

# Dissolution of cemented carbide powders in artificial sweat: implications for cobalt sensitization and contact dermatitis†

Aleksandr B. Stefaniak,\* Christopher J. Harvey, M. Abbas Virji and Gregory A. Day

Received 4th June 2010, Accepted 3rd August 2010

DOI: 10.1039/c0em00269k

Skin exposure to cobalt-containing materials can cause systemic immune sensitization and upon repeat contact, elicitation of allergic contact dermatitis (ACD). Data on cobalt dissolution rates are needed to calculate uptake through skin and for development of models to understand risk of sensitization or dermatitis. The purpose of this research was to measure the dissolution kinetics of feedstock and process-sampled powders encountered in the production of hard metal alloys using artificial sweat. The physicochemical properties of each material were characterized prior to evaluation of dissolution behavior. Variations in artificial sweat solvent pH and chemistry were used to understand critical factors in dissolution. Dissolution of cobalt, tungsten, and tungsten carbide was often biphasic with the initial rapid phase being up to three orders of magnitude faster than the latter long-term phase. Artificial sweat pH did not influence dissolution of cobalt or tungsten carbide. Solvent composition had little influence on observed dissolution rates; however, vitamin E suppressed the dissolution of cobalt and tungsten carbide from sintered particles obtained from a chamfer grinder. There was no effect of particle size on dissolution of feedstock cobalt, tungsten, tungsten carbide, and admixture powders. Particle physicochemical properties influenced observed dissolution rates with more cobalt and tungsten carbide dissolving from chamfer grinder particles compared to the feedstock powders or admixture powder. Calculations using the observed dissolution rates revealed that skin exposure concentrations were similar to concentrations known to induce cobalt sensitization and elicit ACD. Observed dissolution rates for cobalt in artificial sweat indicate that dermal uptake may be sufficient to induce cobalt sensitization and allergic dermatitis.

## Introduction

Cemented tungsten carbides or “hard metals” are refractory, wear resistant alloy materials composed of tungsten carbide (WC) particles embedded in a cobalt (Co) matrix. During the manufacture and handling of cemented carbides and other cobalt-containing products, workers’ hands may become contaminated with Co, which is difficult to remove, even with hand washing.<sup>1–4</sup> Skin exposure to Co is associated with development of sensitization and allergic contact dermatitis.<sup>5–8</sup> Life-

long avoidance of the offending agent is often necessary to prevent elicitation of allergic contact dermatitis (ACD).<sup>9</sup> As such, people with cobalt-induced ACD are often forced to change jobs to avoid exposure, which may impact income-earning potential and quality of life.

Uptake is regarded as the ultimate metric of dermal exposure.<sup>10</sup> To understand uptake through intact skin requires knowledge of: 1) the mass of contaminant in contact with an area of the skin surface, 2) the rate at which the contaminant dissolves on the skin surface, and 3) the rate at which solubilized ions permeate through the skin. Day *et al.*<sup>3</sup> determined that hands and necks of workers engaged in the manufacture of cemented carbides were heavily contaminated with Co after just a few hours of work. Field studies confirm that skin becomes contaminated with cobalt during work.<sup>1,3,4</sup> Elevated cobalt levels have been measured in urine of workers exposed through the

National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Mailstop H-2800, Morgantown, WV, 26505. E-mail: AStefaniak@cdc.gov; Fax: +304.285.6321; Tel: +304.285.6302

† Competing interests: two authors (ABS and CJH) are co-applicants on a pending U.S. patent of the skin surface film liquids formulation, though no commercial benefit is being received from the patent.

## Environmental impact

Skin exposure to metal allergens may cause systemic sensitization and upon subsequent exposure to the same metal, elicit allergic contact dermatitis (ACD). Dermal exposures to allergenic metals are assessed without knowledge of uptake through skin which precludes meaningful risk assessment. Our goal was to measure the dissolution kinetics of cobalt, a known dermal sensitizer, in artificial sweat to bridge the gap between exposure and dose. The dissolution kinetic data indicate that cobalt particulate is oxidized in artificial sweat and initially releases cobalt ions very rapidly. These kinetic data, in conjunction with published estimates of cobalt skin loading and permeation rates, enabled calculation of cobalt uptake through skin and risk of ACD. Calculated uptake levels were comparable to levels sufficient to elicit ACD.

dermal and inhalation routes.<sup>1,11–14</sup> Recent in vitro laboratory studies using cobalt powder suspended in artificial sweat and applied to the surface of human skin have provided estimates for cobalt permeation rates. Cobalt was observed to permeate intact skin and rates were significantly increased if skin was damaged by scratching the stratum corneum.<sup>15–17</sup> However, the kinetics and factors that influence dissolution of cobalt compounds on the skin surface are not well understood, making it difficult to accurately model uptake, develop risk assessments, and derive occupational dermal exposure limits.

The purpose of this study was to investigate the dissolution of particulate Co compounds in artificial skin surface film liquids. Specifically, we 1) evaluated the influence of solvent properties (chemical composition, pH) and particle physicochemical properties (surface chemistry, bulk homogeneity and heterogeneity) on dissolution, and 2) evaluated dissolution kinetics and derived chemical dissolution rate constants for powders encountered during the production of cemented carbides.

## Materials and methods

### Powder preparation and characterization

**Study powders.** Five bulk powders encountered during the manufacture of cemented carbides were chosen for study: feedstock tungsten (W), WC, and Co metal powders; pre-sintered powder from a spray dryer; and dust from a chamfer grinder used for final machining of post-sintered product.<sup>18</sup> Each bulk powder was aerodynamically size-separated in the laboratory using a dry powder disperser (Model 175, DeVilbiss, Somerset, PA) and five-stage aerosol cyclone operated at 10 L min<sup>-1</sup>.<sup>19</sup> A sixth study powder was prepared as an admixture by mechanically blending aerodynamically size-separated WC and Co powders at a ratio of 94 : 6 by mass.<sup>20</sup> The admixture was prepared for the purpose of comparing dissolution of loosely-associated Co and WC particles to the individual feedstock powders and heterogeneous process-sampled powders. We evaluated solubility of the bulk feedstock powders and materials collected in stages 1, 2, and/or 3 of the aerosol cyclone, having aerodynamic sizes of >10, 4.3–10, and 2.9–4.3  $\mu\text{m}$ , respectively. These large particles were chosen for study because they were most likely to deposit on surfaces or skin.

**Static dissolution technique.** Dissolution of study powders was evaluated in two solvents: 1) artificial skin surface film liquids (SSFL) consisting of sweat and sebum with composition that closely approximated human sweat, and 2) a widely used sweat solvent developed by Pedersen *et al.*<sup>21</sup> For each dissolution experiment, triplicate samples were prepared for each bulk or size fraction of each study powder.

The artificial human sweat component of SSFL (U.S. Patent Pending) contained seven electrolytes, 13 ionic constituents, nine organic acids and carbohydrates, 16 amino acids, five nitrogenous substances, and 11 vitamins.<sup>22</sup> The sweat solution pH was adjusted to the desired value (see below) by addition of 0.1N sodium hydroxide. The artificial human sebum component (U.S. Patent Pending) was prepared by dissolving lipids in a 2 : 1 co-solvent mixture of chloroform and methanol as described previously.<sup>23</sup>

The formulation developed by Pedersen *et al.*<sup>21</sup> is specified in EN 1811 : 1998 – Reference Test Method for Release of Nickel from Products Intended to come into Direct and Prolonged Contact with the Skin.<sup>24</sup> The sweat formulation consists of sodium chloride (0.5% M/M), lactic acid (0.1% M/M), and urea (0.1% M/M) buffered to pH 6.5 using ammonium hydroxide.

Powder dissolution in artificial sweat was evaluated using the well-established static dissolution techniques as described previously.<sup>25</sup> Briefly, a known mass of each study powder was weighed (Model XS205, Mettler-Toledo, Greifensee, Switzerland) onto a pre-weighed sebum-coated filter that rested on top of a 0.025  $\mu\text{m}$  pore size 47 mm diameter nitrocellulose filter. The material was covered with a second 47 mm filter to form a “sandwich” which was secured tightly in a static dissolution chamber (InTox Products, Moriarity, NM). Each chamber was placed in a polypropylene plastic beaker and immersed in 80 mL of artificial sweat solvent and maintained at 36.3 °C in a water-jacketed incubator (Model 460, Barnstead International, Dubuque, IA) to mimic human skin temperature. At each pre-designated time point (see below), the chamber was removed from its beaker, the fluid was transferred to a borosilicate glass jar, and fresh equilibrated fluid was added to the sample beaker before being returned to the incubator.

**Bulk powder experiments.** Bulk feedstock W, WC, and Co powder materials were used to investigate the influence of the following solvent factors on dissolution: 1) pH, 2) presence or absence of glycine, 3) presence or absence of phosphate, and 4) overall chemical composition. Bulk feedstock powders were used for these studies to permit investigation of solvent factors on dissolution of individual hard metal chemical constituents. To evaluate the influence of solvent pH, dissolution of bulk feedstock W, WC, and Co powders were evaluated using sweat solvent buffered to pH 5.3, 5.9, and 6.5. The pH value of 5.3 was chosen to match the median for whole body sweat,<sup>22</sup> the pH value of 6.5 was chosen to facilitate comparison of data to the Pedersen *et al.*<sup>21</sup> formulation, and the pH level of 5.9 was chosen as an intermediate value. Takahashi and Koshi<sup>26</sup> report that glycine may influence dissolution of Co. Lison and Lauwerys<sup>27</sup> report that phosphate may react with solubilized Co to form a very insoluble salt, cobalt phosphate [Co<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>]. Thus, dissolution of bulk feedstock Co powder were evaluated in the novel artificial SSFL (buffered to pH 5.3) with and without glycine or phosphate. The influence of overall solvent composition was evaluated by comparing dissolution in SSFL (buffered to pH 6.5) to the Pedersen *et al.*<sup>21</sup> formulation (pH 6.5).

Pilot studies were performed to evaluate the shapes of dissolution curves; solvents were changed at 1, 4, 8, 12, 16, 20, 24, 32, 40, and 48 h. Preliminary results indicated that Co dissolution was biphasic while W and WC dissolution consisted of a single phase. As such, for the full-scale studies we used a reduced number of solvent changes and increased duration of time between changes, *i.e.*, 1, 3, 5, 8, 12, 24, 48, and 72 h.

**Size-separated powder experiments.** The study powders can generate hydroxyl radicals in artificial sweat.<sup>19</sup> As such, large (>10  $\mu\text{m}$ ) sizes of the six study powders were used to investigate whether vitamin E, an antioxidant component of sebum, depressed material dissolution in SSFL. To investigate effects of

particle size, each size fraction of feedstock powder was used to estimate material-specific dissolution rates for comparison with corresponding values estimated from the bulk powders. All six study powders were used to elucidate the influence, if any, of particle composition on dissolution. For these studies, SSFL buffered to pH 5.3 was changed at 1, 3, 5, 8, 12, 24, 48, and 72 h.

### Cobalt and tungsten analyses and quality control

All experimental and quality control samples were analyzed by US Occupational Safety and Health Administration (OSHA) Method ID-213: Tungsten and cobalt in workplace atmospheres (Inductively Coupled Plasma-Atomic Emission Spectroscopy). Artificial SSFL liquid samples, which contained dissolved Co and/or W, were analyzed without digestion to quantify the mass of dissolved metals ( $M_D$ ). The limits of detection (LOD) and quantification (LOQ) for Co were 0.6 and 2 mg/L (SSFL formulation) and 0.3 and 0.83 mg/L (Pedersen formulation). For W, the LOD and LOQ values were 0.3 and 1.1 mg/L (SSFL formulation) and 0.3 and 1 mg/L (Pedersen formulation). Filter samples containing masses of undissolved (M) particulate Co and/or W were digested prior to analysis; the LOD and LOQ values were 0.08 and 0.25  $\mu\text{g}/\text{sample}$  for Co and 0.4 and 1.3  $\mu\text{g}/\text{sample}$  for W.

### Statistical analyses

All statistical analyses were performed using SAS<sup>®</sup> version 9.1 software (SAS Institute, Cary, NC) using a surface-area-limited dissolution model (Stefaniak *et al.*, 2010).<sup>25</sup> Briefly, values of the mass fraction of material remaining ( $M/M_0$ ), which is the ratio of undissolved metal mass to initial metal mass, were plotted *versus* time (t) in sweat for each sample. Single- or multiple-component negative exponential functions were fitted to the plotted data to obtain estimates of  $f_i$ , the fraction of material dissolved in each dissolution component, and  $\lambda_i$  from which  $t_{1/2}$ , the dissolution half-time ( $0.693/\lambda_i$ ), and  $k_i$ , the chemical dissolution rate constant, ( $-\lambda_i/\text{SSA}$ ) were calculated for each sample.<sup>28</sup>

Functions were fitted using non-linear regression models (proc NLIN) in SAS with the optimum number of components determined using model convergence and the F-ratio test.<sup>25</sup> One-way analysis of variance (ANOVA) models were then developed to investigate the impact of the fixed effects of solvent composition and industrial bulk and size separated powders on the dissolution parameters ( $f_i$ ,  $t_{1/2}$ , and  $k_i$ ) for Co, W, and WC using the GLM procedure in SAS. Specifically, the ANOVA models were used to investigate the fixed effects of: 1) solvent composition and pH on dissolution of bulk feedstock Co, W, and WC powders; 2) process-sampled powder composition (and their differing physicochemical characteristics: bulk, admixture, chamfer grinder and spray dryer) on the dissolution of Co and WC; and 3) powder particle size on the dissolution of Co, W, and WC. Tukey's test option was specified for multiple comparisons.

## Results

The SSFL solvent pH was  $5.3 \pm 0.1$ ,  $5.9 \pm 0.1$ , or  $6.5 \pm 0.1$  for the respective experiments. For the bulk powder experiment using the Pedersen *et al.*<sup>21</sup> formulation, pH was  $6.5 \pm 0.2$ . Levels of

dissolved Co and W in reagent and field blank samples were below analytical LODs.

### Influence of solvent properties on dissolution

**Solvent pH.** Dissolution of each bulk feedstock powder (Co, W, WC) in SSFL was biphasic, meaning the data was best described by two negative exponential functions, and consisted of an initial rapid phase and a latter long-term phase for the three pH values tested. Fig. 1 is a plot of cobalt dissolution and is representative of the biphasic dissolution behavior observed for all feedstock study materials. For each feedstock material at each pH value, parameter estimates for the initial rapid dissolution phase were two to three orders of magnitude shorter ( $t_{1/2}$ ) and faster ( $k$ ) compared to the latter long-term phase (Table 1);  $p < 0.05$ . Generally, less than 20% of the dissolved material was released during the initial rapid dissolution phase with the balance released during the latter long-term phase. Dissolution of Co was generally independent of solvent pH in the range tested, the one exception being at pH 5.3 where  $t_{1/2}$  for the initial rapid phase was longer ( $p < 0.05$ ) compared to pH 5.9 and pH 6.5. For W, values of  $t_{1/2}$  and  $k$  in SSFL buffered to pH 5.9 differed from values observed at pH 5.3 or pH 6.5 ( $p < 0.05$ ). The dissolution of WC powder was independent of solvent pH over the range of tested values.

**Solvent chemical composition.** Bulk feedstock Co powder dissolved in SSFL buffered to pH 6.5 but levels were below the LOD in the Pedersen *et al.*<sup>21</sup> formulation buffered to the same pH (Table 1). Glycine or phosphate did not influence dissolution of bulk feedstock Co in SSFL. For the bulk feedstock W and WC powders, solvent chemical composition (SSFL pH 6.5 *versus* Pedersen *et al.* 1974) did not influence dissolution.

**Vitamin E.** Generally, dissolution of the size-separated powders was biphasic and consisted of a rapid initial phase and latter long-term phase; however, a few samples had only one

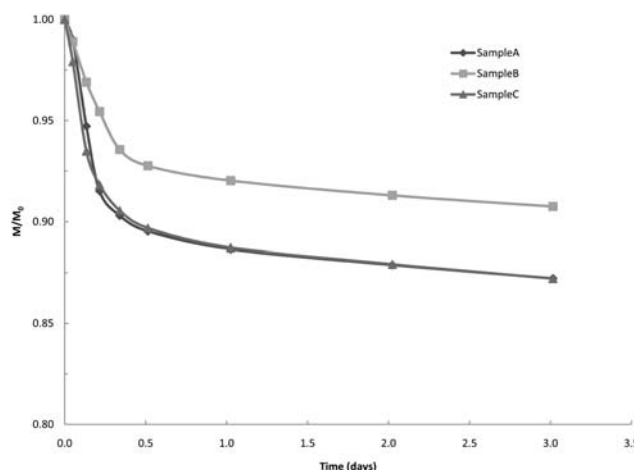


**Fig. 1** Plot of mass fraction remaining ( $M/M_0$ ) *versus* time for  $n = 3$  samples (identified in figure as A, B, and C) of cobalt metal powder in artificial skin surface film liquids (pH 5.9) illustrating biphasic dissolution behavior. Dissolution consisted of an initial rapid phase and a latter long-term phase.

**Table 1** Influence of solvent pH and composition on dissolution of bulk tungsten, tungsten carbide, and cobalt powders

| Powder <sup>b</sup> | Solvent <sup>c</sup>    | pH  | M <sub>0</sub> (mg) <sup>d</sup> | ΣM <sub>D</sub> (mg) | Dissolution function parameters (Mean ± Standard Deviation) <sup>a</sup> |                                      |                                                       |                        |                         |                                          |
|---------------------|-------------------------|-----|----------------------------------|----------------------|--------------------------------------------------------------------------|--------------------------------------|-------------------------------------------------------|------------------------|-------------------------|------------------------------------------|
|                     |                         |     |                                  |                      | Initial Rapid Phase                                                      |                                      |                                                       | Latter Long-Term Phase |                         |                                          |
|                     |                         |     |                                  |                      | f <sub>1</sub> (%)                                                       | t <sub>1/2</sub> (days) <sup>f</sup> | k <sub>1</sub> [g/(cm <sup>2</sup> day)] <sup>g</sup> | f <sub>2</sub> (%)     | t <sub>1/2</sub> (days) | k <sub>2</sub> [g/(cm <sup>2</sup> day)] |
| Co                  | SSFL                    | 5.3 | 3.17 ± 0.10                      | 0.71 ± 0.02          | 18 ± 2                                                                   | 0.21 ± 0.02                          | 4.4 ± 0.4 × 10 <sup>-5</sup>                          | 82 ± 2                 | 26.3 ± 10.5             | 4.0 ± 1.0 × 10 <sup>-7</sup>             |
|                     | SSFL                    | 5.9 | 3.30 ± 0.39                      | 0.39 ± 0.10          | 10 ± 2                                                                   | 0.14 ± 0.03                          | 7.1 ± 1.6 × 10 <sup>-5</sup>                          | 90 ± 2                 | 116.8 ± 35.2            | 0.9 ± 0.3 × 10 <sup>-7</sup>             |
|                     | SSFL                    | 6.5 | 3.36 ± 0.25                      | 0.51 ± 0.12          | 12 ± 3                                                                   | 0.15 ± 0.03                          | 6.3 ± 1.3 × 10 <sup>-5</sup>                          | 88 ± 3                 | 78.3 ± 33.1             | 1.4 ± 0.5 × 10 <sup>-7</sup>             |
|                     | SSFL no Gly             | 5.3 | 3.26 ± 0.33                      | 0.67 ± 0.08          | 19 ± 2                                                                   | 0.26 ± 0.02                          | 3.7 ± 0.2 × 10 <sup>-5</sup>                          | 81 ± 2                 | 105.2 ± 56.1            | 2.8 ± 2.5 × 10 <sup>-7</sup>             |
|                     | SSFL no PO <sub>4</sub> | 5.3 | 3.30 ± 0.29                      | 0.60 ± 0.13          | 14 ± 2                                                                   | 0.20 ± 0.03                          | 4.8 ± 0.7 × 10 <sup>-5</sup>                          | 86 ± 5                 | 34.0 ± 5.5              | 2.8 ± 0.5 × 10 <sup>-7</sup>             |
| W                   | Pedersen                | 6.5 | 3.30 ± 0.24                      | ND <sup>e</sup>      | ND                                                                       | ND                                   | ND                                                    | ND                     | ND                      | ND                                       |
|                     | SSFL                    | 5.3 | 46.47 ± 0.35                     | 4.71 ± 0.30          | 1 ± 0                                                                    | 0.22 ± 0.03                          | 4.7 ± 0.6 × 10 <sup>-4</sup>                          | 99 ± 0                 | 21.7 ± 1.7              | 4.7 ± 0.4 × 10 <sup>-6</sup>             |
|                     | SSFL                    | 5.9 | 46.40 ± 0.30                     | 3.62 ± 0.65          | 7 ± 1                                                                    | 0.76 ± 0.11                          | 1.3 ± 0.2 × 10 <sup>-4</sup>                          | 93 ± 1                 | 137.0 ± 61.5            | 0.9 ± 0.4 × 10 <sup>-6</sup>             |
|                     | SSFL                    | 6.5 | 46.53 ± 0.46                     | 4.43 ± 0.25          | 1 ± 0                                                                    | 0.15 ± 0.01                          | 6.9 ± 0.6 × 10 <sup>-4</sup>                          | 99 ± 0                 | 23.7 ± 2.1              | 4.3 ± 0.4 × 10 <sup>-6</sup>             |
| WC                  | Pedersen                | 6.5 | 46.23 ± 0.38                     | 2.02 ± 0.09          | 1 ± 1                                                                    | 0.17 ± 0.08                          | 7.2 ± 3.3 × 10 <sup>-4</sup>                          | 99 ± 1                 | 41.9 ± 10.2             | 2.5 ± 0.6 × 10 <sup>-6</sup>             |
|                     | SSFL                    | 5.3 | 46.19 ± 0.22                     | 0.47 ± 0.01          | 1 ± 0                                                                    | 0.10 ± 0.03                          | 1.2 ± 0.4 × 10 <sup>-3</sup>                          | 99 ± 0                 | 869 ± 177               | 1.2 ± 0.2 × 10 <sup>-7</sup>             |
|                     | SSFL                    | 5.9 | 46.12 ± 0.20                     | 0.41 ± 0.01          | 1 ± 0                                                                    | 0.16 ± 0.00                          | 0.6 ± 0.1 × 10 <sup>-3</sup>                          | 99 ± 0                 | 1973 ± 272              | 0.5 ± 0.1 × 10 <sup>-7</sup>             |
|                     | SSFL                    | 6.5 | 46.19 ± 0.14                     | 0.42 ± 0.01          | 1 ± 0                                                                    | 0.17 ± 0.07                          | 0.7 ± 0.2 × 10 <sup>-3</sup>                          | 99 ± 0                 | 1985 ± 1239             | 0.7 ± 0.4 × 10 <sup>-7</sup>             |
|                     | Pedersen                | 6.5 | 46.15 ± 0.19                     | 0.46 ± 0.04          | 1 ± 0                                                                    | 0.20 ± 0.07                          | 0.6 ± 0.2 × 10 <sup>-3</sup>                          | 99 ± 0                 | 711 ± 58                | 1.5 ± 0.1 × 10 <sup>-7</sup>             |

<sup>a</sup> Values were obtained from one- or two-component negative exponential functions fitted by nonlinear least squares regression to each of three samples for each powder, dissolution solvent, and solvent pH; *f* = fraction of material dissolved in each dissolution phase, *t*<sub>1/2</sub> = dissolution half-time, and *k* = chemical dissolution rate constant. <sup>b</sup> Co = cobalt, W = tungsten, and WC = tungsten carbide. <sup>c</sup> SSFL = skin surface film liquids.<sup>22</sup> Gly = glycine, PO<sub>4</sub> = phosphate, Pedersen = sweat.<sup>21</sup> <sup>d</sup> M<sub>0</sub> = average initial mass of metal (*n* = 3 replicate samples); ΣM<sub>D</sub> = average sum of the dissolved masses of metal. <sup>e</sup> ND = not detected (levels of dissolved cobalt were below the analytical limit of detection). <sup>f</sup> For each combination of powder, solvent, and pH, all *t*<sub>1/2</sub> values during the initial rapid phase were significantly shorter than the latter long-term phase; *p* < 0.05. <sup>g</sup> For each combination of powder, solvent, and pH, all *k*<sub>1</sub> values during the initial rapid phase were significantly faster than the latter long-term phase; *p* < 0.05.

phase, meaning the data was best described by one negative exponential function, and consisted of a single long-term phase (Table 2). The feedstock W and admixture WC constituent each

exhibited a single long-term dissolution phase; vitamin E in SSFL did not influence their dissolution. Feedstock Co and WC powders and the admixture Co constituent each exhibited an

**Table 2** Influence of particle size and vitamin E on dissolution of size-separated cobalt, tungsten, tungsten carbide, and admixture powders

| Powder <sup>b</sup> | Stage <sup>c</sup> | Solvent <sup>d</sup> | M <sub>0</sub> (mg) | ΣM <sub>D</sub> (mg) | Dissolution function parameters (Mean ± Standard Deviation) <sup>a</sup> |                                      |                                                       |                        |                         |                                          |
|---------------------|--------------------|----------------------|---------------------|----------------------|--------------------------------------------------------------------------|--------------------------------------|-------------------------------------------------------|------------------------|-------------------------|------------------------------------------|
|                     |                    |                      |                     |                      | Initial Rapid Phase                                                      |                                      |                                                       | Latter Long-Term Phase |                         |                                          |
|                     |                    |                      |                     |                      | f <sub>1</sub> (%)                                                       | t <sub>1/2</sub> (days) <sup>g</sup> | k <sub>1</sub> [g/(cm <sup>2</sup> day)] <sup>h</sup> | f <sub>2</sub> (%)     | t <sub>1/2</sub> (days) | k <sub>2</sub> [g/(cm <sup>2</sup> day)] |
| Co                  | 1                  | SSFL w/Vit E         | 3.49 ± 0.20         | 0.23 ± 0.08          | 5 ± 2                                                                    | 0.04 ± 0.01                          | 2.7 ± 0.2 × 10 <sup>-4</sup>                          | 95 ± 2                 | 159 ± 65                | 8.3 ± 4.1 × 10 <sup>-8</sup>             |
|                     | 1                  | SSFL                 | 3.71 ± 0.78         | 0.16 ± 0.08          | 3 ± 1                                                                    | 0.04 ± 0.02                          | 3.0 ± 1.0 × 10 <sup>-4</sup>                          | 97 ± 1                 | 259 ± 92                | 4.8 ± 1.6 × 10 <sup>-8</sup>             |
|                     | 2                  | SSFL                 | 3.39 ± 0.39         | 0.06 ± 0.01          | — <sup>f</sup>                                                           | —                                    | —                                                     | 100 ± 0                | 255 ± 26                | 4.4 ± 0.5 × 10 <sup>-8</sup>             |
|                     | 3                  | SSFL                 | 3.84 ± 0.63         | 0.16 ± 0.04          | 3 ± 1                                                                    | 0.03 ± 0.02                          | 1.6 ± 2.3 × 10 <sup>-3</sup>                          | 97 ± 1                 | 194 ± 36                | 6.1 ± 1.0 × 10 <sup>-8</sup>             |
| W                   | 1                  | SSFL w/Vit E         | 45.83 ± 0.15        | 4.22 ± 0.54          | —                                                                        | —                                    | —                                                     | 100 ± 1                | 21 ± 2                  | 5.5 ± 0.6 × 10 <sup>-6</sup>             |
|                     | 1                  | SSFL                 | 46.20 ± 0.30        | 3.12 ± 0.51          | —                                                                        | —                                    | —                                                     | 100 ± 0                | 28 ± 5                  | 4.2 ± 0.6 × 10 <sup>-6</sup>             |
|                     | 2                  | SSFL                 | 46.60 ± 0.30        | 3.94 ± 0.68          | 10 ± 9                                                                   | 1.77 ± 1.08                          | 0.6 ± 0.3 × 10 <sup>-4</sup>                          | 90 ± 9                 | 202 ± 239               | 1.3 ± 1.6 × 10 <sup>-6</sup>             |
|                     | 3                  | SSFL                 | 45.87 ± 0.25        | 5.16 ± 0.13          | 5 ± 2                                                                    | 0.47 ± 0.19                          | 1.6 ± 0.6 × 10 <sup>-4</sup>                          | 95 ± 2                 | 33 ± 11                 | 2.2 ± 0.7 × 10 <sup>-6</sup>             |
| WC                  | 1                  | SSFL w/Vit E         | 46.56 ± 0.11        | 0.53 ± 0.05          | 1 ± 0                                                                    | 0.24 ± 0.08                          | 3.4 ± 1.4 × 10 <sup>-4</sup>                          | 99 ± 0                 | 544 ± 264               | 0.3 ± 0.1 × 10 <sup>-6</sup>             |
|                     | 1                  | SSFL                 | 46.06 ± 0.41        | 0.51 ± 0.07          | 1 ± 0                                                                    | 0.27 ± 0.17                          | 6.4 ± 4.8 × 10 <sup>-4</sup>                          | 99 ± 0                 | 456 ± 250               | 3.4 ± 1.8 × 10 <sup>-7</sup>             |
|                     | 2                  | SSFL                 | 46.19 ± 0.05        | 0.58 ± 0.08          | 1 ± 0                                                                    | 0.36 ± 0.19                          | 4.2 ± 0.0 × 10 <sup>-4</sup>                          | 99 ± 0                 | 835 ± 163               | 1.3 ± 0.3 × 10 <sup>-7</sup>             |
|                     | 3                  | SSFL                 | 46.81 ± 0.09        | 0.69 ± 0.03          | 1 ± 0                                                                    | 0.29 ± 0.13                          | 3.4 ± 1.5 × 10 <sup>-4</sup>                          | 99 ± 0                 | 869 ± 434               | 1.2 ± 0.8 × 10 <sup>-7</sup>             |
| Admix-Co            | 1                  | SSFL w/Vit E         | 2.97 ± 0.05         | 0.10 ± 0.01          | 2 ± 0                                                                    | 0.10 ± 0.10                          | 1.7 ± 1.1 × 10 <sup>-4</sup>                          | 98 ± 1                 | 154 ± 27                | 7.6 ± 1.0 × 10 <sup>-8</sup>             |
|                     | 1                  | SSFL                 | 3.01 ± 0.00         | 0.06 ± 0.04          | 1 ± 1                                                                    | 0.05 ± 0.05                          | 3.1 ± 5.1 × 10 <sup>-3</sup>                          | 99 ± 1                 | 227 ± 89                | 5.7 ± 2.4 × 10 <sup>-8</sup>             |
|                     | 2                  | SSFL                 | 2.97 ± 0.04         | 0.15 ± 0.05          | 3 ± 1                                                                    | 0.07 ± 0.01                          | 1.7 ± 0.2 × 10 <sup>-4</sup>                          | 97 ± 1                 | 111 ± 20                | 1.0 ± 0.2 × 10 <sup>-7</sup>             |
|                     | 3                  | SSFL                 | 2.94 ± 0.01         | 0.19 ± 0.03          | 4 ± 1                                                                    | 0.06 ± 0.02                          | 2.1 ± 0.7 × 10 <sup>-4</sup>                          | 96 ± 1                 | 94 ± 21                 | 1.3 ± 0.3 × 10 <sup>-7</sup>             |
| Admix-WC            | 1                  | SSFL w/Vit E         | 46.47 ± 0.72        | 0.51 ± 0.04          | —                                                                        | —                                    | —                                                     | 100 ± 0                | 204 ± 13                | 6.2 ± 0.4 × 10 <sup>-7</sup>             |
|                     | 1                  | SSFL                 | 47.22 ± 0.05        | 0.42 ± 0.06          | —                                                                        | —                                    | —                                                     | 100 ± 0                | 228 ± 14                | 5.5 ± 0.4 × 10 <sup>-7</sup>             |
|                     | 2                  | SSFL                 | 46.50 ± 0.55        | 0.66 ± 0.02          | 1 ± 0                                                                    | 0.08 ± 0.05                          | 1.7 ± 1.1 × 10 <sup>-3</sup>                          | 99 ± 0                 | 376 ± 26                | 2.8 ± 1.9 × 10 <sup>-8</sup>             |
|                     | 3                  | SSFL                 | 46.09 ± 0.14        | 0.47 ± 0.02          | 1 ± 0                                                                    | 0.08 ± 0.02                          | 1.1 ± 0.2 × 10 <sup>-3</sup>                          | 99 ± 0                 | 333 ± 66                | 2.6 ± 0.3 × 10 <sup>-8</sup>             |

<sup>a</sup> Values were obtained from one- or two-component negative exponential functions fitted by nonlinear least squares regression to each of three samples for each powder and dissolution solvent; *f* = fraction of material dissolved in each dissolution phase, *t*<sub>1/2</sub> = dissolution half-time, and *k* = chemical dissolution rate constant. <sup>b</sup> Co = cobalt, W = tungsten, WC = tungsten carbide, Admix = Admixture. <sup>c</sup> From five-stage aerosol cyclone operated at 10 L min<sup>-1</sup>; <sup>d</sup> the D<sub>50</sub> were: stage 1, >10 μm; stage 2, 4.3–10 μm, and stage 3, 2.9–4.3 μm. <sup>e</sup> SSFL = skin surface film liquids (pH 5.3), Vit E = vitamin E. <sup>f</sup> M<sub>0</sub> = average initial mass of metal (*n* = 3 replicate samples); ΣM<sub>D</sub> = average sum of the dissolved masses of metal. <sup>g</sup> Not applicable for samples best described by a single long-term dissolution phase. <sup>h</sup> For each combination of powder, solvent, and pH exhibiting biphasic dissolution, all *t*<sub>1/2</sub> values during the initial rapid phase were significantly shorter than the latter long-term phase; *p* < 0.05. <sup>i</sup> For each combination of powder, solvent, and pH exhibiting biphasic dissolution, all *k*<sub>1</sub> values during the initial rapid phase were significantly faster than the latter long-term phase; *p* < 0.05.

initial rapid and latter long-term dissolution phase that was not influenced by the presence of vitamin E.

Regarding the process-sampled materials, the presence of vitamin E did not influence dissolution of Co or WC from the chemically heterogeneous spray dryer powder (Fig. 2). Initially, the presence of vitamin E did not influence dissolution of Co from heterogeneous chamfer grinder particles (Fig. 3). However, by day 1 vitamin E began to suppress the dissolution of Co,  $5.5 \pm 1.5\%$  versus  $2.6 \pm 0.7\%$  ( $p < 0.05$ ). After just three hours in SSFL, vitamin E began to suppress the dissolution of WC from chamfer grinder particles,  $2.7 \pm 0.2\%$  versus  $1.0 \pm 0.2\%$  ( $p < 0.05$ ).

### Influence of particle physicochemical properties

For each feedstock material, there were no differences in initial rapid or latter long-term dissolution phase parameter estimates among the three particle sizes (samples for which dissolution was single phase were excluded from this analysis) immersed in SSFL buffered to pH 5.3 (Table 2). Similarly, among the three sizes of mechanically blended admixture powder there were no differences in Co and WC dissolution parameter estimates.

For each size of admixture powder, values of  $t_{1/2}$  and  $k$  for the Co and WC constituents generally did not differ from the corresponding values for the same size of feedstock powder. The

exception was for stage 3 admixture powder where  $k_2$  for Co was faster than the feedstock and  $t_{1/2}$  and  $k$  for WC differed from the feedstock. Overall, these data indicate that dissolution of the Co and WC constituents were generally independent in this powder mixture.

Table 3 summarizes the total amounts of Co and WC dissolved from process-sampled powders and the admixture material in 72 h. Total dissolution of Co from spray dryer particles was comparable to that observed for the corresponding sizes of admixture Co:  $1.9 \pm 0.8\%$  versus  $2.0 \pm 1.3\%$  ( $>10 \mu\text{m}$  size) and  $2.1 \pm 1.0\%$  versus  $4.9 \pm 1.5\%$  ( $4.3\text{--}10 \mu\text{m}$  size). Total masses of WC dissolved from spray dryer particles were higher than observed for the corresponding sizes of admixture WC:  $3.5 \pm 0.1\%$  versus  $0.9 \pm 0.1\%$  ( $>10 \mu\text{m}$  size) and  $2.9 \pm 0.6\%$  versus  $1.1 \pm 0.1\%$  ( $4.3\text{--}10 \mu\text{m}$  size). Total dissolution of Co ( $7.4 \pm 2.2\%$ ) and WC ( $4.0 \pm 0.4\%$ ) from large ( $>10 \mu\text{m}$  size) chamfer grinder particles were higher than observed for the admixture ( $p < 0.05$ ).

### Discussion

Skin surface pH may vary due to endogenous (race, skin temperature, *etc.*) and exogenous factors (hygiene practices, use of topical products, *etc.*). Among healthy adults, skin pH is

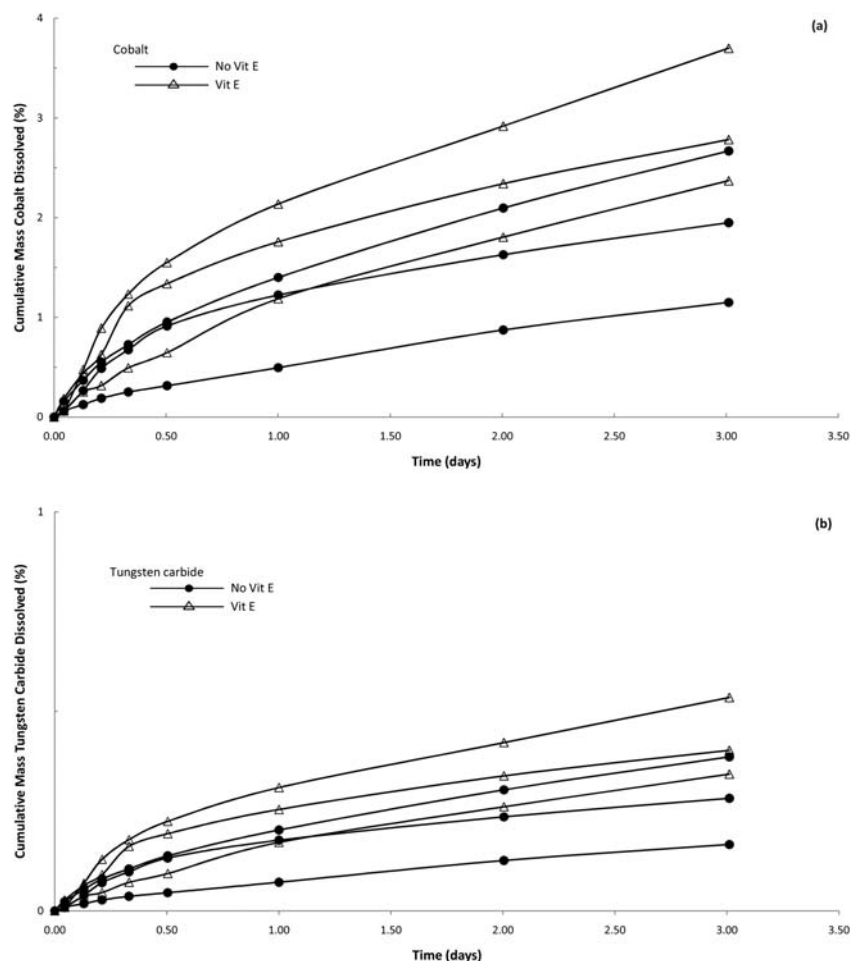


**Fig. 2** Cumulative masses of (a) cobalt and (b) tungsten carbide released from stage 1 spray dryer powder in artificial skin surface film liquids buffered to pH 5.3. Dissolution of both cobalt and tungsten carbide were biphasic and were not influenced by the presence of vitamin E.

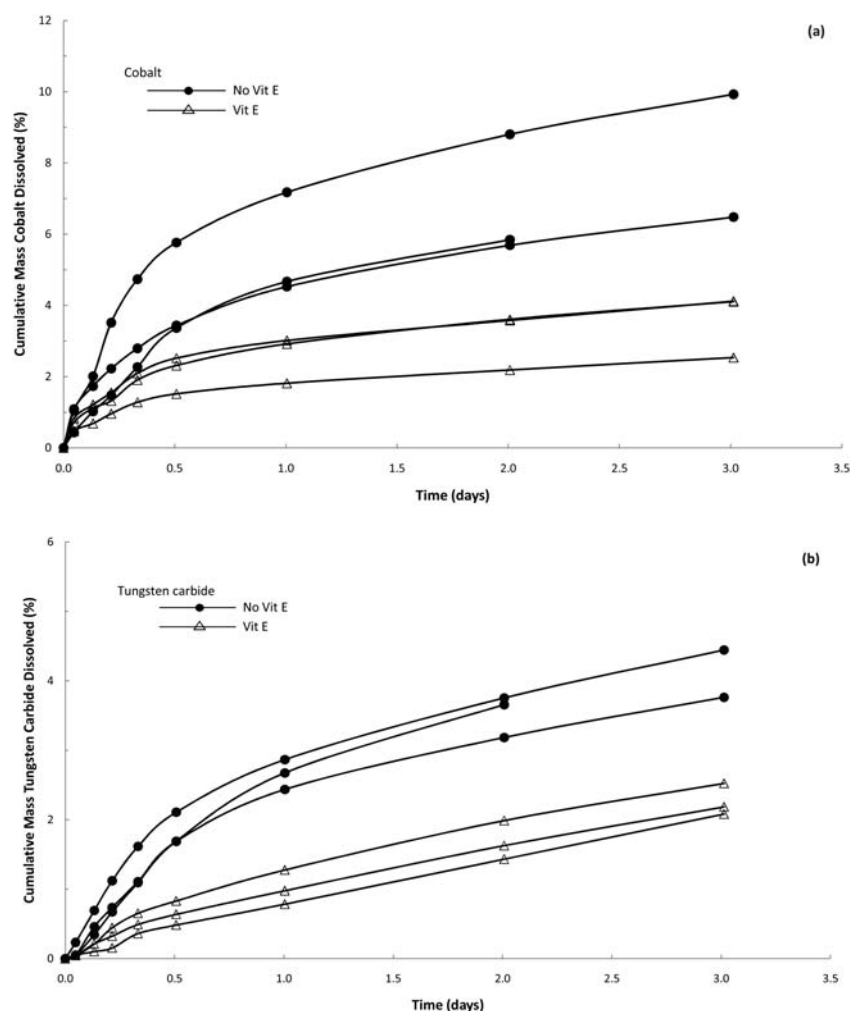


**Fig. 3** Cumulative masses (a) cobalt and (b) tungsten carbide released from stage 1 chamfer grinder powder in artificial skin surface film liquids buffered to pH 5.3. Dissolution of both cobalt and tungsten carbide were biphasic. Vitamin E suppressed dissolution of cobalt within 24 h and tungsten carbide within three hours ( $p < 0.05$ ).

**Table 3** Dissolution of admixture and size-separated process-sampled powders in skin surface film liquids (pH 5.3)

| Powder          | Constituent <sup>a</sup> | Dissolved, % (Mean $\pm$ Standard Deviation) |                      |
|-----------------|--------------------------|----------------------------------------------|----------------------|
|                 |                          | Stage 1 <sup>b</sup>                         | Stage 2 <sup>b</sup> |
| Admixture       | Co                       | 2.0 $\pm$ 1.3                                | 4.9 $\pm$ 1.5        |
|                 | WC                       | 0.9 $\pm$ 0.1                                | 1.1 $\pm$ 0.1        |
| Spray drier     | Co                       | 1.9 $\pm$ 0.8                                | 2.1 $\pm$ 1.0        |
|                 | WC                       | 3.5 $\pm$ 0.1 <sup>d</sup>                   | 2.9 $\pm$ 0.6        |
| Chamfer grinder | Co                       | 7.4 $\pm$ 2.2 <sup>e</sup>                   | — <sup>c</sup>       |
|                 | WC                       | 4.0 $\pm$ 0.4 <sup>d</sup>                   | —                    |

<sup>a</sup> Co = cobalt, WC = tungsten carbide. <sup>b</sup> From five-stage aerosol cyclone operated at 10 L min<sup>-1</sup>; <sup>19</sup> the D<sub>50</sub> were: stage 1, >10  $\mu$ m and stage 2, 4.3–10  $\mu$ m. <sup>c</sup> — = Insufficient mass of material available in this size-fraction for dissolution experiment. <sup>d</sup> Value is significantly higher compared to stage 1 admixture-WC powder. <sup>e</sup> Value is significantly higher compared to stage 1 spray drier-Co and admixture-Co powders.

generally 4.2 to 6.1,<sup>29</sup> with median of pH 5.3.<sup>22</sup> In our study, pH had little effect on Co dissolution. In contrast, dissolution of beryllium<sup>25</sup> and nickel<sup>30</sup> particles is significantly influenced by

artificial sweat pH. Lunk and Roychowdhury<sup>31</sup> reported that dissolution of W increased as pH in oxygen-containing water increased. We observed that dissolution of W increased as artificial sweat pH increased from 5.3 to 5.9; however, for reasons that we cannot explain, dissolution decreased as SSFL pH increased from 5.9 to 6.5. Andersson and Bergström<sup>32</sup> reported that dissolution of WC in water was independent over the range pH 3 to 11 due to a thin layer of tungsten trioxide (WO<sub>3</sub>) that formed on particle surfaces and passivated the material. The surface of our WC powder was rich in oxygen, a condition consistent with formation of WO<sub>3</sub> by oxidation in the presence of ambient water, which may explain why SSFL pH did not influence dissolution of this material.<sup>19</sup>

Co dissolved in SSFL (pH 6.5) but not the Pedersen *et al.*<sup>21</sup> formulation (pH 6.5) which suggests that solvent composition was an important experimental factor. However, the LOD for Co in SSFL was two times higher than in the Pedersen formulation. Additionally, Filon *et al.*<sup>15</sup> previously reported that a Co metal dissolved in the Pedersen *et al.*<sup>21</sup> formulation. Masses of Co metal used in their study (2.5 g) were higher than in our study (0.003 g) and their analytical detection limit (0.2  $\mu$ g/L) was lower than our

study (300 µg/L). Thus, the apparent role of solvent composition in Co dissolution in our study could be an artifact due to experimental and analytical factors. Dissolution of W and WC was not influenced by overall solvent composition. Lunk and Roychowdhury<sup>31</sup> reported that the presence of 0.5% to 1% calcium sulfate in oxygen-containing water suppressed dissolution of W in the range pH 5.8 to pH 8.4 by forming poorly soluble calcium tungsten precipitate. The concentration of calcium in SSFL was 0.002% and was too low to cause formation of calcium precipitates.

Lison *et al.*<sup>33</sup> demonstrated that particulate Co in the presence of particulate WC generates reactive oxygen species (ROS) and suggested that dissolved Co is released as a byproduct from the oxidation reaction. Previously, we demonstrated that chamfer grinder particles generated higher levels of ROS in artificial sweat compared to the feedstock, admixture, and spray dryer powders.<sup>19</sup> As such, we expected chamfer grinder particles would release high levels of dissolved Co in artificial sweat. Results from our experiments confirmed that among study powders, total Co dissolution in artificial sweat was  $7.4 \pm 2.2\%$  for chamfer grinder particles (Fig. 3) compared to  $4.1 \pm 1.4\%$  for feedstock Co (Table 2),  $2.0 \pm 1.3\%$  for admixture Co (Table 2), and  $1.9 \pm 0.8\%$  for spray dryer powder (Fig. 2). However, when vitamin E, an antioxidant, was added to artificial sweat at 0.1% by weight, dissolution of Co from chamfer grinder particles was suppressed to  $3.5 \pm 1.1\%$  (Fig. 3) which supports the premise that dissolved Co was a byproduct of ROS generation.

Finally, we investigated possible effects of particle physical and/or chemical properties on dissolution. Particle size did not influence dissolution of feedstock Co, W, and WC powders. Andersson and Bergström<sup>32</sup> reported that presence of Co (8% by volume) reduced dissolution of WC in water (pH 5) by a factor of 10 because dissolved Co passivated the WC particle surfaces. In our admixture powder, Co was 0.03% by volume and dissolution of Co and WC were generally independent of one another. For the spray dryer powder, which consists of Co and WC powders which have been wet milled, dissolution of Co was similar to that observed for the admixture whereas dissolution of WC was higher. The observed dissolution pattern was attributed to the surface area and surface chemistry of spray dryer particles.<sup>19</sup> Spray dryer particles have higher surface area than WC particles in admixture powder and their surfaces are composed of carbon. Thus, for surface-area-limited dissolution, more WC will dissolve from spray dryer particles than WC particles in the admixture. Upon dissolution of the WC surface layer on spray dryer particles, the underlying Co would be made available for dissolution. As noted above, for chamfer grinder particle which contain Co and WC that are chemically bonded, dissolution of constituents were higher than observed for the feedstock, admixture, and spray dryer powders. Chamfer grinder particles contain both Co and WC on their surface and the chemical bonds between these constituents enhances dissolution.

### Risk of cobalt sensitization and allergic contact dermatitis

To cause sensitization, an allergenic metal that deposits on the skin must dissolve in skin surface liquids, permeate through the stratum corneum layer of the epidermis, and initiate an immune response in the underlying viable tissue. Once a person becomes

sensitized, repeat exposure to the same chemical will elicit ACD.<sup>9</sup> Values of  $t_{1/2}$  and  $k_1$  were orders of magnitude shorter and faster, respectively during the initial rapid Co dissolution phase compared to the long-term phase. As such, given the initial rapid dissolution rate of cobalt and duration of a standard work shift (8 h), short-term skin contact may contribute more bioaccessible Co than long (*e.g.*, days) contact times (Tables 1 and 2).

During production of cemented carbides, Co and WC powders are combined in a powder mixing operation, which is a dusty job.<sup>3</sup> The fraction of cobalt dissolved in a given time can be calculated from our dissolution data for Co powder in SSFL with pH 5.3 (Table 1):

$$\frac{M_D}{M_0} = 1 - f_1 \exp(-k_1 \text{SSA}t) + f_2 \exp(-k_2 \text{SSA}t)$$

$f_i$  = fraction of material available for dissolution in the initial rapid or latter long-term phase,  $k_i$  = chemical dissolution rate constant for the initial rapid or latter long-term phase, g/(cm<sup>2</sup> day), SSA = Initial powder specific surface area, cm<sup>2</sup>/g,  $t$  = time, day.

Multiplying this dissolved fraction by a previously measured mass loading of 1.33 mg particulate Co on two hands for a powder mixing worker,<sup>3</sup> the masses of dissolved Co on hands would be 60, 100, 140, and 170 µg at two, four, six, and eight hours, respectively. Normalizing these masses of dissolved Co to 840 cm<sup>2</sup>, the area of two hands,<sup>34</sup> yields exposure concentrations of 0.07, 0.12, 0.17, and 0.20 µg/cm<sup>2</sup>, respectively. Yanagi *et al.*<sup>35</sup> induced sensitization in guinea pigs by topical application of cobalt sulfate to abraded skin and reported that 100% of test animals exhibited a skin reaction when challenged with the equivalent of 0.14 µg Co/cm<sup>2</sup> which is comparable to levels calculated for a two hour exposure in our study. Julander *et al.*<sup>4</sup> reported that a strong positive ACD reaction was elicited in cobalt-sensitized persons when patch tested with cobalt chloride at an equivalent concentration of 0.5 µg Co/cm<sup>2</sup>. Previously, Allenby and Basketter<sup>36</sup> reported that ACD was elicited from persons with mildly inflamed or occluded skin upon exposure to cobalt chloride at the equivalent of 0.5 µg Co/cm<sup>2</sup>. In addition to using cobalt chloride, Julander *et al.*<sup>4</sup> also patch tested sensitized persons using solid cemented carbide discs. The authors investigated dissolution of Co from the cemented carbide discs using the Pedersen *et al.*<sup>21</sup> artificial sweat formulation buffered to pH 6.5. Strong positive allergic reactions were observed for all volunteers that were patch tested using discs that leached 0.2 to 2.7 µg Co/cm<sup>2</sup> in two minutes. Thus, in our example, the skin exposure level predicted for a powder mixing worker is within the range known to elicit cobalt ACD in sensitized persons challenged with soluble cobalt chloride or solid cemented carbide material.

The exact mechanism by which Co initiates an immune response in the underlying epidermis is unclear; however, dermal uptake of Co ions is prerequisite for an immune reaction. Using four hours (mid-shift) as a representative exposure time and fractional absorption rates of 0.001 to 0.15% per hour,<sup>16</sup> 0.1 and 15 µg of cobalt would be absorbed through the hands of a powder mixing worker with intact and abraded skin, respectively. Once in the viable epidermis layer, Co ions may bind with proteins to form haptens and elicit sensitization by interacting with T-cells.<sup>37</sup> Alternatively, Van den Broeke *et al.*<sup>38</sup> hypothesized that cobalt ions could mediate redox reactions to generate free radicals,

thereby altering biomolecules to form antigen and elicit sensitization. Results from studies by Lison *et al.*<sup>33</sup> and our laboratory suggest that dissolved Co can be produced during ROS generation. However, Gäfvert *et al.*<sup>39</sup> reported that pre-treatment of skin with 10% vitamin E had no effect on induction of cobalt sensitization or elicitation of cobalt ACD, which suggests that ROS are not involved in antigen formation for Co allergy.

## Conclusion

A novel artificial sweat solvent with chemical composition and pH that matches human sweat was used to investigate the dissolution of metals encountered during the production of cemented tungsten carbides. Observed cobalt dissolution rates, coupled with knowledge of skin permeation rates, allowed for modeling of dermal uptake and can be used to develop models for risk of cobalt sensitization and cobalt ACD.

## Acknowledgements

The authors thank M. Duling, N. Edwards, R. Lawrence, D. Sbarra, and B. Tift at NIOSH for their assistance and G. S. Dotson and S. Anderson for critical review of this manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

## References

- 1 M. Linnainmaa and M. Kiilunen, *Int. Arch. Occup. Environ. Health*, 1997, **69**, 193–200.
- 2 C. Lidén, L. Skare, B. Lind, G. Nise and M. Vahter, *Contact Dermatitis*, 2006, **54**, 233–238.
- 3 G. A. Day, M. A. Virji and A. B. Stefaniak, *J. Exposure Sci. Environ. Epidemiol.*, 2009, **19**, 423–434.
- 4 A. Julander, L. Skare, M. Mulder, M. Grandt, M. Vahter and C. Lidén, *Ann. Occup. Hyg.*, 2010, **54**, 340–350.
- 5 T. Fischer and I. Rystedt, *Am. J. Ind. Med.*, 1985, **8**, 381–394.
- 6 L. Kanerva, T. Estlander and R. Jolanki, *Int. Arch. Occup. Environ. Health*, 1988, **60**, 89–94.
- 7 Ed. J. E. Walberg, L. In Kanerva, P. Elsner, J. E. Wahlberg, and H. I. Maibach, in *Handbook of Occupational Dermatology*, Springer-Verlag, Berlin, 2000, pp. 551.
- 8 A. Julander, M. Hindsen, L. Skare and C. Lindén, *Contact Dermatitis*, 2009, **60**, 165–170.
- 9 G. Forte, F. Petrucci and B. Bocca, *Inflammation Allergy: Drug Targets*, 2008, **7**, 145–162.
- 10 T. Schneider, J. W. Cherrie, R. Vermeulen and H. Kromhout, *Ann. Occup. Hyg.*, 2000, **44**, 493–499.
- 11 P. Apostoli, S. Porru and L. Alessio, *Sci. Total Environ.*, 1994, **150**, 129–132.
- 12 G. Mosconi, M. Bacis, M. T. Vitali, P. Leghissa and E. Sabbioni, *Sci. Total Environ.*, 1994, **150**, 133–139.
- 13 G. Scansetti, G. C. Botta, P. Spinelli, L. Reviglione and C. Ponzetti, *Sci. Total Environ.*, 1994, **150**, 141–144.
- 14 G. Scansetti, G. Maina, G. C. Botta, P. Bambace and P. Spinelli, *Int. Arch. Occup. Environ. Health*, 1998, **71**, 60–63.
- 15 F. L. Filon, G. Maina, G. Adami, M. Venier, N. Coceani, R. Bussani, M. Massiccio, P. Barbieri and P. Spinelli, *Int. Arch. Occup. Environ. Health*, 2004, **77**, 85–89.
- 16 F. L. Filon, F. D'Agostin, M. Crosera, G. Adami, M. Bovenzi and G. Maina, *Toxicol. in Vitro*, 2009, **23**, 574–579.
- 17 F. Larese, A. Gianpietro, M. Venier, G. Maina and N. Renzi, *Toxicol. Lett.*, 2007, **170**, 49–56.
- 18 A. B. Stefaniak, G. A. Day, C. J. Harvey, S. S. Leonard, D. E. Schwegler-Berry, S. J. Chipera, N. M. Sahakian and W. P. Chisholm, *Ind. Health*, 2007, **45**, 793–803.
- 19 A. B. Stefaniak, C. J. Harvey, V. C. Bukowski and S. S. Leonard, *J. Occup. Environ. Health*, 2010, **7**, 23–34.
- 20 K. J. A. Brookes, in *Cemented carbides for engineers and tool users*, International Carbide Data, East Barnet, UK, 1983. pp. 1–19.
- 21 N. B. Pedersen, S. Fregert, P. Brodelius and B. Gruvberger, *Acta Dermatovener (Stockh.)*, 1974, **54**, 231–234.
- 22 C. J. Harvey, R. F. LeBouf, and A. B. Stefaniak, *Toxicol. In Vitro*, In Press., DOI: 10.1016/j.tiv.2010.06.016.
- 23 A. B. Stefaniak, C. J. Harvey and P. W. Wertz, *Int. J. Cosmet. Sci.*, 2010, DOI: 10.1111/j.1468-2494.2010.00561.
- 24 European Committee for Standardization, *Reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin*, 1998, EN1811:1998.
- 25 A. B. Stefaniak, M. A. Virji and G. A. Day, *Ann. Occup. Hyg.*, 2010, DOI: 10.1093/annhyg/meq057.
- 26 H. Takahashi and K. Koshi, *Ind. Health*, 1981, **19**, 47–59.
- 27 D. Lison and R. Lauwerys, *Toxicol. Lett.*, 1992, **60**, 203–210.
- 28 G. L. Finch, J. A. Mewhinney, A. F. Edison, M. D. Hoover and S. J. Rothenberg, *J. Aerosol Sci.*, 1988, **19**, 333–342.
- 29 P. Agache, in *Measuring the Skin*, ed. P. Agache and P. Humbert, Springer-Verlag, Germany, 2004, pp. 21–32.
- 30 J. D. Hemingway and M. M. Molokhia, *Contact Dermatitis*, 1987, **16**, 99–105.
- 31 H.-J. Lunk and S. Roychowdhury, *Proceedings of the Sixth International Conference of Tungsten, Refractory and Hard Metals*, Metal Powder Industries Federation, Princeton, NJ, 2006, pp. 107–115, ISBN: 0-9762057-4-2.
- 32 K. M. Andersson and L. Bergström, *Int. J. Refract. Met. Hard Mater.*, 2000, **18**, 121–129.
- 33 D. P. Lison, P. Carbonnelle, L. Mollo, R. Lauwerys and B. Fubini, *Chem. Res. Toxicol.*, 1995, **8**, 600–606.
- 34 U.S. Environmental Protection Agency, *Exposure Factors Handbook*, 1997.
- 35 M. Yanagi, M. Hoya, M. Mori and Y. Katsumura, *Contact Dermatitis*, 2001, **44**, 140–145.
- 36 C. F. Allenby and D. A. Basketter, *Contact Dermatitis*, 1989, **20**, 185–190.
- 37 M. Goebeler, G. Meinardus-Hager, J. Roth, S. Goerdt and C. Sorg, *J. Invest. Dermatol.*, 1993, **100**, 759–765.
- 38 L. T. Van den Broeke, A. Gräslund, J. L. G. Nilsson, J. Wahlberg, A. Schevnius and A. T. Karlberg, *Eur. J. Pharm. Sci.*, 1998, **6**, 279–286.
- 39 E. Gäfvert, J. L. G. Nilsson, G. Hagelthorn and A.-T. Karlberg, *Br. J. Dermatol.*, 2002, **146**, 649–656.