Cytotoxicity and Solubility of Beryllium Particles

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List of Abbreviations

ABDC: alkylbenzyldimethylammonium chloride
CBD: chronic beryllium disease
CuO: copper (II) oxide
Cu_2O: copper (I) oxide
ECF: extracellular lung fluid
ICP-MS: inductively coupled plasma-mass spectroscopy
k: chemical dissolution rate constant
MCE: mixed cellulose ester
MHC: major histocompatability complex
M_0: initial mass of beryllium particulate
M_D: mass of dissolved beryllium
M: mass of particulate beryllium remaining
PSF: phagolysosomal simulant fluid
SUF: serum ultrafiltrate
SSA: specific surface area
SRM: standard reference material
XRD: X-ray diffraction

Abstract

The long-term goal of this research is to develop more protective industrial hygiene metrics of exposure that are related to bioavailability, and therefore, potential risk of immune-mediated diseases caused by exposure to metals. We chose chronic beryllium disease (CBD) as a model for this research because it is a potentially fatal cell-mediated immune disease of the lung with no known cure that continues to be diagnosed among workers exposed to beryllium and former workers no longer exposed to beryllium. CBD is associated with exposure to beryllium-containing particles; however, at the cellular level a dissolved beryllium species is the hypothesized input to the immune reaction that drives development of CBD. As beryllium containing particles dissolve in phagocytic lung cells, a fraction of the dissolve material may be consumed in the formation of the beryllium antigen, which is hypothesized to cause CBD. Little is known about the physicochemical properties or dissolution behavior of respirable beryllium aerosols, suggesting that the relationship between exposure to beryllium and dose is not well understood.

Dissolution (conversion from particle to a dissolved species) is a physicochemical process. Physicochemical properties of four materials were characterized utilizing a suite of analytical techniques: finished product beryllium metal powder and beryllium oxide powder, and particles sampled from manufacturing processes during production of beryllium oxide and copper-beryllium alloy. All study materials are associated with elevated prevalence of CBD. Characterization of study materials included aerodynamic size-separation and microscopy to understand size and morphology, x-ray diffraction (XRD) to determine crystalline chemical composition, and surface area analysis to determine specific surface area (SSA). Lacking a model of the acidic liquid environment of the pulmonary alveolar macrophage phagolyososome, the hypothesized site of beryllium dissolution, a solvent model termed phagolysosomal simulant fluid (PSF) was refined and characterized. Efforts to characterize the solvent included comparison of beryllium dissolution in PSF to dissolution in the J774A.1 murine monocyte-macrophage cell line. No difference in beryllium dissolution was observed in PSF compared to the J774A.1 cell line, thus PSF was used to determine the chemical dissolution rate constant (k), having units of $g/(cm^2 \cdot day)$, for each material. The k value is a constant unique to a given chemical form of beryllium, and permits intercomparison of dissolution rate data.

Results indicated that physicochemical properties, and in turn, values of k, differed among the study materials. The ranking of materials by solubility (from greatest to least) was beryllium metal powder> beryllium oxide powder ≈ beryllium oxide particles sampled from a production process. For the copper-beryllium particles studied, measured SSA for the entire sample did not govern beryllium dissolution from the beryllium oxide component of the particles. Additional studies of this material showed that dissolution did not vary with measured SSA of the entire sample as expected from dissolution theory. Using k determined for beryllium oxide, the effective beryllium oxide SSA was calculated for the copper-beryllium material using a technique developed as part of this project. To account for measured dissolution, the SSA of the beryllium oxide component of the copper-beryllium particles must be several orders of magnitude higher than the SSA determined for the total particle sample.

Research performed as part of this project improved understanding of how physicochemical properties of beryllium aerosols relate to solubility by using a solvent of phagolysosomal fluid. An understanding of beryllium particle dissolution is important because the rate of dissolved beryllium production may be the rate limiting step in the activation of the CBD immune response. Data collected indicate that k values of beryllium materials with capacity to cause CBD may vary by an order of magnitude or more. Thus, an improved approach for regulating exposure might categorize chemical forms of beryllium according to solubility characteristics and SSA, with those materials producing dissolved beryllium at a rate sufficient for activation and maintenance of the CBD immune response posing the highest risk.

Significant Findings

The purpose of the first phase of this project is to characterize the physicochemical properties of finished product beryllium metal powder and beryllium oxide, and particles sampled during production of beryllium oxide and copper-beryllium alloy materials. These materials are associated with elevated prevalence of chronic beryllium disease (CBD). In the second phase of the project, the dissolution rate of each material was determined to test the hypothesis that dissolution of beryllium from particles depends on the physicochemical properties of the particles.

With respect to characterization of the physicochemical properties of aerodynamically size-separated powders and process-sampled particles of beryllium, the significant findings are:

- Process-related factors influence the morphology and specific surface area (SSA) of beryllium materials
- The mechanisms of particle formation should be understood and the SSA of beryllium particles should be measured directly
- Use of a geometric model rather than direct measurement of SSA results in order of magnitude errors, which translate directly into order of magnitude errors in predictions of bioavailability of beryllium
- All particles and powders studied, regardless of source, contain at least some beryllium in the form of beryllium oxide
- Beryllium aerosol properties must be thoroughly characterized when performing exposure assessments
- Differences in particle chemical composition, size, number, and surface area may influence bioavailability of beryllium and contribute to risk of CBD

With respect to characterization of phagolysosomal simulant fluid (PSF) for use in a static dissolution technique, the significant findings are:

- Beryllium dissolution in PSF is not different from dissolution in a murine monocyte-macrophage cell line ($p = 0.78$) or from dissolution in another simulant having the same pH but different ionic composition ($p = 0.73$)
- PSF is a useful and appropriate model of *in vitro* beryllium dissolution when using a static dissolution technique
- The critical approach used to evaluate and adjust the composition of PSF is useful for designing a simulant of the phagolysosomal environment for dissolution studies of other metal and oxide particles

With respect to measuring the dissolution of study materials to test the hypothesis that dissolution of beryllium depends on the physicochemical properties of the particles, the significant findings are:

- Distinct values of k are observed for each chemical form of aerosol material. For beryllium metal powder, the mean value of k was an order of magnitude faster than for beryllium oxide ($p<0.0001$)
- For the copper-beryllium aerosol material, which contained beryllium in the form of beryllium oxide, k was nearly two orders of magnitude faster than k for beryllium oxide powder and screener oxide particles. This finding suggests that measured SSA for the entire particle sample differs from that governing beryllium dissolution. Additional dissolution studies demonstrated that dissolution of the copper-beryllium aerosol material did not vary with SSA of the entire sample as expected
- Improved understanding that beryllium dissolution is dependent upon the physicochemical properties of the beryllium material may aid in developing dose – response models for CBD, which in turn, could improve understanding of the risk of disease

Translation of Findings

An understanding of beryllium particle dissolution is important because the rate of production of dissolved beryllium may be rate limiting in the CBD immune response. The chronic pulmonary inflammation associated with development of CBD may be supported by long particle dissolution lifetimes or chronic exposure regimens to particles with short dissolution lifetimes (Stefaniak et al., 2003). Compliance with current mass concentration exposure limit and guidelines, which utilize the concept of controlling exposure to the airborne mass of beryllium, have not been ineffective at reducing the prevalence of CBD (Eisenbud, 1998). This regulatory strategy ignores the concept that all particles regardless of size and chemical form of beryllium may not be equally toxic. Therefore, an improved exposure metric might also account for particle size considerations for deposition in the respiratory tract and beryllium form-specific particle dissolution kinetics that affect bioavailability. Such a metric might categorize chemical forms of beryllium according to solubility characteristics and SSA, with those materials producing dissolved beryllium at a rate sufficient for activation and maintenance of the CBD immune response posing the highest risk. This alternative metric could augment or replace the current beryllium particle mass concentration approach used to regulate exposure.

Prior to undertaking this project, very little data existed describing the physicochemical properties of beryllium materials, especially for materials associated with elevated prevalence of CBD that were sampled from the workplace or had heterogeneous composition. Additionally, there were no data describing the dissolution of various chemical forms of beryllium at phagolysosomal pH. Experiments performed as part of this project helped filled these gaps in understanding beryllium dissolution by 1) characterizing the physicochemical properties of beryllium metal, beryllium oxide, and copper-beryllium alloy materials associated with prevalence of CBD, and 2) estimating the k value for each material using a simulant model with pH that matches phagolysosomal fluid of pulmonary alveolar macrophage cells. This project demonstrated that differences in physicochemical properties of beryllium aerosols translated into differences in dissolution rates in a simulant of pulmonary alveolar macrophage phagolysosomal fluid.

Data collected as part of this project are not sufficient in and of themselves to abandon the current mass concentration approach to regulating beryllium exposure. However, the data do clearly demonstrate the need to thoroughly characterize beryllium aerosol exposure materials and provide evidence to suggest that particle physicochemical properties may play a role in development of beryllium sensitization and CBD. Additional research by investigators on this project, and in collaboration with others, will provide additional data needed to further define the role of particle physicochemical properties in development of CBD. Current and planned projects include:

- The well characterized beryllium oxide powder has been supplied to collaborators at Los Alamos National Laboratory to repeat experiments on responses (cytokine expression, etc.) of primary cells to soluble beryllium salts (Chaudhary et al., 2004) with particles.
- Using the methodological framework outlined in this project, the physicochemical properties and dissolution behavior of beryllium materials not yet associated with causing

CBD, e.g. bertrandite ore dust (Deubner et al., 2001) will be determined and compared to data for materials associated with causing CBD. For this study, bertrandite ore dust was donated by Brush-Wellman Inc. and beryl ore was collected from an abandoned mine in collaboration with University of New Mexico Geology Department faculty.

- A well-characterized, commercially available, high-fired beryllium oxide material is being sought for national and international use as an analytical standard reference material (SRM) for insoluble beryllium oxide particulate material. Work on characterization of the physicochemical properties of the final product beryllium oxide powder for the funded project 1R03 OH007447-01 has led to this material being considered a possible SRM. Work on evaluating this material as an SRM are continuing in collaboration with the National Institute for Occupational Safety and Health, the U.S. Department of Energy and its prime contractor organizations, the National Institute for Standards and Technology, Brush Wellman, Inc., and others.

Results of the studies from the funded project were presented to employees (production through upper management) of Brush Wellman Inc. at the annual National Institute for Occupational Safety and Health – Brush-Wellman Inc. sponsored Program Leadership Team meeting held November 3 – 4, 2004 in Morgantown, WV. Brush Wellman Inc. is the sole primary producer of beryllium in North America. Employees from the companies Tucson, AZ, Elmore, OH, and Reading, PA plants attended the meeting. The purpose of this meeting was for researchers to present findings to the company, with emphasis on communicating to the production employees. Production employees presented on how information they learned at the previous years meeting was communicated and utilized in their workplace. Production employees were provided with copies of the current meeting presentations, which included results from this project, and will communicate the findings to their co-workers. At the next annual meeting, a different group of production employees will present on how information from the 2004 meeting was utilized in their workplace.

Scientific Report

Specific Aims

Research funds were requested to conduct chemical simulant studies of the solubility of beryllium metals, copper alloys, and oxides. The goals of this project were to:

1. obtain finished product and process-sampled beryllium aerosol materials using size-selective sampling,
2. characterize the materials with respect to surface area and chemical composition,
3. refine a simulant model of the intracellular dissolution environment, and
4. utilize this model to investigate beryllium particle solubility

to test the hypothesis that beryllium particle surface area, size, and chemical composition is predictive of solubility.

Knowledge of solubility is critical to understanding beryllium disease because the dissolved beryllium species is hypothesized to be the exogenous input to the immune reaction resulting in CBD. Benefits of this project to industrial hygiene include gaining a better understanding of beryllium particle solubility in the context of measurable exposure parameters (particle surface area, size, and chemical form). This solubility data can be used to study immune lymphocyte cell activation rates to better understand beryllium exposure-response relationships, a priority for Exposure Assessment Methods research in the National Occupational Research Agenda.

Background

The continued prevalence of CBD and sensitization (Kreiss et al., 1996), despite great reductions in total aerosol mass exposure and the lack of a clear dose-response relationship (Kreiss et al., 1997), has lead some investigators to the conclusion that aside from beryllium mass, factors such as particle size and surface properties are important in risk of CBD (Eisenbud, 1998; Martyny et al., 1999).

At the cellular level, several observations support the hypothesis that CBD is a cell-mediated immune disease. First, macrophage cells isolated from the blood of CBD patients that phagocytize beryllium oxide particles induce lymphocyte proliferation in a dose-response manner *in vitro* (Hanifin et al., 1970). Secondly, bronchoalveolar lavage of persons with CBD reveals a significantly higher proportion of T-lymphocyte cells, specifically helper T-lymphocytes, than disease-free controls or persons with sarcoidosis (Rossman et al., 1988; Saltini et al., 1989). Finally, the *in vitro* proliferation of T-lymphocyte cells in response to beryllium is restricted to CD4+ T-lymphocyte cells and is dependent upon presentation of antigen by major histocompatabiliy (MHC) class II proteins (Saltini et al., 1989; Fontenot et al., 2000). Thus, beryllium particle solubility within the macrophage cell phagolysosome may be an important pathway in lung clearance, giving rise to ionic beryllium that can provoke a transformation response from lymphocytes via an antigen.

To better understand risk of CBD research is needed to characterize solubility of beryllium exposure materials in the context of particle physicochemical properties. Using an acellular static dissolution technique, this research investigated the role of particle size, surface area, and chemical form in solubility of beryllium.

Particle Size

A recent study of beryllium machinists' exposure (Martyny et al., 1999) indicates that more than 50% of beryllium particles in the workers breathing zone may be respirable (<10 µm in aerodynamic diameter). Based on median size approximately 6% to 8% of particles sampled were predicted to deposit in the alveolar and lung region. Thus, prior to characterization of physicochemical properties or dissolutions studies all materials were aerodynamically size-separated into 6 size-fractions, all in the respirable size range.

Surface Area

Theoretically, the rate of dissolved mass from a particle surface is proportional to available surface area (Mercer, 1967). Given the potential importance of surface area, surface area was determined for materials sampled at different stages of various beryllium manufacturing processes and for final product powders.

Particle Chemical Composition

Using a simulant of extracellular lung fluid (neutral pH), Finch et al. (1988) demonstrated that beryllium metal dissolves faster than beryllium oxide. This result implicates chemical form as an important parameter in predicting beryllium solubility. However, the role of chemical form in solubility has not been extended to include beryllium-copper alloys, comparison of commercially available to process sampled metal and oxide particles, or relative differences among real-world metal, oxide, and copper alloy exposure aerosols.

Acellular Dissolution Techniques

Acellular chemical dissolution techniques are often used to compare the relative solubility of materials, and to compare results with *in vitro* or *in vivo* models. The pH of simulants commonly used in dissolution studies is either 1 or 7.4; however, macrophage intracellular particle dissolution occurs at pH 4.5 – 5.5 (Kreyling et al., 1991). Therefore, solubility experiments in this proposed research will employ a static dissolution technique but use a simulant that has pH 4.5 to more accurately mimic the intracellular dissolution environment.

Experimental

Particle Size

Two types of beryllium materials were used in this project: process sampled particles and final product powders. Process-sampled particles were collected during the manufacturing processes themselves and represent aerosols to which workers may be exposed. We collected these particles from specific unit operations of beryllium oxide and copper beryllium alloy production lines where determinations of sensitization and disease prevalence have been reported (Kreiss et al., 1997). The final products of beryllium metal and oxide production lines are powders of beryllium metal and beryllium oxide. These powders represent standard feed materials for manufacturing processes. For example, beryllium oxide powder is typically pressed into solid pieces (known as "green" ware), machined to near-net size, heat-treated, then polished or lapped to final dimensional specifications.

Preparation of aerodynamically size-separated powders

Commercially available beryllium metal (Product Type I-400, Brush Wellman, Inc., Elmore, OH) and beryllium oxide (Product Type UOX-125, Brush Wellman, Inc.) powders were aerodynamically size-separated at Lovelace Respiratory Research Institute (LRRI, Albuquerque, NM). Details of the aerosolization and size separation procedure have been described (Hoover et al., 1989). Briefly, aerosols were dispersed from the beryllium powders using a DeVilbis dry powder blower (Model 175, DeVilbis, Inc., Somerset, PA), and aerodynamically size-separated using a five-stage aerosol (Smith et al., 1979). The cyclone system was operated at 20°C with a flow rate of 24 actual liter per minute and was followed by a backup concentric electrostatic precipitator (Mine Safety Appliances, Pittsburgh, PA). Table I lists the cyclone stages and corresponding aerodynamic diameter cutoffs.

Collection of process-sampled particles

Process-sampled particles were collected from the ventilation ducts of the beryllium metal, oxide, and copper alloy production lines denoted in Figure 3-2 using an isokinetic sample probe (Model 280N, Graseby-Andersen, Smyrna, GA) operated at 28 actual liter per minute. The probe was connected to a sampling system similar to that used for separation of the powders at LRRI: a five-stage aerosol cyclone (Model 285, Graseby-Andersen) followed by an electrostatic precipitator with a backup thimble filter. The aerodynamic cutoff diameters for the process sampling conditions are also given in Table I.

Surface Area

Determination of chemical composition and morphology

Chemical composition of the beryllium materials was determined by x-ray diffraction (XRD, Model XDS2000 powder diffractometer, Scintag, Inc., Sunnyvale, CA). Morphology was observed using transmission electron microscopy (Model CM30 electron microscope, Philips Electron Optics, Eindhoven, Netherlands) or scanning electron microscopy (Model 6300FXV, JEOL USA).

Determination of specific surface area

Specific surface area was determined by gas adsorption using the single point Brunauer, Emmett, and Teller method (Monosorb Model MS-16 Automated Direct Reading Surface Area Analyzer, Quantachrome Corp., Syossett, NY). Each powder sample was loaded into a pre-weighed glass cell (Quantachrome Model 74000 standard cells or Model 74001 Micro cells). The mass of powder was selected to provide significantly more surface area than the internal surface of the cell, i.e. 0.01 m^2 for the micro-cell. Prior to analysis the samples were outgassed for a minimum of 5 hours and maximum of 24 hours at 250°C with flowing ultrahigh purity helium (Quantector Outgassing Station, Quantachrome).

The surface area analyzer was calibrated daily before use by injecting one cubic centimeter of nitrogen gas into the calibration port of the instrument using a gas-tight syringe (Quantachrome). Triplicate surface area measurements were performed for each sample using ultra high purity nitrogen (30% molar volume) in a helium carrier gas. A value of 16.2 $Å^2$ was used for the area of a nitrogen molecule. The dry mass of powder in the sample was determined by subtracting the tare weight of the cell from the gross weight of the sample and the cell. Surface area measurements (m^2) were corrected for temperature and pressure, then normalized by powder mass to obtain SSA (m^2/g).

Results

Table II presents the mean and standard deviation of triplicate determinations of the measured SSA of the size-separated beryllium metal and beryllium oxide powders, and of the screener oxide, and master alloy particles. The average primary particle sizes from XRD for the beryllium oxide powder and screener oxide particles are also included in the table.

Figure 1 displays plots of measured SSA as a function of aerodynamic particle diameter of stages 2 through 5 of the a) size-separated metal powder, b) size-separated beryllium oxide powder and screener beryllium oxide, and c) the master copper-beryllium alloy. For the purposes of these plots, the mid-point of each aerodynamic size range was used as the particle diameter. Theoretical SSA curves for solid spherical beryllium metal (Figure 1a) and oxide particles (Figure 1b) where surface area is predictable from an external characteristic dimension are also plotted (assuming metal density of 1.85 g/cm^3 and oxide density of 3.0 g/cm^3).

Discussion

Results presented above confirm that both compact-particle and cluster-particle regimes of surface characteristics apply for beryllium particles found in the workplace. Therefore, direct measurements were necessary to accurately characterize SSA. Metal particles fall into the compact class of particles in which SSA decreases with increasing particle diameter. Beryllium oxides and particles sampled from the master alloy process fall into the cluster-particle regime, in which the SSA is approximately constant with particle size. These results suggest that mechanisms of aerosol formation under industrial process conditions can produce aerosol materials with surface areas that depend on the morphology and internal surface or surface roughness.

Particle Chemical Composition

Determination of crystalline chemical composition

XRD was utilized to identify crystalline chemical constituents of aerosol samples. Diffraction patterns were generated using a Model XDS2000 powder diffractometer (Scintag, Inc., Sunnyvale, CA) with a sealed-tube x-ray source (Cu k-alpha, 45 kV, 40mA). Samples were prepared by adding a thin layer of beryllium material to a one-inch diameter single crystal plate coated in a thin film of vacuum grease. Analysis of diffraction patterns to determine the phase composition of crystalline constituents was performed using the General Structure Analysis System computer software program (Larson and Von Dreele, 2000; Toby, 2001). The identity of constituents was determined by comparing diffraction patterns to reference patterns in the International Centre for Diffraction Data Powder Diffraction Files database (ICDD, Newton Square, PA).

Results

The x-ray diffractogram of finished product powder only contained beryllium metal. As shown in Figure 2, x-ray diffractograms from screener beryllium oxide particles and finished product beryllium oxide powder were consistent with high-purity beryllium oxide. The x-ray diffractograms of master alloy (stage 3) particles were complex. Substances identified from diffractograms of master alloy particles were similar for stage 1 and stage 3 particles, i.e. beryllium oxide, copper (I) oxide (Cu_2O), copper (II) oxide (CuO), and calcium fluoride. Copper and C were identified in stage 1 but not stage 3 particles. Calcium sulfate may be present in stage 3 particles; the locations of diffraction peaks match a reference pattern but their intensities do not. Crystalline constituents were not detected in the electrostatic precipitator size fraction of master alloy particles.

Discussion

The XRD system used to characterize materials is capable of detecting crystalline constituents present at 1% by weight or more. Because of this limit of detection, the x-ray diffractogram for finished product metal powder identified only beryllium metal; however, previous work by Hoover et al. (1989) demonstrated that a thin layer of beryllium oxide, formed under ambient conditions, is present on the surface of this powder. X-ray diffractograms of the oxides indicate the process-sampled particles and finished product powder had identical composition, i.e. high purity beryllium oxide. Substances identified from diffractograms of master alloy particles were consistent with the raw materials input to the unit operation. Crystalline constituents were not detected in the electrostatic precipitator size fraction of master alloy particles probably because of the small primary particle size of particles. As primary particle size decreases the number of crystalline planes diffracting decreases resulting in a broadening of diffraction peaks, which in turn precludes identification of constituents. Note again that beryllium metal was not detected in these process-sampled particles. Workers in the master alloy furnace area are therefore exposed to beryllium oxide not beryllium metal.

Acellular Dissolution Studies

Formulation of the phagolysosomal simulant fluid (PSF)

Many of the enzyme and protein constituents of phagolysosomes are known (Tappel, 1968); however, our literature searches could not find any measurements of the ionic composition of phagolysosomes. Absent data describing the ionic composition of phagolysosomes we assumed it was the same as extracellular lung fluid (ECF) and therefore chose and modified a serum ultrafiltrate (SUF) developed by Guldberg et al. (1998) as the basis for our PSF.

Ionic composition of the Guldberg et al. (1998) SUF was the same as that of ECF for phosphate, organic acids, and sulfate, but differed in concentration for many of the other constituents. Although ionic composition may not be a factor in dissolution, we adjusted the concentration of several constituents to match Gamble's (1967) measurements of ECF, and we eliminated constituents such as ammonium ions that had been added to maintain pH 7.3 (Kanapilly et al., 1973), but were not relevant to the phagolysosome. The Guldberg et al. (1998) SUF used 0.01-M potassium hydrogen phthalate (KHP) buffer to maintain stable pH at 4.5 for several weeks. Pilot studies suggested that beryllium particles could alter pH of a 0.01-M KHP solution so the buffer concentration of PSF was doubled to 0.02-M KHP to limit variation in pH to less than ± 0.10 during dissolution studies.

The chemical composition of PSF is presented as Table III. Analytical grade chemicals (Fisher Scientific, Fairlawn, NJ) were used to prepare the PSF. As in previous dissolution studies, 50-ppm ABDC was added as an antifungal agent (Finch et al., 1988). The pH of the 0.02-M potassium hydrogen phthalate-buffered solution was adjusted to 4.55 by addition of 0.1-M sodium hydroxide while monitoring with a calibrated pH electrode connected to a meter (US EPA, 1996). To verify complete transfer and mixing of representative salt constituents, the chloride concentration of PSF was measured at 37.0°C according to EPA Method 9212: Potentiometric determination of chloride in aqueous samples with ion-selective electrode (US EPA, 1996) using a calibrated combination ion selective electrode (Model 96-17, Orion Research Inc., Beverly, MA) with separate temperature probe connected to a pH meter (Model 15, Fisher Scientific).

An array of experiments was developed to test the suitability of our PSF formulation for *in vitro* studies of beryllium particles:

- To test the assumption that the selected PSF is an appropriate phagolysosomal simulant, beryllium dissolution in PSF was compared to that observed using the J774A.1 murine monocyte-macrophage cell line (Ralph and Nakoinz, 1975).
- To determine if variations in ionic concentration influences beryllium dissolution, PSF was compared to a simulant fluid having the same pH but ionic concentrations that match intracellular fluid of leukocytes.
- To ensure that buffer strength was adequate to maintain pH without altering the basic rate of beryllium dissolution, beryllium dissolution in PSF was compared at three buffer concentrations.
- Finally, dissolution was compared in PSF with alkylbenzyldimethylammonium chloride (ABDC) and PSF lacking ABDC to ensure that the presence of this compound, often added to simulants to prevent fungal growth (Finch et al., 1988), does not influence beryllium dissolution.

Results

PSF as a phagolysosomal simulant

Comparison of beryllium dissolution in PSF and the J774A.1 cell line illustrated that median levels were similar. The median percent of beryllium mass dissolved from stage-3 beryllium oxide powder in PSF, 0.9 ± 0.5%, was not significantly different ($p = 0.78$) from 1.0 ± 0.5% the median percent of beryllium mass dissolved by the J774A.1 cell line for the pooled trials ($n = 10$). The data suggest PSF may be a useful and appropriate model of *in vitro* beryllium dissolution when using a static dissolution technique.

Influence of simulant ionic composition on dissolution

The median percent of beryllium mass dissolved from stage 4 metal powder in PSF was 34.1% and in a simulant fluid having the same pH but ionic concentrations that match intracellular fluid of leukocytes it was 35.7%; this difference in medians was not statistically significant ($p = 0.73$).

Influence of buffer strength on dissolution in PSF

The beryllium mass dissolved from stage-3 beryllium oxide powder in PSF prepared at the three buffer strengths was 1.5% in PSF with 0.01-M KHP, 1.8% in PSF with 0.02-M KHP, and 3.4% in PSF with 0.04-M KHP. The median percent of beryllium mass dissolved in PSF with 0.01-M KHP was not statistically different from the median in PSF with 0.02-M KHP ($p = 0.12$). The median percent of beryllium mass dissolved in PSF with 0.04-M KHP was statistically different from median dissolved beryllium in PSF with 0.01-M KHP ($p = 0.001$) and 0.02-M KHP ($p = 0.02$).

Influence of antifungal additive on dissolution in PSF

Median percent of beryllium mass dissolved from stage-3 metal powder in PSF containing 50-ppm ABDC ($n = 9$) was 18.3% and in PSF without ABDC ($n = 10$) it was 15.1%. The difference in median levels of dissolved beryllium between these treatment groups was not statistically significant ($p = 0.35$).

Discussion

Comparison of beryllium dissolution in PSF and the J774A.1 cell line illustrated that median levels were similar. The data suggest PSF may be a useful and appropriate model of *in vitro* beryllium dissolution when using a static dissolution technique. The median percent of beryllium mass dissolved in PSF was similar to that in a simulant fluid having the same pH but ionic concentrations that match intracellular fluid of leukocytes, suggesting that pH is a more important variable to control in a simulant than ionic composition. Data on the beryllium mass dissolved from stage-3 beryllium oxide powder in PSF prepared at the three buffer strengths support the assumption that doubling buffer strength to 0.02-M KHP to limit variation of pH to no more than ±0.10 did not result in buffer driving beryllium dissolution. However, dissolution was clearly higher at 0.04-M KHP, suggesting that this increased level of buffer was altering the dissolution behavior of PSF. Results also indicated that the presence of ABDC did not influence beryllium dissolution in PSF. PSF appears to be a useful and appropriate model of *in vitro* beryllium dissolution when using a static dissolution technique.

Static dissolution measurements

Acellular dissolution experiments were performed using a static dissolution technique developed by Kanapilly et al. (1973). A static technique was chosen because it is among the easiest and most widely implemented *in vitro* systems and is not subject to filter clogging, maintaining constant simulant pressure and flow, and production of large volumes of simulant associated with other methods such as the flow-through system (Ansoborlo et al., 1999). Briefly, a beryllium particle dose was placed on a filter and the filter inserted between two other filters that serve as barriers to particle migration into the dissolution medium (Figure 3). Specifically, this "sandwich" consisted of two 0.025-μm pore size 47-mm diameter mixed cellulose ester (MCE) filters (Millipore, Bedford, MA) with a beryllium-dosed 37-mm diameter cellulose sample filter inserted in between. The sample filter can be any convenient filter media provided it is readily digestible for spectroscopic analysis of beryllium. In these studies a 37-mm filter support pad (stock number AP10, Millipore) was used because its high porosity was amenable to loading the dissolution chamber. The filter sandwich was then secured tightly in a static dissolution chamber (Intox Products, Moriarity, NM). Four nylon screws with two O-rings were used to provide a particle tight seal around the edge of the sandwich. The chamber was immersed in 80-mL of PSF contained in a polypropylene plastic beaker and maintained at 37°C for the duration of the study period using water baths (Model 286, Precision, Winchester, VA; Model G76, New Brunswick Scientific Co., Inc., Edison, NJ). The pH was monitored daily using a calibrated pH electrode connected to a meter (Model AB15, Fisher Scientific) according to United States Environmental Protection Agency Method 9040B: pH electrometric measurement (US EPA, 1996). At the end of the study period each chamber was removed from the beaker and the PSF analyzed for dissolved beryllium (M_D) content using inductively coupled plasma-mass spectroscopy (ICP-MS) as described below.

The MCE filter pore size was selected to be sufficiently smaller than 0.19 ± 0.04 μm, the average diameter of individual beryllium oxide primary particles (Stefaniak et al., 2003), to prevent particle migration into the dissolution medium, thereby biasing measurements of M_D.

For each beryllium powder, the amount needed to dose all filter sandwiches for a given experiment was weighed (Model AX205 microbalance, Metler-Toledo) into a clean, dry 20-mL borosilicate glass scintillation vial (Kimble Glass Inc., Vineland, NJ), with a foil-lined screw top cap. Approximately 20-mL of 0.0095-M phosphate buffered saline (PBS, GIBCO, Grand Island, NY) was added to the vial, and the vial reweighed to determine the total mass. From these measurements the concentration of each suspension was calculated. The suspension was then stored frozen until just before use.

Each suspension was thawed, briefly subjected to ultrasonic agitation, and then shaken vigorously prior to dosing filter sandwiches. The volume of suspension needed to achieve a target dose (M_0) was pipetted onto a 37-mm diameter cellulose filter support pad (Millipore) using a precision micropipetter (Model E2-1000, Rainin, Inc., Emeryville, CA) and the pad inserted between two 47-mm diameter filters (Millipore) to form a sandwich. Dose levels were selected to ensure that dissolved beryllium levels would exceed method analytical limits of detection for ICP-MS. To confirm M_0 in the sandwiches estimated by pipetting, replicate filter sandwiches of each material were prepared at the time of experimental setup and submitted for analysis by ICP-MS.

According to Mercer (1967), for $\frac{M}{M_0} > 0.3$ and σ from 0 to 1 (σ_g from 1 to 2.72), dissolution of a material can be expressed as a single exponential:

Equation 1. $$\frac{M}{M_0} = \exp\left\{-\lambda k t SSA \exp -\frac{\sigma^2}{2}\right\}$$

where, M = mass of material remaining at time t

$\lambda = 1.18$

k = chemical dissolution rate constant

t = time

SSA = specific surface area that governs dissolution

$\sigma = 0.5$

which can be rearranged to solve for k:

Equation 2. $$k = \frac{\ln\left(\frac{M}{M_0}\right)}{-1.04 SSA t}$$

In the first experiment, values of k were determined for each study material. For each of the four study materials, k was estimated at three different initial beryllium dose masses (M_0). Each M_0 sample was taken from the same cyclone stage to ensure constant SSA. Static dissolution chambers were prepared by dosing filter sandwiches as previously described. For each material n = 11 dissolution chambers were loaded with a low dose of beryllium stage-2 metal powder, stage-3 beryllium oxide powder, stage-3 screener beryllium oxide particles, or stage-3 master copper-beryllium alloy particles (N = 44). Two field blank dissolution chambers consisting of undosed filter sandwiches were also prepared.

PSF was prepared as described previously. Each dissolution chamber was placed in a separate beaker containing 80 ml of PSF, covered, and maintained at 37 °C in water baths (Model 286, Precision, Winchester, VA; Model G76, New Brunswick Scientific Co., Inc., Edison, NJ). The pH in each beaker (N = 46) was monitored daily using a calibrated pH electrode to ensure it remained at a pH of 4.55 ± 0.10. After 10 days the dissolution chamber was removed from each beaker and the liquid fractions transferred to separate borosilicate glass sample jars (Qorpak, Bridgeville, PA) and stored frozen at –5 °C until analysis for beryllium content using ICP-MS as described below. This protocol was then repeated for a medium dose mass then a high dose mass. Separate batches of PSF were prepared for each dose trial.

As noted below, the rate of beryllium dissolution from the multi-component aerosol material was greater than expected compared to dissolution of the single-component materials. This finding prompted a follow-on experiment in which additional details about the bulk and surface composition of the study materials were used to account for observed beryllium dissolution from the multi-component material. In this experiment, dissolution of beryllium from master alloy particles and single-component beryllium oxide powder was measured for three different size-fractions (stage-2, 3, and 4). For each material and size fraction, 14 static dissolution chambers were prepared by dosing filter sandwiches as previously described. Field blank dissolution chambers consisting of undosed filter sandwiches were also prepared. Dissolution of beryllium was measured using the protocol described above.

Results

Estimation of chemical dissolution rate constants (k)

In general, dissolution data were not normally distributed so medians were used for statistical comparisons. Within each beryllium material median values of k estimated at the low, medium, and high M_0 dose values were not statistically different. As a result, for each material data from the three trials were pooled. Median values of k, along with the range, are reported in Table IV for beryllium metal powder, beryllium oxide powder, and screener oxide particles.

The median value of k for metal powder, 6.1×10^{-8} g/(cm$^2 \cdot$ day), was significantly higher ($p<0.0001$) than k for the oxide materials. Median values of k were 1.1×10^{-8} g/(cm$^2 \cdot$ day) and 8.5×10^{-9} g/(cm$^2 \cdot$ day) for oxide powder and screener oxide particles, respectively. The difference in median values of k was not statistically significant ($p = 0.52$) and pooling data for the two materials yielded an overall median $k = 9.0 \times 10^{-9}$ g/(cm$^2 \cdot$ day) for oxide.

The median value k estimated for master alloy particles using SSA (Table II) was 2.1×10^{-7} g/(cm$^2 \cdot$ day) (range 7.9×10^{-8} to 3.3×10^{-7} g/(cm$^2 \cdot$ day)). This estimate of k was significantly ($p<0.0001$) faster than $k = 9.0 \times 10^{-9}$ g/(cm$^2 \cdot$ day) determined for single-component oxide aerosol materials, despite the fact the beryllium was present only in the form of oxide.

Estimating beryllium specific surface area available for beryllium dissolution from multi-component materials

The mass fraction dissolved for beryllium oxide powder was nearly constant regardless of particle size tested. In contrast, the dissolution of beryllium from master alloy particles did not follow the expected relation between mass fraction dissolved and SSA, i.e. much more beryllium dissolved from beryllium oxide in master alloy particles than from oxide powder. To account for this excess beryllium dissolution, equation 1 dictates that beryllium oxide in master alloy particles must be more finely divided than the oxide powder. Estimates of the oxide specific surface area were made using equation 1 with measured values of M and M_0 from the master alloy particles, and the pooled value of k from the oxides. The effective beryllium oxide specific surface area for the master alloy particles was calculated to be 75, 125, and 174 m^2/g for stage 2, 3, and 4 particles, respectively.

Discussion

Estimates of k in HCl and SUF for metal powder by Finch et al. (1988) are also faster than k for BeO, indicating metal dissolution is intrinsically higher than BeO. Beryllium metal powder studied here is from the same production batch as the metal powder used by Finch et al. (1988) to estimate values of k in HCl (pH 1) and SUF (pH 7.3). The median value of k estimated in this study using PSF (pH 4.55), was nearly an order of magnitude lower than the average value estimated by Finch et al. (1988) in HCl having pH 1 ($k = 4.1 \pm 0.2 \times 10^{-7}$ g/(cm^2· day)) and nearly an order of magnitude higher than the average value estimated in SUF having pH 7.3 ($k = 1.5 \pm 0.8 \times 10^{-9}$ g/(cm^2· day)).

Both beryllium oxide materials studied are single component aerosol materials composed of agglomerate particles with nearly identical SSA controlled by primary particle size (Stefaniak et al., 2003), thus no difference in values of k were expected between these materials. The median value of k for BeO ($k = 9.0 \times 10^{-9}$ g/(cm^2· day)) in PSF was nearly identical to that determined *in vivo* using beagle dogs, $k = 6.7 \times 10^{-9}$ g/(cm^2· day) (Finch et al., 1990). In addition, our value of k was bounded by values estimated by Finch et al. (1988) for BeO in HCl ($k = 6.1 \pm 2.2 \times 10^{-8}$ g/(cm^2· day)) and SUF ($k = 3.7 \pm 1.2 \times 10^{-9}$ g/(cm^2· day)).

The median value k estimated for master alloy particles using SSA (Table II), 2.1×10^{-7} g/(cm^2· day), should not have differed by over an order of magnitude from $k = 9.0 \times 10^{-9}$ g/(cm^2· day) determined for single-component oxide aerosol materials. Thus, SSA determined for the total particle sample may not equal SSA that governs beryllium dissolution for this multi-component material. One explanation for the greater than expected solubility of beryllium from the arc furnace particles was that copper rapidly dissolved from the particles, thereby exposing the small inclusions of beryllium oxide. These beryllium inclusions would have higher SSA than measured for the total particle sample and would therefore dissolve at a proportionally higher rate. Such a mechanism for enhanced dissolution has been proposed by Brown et al. (1982) to explain the observation that more gold dissolves from higher-copper-content gold-copper alloys than from lower-copper-content gold-copper alloys in a 0.1M glycine solvent. Hirano et al. (1993) observed that respirable, single-component CuO particles intratracheally instilled in rats cleared the lung with a half-time of 37 hours. Benson et al. (2000) observed that 90% of copper in respirable particles of copper-beryllium alloy intratracheally instilled in rats cleared the lung with a half-time of 12 to 48 hours, whereas the beryllium content of the particles cleared more slowly (half-time of several weeks).

The mass fraction dissolved for beryllium oxide powder was nearly constant regardless of particle size tested as expected from its SSA and equation 1. In contrast, the dissolution of master alloy particles did not follow the expected relation between fraction dissolved and SSA in equation 1. The calculated values of effective SSA were approximately seven to 16 times the SSA of the oxide materials. Values of effective oxide SSA in the range predicted are not unreasonable. For high-purity beryllium oxide, Finch et al. (1988) reported measured values in the range from 45 – 146 m^2/g.

Conclusions

Dissolution rates were measured for single-component and multi-component beryllium aerosol materials and modeled using a surface-area-proportional dissolution model. Beryllium materials known to be associated with beryllium sensitization and CBD have a range of physicochemical properties, which translate into different dissolution behaviors and different degrees of bioavailability. Consistent with previously published observations of the relative solubility of beryllium metal and beryllium oxide in a variety of solvents, values of the dissolution constant k for single-component beryllium metal in the simulated phagolysosomal fluid were significantly greater than for beryllium oxide. As expected, dissolution of both materials was greater in the simulated phagolysosomal fluid than previously published values in the more pH-neutral fluid of the extracellular lung environment, and less than dissolution in a more acidic HCl solvent. In addition, values of k were independent of the initial mass of material in the dissolution chamber, demonstrating the flexibility of requirements for initial mass and analytical sensitivity in the *in vitro* dissolution technique.

The rate of beryllium dissolution from the multi-component arc furnace particles was greater than expected compared to dissolution of the single-component beryllium oxide powder, despite that fact that the chemical form of beryllium in the arc furnace particles was beryllium oxide. This greater-than-expected beryllium solubility appears to stem from the bulk and surface composition of the study materials: it is likely that copper rapidly dissolves from the particles, thereby exposing the small inclusions of BeO which have higher SSA than measured for the total particle sample and therefore dissolve at a proportionally higher rate.

An understanding of beryllium particle dissolution is important because the rate of dissolved beryllium production may be the rate limiting step in the activation of the CBD immune response. Data collected indicate that k values with capacity to cause CBD may vary by an order of magnitude or more. Thus, an improved approach for regulating exposure might consider particle size for aerosol deposition in the respiratory tract and categorize chemical forms of beryllium according to solubility characteristics and SSA, with those materials producing dissolved beryllium at a rate sufficient for activation of the CBD immune response posing the highest risk.

Table I. Aerodynamic diameter cutoffs for cyclone systems used to aerodynamically size-separate beryllium aerosols

	Aerodynamic Diameter (μm)	
Stage	Separation of Powders[A]	Sampling of Process Particles[B]
1	> 6	> 5.7
2	2.5	2.3
3	1.7	1.5
4	0.9	0.7
5	0.4	0.4
ESP[C] (backup)	≤ 0.4	≤ 0.4

[A] Size segregated using a Southern Research Institute five-stage aerosol cyclone (Smith et al., 1979) operated at 20°C with a flow rate of 24 actual liters per minute

[B] Size segregated using a Graseby-Andersen Model 285 version of the five-stage aerosol cyclone (Parsons and Felix, 1980) operated at 40°C with a flow rate of 28 actual liters per minute

[C] ESP = Electrostatic precipitator

Table II. Specific surface area (m^2/g) of powders and process-sampled respirable beryllium aerosols

Size Classification	Metal Powder[A]	Oxide Powder[B]	Screener Oxide[C]	Master Alloy
Bulk	5.6± 0.02	11.03± 0.00	NA	NA
Stage 1	4.0± 0.01	11.43± 0.06	11.88± 0.02	5.29± 0.01
Stage 2	7.6± 0.06	10.96± 0.00	11.72± 0.02	3.66± 0.02
Stage 3	13.7± 0.05	11.14± 0.00	11.90± 0.00	4.44± 0.02
Stage 4	19.4± 0.17	10.72± 0.00	--[D]	3.85± 0.02
Stage 5	20.4± 0.57	13.48± 0.03	--[D]	5.31± 0.03
ESP	20.8± 0.44	13.12± 0.00	--[D]	4.59± 0.00

[A] These data were first reported in Hoover et al. (1989)

[B] Average primary particle diameter 0.19 μm

[C] Average primary particle diameter 0.22 μm

[D] Insufficient mass of material for surface area analysis

NA = Not applicable for unit operations sampled with aerosol cyclone

Table III. Composition of 0.02-M potassium hydrogen phthalate buffered phagolysosomal simulant fluid (PSF)

Constituent	mg/L
Sodium phosphate dibasic anhydrous (Na_2HPO_4)	142.0
Sodium chloride (NaCl)	6650.0
Sodium sulfate anhydrous (Na_2SO_4)	71.0
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	29.0
Glycine ($C_2H_5NO_2$)[A]	450.0
Potassium hydrogen phthalate (1-(HO_2C)-2-(CO_2K)-C_6H_4)	4084.6
Alkylbenzyldimethylammonium chloride (ABDC)[B]	50 ppm

	Concentration of ionic constituent species						
	Sodium	Potassium	Calcium	Chloride	Phosphate	Sulfate	Glycine
mmol/L	116.8	25[C]	0.2	114.2	1.0	0.5	6.0

[A] Representative of organic acids

[B] Added as an antifungal agent

[C] Includes the contribution from 0.1-M potassium hydroxide used to adjust the 0.02-M potassium phthalate buffer to pH 4.55

Table IV. Chemical dissolution rate constants (k)

Material	Dose level	M_0 (μg Be)	k [g/(cm^2· day)]^A Median	Minimum	Maximum
Metal powder	Low	368.0 ± 14.8	6.1×10^{-8}	4.5×10^{-9}	5.4×10^{-7}
	Medium	1043.3 ± 92.9			
	High	2110.0^B			
Oxide powder	Low	56.3 ± 1.2	1.1×10^{-8}	3.1×10^{-9}	2.2×10^{-8}
	Medium	184.6 ± 10.7			
	High	300.4 ± 20.5			
Screener oxide	Low	59.5 ± 1.9	8.5×10^{-9}	2.6×10^{-9}	1.1×10^{-7}
	Medium	163.4 ± 5.6			
	High	261.8 ± 27.3			

[A] Median calculated using data pooled from the low, medium, and high initial dose level (M_0) trials.

[B] Only one replicate sample to estimate M_0 at this dose level.

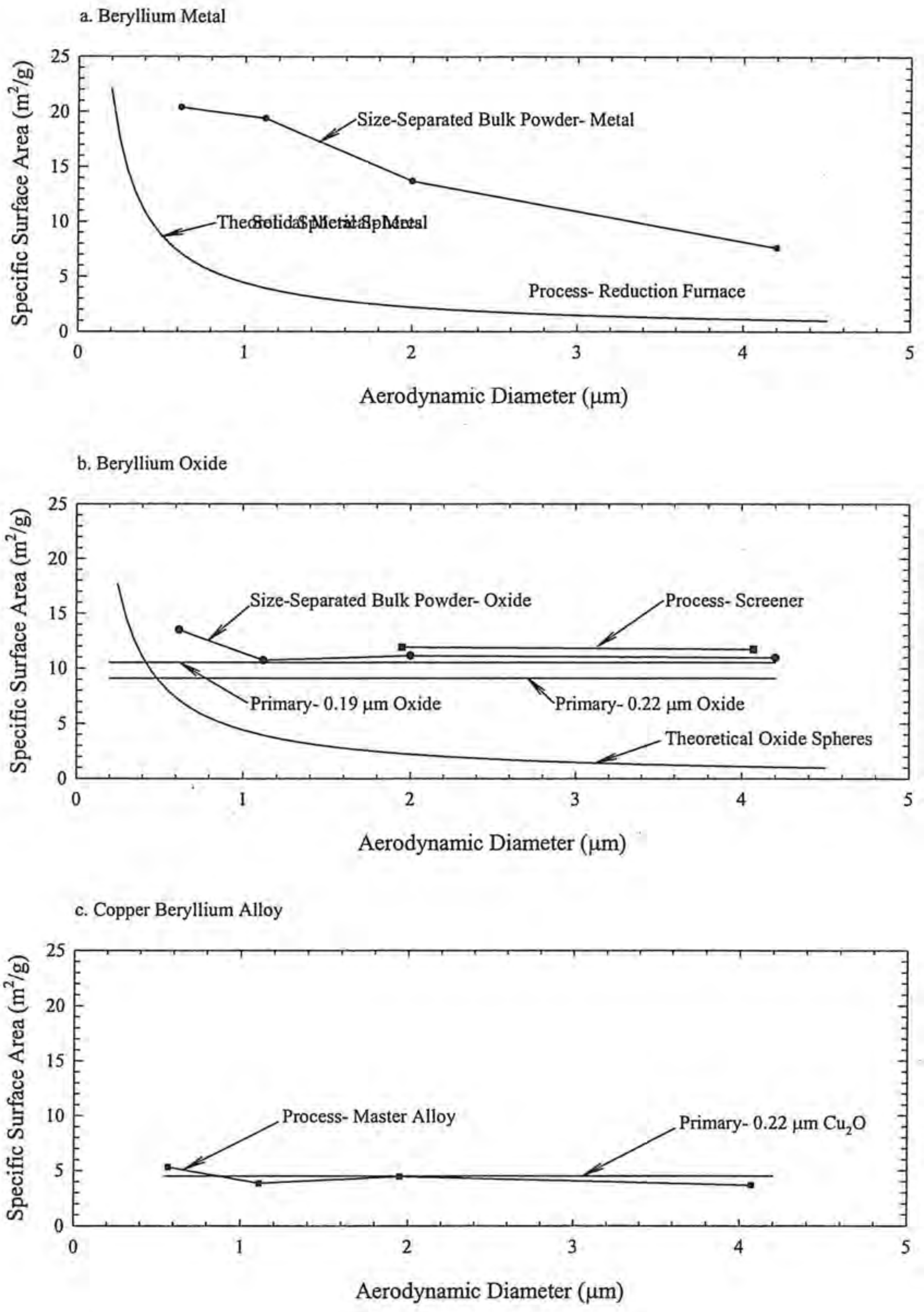

Figure 1. Plots of specific surface area versus aerodynamic diameter for (a) beryllium metal, (b) beryllium oxides, and (c) copper-beryllium alloy.

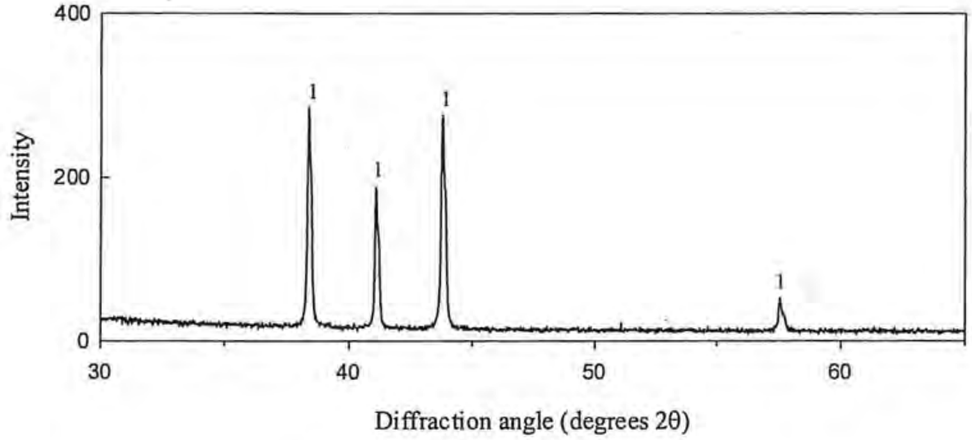

Figure 2. X-ray diffractogram illustrating the high chemical purity of beryllium oxide particles and powders. Peaks labeled 1 = beryllium oxide. Material in this example is beryllium oxide powder from stage 1 of the aerosol cyclone.

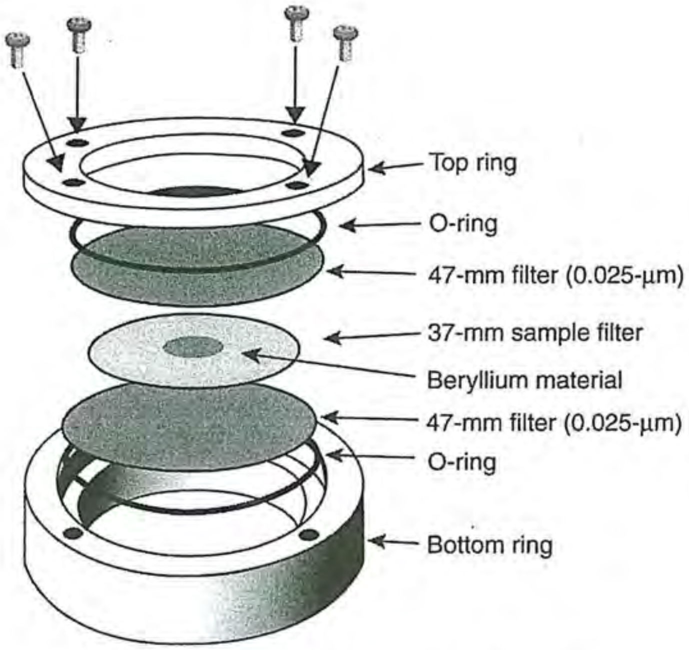

Figure 3. Static dissolution chamber assembly.

Publications

Stefaniak AB, Hoover MD, Dickerson RM, Peterson EJ, Day GA, Breysse PN, Kent MS, Scripsick RC: Surface Area of Respirable Beryllium Metal, Oxide, and Copper Alloy Aerosols and Implications for Assessment of Exposure Risk of Chronic Beryllium Disease. *Am. Ind. Hyg. Assoc. J.* 64:297-305 (2003).

* Dissolution is a physicochemical process. In this paper, a gas adsorption technique was used to determine the specific surface area of several different finely divided beryllium-containing materials, including the finished product beryllium metal powder and beryllium oxide powder, and particles sampled from manufacturing processes during production of beryllium oxide and copper-beryllium alloy that were studied in this project. This publication was given the David Swift Memorial Award for 2003 by the American Industrial Hygiene Association. This award is given annually for the best aerosol-related paper published in the *American Industrial Hygiene Association Journal* or *Journal of Applied Occupational and Environmental Hygiene* (now merged into the *Journal of Occupational and Environmental Health*).

Stefaniak AB, Hoover MD, Day GA, Dickerson RM, Peterson EJ, Kent MS, Schuler CR, Breysse PN, Scripsick RC: Characterization of Physicochemical Properties of Beryllium Aerosols Associated with Prevalence of Chronic Beryllium Disease. *J. Environ. Monit.* 6(6):523-532 (2004).

* Dissolution is a physicochemical process. In this paper, a suite of analytical techniques were used to determine the chemical (elemental, crystalline, and bulk) properties of several different finely divided beryllium-containing materials, including the materials that were studied in this project. This publication was named a Hot Article by the *Journal of Environmental Monitoring* and was featured on the journal web page.

Day GA, **Stefaniak AB**, Hoover MD, Dickerson RM, Peterson EJ, Esmen NA, Scripsick RC: Intracellular Behavior of Beryllium Oxide Particles: An *In vitro* Study in Murine Macrophages. *Am. J. Respir. Crit. Care Med.*169(7):A640 (2004). [Abstract]

* This abstract reported the results from a study of the dissolution behavior of finished product beryllium oxide powder in the J774A.1 murine-monocyte cell line. The experiment utilized methodology developed as part of the NIOSH funded 1R03 OH007447-01 project.

Stefaniak AB, Guilmette RA, Day GA, Hoover MD, Breysse PN, Scripsick RC: Characterization of phagolysosomal fluid for study of beryllium aerosol particle dissolution. *Toxicol. In vitro.* (*In Press*).

* Lacking a model of the acidic liquid environment of the pulmonary alveolar macrophage phagolyososome, the hypothesized site of beryllium dissolution, a solvent model, termed phagolysosomal simulant fluid (PSF), was refined and characterized as part of the NIOSH funded 1R03 OH007447-01 project. Efforts to characterize the solvent included comparison of beryllium dissolution in PSF to beryllium dissolution in the J774A.1 murine monocyte-macrophage cell line, and evaluation of the effect of ionic composition, buffer strength, and the presence of an antifungal agent on beryllium dissolution in PSF. Results indicated that PSF was a robust solvent that gave results similar to the J774A.1 cell line, and could be used for the planned dissolution studies.

Day GA, Hoover MD, **Stefaniak AB**, Dickerson RM, Peterson EJ, Esmen NA, Scripsick RC: Intracellular Behavior of Beryllium Oxide Particles: An *In vitro* Study in Murine Macrophages. *Exp. Lung Res.* (*In Press*).

* This article reported results from a study of the dissolution behavior of finished product beryllium oxide powder in the J774A.1 murine-monocyte cell line. The experimental methodology utilized was based on methods developed as part of the NIOSH funded 1R03 OH007447-01 project. Results of this study independently corroborated some of the findings of Stefaniak et al. *Exp. Lung Res.* (*In Press*).

Stefaniak AB, Day GA, Hoover MD, Breysse PN, RC Scripsick: Differences in dissolution behavior in a phagolysosomal simulant fluid for single-component and multi-component materials associated with beryllium sensitization and chronic beryllium disease. (*In Preparation for submission to J Aerosol Sci*).

* Little is known about the physicochemical properties or dissolution behavior of respirable beryllium aerosols, suggesting that the relationship between exposure to beryllium and dose is not well understood. This article reports that physicochemical properties, and in turn, values of k, of beryllium materials with capacity to cause CBD may vary by an order of magnitude or more. These data suggest an improved approach for regulating exposure might categorize chemical forms of beryllium according to solubility characteristics and SSA, with those materials producing dissolved beryllium at a rate sufficient for activation and maintenance of the CBD immune response posing the highest risk.

Platform Presentations

2^{nd} *Annual NIOSH Nanoaerosols Workshop*, Oglebay, WV, August 2004
Comprehensive Aerosol Characterization: Knowledge from Studies of Respirable Beryllium Aerosols. **Stefaniak AB**.

Beryllium Research Symposium: Basic Mechanisms & Human Health, Bethesda, MD, June 2002
Surface Area of Respirable Beryllium Metal, Oxide, and Copper Alloy Aerosols and Implications for Assessment of Exposure Risk of Chronic Beryllium Disease. **Stefaniak AB**, Hoover MD, Dickerson RM, Peterson EJ, Day GA, Breysse PN, Kent MS, Scripsick RC.

Beryllium Research Symposium: Basic Mechanisms & Human Health, Bethesda, MD, June 2002
In vitro Transformation of Phagocytized Beryllium Oxide Particles in the Murine J774a.1 Cell. Day GA, **Stefaniak AB**, Hoover MD, Dickerson RM, Peterson EJ, Esmen NA, Scripsick RC.

Poster Presentations

1st International Symposium on Occupational Health Implications of Nanomaterials, October 2004

Comprehensive Characterization Strategies for Ultrafine Particles: Lessons from Beryllium Health and Safety Studies. **Stefaniak AB**, Day GA, Scripsick RC, and Hoover MD.

100th International Conference of the American Thoracic Society, May 2004

Intracellular Behavior of Beryllium Oxide Particles: An *In vitro* Study in Murine Macrophages. Day GA, **Stefaniak AB**, Hoover MD, Dickerson RM, Peterson EJ, Esmen NA, Scripsick RC.

American Industrial Hygiene Conference and Exposition, May 2003

Influence of Surface Area on Dissolution of Beryllium from Respirable Beryllium Metal, Beryllium Oxide, and Copper-Beryllium Aerosols Associated with Chronic Beryllium Disease. **Stefaniak AB**, Scripsick RC, Breysse PN, Day GA, Hoover MD.

American Industrial Hygiene Conference and Exposition, June 2002

In vitro Transformation of Phagocytized Beryllium Oxide Particles in the Murine J774a.1 Cell Line. Day GA, **Stefaniak AB**, Hoover MD, Dickerson RM, Peterson EJ, Esmen NA, Scripsick RC.

Microscopy and Microanalysis, August 2001

TEM of BeO Aerosols: Materials Aspects of Method Development in Studying Chronic Beryllium Disease. Dickerson RM, Scripsick RC, Day GA, **Stefaniak AB**, Peterson EJ.

American Industrial Hygiene Conference and Exposition, June 2001

Surface Area of Beryllium Metal, Oxide, and Copper Alloy Aerosol Particles. **Stefaniak AB**, Hoover MD, Day GA, Scripsick RC.

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Final Report Abstract:

The long-term goal of this research is to develop more protective industrial hygiene metrics of exposure that are related to bioavailability, and therefore, potential risk of immune-mediated diseases caused by exposure to metals. We chose chronic beryllium disease (CBD) as a model for this research because it is a potentially fatal cell-mediated immune disease of the lung with no known cure that continues t be diagnosed among workers exposed to beryllium and foffi1er workers no longer exposed to beryllium. CBD is associated with exposure to beryllium- containing particles; however, the cellular level a dissolved beryllium species is the hypothesized input to the immune reaction that drives development of CBD. As beryllium containing particles dissolve in phagocytic lung cells, a fraction of the dissolve material may be consumed in the formation of the beryllium antigen, which is hypothesized to cause CBD. Little is known about the physicochemical properties or dissolution behavior of respirable beryllium aerosols, suggesting that the relationship between exposure to beryllium and dose is not well understood.

Dissolution (conversion from particle to a dissolved species) is a physicochemical process. Physicochemical properties of four materials were characterized utilizing a suite of analytical techniques: finished product beryllium metal powder and beryllium oxide powder, and particles sampled from manufacturing processes during production of beryllium oxide and copper-beryllium alloy. All study materials are associated with elevated prevalence of CBD. Characterization of study materials included aerodynamic size-separation and microscopy to understand size and morphology, x-ray diffraction (XRD) to determine crystalline chemical composition, and surface area analysis to determine specific surface area (SSA). Lacking a model of the acidic liquid environment of the pulmonary alveolar macrophage phagolyososome, the hypothesized site of beryllium dissolution, a solvent model termed phagolysosomal simulant fluid (PSF) was refined and characterized. Efforts to characterize the solvent included comparison of beryllium dissolution in PSF to dissolution in the J774A.l murine monocyte- macrophage cell line. No difference in beryllium dissolution was observed in PSF compared to the J774A.l cell line, thus PS was used to determine the chemical dissolution rate constant (k), having units of g/(cm2.day), for each material. The k value is a constant unique to a given chemical form of beryllium, permits intercomparison of dissolution rate data.

Results indicated that physicochemical properties, and in turn, values of k, differed among the study materials. The ranking of materials by solubility (from greatest to least) was beryllium metal powder> beryllium oxide powder beryllium oxide particles sampled from a production process. For the copper-beryllium particles studied, measured SSA for the entire sample did not govern beryllium dissolution from the beryllium oxide component of the

particles. Additional studies of this material showed that dissolution did not vary with measured SSA of the entire sample as expected from dissolution theory. Using k determined for beryllium oxide, the effective beryllium oxide SS was calculated for the copper-beryllium material using a technique developed as part o this project. To account for measured dissolution, the SSA of the beryllium oxide component o the copper-beryllium particles must be several orders of magnitude higher than the SS determined for the total particle sample. Research performed as part of this project improved understanding of how physicochemical properties of beryllium aerosols relate to solubility by using a solvent of phagolysosomal fluid. An understanding of beryllium particle dissolution is important because the rate of dissolved beryllium production may be the rate limiting step in the activation of the CBD immune response. Data collected indicate that k values of beryllium materials with capacity to cause CBD may vary by an order of magnitude or more. Thus, an improved approach for regulating exposure might categorize chemical forms of beryllium according to solubility characteristics and SSA, with those materials producing dissolved beryllium at a rate sufficient for activation and maintenance of the CBD immune response posing the highest risk.

Publications:

Stefaniak AB, Hoover MD, Dickerson RM, Peterson EJ, Day GA, Breysse PN, Kent MS and Scripsck RC: Surface Area of Respirable Beryllium Metal, Oxide, and Copper Alloy Aerosols and Implications for Asssessment of Exposure Risk of Chronic Beryllium Disease. Am. Ind. Hyg. Assoc. J. 64:297-305 (2003)
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