Release of Beryllium from Beryllium-Containing Materials in Artificial Skin Surface Film Liquids

ALEKSANDR B. STEFANIAK*, M. ABBAS VIRJI and GREGORY A. DAY

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV 26505, USA

Purpose: Skin exposure to soluble beryllium compounds causes systemic sensitization in humans. Penetration of poorly soluble particles through intact skin has been proposed as a mechanism for beryllium sensitization; however, this mechanism is controversial. The purpose of this study was to investigate the hypothesis that particulate beryllium compounds in contact with skin surface release ions via dissolution in sweat.

Methods: Dissolution of 11 particulate beryllium materials (hydroxide, metal, oxides and copper–beryllium fume), 3 copper–beryllium alloy reference materials (chips and solid block), and 4 copper–beryllium alloy tools was measured over 7 days in artificial sweat buffered to pH 5.3 and pH 6.5.

Results: All test materials released beryllium ions in artificial sweat. Particulate from a reduction furnace that contained both crystalline and amorphous beryllium was the most soluble compound—40% dissolved in 8 h. Rates of beryllium release from all other particulate and reference materials were faster at pH 5.3 than at pH 6.5 ($P < 0.05$). At pH 5.3, values of the chemical dissolution rate constant, k [g (cm^2 day)$^{-1}$] differed significantly for hydroxide, metal, and oxide $-1.7 \pm 0.0 \times 10^{-7}$, $1.7 \pm 0.6 \times 10^{-8}$, and $1.0 \pm 0.5 \times 10^{-9}$, respectively ($P < 0.05$). Up to 30 µg of beryllium was released from the alloy tools within 1 h. Dissolution rates in artificial sweat were equal to or faster than values previously determined for these materials in lung models.

Conclusions: Poorly soluble beryllium materials undergo dissolution in artificial sweat, suggesting that skin exposure is a biologically plausible pathway for development of sensitization. Skin surface acidity, which is regulated by sweat chemistry and bacterial hydrolysis of sebum lipids varies by anatomical region and may be an exposure-modifying factor for beryllium particle dissolution.
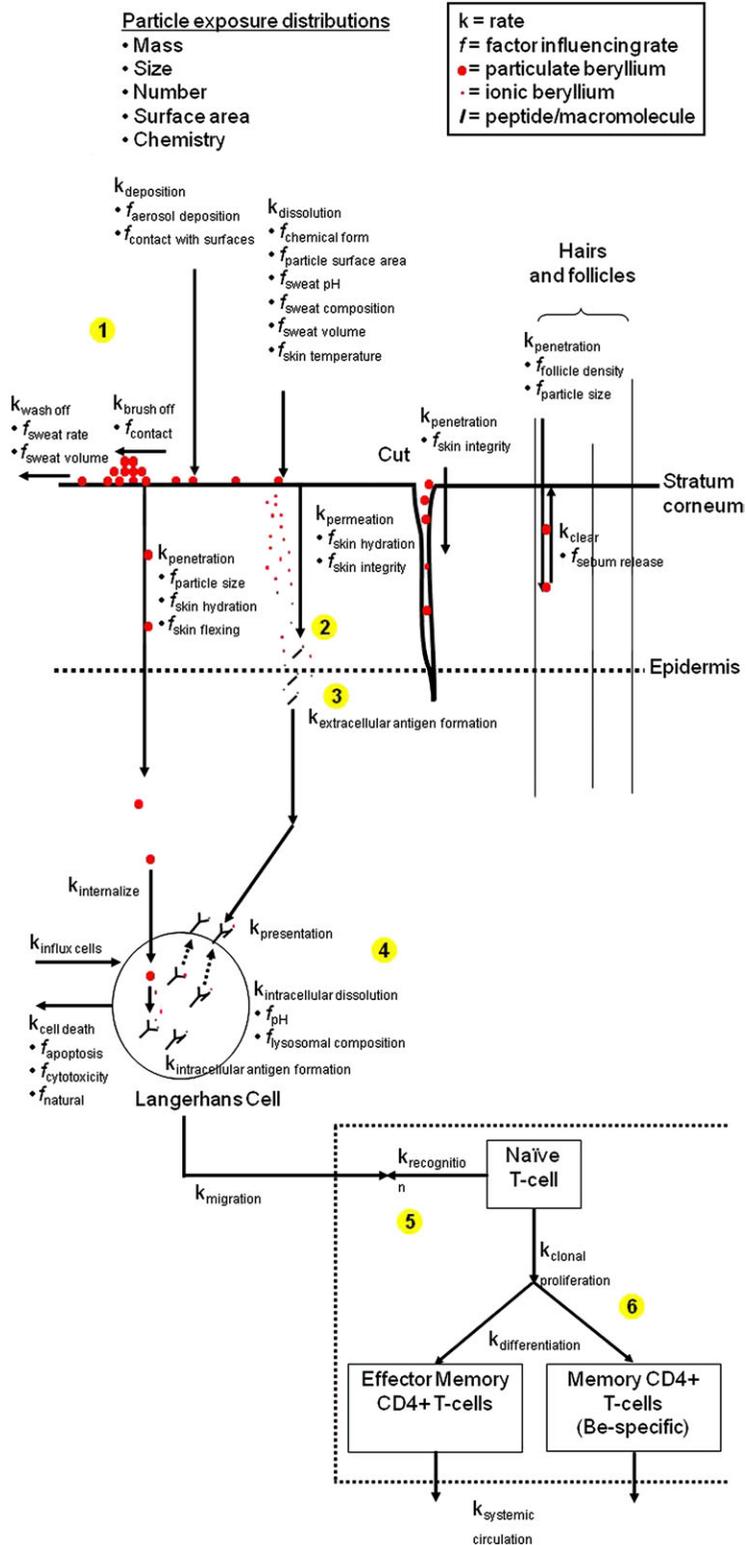
Keywords: beryllium sensitization; bioaccessibility; particle dissolution; skin

INTRODUCTION

Skin exposure to soluble beryllium salts is known to cause systemic immunologic sensitization in humans and animals (Curtis, 1951; Zissu *et al.*, 1996). Tinkle *et al.* (2003) applied micron-sized particulate beryllium oxide (BeO), a poorly soluble compound, to the skin of mice. One week later, they challenged the skin (ears) of the same mice and control mice with soluble beryllium sulfate. Ear swelling in the pre-

treated mice, but not in the control mice, indicated that the BeO particles had induced beryllium sensitization (BeS). The authors suggested that the mechanism of BeS was BeO particle penetration through the outer stratum corneum (SC) layer of the epidermis to the inner immunologically active layer of the epidermis. The concept of particle penetration through intact skin is controversial, even at the nanoscale (Baroli, 2010), where it appears that surface coatings and skin motion are important factors. However, many allergenic metals such as nickel are known to dissolve on the skin surface (Flint, 1998). Thus, release of ions from beryllium particulate

*Author to whom correspondence should be addressed.
Tel: +1-304-285-6302; fax:+1-304-285-6321;
e-mail: astefaniak@cdc.gov

Particle exposure distributions
• Mass
• Size
• Number
• Surface area
• Chemistry

k = rate
f = factor influencing rate
● = particulate beryllium
· = ionic beryllium
l = peptide/macromolecule

$k_{deposition}$
• $f_{aerosol\ deposition}$
• $f_{contact\ with\ surfaces}$

$k_{dissolution}$
• $f_{chemical\ form}$
• $f_{particle\ surface\ area}$
• $f_{sweat\ pH}$
• $f_{sweat\ composition}$
• $f_{sweat\ volume}$
• $f_{skin\ temperature}$

Hairs and follicles

$k_{penetration}$
• $f_{follicle\ density}$
• $f_{particle\ size}$

1

$k_{wash\ off}$
• $f_{sweat\ rate}$
• $f_{sweat\ volume}$

$k_{brush\ off}$
• $f_{contact}$

$k_{penetration}$
• $f_{skin\ integrity}$

Cut

Stratum corneum

$k_{permeation}$
• $f_{skin\ hydration}$
• $f_{skin\ integrity}$

k_{clear}
• $f_{sebum\ release}$

$k_{penetration}$
• $f_{particle\ size}$
• $f_{skin\ hydration}$
• $f_{skin\ flexing}$

2

3

$k_{extracellular\ antigen\ formation}$

Epidermis

$k_{internalize}$

$k_{influx\ cells}$

$k_{presentation}$

4

$k_{intracellular\ dissolution}$
• f_{pH}
• $f_{lysosomal\ composition}$

$k_{cell\ death}$
• $f_{apoptosis}$
• $f_{cytotoxicity}$
• $f_{natural}$

$k_{intracellular\ antigen\ formation}$

Langerhans Cell

$k_{recognition}$

Naïve T-cell

$k_{migration}$

5

$k_{clonal\ proliferation}$

6

$k_{differentiation}$

Effector Memory CD4+ T-cells

Memory CD4+ T-cells (Be-specific)

$k_{systemic\ circulation}$

compounds through dissolution in sweat on the skin surface may be an alternative pathway for inducing BeS in exposed individuals (Day *et al.*, 2006).

Potential exists in the work environment for skin exposure to particulate beryllium compounds (Cummings *et al.*, 2007; Day *et al.*, 2007; Emond *et al.*, 2007; Dufresne *et al.*, 2009). Figure 1 presents our conceptual model of the interaction of particulate beryllium with skin. Upon contact with the outer SC, particles are immersed in skin surface film liquids (SSFL), a complex mixture of mainly aqueous sweat and oily sebum lipids. Many deposited particles are rapidly cleared off the skin, others are hypothesized to penetrate the skin, and some may be trapped and retained in the microtopography of the SC and made available for dissolution. About half of the ions released in artificial sweat will be in the form of free Be^{2+} with the rest forming complexes (Sutton and Burastero, 2003). Soluble beryllium formed on the skin surface may diffuse through the SC to the immunologically active layer of the epidermis. The exact mechanism by which beryllium induces sensitization is unknown; however, one plausible mechanism is that soluble ions bind to macromolecules to form hapten complexes (antigen). Once haptens are internalized, Langerhans cells (LCs) become activated and migrate to the draining lymph nodes. During migration, LCs undergo phenotypic alterations, including increased expression of major histocompatibility complex (MHC) class II molecules. Within the draining lymph nodes, LCs present antigen on the cell surface via MHC class II proteins to naive CD4+ T cells. Upon recognition of antigen, these naive T cells undergo stimulation and differentiation to form antigen-specific T effector cells and memory CD4+ T-helper cells that subsequently circulate in the blood and lymph nodes, i.e. an individual becomes sensitized (Arts *et al.*, 2006; Toebak *et al.*, 2009).

The focus of this study was to investigate the hypothesis that particulate beryllium compounds will dissolve in artificial sweat. We used a novel model artificial SSFL with composition and constituent concentrations that mimic human sweat to evaluate the dissolution of 18 beryllium-containing materials.

MATERIALS AND METHODS

Test materials

Particulate beryllium materials were collected from industrial processes that spanned extraction of beryllium from ore through handling of finished product (Table 1). These materials were bulk powders or airborne particles collected from process local exhaust ventilation using a multistage aerosol sampler. For airborne particles, dissolution was evaluated using material with aerodynamic diameter approximately >6 μm because this large size is likely to settle onto work surfaces or exposed skin. With the exception of reduction furnace and arc furnace aerosols, all airborne particles were chemically homogeneous materials. During beryllium metal production, the reduction furnace generates aerosol with branched-chain morphology, i.e. aggregates of individual 'primary' particles, which are composed of BeO (40% by weight), crystalline magnesium fluoride, and possibly amorphous beryllium fluoride (Stefaniak *et al.*, 2003, 2004). During copper–beryllium alloy production, a 'master' alloy ingot is produced using an arc furnace; branch chain particles composed of BeO and copper oxides are generated during this production process. Details of the production process chemistry, operating conditions, particulate sample collection, and measured physicochemical properties were previously described (Stefaniak *et al.*, 2003, 2004, 2008a).

Three US National Institute of Standards and Technology (NIST) standard reference materials (SRMs®) were evaluated: SRM 458—copper–beryllium (CuBe) alloy chips (0.36% beryllium), SRM 459—CuBe alloy chips (1.82% beryllium), and SRM C1122—CuBe chill cast block (1.75% beryllium). The chip SRMs were used to simulate skin contact with shavings (millimeter-size pieces) of CuBe alloy and the block SRM was used to simulate skin contact with solid rod, bar, and plate product.

CuBe is used to make nonsparking corrosion-resistant tools for use in combustible atmospheres such as mines. Four tools (claw hammer, ball-peen hammer, pipe wrench, and pliers) were evaluated to determine beryllium release rates; the hammers

---

Fig. 1. (1) Beryllium particles deposit on skin and are cleared, penetrate as particles through intact and/or compromised skin, and/or undergo dissolution in SSFL; (2) Particle dissolution yields beryllium ions, which diffuse across the SC toward the immunologically active epidermis and dermis; (3) Beryllium ions bind to macromolecules (peptides and proteins) to form hapten antigen; (4) LC internalize beryllium antigen, become activated, and migrate to the draining lymph nodes; (5) LCs present beryllium antigen on surface MHC class II proteins to naïve CD4+ T lymphocytes; (6) Upon recognition of antigen, naïve T lymphocytes undergo MHC-restricted stimulation and differentiation to form hapten-specific T effector lymphocytes and memory CD4+ T-helper lymphocytes, which circulate in the blood and lymph nodes.

Table 1. Industrial sources of beryllium-containing particulate materials

Facility	Sample location	High risk for BeS?	Reference
Ore mill	Beryllium hydroxide drumming	—[a]	Deubner *et al.* (2001)
Primary production	Reduction furnace	Yes	Kreiss *et al.* (1997)
	Finished product metal powder[b]	Yes	Stange *et al.* (1996)
	Beryllium oxide screener	Yes	Kreiss *et al.* (1997)
	Finished product oxide powder[b]	Yes	Henneberger *et al.* (2001)
	Master alloy arc furnace	Yes	Kreiss *et al.* (1997)
Oxide machining	Receiving	No	Kreiss *et al.* (1996)
	Material preparation	No	Kreiss *et al.* (1996)
	Pressing deck	No	Kreiss *et al.* (1996)
	Green machining	No	Kreiss *et al.* (1996)
	Postsintered machining	Yes	Kreiss *et al.* (1996)

[a]No estimates of material- or process-related risk available for this material.
[b]Obtained from an industrial producer and aerodynamically size-separated in the laboratory (Hoover *et al.* 1989).

had wooden handles so the copper–beryllium head was tested. The two hammers and pipe wrench were reported by the manufacturer to contain 1.8% beryllium by weight. The exact beryllium content of the pliers was unknown, though tools from this manufacturer contain <2% beryllium.

Dissolution test method

Artificial SSFL. Artificial human sweat (US Patent Pending) was prepared by dissolving 7 electrolytes, 13 ionic constituents, 9 organic acids and carbohydrates, 16 amino acids, 5 nitrogenous substances, and 11 vitamins in fully aerated distilled and deionized water and buffered to pH 5.3 (Harvey *et al.*, 2010). Artificial human sebum (US Patent Pending) was prepared by dissolving 10 lipids in a 2:1 cosolvent mixture of chloroform and methanol and then allowing the cosolvents to evaporate off, as described previously (Stefaniak *et al.*, 2010a).

Among healthy adults, the median skin surface pH is 5.3 (Stefaniak and Harvey, 2006) and we buffered our artificial human sweat formulation to match this value. Historically, the most widely used artificial sweat is based on a formulation by Pedersen *et al.* (1974) which is buffered to pH 6.5; this formulation was subsequently adopted for use in the European reference test method EN1811 (CEN, 1998) to assess nickel release from consumer products. Thus, to test the influence of sweat pH on dissolution, each material was evaluated in artificial sweat solution buffered to pH 5.3 and pH 6.5.

Dissolution tests. To simulate the human skin surface, we evaluated dissolution in the presence of both artificial sebum and sweat. Given the difficulty of dispersing lipids in aqueous solvents for *in vitro* testing (Hemingway and Molokhia, 1987), each beryllium material was placed in contact with dry sebum lipids and then immersed in artificial sweat. The assembly of the static dissolution chamber has been described previously (Stefaniak *et al.*, 2005). Briefly, a preweighed (Model XS205; Mettler-Toledo, Greifensee, Switzerland) 47-mm ashless filter was dipped into the artificial sebum solution and air-dried to allow the solvent to evaporate. A known mass of beryllium-containing powder or chips was weighed onto a sebum-coated filter that rested on top of a 0.025-µm pore size, 47-mm diameter nitrocellulose filter, and covered with a second 47-mm filter to form a 'sandwich'. The outer few millimeters of each 47-mm diameter sandwich was securely clamped in the o-ring seal between the retaining rings of a static dissolution chamber (Intox Products, Moriarty, NM, USA), which allowed for free contact and diffusion of the solvent through both the top and the bottom surfaces of the filter sandwich. Because it was too large to fit in a dissolution chamber, the SRM C1122 block was placed on top of a sebum-coated filter. Each sample was placed into a separate polypropylene plastic beaker and immersed in 80 ml of artificial sweat, which was sufficient to completely cover the dissolution chamber or SRM C1122 block. Triplicate samples were prepared for each beryllium-containing material at each pH value.

Each tool was evaluated only in artificial sweat buffered to pH 5.3. The CuBe heads of the hammers and pliers and the handle of the pipe wrench were exposed to 100 ml of artificial sweat that covered the tools to a depth of ∼2 cm (hammers), 3 cm (pliers), or 4–5 cm (pipe wrench) in polypropylene containers, the inside surfaces of which had been previously coated with artificial sebum, and then allowed to air dry.

While the temperature of some anatomical regions, including fingers, is close to 33°C, we maintained

all samples at 36.3°C in a water-jacketed incubator to mimic the median whole-body human skin temperature (Stefaniak and Harvey, 2006). At 1, 4, 8, 12, 24, 48, 72, and 168 h, each test material was removed from its beaker, the fluid transferred to a glass jar, and fresh temperature-equilibrated fluid was added to the sample beaker before it was returned to the incubator. The pH of the artificial sweat remained within ±0.1 units throughout all experiments.

Analytical methods and quality control

Experimental and quality control samples were analyzed by US National Institute for Occupational Safety and Health Method 7303: elements by inductively coupled plasma (ICP) (NIOSH, 2003). All samples were analyzed using a Perkin Elmer Optima 3200XL ICP at a sample flow rate of 2 ml min^{-1} and results expressed as the average of three exposures per analyses. Liquid samples were analyzed without digestion to quantify the mass of dissolved metals (M_D). The limits of detection (LOD) and quantification (LOQ) were, respectively, 0.3 and 1.1 µg beryllium l^{-1} and 10 and 34 µg copper l^{-1} (pH 5.3) and 0.2 and 0.71 µg beryllium l^{-1} and 10 and 33 µg copper l^{-1} (pH 6.5). The triplicate filter samples of each material were digested and analyzed to quantify masses of residual (M) particulate beryllium and/or copper; LOD and LOQ values were, respectively, 0.1 and 0.33 µg beryllium per sample and 0.4 and 1.4 µg copper per sample. All samples were background corrected for masses of beryllium and/or copper in field and reagent blanks as appropriate prior to statistical analyses.

Statistical analyses

The initial mass of beryllium (or copper), i.e. M_0, is equal to the sum of M_D and M. In our study, M_0 for particulate was measured directly by weighing and accounting for the beryllium and/or copper fraction in the material that was previously measured using quantitative X-ray diffraction. For the SRMs, M_0 was taken to be the certified values of beryllium and copper provided by NIST. We chose this gravimetric approach because many acid digestion protocols are insufficient to completely dissolve poorly soluble forms of particulate beryllium such as BeO (Stefaniak et al., 2008b), which could result in underestimation of M_0. All statistical analyses were performed using SAS® software version 9.1 (SAS Institute, Cary, NC, USA) as described previously (Stefaniak et al., 2010b). A fully factorial experimental design was employed, enabling experimental investigation of all factor-level combinations. For each experiment, the mass fraction of material remaining (M/M_0) versus time (t) immersed in artificial sweat were plotted (Finch et al., 1988):

$$y = \sum_{i=1}^{n} f_i \exp(-\lambda_i t), \tag{1}$$

where y is M/M_0

$$\sum_{i=1}^{n} f_i = 100\%,$$

where i = number of dissolution components (one or two); f_i = fraction of material available for dissolution in the ith component; $\lambda_i = k_i \mathrm{SSA}$; k_i = chemical dissolution rate constant, g (cm^2 d)$^{-1}$; SSA = specific surface area, cm^2 g^{-1}.

For beryllium hydroxide [Be(OH)$_2$], beryllium metal, and BeO, negative exponential functions were fitted to the plotted data using nonlinear regression models first with a single component (assuming a single dissolution phase) and then with two components (allowing for two dissolution phases). Starting values were provided based on previous research and/or estimates from the data; the F-ratio test was used to determine the optimum number of components that best described the data. The chemical dissolution rate constant ($k_i = \lambda_i/\mathrm{SSA}$) was calculated for each sample. Repeated measures one-way analysis of variance (ANOVA) models were developed to investigate the impact of the fixed effects of solvent pH and beryllium chemical form on k values. ANOVA F-statistics were used to note overall differences in the means for dissolution parameters among the sweat solvents and powder forms. Tukey's test was used to identify specific paired differences between different means.

For the chemically heterogeneous experimental materials (i.e. reduction furnace, master alloy, and alloy reference materials), k_i values could not be calculated according to the model specified in equation 1 because the beryllium surface area of these materials could not be estimated. The mass fraction of beryllium dissolved (1-(M/M_0)) data were log-transformed and offered as the dependent variable in repeated measures ANOVA models with solution pH and time as fixed effects (for particulates from the master alloy and reduction furnaces) and an additional fixed effect of material (for SRMs).

RESULTS

Dissolution of process-sampled particulate

Beryllium dissolution from all tested process particulates except for the reduction furnace particles

was best described by a one-component negative exponential function at both pH values evaluated. The k value for the following materials were significantly ($P < 0.05$) greater in artificial sweat buffered to pH 5.3 than in artificial sweat buffered to pH 6.5: $Be(OH)_2$ (Table 2), beryllium metal (Table 3), and BeO (Table 4). There was no significant difference in k values among the seven BeO materials evaluated at either pH value. In artificial sweat buffered to pH 5.3, the rank order of dissolution rates for the chemically homogenous particles (from fastest to slowest) was $Be(OH)_2$ > beryllium metal > all BeO; differences were significant ($P < 0.05$). In artificial sweat buffered to pH 6.5, the rank order was $Be(OH)_2 \approx$ beryllium metal > all BeO; differences were significant ($P < 0.05$).

Among chemically heterogeneous particles, reduction furnace particles contained reactive fluoride, precluding accurate measurement of surface area (Stefaniak *et al.*, 2003) and, therefore, estimation of a k value. Dissolution of beryllium from reduction furnace particles was biphasic. Figure 2 is a plot of the mass fraction of beryllium remaining versus time; the mass fraction of beryllium remaining decreased

rapidly and then it decreased more slowly (Fig. 2). The fractions of beryllium dissolved at each time point are summarized in Table 5. Within the first hour, 10–20% of the beryllium dissolved and by the eighth hour, the duration of a work shift, >40% (10 mg) of beryllium dissolved from these particles. For each dissolution phase, the amount of beryllium dissolved from reduction furnace particles was significantly faster in artificial sweat buffered to pH 5.3 than in artificial sweat buffered to pH 6.5 ($P < 0.05$).

Dissolution of beryllium from the chemically heterogeneous master alloy arc furnace particles was biphasic (Fig. 3) and was faster in artificial sweat buffered to pH 5.3 than in artificial sweat buffered to pH 6.5 ($P < 0.05$). The cumulative fractions of beryllium released at 8 h were 0.9% (107 μg) and 0.1% (9.4 μg) for particles immersed in artificial sweat buffered to pH 5.3 and pH 6.5, respectively (Table 5). In contrast with beryllium release, the dissolution rate for copper was higher for the higher pH level tested; at 7 days, the average cumulative fractions dissolved were 24.2% (5350 μg) and 36.9% (20 410 μg) in artificial sweat buffered to pH 5.3 and pH 6.5, respectively.

Table 2. Dissolution of beryllium hydroxide after 168-h immersion in artificial sweat

Powder	SSA (m² g⁻¹)[a]	pH	Sample	M_0 (mg)[b]	ΣM_D (mg)[c]	k g (cm² day)⁻¹	
						Sample	Average ± SD
$Be(OH)_2$	0.3	5.3	A	162.0633	0.5496	1.8×10^{-7}	$1.7 \pm 0.0 \times 10^{-7}$[d]
			B	161.7168	0.5309	1.7×10^{-7}	
			C	161.5551	0.5217	1.7×10^{-7}	
		6.5	A	162.1221	0.0052	1.5×10^{-9}	$1.1 \pm 0.4 \times 10^{-9}$
			B	161.4942	0.0033	0.9×10^{-9}	
			C	161.5866	0.0026	0.8×10^{-9}	

[a]Value from Stefaniak *et al.* (2008a).
[b]M_0 = mass of beryllium at time = 0.
[c]ΣM_D = sum of masses of beryllium dissolved at all time points.
[d]Significantly faster dissolution at pH 5.3 than at pH 6.5 ($P < 0.05$).

Table 3. Dissolution of beryllium metal after 168-h immersion in artificial sweat

Powder	SSA (m² g⁻¹)[a]	pH	Sample	M_0 (mg)[b]	ΣM_D (mg)[c]	k g (cm² day)⁻¹	
						Sample	Average ± SD
Be	5.6	5.3	A	7.9838	0.0674	2.2×10^{-8}	$1.7 \pm 0.6 \times 10^{-8}$[d]
			B	9.1585	0.0368	1.0×10^{-8}	
			C	8.9293	0.0654	1.9×10^{-8}	
		6.5	A	10.3140	0.0060	1.4×10^{-9}	$1.5 \pm 0.1 \times 10^{-9}$
			B	7.9647	0.0046	1.4×10^{-9}	
			C	7.5732	0.0050	1.7×10^{-9}	

[a]Value from Hoover *et al.* (1989).
[b]M_0 = mass of beryllium at time = 0.
[c]ΣM_D = sum of masses of beryllium dissolved at all time points.
[d]Significantly faster dissolution at pH 5.3 than at pH 6.5 ($P < 0.05$).

Table 4. Dissolution of beryllium oxide materials after 168-h immersions in artificial sweat

Powder	SSA $(m^2\ g^{-1})$[a]	pH	Sample	M_0 (mg)[b]	ΣM_D (mg)[c]	k g $(cm^2\ day)^{-1}$	
						Sample	Average ± SD
Screener	11.9	5.3	A	35.8992	0.0117	3.9×10^{-10}	$6.0 \pm 3.0 \times 10^{-10d}$
			B	36.0252	0.0148	4.8×10^{-10}	
			C	35.2188	0.0270	9.3×10^{-10}	
		6.5	A	35.2296	0.0014	4.7×10^{-11}	$4.9 \pm 0.3 \times 10^{-11}$
			B	36.5076	0.0016	5.2×10^{-11}	
			C	35.9712	0.0015	4.9×10^{-11}	
Finished product	11.4	5.3	A	35.6904	0.0371	1.3×10^{-9}	$1.2 \pm 0.1 \times 10^{-9d}$
			B	35.2368	0.0330	1.2×10^{-9}	
			C	35.5428	0.0304	1.1×10^{-9}	
		6.5	A	35.4060	0.0016	5.3×10^{-11}	$5.3 \pm 0.2 \times 10^{-11}$
			B	35.0064	0.0014	5.1×10^{-11}	
			C	35.3268	0.0019	5.6×10^{-11}	
Received	9.6	5.3	A	35.3556	0.0377	1.6×10^{-9}	$1.3 \pm 0.3 \times 10^{-9d}$
			B	35.7444	0.0286	1.2×10^{-9}	
			C	35.5932	0.0225	9.6×10^{-10}	
		6.5	A	36.1692	0.0013	5.4×10^{-11}	$5.8 \pm 2.2 \times 10^{-11}$
			B	35.5428	0.0009	3.8×10^{-11}	
			C	35.2044	0.0019	8.1×10^{-11}	
Material prep	8.6	5.3	A	35.2116	0.0173	7.7×10^{-10}	$8.0 \pm 0.3 \times 10^{-10d}$
			B	35.5464	0.0181	8.0×10^{-10}	
			C	37.5372	0.0195	8.3×10^{-10}	
		6.5	A	41.0760	0.022	8.4×10^{-11}	$9.7 \pm 1.4 \times 10^{-11}$
			B	40.7016	0.0025	9.5×10^{-11}	
			C	35.0712	0.0011	1.1×10^{-10}	
Press deck	8.1	5.3	A	36.5256	0.0194	8.4×10^{-10}	$8.2 \pm 0.3 \times 10^{-10d}$
			B	42.6060	0.0206	7.9×10^{-10}	
			C	41.6520	0.0214	8.3×10^{-10}	
		6.5	A	35.9532	0.0025	1.1×10^{-10}	$1.2 \pm 0.1 \times 10^{-10}$
			B	43.4052	0.0035	1.3×10^{-10}	
			C	39.8304	0.0031	1.2×10^{-10}	
Green machined	6.9	5.3[e]	A	35.1432	0.0122	7.0×10^{-10}	$6.7 \pm 0.4 \times 10^{-10d}$
			B	35.5320	0.0166	6.4×10^{-10}	
		6.5	A	35.0244	0.0049	2.8×10^{-10}	$3.1 \pm 2.5 \times 10^{-10}$
			B	35.8920	0.0047	7.0×10^{-11}	
			C	35.1252	0.0056	5.8×10^{-10}	
Sintered	4.6	5.3	A	50.5728	0.0225	1.4×10^{-9}	$1.8 \pm 0.7 \times 10^{-9d}$
			B	51.7248	0.0423	2.6×10^{-9}	
			C	52.0164	0.0238	1.4×10^{-9}	
		6.5	A	51.1920	0.0023	1.4×10^{-10}	$1.3 \pm 0.1 \times 10^{-10}$
			B	50.6952	0.0024	1.4×10^{-10}	
			C	51.1704	0.0022	1.3×10^{-10}	

[a]Value for finished product powder from Hoover et al. (1989).
[b]M_0 = mass of beryllium at time = 0.
[c]ΣM_D = sum of masses of beryllium dissolved at all time points.
[d]Significantly faster dissolution at pH 5.3 than at pH 6.5 ($P < 0.05$).
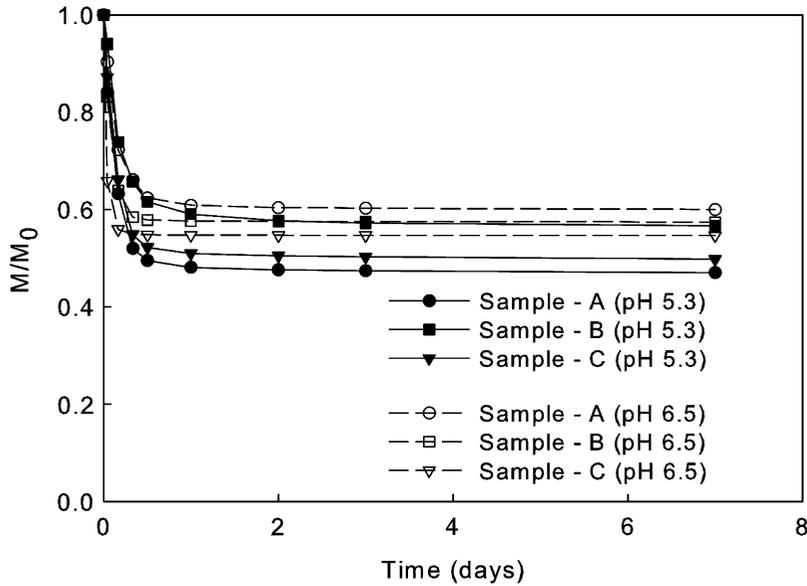[e]$n = 2$ (insufficient mass of material to prepare triplicate samples at this pH value).

Fig. 2. Dissolution kinetics of beryllium from reduction furnace particles, illustrating biphasic behavior in artificial sweat buffered to pH 5.3 and pH 6.5.

Table 5. Cumulative percentage of beryllium released from process-sampled reduction furnace particles and master alloy arc furnace particles in artificial sweat

Time (h)	Average ± SD (%)			
	Reduction furnace		Master alloy arc furnace	
	pH 5.3	pH 6.5	pH 5.3	pH 6.5
1	11.6 ± 5.1	20.3 ± 12.6	0.1 ± 0.0	0.1 ± 0.0
4	32.3 ± 5.5	36.0 ± 8.2	0.5 ± 0.1	0.1 ± 0.0
8	42.5 ± 7.2	40.2 ± 5.7	0.9 ± 0.3	0.1 ± 0.1
12	45.6 ± 6.3	41.7 ± 3.8	1.4 ± 0.3	0.1 ± 0.1
24	47.3 ± 5.7	42.3 ± 3.1	2.2 ± 0.5	0.1 ± 0.1
48	48.1 ± 5.2	42.5 ± 2.8	3.0 ± 0.5	0.1 ± 0.1
72	48.4 ± 5.0	42.5 ± 2.8	3.5 ± 0.5	0.2 ± 0.1
168	48.9 ± 5.0	42.7 ± 2.7	4.5 ± 0.7	0.3 ± 0.1
M_0 (mg)[a]	25.74 ± 0.40	26.27 ± 1.62	11.30 ± 1.30	10.69 ± 0.57

[a] M_0 = mass of beryllium at time = 0.

Dissolution of SRMs

Dissolution patterns observed for beryllium and copper released from the SRMs were biphasic (plot not shown). The dissolution of beryllium from each SRM (Table 6) was faster in artificial sweat buffered to pH 5.3 than in sweat buffered to pH 6.5 ($P < 0.05$). Between the two chip materials, SRM 458 was more soluble than SRM 459. Initially, dissolution of copper was higher in artificial sweat with pH 5.3. At the end of 8 h, the masses of copper dissolved at pH 5.3 and pH 6.5 were 0.01% (1500 μg) and 0.01% (1326 μg)

for SRM 458; 0.01% (850 μg) and 0.003% (312 μg) for SRM 459; and 0.001% (930 μg) and 0.0003% (400 μg) for SRM C1122. However, by the end of the 7-day study, the total fractions of copper dissolved were higher in artificial sweat with pH 6.5: 0.13% (1300 μg) and 0.33% (3240 μg) for SRM 458; 0.10% (980 μg) and 0.17% (1670 μg) for SRM 459; and 0.10% (10 200 μg) and 0.17% (16 300 μg) for SRM C1122.

Dissolution of tools

By 7 days, the tool surfaces immersed in artificial sweat turned a dull brownish color (Fig. 4a) and a green precipitate adhered to the surfaces of the tools and the sample container (Fig. 4). The precipitates indicate that our dissolution data likely underestimate the actual masses of copper and beryllium released. Masses of beryllium released from the one pair of pliers were 15 μg (1 h), 56 μg (8 h), and 610 μg (7 days). Masses of beryllium released from the one pipe wrench were similar: 24 μg (1 h), 61 μg (8 h), and 668 μg (7 days). Initial masses of beryllium released from the two hammers into artificial sweat were similar to the other tools, 15 and 30 μg (1 h) and 50 and 150 μg (8 h). However, the total measured mass of beryllium released in 7 days from the two hammers, 1600 and 2660 μg, were much higher. The mass of dissolved copper totaled *ca.* 11 000 μg for each tool during the first 72 h of the study.
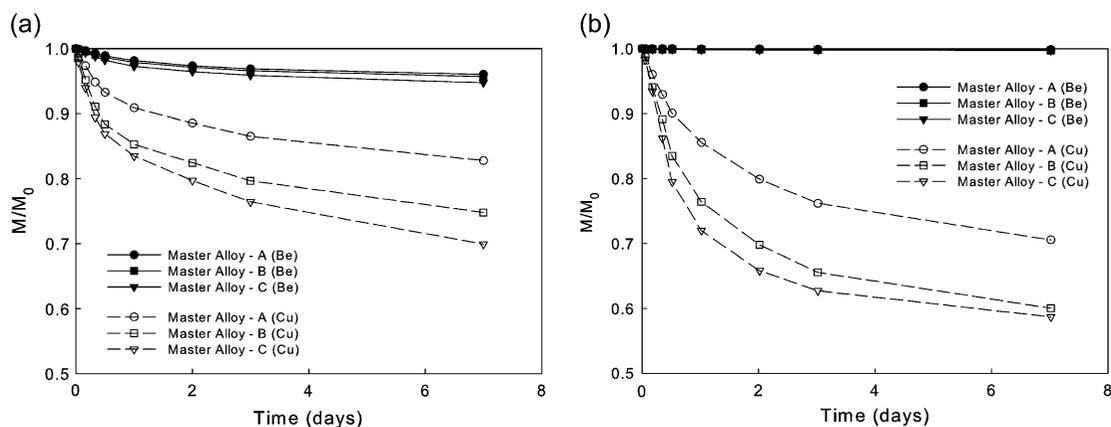
Fig. 3. Dissolution kinetics of beryllium and copper from master alloy arc furnace particles, illustrating biphasic behavior in artificial sweat buffered to (a) pH 5.3 and (b) pH 6.5. Dissolution of beryllium was increased at the lower sweat pH, whereas dissolution of copper was decreased at the lower sweat pH.

Table 6. Cumulative percentage of beryllium released from SRMs in artificial sweat

Time (h)	Average ± SD					
	SRM 458		SRM 459		SRM C1122	
	pH 5.3	pH 6.5	pH 5.3	pH 6.5	pH 5.3	pH 6.5
1	0.002 ± 0.001	0.000 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	$0.9 ± 0.8 × 10^{-6}$
4	0.008 ± 0.005	0.001 ± 0.001	0.004 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	$3.6 ± 1.0 × 10^{-6}$
8	0.02 ± 0.01	0.002 ± 0.001	0.011 ± 0.002	0.000 ± 0.000	0.001 ± 0.000	$7.4 ± 2.8 × 10^{-6}$
12	0.03 ± 0.00	0.003 ± 0.001	0.019 ± 0.002	0.000 ± 0.000	0.001 ± 0.000	$1.1 ± 0.4 × 10^{-5}$
24	0.07 ± 0.00	0.005 ± 0.002	0.042 ± 0.003	0.001 ± 0.000	0.003 ± 0.000	$3.8 ± 1.8 × 10^{-5}$
48	0.14 ± 0.00	0.009 ± 0.003	0.073 ± 0.004	0.001 ± 0.001	0.005 ± 0.000	$8.3 ± 3.9 × 10^{-5}$
72	0.21 ± 0.00	0.012 ± 0.003	0.11 ± 0.00	0.002 ± 0.001	0.007 ± 0.000	$1.4 ± 0.6 × 10^{-4}$
168	0.40 ± 0.01	0.018 ± 0.014	0.19 ± 0.00	0.004 ± 0.001	0.012 ± 0.000	$2.7 ± 0.9 × 10^{-4}$
M_0 (mg)[a]	37.15 ± 1.46	36.13 ± 0.08	182.79 ± 0.45	182.66 ± 0.63	2798.93 ± 4.55	2803.71 ± 7.49

[a]M_0 = mass of beryllium at time = 0.

DISCUSSION

Beryllium dissolution rates

In vitro dissolution data for beryllium-containing materials provide information on their relative bio-accessibility (amount dissolved and available for absorption). Water-soluble forms of beryllium are known to dissolve on the skin surface (Curtis, 1951; Zissu *et al.*, 1996). Historically, exposure research has focused on dissolution of inhaled beryllium particles because of adverse health effects to the lung such as chronic beryllium disease while less emphasis was placed on dermal exposure. As summarized in Table 7, we observed that particulate forms of beryllium dissolved in artificial sweat at rates that were similar to or faster than corresponding rates reported in lung fluids (Finch *et al.*, 1988,

1990; Day *et al.*, 2005; Stefaniak *et al.*, 2006). While these relative dissolution rates provide perspective, it must be noted that similar dissolution rates among biological compartments do not translate directly into similar bioavailability (amount of dissolved material that can cross a biological barrier to reach a target organ or site) and potential for interaction with the immune system.

The high solubility we observed for beryllium from reduction furnace particles in artificial sweat may be due to rapid dissolution of nanoscale BeO primary particles and the water-soluble nature of beryllium fluoride. In a case study, Cummings *et al.* (2009) reported on a beryllium production plant worker who developed a skin rash ~2 weeks after beginning work as a reduction furnace operator, which suggests the rash was immune mediated. He
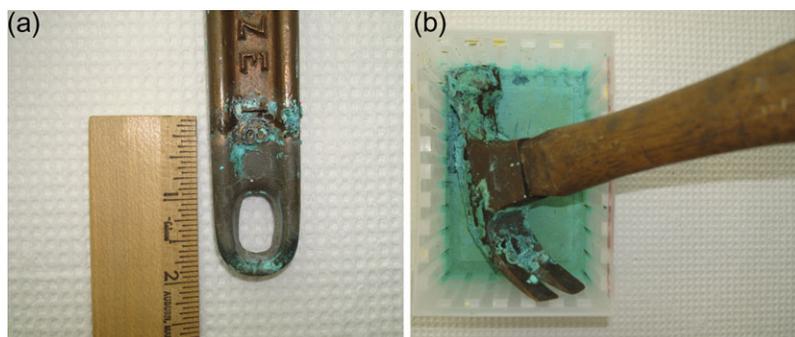
Fig. 4. Photographs of copper–beryllium alloy tools after immersion in artificial sweat buffered to pH 5.3 for 7 days. (a) Visible discoloration of a wrench handle and presence of green precipitate after exposure to the solvent. (b) Substantial masses of residual green precipitate adhered to the head of a claw hammer and the sample container after completion of the experiment.

Table 7. Chemical dissolution rate constant values (k) for various beryllium compounds

Powder	Chemical dissolution rate constant g $(cm^2\ day)^{-1}$				
	Sweat (pH 5.3)[a]	PSF (pH 4.5)[b]	SUF (pH 7.4)[c]	J774A.1 cell[d]	Beagle dogs[e]
$Be(OH)_2$	1.7×10^{-7}	1.3×10^{-7}	—[f]	—	—
Be	1.7×10^{-8}	1.1×10^{-7}	1.5×10^{-9}	—	—
BeO	1.0×10^{-9}	1.1×10^{-8}	3.7×10^{-9}	2.3×10^{-8}	0.7×10^{-8}

[a]Current study; value for BeO is average of all seven BeO materials.
[b]PSF = alveolar macrophage phagolysosomal stimulant fluid; values for Be and BeO (Stefaniak *et al.* 2006) and value for $Be(OH)_2$ (AB, Stefaniak, unpublished data).
[c]SUF = serum ultrafiltrate, a model airway epithelial lining fluid; values from Finch *et al.* (1988).
[d]Murine J774A.1 monocyte–macrophage cell line (Day *et al.* 2005).
[e]Finch *et al.* (1990).
[f]Material not evaluated for this compound in this solvent model.

was placed on medical leave, but the rash recurred when the employee returned to work in the metal production department. The worker continued in metal production, a high-risk process for BeS (Kreiss *et al.*, 1997), for 10 months until being placed on medical leave. One month later, he was determined by a blood beryllium lymphocyte proliferation test to be sensitized to beryllium; it is unknown whether sensitization was due to inhalation exposure, dermal exposure, or a combination of these pathways.

BeS has also been reported among workers who form or machine parts made from beryllium metal powder (Stange *et al.*, 1996). Although the extent of dermal exposure in these workers has not been documented, our observation that the rate of dissolution of beryllium metal powder was intermediate among the materials we tested suggests that it would be plausible that skin exposure could account for BeS reported among these workers.

Production of BeO powder (Kreiss *et al.*, 1997) and machining sintered parts at a ceramics facility are other processes associated with high risk of

BeS (Kreiss *et al.*, 1996; Henneberger *et al.*, 2001). In contrast, handling and pressing presintered BeO powder have not been associated with high risk of BeS (Kreiss *et al.*, 1996). The BeO particles encountered during primary production, handling, and pressing are branched-chain aggregates of primary particles. Each primary particle is a single BeO crystal. During sintering, the individual primary particles fuse together to form larger particles composed of many BeO crystals. We did not observe any difference in solubility between presintered and postsintered BeO particles in artificial sweat that would explain the reported differences in BeS risk; whether BeO crystallinity influences dissolution in lung fluids is unknown.

Zissu *et al.* (1996) reported that challenge with CuBe alloy elicited a delayed skin hypersensitivity reaction in guinea pigs that had been previously sensitized to beryllium. Schuler *et al.* (2005) reported elevated prevalence of BeS among workers in alloy finishing, despite air exposure levels historically well below the 2 μg m^{-3} occupational exposure limit. In our study, master alloy arc furnace particles,

SRMs, and tools released beryllium in artificial sweat, which suggests that skin contact with CuBe alloys may result in bioaccessible beryllium on skin. However, the beryllium dissolution data for the hammers should be interpreted with caution because we continuously immersed the heads in sweat whereas intermittent handling is more likely in the work environment. A simple modification to the handles of the pliers and pipe wrench such as coating the handles in plastic would help to avoid skin contact with beryllium. SRM 458 had lower beryllium content than SRM 459 but released twice as much beryllium in artificial sweat, which indicates that beryllium content of an alloy material may not be a useful predictor of dermal exposure potential. While the observed dissolution pattern was not predicted by beryllium content, release rates were consistent with the higher surface area of SRM 458 (0.012 m^2 g^{-1}) compared to SRM 459 (0.003 m^2 g^{-1}). Release rate is known to be more important than content for development of allergic contact dermatitis to metals such as nickel (Lidén *et al.*, 1996).

In our study, Be(OH)$_2$, beryllium metal, BeO, and CuBe alloys released tens to thousands of micrograms of beryllium in artificial sweat over a 7-day period. Our observations support the hypothesis that release of beryllium ions from particulate beryllium compounds in contact with the skin surface may be a biologically plausible exposure pathway for inducing BeS. In workplaces where beryllium-containing materials are handled or processed, efforts should be made to prevent skin contact and to maintain clean skin while at work and upon leaving work. Additionally, given the difference in dissolution between the two pH values we evaluated, we hypothesize that skin surface pH may be an exposure-modifying factor in the development of BeS. Other physiological exposure-modifying factors of concern for workers exposed to metal sensitizers include skin hydration and barrier integrity (Du Plessis *et al.*, 2010).

The observed influence of artificial sweat pH on dissolution of beryllium is consistent with studies, which have shown that the dissolution rate of nickel decreases as pH increases from 3.5 to 6.5 (Hemingway and Molokhia, 1987). Bocca *et al.* (2007) reported that 10 years after the introduction of a regulatory limit for release of nickel from consumer products, assessed using the Pedersen sweat formulation (pH 6.5) as specified in the European reference test method EN1811 (CEN, 1998), the frequency of nickel allergy due to earrings did not decrease in Italy. Among healthy adults, skin pH is generally 4.2–6.1, with median of pH 5.3 (Stefaniak and Harvey, 2006). Thus, use of an artificial sweat

solvent with pH >6 for risk assessment, as is done under the European method, could result in underestimation of metal dissolution rates. However, other factors such as delays to implement the regulatory release limit for nickel in Italy could also explain the persistence of nickel allergic dermatitis (Rui *et al.*, 2010).

Implications for exposure

Particulate beryllium compounds that contact the skin surface may undergo dissolution in sweat (Fig. 1, Step 1). No information is available regarding diffusion of beryllium across the SC, though absorption of divalent metal sensitizers such as cobalt and nickel ranged from 0.001 to 0.15% per hour for intact and abraded skin, respectively (Larese-Filon *et al.*, 2009). For a hypothetical 1-h skin exposure to reduction furnace particles totaling 50 µg of beryllium, 11.6% is likely to be dissolved (see Table 5) and 50% of the dissolved beryllium is likely to be in the form of Be^{2+} (Sutton and Burastero, 2003), leading to an estimate that 30 pg of beryllium could be absorbed across intact SC (M$_0$ Be $\times$ F$_{dissolved}$ $\times$ F$_{Be^{+2}}$ $\times$ F$_{absorbed}$ $\times$ Conversion Factor): $50\mu g \times 0.116 \times 0.50 \times 0.00001 \times 10^6 \frac{pg}{\mu g} = 30$ pg.

For abraded skin, the estimated mass of beryllium absorbed would be 4350 pg. Assuming the form of beryllium to which workers were exposed in the studies by Day *et al.* (2007), Emond *et al.* (2007), and Dufresne *et al.* (2009) was metal, a skin loading mass of 43 µg beryllium on two hands (Day *et al.*, 2007), applying $k = 1.7 \times 10^{-8}$ g (cm^2 day)$^{-1}$ and specific surface area of 5.6 m^2 g^{-1} (Table 3), would result in 0.01 pg and 1 pg Be^{+2} passing through intact and abraded skin SC, respectively, in 1 h. For BeO, a skin exposure to 46 µg beryllium on two hands (Cummings *et al.*, 2007) would result in 0.001 and 0.2 pg Be^{+2} passing through the SC of intact and abraded skin, respectively, in 1 h (Fig. 1, Step 2). While this range of masses, 0.001–4350 pg Be^{+2}, is seemingly small, these values are based on exposure to hands only and may underestimate historical exposures in workplaces where other parts of the skin were exposed. The estimated masses of beryllium that could penetrate the SC are equivalent to 10^7–10^{14} Be^{+2} ions available to form hapten complexes in the epidermis (Fig. 1, Step 3).

Each antigen-presenting cell has up to 400 antigen–MHC complexes on its surface (Davis *et al.*, 2003). Thus, the numbers of Be^{+2} ions that could reach the viable epidermis may yield 10^5–10^{11} LCs displaying the maximum number of antigen–MHC complexes per cell surface (assuming one Be^{+2} ion per antigen and one antigen per MHC molecule).

Each antigen-presenting cell may contact up to 5000 T cells per hour (Dustin, 2008), which translates to 10^8–10^{15} antigen–MHC/T-cell interactions per hour (Fig. 1, Steps 4 and 5). Proliferation of T cells in response to an unknown amount of presented antigen can initiate BeS (Fig. 1, Step 6).

Though the amount of beryllium needed to initiate BeS via skin exposure is unknown, data from human patch testing provides clues to levels sufficient to elicit dermatitis in persons with BeS. Curtis (1951) patch tested 13 workers with BeS and reported that a weak positive reaction was elicited from 38% (5/13) of patients exposed to aqueous beryllium fluoride and 23% (3/13) of patients exposed to beryllium metal powder; no response was observed from patients exposed to beryllium oxide powder. At the time of this historical study, protocols for patch testing were not standardized, precluding calculation of the actual skin exposure concentration. More recently, Fontenot et al. (2002) patch tested three workers with BeS and reported that a strong positive dermatitis reaction was elicited from 100% (3/3) patients exposed to soluble beryllium sulfate. In this study, patients were tested in accordance with a standard protocol for 48 h using the equivalent of 0.026 µg Be cm^{-2} skin (assuming an 8-mm chamber for applying the test agent). Using the dissolution rate data from our penetration calculations (see above), after 48 h, the concentrations of dissolved beryllium on two hands having total surface area of 405 cm^2 (Agache, 2004) would be 0.030, 0.001, and 0.00001 µg Be cm^{-2} for reduction furnace, beryllium metal, and beryllium oxide powders, respectively. The concentration of beryllium capable of eliciting dermatitis is consistent with the calculated beryllium exposure concentration for reduction furnace particles and observations from a case report of reoccurring skin rash in a worker exposed to this material (Cummings et al., 2009). The elicitation concentration is higher than the calculated exposure concentrations for beryllium metal and beryllium oxide, which is consistent with Curtis (1951) who reported that 23% of patients patch tested with beryllium metal powder had a positive response and none responded to beryllium oxide powder.

In conclusion, beryllium was released from beryllium-containing materials that were immersed in artificial sweat, indicating a plausible exposure pathway for inducing BeS via skin exposure. Measured beryllium dissolution rates were pH-dependent, with more material dissolving in artificial sweat buffered to pH 5.3 than in artificial sweat buffered to pH 6.5. Dissolution rates were similar to or faster than rates previously reported for beryllium materials in artificial lung fluid models. These data support efforts to prevent skin contact with beryllium-containing materials and to promote good skin hygiene practices, such as hand washing and showering, in workplaces where such materials are handled and/or processed.

REFERENCES

Agache P. (2004) Presentation of the skin surface microsystem. In Agache P and Humbert P, editors. Measuring the skin. Berlin, Germany: Springer-Verlag; pp. 21–32.

Arts JH, Mommers C, de Heer C. (2006) Dose-response relationships and threshold levels in skin and respiratory allergy. Crit Rev Toxicol; 36: 219–5.

Baroli B. (2010) Penetration of nanoparticles and nanomaterials in the skin: fiction or reality? J Pharm Sci; 99: 21–50.

Bocca B, Forte G, Senofonte O et al. (2007) A pilot study on the content and the release of Ni and other allergenic metals from cheap earrings available on the Italian market. Sci Total Environ; 388: 24–34.

CEN. (1998) EN1811: 1998. Reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin. London, UK: BSI.

Cummings KJ, Deubner DC, Day GA et al. (2007) Enhanced preventive programme at a beryllium oxide ceramics facility reduces beryllium sensitisation among new workers. Occup Environ Med; 64: 134–40.

Cummings KJ, Stefaniak AB, Virji MA et al. (2009) Acute beryllium disease: reconsideration of a historical entity. Environ Health Perspect; 117: 1250–6.

Curtis GH. (1951) Cutaneous hypersensitivity due to beryllium: a study of thirteen cases. Arch Derm Syphilol; 64: 470–82.

Davis MM, Krosgaard M, Huppa JB et al. (2003) Dynamics of cell surface molecules during T cell recognition. Annu Rev Biochem; 72: 717–42.

Day GA, Dufresne A, Stefaniak AB et al. (2007) Exposure pathway assessment at a copper-beryllium alloy facility. Ann Occup Hyg; 51: 67–80.

Day GA, Hoover MD, Stefaniak AB et al. (2005) Intracellular behavior of beryllium oxide particles: an in vitro study in murine macrophages. Exp Lung Res; 31: 341–60.

Day GA, Stefaniak AB, Weston A *et al.* (2006) Beryllium exposure: dermal and immunological considerations. Int Arch Occup Environ Health; 79: 161–4.

Deubner D, Kelsh M, Shum M *et al.* (2001) Beryllium sensitization, chronic beryllium disease, and exposures at a beryllium mining and extraction facility. Appl Occup Environ Hyg; 16: 579–92.

Dufresne A, Dion C, Viau S *et al.* (2009) Beryllium decontamination with different solvents on different structures. Ann Occup Hyg; 53: 669–75.

Du Plessis JL, Eloff FC, Badenhorst CJ *et al.* (2010) Assessment of dermal exposure and skin condition of workers exposed to nickel at a South African base metal refinery. Ann Occup Hyg; 54: 23–30.

Dustin ML. (2008) T-cell activation through immunological synapses and kinapses. Immunol Rev; 221: 77–89.

Emond C, Robin JP, Breton R *et al.* (2007) Dermal exposure to beryllium: a pilot case study. J Toxicol Environ Health Part A; 70: 529–33.

Finch GL, Mewhinney JA, Eidson AF *et al.* (1988) In vitro dissolution characteristics of beryllium oxide and beryllium metal aerosols. J Aerosol Sci; 19: 333–42.

Finch GL, Mewhinney JA, Hoover MD *et al.* (1990) Clearance, translocation and excretion of beryllium following acute inhalation of beryllium oxide by beagle dogs. Fundam Appl Toxicol; 15: 231–41.

Flint GN. (1998) A metallurgical approach to metal contact dermatitis. Contact Derm; 39: 213–21.

Fontenot AP, Maier LA, Canavera SJ *et al.* (2002) Beryllium skin patch testing to analyze T cell stimulation and granulomatous inflammation in the lung. J Immunol; 168: 3627–34.

Harvey CJ, LeBouf RF, Stefaniak AB. (2010) Formulation and stability of a novel artificial human sweat under conditions of storage and use. Toxicol In Vitro; doi: 10.1016/j.tiv.2010.06.016.

Hemingway JD, Molokhia MM. (1987) The dissolution of metallic nickel in artificial sweat. Contact Derm; 16: 99–105.

Henneberger PK, Cumro D, Deubner DD *et al.* (2001) Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. Int Arch Occup Environ Health; 74: 167–76.

Hoover MD, Castorina BT, Finch GL *et al.* (1989) Determination of the oxide layer thickness on beryllium metal particles. Am Ind Hyg Assoc J; 50: 550–3.

Kreiss K, Mroz MM, Newman LS *et al.* (1996) Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m^3. Am J Ind Med; 30: 16–25.

Kreiss K, Mroz M, Zhen B *et al.* (1997) Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. Occup Environ Med; 54: 605–12.

Larese-Filon F, D'Agostin F, Crosera M *et al.* (2009) In vitro absorption of metal powders through intact and damaged skin. Toxicol In Vitro; 23: 574–9.

Lidén C, Menné T, Burrows D. (1996) Nickel-containing alloys and plating and their ability to cause dermatitis. Br J Dermatol; 134: 193–8.

NIOSH. (2003) Elements by ICP: method 7303. Available at http://www.cdc.gov/niosh/nmam/pdfs/7303.pdf. NIOSH Manual of Analytical Methods (NMAM®). 4th edn. 3rd supplement. Cincinnati, OH: National Institute for Occupational Safety and Health. Accessed 21 March, 2010.

Pedersen NB, Fregert S, Brodelius P *et al.* (1974) Release of nickel from silver coins. Acta Derm Venereol (Stockholm); 54: 231–4.

Rui F, Bovenzi M, Prodi A *et al.* (2010) Nickel, cobalt and chromate sensitization and occupation. Contact Derm; 62: 225–31.

Schuler CR, Kent MS, Deubner DC *et al.* (2005) Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. Am J Ind Med; 47: 195–205.

Stange AW, Hilmas DE, Furman FJ. (1996) Possible health risks from low level exposure to beryllium. Toxicology; 111: 213–24.

Stefaniak AB, Chipera SJ, Day GA *et al.* (2008a) Physicochemical characteristics of aerosol particles generated during the milling of beryllium silicate ores: implications for risk assessment. J Toxicol Environ Health Part A; 71: 1468–81.

Stefaniak AB, Day GA, Hoover MD *et al.* (2006) Differences in dissolution behavior in a phagolysosomal simulant fluid for single-component and multi-component materials associated with beryllium sensitization and chronic beryllium disease. Toxicol In Vitro; 20: 82–95.

Stefaniak AB, Guilmette RA, Day GA *et al.* (2005) Characterization of phagolysosomal fluid for study of beryllium aerosol particle dissolution. Toxicol In Vitro; 19: 123–34.

Stefaniak AB, Harvey CJ. (2006) Dissolution of materials in artificial skin surface film liquids. Toxicol In Vitro; 20: 1265–83.

Stefaniak AB, Harvey CJ, Wertz PW. (2010a) Formulation and stability of a novel artificial sebum under conditions of storage and use. Int J Cosmet Sci; doi: 10.1111/j.1468-2494.2010.00561.x.

Stefaniak AB, Hoover MD, Day GA *et al.* (2004) Characterization of physicochemical properties of beryllium aerosols associated with prevalence of chronic beryllium disease. J Environ Monit; 6: 523–32.

Stefaniak AB, Hoover MD, Dickerson RM *et al.* (2003) 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.

Stefaniak AB, Turk GC, Dickerson RM *et al.* (2008b) Size-selective poorly soluble particulate reference materials for evaluation of quantitative analytical methods. Anal Bioanal Chem; 391: 2071–7.

Stefaniak AB, Virji MA, Harvey CJ *et al.* (2010b) Influence of artificial gastric juice composition on bioaccessibility of cobalt- and tungsten-containing powders. Int J Hyg Environ Health; 213: 107–15.

Sutton M, Burastero SR. (2003) Beryllium chemical speciation in elemental human biological fluids. Chem Res Toxicol; 16: 1145–54.

Tinkle SS, Antonini JM, Rich BA *et al.* (2003) Skin as a route of exposure and sensitization in chronic beryllium disease. Environ Health Perspect; 111: 1202–8.

Toebak MJ, Gibbs S, Bruynzeel DP *et al.* (2009) Dendritic cells: biology of the skin. Contact Derm; 60: 2–20.

Zissu D, Binet S, Cavelier C. (1996) Patch testing with beryllium alloy samples in guinea pigs. Contact Derm; 34: 196–200.