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# Influence of artificial gastric juice composition on bioaccessibility of cobalt- and tungsten-containing powders

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ABSTRACT

The dissolution of metal-containing particles in the gastric compartment is poorly understood. The purpose of this study was to elucidate the influence of artificial gastric juice chemical composition on bioaccessibility of metals associated with ingestion-based health concerns. Dissolution rates were evaluated for well-characterized feedstock cobalt, tungsten metal, and tungsten carbide powders, chemically bonded pre-sintered (spray dryer material) and post-sintered (chamfer grinder) cemented tungsten carbide materials, and an admixture of pure cobalt and pure tungsten carbide, prepared by mechanically blending the two feedstock powders. Dissolution of each study material was evaluated in three different formulations of artificial gastric juice (from simplest to most chemically complex): American Society of Testing Materials (ASTM), U.S. Pharmacopoeia (USP), and National Institute for Occupational Safety and Health (NIOSH). Approximately 20% of cobalt dissolved in the first dissolution phase ($t_{1/2}$ = 0.02 days) and the remaining 80% was released in the second long-term dissolution phase ($t_{1/2}$ = 0.5 to 1 days). Artificial gastric juice chemical composition did not influence dissolution rate constant values ($k$, g/cm$^2$ day) of cobalt powder, either alone or as an admixture. Approximately 100% of the tungsten and tungsten carbide that dissolved was released in a single dissolution phase; $k$-values of each material differed significantly in the solvents: NIOSH > ASTM > USP ($p < 0.05$). The $k$-values of cobalt and tungsten carbide in pre- and post-sintered cemented tungsten carbide powders were significantly different from values for the pure feedstock powders. Solvent composition had little influence on oral bioaccessibility of highly soluble cobalt and our data support consideration of the oral exposure route as a contributing pathway to total-body exposure. Solvent composition appeared to influence bioaccessibility of the low soluble tungsten compounds, though differences may be due to variability in the data associated with the small masses of materials that dissolved. Nonetheless, ingestion exposure may not contribute appreciably to total body burden given the short residence time of material in the stomach and relatively long dissolution half-times of these materials ($t_{1/2}$ = 60 to 380 days).

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## Introduction

An understanding of the fate of metals ingested by humans is of great importance in environmental and occupational health. The two limiting factors in oral bioavailability of a metal are dissolution and absorption (Dean and Ma, 2007; Ellickson et al., 2001). Dissolution of ingested metal-containing particles is often estimated using cell-free artificial saliva-gastro-intestinal fluids and in vitro test methods as surrogates for in vivo measurements (Ellickson et al., 2001; Stopford et al., 2003). In vitro test methods provide estimates of bioaccessibility, i.e., the amount of material that dissolves in the artificial biological fluid and is available for absorption (Ruby et al., 1999), though do not account for transport of ions across biological barriers (Dean and Ma, 2007). Ideally, in vivo measurements provide estimates of material bioavailability, i.e., the rate and extent of material uptake at a target organ (Gibaldi, 1984). Due to the absence of non-invasive measurement methods, bioavailability is also defined as the rate and extent of absorption into the systemic circulation that can be measured, e.g., in blood.

Cobalt is a specific example of a metal for which ingestion is associated with development of adverse health effects, including gastrointestinal problems, liver injury, and cardiomyopathy among humans who consumed large quantities of soluble cobalt-containing beer (Alexander, 1969, 1972; Kennedy et al., 1981; Morin et al., 1971). The potential for hand-to-mouth

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contact increases the likelihood for ingestion as a major route of exposure (Cherrie et al., 2006). For example, Day et al. (2009) measured cobalt on workers' hands during the production of cemented tungsten carbides; Christensen and Poulsen (1994) also noted contamination of the hands of Danish pottery painters who used cobalt-containing paints and glazes. The potential for tungsten-induced adverse health effects from ingestion is poorly understood. Rubin et al. (2007) reported elevated concentrations of tungsten in drinking water and in the urine of residents in Churchill County, Nevada, an area with excess cases of childhood acute lymphoblastic leukemia. The authors could not, however, associate ingestion exposure with development of leukemia.

The residence time of metals in the mouth is usually short; therefore, dissolution in saliva is often considered negligible. Rather, most bioaccessibility studies aim to mimic dissolution in the stomach, where material is subjected to pepsin at pH values of 1 to 4, and in the small intestines at pH values of 4 to 8 (Dean and Ma, 2007). Stopford et al. (2003) evaluated the bioaccessibility of several cobalt-containing compounds in various artificial biological fluids over 72 hours. In a simple formulation of artificial gastric juice (pH 1.5), solubility of cobalt was reported to be 100% whereas in artificial intestinal fluid (pH 7.4) solubility of the same material was 4%. Pre- and post-sintered cobalt tungsten carbide materials followed a similar trend, with solubility in gastric juice being about 25% and solubility in intestinal fluid about 10%. Overall, this study demonstrated that among fluids, pH influenced cobalt dissolution, with rates increasing as pH decreased. In contrast, little attention has been given to the influence of artificial gastric juice solvent chemistry on bioaccessibility of metals. Additionally, data describing the dissolution kinetics of metals in the stomach are needed to understand the contribution of oral exposure to total body exposure.

In humans, up to 97% of dissolved cobalt is absorbed across the gastrointestinal tract depending on the type and dose of compound (oxide, salt, etc), gender, and nutritional status of the individual (Christensen et al., 1993; Christensen and Poulsen, 1994; Leggett, 2008; WHO, 2006). In animals, up to 25% of tungsten is absorbed across the gastrointestinal tract from tungsten oxide and from 40% to 90% from sodium tungstenate depending on the type of dose and animal species (Keith et al., 2007).

The purpose of this study was to elucidate influences of the chemical composition of artificial gastric juices and the physicochemical properties of metal-containing particles on ingestion bioaccessibility of cobalt and tungsten. Specifically, we: 1) estimated chemical dissolution rate constants ($k$-values) in artificial gastric juices for industrial powders encountered during the manufacture of cemented tungsten carbides; 2) investigated whether $k$-values determined for bulk powder samples were equivalent to $k$-values determined for size-separated fractions of the same powders; 3) elucidated the influence, if any, of multi-chemical constituent particle composition on the dissolution of individual chemical constituents; and 4) investigated the influence of artificial gastric juice chemical composition on bioaccessibility.

## Materials and methods

### *Study overview*

We evaluated the influence of gastric juice formulation on bioaccessibility using well-characterized bulk and aerodynamically size-separated cobalt- and tungsten-containing materials. Dissolution results were modeled by fitting single or multiple component negative exponential functions to the data assuming a surface-area-proportional model (Finch et al., 1988).

### *Artificial gastric juice formulations*

Three different formulations of artificial gastric juice were used in this study. To clearly understand the influence of artificial gastric juice chemical composition, none of the samples was subjected to agitation to mimic the peristaltic movements that occur in the gastrointestinal tract. Agitation may increase dissolution, as such the absence of agitation in our system may bias low the absolute values for fraction absorbed and dissolution rate for a particular test material. The first formulation was a simple solution of 0.07 N hydrochloric acid with initial pH 1.5 at 37.0 °C specified by American Society of Testing Materials (ASTM) D5517: A Standard Method of Determining the Solubility of Metals in Art Materials (ASTM, 2003). The second formulation consisted of 0.03 M sodium chloride, 0.084 M hydrochloric acid, and 0.32% (w/v) pepsin with initial pH 1.4 at 37.0 °C, recommended by the U.S. Pharmacopoeia (USP) (Ellickson et al., 2001). The third formulation, developed at the National Institute for Occupational Safety and Health (NIOSH formulation), was the most chemically comprehensive formulation and was tailored for the purposes of the current study (Table 1) and had initial pH 1.5 at 37.0 °C. Because metals such as cobalt (Carson et al., 1986) react with thiol-containing amino acids, a modified-NIOSH (modNIOSH) formulation was also prepared without L-cystine and L-methionine.

The constituent concentrations are representative of minimum normal fasting values for adult humans. The NIOSH formulation had a solvent pH of 1.5 at 37.0 °C. Analytical grade chemicals (all from Fisher Scientific, Fairlawn, NJ, except sialic acid, which was from Rose Scientific Ltd., Edmonton, Canada) were used to prepare the liquid. To facilitate data comparison, the value of pepsin in the NIOSH formulation was chosen to match the USP formulation.

### *Static dissolution technique*

Bioaccessibility of cobalt and tungsten were evaluated using a static dissolution technique (Kanapilly et al., 1973), which is a well-established technique that has been used to evaluate the solubility of metals, including uranium and uranium oxide compounds (Ansoborlo et al., 1992, 1998; Eidson and Mewhinney, 1980), beryllium compounds (Finch et al., 1988; Stefaniak et al., 2006), and montmorillonite clay and curium oxide (Ansoborlo et al., 1999). Static dissolution chambers were prepared by weighing a known mass of powder (Model XS205, Mettler-Toledo, Greifensee, Switzerland) on a 0.025-μm pore size 47-mm diameter nitrocellulose filter (Cat. No. VSWP04700, Millipore, Billerica, MA), covering the powder with a second 0.025-μm pore size 47-mm filter to form a sandwich, and securely clamping the sandwich between the retaining rings of a static dissolution chamber (Cat. No. 06-401, Intox Products, Moriarity, NM). Each sandwich was then placed in a separate autoclaved polypropylene cup (Cat. No. 06100080.03S, SKS, Watervliet, NY) containing 80 mL of appropriate artificial gastric juice. Each cup was covered with a screw-top lid and the dissolution fluid maintained at 37 °C in a water-jacketed incubator (Model 460, Barnstead International, Dubuque, IA). Solvents were changed at 1, 3, 5, and 8 hours after placing the dissolution chambers in an artificial gastric juice; these time points mimic material residence times in the stomach (Dean and Ma, 2007) and allow for elucidation of material dissolution kinetics. At each solvent change, the pH of the artificial gastric juice in each storage bottle was measured at 37.0 °C using a calibrated electrode (Mettler-Toledo). Solvent samples were stored frozen at −10 °C until analysis for dissolved cobalt and/or tungsten content. After the final solvent change,

**Table 1**
Composition of comprehensive artificial gastric juice (NIOSH formulation).

| Constituents | Mass (g/L) |
|---|---|
| *Primary electrolytes and ionic constituents* | |
| Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$) | 0.2646 |
| Magnesium Chloride Hexahydrate ($MgCl_2 \cdot 6H_2O$) | 0.1525 |
| Potassium Chloride (KCl) | 0.8647 |
| Sodium Chloride (NaCl) | 2.8559 |
| 0.04 M Hydrochloric acid (HCl) | 1.4263 |
| Sodium bromide (NaBr) | 0.0008 |
| Copper (II) Chloride Dihydrate ($CuCl_2 \cdot 2H_2O$) | 0.0003 |
| Sodium fluoride (NaF) | 0.0009 |
| Phosphorous Pentachloride ($PCl_5$) | 0.4707 |
| *Organic acids and carbohydrates* | |
| D(+)-Fucose ($C_6H_{12}O_6$) | 0.1380 |
| D(+)-Glucose ($C_6H_{12}O_6$) | 0.3500 |
| D(+)-Glucuronic acid, sodium salt, monohydrate ($C_6H_9NaO_7 \cdot H_2O$) | 0.0241 |
| Sialic acid ($C_{11}H_{19}NO_9$) | 0.0731 |
| *Amino acids* | |
| DL-Alanine ($C_3H_7NO_2$) | 0.0287 |
| L-(+)-Arginine ($C_6H_{14}N_4O_2$) | 0.0330 |
| L-(+)-Aspartic acid ($C_4H_7NO_4$) | 0.0170 |
| L-Cystine ($C_6H_{12}N_2O_4S_2$) | 0.0180 |
| L-(+)-Glutamic acid ($C_5H_9NO_4$) | 0.0200 |
| Glycine ($C_2H_5NO_2$) | 0.0130 |
| L-Histidine ($C_6H_9N_3O_2$) | 0.0130 |
| L-Isoleucine ($C_6H_{13}NO_2$) | 0.0070 |
| L-Leucine ($C_6H_{13}NO_2$) | 0.0120 |
| L-(+)-Lysine Monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$) | 0.0175 |
| L-Methionine ($C_5H_{11}NO_2S$) | 0.0080 |
| L-Phenylalanine ($C_9H_{11}NO_2$) | 0.0080 |
| L-(-)-Proline ($C_5H_9NO_2$) | 0.0170 |
| DL-Serine ($C_3H_7NO_3$) | 0.0160 |
| L-Threonine ($C_4H_9NO_3$) | 0.0150 |
| L-(−)-Tryptophan ($C_{11}H_{12}N_2O_2$) | 0.0140 |
| L-Tyrosine ($C_9H_{11}NO_3$) | 0.0100 |
| *Nitrogenous Substances* | |
| 1 M Ammonium hydroxide ($NH_4OH$) | 0.1996 |
| Urea ($CH_4N_2O$) | 0.0840 |
| Uric acid ($C_5H_4N_4O_3$) | 0.0080 |
| *Vitamins* | |
| L-(+)-Ascorbic Acid ($C_6H_8O_6$) | 0.9500 |
| Cobalamine concentrate ($C_6H_9N(AO_7) \cdot H_2O$) | 0.000006 |
| Histamine Dihydrochloride ($C_5H_9N_3 \cdot 2HCl$) | 0.0002 |
| *Pepsin* | |
| Pepsin | 3.2000 |

each filter sandwich was recovered and stored at −10 °C until analysis to quantify the remaining mass of insoluble particulate cobalt and/or tungsten (described below).

### *Study materials*

Five bulk powder samples were collected at a company that manufactures cemented tungsten carbides. The bulk powders contain all particle sizes and may deposit on surfaces from spills, be transferred to hands upon contact, and subsequently ingested via hand-to-mouth contact (e.g., smoking or eating with contaminated hands). The samples represent the spectrum of materials encountered during the manufacture of cemented tungsten carbides: feed-stock powders (pure cobalt, tungsten metal, and tungsten carbide), intermediate pre-sintered "green" material (powder discharged from a spray dryer after wet-milling), and dust from sintered product (from a dust collector connected to a chamfer grinder used for final machining of sintered parts). The feedstock and pre-sintered materials were produced prior to addition of paraffin wax as a binder for pressing and during sintering any wax melts from the part (Stefaniak et al., 2007). The morphology and chemistry of these powders were qualitatively similar to airborne particles to which workers were potentially exposed during the manufacture of cemented tungsten carbides (Stefaniak et al., 2007).

A thoracic fraction (4.3 – 10 μm) of each powder was obtained from Stage 2 of a multi-stage aerosol cyclone (Smith et al., 1992) operated at 10 Lpm following aerosolization using a dry powder disperser (Model 175, DeVilbiss, Somerset, PA) as previously described (Hoover et al., 1989). Particles in the thoracic fraction are encountered during the manufacture of cemented tungsten carbides (Stefaniak et al., 2009) and have aerodynamic sizes that may deposit in the conducting airways of the lung and subsequently be transported up the mucociliary escalator, swallowed, and ingested. To better understand dissolution of constituents alone and during co-exposure, a sixth material was prepared as an admixture by mechanically blending the stage 2 aerodynamically size-separated pure tungsten carbide and cobalt powders at a 94:6 ratio (by mass) to match common industrial grade powder (Brookes, 1983).

### *Powder characterization*

Powder x-ray diffraction (XRD) (Siemens Model D500, Bruker AXS, Inc., Madison, WI) was used to identify crystalline constituents of the study materials by comparing diffraction patterns to reference patterns in the Inorganic Crystal Structure Database (ICSD, 2003–2005). Relative abundances of crystalline phases were obtained from Rietveld (Bish and Howard, 1988) and full-pattern fitting methods (Chipera and Bish, 2002). The Brunauer, Emmett, and Teller (BET) method was used to determine specific surface area (SSA, $m^2/g$) by nitrogen gas adsorption (NOVA 2000e, Quantachrome Instruments, Boynton Beach, FL); the cross-sectional area of a nitrogen gas adsorbate molecule was taken to be 16.2 $Å^2$. Prior to measurement of surface area ($m^2$), all samples were outgassed for at least three hours at 150 °C to 200 °C under light vacuum (Flovac™ Degasser, Quantachrome) to remove water from the powder surface.

### *Bulk powder studies*

Bulk cobalt, tungsten metal, and tungsten carbide powders were used to estimate material-specific *k*-values and to investigate the influence of artificial gastric juice composition (ASTM, USP, and NIOSH formulation) on material bioaccessibility. Metals such as cobalt (Carson et al., 1986) react with thiol-containing amino acids; therefore, the dissolution of bulk cobalt powder was also evaluated in the modified NIOSH artificial gastric juice that was prepared without L-cystine and L-methionine.

Static dissolution chambers were prepared in triplicate for each bulk powder for evaluation of dissolution in each artificial gastric juice formulation. Determination of the initial mass of material in a dissolution chamber can be made in one of two ways: gravimetrically or by quantifying and summing the masses of material dissolved and particulate remaining using atomic spectroscopy, radioisotope counting, etc. To avoid error associated with incomplete digestion of polydisperse particles and subsequent under reporting of mass by atomic spectroscopy (Oomen et al., 2002; Stefaniak et al., 2008), the minimum initial masses of powder in each static dissolution chamber were determined gravimetrically: 0.0032 g (cobalt), 0.0460 g (tungsten), and 0.0489 g (tungsten carbide). The actual initial mass of metal (i.e., $M_0$) in each chamber was calculated by multiplying the mass of bulk powder determined gravimetrically by the mass fraction of metal in the powder determined from

**Table 2**
Physicochemical properties of bulk and aerodynamically size-separated powders for investigation of dissolution in artificial gastric juices.

| Powder | $D_{ae}$ (μm)[a] | Composition (weight %)[b] | Surface area ($m^2$/g) |
|---|---|---|---|
| Cobalt | Bulk | Co (87%), $Co_3O_4$ (13%) | 7.30 ± 0.24 |
| | 4.3-10 | Co (major) | 6.05 ± 0.87 |
| Tungsten | Bulk | W (100%) | 0.68 ± 0.09 |
| | 4.3-10 | W (major) | 0.87 ± 0.05 |
| Tungsten carbide | Bulk | WC (98.3%), $W_2C$ (1.7%) | 0.66 ± 0.01 |
| | 4.3-10 | WC (major), $W_2C$ (minor) | 0.65 ± 0.03 |
| Admix | Bulk | N/A | N/A |
| | 4.3-10 | WC (major), Co (minor), $W_2C$ (minor) | 0.84 ± 0.10 |
| Spray dryer | Bulk | WC (82.6%), Co (11.8%), W (4.6%), $W_2C$ (1.0%) | 2.26 ± 0.21 |
| | 4.3-10 | WC (major), Co (minor) | 0.79 ± 0.11 |
| Chamfer grinder | Bulk | WC (84.7%), Co (11.2%), C (4.1%) | 0.42 ± 0.02 |
| | 4.3-10 | WC (major), Co (minor) | 0.61 ± 0.09 |

N/A=Not applicable.

[a] $D_{ae}$ = aerodynamic cutoff diameter interval for stage 2 of the multi-stage aerosol cyclone.
[b] Data for bulk materials are crystalline composition (relative mass percents) determined using quantitative XRD analysis. Data for size-separated materials are crystalline composition (relative amounts) determined using qualitative XRD analysis. Co = cobalt, $Co_3O_4$ = cobalt oxide, W = tungsten, WC = tungsten monocarbide, $W_2C$ = tungsten carbide, C = carbon.

quantitative XRD analysis which does not require sample preparation techniques such as acid digestion. For example, cobalt powder was 87% cobalt and 13% cobalt oxide ($Co_3O_4$) by weight (see Table 2). Using the molecular weight of cobalt (58.9 g/mol) and formula weight of cobalt oxide (240.8 g/mol), cobalt powder was: ((0.87 × (58.9/58.9)) + ((0.13 × (176.8/240.8)) = 0.96 g cobalt/g powder. Thus, 0.0032 g powder/chamber × 0.96 g cobalt/g powder = 0.0031 g cobalt/chamber.

In addition to consideration of the minimum amount needed to obtain a detectable concentration in the test solvent, selection of an appropriate test mass requires consideration of the maximum amount permissible to avoid saturation of the test solvent (Stopford et al., 2003). For the purposes of this study, data from Johansson et al. (1980) and Krauss et al. (2001) were used to determine the feasibility of obtaining desired dissolution information during a short-term test and to provide insight into appropriate time-points. Johansson et al. (1980) evaluated dissolution of cobalt powder in artificial extracellular lung fluid having neutral pH. Using solubility data and particle physical characteristics data reported by Johansson et al. (1980), we estimated $k = 2 \times 10^{-7}$ g/($cm^2$ day) for cobalt in extracellular lung fluid, assuming single phase surface-area-limited particle dissolution (Mercer, 1967). For the tungsten-containing materials, we assumed the upper-limit *k*-value to be one order of magnitude slower than for cobalt, an assumption consistent with the poorer *in vivo* solubility of tungsten compounds (Kraus et al., 2001). In this manner, we predicted that a minimum solvent contact time of one hour was adequate to ensure that an adequate amount of material would dissolve from an initial powder mass of several milligrams to exceed the analytical limit of detection (see below) but remain less than about 5 ppm (Stopford et al., 2003) to avoid saturation of the gastric juice solvent during this time.

*Aerodynamically size-separated powder studies*

The aerodynamically size-separated powders were used in dissolution studies to estimate *k* values for comparison with the bulk feedstock powders. The aim was to elucidate the influence, if any, of particle size and multi-chemical constituent particle composition on the bioaccessibility of individual chemical constituents in artificial gastric juice.

Static dissolution chambers were prepared in triplicate for each powder for evaluation of dissolution in the NIOSH artificial gastric juice formulation. The minimum masses of powder in each dissolution chamber were: 0.0032 g (cobalt), 0.0460 g (tungsten), 0.0489 g (tungsten carbide; 0.0489 g admixture; 0.0252 g (spray dryer); and 0.0278 g (chamfer grinder). The initial masses of metal in each static dissolution chamber were calculated as described for the bulks.

*Cobalt and tungsten analysis and quality control*

All samples and quality control samples were analyzed in accordance with U.S. Occupational Safety and Health Administration (U.S. OSHA) Method ID-213: Tungsten and cobalt in workplace atmospheres (ICP analysis) (US OSHA, 2008). Liquid samples containing dissolved cobalt and/or tungsten were analyzed without digestion to quantify the masses of dissolved metals ($M_D$). For cobalt, matrix-specific method limits of detection (LOD) and quantification (LOQ) were 0.02 and 0.25 mg/L (ASTM and USP formulations) and 0.01 and 0.25 mg/L (NIOSH formulations). For tungsten, LODs and LOQs were 0.02 and 0.25 mg/L (all gastric juice formulations).

Filter samples containing residual particulate cobalt and/or tungsten (i.e., M) were digested and analyzed according to a modified OSHA ID-213. Analytical LODs and LOQs were 0.2 and 0.78 μg for cobalt and 1 and 3.4 μg for tungsten.

*Data analyses*

All statistical analyses were performed using PC-SAS version 9.1 (SAS Institute, Cary, NC). A fully factorial experimental design was employed, enabling experimental investigation of all factor-level combinations for evaluation of joint effects. The data analyses strategy employed non-linear regression followed by ANOVA models. For each experiment, the $\log_{10}$-transformed values of the mass fraction of material remaining ($M/M_0$) versus time (t) in gastric juice were plotted. Single- or multiple-component negative exponential functions were fitted to values of the mass fraction of material remaining ($M/M_0$) versus time (t) in gastric juice (Finch et al., 1988):

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

where y is $\frac{M}{M_0}$

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

$i$ = number of dissolution components (one or two)
$f_i$ = fraction of material dissolved in the $i^{th}$ component

$\lambda_i = k_i SSA$

$k_i$ = chemical dissolution rate constant, $\frac{g}{(cm^2 \cdot d)}$

SSA = Specific surface area, $\frac{cm^2}{g}$

Functions were fitted using the non-linear regression models (proc NLIN) in SAS first with a single component, then with two components. Starting values were provided based on previous research and/or estimates from the data. The models were allowed to run for up to 500 iterations to reach convergence or until no further improvement in reaching the convergence criteria was observed. The optimum number of components required to best describe the data was determined using model convergence and the F-ratio test comparing the model with two components to the model with one component. The model outputs ($f_i$ and $\lambda_i$) were used to calculate the percentage of material dissolved ($f_i \times 100$); $t_{1/2}$, the dissolution half-time ($0.693/\lambda_i$); and, $k_i$, the chemical dissolution rate constant, ($\lambda_i$/SSA) for each sample. One-way analysis of variance (ANOVA) models were then developed to investigate the impact of the fixed effects of gastric juice composition and industrial bulk and size separated powders on the dissolution parameters for cobalt, tungsten metal, and tungsten carbide. The GLM procedure in SAS was used to fit models with $f_i$, $t_{1/2}$, and $k_i$ as the dependent variables and the fixed effects under experimental investigation as the independent factors. One-way ANOVA models were used to investigate the fixed effects of: 1) gastric juice composition on dissolution of feedstock cobalt, tungsten metal, and tungsten carbide; 2) industrial powder composition (and their differing physicochemical characteristics: bulk, admixture, chamfer grinder and spray dryer) on the dissolution of cobalt and tungsten carbide; and 3) powder particle size on the dissolution of cobalt, tungsten metal, and tungsten carbide. In these procedures, the Tukey's test option was specified for multiple comparisons. ANOVA F-statistics were used to note the overall differences in the means for dissolution parameters among the gastric juice and industrial powder composition and size, while Tukey's test was used to identify specific paired differences between the different means.

## Results

The feedstock powders were single-chemical constituent materials whereas the admixture and process-sampled materials were multi-chemical constituent materials (Table 2). Values of SSA were highest for feedstock cobalt powder. SSA values were less than 1 $m^2/g$ for all powders except for the feedstock cobalt and bulk spray dryer powders. Process knowledge coupled with examination of the study materials by electron microscopy revealed aggregate particles (Stefaniak et al., 2007); as expected, the size of the particles collected in the aerosol cyclone decreased with decreasing aerodynamic diameter (data not shown).

### *Dissolution experiments*

Throughout all experiments with the bulk and size-separated powders, the pH of each gastric juice formulation remained within $\pm 0.1$ of its initial value. As expected with methods that use acid digestion to dissolve poorly soluble polydisperse particles, average recovery rates (the sum of the masses of dissolved metal and residual particulate metal determined by spectroscopy and divided by the initial gravimetric metal mass) for the study materials were highly variable and less than quantitative: cobalt, 68 $\pm$ 12% (range 48 to 87%); tungsten, 68 $\pm$ 12% (range 45 to 88%); and tungsten carbide, 70 $\pm$ 22% (range 35 to 100%). As such, to minimize error in dissolution parameter estimates, only the initial masses of metal from the gravimetric and quantitative XRD data and the masses of soluble metals determined by spectroscopy were used in calculations.

### *Bulk powders*

Fig. 1 is a plot of the dissolution kinetics of bulk cobalt, tungsten, and tungsten carbide powders in the NIOSH artificial gastric juice formulation. Cobalt exhibited biphasic dissolution, consisting of an initial rapid dissolution phase followed by a second long-term dissolution phase. Tungsten and tungsten carbide powders exhibited a single dissolution phase in this solvent. Table 3 summarizes the fitted parameters of functions describing the dissolution of bulk study powders in different formulations of artificial gastric juice. Each cobalt data set was best described by a two-component negative exponential function, whereas a one-component negative exponential was adequate to describe tungsten metal and tungsten carbide dissolution. Of the total mass of cobalt that dissolved from the bulk powder, approximately 20% was released with a half-time of 0.02 days in the first dissolution phase and the remaining 80% was released with a half-time of 0.5 to 1 days in the latter long-term dissolution phase. Note that $f_i$ is the percentage of the total material that dissolved in a given phase, i.e., amount of material available for absorption, not the total mass of all material that dissolved. For cobalt, there were no differences in the fractions of material dissolved ($f_1$ or $f_2$), half-times ($t_{1/2}$), or chemical dissolution rate constants ($k_1$ or $k_2$) among the four gastric juice formulations based on the ANOVA models. For bulk tungsten metal and tungsten carbide, all of the material that dissolved was released in the first dissolution phase ($f_1$). For tungsten, there were no differences in estimates of $f_1$; however, $t_{1/2}$ and $k_1$ differed among the solvent formulations. Tukey's multiple comparison test indicated that tungsten $t_{1/2}$ = 381 $\pm$ 84 days was longer in the USP formulation than in the ASTM and NIOSH formulations ($p < 0.05$). Values of $k_1$ for tungsten metal were significantly different among all solvent formulations ($p < 0.05$): NIOSH ($k_1$ = 1.7 $\pm$ 0.1 $\times$ $10^{-6}$ $g/cm^2day$) > ASTM ($k_1$ = 8.8 $\pm$ 0.3 $\times$ $10^{-7}$ $g/cm^2day$) > USP ($k_1$ = 2.8 $\pm$ 0.6 $\times$ $10^{-7}$ $g/cm^2day$). For bulk tungsten carbide, there were no differences in $f_1$ among the solvent formulations. Using Tukey's multiple comparison, $t_{1/2}$ (292 $\pm$ 30 days) for tungsten carbide in the USP formulation was significantly slower than in the ASTM and NIOSH solvent formulations ($p < 0.05$). Values of $k_1$ for tungsten carbide were significantly different among all formulations ($p < 0.05$): NIOSH > ASTM > USP. In each of the gastric juice formulations, values of all dissolution parameters for bulk cobalt powder differed from bulk tungsten metal and tungsten carbide powders based on ANOVA models ($p < 0.05$).

### *Aerodynamically size-separated powders*

Table 4 summarizes the fitted dissolution parameters for the aerodynamically size-separated powders in the NIOSH formulation of artificial gastric juice. There were no differences in values of $f$, $t_{1/2}$, and $k$ among the size-separated cobalt, tungsten metal, and tungsten carbide powders and their corresponding bulk powder. Regarding the cobalt constituent of the heterogeneous powders, the value of $f_1$ for the admixture (30%) was higher than pure cobalt powder ($p < 0.05$) and the value of $f_2$ (70%) was lower ($p < 0.05$); no differences were observed for spray dryer and chamfer grinder powders. In the initial dissolution phase, there was no difference in cobalt $t_{1/2}$ among any of the heterogeneous materials and pure cobalt. In the second long-term cobalt dissolution phase, $t_{1/2}$ and $k_2$ for chamfer grinder powder and $k_2$ for spray dryer powder differed from pure cobalt

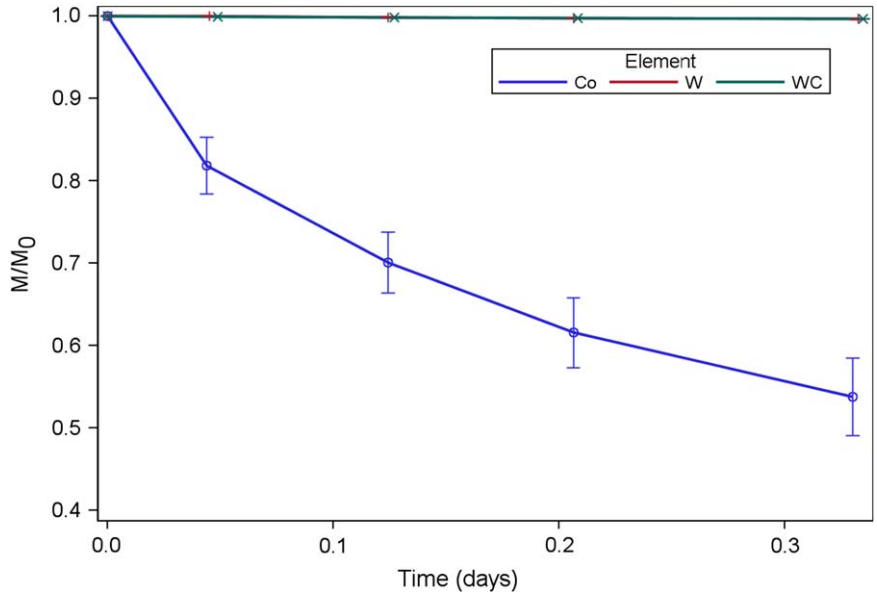


**Fig. 1.** Dissolution of bulk cobalt powder (n=3), tungsten powder (n=3), and tungsten carbide powder (n=3) in the NIOSH artificial gastric juice formulation. Cobalt exhibited biphasic dissolution behavior consisting of a rapid initial phase followed by a second long-term phase whereas both tungsten and tungsten carbide exhibited a single dissolution phase.

**Table 3**
Parameters and dissolution rate constants of bulk powder materials in different artificial gastric juice formulations.

| | | Dissolution function parameters (Mean ± Standard Deviation)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | | | II | | |
| Material | Formulation | $f_1$ (%) | $t_{1/2}$ (days) | $k_1$ (g/cm$^2$day) | $f_2$ (%) | $t_{1/2}$ (days) | $k_2$ (g/cm$^2$day) |
| Cobalt | ASTM[b] | 18 ± 4 | 0.02 ± 0.00 | $4.3 \pm 0.3 \times 10^{-4}$ | 82 ± 4 | 1.1 ± 0.6 | $1.0 \pm 0.5 \times 10^{-5}$ |
| | USP | 23 ± 2 | 0.02 ± 0.00 | $4.5 \pm 0.6 \times 10^{-4}$ | 77 ± 2 | 0.6 ± 0.2 | $1.5 \pm 0.1 \times 10^{-5}$ |
| | NIOSH | 21 ± 2 | 0.03 ± 0.02 | $3.6 \pm 1.6 \times 10^{-4}$ | 79 ± 2 | 0.6 ± 0.0 | $1.7 \pm 0.6 \times 10^{-5}$ |
| | modNIOSH[c] | 20 ± 7 | 0.02 ± 0.00 | $4.3 \pm 0.9 \times 10^{-4}$ | 80 ± 7 | 0.7 ± 0.1 | $1.4 \pm 0.2 \times 10^{-5}$ |
| Tungsten | ASTM | 100 ± 0 | 116 ± 4 | $8.8 \pm 0.3 \times 10^{-7}$ | – | – | – |
| | USP | 100 ± 0 | 381 ± 84 | $2.8 \pm 0.6 \times 10^{-7}$ | – | – | – |
| | NIOSH | 100 ± 0 | 60 ± 2 | $1.7 \pm 0.1 \times 10^{-6}$ | – | – | – |
| Tungsten carbide | ASTM | 100 ± 0 | 129 ± 27 | $8.3 \pm 1.5 \times 10^{-7}$ | – | – | – |
| | USP | 100 ± 0 | 292 ± 30 | $3.6 \pm 0.4 \times 10^{-7}$ | – | – | – |
| | NIOSH | 100 ± 0 | 73 ± 9 | $1.4 \pm 0.2 \times 10^{-6}$ | – | – | – |

– indicates parameters that are not applicable for materials with one-component dissolution behavior.

[a] 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.

[b] Results are for n = 2 samples. Inclusion results from of a suspect third sample yielded mean $k_1 = 1.2 \pm 1.4 \times 10^{-3}$ and $k_2 = 1.2 \pm 0.5 \times 10^{-5}$ g/(cm$^2$ day).

[c] Results are for n = 2 samples. Inclusion of results from a suspect third sample yielded mean $k_1 = 2.3 \pm 3.2 \times 10^{-3}$ and $k_2 = 1.1 \pm 0.5 \times 10^{-5}$ g/(cm$^2$ day).

powder. There were no differences in $f_1$ for the tungsten carbide constituent of the heterogeneous powders relative to the size-separated pure material; however, $t_{1/2}$ for tungsten carbide in the spray dryer material was longer ($p < 0.05$). There was no difference in $k_1$ between pure tungsten carbide powder and the admixture, whereas values were slower for the spray dryer ($p < 0.05$) and faster for the chamfer grinder ($p < 0.05$) material.

## Discussion

### *Bioaccessibility of cobalt and tungsten*

Using different formulations of artificial gastric juice with varying chemical complexity, we observed that both cobalt and tungsten were bioaccessible. The dissolution rate of pure cobalt was at least two orders of magnitude faster than either pure tungsten metal or tungsten carbide due to the unique physico-chemical properties of the cobalt material, including higher SSA. All dissolution parameters determined for the feedstock bulk cobalt, tungsten metal, and tungsten carbide powders were the same for the corresponding aerodynamically size-separated materials indicating that bulk powders were representative materials for *in vitro* studies of bioaccessibility. Based on the design parameters of test mass and solvent volume and to simplify the experimental approach used in this study, none of the samples were agitated during the extraction period. Though it is generally believed that agitation may increase dissolution in a test system, the exact influence of solvent or sample movement on dissolution does not appear to be well understood. Indirect evidence from existing research on estimating oral bioaccessibility suggests that sample movement may be less important than solvent pH (Oomen et al., 2002) or separation protocols (Van de Wiele et al., 2007). For example, Oomen et al. (2002) performed an inter-laboratory study using five different in vitro digestion models; each used a different technique to simulate movement in

**Table 4**
Parameters and dissolution rate constants of aerodynamically size-separated (multi-stage aerosol cyclone stage 2) powder materials in NIOSH artificial gastric juice formulation.

| | | Dissolution function parameters (Mean ± Standard Deviation)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | | | II | | |
| Material | Constituent | $f_1$ (%) | $t_{1/2}$ (days) | $k_1$ (g/cm$^2$day) | $f_2$ (%) | $t_{1/2}$ (days) | $k_2$ (g/cm$^2$day) |
| Cobalt | Co | 19 ± 2 | 0.02 ± 0.00 | 6.0 ± 0.5 × $10^{-4}$ | 81 ± 2 | 0.7 ± 0.1 | 1.8 ± 0.4 × $10^{-5}$ |
| Tungsten | W | 99 ± 1 | 51 ± 10 | 1.5 ± 0.7 × $10^{-6}$ | – | – | – |
| Tungsten carbide | WC | 100 ± 0 | 93 ± 11 | 1.0 ± 0.0 × $10^{-6}$ | – | – | – |
| Admixture | Co | 29 ± 2 | 0.01 ± 0.00 | 1.3 ± 0.5 × $10^{-3}$ | 71 ± 2 | 0.5 ± 0.2 | 2.4 ± 1.3 × $10^{-5}$ |
| | WC | 100 ± 0 | 124 ± 12 | 1.0 ± 0.0 × $10^{-6}$ | – | – | – |
| Spray dryer | Co | 13 ± 5 | 0.03 ± 0.02 | 3.4 ± 1.6 × $10^{-3}$ | 87 ± 5 | 0.9 ± 0.2 | 1.0 ± 0.3 × $10^{-4}$ |
| | WC | 100 ± 0 | 494 ± 112 | 1.8 ± 0.5 × $10^{-7}$ | – | – | – |
| Chamfer grinder | Co | 11 ± 3 | 0.01 ± 0.01 | 2.7 ± 3.4 × $10^{-2}$ | 89 ± 3 | 1.7 ± 0.3 | 7.0 ± 1.2 × $10^{-5}$ |
| | WC | 100 ± 0 | 51 ± 11 | 2.5 ± 0.7 × $10^{-6}$ | – | – | – |

– indicates parameters that are not applicable for materials with one-component dissolution behavior.

[a] Values were obtained from one- or two-component negative exponential functions fitted by nonlinear least squares regression to each constituent of three samples for each powder.

the stomach, i.e., rotation (30 or 50 rpm), agitation (200 rpm), stirring (150 rpm), or peristaltic movements. The authors concluded that the wide range of bioaccessibility estimates for arsenic, cadmium, and lead in soil was explained on the basis of gastric solvent pH employed in each model, not movement of the samples. In a study of lead bioaccessibility, Van de Wiele et al. (2007) concluded that the laboratory separation method (i.e., centrifugation, filtration, ultrafiltration) employed to separate the bioaccessible fraction from the non-bioaccessible fraction largely influenced results. Given uncertainty of the influence of agitation, or lack thereof, some caution is warranted when interpreting our our absolute measures of bioaccessibility.

The focus of the current study was on the role, if any, of gastric juice chemistry on bioaccessibility. In our study, dissolution of cobalt was not influenced by the chemical composition of the solvents. The NIOSH and the modified NIOSH formulations contained levels of cystine, methionine, and glycine consistent with human gastric juice; however, these levels may be too low to influence dissolution. For example, the NIOSH gastric juice formulation contained 2 × $10^{-4}$ M glycine to match adult human gastric juice, whereas 1 × $10^{-2}$ M glycine was the lowest tested concentration observed by Takahashi and Koshi (1981) to influence cobalt dissolution in simulated lung fluid.

In our study, 45% of cobalt metal powder (18% at 1 hour, 31% at 3 hours, and 41% at 6 hours), 33% of the total cobalt in the pre-sintered spray dryer material, and 23% of the cobalt in the post-sintered chamfer grinder material dissolved in eight hours. Stopford et al. (2003) reported that 100% of cobalt metal, 25% of total cobalt in a pre-sintered material, and 24% of cobalt in a post-sintered cobalt cemented carbide material dissolved in 72 hours in the ASTM gastric juice formulation. Direct comparison of results between studies is difficult because Stopford et al. used a protocol in which if a material was >50% soluble at 2, 5, or 24 hours, the authors stopped the test prior to the scheduled 72 hour endpoint and assumed the material was 100% soluble. Nonetheless, results from their experiments and the current experiments agree that cobalt metal and hard metals are highly soluble in gastric juice during physiologically-relevant residence times in the stomach.

Dissolution rates of the poorly soluble bulk tungsten metal and tungsten carbide powders materials in the chemically-complex NIOSH formulation were an order of magnitude faster than observed in the ASTM and USP formulations indicating that NIOSH solvent composition was an important experimental factor. Dissolution rates of tungsten and tungsten carbide were significantly faster in the ASTM formulation relative to the USP formulation. The USP formulation included pepsin, which is thought to be important for dissolution in the stomach (Dean and Ma, 2007); however, dissolution was slower relative to the ASTM formulation. In water, tungsten is oxidized to form tungstate ion, which forms insoluble amorphous tungstic acid precipitates as pH approaches 1 (Lassner and Schubert, 1999). As such, the slight difference in pH between these solvents may have contributed to formation of varying amounts of tungstic precipitate. Alternatively, the observed difference may be the result of variability in the data due to the small masses (< 0.4%) of materials dissolved in these solvents. It is important to note that among all gastric juice formulations, the dissolution half-times for tungsten and tungsten carbide were long, i.e., 60 to 380 days, and the associated k-values were slow. With respect to residence times in the stomach, 0.06, 0.2, and 0.3% of tungsten and 0.04, 0.1, and 0.2% of tungsten carbide would dissolve in 1, 3, and 6 hours, respectively. Thus, relative to the short residence time of materials in the stomach, these observed statistical differences in dissolution among solvent formulations may not be relevant.

As expected, values of $k$ for the cobalt and tungsten carbide constituents of the admixture powder did not differ from the corresponding feedstock powders. This outcome supports our assumption that dissolution of each constituent was independent despite the physical association of these constituents in the agglomerated powder. For the spray dryer powder, the value of $k_1$ for tungsten carbide was slower and the value of $k_2$ for cobalt was faster relative to the feedstock powders, suggesting that dissolution of these constituents may not be independent in this chemically heterogeneous material. For chamfer grinder powder, both $k_1$ for tungsten carbide and $k_2$ for cobalt were faster relative to the pure powders. These differences in dissolution rates may be attributable to the chemically bonded cobalt and tungsten carbide on the grinder particle surfaces that enhance the dissolution of each constituent. Additionally, the observed differences in $k$-values may be due to the assumption that the total powder surface area measured by gas adsorption (a non-chemical specific technique) was equal to cobalt and tungsten carbide chemistry. From Equation 1, if the surface were a mixture of these two chemical constituents, only a portion of the total particle surface area would be cobalt (or tungsten carbide), resulting in artificially fast $k$-value estimates for both constituents.

*Implications for occupational and environmental health*

The high bioaccessible fraction of cobalt and short dissolution life-time, coupled with near complete absorption of solubilized cobalt across the gastrointestinal tract (Christensen et al., 1993; Christensen and Poulsen, 1994; Leggett, 2008; WHO, 2006) will influence internal dose. Currently, use of bioavailability data for environmental risk assessment is focused on the extent to which material is absorbed rather than the rate. In this study, *f*, the fraction of material that dissolved and was available for absorption, was estimated in vitro as a surrogate for bioavailability. Validation of these data for environmental risk assessment would require correlating the fraction dissolved to the fraction of material absorbed in appropriate animal models. In contrast, for the work environment, knowledge of the rate at which a material dissolves ($k_i$) has critical implications for biomonitoring using urine or blood. A rapid dissolution rate corresponds to a short dissolution half-time which influences the timing of biological sample collection. For example, the current practice for inhalation exposure is to collect a urine sample at the end of the work week; however, following an accidental cobalt ingestion event it may be more prudent to sample mid-shift or end-of-shift. But it should also be noted that peak concentrations in blood or urine are influenced by other toxicokinetic parameters and that bioaccessibility kinetics are influenced by parameters not investigated in this study such as a person's diet.

Note also that the ingestion of equal masses of different sized material can result in substantially different bodily exposure. For example, monodisperse cobalt particles with physical diameters of 0.1, 1, 10, and 100 μm may each have an initial characteristic *k* value on the order of $4 \times 10^{-4}$ g/cm$^2$day (based on the dissolution results for artificial gastric juice formulations used in this study), but can be expected to have the substantially differing dissolution half-times of 0.03, 0.3, 3, and 30 days, respectively. Therefore, given the exponential nature of the surface-proportional dissolution model (Equation 1), the approximate fraction of material dissolved for each of these particle sizes during a brief residence time in the stomach (up to 3 hour) would be 95, 25, 3, and only 0.3%, respectively. Exposure assessment and occupation health protection requires an understanding of both the intrinsic dissolution behavior of the ingested material as well as of the particle size distribution of the ingested material (as it affects the available SSA of the particles). Our findings support efforts to mitigate occupational and environmental ingestion of cobalt to reduce total-body exposures.

In this in vitro study, the particulate tungsten compounds had relatively low bioacessibilty, with $<0.4\%$ of the total mass of material dissolving in eight hours. Keith et al. (2007) reviewed the health effects of tungsten and reported that following oral administration, absorption of sodium tungstenate was 40% to 90% in rats and dogs. For tungsten oxide, about 25% was absorbed from the gastrointestinal tract of the dog. No quantitative data are available to describe absorption of tungsten in humans following oral exposure; however, one study reported excretion rates for non-essential elements including tungsten and estimated that 60% of total tungsten (chemical forms unknown) was absorbed across the gastrointestinal tract. Several reasons may explain why our tungsten-containing study materials had relatively low bioacessibilty in gastric juices. One major factor is the chemical form of these tungsten compounds. Sodium tungstenate is a soluble material that readily dissociates to form ions that are available for absorption during the short material residence time in the gastrointestincal tract. As expected, particulate tungsten oxide had lower absorption than tungstenate, though still higher than the metal and carbide compounds evaluated in this study. The lower solubility of our tungsten metal and carbide compounds relative to tungsten oxide may be due to differences in crystallinity, surface area, and other physicochemical properties and/or the composition of our dissolution solvent relative to the gastric juice of the dog. A second factor may be follow-up time after exposure. In these animal studies, urine and/or fecal samples were collected at several time points up to 24 hours and for as long as 13 weeks. In the current study, dissolution was only followed for 8 hours in the gastric compartment. Finally, this comparison of in vitro bioaccessibility and in vivo bioavailability estimates raise an interesting question as to whether any short-term (*ca.* hours) in vitro test system, including the one used in this study, are appropriate for modeling gastrointestinal dissolution of low solubility materials such as tungsten compounds. Although our explanations are plausible, the exact reason why bioaccessibility of tungsten varies among compounds is not clearly understood. As such, incorporation of these tungsten metal and carbide bioaccessibility values into human risk assessment should be done cautiously. Additionally, a limitation of in vitro test systems in general is that these systems cannot accurately mimic all conditions of the human gastrointestinal tract and caution should be used when using any in vitro data for risk assessment. For example, in this study dissolution half-times of 60 to 380 days were calculated from an eight hour study and therefore are only indications of the likely longer-term behavior. Nonetheless, in vitro test systems still provide useful data on dissolution behavior of poorly soluble materials that can be used for risk assessment or design of in vivo studies.

Despite the general limitations of any in vitro test system described above, our bioaccessibility data for tungsten metal and tungsten carbide in artificial gastric juice may still help to inform exposure and biomonitoring measurements, such as those reported for cemented tungsten carbide workers (Kraus et al., 2001) and residents of Churchill County, Nevada (Rubin et al., 2007). Results reported in the current study indicate that particulate forms of tungsten metal and tungsten carbide are poorly soluble in the NIOSH artificial gastric juice formulation (*ca.* $1 \times 10^{-6}$ g/cm$^2$ day) which corresponds to a low bioaccessible fraction of material and dissolution life-time. Animal studies indicate that absorption of tungsten compounds in the gastrointestinal tract is 25 to 90%, depending on the chemical form of the compound (Keith et al., 2007). If we assume that particulate tungsten was present in the drinking water, then as described previously, we can also assume that $<0.3\%$ would dissolve given $t_{1/2}$ of 60 to 380 days and k on the order of $1 \times 10^{-6}$ g/cm$^2$day during a short residence time (up to six hours) in the stomach . Thus, a negligible amount of dissolved tungsten would be available for absorption across the gastrointestinal tract. As such, it is plausible that ingestion of particulate tungsten metal or carbide may not contribute appreciably to dose and subsequently to urinary levels. Alternative exposure pathways such as inhalation of anthropogenic tungsten carbide particles (Sheppard et al., 2007) may be relevant to understanding tungsten exposure and biomonitoring results among residents in Churchill County (Rubin et al., 2007) and among occupationally exposed workers (Kraus et al., 2001).

## Summary

Careful attention should be given not only to the chemical composition of artificial gastric juice but also to the type of metal when estimating oral bioaccessibility of poorly soluble metal compounds such as tungsten metal and tungsten carbide. In contrast, more simplistic formulations of artificial gastric juice may be appropriate when estimating oral bioaccessibility of highly soluble metals such as cobalt because the composition of artificial gastric juice appears to be less influential. The dissolution behavior of a material can be described in terms of fraction of dissolved material, dissolution half-time, and dissolution rate. Accurate understanding of the fraction (*f*), $t_{1/2}$, and rate (*k*) at which a material dissolves is important for understanding absorption and biomonitoring, respec-

tively. For highly soluble cobalt, our bioaccessibility data support consideration of the oral exposure route as a contributing pathway to total-body exposure whereas for poorly soluble tungsten and tungsten carbide, ingestion exposure may not contribute appreciably to total tungsten body burden given the short residence time of material in the stomach and relatively long dissolution half-times of these materials.

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## References

Alexander, C.S., 1969. Cobalt and the heart. Ann. Intern. Med. 70, 411–413.
Alexander, C.S., 1972. Cobalt-beer cardiomyopathy. A clinical and pathological study of twenty-eight cases. Am. J. Med. 53, 395–417.
American Society of Testing Materials, 2003. D5517-03: A Standard Method of Determining the Solubility of Metals in Art Materials. ASTM International, West Conshohocken, PA.
Ansoborlo, E., Chalabreysse, J., Hengé-Napoli, M.-H., Pujol, E., 1992. In vitro chemical and cellular tests applied to uranium trioxide with different hydration states. Environ. Health Perspect. 97, 139–143.
Ansoborlo, E., Guilmette, R.A., Hoover, M.D., Chazel, V., Houpert, P., Hengé-Napoli, M.-H., 1998. Application of in vitro dissolution tests to different uranium compounds and comparison with in vivo data. Rad. Protect. Dosim. 79, 33–37.
Ansoborlo, E., Hengé-Napoli, M.-H., Chazel, V., Gibert, R., Guilmette, R.A., 1999. Review and critical analysis of available in vitro dissolution tests. Health Phys. 77, 638–645.
Bish, D.L., Howard, S.A., 1988. Quantitative phase analysis using the Rietveld method. J. Appl. Crystallogr. 21, 86–91.
Brookes, K.J.A., 1983. Cemented Carbides for Engineers and Tool Users. International Carbide Data, East Barnet, UK, pp. 1–19.
Carson, B.L., Ellis, H.V., McCann, J.L., 1986. Toxicology and Biological Monitoring of Metals in Humans. Lewis Publishers Inc., Chelsea, MI, pp. 85.
Cherrie, J.W., Semple, S., Saleem, A., Hughson, G.W., Philips, A., 2006. How important is inadvertent ingestion of hazardous substances at work? Ann. Occup. Hyg. 50, 693–704.
Chipera, S.J., Bish, D.L., 2002. FULLPAT: a full-pattern quantitative analysis program for X-ray powder diffraction using measured and calculated patterns. J. Appl. Crystallogr. 35, 744–749.
Christensen, J.M., Poulsen, O.M., Thomsen, M., 1993. A short-term cross-over study on oral administration of soluble and insoluble cobalt compounds: sex differences in biological levels. Int. Arch. Occup. Environ. Health 65, 233–240.
Christensen, J.M., Poulsen, O.M.A., 1994. 1982–1992 surveillance programme on Danish pottery painters. Biological levels and health effects following exposure to soluble or insoluble cobalt compounds in cobalt blue dyes. Sci. Total Environ. 150, 95–104.
Day, G.A., Virji, M.A., Stefaniak, A.B., 2009. Characterization of exposures among cemented tungsten carbide workers. Part II: Assessment of surface contamination and skin exposures to cobalt, chromium and nickel. J. Expo. Sci. Environ. Epidemiol. 19, 423–434.
Dean, J.R., Ma, R., 2007. Aproaches to assess the oral bioaccessibility of persistent organic pollutants: a critical review. Chemosphere 68, 1399–1407.
Eidson, A.F., Mewhinney, J.A., 1980. In vitro solubility of uranium yellowcake samples from four uranium mills and the implications for bioassay interpretation. Health Phys. 39, 893–902.
Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B.T., Lioy, P.J., 2001. Oral bioavailability of lead and arsenic from a NIST standard reference material. Arch. Environ. Contam. Toxicol. 40, 128–135.
Finch, G.L., Mewhinney, J.A., Eidson, A.F., Hoover, M.D., Rothenberg, S.J., 1988. In vitro dissolution characteristics of beryllium oxide and beryllium metal aerosols. J. Aerosol Sci. 19, 333–342.
Gibaldi, M., 1984. Biopharmaceutics and Clinical Pharmacokinetics 3rd ed. Lea and Febiger, Philadelphia, PA.
Hoover, M.D., Castorina, B.T., Finch, G.L., Rothenberg, S.J., 1989. Determination of the oxide layer thickness on beryllium metal particles. Am. Ind. Hyg. Assoc. J. 50, 550–553.
Inorganic Crystal Structure Database, Copyright 2003–2005 by Fachinformationszentrum (FIZ), Karlsruhe, http://icsdweb.fiz-karlsruhe.de/index.php (last accessed January 26, 2009).
Johansson, A., Lundborg, M., Hellström, P.-Å., Camner, P., Keyser, T.R., Kirton, S.A., et al., 1980. Effect of iron, cobalt, and chromium dust on rabbit alveolar macrophages: a comparison with the effects of nickel dust. Environ. Res. 21, 165–176.
Kanapilly, G.M., Raabe, O.G., Goh, C.H.T., Chimenti, R.A., 1973. Measurement of in vitro dissolution of aerosol particles for comparison to in vivo dissolution in the lower respiratory tract after inhalation. Health Phys. 24, 497–507.
Keith, L.S., Moffett, D.B., Rosemond, Z.A., Wohlers, D.W., 2007. ATSDR evaluation of health effects of tungsten and relevance to public health. Toxicol. Ind. Health 23, 347–387.
Kennedy, A., Dornan, J.D., King, R., 1981. Fatal myocardial disease associated with industrial exposure to cobalt. Lancet 1, 412–414.
Kraus, T., Schramel, P., Schaller, K.H., Zöbelein, P., Weber, A., Angerer, J., 2001. Exposure assessment in the hard metal manufacturing industry with special regard to tungsten and its compounds. Occup. Environ. Med. 58, 631–634.
Lassner, E., Schubert, W.-D., 1999. Tungsten: Properties, Chemistry, Technology of the Element, Alloys, and Chemical Compounds. Kluwer Academic/Plenum Publishers, New York, pp. 86–123.
Leggett, R.W., 2008. The biokinetics of inorganic cobalt in the body. Sci. Total Environ. 389, 259–269.
Mercer, T.T., 1967. On the role of particle size in the dissolution of lung burdens. Health Phys. 13, 1211–1221.
Morin, Y., Têtu, A., Mercier, G., 1971. Cobalt cardiomyopathy: clinical aspects. Br. Heart J. 33 (Suppl.), 175–178.
Oomen, A.G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., et al., 2002. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. Environ. Sci. Technol. 36, 3326–3334.
Rubin, C.S., Holmes, A.K., Belson, M.G., Jones, R.L., Flanders, W.D., Kieszak, S.M., et al., 2007. Investigating childhood leukemia in Churchill County, Nevada. Environ. Health Perspect. 115, 151–157.
Ruby, M.V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., et al., 1999. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. Environ. Sci. Technol. 33, 3697–3705.
Sheppard, P.R., Toepfer, P., Schumacher, E., Rhodes, K., Ridenour, G., Witten, M.L., 2007. Morphological and chemical characteristics of airborne tungsten particles of Fallon, Nevada. Microsc. Microanal. 13, 296–303.
Smith, W.B., Wilson, R.R., Harris, D.B., 1992. A five-stage cyclone system for in situ sampling. Environ. Sci. Technol. 13, 1387–1392.
Stefaniak, A.B., Day, G.A., Hoover, M.D., Breysse, P.N., Scripsick, R.C., 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, A.B., Day, G.A., Harvey, C.J., Leonard, S.S., Schwegler-Berry, D.E., Chipera, S.J., et al., 2007. Characteristics of dusts encountered during the production of cemented tungsten carbides. Ind. Health 45, 793–803.
Stefaniak, A.B., Turk, G.C., Dickerson, R.M., Hoover, M.D., 2008. Size-selective poorly soluble particulate reference materials for evaluation of quantitative analytical methods. Anal. Bioanal. Chem. 391, 2071–2077.
Stefaniak, A.B., Virji, M.A., Day, G.A., 2009. Characterization of exposures among cemented tungsten carbide workers. Part I: Size-fractionated exposures to airborne cobalt and tungsten particles. J. Expo. Sci. Environ. Epidemiol. 19, 475–491.
Stopford, W., Turner, J., Cappellini, D., Brock, T., 2003. Bioaccessibility testing of cobalt compounds. J. Environ. Monit. 5, 675–680.
Takahashi, H., Koshi, K., 1981. Solubility and cell toxicity of cobalt, zinc and lead. Ind. Health 19, 47–59.
United States Occupational Safety & Health Administration (US OSHA), 2008. Method Number ID-213: Tungsten and cobalt in workplace atmospheres (ICP analysis). http://www.osha.gov/dts/sltc/methods/inorganic/id213/id213.html, (accessed November 13, 2008).
Van de Wiele, T.R., Oomen, A.G., Wragg, J., Cave, M., Minekus, M., Hack, A., et al., 2007. Comparison of five in vitro digestion models to in vivo experimental results: lead bioaccessibility in the human gastrointestinal tract. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 15, 1203–1211.
World Health Organization, 2006. Concise International Chemical Assessment Document No. 69: Cobalt and Inorganic Cobalt Compounds. WHO Press, Geneva, Switzerland 13 pp.