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ORIGINAL PAPER

Release of beryllium from mineral ores in artificial lung and skin surface fluids

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Abstract Exposure to some manufactured beryllium compounds via skin contact or inhalation can cause sensitization. A portion of sensitized persons who inhale beryllium may develop chronic beryllium disease (CBD). Little is understood about exposures to naturally occurring beryllium minerals. The purpose of this study was to assess the bioaccessibility of beryllium from bertrandite ore. Dissolution of bertrandite from two mine pits (Monitor and Blue Chalk) was evaluated for both the dermal and inhalation exposure pathways by determining bioaccessibility in artificial sweat (pH 5.3 and pH 6.5), airway lining fluid (SUF, pH 7.3), and alveolar macrophage phagolysosomal fluid (PSF, pH 4.5). Significantly more beryllium was released from Monitor pit ore than Blue Chalk pit ore in artificial sweat buffered to pH 5.3 (0.88 ± 0.01% vs. 0.36 ± 0.00%) and pH 6.5 (0.09 ± 0.00% vs. 0.03 ± 0.01%). Rates of beryllium released from the ores in artificial sweat were faster than previously measured for manufactured forms of beryllium (e.g., beryllium oxide), known to induce sensitization in mice. In SUF, levels of beryllium were below the analytical limit of detection. In PSF, beryllium dissolution was biphasic (initial rapid diffusion followed by latter slower surface reactions). During the latter phase, dissolution half-times were 1,400 to 2,000 days, and rate constants were $\sim 7 \times 10^{-10}$ g/(cm^2·day), indicating that bertrandite is persistent in the lung. These data indicate that it is prudent to control skin and inhalation exposures to bertrandite dusts.

Keywords Beryllium · Bioaccessibility · Minerals · Sensitization · Chronic beryllium disease · Exposure

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Introduction

Beryllium is a lightweight metal, which is commercially extracted from two silicate minerals, bertrandite ($Be_4Si_2O_7(OH)_2$) and beryl ($Be_3Al_2Si_6O_{18}$). Processed beryllium compounds (metal, oxide, alloys) possess unique physical, mechanical, and nuclear properties that are commercially valuable in a range of industries, including aerospace, telecommunications, defense, and automotive manufacturing (Stonehouse 1986; Weston et al. 2005). Some individuals exposed to beryllium (via inhalation and/or skin contact) become

sensitized, meaning that their immune systems respond to beryllium. A portion of sensitized individuals who inhale beryllium may progress to develop chronic beryllium disease (CBD), a debilitating respiratory disease (Sawyer and Maier 2011). Cases of CBD have been reported among workers in beryllium manufacturing facilities (Kreiss et al. 2007) and among residents living in communities surrounding manufacturing facilities (Maier et al. 2008). Development of sensitization may occur via inhalation and/or skin contact with some beryllium compounds (Curtis 1951; Kreiss et al. 2007). Development of CBD is associated with subsequent inhalation of beryllium compounds that are persistent in the lung (Sterner and Eisenbud 1951; Kreiss et al. 2007).

Research is ongoing to investigate linkages among beryllium exposure to material physicochemical properties, bioaccessibility, and development of sensitization and CBD (Finch et al. 1988a; Hoover et al. 1989; Stefaniak et al. 2006, 2011; Huang et al. 2011). These efforts have largely focused on processed beryllium compounds, some of which have epidemiological associations with sensitization and disease. The properties and solubility of naturally occurring forms of beryllium are poorly understood, in part due to the paucity of toxicological (Wagner et al. 1969) and epidemiological (Frommel et al. 1993; Wegner et al. 2000; Deubner et al. 2001) studies of these materials. Huang et al. (2011) recently evaluated dissolution of a bertrandite ore and a beryl ore in artificial human lung fluids. At pH 4.5, dissolution of bertrandite was generally greater than beryl. At pH 7.2, dissolution of bertandite was initially greater than beryl, though at latter time points beryl dissolution continued to increase, while bertrandite dissolution rapidly decreased. One historical study reported that rodents chronically exposed to bertrandite dust, but not beryl, developed lesions in the lung consisting of tightly packed dust-laden macrophages (Wagner et al. 1969). In a study of workers who cut and ground beryl gemstones (aquamarine and emerald), one of 57 was found to be sensitized (Wegner et al. 2000). In another study, three of 75 workers at a bertrandite/beryl extraction mill were sensitized, though none of the four bertrandite miners was sensitized. Of the three sensitized mill workers, one was diagnosed with CBD, though this person previously worked in a beryllium primary production facility (Deubner et al. 2001). Frommel et al. (1993) investigated elevated prevalence of podoconiosis among farmers in Ethiopia and attributed granuloma formation in lymphoid tissue to dermal exposure to soil containing high levels of beryllium (chemical form not determined). Hence, whether exposure to naturally occurring beryllium-containing ore material such as bertrandite can cause sensitization and CBD remains an open question due to limited toxicology studies and low statistical power in these epidemiology studies. The answer to this question is important for determining whether dust controls are needed for worker and public health protection. As a first step toward understanding the hazard potential of beryllium silicates, we investigated the dissolution behavior of bertrandite ore from two open-pit mines. Dissolution of bertrandite was evaluated for both the inhalation and dermal exposure pathways.

Materials and methods

Test materials and characterization

Bulk samples of bertrandite ore feedstock material from two open-pit mines (referred to as the Monitor and the Blue Chalk pits) located in Delta, Utah, USA were studied. This mine is the only domestic source of ore for commercial extraction. Ore is sent to a nearby mill, which extracts bertrandite and beryl (imported) to produce beryllium hydroxide. The hydroxide powder is shipped to another facility where it is used as the feedstock for production of beryllium metal, oxide, and alloy products (Stonehouse 1986).

The ore materials consisted of millimeter-size coarse particles. To obtain biologically relevant particle sizes, one kilogram of each ore was pulverized using a tungsten carbide shatterbox mill (SPEX Sample Prep, Metuchen, NJ), prior to characterization or solubility testing. A 10-mg aliquot of each pulverized ore powder was suspended in deionized water and analyzed for particle size using a liquid particle size distribution analyzer (CAPA500, Horiba, Edison, NJ). On a volume basis, greater than 50% of particles were less than 5 μm in physical diameter. The calculated mass median diameters were 25 μm (Monitor pit) and 26 μm (Blue Chalk pit), corresponding to an aerodynamic equivalent diameter of

approximately 40 μm. At the mill in Utah, the mass median aerodynamic diameter of airborne dust in the bertrandite ore crushing work area is 10.5 μm (Stefaniak et al. 2008). Hence, the size of the prepared ore powders was sufficient to be inhaled by workers and included sizes observed in the workplace atmosphere.

Physical characterization of powders included measurement of surface area and density. The Brunauer, Emmett, and Teller (BET) surface area was measured for each powder using nitrogen gas adsorption (ASTM 2002). Samples were vacuum degassed ($P \sim 0.015$ Torr) for a minimum of 4 h at 200°C to remove physisorbed material (e.g., water), prior to surface area measurement (Flovac™ Degasser, Product No. 05076, Quantachrome, Boca Raton, FL). Nitrogen gas adsorption isotherms were measured using a NOVA 2000e surface area analyzer (Quantachrome). Surface area values were calculated from five adsorption points in the relative pressure range $p/p_0 = 0.05$–0.35, and assuming 16.2 $Å^2$ for the cross-sectional area of a nitrogen molecule. Surface area values (m^2) were normalized by dry powder mass (determined gravimetrically) to obtain specific surface area (SSA) with units of m^2/g. Density was measured by gas displacement using a multipycnometer (Product no. 03035, Quantachrome) with ultra high-purity helium gas.

The crystalline chemical composition of the bulk powders was determined using powder x-ray diffraction (XRD, Siemens D500, Bruker AXS, Inc., Madison, WI) with CuKα radiation, incident- and diffracted-beam Soller slits, and a Kevex Si(Li) solid-state detector. Samples were run from 2 to 70° 2θ, using 0.02° steps, and counting for at least 4 s/step. To prepare the samples, a small portion of each sample (~0.8 g) was mixed with 1.0-μm corundum (Al_2O_3) internal standard in the ratio 80% sample to 20% Al_2O_3 by weight. Each sample was then ground under acetone in an automatic Brinkmann Micro-Rapid mill (fitted with an agate mortar and pestle) for a time greater than 10 min. This procedure yielded a sample with an average particle size of less than 5 μm and ensured thorough mixing of sample and internal standard. Quantitative mineral abundances were determined using FULLPAT, a quantitative X-ray powder diffraction program and method developed at Los Alamos National Laboratory (Chipera and Bish 2002).

Dissolution studies

To understand beryllium bioaccessibility, dissolution of the ore powders was evaluated in artificial biological fluids (skin surface and lung).

Artificial biological fluids

On the skin surface, human sweat contains at least 61 different chemical constituents and has a median pH of 5.3, ranging from 4.3 to 6.5 (Stefaniak and Harvey 2006). An artificial sweat that mimics human sweat was prepared using the formulation described by Harvey et al. (2010) and buffered to pH 5.3 or 6.5 by addition of 5 N sodium hydroxide.

In the human lung, the airway epithelial lining is coated with a fluid rich in complexing agents that has near-neutral pH. Upon deposition in the lung, particles are immersed in this fluid. We used serum ultrafiltrate (SUF), a model of airway epithelial lining fluid to evaluate dissolution. This model is widely used to evaluate solubility of metals such as uranium (Ansoborlo et al. 1999). SUF was prepared as described by Finch et al. (1988a), buffered to pH 7.3 using sodium bicarbonate, and maintained at this pH throughout studies by blanketing the sample headspace with 95% air/5% CO_2 gases.

Fine particles that deposit in the lung alveoli (gas exchange region) are rapidly engulfed by phagocytic cells such as macrophages. Within these cells, vesicles containing engulfed particles merge with lysosomes to form phagolysosomes, which contain oxidizers at acidic pH and function to break down foreign material. Lung alveolar macrophage phagolysosomal simulant fluid (PSF) was prepared as described by Stefaniak et al. (2005) and buffered to pH 4.5 using 0.02 M potassium hydrogen phthalate. PSF is widely used to evaluate the dissolution of inorganic materials including asbestos (Turci et al. 2009), carbon nanotubes (Liu et al. 2010; Russier et al. 2011), and atmospheric particles (Julien et al. 2011).

Experimental setup

Each experimental setup represented two ore materials and one of the three artificial biological fluids; in each setup, a nominal mass of 4.5 g of powder was used. The beryllium mass fraction in each ore

material was determined by microwave-assisted digestion of the powders using HF, HCl, and HNO_3 acids followed by beryllium analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ATS Laboratories, Marietta, GA). The actual mass of beryllium in each sample was calculated as the product of the ore powder mass times the appropriate beryllium mass fraction. Triplicate samples were prepared for each ore material in each artificial biological fluid.

A sterile beaker was used to add 50 mL of artificial biological fluid to appropriately labeled centrifuge tubes containing ore powder. Each tube was sealed using a screw-top lid and placed on a rotisserie (Thermo Scientific, Asheville, NC) at 32 rpm to ensure uniform particle contact with dissolution solvents. The rotisserie with tubes was placed in an incubator maintained at either 36.3°C to mimic skin surface temperature or 37°C to mimic lung temperature. In each experimental setup, a field blank consisting of a centrifuge tube containing only solvent was handled in the same manner and run alongside the experimental samples for each experiment.

At pre-designated time points, each centrifuge tube was removed from the rotisserie and centrifuged at 2,500 rpm (1300×*g*) for 22 min (Sorvall RT7, Kendo Laboratory Products, Newtown, CT) to form a particle pellet and liquid supernatant. For artificial sweat and SUF, samples were collected at 0.04, 0.17, 0.33, 0.5, 1, 2, 3, and 7 days. For PSF, samples were collected at 0.08, 0.21, 0.33, 0.5, 1, and 3 days, then twice weekly to 31 days. The centrifugation conditions were calculated based on the terminal settling velocity of the ore particles in water (Hinds 1999) and were sufficient to separate particles as small as 0.2 μm from the liquid. The supernatant from each tube was poured into a 60-ml luer-lock syringe and filtered through a 0.2-μm pore-size nylon membrane to ensure that particles were not present in the dissolved fraction. Fresh equilibrated solvent was added to each centrifuge tube, which was capped and agitated (Genie Vortex, Fisher Scientific) to thoroughly disperse and re-suspend the sediment prior to returning to the rotisserie in the incubator.

The pH of solvent in each sample was measured at 36°C (sweat) or 37°C (PSF and SUF) using a calibrated electrode. The probe was rinsed with distilled and deionized water and blotted dry between each sample measurement. Liquid samples containing soluble beryllium and quality control samples were diluted and analyzed without digestion by ICP-AES. The analytical limit of detection (LOD) for beryllium for all solvents was 0.2 μg/L, except sweat buffered to pH 6.5 (LOD = 0.4 μg/L).

Statistical analysis

All statistical analyses were performed using PC-SAS version 9.1 (SAS Institute, Cary, NC, USA). Based on the low solubility of these ores in dissolution fluids, we assumed that particles had constant surface area during the study period. A two-part segmented regression model was used to fit parameters to the observed dissolution data:

$$\text{If} \quad t \le x, 1 - \frac{M}{M_0} = b\sqrt{t} \tag{1}$$

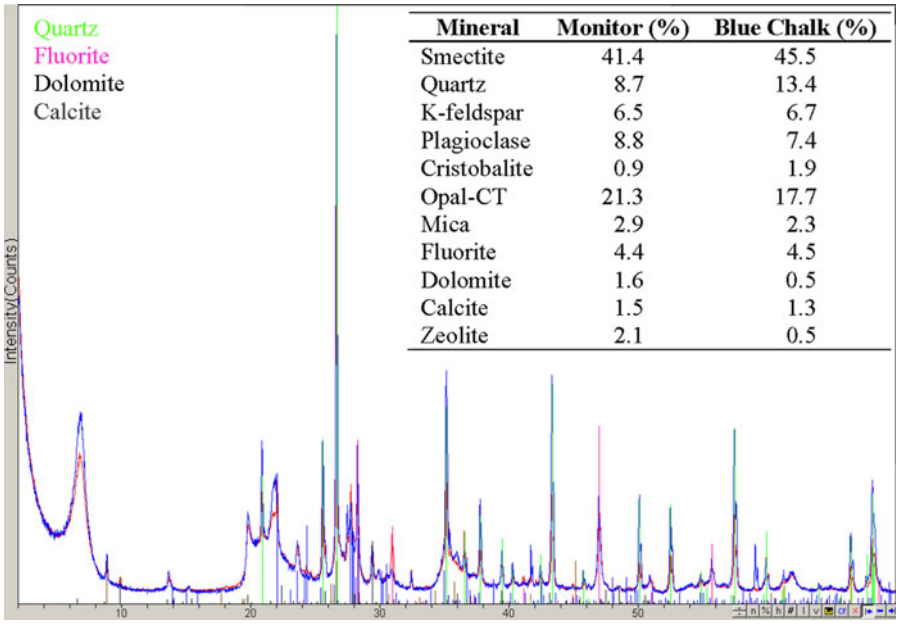
$$\text{If} \quad t \ge x, 1 - \frac{M}{M_0} = a + bt \tag{2}$$

where, $x = 7$ days (sweat) or 2 days (lung fluids); a = the extracted fraction due to initial exchange of surface molecules; b = slope; t = time (day).

Equation 1 was used to evaluate dissolution due to extraction of beryllium molecules from within a particle, and Eq. 2 was used to evaluate dissolution of beryllium from particle surfaces (Moss and Kanapilly 1980). For each solvent, values of $1 - M/M_0$ were plotted versus $\sqrt{t}$ (Eq. 1) and t (Eq. 2) and the slope determined for the linear portion of each curve. The slope value, b, for this line was used to calculate the beryllium dissolution half-time ($t_{1/2} = -0.693/b$) and normalized by SSA to estimate k, the chemical dissolution rate constant (b/SSA). The Student's t test was used to compare specific paired differences between means of the ores within dissolution solvents.

Results

From powder x-ray diffraction analysis, the Monitor and Blue Chalk pit ore materials were mainly comprised of smectite, opal-CT, and quartz with minor amounts of feldspar, calcite, dolomite, fluorite, and mica (Fig. 1). A definitive identification of a beryllium-bearing phase could not be determined from the diffraction patterns. The elemental beryllium content of the ore materials determined using ICP



Mineral	Monitor (%)	Blue Chalk (%)
Smectite	41.4	45.5
Quartz	8.7	13.4
K-feldspar	6.5	6.7
Plagioclase	8.8	7.4
Cristobalite	0.9	1.9
Opal-CT	21.3	17.7
Mica	2.9	2.3
Fluorite	4.4	4.5
Dolomite	1.6	0.5
Calcite	1.5	1.3
Zeolite	2.1	0.5

Fig. 1 X-ray diffraction patterns from the Monitor pit (*red pattern*) and Blue Chalk pit (*blue pattern*) bertrandite ore powders and phases identified from reference patterns. No beryllium-containing phases were definitively identified in the ore powders. The corundum phase identified in the diffraction pattern was from the internal standard and not part of the primary sample. (color figure online)

analysis was 0.307 ± 0.001% (Monitor pit) and 0.216 ± 0.010% (Blue Chalk pit). Values of BET specific surface area were 64.1 ± 4.7 m^2/g (Monitor pit) and 55.2 ± 4.0 m^2/g (Blue Chalk pit). Powder densities were 2.60 ± 0.01 g/cm^3 (Monitor pit) and 2.63 ± 0.05 g/cm^3 (Blue Chalk pit).

Dissolution in artificial sweat

On a mass basis, more beryllium was released from Monitor pit ore than Blue Chalk pit ore in artificial sweat buffered to pH 5.3 (0.88 ± 0.01% vs. 0.36 ± 0.00%) and pH 6.5 (0.09 ± 0.00% vs. 0.03 ± 0.01%); $p < 0.05$. For a given ore pit, the amount of beryllium released in artificial sweat was a factor of 10 higher at pH 5.3 than pH 6.5; $p < 0.05$.

Both ore materials exhibited biphasic beryllium dissolution in artificial sweat consisting of an early rapid phase followed by a latter slower phase (Fig. 2a). When plotted versus $\sqrt{t}$, the beryllium dissolution data were more linear (Fig. 2b), which is indicative of diffusion of molecules from within particles (Moss and Kanapilly 1980). Hence, parameter estimates were fitted to only the linear portion of the dissolution data using Eq. 1 for the ore materials in artificial sweat (Table 1). For Monitor pit ore in artificial sweat buffered to pH 5.3 and pH 6.5, values of $t_{1/2}$ were faster, and values of k were shorter than Blue Chalk pit ore ($p < 0.05$). For both ore pit materials, values of $t_{1/2}$ and k were significantly shorter and faster in artificial sweat buffered to pH 5.3 compared to pH 6.5 ($p < 0.05$).

Dissolution in artificial lung fluids

Amounts of beryllium released from both Monitor and Blue Chalk pit ores in SUF were <LOD. Figure 3 summarizes the dissolution results for the ore materials in the more acidic PSF. The total amount of beryllium released from Monitor pit ore (1.88 ± 0.01%) was higher than from Blue Chalk pit ore (1.34 ± 0.01%); $p < 0.05$.

On a linear time scale, both ore materials exhibited biphasic beryllium dissolution in PSF consisting of an early rapid phase followed by a latter slower phase. When beryllium dissolution data were plotted versus $\sqrt{t}$ (Eq. 1), the initial rapid phase became linear over the first 2 days of the study (data not shown). Hence, for beryllium release in PSF, Eq. 1 was used to evaluate initial dissolution due to extraction of beryllium molecules from within a particle, and Eq. 2 was used to evaluate steady state dissolution of beryllium from particle surfaces during the latter phase (Moss and Kanapilly 1980). Table 1 summarizes the fitted dissolution parameters for Monitor and Blue Chalk pit ores in PSF. For each ore material, the dominant dissolution mechanism in PSF was diffusion during the initial 2 days of the

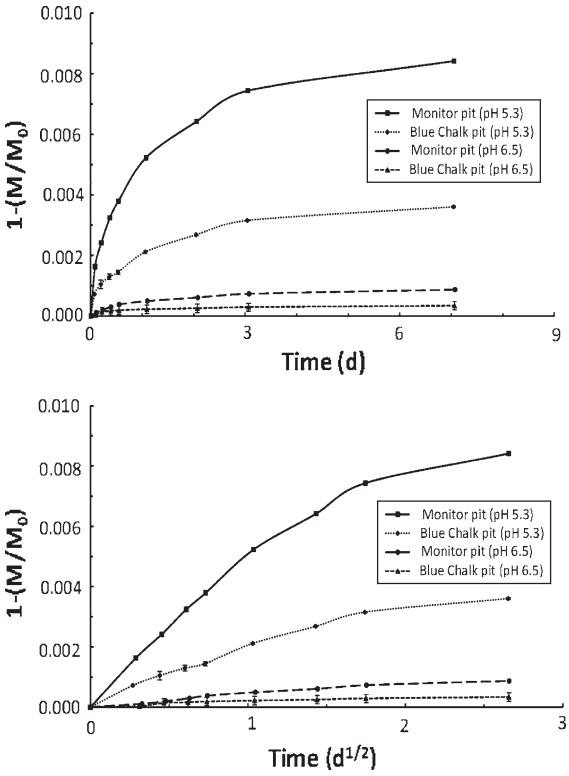


Fig. 2 Release of beryllium from Monitor and Blue Chalk pit bertrandite ores in artificial sweat was biphasic consisting of (**a**) an early rapid phase followed by a latter long-term phase, when plotted versus *t,* and (**b**) linear over 4–7 days, when plotted versus $\sqrt{t}$, indicating that the dominant mechanism of dissolution during this experiment was diffusion of molecules from within particles. *Error bars* represent one standard deviation from the mean

study. The beryllium $t_{1/2}$ value for Monitor pit ore was shorter ($p < 0.05$) than Blue Chalk pit ore during the initial rapid dissolution phase (diffusion), though there was no difference in k values. The value of $t_{1/2}$ was faster and k was shorter for Monitor pit ore than Blue Chalk pit ore during the latter slower phase (surface reactions); $p < 0.05$.

Discussion

For both pit ore materials, dissolution rates were faster in the more acidic solvents (PSF, pH 4.5; sweat, pH 5.3) compared to sweat (pH 6.5); dissolution in SUF at pH 7.3 was below the analytical LOD. This observed trend could be due to the crystalline phases of ore constituents. The predominant mineral component of both ores is smectite (Fig. 1). The steady state dissolution rates for smectite is inversely proportional to solvent pH (Amram and Ganor 2005; Golubev et al. 2006). Hence, increased smectite dissolution at lower pH values could yield more silica dissolution, thereby exposing more beryllium for dissolution.

The mass fraction of beryllium dissolved from Monitor pit ore was higher than Blue Chalk pit ore in artificial sweat and PSF. Surface area values and matrices of the ores were similar; hence, the observed differences in masses of beryllium released from these two ores were likely due to the higher naturally occurring beryllium content of the Monitor pit ore (0.307%) relative to the Blue Chalk pit ore (0.216%).

Exposure of the skin to soluble beryllium salts is known to cause immunologic sensitization in humans (Curtis 1951) and animals (Zissu et al. 1996; Tinkle et al. 2003). Skin contact with soil containing elevated levels of beryllium is implicated with elevated prevalence of podoconiosis in humans (Frommel et al. 1993). Topical skin application of beryllium oxide particles has been shown to induce sensitization in mice (Tinkle et al. 2003). Beryllium oxide dissolution rates in artificial sweat are $1.2 \pm 0.1 \times 10^{-9}$ and $5.3 \pm 0.2 \times 10^{-11}$ g/(cm^2·day) at pH 5.3 and 6.5, respectively (Stefaniak et al. 2011). The beryllium dissolution rates for the Monitor and Blue Chalk pit bertrandite ores in artificial sweat (Table 1) buffered to pH 5.3 are a factor of three to six times faster than beryllium oxide, whereas at pH 6.5, rates for these ores are an order of magnitude faster. Hence, release of beryllium from bertrandite ore particles immersed in sweat on the skin surface with subsequent absorption to the underlying immunologically active epidermis could be a plausible pathway for inducing sensitization.

With regard to inhalation of bertrandite ore dusts, lung fluid release rates provide insight as to how these particles will behave once deposited in the lung. The specific chemistry of lung fluids that particles encounter will vary by lung region: large particles that deposit in the conducting airways are immersed in the airway epithelial lining fluid having near-neutral pH, and fine particles that deposit in the alveoli are rapidly internalized by phagocytic cells and sequestered in phagolysosomes having acidic pH. An understanding of beryllium dissolution behavior is important due to the extensive contribution of lung exposures to sensitization and ultimately CBD.

Table 1 Fitted dissolution parameter estimates for Monitor pit ore and Blue Chalk pit ore in artificial lung fluids (PSF, SUF) and skin surface liquids (sweat)

Ore	Solvent[a]	Diffusion		Surface reactions	
		$t_{½}$ (days)	k_d [g/(cm^2·day)]	$t_{½}$ (days)	k_s [g/(cm^2·day)]
Mon Pit	$Sweat_{5.3}$	151 ± 1	$7.2 \pm 0.1 \times 10^{-9}$	–[b]	–
	$Sweat_{6.5}$	1,593 ± 39	$6.8 \pm 0.2 \times 10^{-10}$	–	–
	PSF	272 ± 9	$4.0 \pm 0.1 \times 10^{-9}$	1,417 ± 15	$7.6 \pm 0.1 \times 10^{-10}$
	SUF	ND[c]	ND	ND	ND
BC Pit	$Sweat_{5.3}$	362 ± 6	$3.5 \pm 0.1 \times 10^{-9}$	–	–
	$Sweat^{d}_{6.5}$	5,458 ± 122	$2.3 \pm 0.1 \times 10^{-10}$	–	–
	PSF	317 ± 19	$4.0 \pm 0.2 \times 10^{-9}$	2,050 ± 10	$6.1 \pm 0.0 \times 10^{-10}$
	SUF	ND	ND	ND	ND

[a] Sweat = skin surface liquid (pH 5.3 or pH 6.5); PSF = alveolar lung macrophage phagolysosomal simulant fluid (pH 4.5); SUF = lung airway epithelial lining fluid (pH 7.3)

[b] Parameter not applicable for material and solvent combination, where dissolution was due to diffusion of molecules from only within particles

[c] ND = levels of beryllium non-detectable

[d] Excludes $n = 1$ sample for which regression model did not converge

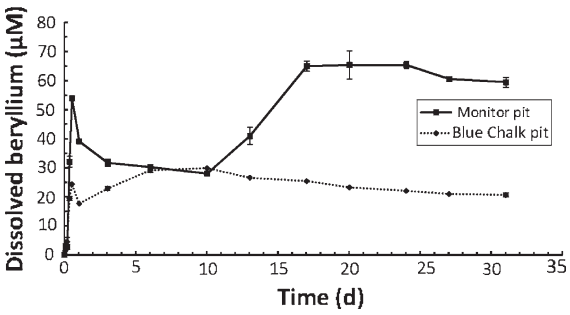


Fig. 3 Release of beryllium from Monitor and Blue Chalk pit bertrandite ores in artificial alveolar macrophage fluid. *Error bars* represent one standard deviation from the mean

The dissolution rates observed in this study indicate that beryllium release from bertrandite is very slow in the conducting airway and alveolar regions of the lung. In our experiments, beryllium release from bertrandite ore in SUF was <0.2 µg/L, which is consistent with the study by Huang et al. (2011). In that study, dissolution of beryllium from bertrandite in SUF was low, but measureable using a more sensitive analytical method. The mechanical clearance (mucociliary-mediated movement of material to the pharynx, where it is swallowed) half-time of particles deposited in the conducting airways is on the order of tens of hours to days (ICRP 1994). Hence, little beryllium is expected to be released by chemical dissolution of bertrandite particles that deposit in the conducting airways during this short residence time.

Beryllium dissolution rates from the bertrandite ores in PSF indicate that fine particles deposited in the alveolar region of the lung will persist for thousands of days. The slow release of beryllium from bertrandite in PSF, coupled with slow mechanical clearance rates on the order of thousands of days from the non-ciliated alveoli (ICRP 1994), suggests that long-term retention of bertrandite dusts would provide a sustained source of soluble beryllium. To provide a sustained source of beryllium, the amount released must not be cytotoxic. While data is not available on cytotoxicity of bertrandite ore dusts, Finch et al. (1988b) reported that the beryllium concentration required to decrease canine pulmonary macrophage cell viability by 50% (EC_{50}) was 3.3×10^{-3} M for beryllium oxide particles. In our study, the total cumulative beryllium concentration released from the Monitor and Blue Chalk ore materials was 2.8 and 5.8×10^{-4} M, respectively. Hence, the slow rates of mechanical clearance and chemical dissolution of bertrandite particles suggest a net accumulation of beryllium in the alveoli with repeat exposure over time, thereby providing continuous release of beryllium ion for interaction (antigen formation) with the immune system. Huang et al. (2011) also reported that dissolution of beryllium from bertrandite was biphasic

in PSF. The long-term bertrandite dissolution rates in PSF (via surface reactions) are a more appropriate indicator of lung persistence (Table 1). Our measured rates were two to three orders of magnitude slower than previously measured for beryllium metal powder ($5.2 \pm 0.2 \times 10^{-7}$ g/(cm^2·day)) and beryllium oxide powder ($3.6 \pm 0.4 \times 10^{-8}$ g/(cm^2·day)) sampled from work processes associated with elevated prevalence of CBD (Stefaniak et al. 2011). Development of CBD is associated with formation of granulomas in the lung alveoli over a period of years (Sawyer and Maier 2011). Hence, whether the reason that CBD has not yet been diagnosed among persons exposed to mineral forms of beryllium is because the solubility rate is too slow to sustain an immune reaction or because aerosols in work areas such as ore crushing are too large (Stefaniak et al. 2008) to penetrate to the alveoli remains unanswered at this time.

The observed beryllium dissolution rates for the Monitor and Blue Chalk pit bertrandite ores in PSF were a factor of two to three times faster (but within the same order of magnitude) than previously determined for a beryl ore obtained from the defunct Harding mine in New Mexico, $2.1 \pm 0.0 \times 10^{-10}$ g/(cm^2·day) (Stefaniak et al. 2011). This beryl mine was operational from 1950 to 1958 and was staffed by four workers who hand-sorted the beryl ore (Jahns and Ewing 1976). Exposure to Harding mine beryl dust has not been evaluated for an association with sensitization or CBD. Huang et al. (2011) also observed that dissolution of beryllium was higher from a bertrandite ore (same mine as our materials, pit source unknown) than an imported beryl ore. During milling of beryl ore at the facility in Utah, beryllium dissolution rates generally increase, as the ore is refined into high-purity product powder (Stefaniak et al. 2011). For example, the dissolution rate for dusts emitted from a heat-treater (after destroying the silicate structure of the beryl ore) is $\sim 1 \times 10^{-8}$ g/(cm^2·day), and the rate for finished product beryllium hydroxide powder is $\sim 2 \times 10^{-7}$ g/(cm^2·day). As noted previously, the low numbers of workers at this mill facility preclude estimates of process-related risk for sensitization and CBD.

Wagner et al. (1969) reported that rodents chronically exposed to bertrandite dust, but not beryl, developed tightly packed dust-laden macrophages in the lung. Our data indicate that long-term dissolution rates of bertrandite and beryl ores are $\sim 10^{-10}$ g/(cm^2·day), though rates for bertrandite are a factor of three times faster. While the slightly faster dissolution rate of beryllium from bertrandite ore may be sufficient to provoke an immune reaction leading to macrophage accumulation in the lung, it is more likely that variability in beryllium content of the bertrandite ore powders used in our study, 0.216 and 0.307%, and the bertrandite ore used by Wagner et al. (1969), 1.4%, explains the formation of clusters of dust-laden macrophages in their animal study.

Elevated prevalence of podoconiosis among Ethiopian farmers who do not wear shoes is attributed to dermal exposure to naturally occurring beryllium (Frommel et al. 1993). In the workplace, sensitization has been reported among workers exposed to beryl dusts during gemstone cutting (Wegner et al. 2000) and among workers exposed to dusts generated during bertandite and beryl extraction (Deubner et al. 2001). Multiple forms of airborne beryllium generated during extraction at the Utah mill include bertrandite, beryl, beryllium oxide, and beryllium hydroxide (Stefaniak et al. 2008). Additionally, these occupational epidemiological studies were based on very small numbers of workers and sensitized cases refused follow-up clinical examination for CBD. Hence, at this time, data are insufficient to exclude CBD as an outcome from inhalation of mineral forms of beryllium.

Summary

Dissolution of beryllium from bertrandite ore occurs in artificial sweat and lung fluids. Rates of beryllium release from the Monitor and Blue Chalk bertrandite ores in artificial sweat were faster than previously measured for beryllium oxide, a form capable of inducing sensitization in mice. Cummings et al. (2007) reported that when an occupational preventive program, which included protecting skin, was instituted in an oxide machining facility, the prevalence of beryllium sensitization among new workers declined, despite similar air exposure levels before and after the program. These data indicate that it is prudent to protect skin from contact with bertrandite dusts and to practice good hygiene (e.g., handwashing, showering) to remove material that contacts skin. The dissolution rates we observed for bertrandite ores in lung fluids were consistent with data from Huang

et al. (2011). Collectively, these studies constitute a reliable data set of dissolution rates in simulated lung fluids, which suggest that it is prudent to control exposures to reduce inhalation of bertrandite dusts based at least on risk of developing sensitization and possibly risk of CBD.

Stefaniak et al. (2008) hypothesized that particle dissolution kinetics limit the rate at which soluble beryllium becomes available to the immune system for inducing sensitization and pathological changes to lung tissue. This hypothesis is based on the premise that soluble beryllium is input to the immune response and either forms hapten for presentation by immune cells or binds directly to cell surface proteins involved in immune reactions. Alternatively, beryllium ion may chemically displace peptide in the binding groove of cell surface proteins and alter the protein conformation, thereby triggering an immune response (Snyder et al. 2008). In our model, sensitization occurs when beryllium particles undergo dissolution, thereby providing sufficient amounts of soluble beryllium to provoke an initial immune reaction (induction of sensitization). To cause disease, a sustained release of beryllium ion via dissolution of particles in alveolar macrophages is needed to support formation of granulomas (tightly packed groups of macrophage cells), chronic inflammation, and progression to fibrotic lung disease. Sawyer and Maier (2011) hypothesized that macrophage cells engulf beryllium particles in the lung during granuloma formation and subsequently undergo apoptosis (programmed cell death). As lung cells attempt to clear these dead macrophages, beryllium interacts with immune cells, which results in release of pro-inflammatory signaling molecules called cytokines. These cytokines function to recruit beryllium-specific immune cells to the site of injury to eliminate the poorly soluble dusts. This cycle of apoptosis followed by release of pro-inflammatory cytokines continues for years if beryllium dusts persist in the lung, thereby leading to inflammation and progression to fibrosis. While the exact mechanism by which beryllium causes chronic lung disease is yet to be fully elucidated, available data indicate a role for ionic beryllium released during dissolution, with persistence of poorly soluble forms of material providing a reservoir to support an immunological response. Results of the current study as well as previous reports support the conclusion that bertrandite and beryl ores are more persistent in lung alveoli than beryllium hydroxide, beryllium metal, beryllium oxide, and copper-beryllium alloys.

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Conflict of interest The authors declare that they have no conflict of interest.

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