part of a serial medical surveillance program which included testing with the BeLPT and resulted in at least 117 individuals diagnosed with CBD and 184 with BeS.[9]

Case-control study recruitment and design

While details on recruitment and study design for this case-control study are presented in Chapter 3, briefly, all workers ever working at RFETS and participating in beryllium medical surveillance were eligible to participate in the study. Participants were recruited through mailings and clinical contact at National Jewish Health. BeS cases by definition had two or more abnormal blood BeLPT results or an abnormal bronchoalveolar lavage (BAL) BeLPT. Some BeS cases had undergone a medical evaluation to rule-out CBD, others had not. CBD cases had evidence of BeS along with either: 1) pathological evidence of granulomatous inflammation on biopsy; or 2) both an abnormal BAL BeLPT and greater than 15% lymphocytes in BAL fluid. Controls were individuals who had worked at RFETS and had at least two normal blood BeLPTs and no abnormal blood BeLPTs. Controls were frequency matched approximately two to one to CBD and BeS cases based on gender, race, and decade of hire at RFETS. The study

protocol was reviewed and approved by the National Jewish Health Institutional Review Board. Written informed consent was obtained from each study participant.

Exposure Assessment Methods

Job exposure matrix (JEM) method

A JEM was created at RFETS in the late 1990s to assess exposure to multiple chemicals including metals and solvents. Details on the creation of this JEM have been presented elsewhere[10], thus, only a brief description is provided here. The payroll department at RFETS recorded monthly job and building assignments for most of the workers on the site from 1952 and 1996. From these RFETS archived payroll records, the job title and work location (building) from one month each year was entered into a database for each of 13,480 workers at the plant. In order to assign chemical exposures to each of the year, job title, and building locations, historical records on chemical use and processes were reviewed for the 83 buildings at the facility. Twenty buildings were identified with potential exposure to chemicals with known acute or chronic health effects. For each of these 20 buildings, at least three workers from each job title within the building were interviewed to determine the potential exposures and task composition of their jobs. Workers were also asked about substantial process or work-practice changes that may have affected exposure in order to categorize tasks into time-periods of similar exposure. Using the task descriptions related to beryllium provided by the interviews, an IH reviewed the available beryllium exposure data from the facility for the specific time period of similar exposure to assign an average exposure in $\mu g/m^3$ to the task. Further, using the task composition or percent time working in each task for the job title, the IH

constructed a yearly average beryllium exposure for the job title. The job title yearly averages were adjusted appropriately based on increases or decreases in production using the estimates of percent time provided in the interviews. Cumulative beryllium exposures were estimated by summing the yearly average beryllium exposures over workers' employment histories. LTWA beryllium exposures were estimated by dividing the cumulative exposure by the total number of work years at the facility. In calculating cumulative and LTWA beryllium exposures, yearly average beryllium exposures were summed up to the year of employment termination or the last year of data availability (1996) for participants who continued to work at least through 1996. Beryllium exposures for BeS and CBD cases incurred after the date of diagnosis were not included in their cumulative exposure. For this case-control study, beryllium exposures were available in the JEM for 86% (332/386) of the participants.

IH rating method

Task exposure questionnaire

A task exposure questionnaire was developed using information from focus groups of RFETS workers. This questionnaire was administered by one of four trained interviewers, with industrial hygiene or exposure assessment experience, using an interview script and work history from the JEM method to help refresh interviewees regarding the start and end dates of each work assignment. Participants were asked to verify the start and end dates for each job title assignment (i.e., machinist, chemical operator, electrician, etc.) along with an estimate of the average number of hours worked per week in the job. Participants were also asked to describe the specific tasks (i.e., lathe,

grind, plating, cleaning, etc.) performed for each job title assignment. For each task, participants were required to categorize their beryllium exposure into one of seven exposure categories listed in order of decreasing qualitative exposure: 1) directly altering a beryllium part; 2) contact with beryllium waste materials; 3) contact with finished and cleaned beryllium parts; 4) work within 5 feet of a beryllium operation with no direct beryllium contact; 5) work in the same room as a beryllium operation with no direct beryllium contact; 6) work in the same building as a beryllium operation with no direct beryllium contact; or 7) no known beryllium exposure. For each of the tasks, the participant provided a percentage of time spent performing the task and a percentage of time spent performing the task with beryllium.

Task exposure assessment

From the exposure questionnaires, a total of 50 unique combinations of exposure category and task were identified (Table IV-I) consisting of 27 combinations with direct beryllium exposure involving direct work with a beryllium part or with beryllium waste materials and 23 combinations with indirect beryllium exposure where there was no direct work with beryllium or beryllium waste materials, but instead proximity to a beryllium operation. Two IHs with each with over five years experience in the beryllium industry were provided with this list of 50 tasks. The IHs were asked to independently provide a single number representative of the arithmetic mean of the beryllium exposure on a $\mu g/m^3$ scale that would have been expected for workers performing the task in the late-1970s and early-1980s. Following the independent assessments, the two IHs met to

describe their rationale in establishing each task exposure estimate and reach consensus on any discordant task exposure estimates.

Participant exposure assessment

The consensus task exposure estimates from the IHs were used to calculate a cumulative beryllium exposure by summing the job specific exposures obtained by multiplying the consensus task exposure estimates by the percent of time working with beryllium in that time period, the percent of time performing the tasks, the number of years spent in the job, and the ratio of the average number of work hours per week to 40 hours. A LTWA exposure was calculated by dividing the cumulative exposure by the total number of work years. In calculating cumulative and LTWA exposures for each participant, beryllium exposure and work time were allowed to accrue over a work history for an individual until the date of employment termination for controls, or until the date of BeS or CBD diagnosis or employment termination for the cases, whichever came first.

IH data method

This method used the same exposure questionnaire data and combinations of exposure category and task described in the IH rating method (Table IV-I) and is described in detail in Chapter 3. Briefly, the 50 combinations of exposure category and task were separated into one to three time periods of similar exposure based on installed controls and plant practices based on published information[11-13] and personal knowledge about beryllium production at RFETS. Each of the unique combinations of exposure type,

task, and time period constituted a cell of the task exposure matrix. Each cell involving direct exposure to beryllium was filled in using the arithmetic mean of the available exposure measurements from published [11-18] and study specific data closest in time period and task composition based on the judgment of the authors. As very little relevant data were available to fill in the cells involving indirect beryllium exposure and realizing that indirect exposures were closely related to direct exposures, we used a conservative method for assigning average exposures to these cells. Using the same established time periods, reported indirect exposures within 5 ft. of a beryllium task were assigned an average of 50% of the task, those in the same room 10% of the task, and those in the same building 1% of the task. Participant cumulative and LTWA exposures were determined using the same method outlined in the discussion on the IH rating method except that time-specific average task exposures were used rather than a single average exposure for each task.

Statistical analysis

We used SAS v. 9.1 (SAS Institute, Inc., Cary, NC) for statistical analyses. Univariate analyses of categorical variables were performed using chi-square and Fisher's exact tests. Continuous unpaired variables were compared across the three groups (controls, BeS, and CBD) using the Kruskal-Wallis test followed by pair-wise comparisons using the Mann-Whitney test when significant. Differences in paired variables, including task exposures and participant exposures, were assessed using the non-parametric Wilcoxon-Rank Sum test. Since there was only a single exposure estimate for each task using the IH rating method and from one to three exposure

estimates for each task using the IH data method, comparisons of task exposures were performed between estimates corresponding to the late-1970s and early-1980s time period. Sensitivity, specificity, and Kappa scores were calculated comparing the exposure estimates from the IH data method to those from the IH rating method and the JEM method. For these calculations, the data were re-coded as a binary variable with zero exposure (0) defined as a LTWA exposure less than or equal to 0.02 $\mu g/m^3$ and exposure (1) defined as a LTWA exposure greater than 0.02 $\mu g/m^3$. Confidence intervals for sensitivity, specificity, and Kappa scores were calculated using a general method outlined by Fleiss[19]. Pearson correlations were computed for the log-transformed task exposures assessed by the IH rating and IH data method and on the log-transformed participant LTWA exposures by the JEM, IH rating, and IH data methods. Spearman rank correlations were computed to assess rank agreement. Relative agreement for the task exposure estimates and participant LTWA exposures comparing the IH data method to the IH rating and JEM methods was assessed overall and by case status using a one-way random single measure of the intraclass correlation coefficient based on the convention of Shrout and Fleiss[20]. Linear regression on log-transformed values was used to assess the relationships between task exposures assessed using the two methods and to compare participant LTWA exposures from the IH rating and JEM methods compared to the IH data method. Unconditional logistic regression was used to model CBD as a function of LTWA beryllium exposure. To facilitate comparison of separate models, only the LTWA exposures from the three different exposure assessment methods were included in the models. Based on the lack of an exposure-response relationship for BeS identified in Chapter 3, logistic models with BeS as an outcome were not attempted.

Results

Study population and demographic characteristics

Study population and demographic characteristics are presented in detail in Chapter 3 including data tables. However, important details are presented briefly here. There were 386 former RFETS workers in the study population including 255 controls with potential beryllium exposure, 61 subjects with confirmed CBD, and 70 individuals classified as BeS due to two abnormal BeLPTs. Participants were predominately male (88.3%), Caucasion (97.7%), and non-smokers (94.0%). The median year of hire at the facility was 1969 and ranged from 1952 to 1998. The typical participant worked at the facility for approximately 15 years, although the minimum time at the facility was less than three months and the maximum more than 40 years. The only important difference in demographic characteristics that may relate to exposure was that BeS cases typically worked fewer years at the facility (median=12.6 years) as compared to controls (median=15.8 years, p=0.020).

Task exposures

Task exposures assigned using the IH data and IH rating method are shown in Table IV-I separated by task and exposure type. Overall, for the 50 task/exposure type combinations, exposures assigned using the IH rating method were higher than those assigned using the IH data method for 74% (37/50) including 70% (19/27) of the direct exposure combinations and 78% (18/23) of the indirect exposure combinations. However, of the 13 exposure combinations where the IH data estimate was higher than the IH rating

estimate, six had estimates much greater than the IH data estimates including: 1) beryllium casting and mold breakout with an IH data estimate of 73 μg/m^3 compared to a rating estimate of 10 μg/m^3; 2) filter replacement and testing with an IH data estimate of 23.9 μg/m^3 compared to an IH rating estimate of 4.0 μg/m^3; and 3) crushing beryllium parts/shapes with an IH data estimate of 36.4 μg/m^3 compared to an IH rating estimate of 3.0 μg/m^3. The mean absolute difference between the paired estimates was 4.1 μg/m^3. However, this difference was skewed by a few large differences as mentioned above. The median absolute difference was 0.43 μg/m^3 which indicates that for half the exposure scenarios the two estimates were within ±0.43 μg/m^3.

Table IV-II shows summary statistics of the task exposures by the two assessment methods. There were significant differences between the pairs of task exposure estimates ($p < 0.001$) by Wilcoxon Rank Sum tests with IH rating estimates generally higher than IH data estimates. The intraclass correlation coefficients between all task exposure estimates using the two methods was 0.17 suggesting poor agreement. However, given that 75% of the exposure time of the participants occurred in 10 tasks, the intraclass correlation coefficient for exposure assignments for those 10 tasks was 0.74 suggesting that for the majority of the exposures in terms of exposure time there was good agreement. In addition, a Pearson correlation coefficient of 0.82 ($p < 0.001$) for the log-transformed task exposure estimates suggests increases and decreases in IH rating estimates were usually accompanied by increases and decreases in IH data estimates of fairly consistent magnitude. The Spearman rank correlation of 0.81 ($p < 0.001$) also suggested similar rank-order of the estimates. A linear regression between the log-

transformed estimates (Figure 4-1) suggested that 67% of the variation in the IH data estimates was explained by the IH rating estimates.

Participant exposures

JEM method

As shown in Table IV-III, while all participants had assigned LTWA exposures using the IH data and IH rating methods, only 86% (332/386) of the participants had assigned exposures using the JEM method which included 48 BeS cases, 38 CBD cases, and 187 controls. In addition, 273 (82%) of the 332 individuals with an assigned LTWA exposure using the JEM method were assigned an exposure of zero. Table IV-IV compares reported exposure characteristics from exposure interviews used in the IH rating and data methods among participants assigned zero and non-zero LTWA exposures using the JEM method. All (100%, 59/59) of the participants that were assigned non-zero exposures using the JEM method were also identified as having been exposed to beryllium through later exposure interviews. Furthermore, those assigned a non-zero exposure were more likely to have directly altered a beryllium part (80.0%, $p < 0.001$) as compared to those assigned a zero exposure (22.7%). Only seven of the participants with non-zero JEM exposure did not have direct exposure to beryllium. Those assigned a non-zero JEM exposure also spent a greater percentage of their time working directly or indirectly with beryllium (43.1% vs. 27.6%, $p < 0.001$) and had a higher LTWA exposure as determined using the IH data method (median=0.17 vs. median=0.02, $p < 0.0001$). Of those assigned zero exposure using the JEM method, nearly 50% had assigned LTWA exposures from the IH data method of less than or equal

to 0.02 μg/m^3 which would have been considered a very low exposure in the late-1990s when the JEM was constructed. When combined, this evidence suggests that the JEM was effective in assigning exposures to those individuals frequently working directly with beryllium and was much less effective at identifying workers with only indirect exposure to beryllium.

Comparing exposures by case status

When comparing LTWA exposures assigned using the IH data and IH rating methods, there were no significant differences between the methods by case status including exposures assigned to controls (medians: 0.03 μg/m^3 and 0.04 μg/m^3, respectively), BeS cases (medians: 0.009 μg/m^3 and 0.01 μg/m^3, respectively), and CBD cases (medians: 0.07 μg/m^3 and 0.10 μg/m^3, respectively). Exposures assigned using the JEM method differed significantly from those assigned using either the IH data or IH rating methods for CBD cases, BeS cases, and controls (median=0 μg/m^3 and $p < 0.0001$ for all). However, when comparing exposures across case status within each individual exposure assessment method, the same conclusion was made regardless of method; namely exposures for CBD cases were significantly higher than exposures for either BeS cases or controls (CBD vs. controls: IH data method p=0.012, IH rating method p=0.024, JEM method p=0.024; CBD vs. BeS: IH data method p=0.002, IH rating method p=0.006, JEM method p=0.011). In addition, there were no differences in LTWA exposures between BeS cases and controls for any of the exposure assessment methods.

Paired exposure comparisons by assessment method

Table IV-V shows the relative and absolute differences in pairs of LTWA exposure assignments by case status and exposure assessment method. Paired LTWA exposure assignments were significantly different ($p< 0.0001$) when comparing either the IH data method to the IH rating or to the JEM methods. This difference was consistent across cases and controls. Typically, these differences were relatively small with median relative differences of less than 0.001 $\mu g/m^3$ when comparing the IH data and IH rating methods and 0.01 $\mu g/m^3$ when comparing the IH data and JEM methods. Figure 4-2 shows the distribution of the relative differences in LTWA exposures between the methods. Compared to the IH data method (Figure 4-2a), the IH rating method tended to overestimate LTWA exposures for BeS cases and controls and underestimate LTWA exposures for CBD cases. Compared to the IH data method (Figure 4-2b), the JEM method tended to underestimate LTWA exposures for all participants. In both cases, there appeared to be more variability in the differences between LTWA exposures assigned to CBD cases than controls or BeS cases. The relative differences in LTWA exposure assignments between the IH data and IH rating methods were significantly greater for CBD cases as compared to controls ($p=0.006$).

Absolute differences in the pairs of assigned LTWA exposures by method are also shown in Table IV-V. Overall, LTWA exposures assigned using the IH rating method were within $\pm$ 0.02 $\mu g/m^3$ of those assigned using the IH data method for 50% of participants and within $\pm$ 0.15 $\mu g/m^3$ for 75% of the participants. However, the absolute differences between the LTWA exposures assigned using the IH data and IH rating methods were typically greater for CBD cases compared to either BeS cases ($p=0.007$) or

controls (p=0.006). LTWA exposures assigned using the JEM method were within ± 0.03 μg/m^3 of those assigned using the IH data method for 50% of participants and within ± 0.21 μg/m^3 for 75% of the participants. The magnitude of these absolute differences did not change by case status.

Sensitivity, specificity, and agreement

Table IV-VI compares the sensitivity, specificity, and relative agreement of the JEM and IH rating methods to the IH data method. In this case, the IH data method was chosen as the "gold standard" due to its use of all available information on exposure at the facility. In order to perform these comparisons, "exposed" was defined as a LTWA exposure greater than 0.02 μg/m^3, and "unexposed" was defined as a LTWA exposure less than or equal to 0.02 μg/m^3. The specificity of the JEM method ranged from 0.93 to 0.96 and did not differ significantly depending on case status. The cost of the relatively high specificity of the JEM was its relatively low sensitivity ranging from 0.19 to 0.40 which appeared to be higher for CBD cases (0.40, 95% CI: 0.30-0.43) as compared to BeS cases (0.19, 95%CI: 0.09-0.23) and controls (0.25, 95% CI: 0.20-0.28). The specificity of the IH rating method ranged from 0.87 to 0.93 with no significant differences by case status. The sensitivity of the IH rating method ranged from 0.81 to 0.90 and also did not differ by case status. In order to measure the relative agreement between the IH data method and the JEM and IH rating methods, a Kappa coefficient was calculated. This Kappa coefficient was compared to previous interpretations classifying the degree of agreement between two raters as outlined by Altman[21]. For the JEM method compared to the IH data method, the Kappa coefficient ranged from 0.16 to 0.27

suggesting “poor” to “fair” agreement between the methods in terms classifying participants as exposed or unexposed. For the IH rating method compared to the IH data method, the Kappa coefficient ranged from 0.71 to 0.77 suggesting “good” agreement between the methods.

Correlation among the three exposure assessment methods

Table IV-VII shows the Pearson and Spearman rank correlations between the assigned LTWA exposures for participants by case status. Pearson correlations were determined using the log-transformed LTWA exposures. Comparing IH data and IH rating methods, significant positive Pearson correlations were identified ranging from 0.62 for controls to 0.68 for CBD cases. Rank order Spearman correlations were higher ranging from 0.91 to 0.96 suggesting very high agreement in the rank ordered LTWA exposures. Correlations between LTWA exposures assigned using the IH data and JEM methods were lower ranging from -0.04 for BeS cases to 0.47 for CBD cases. Rank order Spearman correlations were also lower ranging from 0.26 for BeS cases to 0.51 for CBD cases. Intraclass correlation coefficients comparing the relative agreement of the IH data and IH rating methods ranged from 0.48 to 0.61 suggesting “moderate” agreement between the methods. When comparing the IH data method to the JEM method using intraclass correlation coefficients, there was “fair” agreement for the CBD cases (ICC=0.26), but “poor” agreement for the BeS cases (ICC= - 0.13) and for the controls (ICC= - 0.01). Together, all these results suggested a much higher level of agreement between the IH data and IH rating methods which was confirmed via the linear regression analyses in Figures 4-3 and 4-4. Figure 4-3 shows the linear regression of the log-

transformed IH data assigned LTWA exposures versus the log-transformed IH rating assigned LTWA exposures with a coefficient of determination (R^2-value) of 0.42. While this analysis identified a significant linear relationship between the two methods, it also indicated that the IH rating method only explained approximately 42% of the variability in the log-transformed LTWA exposures assigned by the IH data method. A similar linear regression between the IH data method and the JEM method is presented in Figure 4-4. This regression suggested the relationship between the two methods, though statistically significant, was not very strong with a coefficient of determination of only 0.09.

Differences in odds ratios by exposure assessment method

Logistic regression models for the odds of CBD as a function of LTWA exposure are presented in Table IV-VIII for the three different exposure assessment methods. All three exposure assessment methods produced LTWA exposures that were significant predictors of CBD odds. The LTWA exposure assigned using the IH data method was the most highly significant in the logistic model ($p < 0.001$). Exposures assigned using the IH rating and JEM methods had similar significance levels ($p=0.032$ and $p=0.034$, respectively). Point estimates of the odds ratios for each unit increase in LTWA exposure ranged from 1.51 for the JEM method to 2.50 for the IH data method. However, the 95% confidence intervals from all three methods overlapped suggesting none of the three models produced a significantly different risk estimate. The output of the logistic regression models in terms of predicted probability of CBD by LTWA exposure level is presented in Figure 4-5. Probability profiles were very similar when using the IH data and IH rating methods with very little difference in the predicted probability of CBD with

increasing LTWA exposure. On the other hand, the curve showing the predicted probability of CBD produced using the JEM LTWA exposures was somewhat flatter with smaller increases in probability with increasing exposure. Odds ratio point estimates for a LTWA exposure of 2.0 $\mu g/m^3$ (Table IV-IX) would likely be interpreted differently with a nearly 6-fold increased odds of CBD using the IH data and IH rating methods and only a 2-fold increased odds of CBD using the JEM method.

Discussion

This report compared three methods of exposure assessment in an occupational case-control study providing evidence suggesting IHs can effectively assign exposures to industrial tasks based on task descriptions with good rank agreement compared to those produced by analyzing historical IH exposure measurements. Furthermore, these IH assigned task-based exposures can be incorporated into participant-reported task histories to create LTWA exposures of sufficient quality to produce very little attenuation in odds ratios for the exposure-disease relationship as compared to those produced using IH measurements. Though there was limited correlation between continuous participant LTWA exposure estimates produced by a JEM method compared to methods using self-reported tasks, exposure-disease relationships produced by this JEM method were not substantially different from those produced using either the IH assigned exposures or those produced using actual IH data.

Most published validation and reliability studies focusing on expert assignment of occupational exposures have focused on the ability of experts to assign a binary exposed/not-exposed rating or a categorical rating based on descriptive information

provided regarding the participant's job title or industry. In these studies, investigators have found significant associations between expert rankings and IH measurements in nearly all studies with the proportion of variance explained ranging from 0.2 to 0.65 with a median of approximately 0.3.[22-25] However, we could identify only a single study that involved evaluation of expert exposures assigned on a continuous airborne concentration scale. Cherrie and Schneider [25] evaluated two IHs' estimates of exposure using a subjective method based on descriptive information about the work environment compared to IH measurements for five different agents and identified Pearson correlation coefficients ranging from 0 to 0.93 with a median of 0.39. The overall correlation between our task exposure estimates from the IH data and IH rating methods was 0.81 which is well within the range identified by Cherrie and Schneider. However, it should be noted that our comparison was between a less formal approach for assigning continuous exposure metrics versus Cherrie and Schneider's structured approach. In addition, we compared our expert assignments to concentrations obtained from analysis of historical data rather than contemporary measurements. Cherrie and Schneider also found that their expert assessments were positively biased with ratios of the expert assessment to the measured levels ranging from 1.3 to 2.2. For our tasks, the expert assignments were positively biased most of the time, but the ratios of the IH assigned task exposure assignments to those based on IH data ranged from 0.04 to 16.7 with a median of 1.8.

Overall, our IH rating method demonstrated that, at least in the beryllium industry, experienced IHs can assign exposures to tasks based solely on their professional judgment with good rank order agreement (rho=0.81) compared to those assigned using analyses of historical data. However, when considered on equivalent $\mu g/m^3$ scales, task

exposures assigned using the IH rating method agreed poorly with those assigned using IH data (ICC=0.17). Much of this disagreement was likely due to our IH raters' lack of familiarity with some rarer tasks such as filter replacement and testing on a beryllium contaminated ventilation system or crushing of beryllium parts or shapes. When considering only the common tasks occurring at the facility, those that comprise over 75% of our participants' exposed work time, there was good agreement on an absolute scale as evidenced by an intraclass correlation coefficient of 0.74.

JEM methods were conceived as an efficient method to assess exposures for case-control studies involving thousands of workers or community members based on job title. As such, the major limitation of JEM methods is the potential misclassification of exposure introduced due to variability of exposure related tasks within a job title.[26] A review on the reliability and validity of common exposure assessment methods for case-control studies indicated that compared to expert assessment or self-reports of exposure, JEMs typically have low sensitivity, most often below 0.5, but fairly good specificity, generally above 0.85.[27] In our evaluation, the JEM method was clearly less sensitive than the IH data method with sensitivities ranging from 0.19 to 0.40. Also concerning, was the differential sensitivity of the JEM method compared to the IH data method. The sensitivity of the JEM was higher for CBD cases (0.40) as compared to either BeS cases (0.19) or controls (0.25). Thus, the use of the JEM method as the primary exposure assessment method may result in differential misclassification of exposures and potential bias away from the null for the case control study. However, in defense of the JEM method, it was much more efficient than the other two methods. The JEM method required conducting only a few hundred interviews to assign exposures to multiple

chemicals to the entire known workforce of more than 13,000 workers. In addition, the method was highly specific with specificities ranging from 0.93 to 0.96 suggesting a high level of confidence that those identified as exposed were truly exposed. Finally, the JEM method, even with its limitations, produced CBD odds estimates that were not substantially different from those produced using the other more time-intensive methods.

Based on the comparability of the task exposure estimates using the IH rating and IH data methods for the tasks comprising the majority of the participants' exposed work time, it was not surprising that incorporation of these task exposures into identical data matrices of worker job and task times produced similar measures of LTWA beryllium exposure. Typically, exposures for the same worker produced by the IH data and IH rating methods were within ± 0.02 $\mu g/m^3$. However, possibly concerning, was that there were larger absolute differences in the two LTWA exposure assignments for CBD cases as compared to BeS cases or controls. This may suggest either differential misclassification for one of the two methods, or the more likely explanation that since CBD cases incurred greater total beryllium exposure, differences in task exposure estimates were magnified for CBD cases as compared to others. Overall measures of sensitivity (0.91), specificity (0.85), and agreement (kappa=0.76) suggested a high degree of comparability between the IH data and rating methods in identifying those truly exposed and those truly not exposed. There was excellent rank correlation between the two methods (rho=0.93) which was likely most responsible for the very similar logistic regression coefficients in evaluating CBD odds. Intraclass correlation coefficients (0.48 to 0.61) also suggest moderate to good agreement on an absolute scale. Overall, LTWA

exposure estimates produced using the IH rating method explained approximately 42% of the variability in the LTWA exposure estimates produced using the IH data method.

The most important aspect of any exposure assessment activity for epidemiology studies is to identify how exposure affects disease status. In general, non-differential misclassification of exposure biases the exposure-response relationship towards the null. In a logistic regression analysis, this bias towards the null is manifested as an attenuated odds ratio for the exposure covariate. Interestingly, in our analyses, use of either the LTWA exposure estimate produced using the IH rating method or using the IH data method resulted in very similar odds ratios for the continuous exposure covariate (2.50, 95% CI: 1.47-4.26 for the IH data method and 2.36, 95% CI: 1.08-5.19 for the IH rating method). Thus, compared to the IH data method, the more efficient IH rating method attenuates the point estimate of the odds ratio by only about 5%. This is interesting considering that the IH rating method relied on a single index or estimate of task exposure for all exposure time periods as compared to the IH data method which had up to three time-based exposure estimates for each task. Use of LTWA exposure assignments produced by the JEM method in a logistic regression model produced a lower odds ratio estimate (1.51, 95% CI: 1.03-2.22). Attenuation of the JEM odds ratio compared to the IH data odds ratio was approximately 40%. This larger attenuation was not surprising due to the low sensitivity of the JEM method. Interpretations of CBD risk covering the relevant range of possible beryllium exposures (0.05 to 2.0 $\mu g/m^3$) were very similar using either the IH rating or IH data methods. However, at the higher average exposure levels, the attenuation of the odds ratio from the JEM method would

likely result in the acceptance of a higher occupational exposure level due to lower identified CBD odds.

Limitations

All three exposure assessment methods likely had significant exposure misclassification that could have impacted our results. Cumulative exposures and resulting LTWA exposures may have been overestimated for BeS/CBD cases using all three methods as exposures accrued until the date of BeS or CBD diagnosis which was likely much later than the date of disease development. The JEM method relied on interviews with only a few individuals from each job title to determine work tasks. This likely resulted in both under and over estimation of beryllium exposures for other individuals within the same job title due to variation in work tasks within a job title. The use of IH judgment to assign exposures to tasks clearly resulted in overestimation of exposures for some individuals and underestimation of exposure for others. The use of IH data from other facilities may have impacted the task exposure estimates in the IH data method. However, in assigning exposure estimates to tasks rather than individuals for all of these methods, exposure misclassification should have been non-differential. Based on the available IH exposure data from the RFETS facility, it is unlikely that we could have reconstructed exposure estimates with less misclassification than that in the IH data method.

Conclusions

This study was designed to evaluate three different methods of retrospective occupational exposure assessment for a case-control study of BeS and CBD in a nuclear weapons facility. We identified that a method of task exposure assessment relying solely on the professional judgment of industrial hygienists performed similarly to a method involving extensive analyses of historical industrial hygiene exposure measurements in terms of rank order assessment of average task exposure. Continuous estimates of task exposure in $\mu g/m^3$ provided by "expert" IH assessors were able to explain 65% of the variability in the task exposures assigned based on historical IH exposure measurements. In addition, we confirmed the widely reported high specificity and low sensitivity of a facility-specific job exposure matrix method. Use of any of the three exposure assessment methods resulted in estimates of increasing odds of CBD with increasing LTWA exposure levels that were not statistically different. Odds ratio point estimates for the odds of CBD for each unit increase in LTWA beryllium exposure were attenuated by approximately 5% using the "expert" assessment method and approximately 40% using the JEM method, both compared to the method using historical IH data. However, regardless of the exposure assessment method, this study demonstrates an exposure-response relationship between LTWA exposure and CBD and the need to reduce the current OSHA permissible exposure limit of 2.0 $\mu g/m^3$.

Table IV-I – Task exposure estimates from IH data method and IH rating method

Description	IH Data Method Time Periods and Exposure Assignments						IH Rating Method
	Time Period One[a]	Task Exposure Time Period One[b]	Time Period Two[a]	Task Exposure Time Period Two[b]	Time Period Three[a]	Task Exposure Time Period Three[b]	Task Exposure Estimate[c]
Assembly/Inspection							
General assembly work with Be parts	‘52-‘05	0.13[e]					1.0
Hand polishing or etching Be parts	’52-‘85	1.0[e]	‘86-‘05	0.14			3.0
Brazing/Welding Be parts	’52-‘85	1.32[e]	‘86-‘05	0.7			1.5
Inspection or handling of Be parts	’52-‘85	0.71[e]	‘86-‘05	0.15			0.2
Work within 5 feet of Be inspection operations[d]	’52-‘85	0.36[e]	‘86-‘05	0.075			0.5
Work in same room as Be inspection operations[d]	’52-‘85	0.07[e]	‘86-‘05	0.015			0.05
Work in same building as Be inspection operations[d]	’52-‘85	0.007[e]	‘86-‘05	0.0015			0.02
Machining							
Cutting Be with a band saw	‘52-‘05	1.78[e]					2.0
Machining Be parts (mill, lathe, bore)	’52-‘74	2.56	‘75-‘85	1.19[e]	‘86-‘05	0.052	1.0
Hand grinding of Be parts	‘52-‘05	0.56[e]					1.5
Machine grinding Be parts	’52-‘74	3.16	‘75-‘05	0.56[e]			1.5
Machining BeCu parts	‘52-‘05	0.09					0.1
Work within 5 feet of a Be machining operation[d]	’52-‘74	1.28	‘75-‘85	0.6[e]	‘86-‘05	0.026	0.5
Work in same room as a Be machining operation[d]	’52-‘74	0.35	‘75-‘85	0.16[e]	‘86-‘05	0.007	0.2
Work in same building as a Be machining operation[d]	’52-‘74	0.035	‘75-‘85	0.016[e]	‘86-‘05	0.0007	0.02
Foundry							
Be casting and mold breakout (old foundry,444)	’52-‘85	73.0[e]	‘86-‘05	2.0			10.0
Be casting and mold breakout (new foundry, 865)	‘52-‘05	2.0[e]					5.0
Work within 5 feet of Be casting (old foundry, 444)[d]	’52-‘85	36.0[e]	‘86-‘05	1.0			1.5
Work within 5 feet of Be casting (new foundry, 865)[d]	‘52-‘05	1[e]					1.5
Work in same room as Be casting (old foundry, 444)[d]	’52-‘85	7.3[e]	‘86-‘05	0.1			1.0
Work in same room as Be casting (new foundry, 865)[d]	‘52-‘05	0.2[e]					1.0

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period from IH data method
[b]Specifies the arithmetic mean of the exposure for the task and time period combination in $\mu g/m^3$ from IH data method
[c]Task exposure estimate from IH rating method.
[d]For these tasks, there was only indirect exposure to beryllium.
[e]Specifies time period estimate relevant to compare with IH rating method

Table IV-I (continued)– Task exposure estimates from IH data method and IH rating method

Description	IH Data Method Time Periods and Exposure Assignments						IH Rating Method
	Time Period One[a]	Task Exposure Time Period One[b]	Time Period Two[a]	Task Exposure Time Period Two[b]	Time Period Three[a]	Task Exposure Time Period Three[b]	Consensus Exposure Estimate
Forming							
Hot pressing of Be parts	'52-'05	1.03[e]					2.0
Rolling Be parts (sheet rolling)	'52-'05	0.18[e]					1.0
Cutting Be using a shear	'52-'05	1.28[e]					1.0
Annealing/Heat treating Be parts	'52-'05	0.2[e]					2.0
Work within 5 feet of Be rolling/pressing[d]	'52-'05	0.3[e]					0.5
Work in same room as Be rolling/pressing[d]	'52-'05	0.06[e]					0.2
Work in same building as a Be rolling/pressing[d]	'52-'05	0.006[e]					0.02
Laboratory							
Metallurgical testing of Be parts	'52-'05	0.16[e]					2.0
Laboratory analysis of Be samples	'52-'85	0.26[e]	'86-'05	0.13			0.5
Work within 5 feet of Be laboratory operation[d]	'52-'05	0.08[e]					0.1
Work in same room as a Be laboratory operation[d]	'52-'05	0.016[e]					0.05
Work in same building as Be laboratory operation[d]	'52-'05	0.002[e]					0.02
Treating/Finishing							
Plating/Chemical milling/Etching beryllium parts	'52-'05	0.32[e]					1.5
Operating metal spray/plasma machine with Be	'52-'05	0.52[e]					2.0
Grit blasting or sand blasting Be parts	'52-'05	0.3[e]					1.5
Work within 5 feet of Be plating/chem. Milling[d]	'52-'05	0.16[e]					0.5
Work in same room as a Be plating/chem. Milling[d]	'52-'05	0.03[e]					0.5
Work in same building as a Be plating/chem. Milling[d]	'52-'05	0.003[e]					0.02

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period from IH data method
[b]Specifies the arithmetic mean of the exposure for the task and time period combination in $\mu g/m^3$ from IH data method
[c]Task exposure estimate from IH rating method.
[d]For these tasks, there was only indirect exposure to beryllium.
[e]Specifies time period estimate relevant to compare with IH rating method

Table IV-I (continued)– Task exposure estimates from IH data method and IH rating method

Description	IH Data Method Time Periods and Exposure Assignments						IH Rating Method
	Time Period One[a]	Task Exposure Time Period One[b]	Time Period Two[a]	Task Exposure Time Period Two[b]	Time Period Three[a]	Task Exposure Time Period Three[b]	Consensus Exposure Estimate[d]
Maintenance and D&D							
Cleaning Be contaminated machines/surfaces	’52-‘85	4.5[e]	‘86-‘94	2.25	‘95-‘05	0.05	2.0
Maintenance on Be contaminated machines/equipment	’52-‘85	1.0[e]	‘86-‘94	0.18	‘95-‘05	0.04	2.0
Filter replacement/testing on Be contaminated systems	‘52-‘05	23.9[e]					4.0
Work in same building as a Maint/D&D operation[d]	’52-‘85	0.045[e]	‘86-‘94	0.023	‘95-‘05	0.0005	0.02
Waste							
Washing Be contaminated laundry	‘52-‘05	0.3[e]					1.5
Collecting Be waste materials (chip collecting)	’52-‘85	23.9[e]	’86-‘05	3.3			2.0
Crushing Be parts/shapes	’52-‘85	36.4[e]	’86-‘05	3.3			3.0
Be waste packaging/re-packaging	’52-‘85	0.6[e]	’86-‘05	0.31			1.0
Miscellaneous							
Oversight within 5 feet of unspecified Be activities[d]	’52-‘74	0.93	‘75-‘85	0.42[e]	‘86-‘05	0.06	0.5
Oversight in same room as unspecified Be activities[d]	’52-‘74	0.18	‘75-‘85	0.075[e]	’86-‘05	0.015	0.15
Oversight in same bldg as unspecified Be activities[d]	’52-‘74	0.026	‘75-‘85	0.012[e]	‘86-‘05	0.001	0.02

[a]Specifies the time period of similar exposure for the task. Tasks did not necessarily occur in every year in the time period from IH data method

[b]Specifies the arithmetic mean of the exposure for the task and time period combination in $\mu g/m^3$ from IH data method

[c]Task exposure estimate from IH rating method.

[d]For these tasks, there was only indirect exposure to beryllium.

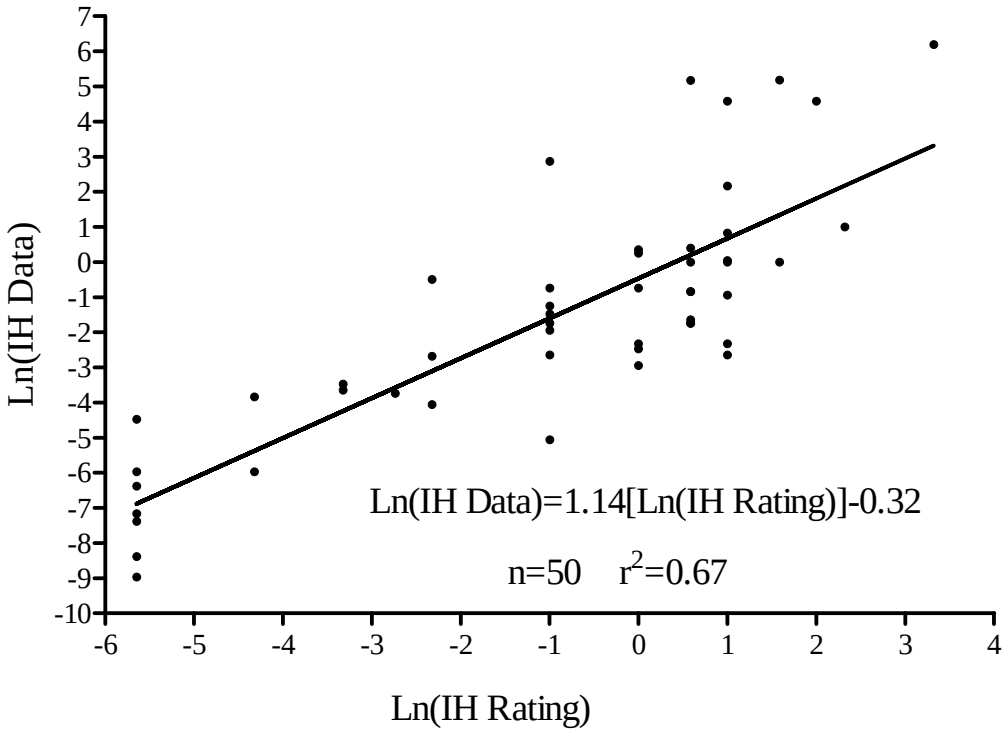
[e]Specifies time period estimate relevant to compare with IH rating method

Table IV-II – Summary of task exposures by IH data and IH rating methods (in µg/m^3)

	IH data method			IH rating method			P-value[a]
Tasks	**Median**	**Range**	**IQR**	**Median**	**Range**	**IQR**	
All tasks (n=50)	0.31	0.002-73.0	0.08-1.03	1.0	0.02-10.0	0.20-2.0	< 0.001
Direct exposure tasks (n=27)	0.71	0.09-73.0	0.30-1.78	1.5	0.10-10.0	1.0-2.0	< 0.001
Indirect exposure tasks (n=23)	0.075	0.002-36.0	0.016-0.36	0.20	0.02-1.5	0.02-0.50	< 0.001

[a]Paired Wilcoxon-Rank sum test for difference between the two methods

Figure 4-1 – Linear regression of the natural log of the IH data task exposure estimates vs. the natural log of the IH rating task exposure estimates showing data, regression line, and predicted IH data estimates in table below



IH rating estimate (µg/m^3)	Predicted IH data estimate (µg/m^3)
0.02	0.0084
0.05	0.024
0.10	0.053
0.20	0.11
1.0	0.73
1.5	1.1
2.0	1.6
5.0	4.5
10	10

Table IV-III – Summary of subjects' lifetime-weighted average beryllium exposures in µg/m^3 by case status and exposure assessment method

Subjects and exposure assessment methods	n	Mean	sd	Median	Min	5th %ile	25th %ile	75th %ile	95th %ile	Max
All subjects[a]										
IH data method	386	0.25	0.79	0.03	0	0	0.001	0.17	1.00	10.7
IH rating method	386	0.18	0.32	0.03	0	0	0.002	0.21	0.86	2.00
JEM method[b]	332	0.19	0.61	0	0	0	0	0	1.16	4.31
Controls[a]										
IH data method	255	0.15	0.36	0.03	0	0	0.002	0.15	0.62	3.14
IH rating method	255	0.16	0.28	0.04	0	0	0.003	0.17	0.76	1.90
JEM method[b]	225	0.16	0.57	0	0	0	0	0	0.92	3.87
BeS cases[a]										
IH data method	70	0.25	0.75	0.009	0	0	0.00003	0.08	1.43	4.58
IH rating method	70	0.19	0.38	0.01	0	0	0.00009	0.14	1.01	2.00
JEM method[b]	54	0.09	0.33	0	0	0	0	0	0.88	2.00
CBD cases[a]										
IH data method	61	0.64	1.61	0.07	0	0	0.004	0.64	2.06	10.7
IH rating method	61	0.25	0.39	0.10	0	0	0.01	0.37	0.79	1.94
JEM method[b]	53	0.38	0.87	0	0	0	0	0.07	2.24	4.31

[a]Medians (or underlying distributions) differ across exposure assessment methods by Kruskal-Wallis test ($p < 0.001$)

[b]Medians (or underlying distributions) from JEM method differ significantly from other exposure assessment methods by Mann-Whitney ($p < 0.001$)

[c]CBD median exposures (or their underlying distributions) differ significantly from controls and BeS cases ($p < 0.05$) by Mann-Whitney pairwise tests. (within each exposure assessment method)

Table IV-IV – Comparison of reported exposure characteristics from exposure interviews used in the IH rating and data methods among participants assigned zero and non-zero lifetime-weighted average exposures using the JEM method

JEM method lifetime-weighted average exposure	**Assigned zero exposure (N=273)**	**Assigned non-zero exposure (N=59)**	**P-Value**
Any reported exposure to Be, n (%)[a]	237 (86.8%)	59 (100%)[e]	< 0.001
Highest reported Be exposure, n (%)[a]			
Any direct Be exposure	161 (59.9%)	52 (88.1%)	< 0.001
a. Directly alter Be part	62 (22.7%)	47 (80.0%)	< 0.001
b. Contact with Be waste materials	70 (25.6%)	5 (8.5%)	0.003
c. Contact with finished Be part	29 (10.6%)	0 (0%)	0.004
Any indirect Be exposure	76 (27.8%)	7 (11.9%)	0.012
d. Work within 5 ft. of Be operation	23 (8.4%)	1 (1.7%)	0.094
e. Work in same room as Be operation	13 (4.8%)	1 (1.7%)	0.478
f. Work in same bldg as Be operation	40 (14.6%)	5 (8.5%)	0.293
No known exposure to Be	36 (13.1%)	0 (0%)	< 0.001
Mean percent of work time exposed to Be (median)[b]			
Directly (categories a – c above)	8.9% (0.9%)	19.0% (9.2%)	< 0.001
Indirectly (categories d – f above)	18.7% (4.4%)	27.7% (17.2%)	0.003
Directly or indirectly	27.6% (13.0%)	46.7% (43.1%)	< 0.001
Ever worked as a Be machinist, n (%)[a]	21 (7.7%)	27 (45.8%)	< 0.001
Lifetime-weighted exposure from IH data method			
Mean (median)[b]	0.21 (0.02)	0.51 (0.17)	< 0.001
≤ 0.02 µg/m^3[a]	136 (49.8%)	9 (15.2%)	< 0.001

[a]Compared using χ^2 or Fisher's exact method
[b]Compared using Mann-Whitney test

Table IV-V- Relative and absolute differences between subjects' lifetime-weighted average exposures by exposure assessment method

Comparison	Relative differences[a]			Absolute differences[b]		
	Mean	**Median**	**IQR[c]**	**Mean**	**Median**	**IQR[c]**
IH data method vs. IH rating method						
Overall	0.07	-0.00006	-0.03-0.17	0.20	0.02	0.0009-0.15
Controls	-0.009	-0.0005[d]	-0.05-0.002	0.13	0.02[e]	0.001-0.12
BeS cases	0.06	0	-0.02-0.0006	0.20	0.009[e]	0.00004-0.17
CBD cases	0.39	0[d]	-0.02-0.13	0.50	0.05[e]	0.005-0.37
IH data method vs. JEM method						
Overall	0.08	0.01	0-0.10	0.32	0.03	0.002-0.21
Controls	0.001	0.01	0.00002-0.09	0.24	0.04	0.003-0.20
BeS cases	0.19	0.007	0-0.05	0.34	0.02	0.0004-0.21
CBD cases	0.30	0.01	0-0.19	0.62	0.07	0.004-0.58

[a]Differences in paired exposures (IH data average exposure minus other method)

[b]Absolute differences in paired exposure (absolute value of IH data average exposure minus other method)

[c]Interquartile range

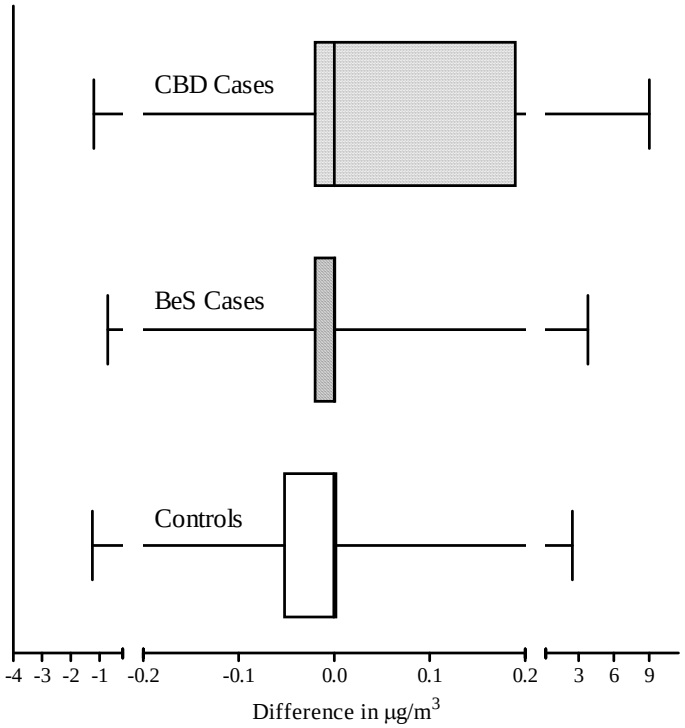
[d]Significant difference in median difference (p=0.006) by Mann-Whitney test.

[e]Significant difference in absolute difference by Mann-Whitney tests (p=0.006 vs. BeS and p=0.010 vs. controls)

[f]CBD cases compared to BeS cases (p=0.006) and controls (p=0.011) by Mann-Whitney tests

Figure 4-2 – Box and whiskers plots showing relative differences in subjects' average exposures by exposure assessment method

a. IH data method minus IH rating method



b. IH data method minus JEM method

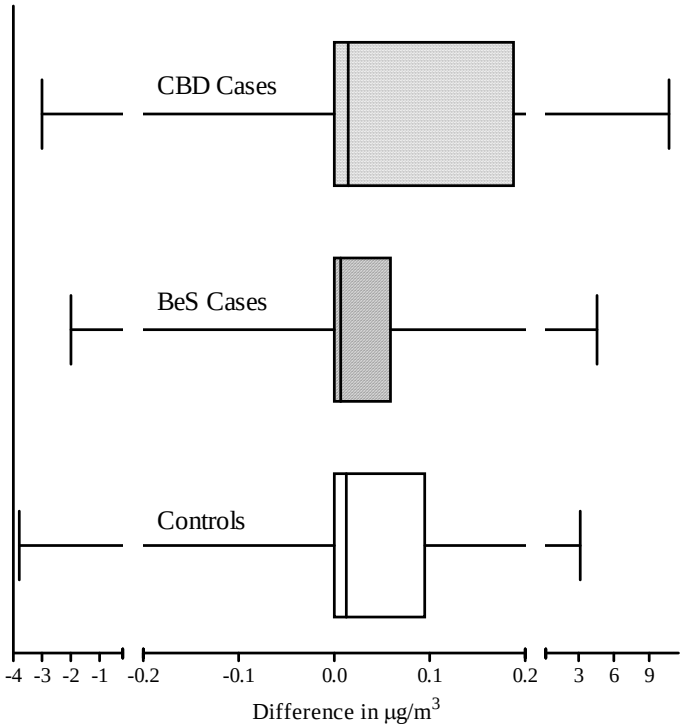


Table IV – VI – Measures of sensitivity and specificity comparing exposure assessment methods using an average exposure cutoff of 0.02 μg/m^3 as zero exposure

Methods and subjects compared	**Sensitivity[a]**	**Specificity[b]**	**Kappa[c]**
IH rating method vs. IH data method			
All subjects (n=386)	0.91 (0.88-0.94)	0.85 (0.81-0.88)	0.76 (0.69-0.82)
CBD cases (n=61)	0.90 (0.81-0.96)	0.81 (0.63-0.92)	0.71 (0.44-0.87)
BeS cases (n=70)	0.87 (0.74-0.94)	0.90 (0.80-0.96)	0.77 (0.54-0.90)
Controls (n=255)	0.93 (0.88-0.96)	0.84 (0.79-0.88)	0.77 (0.67-0.84)
JEM vs. IH data method			
All subjects (n=332)	0.27 (0.23-0.29)	0.94 (0.89-0.97)	0.19 (0.11-0.24)
CBD cases (n=53)	0.40 (0.30-0.43)	0.94 (0.75-1.0)	0.27 (0.04-0.33)
BeS cases (n=54)	0.19 (0.09-0.23)	0.96 (0.87-1.0)	0.16 (-0.04-0.23)
Controls (n=225)	0.25 (0.20-0.28)	0.93 (0.87-0.97)	0.16 (0.06-0.22)

[a]Sensitivity with 95% confidence interval
[b]Specificity with 95% confidence interval
[c]Kappa score with 95% confidence interval
[e]Subjects reporting highest exposure in this category

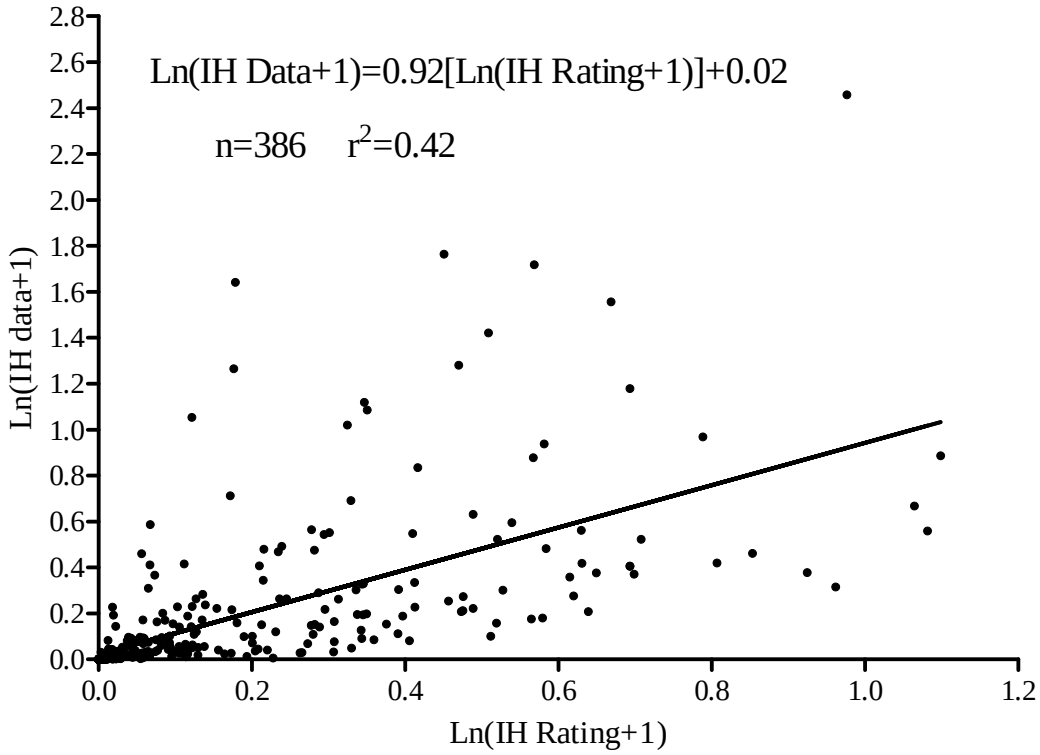
Table IV-VII – Correlations between subjects' lifetime-weighted average exposures by exposure assessment method and case status

Methods and subjects compared	**Pearson r[a] (log-scale)**	**Spearman rho**	**ICC[b]**
IH data method vs.IH rating method			
All subjects (n=386)	0.65	0.93	0.54
CBD cases (n=61)	0.68	0.91	0.61
BeS cases (n=70)	0.75	0.96	0.48
Controls (n=255)	0.62	0.91	0.56
IH data method vs. JEM method			
All subjects (n=332)	0.31	0.34	0.06
CBD cases (n=53)	0.47	0.51	0.26
BeS cases (n=54)	-0.04	0.26	-0.13
Controls (n=225)	0.25	0.29	-0.01

[a]Based on log-transformed exposure measures

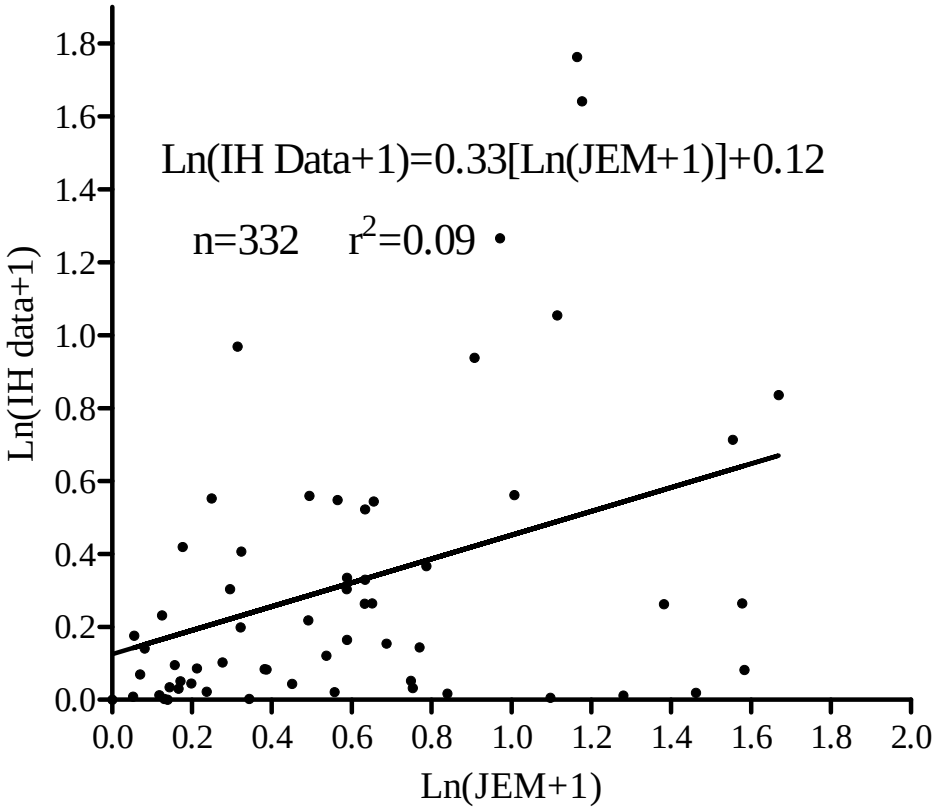
[b]Intraclass correlation coefficient (one-way random effects)

Figure 4-3 – Linear regression of the natural log of the IH data lifetime-weighed average exposure estimates vs. the natural log of the IH rating lifetime-weighted average exposure estimates showing data, regression line, and predicted IH data lifetime-weighted average exposure estimates in table below



IH rating estimate (µg/m^3)	Predicted IH data estimate in µg/m^3 (95% CI)
0	0.02 (-0.006-0.05)
0.02	0.04 (0.01-0.07)
0.05	0.07 (0.04-0.09)
0.1	0.11 (0.09-0.13)
0.2	0.19 (0.17-0.21)
0.5	0.39 (0.36-0.43)
1.0	0.66 (0.59-0.72)
2.0	1.03 (0.93-1.14)
5.0	1.67 (1.49-1.85)

Figure 4-4 – Linear regression of the natural log of the IH data lifetime-weighed average exposure estimates vs. the natural log of the JEM lifetime-weighted average exposure estimates showing data, regression line, and predicted IH data lifetime-weighted average exposure estimates in table below



JEM estimate (µg/m^3)	Predicted IH data estimate in µg/m^3 (95% CI)
0	0.12 (-0.09-0.16)
0.02	0.13 (0.10-0.16)
0.05	0.14 (0.11-0.17)
0.1	0.16 (0.12-0.19)
0.2	0.18 (0.15-0.22)
0.5	0.26 (0.21-0.30)
1.0	0.35 (0.28-0.42)
2.0	0.48 (0.37-0.60)
5.0	0.71 (0.52-0.90)

Table IV-VIII– Multivariate logistic regression models for odds of CBD considering lifetime-weighted average exposure by three different exposure assessment methods

Exposure assessment method and independent variables	**Regression coefficient**	**Standard error**	**P value**	**OR (95% CI)**
IH data method				
Intercept	-1.68	0.16	< 0.001	
Per unit increase in average Be exposure (µg/m^3)	0.92	0.27	< 0.001	2.50 (1.47-4.26)
IH rating method				
Intercept	-1.60	0.17	< 0.001	
Per unit increase in average Be exposure (µg/m^3)	0.86	0.40	0.032	2.36 (1.08-5.19)
JEM method				
Intercept	-1.55	0.16	< 0.001	
Per unit increase in average Be exposure (µg/m^3)	0.41	0.19	0.034	1.51 (1.03-2.22)

Figure 4-5 – Logistic regression output for odds of CBD (models shown in Table IV-VII) showing predicted probability of CBD by lifetime-weighted average beryllium exposure. Each line shows prediction of separate model

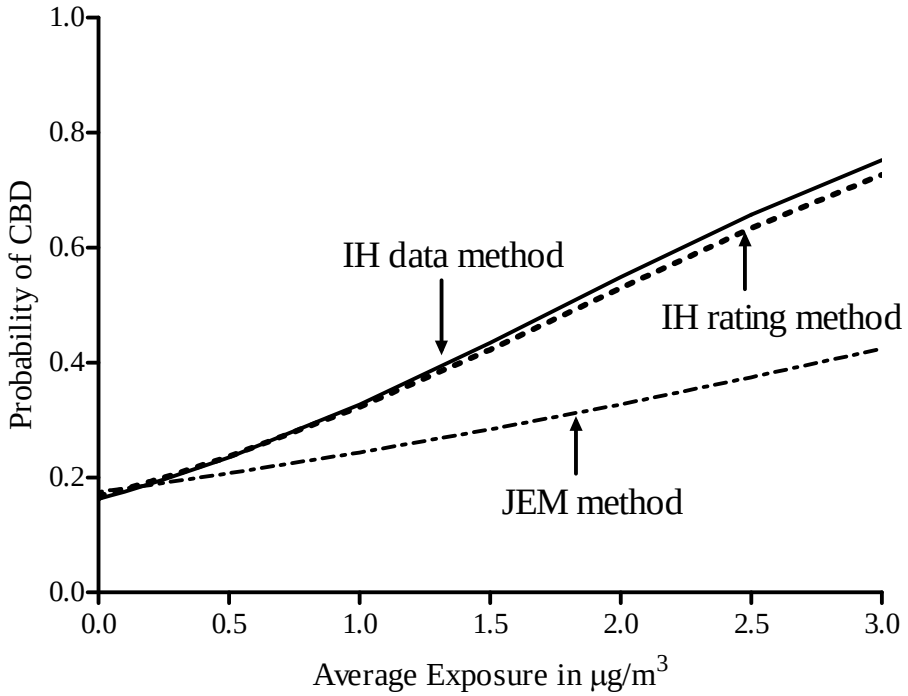


Table IV-IX – Odds ratio estimates by lifetime-weighted average exposure from logistic regression models for three different exposure assessment methods

Average exposure (μg/m^3)	**IH data method model**	**IH rating method model**	**JEM method model**
0.05 μg/m^3	1.05 (1.02-1.07)	1.04 (1.00-1.09)	1.02 (1.00-1.04)
0.10 μg/m^3	1.10 (1.04-1.16)	1.09 (1.01 -1.18)	1.04 (1.00-1.08)
0.20 μg/m^3	1.20 (1.08-1.34)	1.19 (1.01-1.39)	1.09 (1.00-1.17)
0.50 μg/m^3	1.58 (1.22-2.06)	1.54 (1.04-2.28)	1.23 (1.02-1.49)
1.0 μg/m^3	2.50 (1.47-4.26)	2.36 (1.08-5.19)	1.51 (1.03-2.22)
2.0 μg/m^3	6.25 (2.15-18.16)	5.59 (1.16-29.95)	2.29 (1.07-4.93)

References

1. **Kreiss, K., L.S. Newman, and M. Mroz:** Blood testing for chronic beryllium disease. *Journal of Occupational Medicine* 33: 1188-1189 (1991).

2. **Kreiss, K., L.S. Newman, M.M. Mroz, and P.A. Campbell:** Screening blood test identifies subclinical beryllium disease. *Journal of Occupational Medicine* 31: 603-608 (1989).

3. **Mroz, M.M., K. Kreiss, D.C. Lezotte, P.A. Campbell, and L.S. Newman:** Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *Journal of Allergy and Clinical Immunology* 88: 54-60 (1991).

4. **Rosenman, K., V. Hertzberg, C. Rice, M.J. Reilly, J. Aronchick, J.E. Parker et al.:** Chronic beryllium disease and sensitization at a beryllium processing facility. *Environmental Health Perspectives* 113: 1366-1372 (2005).

5. **Kreiss, K., M.M. Mroz, L.S. Newman, J. Martyny, and B. Zhen:** Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. *American Journal of Industrial Medicine* 30: 16-25 (1996).

6. **Kreiss, K., M.M. Mroz, B. Zhen, J.W. Martyny, and L.S. Newman:** Epidemiology of beryllium sensitization and disease in nuclear workers. *American Review of Respiratory Disease* 148: 985-991 (1993).

7. **Kreiss, K., M.M. Mroz, B. Zhen, H. Wiedemann, and B. Barna:** Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occupational and Environmental Medicine* 54: 605-612 (1997).

8. **U.S. Department of Energy, O.o.H., Safety and Security:** "The Department of Energy Former Worker Medical Surveillance Program". Washington, D.C., 2008.

9. **Stange, A.W., F.J. Furman, and D.E. Hilmas:** The beryllium lymphocyte proliferation test: Relevant issues in beryllium health surveillance. *American Journal of Industrial Medicine* 46: 453-462 (2004).

10. **Ruttenber, A.J., M. Schonbeck, J. McCrea, D. McClure, and J. Martyny:** Improving estimates of exposures for epidemiologic studies of plutonium workers. *Occupational Medicine* 16: 239-258 (2001).

11. **Richen, M.J.:** Surprises during beryllium exposure and operation assessment at Rocky Flats 1984-1986. In U.S. Department of Energy Occupational Exposure Assessment and Chronic Beryllium Disease Prevention Program Implementation Combined Workshop. Northglenn, Colorado, 1997.

12. **Barnard, A.E., J. Torma-Krajewski, and S.M. Viet:** Retrospective beryllium exposure assessment at the Rocky Flats Environmental Technology Site. *American Industrial Hygiene Association Journal* 57: 804-808 (1996).

13. **Viet, S.M., J. Torma-Krajewski, and J. Rogers:** Chronic beryllium disease and beryllium sensitization at Rocky Flats: a case-control study. *American Industrial Hygiene Association Journal* 61: 244-254 (2000).

14. **Johnson, J.S., K. Foote, M. McClean, and G. Cogbill:** Beryllium Exposure Control Program at the Cardiff Atomic Weapons Establishment in the United Kingdom. *Applied Occupational and Environmental Hygiene* 16: 619-630 (2001).

15. **Kelleher, P.C., J.W. Martyny, M.M. Mroz, L.A. Maier, A.J. Ruttenber, D.A. Young et al.:** Beryllium particulate exposure and disease relations in a beryllium machining plant. *Journal of Occupational and Environmental Medicine* 43: 238-249 (2001).

16. **Madl, A.K., K. Unice, J.L. Brown, M.E. Kolanz, and M.S. Kent:** Exposure-response analysis for beryllium sensitization and chronic beryllium disease among workers in a beryllium metal machining plant. *J Occup Environ Hyg* 4: 448-466 (2007).

17. **OSHA:** "H005C-6-9-5-8. U.S. Department of Labor, Occupational Safety and Health Administration. Beryllium Docket No. H005C. Exhibit No. 6-9-5-8. Docket Title: Attachment 2.7 Facilities Machining Copper Beryllium. Comments received in response to Federal Register of November 26, 2002." 2002.

18. **OSHA:** "H005C-6-9-5-3. U.S. Department of Labor, Occupational Safety and Health Administration. Beryllium Docket No. H005C. Exhibit No. 6-9-5-3. Docket Title: Attachment 2.1 Primary Beryllium Manufacturing and Processing Facility. Comments received in response to Federal Register of November 26, 2002." 2002.

19. **Fleiss, J.L.:** *Statistical Methods for Rates and Proportions*. New York: John Wiley & Sons, 1981.

20. **Shrout, P.E., and J.L. Fleiss:** Intraclass correlations: uses in asessing rater reliability. *Psychological Bulletin* 86: 420-428 (1979).

21. **Altman, D.G.:** *Practical Statistics for Medical Research*. London, England: Chapman and Hall, 1991.

22. **Kromhout, H., Y. Oostendorp, D. Heederik, and J.S. Boleij:** Agreement between qualitative exposure estimates and quantitative exposure measurements. *American Journal of Industrial Medicine* 12: 551-562 (1987).

23. **Post, W., H. Kromhout, D. Heederik, D. Noy, and R.S. Duijzentkunst:** Semiquantitative estimates of exposure to methylene chloride and styrene: the influence of quantitative exposures. *Applied Occupational and Environmental Hygiene* 6: 197-204 (1991).

24. **de Cock, J., H. Kromhout, D. Heederik, and J. Burema:** Experts' subjective assessment of pesticide exposure in fruit growing. *Scandinavian Journal of Work, Environment and Health* 22: 425-432 (1996).

25. **Cherrie, J.W., and T. Schneider:** Validation of a new method for structured subjective assessment of past concentrations. *Annals of Occupational Hygiene* 43: 235-245 (1999).

26. **Miligi, L., and G. Masala:** Methods of exposure assessment for community-based studies: Aspects inherent to validation of questionnaires. *Applied Occupational and Environmental Hygiene* 6: 502-507 (1991).

27. **Teschke, K., A.F. Olshan, J.L. Daniels, A.J. De Roos, C.G. Parks, M. Schulz et al.:** Occupational exposure assessment in case-control studies: opportunities for improvement. *Occupational and Environmental Medicine* 59: 575-593; discussion 594 (2002).

CHAPTER 5

CONCLUSIONS

Introduction

The overall goal of this dissertation research was to identify the contribution of exposure and genetic effects in the development of beryllium sensitization (BeS) and chronic beryllium disease (CBD) to better understand disease pathogenesis and provide important information to policy makers considering a new beryllium exposure standard. The first case-control study (Chapter 2) evaluated carriage of any glutamic acid at position 69 (E69) of the HLA-DPB1 gene in combination with beryllium exposure assessed using a method that combined individual subject interviews with "expert" assessment of task exposures by industrial hygienists. This study was performed with participants from an active nuclear weapons manufacturing facility, Y-12 in Oak Ridge, TN. A second, larger, case-control study was performed (Chapter 3) involving former workers from a decommissioned nuclear weapons manufacturing facility, Rocky Flats Environmental Technology Site (RFETS) in Arvada, CO. This study evaluated carriage of any E69 allele in addition to specific E69 alleles in combination with beryllium exposure assessed using a more intensive method that combined individual subject interviews with analyses of facility-specific and industry-wide industrial hygiene exposure measurements. The third study (Chapter 4) evaluated three methods of

beryllium exposure assessment including the methods used in Chapters 2 and 3 in addition to a more traditional job exposure matrix (JEM) method. The goals of this study were to determine the comparability of odds ratio estimates produced in previous chapters and to establish the most efficacious exposure assessment method for future studies on larger populations.

Summary and significance of each study

Exposure and genetics increase risk of beryllium sensitization and chronic beryllium disease at the Y-12 National Security Complex

This case-control study of beryllium-exposed workers in the nuclear weapons industry was the first to examine the individual contributions of quantitative beryllium exposure estimates and HLA DPB1 E69 status. The study confirmed increased susceptibility to BeS/CBD among carriers of the HLA DPB1 E69 with genotype frequencies similar to those reported in previous cross-sectional studies [(1-8)]. In addition, similar HLA DPB1 E69 genotype frequencies were noted for cases of CBD and BeS supporting the notion that E69 status is likely important for antigen presentation and not a relevant marker for progression from BeS to CBD [(9-11)]. We found evidence of an exposure-response for beryllium exposure and BeS/CBD in a combined group of BeS and CBD cases. We also confirmed that BeS cases occurred among workers with very low estimated exposures, as demonstrated by previous reports[(12, 13)]. Most importantly, we demonstrated that high exposure and genetic susceptibility via HLA DPB1 E69 each individually conferred increased odds of BeS/CBD of similar magnitude and added new information suggesting that together these odds appear to be additive. The most important

practical implications were the confirmation that high exposure to beryllium conferred increased odds of BeS/CBD even in the absence of genetic susceptibility and that reductions in exposure should reduce the odds of BeS/CBD among carriers and non-carriers of the E69 susceptibility marker. The study implied that establishment of new occupational exposure levels should be aimed at the most susceptible population, those with the E69 genetic variant, due to their higher risk of BeS/CBD at lower exposure levels.

Exposure and genetics in beryllium sensitization and chronic beryllium disease: a case-control study at Rocky Flats Environmental Technology Site

This larger case-control study, involving twice as many cases of BeS and three times as many cases of CBD as compared to the study in Chapter 2, had additional statistical power to evaluate not only carriage of any E69 allele, but also carriage of specific E69 alleles. These genetic factors could be evaluated in combination with beryllium exposure assessed in a more comprehensive manner than in Chapter 2 due to the large number of industrial hygiene exposure measurements available from RFETS. In addition, this study was able to model the odds of BeS and CBD separately due to the larger numbers of cases. This was the largest case-control study of beryllium exposed workers to date. Unlike the study in Chapter 2, this study identified increased beryllium exposure for CBD cases compared with controls and BeS cases which was evident whether considering self-reported exposure assessments or quantitative exposure reconstructions. In contrast, there were no significant differences in exposure between BeS cases and controls. While increased odds of both BeS and CBD was conferred by

carriage of any E69 allele, these odds appeared to be differentially distributed based on E69 genotype with greater odds among carriers of the rarer non-*02 HLA-DPB1 E69 alleles, and among HLA-DPB1 E69 homozygotes. We also found evidence supporting the additive relationship between exposure and genetic susceptibility via E69 in the odds of CBD as identified in Chapter 2 and provided evidence suggesting an exposure-response relationship for CBD and lack thereof for BeS after adjusting for E69 genetic risk factors.

The finding of an exposure-response relationship and the additive relationship between exposure and genetics in the risk of CBD has implications for standard setting in workplace, at a time when OSHA is reconsidering revising the currently out of date beryllium exposure standard. The models from this case-control study can be roughly extrapolated to project the probability of CBD for workers at Rocky Flats given the facility prevalence of CBD of 1.7% identified in a stratified sample of Rocky Flats workers by Kreiss et al.[13] and assuming the E69 genotypes of controls are representative of the entire workforce. Extrapolation of our models indicated the expected prevalence of CBD resulting from a 0.2 $\mu g/m^3$ lifetime weighted average exposure was 1.5%.

Comparison of three methods of retrospective exposure assessment in a case-control study of beryllium sensitization and chronic beryllium disease

The third study compared three different, but related, retrospective exposure assessment methods applied to the participants of the case-control study in the second study. This study compared the exposure assessment methods used in Chapters 2 (IH rating method) and 3 (IH data method) and a more traditional job exposure matrix

method (JEM method). Results from this study suggested that a method of task exposure assessment relying primarily on the professional judgment of industrial hygienists (IH rating method) performed similarly to a method involving extensive analyses of historical industrial hygiene measurements (IH data method) in terms of rank order assessment of average task exposure. Participant exposure assignments using all three of the methods were significant predictors of increased CBD risk. Exposure misclassification likely attenuated the odds ratio point estimates for the risk of CBD by approximately 5% using the IH rating method and approximately 40% using the JEM method. These results imply that, in the case of beryllium exposures, using the combination of industrial hygienist assigned task-based exposures and participant-reported task histories can be an effective strategy to assign long-term exposure estimates to participants in an epidemiology study. These "expert" based long-term exposure estimates appear to only minimally attenuate the odds ratios for the exposure-disease relationship as compared to those produced using a large number of industrial hygiene measurements.

Conclusions

These three studies confirm the importance of both beryllium exposure and E69 genotype in the risk of CBD suggesting an additive relationship between the two. Furthermore, it appears that BeS and CBD risk is differentially distributed among E69 genotypes with carrriers of rarer non-*02 E69 alleles at higher risk. These studies also provide additional evidence on the importance of extremely low beryllium exposures in the risk of BeS even after adjusting for genetic susceptibility. Finally, the studies provide evidence to validate a more efficient exposure assessment method based on task

exposures assessed using “expert” industrial hygiene assessment rather than resource-intensive compilation and analysis of thousands of exposure measurements.

Future Studies

Findings of this dissertation raised several questions for future investigation including:

1. *Investigation of other exposure metrics and their relationship to the development of BeS:* These studies identified trends showing lower cumulative and lifetime-weighted average beryllium exposures among BeS cases compared to controls. This information combined with the frequent anecdotal reports of BeS occurring at extremely low exposures suggest current exposure assessment strategies have not identified the appropriate exposure metric to predict BeS. As has been suggested by others [14], dermal exposure metrics should be further explored to evaluate the importance of alternate exposure pathways in the development of BeS.
2. *Investigation of alternate susceptibility genes in combination with exposure among cases without the E69 variant:* Others [3, 6, 8] have identified a glutamic acid substitution at position 71 of the HLA-DRB1 gene as a potential alternate pathway for the development of BeS and CBD. Carriage of this gene has not been evaluated in combination with exposure.
3. *Investigation of the exposure effects on the severity of CBD:* A diagnosis of CBD represents a wide spectrum of clinical effects ranging from the development of granulomas on biopsy without evidence of symptoms or impairment in lung

function to severe, disabling gas-exchange and other lung function abnormalities requiring medical treatment. Researchers have long hypothesized that CBD severity is related to the total lung burden of beryllium. However, to date, an effective scale for classifying CBD severity has not been developed.

4. *Investigation of alternate exposure-response relationships for CBD:* Logistic regression models in analyses in this dissertation assumed a linear relationship between the log-odds of CBD and lifetime-weighted average exposure. Further analyses using methods allowing for non-linearity, such as fitting splines to the exposure-response relationship, should be performed to explore alternate relationships.

References

1. **Richeldi, L., K. Kreiss, M.M. Mroz, B. Zhen, P. Tartoni, and C. Saltini:** Interaction of genetic and exposure factors in the prevalence of berylliosis. *American Journal of Industrial Medicine* 32: 337-340 (1997).

2. **Richeldi, L., R. Sorrentino, and C. Saltini:** HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 262: 242-244 (1993).

3. **Rossman, M.D., J. Stubbs, C.W. Lee, E. Argyris, E. Magira, and D. Monos:** Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *American Journal of Respiratory and Critical Care Medicine* 165: 788-794 (2002).

4. **Wang, Z., G.M. Farris, L.S. Newman, Y. Shou, L.A. Maier, H.N. Smith et al.:** Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology* 165: 27-38 (2001).

5. **Wang, Z., P.S. White, M. Petrovic, O.L. Tatum, L.S. Newman, L.A. Maier et al.:** Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. *Journal of Immunology* 163: 1647-1653 (1999).

6. **Maier, L.A., D.S. McGrath, H. Sato, P. Lympany, K. Welsh, R. Du Bois et al.:** Influence of MHC CLASS II in susceptibility to beryllium sensitization and chronic beryllium disease. *Journal of Immunology* 171: 6910-6918 (2003).

7. **McCanlies, E.C., J.S. Ensey, C.R. Schuler, K. Kreiss, and A. Weston:** The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *American Journal of Industrial Medicine* 46: 95-103 (2004).

8. **Saltini, C., L. Richeldi, M. Losi, M. Amicosante, C. Voorter, E. van den Berg-Loonen et al.:** Major histocompatibility locus genetic markers of beryllium sensitization and disease. *European Respiratory Journal* 18: 677-684 (2001).

9. **Snyder, J.A., E. Demchuk, E.C. McCanlies, C.R. Schuler, K. Kreiss, M.E. Andrew et al.:** Impact of negatively charged patches on the surface of MHC class II antigen-presenting proteins on risk of chronic beryllium disease. *J R Soc Interface* 5: 749-758 (2008).

10. **Snyder, J.A., A. Weston, S.S. Tinkle, and E. Demchuk:** Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. *Environmental Health Perspectives* 111: 1827-1834 (2003).

11. **Fontenot, A.P., M. Torres, W.H. Marshall, L.S. Newman, and B.L. Kotzin:** Beryllium presentation to CD4+ T cells underlies disease-susceptibility HLA-DP alleles in chronic beryllium disease. *Proceedings of the National Academy of Sciences of the United States of America* 97: 12717-12722 (2000).

12. **Kreiss, K., M.M. Mroz, L.S. Newman, J. Martyny, and B. Zhen:** Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. *American Journal of Industrial Medicine* 30: 16-25 (1996).

13. **Kreiss, K., M.M. Mroz, B. Zhen, J.W. Martyny, and L.S. Newman:** Epidemiology of beryllium sensitization and disease in nuclear workers. *American Review of Respiratory Disease* 148: 985-991 (1993).

14. **Day, G.A., A.B. Stefaniak, A. Weston, and S.S. Tinkle:** Beryllium exposure: dermal and immunological considerations. *International Archives of Occupational and Environmental Health* 79: 161-164 (2006).