Beryllium, an Environmental Exposure Factor as Cause for Reconsidering Sarcoidosis Diagnoses

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ABSTRACT

Paul G. James: Beryllium, an Environmental Exposure Factor as Cause for Reconsidering Sarcoidosis Diagnoses
(under the direction of Woodhall Stopford)

Several years ago a cluster of sarcoidosis cases occurred in a neighborhood in the southwestern part of North Carolina. The neighborhood was located near a large beryllium reserve and a few miles away from a quarry in which one of the sarcoidosis patients was employed. Background information included the facts that gravel from the local mine(s) had been used on some of the neighborhood roads prior to the time roads were paved, and that there was also agricultural activity in the area. Considering that these sarcoidosis cases were in a residential neighborhood, the proximity of quarries to the neighborhood, the large beryllium reserve in the area, and the fact that one of the sarcoidosis patients was employed at the quarry led to the question of whether chronic beryllium disease (berylliosis) due to an environmental exposure should be considered as a diagnosis for these individuals instead of sarcoidosis.
ACKNOWLEDGEMENTS

I would like to acknowledge Dr Ricky Langley of the North Carolina Department of Health and Human Services, and a former intern with the same organization, Ms Amy Stopford, for providing background information from interviews with the sarcoidosis cluster patients as well as the descriptions of the neighborhood and proximity to the local mines. Dr Langley also provided interesting discussion of cluster investigations and provided data regarding North Carolina sarcoidosis deaths.

I would also like to acknowledge Dr Dennis Darcey of Duke University who contacted the sarcoidosis patients and hospital(s) to determine the availability of biopsy samples and updated the willingness of patients to remain involved with or further the investigation of their sarcoidosis diagnoses. Dr Darcey along with Dr Woodhall Stopford, my thesis advisor, have provided medical and research based opinions on the adequacy of sarcoidosis/berylliosis diagnostics and have provided input for future research considerations.

I would like to thank members of my technical report committee. Dr Michael Flynn, who also served as my academic advisor, provided the guidance and contact for obtaining a practicum rotation through the The Hamner Institutes for Health Sciences where Rich Cravener served as preceptor. Rich made the experience productive and enjoyable, and the The Hamner was a cooperative and gracious host for the practicum.

Finally I would like to thank Dr Swenberg, a member of my committee who was also my Advanced Toxicology instructor. Dr Swenberg provided useful discussions and upon hearing my brief overview for a beryllium study offered insights into analytical capabilities regarding
beryllium detection and later provided a UNC contact who was able to discuss analysis requirements for the samples I had hoped to provide.
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<tr>
<td>ATW EDXA</td>
<td>Atmospheric Thin Window Energy Dispersive X-ray Analysis</td>
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<tr>
<td>BEI</td>
<td>Backscattered Electron Imaging</td>
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<tr>
<td>BeLPT</td>
<td>Beryllium Lymphocyte Proliferation Test</td>
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<tr>
<td>BeS</td>
<td>Beryllium Sensitization</td>
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<tr>
<td>CA-CBD</td>
<td>Community Acquired-Chronic Beryllium Disease</td>
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<td>CBD</td>
<td>Chronic Beryllium Disease</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
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<tr>
<td>DOE</td>
<td>Department of Energy</td>
</tr>
<tr>
<td>GLU</td>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte antigen</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma-Mass Spectrometry</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>NC</td>
<td>North Carolina</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program</td>
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<tr>
<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
</tr>
<tr>
<td>TLV-STEL</td>
<td>Threshold Limit Value-Short Term Exposure Limit</td>
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<tr>
<td>µg</td>
<td>microgram</td>
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<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1: INTRODUCTION

Beryllium is a naturally occurring element which is present in rocks, coal, oil, soil, and volcanic dust, and it is also the lightest metal. (ATSDR, 2002) It is a silver-gray to grayish-white Group II metallic element with an atomic weight of 9.01 which is lighter than aluminum, but 40% more rigid, and about one-third more elastic than steel. (DHHS, 2005) It is present at about 6mg/kg in the earth’s crust and at 0.03-0.06 ng/m$^3$ in the atmosphere in rural areas of the US. (WHO, 1990) The source of the largest amount of naturally occurring atmospheric beryllium and beryllium compounds is windblown dust, 5 metric tons/year, and the largest anthropogenic sources are electric utilities, 3.5 metric tons/year. (DHHS, 2005) The most common form for naturally occurring beryllium is the beryllium aluminum silicate (beryl), $3\text{BeO}\cdot\text{Al}_2\text{O}_3\cdot6\text{SiO}_2$. (Cooper & Harrison, 2009)

Beryllium is critical to nuclear, aerospace, and electronics industries among others. (Kreiss, et al., 2007) Its value, in addition to being a light metal with a high melting point, is that when used as an alloy it increases thermal and electrical conductivity and strength. (DHHS, 2005; IARC, 1993) In addition, beryllium has a high affinity for oxygen which allows its surface films to provide high resistance to corrosion, water, and cold oxidizing acids. (WHO, 1990) The most commonly used beryllium products are beryllium-copper alloys though beryllium-aluminum alloys containing up to 65% beryllium are becoming more important. (McNeil, 2001) The strength of copper is increased 6-fold by the addition of 2% beryllium. (Cooper & Harrison, 2009) (IARC, 1993)
DISTRIBUTION AND MINING

Most beryllium mining in the United States is carried out in the Topaz-Spor Mountains in Utah and most of the beryllium manufacturing is done by Brush Wellman in Ohio. (Brush Wellman) A recent business article states that the mine site in Utah produces 80,000 tons of bertrandite ore annually. (Rattle, 2007) Bertrandite is one of two economically important beryllium minerals, notwithstanding the gemstones emerald and aquamarine, and it contains less than 1% beryllium. (WHO, 1990) Beryl is the other mineral and it can have as much as 4% beryllium (Ibid). It is thought that “shallow epithermal environments” such as the one at the Utah site are favorable for the deposition of beryllium. (Lindsey, 1977) Through the year 2004 the US was producing about 80% of the world’s beryllium output with China, Brazil, and Mozambique providing most of the remainder. (McNeil, D.)

As might be expected, beryllium levels in soil vary from place to place. Overall, beryllium in soils is found at levels of 0.1-40 mg/kg. (Sutton, et al., 2012; Taylor et al., 2003; Anderson, et al., 1990; Shireman, Mayila, et al., 1995) The beryllium at 3 sites in the southeastern US was measured at levels as high as 17.8 mg/kg, but the mean level was only 0.15 mg/kg. (Sutton, et al., 2012; Aelion, et al., 2009)

BERYLLIUM TOXICOLOGY

Beryllium can cause both acute and chronic health problems, usually from breathing beryllium mists, dusts, or fumes. (DOE) It has been known since the 1930’s that beryllium was a lung hazard, and it was subsequently found that chronic lung disease developed in beryllium manufacturer workers and other workers exposed to beryllium materials. (Kreiss et al., 2007; Gelman, 1936; Hardy & Tabershaw, 1946) For the purposes of the Department of Energy, regulation 10 CFR 850 of the Chronic Beryllium Disease Prevention Program defines beryllium as: “elemental beryllium and any insoluble beryllium compound or alloy containing 0.1 percent
beryllium or greater that may be released as an airborne particle”. (DOE)

Beryllium sensitization among workers is estimated at 1-16% (Saltini et al., 2001) and approximately 50% of beryllium sensitized individuals already have chronic beryllium disease upon clinical examination. (Newman, et. al., 2005) The progression of beryllium sensitization (BeS) to CBD is 6 to 8% per year after the initial diagnosis. One estimate that is now several years old is that up to 800,000 workers in the U.S. have already been exposed to beryllium (Barna, et al., 2003), so there could potentially be a large number of affected individuals. The addition of a 2µg/m³ standard in 1948, which was accepted by the Atomic Energy Commission in 1949 and by OSHA in 1971, was considered important, but in 1998 the OSHA Assistant Secretary admitted that a 2 µg/m³ PEL did not protect beryllium exposed workers from developing chronic beryllium disease. (Michaels & Monforton, 2008)

The basis of the initial beryllium standard was not epidemiological findings, rather it was based on the atomic weight of beryllium compared to other toxic metals. (Kreiss et al., 2007; Eisenbud, 1982; Eisenbud, 1998) NIOSH has established a workplace air advisory of 0.5µg/m³ 10hr TWA, OSHA has a 2µg/m³ regulation for PEL as TWA and 5µg/m³ ceiling, and there are other agency regulations as well. (ATSDR, 2008) Brush Wellman uses a 0.2 µg/m³ per 8 hour time weighted average standard. (Carey, 2005)

Acute beryllium disease in which beryllium acts as chemical irritant, as thought by some, is no longer very common due to the industrial efforts to improve safety, though chronic beryllium disease (CBD) continues to occur. (Dweik, 2008) There are some; however, who think that the dermatitis associated with acute beryllium disease is not due to irritative effects, but instead may be a result of delayed-type hypersensitivity, and that acute beryllium disease may, like chronic beryllium disease, be immune-mediated. (Cummings, et al., 2009) According to Dweik, the pathogenesis of CBD proceeds as a delayed-type hypersensitivity reaction with
beryllium acting as a hapten and a class II restricted antigen. (Dweik, 2008) It has been described as a “progressive, noncaseating granulomatous disease of the lung” which can cause death. (Haley, 1991) It has been reported that the soluble forms of beryllium are the most likely culprits for sensitization and could occur by respiratory, dermal, or oral routes of exposure. (Deubner et al., 2001) The same authors report that CBD is most often associated with beryllium oxide, but it was unknown whether this was due to chemical properties or due to its tendency to form small particles (0.1um) during manufacturing. The same was true for beryllium metal which had the second most common association with CBD.

It has been suggested that the low prevalence of chronic beryllium disease may be due to genetics, and immunogenetic research has shown support for this concept. (Haley, 1991), (Lombardi et al., 2001) HLA DP presents beryllium to lung-derived CD4+ T cells from CBD patients, and this appears to be a selective ability when compared to HLA DR and HLA DQ. (Fontenot, et al., 2000) HLA DP Glu69 has been found to be associated with berylliosis, and its gene has been shown to have a role in the disease pathogenesis. (Saltini, et al., 1998) However, the high prevalence of this HLA type in the population and the low prevalence of Chronic Beryllium Disease means that it has poor positive predictive value. (Kreiss et al., 2007)

One of the diagnostic tools available for the diagnosis of CBD is the beryllium lymphocyte proliferation test (BeLPT), and it can be used to detect beryllium sensitization prior to pulmonary disease. (Middleton & Kowalski, 2010) In this test, beryllium sulfate is used to stimulate sensitized T-lymphocyte uptake of tritiated thymidine and then compared to T cells not artificially exposed to beryllium sulfate. The BeLPT is used to help distinguish chronic beryllium disease from sarcoidosis. (Maier, 2002)
OBJECTIVES

1. Provide a literature review of beryllium and chronic beryllium disease (berylliosis)
2. Provide sufficient information about sarcoidosis that it can be compared to berylliosis
3. Discuss a cluster of sarcoidosis cases which occurred in Cleveland county, North Carolina
4. Discuss environmental beryllium in the context as to whether it could have played a role in the cluster of cases and and whether sarcoidosis in the cluster cases could be a misdiagnosis
5. Discuss future research activities for beryllium

BACKGROUND:

SARCOIDOSIS AND BERYLLIOSIS

Sarcoidosis is a multisystem granulomatous disease which was first identified in 1899. (Iannuzzi & Fontana, 2011) Noncaseating granulomas without organisms or particles is characteristic of sarcoidosis, but there is no single diagnostic test for sarcoidosis. This disorder is most prevalent in northern Europe at an incidence of 5 to 40 cases per 100,000 people, but exists in many countries. (Iannuzzi, et al., 2007) Sarcoidosis associations with HLA Class II genes differ with cohort, ethnicity, and race. (Spagnola & du Bois, 2007) In the US sarcoidosis affects black individuals approximately 3 times more often than whites and is also more likely to be fatal and chronic in black individuals. (Iannuzzi et al., 2007; Rybicki et al., 1997) Peak incidence usually occurs between the ages of 20 and 39 although the peak for black Americans will be their 40's. Sarcoidosis has an unknown etiology but these authors suggest that much consideration is being given to environmental exposure to airborne antigen due to the lung, eye, and skin involvement. Heffner argues that sarcoidosis has as a cofactor a genetic-based dysregulated hyperimmune response that operates along with its other cofactor, the presence of noninfectious, common, mineral nanoparticles to cause disease. (Heffner, 2007)

The difficulty in distinguishing sarcoidosis from berylliosis can be seen from a
prospective case study in which upon re-evaluation, 34 of 84 sarcoidosis patients were diagnosed with chronic beryllium disease. (Muller-Quernheim, et al., 2006) In this study the authors used the Beryllium Lymphocyte Proliferation Test, occupational history, and upon finding positive BeLPTs, furthered support of their diagnosis of CBD with an occupational evaluation of the workplaces. The difficulty in distinguishing berylliosis from sarcoidosis by laboratory or clinical analyses can be seen in Figure 1 which uses data derived from published tabulated data of Carrington et al. (Carrington et al., 1976)
Figure 1: Berylliosis and Sarcoidosis Comparison; data from Carrington et al. used with permission from John Wiley and Sons; (Carrington et al., 2006) (top left, findings based on physiology; top right, radiology findings; bottom left, histology findings; and bottom right, granuloma composition; plotted using R (RCoreTeam, 2013).
BERYLLIUM AND CANCER

Beryllium is listed as a Group 1 carcinogen by IARC, i.e. probably carcinogenic to humans, and IARC also concluded evidence of carcinogenicity was sufficient for animals .(Beyersmann & Hartwig, 2008; IARC, 1993) It has been categorized as a “known human carcinogen” since 2002 by the National Toxicology Program (NTP, 2011) due in part to epidemiological studies which indicate there is an increased risk of occupational lung disease when working with beryllium.(Steenland & Ward, 1991) One of the possible causalities for carcinogenesis is infidelity of DNA replication(NTP, 2011; Leonard & Lauwerys, 1987).

Beryllium competes with Mg$^{2+}$ for nucleic acid phosphate binding sites, it activates mitogenic signaling pathways(Beyersmann & Hartwig, 2008; Misra, et al., 2002), it induces the expression of proto-oncogenes in vitro(Beyersmann & Hartwig, 2008; Kesheva et al., 2001), and it induces hypermethylation of DNA for a tumor suppressor gene which results in less gene product(Beyersmann & Hartwig, 2008), (Belinsky et al., 2002). Studies have shown that beryllium salts are not mutagenic in bacteria, but studies have also shown that beryllium salts affect chromosomes and cause mutations in mammalian cells.(Steenland & Ward, 1991; Gordon & Bowser, 2003) Lung cancer has also been found by some to be strongly correlated to mean, maximum, and cumulative beryllium exposures for male workers in beryllium processing plants.(Schubauer-Berigan, et al., 2011)

However, a literature-based weight of evidence study for beryllium as a cause of lung cancer concluded, using Bradford Hill criteria(Hill, 1965), that the carcinogenicity of beryllium in humans should be considered either inadequate or marginally suggestive.(Hollins et al., 2009) The authors of the weight of evidence study attribute some of the lung cancer cases to very high doses of beryllium that are no longer typical of industrial exposures.
BERYLLIUM CLEARANCE

Mucociliary escalator transport, translocation to tracheobronchial lymph nodes, alveolar macrophage clearance to the tracheal region, and solubilization of beryllium are mechanisms which may contribute to the clearance of beryllium. (EPA, 1998) Generally, phagocytosis is a rapid process with 50-70% of particles engulfed by the macrophage within 2-3 hours and approximately 100% internalization after 24 hours. (Geiser, 2010) However, phagocytosis of inhaled nanoparticles only occurs at a rate of approximately 0.1% per 24 hours and appears to occur sporadically. Nanoparticles in the lung are cleared primarily by movement up the respiratory tree via mucociliary transport, but the macrophages can also transport the particles from alveolar epithelium to the interstitium or to the circulating blood. (Ng, et al., 2004) Contact of the insoluble particles with epithelial cells followed by uptake into underlying cells is likely to occur if the particles have escaped elimination by the mucociliary escalator or by the alveolar/airway macrophages. (Churg, 1996; Adamson & Bowden, 1981) The skeleton is the ultimate beryllium storage site following its clearance from the lungs although clearance from the lung to the tracheobronchial lymph nodes or to liver, kidney, spleen or other tissues will occur. (Muller et al., 2010; Finch et al., 1990)

BERYLLIUM SOLUBILITY

Dissolution rates in phagolysosomal simulant fluid were determined to have rate constants on the order of $10^{-8} \text{g/cm}^2\text{-day}$ for beryllium oxide, and $10^{-7} \text{g/cm}^2\text{-day}$ for beryllium. (Stefaniak, et al., 2006) These rate constants were independent of the initial mass and provide insight into the amount of dissolved beryllium that macrophage lysosomes may produce. Beryl ore dusts emitted from a heat-treater, in which the silicate structure has been destroyed, have dissolution rates of approximately $1 \times 10^{-8} \text{g/cm}^2\text{-day}$, and the beryllium hydroxide powder has a rate of approximately $2 \times 10^{-7} \text{g/cm}^2\text{-day}$. 
A dissolution rate of $7.8 \times 10^{-5}$ of the initial amount of added beryllium was obtained after approximately 30 hours (constant rate achieved) by passing a serum ultrafiltrate simulant over beryllium particles. (Andre et al., 1987) In vivo beryllium solubility rates in baboons and rats following intratracheal injection were $5 \times 10^{-6}$ for metal and $5 \times 10^{-5}$ for hot-pressed particles. When poorly soluble particulate beryllium compounds were assessed for solubility in artificial macrophage phagolysosomal fluid, it was found that the beryllium materials associated with elevated disease levels had faster dissolution rates than beryllium materials which were not associated with higher disease levels (p<0.05). (Stefaniak et al., 2011a)

By comparison to another material, plasma solubilized approximately 0.48% of the available silica from 5 µm quartz dust particles over 8 days (243 µg from 50 mg), and approximately the same amount was solubilized with serum (233 µg). (Rahman et al., 1975) This gives a rate of approximately $3.0 \times 10^{-5}$ g silica solubilized/day or $1.2 \times 10^{-6}$ g/cm$^2$-day. The $K_a$ which defines the dissociation of SiOH to SiO$^{-}$ H$^+$ at 25 °C is $10^{-6.8}$. (Dove, 1994; Schindler & Kamber, 1968)

Particles larger than 5-10 µm in the lung will be cleared by the mucociliary escalator and phagocytosis, and soluble beryllium is cleared by dissolution in the pulmonary fluid. (Paustenbach et al., 2001; McClellan & Henderson, 1995) Inhaled water-soluble beryllium salts are primarily excreted via the kidneys with a half-life of 2-8 weeks. (Muller et al., 2010; Zorn et al., 1986) Intracellular solubility following phagocytosis is another potential toxicity issue. (Finch et al., 1988)

Some studies report that absorption through intact skin is negligible although injured skin could be vulnerable to higher absorption. (Deubner et al., 2001; Ivannikov et al., 1982) Other studies have indicated that skin exposure to soluble beryllium salts is a cause of systemic immunological sensitization. (Stefaniak et al., 2011b; Zissu et al., 1996) Rates of beryllium
dissolution in sweat are $\sim 1.2 \times 10^{-9} \text{ g/cm}^2\text{-day}$ at pH 5.3 and $5.3 \times 10^{-11} \text{ g/cm}^2\text{-day}$ at pH 6.5. (Duling et al., 2012)

Ingestion is not the major route for beryllium exposure, but long ago the U.S. Public Health service set a value of 0.006% for gastrointestinal absorption though other research only suggests a general level of <1%. (Deubner et al., 2001)

**BERYLLIUM EXPOSURE**

A comparison of personal breathing zone samplers obtained over a four year span revealed that bertrandite foot miners had one of the lower overall beryllium exposures (0.07 µg/m$^3$) among mining processes while workers involved in loading, crushing, and grinding bertrandite ore had levels of exposure 0.55 µg/m$^3$, beryl furnace workers 0.64 µg/m$^3$, furnace pour, heat treat, grinding workers 0.99 µg/m$^3$ and those involved in the precipitation and drumming of Be(OH)$_2$ 0.76 µg/m$^3$. (Deubner et al., 2011) A separate study of different work process areas such as crushing, melting, heat treating, grinding, and drumming showed that particles had specific surface areas ranging from 0.34-25.87 m$^2$/g. The smallest particle surface areas were associated with drumming (0.34-0.4 m$^2$/g), grinding (beryl) work areas had surface areas of 4.32-6.41 m$^2$/g, heat treating 8.94 m$^2$/g, melting 7.14-13.39 m$^2$/g, and crushing (beryl) 7.03-25.87 m$^2$/g. (Stefaniak et al., 2008)

Exposure estimates from beryllium workdays include hand to mouth, tracheobronchial mucociliary-aided ingestion, and head airways ingestion with a total ingestion estimate of 0.0365-4.11 µg/day and an inhalation estimate of 0.116-1.63 µg/day. (Deubner et al., 2001)

The temperature at which beryllium oxide is calcined is a factor in its toxicity, and attributes such as particle size or surface area and solubility of the beryllium oxide may be contributing factors to the toxicity. (EPA, 1998; Finch, et al., 1988) Beryllium oxide calcined at 500 °C is reported to be more toxic than that calcined at 1000 °C and this difference may be due
to the greater surface area of the 500 °C BeO.

**INSTRUMENTAL METHODS FOR TISSUE DETECTION OF METALS IN TISSUES**

An analytical method which can be used for the quantitative detection of metals in formalin-fixed paraffin-embedded tissue is high resolution inductively coupled plasma mass-spectrometry (ICP-MS). (Sarafanov et al., 2008) Metals Fe, Zn, Se, and Cd were measured in tissues following deparaffinization by machine based xylene for one week at 55 °C or by handling based deparaffinization by hexane at 20 °C for one week. The quantitative recovery of the metals ranged from 24-97%.

A separate study compared Cu, Fe, and Zn levels of fresh, frozen, and paraffin embedded bovine liver samples by ICP. (Bischoff, et al., 2008) A higher value for the metals was consistently found in the paraffin embedded samples and the authors found this difficult to explain. Their overall view was that results obtained from formalin-fixed samples with or without paraffin embedding were useful but should be interpreted with caution and that fresh or frozen samples were preferred.

Flame atomic absorption has also been used as a detection system to compare metal quantities in tissues which had been deparaffinized versus levels in fresh frozen tissues.(Johnston, et al., 2009) The findings for canine liver levels of Cu, Fe, and Zn revealed that while there were differences between fresh frozen tissue and deparaffinized tissue there were no changes in the clinical severity scoring based which typically included a broad range of concentration values for each severity level.

Laser ablation ICP-MS typically uses cryo cut native samples with sample sizes ranging from 1 square mm to 100 mm x 100 mm with thicknesses of 10-100 micrometers. (Becker, 2013) The technique has sensitivities in the range of 0.001-1 ug/g, it can support simultaneous scanning of up to 40 elements, and it can be used to image samples such as a brain hemisphere.
BERYLLIUM DETERMINATION IN LUNG TISSUE OF A
NUCLEAR WEAPONS WORKER

Beryllium has been detected in the human lung tissue of a female with occupational
exposure to beryllium at a nuclear weapons testing facility. (Butnor et al., 2003) Local areas of
beryllium in the tissue could be identified by negative backscattered electron imaging (BEI) in
which the beryllium had a light appearance against a dark background. Confirmation of the
substance as beryllium was accomplished using paraffin sections of lung tissue and the
nondestructive technique of atmospheric thin-window energy-dispersive X-ray analysis (ATW
EDXA) which generated a spectrum with discrete beryllium peaks.

This case study patient's medical history revealed she had already developed a cough with
dyspnea on exertion, and biopsy revealed nonnecrotizing, granulomatous inflammation. The
occupational history, biopsy, positive beryllium lymphocyte proliferation test, and the presence
of beryllium in the lungs are major factors which can support a diagnosis of berylliosis. The
ATW EDXA technique provided confirmation that beryllium was present in lung tissues and thus
helped provide diagnostic distinction between sarcoidosis and berylliosis that histology and
clinical testing alone did not provide.

HISTORICAL LEVELS OF BERYLLIUM DETECTION DUE TO ACUTE,
OCCUPATIONAL, AND NONOCCUPATIONAL EXPOSURES

An older study that looked at tissue, urine, sputum, and blood samples from individuals
with beryllium exposure had several findings in the tissue samples of the study subjects. (Dutra,
et al., 1949) They measured 9-220 ug beryllium per 100 g lung tissue in subjects with acute
beryllium disease, 1.7-78 ug per 100 g lung tissue for subjects with chronic disease and
occupational exposure, and in 3 of 4 non occupationally exposed individuals with chronic
disease they found 0.24-1.6 ug beryllium per 100 g lung tissue. Beryllium was also detected in
kidney stones of the subjects.
WORK SITE EXPOSURES TO BERYLLIUM CONTINUE

Work sites continue to be a source of numerous cases of chronic beryllium disease. For instance the Y-12 national lab in Tennessee had 17 active workers with chronic beryllium disease in the year 2007 and 40 additional workers had tested positive for beryllium sensitization at that time. (Munger, 2007) Even OSHA inspectors have been vulnerable with 10 of its 271 inspectors sensitized to beryllium. (Carey, 2005)

COMMUNITY ACQUIRED CHRONIC BERYLLIUM DISEASE

Though no new cases of community-acquired chronic beryllium disease (CA-CBD) have been reported since 1969, a more recent (2008) journal article takes a look back at the occurrences near the Lorraine, Ohio and Reading, Pennsylvania beryllium facilities. (Maier, et al., 2008) During the early years of operation in Lorraine, Ohio the community based rates of CBD were similar those found in the beryllium plant so it was considered that airborne particles could be responsible. In Ohio all but one case occurred in residents living within 0.75 mile radius of the plant, but in Reading, Pennsylvania the cases were found up to 5.3 miles from the plant. In this publication, the authors report on their reexamination of some of the patients in the Reading area and also on additional people from that area that came forward.

They found 8 cases to investigate with confirmed CBD in five cases and three probable cases. Their study excluded individuals with occupational or para-occupational exposure, i.e. exposure of family members to workplace chemicals via dust in worker's clothes, shoes, hair. (Donovan et al., 2012) In contrast to the study done many years earlier in which affected residents lived as far as 5.3 miles away with over half living over 4 miles away from the beryllium facility, the patients in this study (diagnosed between 1999 and 2002) lived within 1.5 miles from the plant and were not in the path of the prevailing wind for the area. Three of the cases were family members suggesting genetic susceptibility.
There have been few, if any, other recently published studies of this nature; however, this paper has indicated that new cases community acquired cases have not been reported since 1969 and so it would appear that the safety at the beryllium plants has improved.

The study described above did not include para-occupational beryllium exposures. In the earlier industrial years however, para-occupational exposures were known to occur as in the finding for 60 neighborhood cases in which the beryllium exposure for 27 of the cases was only through contaminated clothing. (Knishkowy & Baker, 1986); Hardy, et al., 1967) One study showed that shaking clothes contaminated with beryllium released an average of 500 µg beryllium/m$^3$ air. (Knishkowy & Baker, 1986); Eisenbud, et al., 1949)

**SARCOIDOSIS FALL LINE AND NORTH CAROLINA**

Sarcoidosis, especially sarcoidosis in the U.S. south was a topic for discussion even in the aftermath of World War II. A study from that era with members of the Armed Forces who had been diagnosed with sarcoidosis used birthplace and sarcoidosis induction residence as part of discussion which makes an argument for soil composition as a contributing factor for sarcoidosis. (Gentry et al., 1955) From the cases they developed a fall line which ran from mid Texas up through Arkansas, the western part of Kentucky and Tennessee before dipping back down into Alabama and Georgia and up through the middle of North Carolina and Virginia and which demarcated a preponderance of cases most of which occurred below the line. The Middle Atlantic Coastal plain was one of the areas described by the authors and, it was associated in part with Red-Yellow Podzolic soils which were present in many states, but the North Carolina black service men had a particularly high sarcoidosis rate of 71.3 cases per 100,000 in that soil region. Based on the study group, the black population was generally far more prone to sarcoidosis than whites no matter where they had lived.

In addition to soil, a number of possible vectors are mentioned as possible etiological
factors for sarcoidosis, and the similarity of sarcoidosis to berylliosis brought in possible associations with beryllium and beryllium/soil composition. The soils of the Middle Atlantic Coastal Plain were said to be derived from granitic rock in the Appalachians which they described as having the major concentration of beryllium surface ore in the U.S.

**ROLE OF STATE HEALTH DEPARTMENTS IN SUSPECTED DISEASE CLUSTERS**

State Health Departments may have many cluster investigation requests, but typically only 1-3% of these requests result in field investigations. (Wartenberg & Greenberg, 1992; Greenberg & Wartenberg, 1991) From the Health Department's point of view, their resources are limited, and epidemiologists have concerns that data provided by residents are biased, usually not worthy of scientific follow-up and usually don't help health officials find true problems.

A finding in a 1991 report was that reporting of disease clusters was stretching health departments resources and not revealing many environmental hazards, and it was the opinion of the authors that cluster investigations were actually biased away from the most serious hazards. (Wartenberg & Greenberg, 1992) A survey of state health departments showed that from those health departments which responded, a noncommunicable cluster investigation was likely to originate from citizen reports (37 states), politician requests (33 states), news articles/media reports (30 states), tracking data (28 states), and physician or health care providers (17 states). (Juzych et al., 2007)

A protocol for investigating disease clusters was developed and published by Wisconsin public health workers. (Fiore, et al., 1990) It includes the following 8 steps: "1) circumscribing the cluster; 2) ascertaining cases; 3) assessing risk of exposed versus a referent population; (4) statistically analyzing disease rates; 5) examining potential exposure; 6) assessing biologic plausibility; 7) determining cluster significance and need for further investigation; and (8) reporting results." This Wisconsin health group did not view the investigation as a purely
scientific endeavor, but viewed it as part of an important interactive public health activity.
CHAPTER 2: BERYLLIOSIS AND SARCOIDOSIS IN NORTH CAROLINA

SARCOIDOSIS CLUSTER

NC Department of Health and Human Services, Division of Public Health contacts (Dr Ricky Langley and Amy Stopford (former intern)) have provided unpublished information regarding a sarcoidosis cluster in Cleveland County, in the western part of North Carolina. The Centers for Disease Control (CDC) forwarded information regarding these cases to NC Division of Public Health after they were contacted by one of the affected residents. There were 9 cases in a neighborhood, and a focus on five of these individuals provided the following profile: they were all black, 3 females, 2 males, sarcoidosis was confirmed by biopsy in all five cases, and the five individuals included a mother-son and a husband-wife pair. At the time of diagnosis one person was employed as a mechanic at the Foote Mineral Company, one was a truck driver, and 3 were in the school system. The neighborhood which was affected included 6 households and was located approximately 2 miles from the Kings Mountain Mica Patterson Plant and approximately 5 miles from Martin Marietta Aggregates (gravel) and Rockwood Lithium Mine.

The Kings Mountain Region contains a large beryllium reserve (Bentzen, 1970). One of the components is a “tin-spodumene” belt which contains beryllium in the form of beryllium oxide and beryl.(Griffitts, 1954; Kesler, 1942) This two mile wide belt of minerals extends from Gaffney, South Carolina toward the northeast. The path goes through the eastern portion of North Carolina's Cleveland county, the northwest section of Gaston County, and ends in the south central portion of Lincoln County. About 40,000 tons of beryl (~12% BeO) are thought to be contained in the minable spodumene ore of Kings Mountain and another 238,000 tons of beryl in
other pegmatite in the area. The Beaverdam portion of the belt which is located further north in Gaston county has about 6,000 tons of beryl and a little over twice that amount in other parts of the Beaverdam area.

The proximity of the neighborhood sarcoidosis cases to environmental beryllium introduces the possibility that these neighborhood sarcoidosis cases might possibly be cases of berylliosis rather than sarcoidosis. Sarcoidosis and berylliosis are difficult to distinguish and further study would be needed with the cooperation of these patients.

There were 445 deaths attributed to sarcoidosis in North Carolina over the years 2005-2009. (State Center for Health Statistics, NC DHHS 2005-2009, raw data contributed by Dr Ricky Langley). There was one North Carolina death attributed to berylliosis during this same period. Of North Carolina's 100 counties, 78 counties reported sarcoidosis deaths during this 5 year span and 22 counties did not. Of those counties that reported sarcoidosis deaths, the total number of deaths ranged from 1 to 31.

Since the counties having larger populations might have a larger number of sarcoidosis cases to report based on their population alone, a death rate was considered a more appropriate statistic. The previously mentioned death totals were used along with the population estimates for each county (North Carolina Revised Population Estimates, 2005-2009) summed over the years 2005-2009 to obtain a death rate. The rate of death due to sarcoidosis in North Carolina counties ranged from 1.1 to 53.9 sarcoidosis deaths per million county residents with a mean death rate of 13.5. The highest rate (53.9) was in Chowan County in northeastern North Carolina. The next 5 highest rates were Hertford (41.5), Bertie (38.9), Nash (31.7), Halifax (29.0), and Bladen (28.9). Three of the counties having the highest rates, Chowan, Hertford, and Bertie are clustered together in the northeastern part of North Carolina.

Cleveland County which had the cluster of sarcoidosis cases which were being
Figure 2: NC Death Rates per County; plotted using R(RCoreTeam, 2013;Brownrigg et al.(R version), 2013)

considered as possible berylliosis cases had a rate of 16.6 sarcoidosis deaths per million residents. The one North Carolina death attributed to berylliosis occurred in Gaston county which is adjacent to Cleveland County.

Even though Cleveland County, North Carolina and the surrounding area has a large beryllium reserve, minerals other than beryllium rather than beryllium itself are currently mined there. It is not known whether locations which are actually involved in mining beryllium might have an elevated sarcoidosis death rate in the local populations either as a consequence of the mining or due to the types of soil in the region.

Utah is one of the states which has been involved in beryllium mining for years, but it is not known whether there was increased sarcoidosis deaths due to mining. The website http://www.us-mining.com/utah/beryllium-mines was useful in identifying some of the Utah counties engaged in beryllium mining. When Utah’s Mortality ICD-10 module (ibis.health.utah.gov) was queried for counties reporting sarcoidosis (ICD code -D86) during years 2008-2012 there were several Utah counties listed as having deaths. Only 4 counties had
reportable counts, and they included the mining counties Davis (5), Tooele (4), Utah (4) and an urban county Salt Lake (11). These values were reported to have coefficients of variation of greater than 30% by the Utah query module. Counties which were listed in the query response as having sarcoidosis deaths but had suppressed counts due to relative standard error of greater than 50% were Box Elder, Duchesne, Uintah, and Washington. The sarcoidosis deaths for Davis, Tooele, and Utah were adjusted for population to get a sarcoidosis death rate and then compared to a nonberyllium mining county, Salt Lake, see Figure 3.

![Utah Sarcoidosis Deaths per Million Residents](http://wonder.cdc.gov)

**Figure 3. Utah Sarcoidosis Deaths per Million Residents**

It is obvious that Tooele County which is located just north of the Spor Mountain area (Juab County) has a higher sarcoidosis rate than those of other counties. It is not known whether this would be due to the mining in the area since other mining counties (Davis and Utah) did not show elevated sarcoidosis deaths compared to an urban county (Salt Lake). There were no berylliosis cases reported in the Utah Ibis module for the years 2008-2012.

The CDC Wonder Compressed Mortality data (http://wonder.cdc.gov) for sarcoidosis of
all types (ICD code D86) revealed that there were 10,348 deaths reported in years 1999-2010. CDC’s query report includes a crude rate per 100,000 population for search topics. The death rate per 100,000 for blacks during these years was 1.3 (6285 deaths) and the rate for whites was 0.1 (3,984 deaths). American Indian or Alaska Natives had a low rate based on a total of 20 deaths and Asian and Pacific Islanders had a total of 23 deaths during this time.

A similar query of CDC Wonder Compressed Mortality data for berylliosis revealed a total of 81 berylliosis deaths (ICD code J63.2) during the years 1999-2010. 79 of those deaths were individuals of the white race. The race(s) of the other two individuals was not specified. The states having the most deaths were Ohio with 16 and Pennsylvania with 21. The states making up the remainder of the deaths were not identified.
Chapter 3: Discussion

Berylliosis shares common characteristics with other lung diseases, most notably sarcoidosis. This literature review has included many of the basic characteristics of both berylliosis and sarcoidosis as well as some of their distinctions.

This project initially had the objective of evaluating individual(s) from a neighborhood cluster of sarcoidosis cases for evidence of berylliosis. The justification for suggesting their diagnosis could be berylliosis was based in part on the clinical similarity of sarcoidosis and berylliosis, and the fact that the quarry region has one of the highest beryllium reserves in the U.S. (Bentzen, 1970) Additionally, the quarry was located a few miles away from the neighborhood in which the quarry worker and several other people lived who had been diagnosed with sarcoidosis. The gravel from local quarries had been used on some of the neighborhood roads before they were paved and thus may have contributed to environmental exposure. Since there were nonquarry workers and multiple households which were affected further investigation of beryllium as the possible causative agent of the respiratory disease would include the rationale that this was an environmental exposure and not just an occupational exposure.

The sarcoidosis patients in this cluster were diagnosed many years ago. Their lung biopsy samples had been stored at local hospitals, and it was thought that with the cooperation of the patients, their paraffin embedded tissues might be analyzed for the presence of beryllium. However, upon re-initiating communications with these patients, and focusing on the most likely and cooperative candidate for beryllium exposure, the quarry worker, we found that the hospital
did not retain the tissue samples long enough for us to carry out this analysis.

Once it became clear that the tissue sample from the most likely candidate for the detection of beryllium was not available, it became necessary to rethink this project. An alternative solution that could have been pursued would have been to obtain fresh blood samples from patients and to have those blood samples analyzed by beryllium lymphocyte proliferation testing (BLPT). A positive BLPT would tell us the patient had been sensitized by beryllium, but a negative test would not necessarily indicate that the patient had not had an environmental exposure. Only a small percentage of patients who are exposed to beryllium become sensitized and/or develop berylliosis. A positive BLPT test; however, would excluded sarcoidosis as a diagnosis; patients suffering from granulomatous diseases other than berylliosis have not produced positive BLPT tests, i.e. the BLPT test has high specificity albeit with a sensitivity that has been reported to vary from low to high levels. (Muller-Quernheim, 2005) The BLPT had been considered early in the project and had been projected to be used as a confirmation test if and when detection of beryllium by analytical means had been achieved.

The cluster which occurred in Cleveland County is still only a small group of diagnosed sarcoidosis individuals, but the occurrence is west of the North Carolina areas associated with the highest death rates according to the data in Figure 2 and also according to the World War II era study which developed associations between sarcoidosis and soil type, induction location, and the Fall Line depicting the majority of the cases. (Gentry et al., 1955)

Older reports of sarcoidosis in the western states indicate that it is not a very common disease in that part of the country. (Beier & Lahey, 1964) Part of their stated reasoning for considering it uncommon was that among birthplaces determined during 2 studies of military servicemen with sarcoidosis, one with 400 Army personnel from 1952-1956 and the other a Navy and Marines study which included 132 servicemen from 1957-1958, showed that none of
the study individuals had originated from Utah. (Cooch, 1961; Gundelfinger, B.F. & Britten, 1961). Although the Beier & Lahey publication reported on 8 white children with sarcoidosis over a 7 year period during that era, it seems that current data also indicates that sarcoidosis remains uncommon there. The increased death rate in Tooele County may be an indication of higher incidence due to mining activity or there could be other unknown reasons. However, the sarcoidosis death rate in a known beryllium mining area such as in Tooele County is lower than many of the death rates seen in North Carolina for sarcoidosis regardless of mining activities.

Further study of chronic beryllium disease and sarcoidosis could proceed in at least two additional directions. One direction would be to determine whether there is hospitalization information for sarcoidosis and/or berylliosis that might help further explain any patterns of sarcoidosis/berylliosis occurring in North Carolina and yet are not well-defined or obvious even when the death rates are considered. One of the most obvious conclusions and one of the most obvious reasons for adding this step is that sarcoidosis death rates might not correlate well with the number of sarcoidosis cases. The analysis of in-patient hospitalizations for sarcoidosis (or berylliosis) could add more detail or perhaps provide a more accurate representation of these diseases. This approach of using in-patient hospitalization data was the basis of a study of North Carolina asbestosis and silicosis cases, and among many findings the research revealed that the highest silicosis rates were in Western North Carolina and that asbestosis cases did not show a geographical pattern. (Dang, et al., 2013)

An introductory look into available resources shows that the University of North Carolina Sheps Center provides a NC Discharge database, but according to their website (http://www.shepscenter.unc.edu/data) these are not public records. Access to their information requires an application, and among the requirements are IRB permission or IRB exemption information. To access more general data the UNC Sheps Center recommends HCUPnet, a free,
searchable service, see website [http://hcupnet.ahrq.gov/](http://hcupnet.ahrq.gov/). The HCUP site includes searchable state data that can be used to determine the number of sarcoidosis discharges (ICD-9, code 135) in a given year, but the site for North Carolina does not provide county specific information using ICD codes. The HCUP site state searches can provide hospital stay/discharge information for a primary diagnosis such as sarcoidosis in terms of age, gender, and metropolitan vs suburban type areas as well as many other possibilities. The site does have a beta system with some county information searchable by Clinical Classification Software which groups numerous ICD codes into a category rather than by ICD code. In order to develop a North Carolina County map of sarcoidosis discharge rates further research would be needed.

The other major research direction which could be taken to further the understanding of the sarcoidosis/berylliosis cases would be to look at environmental factors in the area of the beryllium deposits by environmental sampling. Soil samples from Cleveland County or other counties of interest could be analyzed for respirable silica and beryllium content to determine whether levels of these minerals correlate with sarcoidosis/berylliosis deaths and/or hospitalizations from the county. A study in which soil metals levels were being evaluated for possible correlation with elevated levels of mental retardation and developmental delay did not show any significant beryllium concentration differences in any regions including control regions for the study.(Aelion et al., 2009) While this study did not contribute to understanding of berylliosis and sarcoidosis it does provide soil sampling techniques and laboratory information that would be useful. For the soil study 20-50 mg of the top 5 cm of soil from each study location was collected and then sent to Pace Analytical Lab in Huntersville, North Carolina. It appears as though the sample collection requirement and the analysis lab would both be convenient for further study of Cleveland County soils.

A study of air concentrations of beryllium in areas ranging from rural farming areas to
highly industrialized areas of Ohio made use of gas chromatography as a tool for analysis of beryllium particulates captured by high volume air samplers. (Ross et al., 1977) Wind blowing from industrialized sites toward sites of interest was shown to increase ambient air concentrations of beryllium between 1 and 1.5 ppm for some locations which had beryllium levels ranging approximately 0.7 to 2.8 ppm. Wind was not a factor in some sites, but with regard to the Cleveland County study, the wind contribution would introduce an additional variable for consideration.

Stream water from numerous North Carolina counties has already been mapped to some extent for beryllium and other minerals, but some of this beryllium data was documented years ago in 1999 (http://www.geology.enr.state.nc.us/NUREgeochem/geochem2.htm). Additional sampling and analysis could provide more current information.

The best possible study might use all of the above approaches to provide a comprehensive overview of sarcoidosis and berylliosis using clinical, epidemiological, and environmental data. These data could provide better understanding of the impact of a geographically enriched beryllium environment on health and possibly help identify environmental conditions which contribute to berylliosis or sarcoidosis.
BIBLIOGRAPHY


State Center for Health Statistics. (Raw data contributed by Dr Ricky Langley, NC DHHS which was retrieved from State Center for Health Statistics, NC DHHS 2005-2009)


APPENDIX

Table 1: North Carolina County Death Rates from Sarcoidosis

<table>
<thead>
<tr>
<th>County</th>
<th>Estimated Population 2005-2009</th>
<th>Total Number of Sarcoidosis Deaths 2005-2009</th>
<th>Sarcoidosis Deaths/Total Population (x 10^{-6})</th>
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<td>Total Number of Sarcoidosis Deaths 2005-2009</td>
<td>Sarcoidosis Deaths/Total Population (x 10^{-6})</td>
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Figure 4: Spodumene-Tin Belt and Other Mineral Regions (Griffitts, 1954)

1. Kings Mountain area
   (Spodumene-beryl pegmatites)
2. Beaverdam Creek area
   (Spodumene-beryl pegmatites)
3. Cherryville area
   (Mio pegmatites)
4. Hiddensite area
   (Mio-spodumene-beryl-graphite pegmatites)
5. Conover area
   (Mio pegmatites)
6. Fallston area
   (Mio pegmatites)
7. Shelby area
   (Mio pegmatites)

Spodumene-rich part of the tin-spodumene belt
Figure 5: North Carolina Counties (RCoreTeam, 2013; Brownrigg et al (R version), 2013)