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Mineralogy affects geoavailability, bioaccessibility and bioavailability of zinc

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Abstract

We correlated mineralogical and particle characteristics of Zn-containing particles with Zn geoavailability, bioaccessibility, and bioavailability following gavage and intranasal (IN) administration in rats. We compared samples of Zn/Pb mine waste and five pulverized pure-phase Zn minerals (<38 μm). Particles were neutron-activated to produce radioactive 65 Zn. We assessed geoavailability using sequential extractions and bioaccessibility using in vitro extraction tests simulating various pH and biological conditions. Zn in vivo bioavailability and in vitro bioaccessibility decreased as follows: mine waste > hydrozincite > hemimorphite > zincite ≈ smithsonite ≫ sphalerite. We found significant correlations among geoavailability, bioaccessibility and bioavailability. In particular, Zn bioavailability post-gavage and post-IN was significantly correlated with bioaccessibility in simulated phagolysosomal fluid and gastric fluid. These data indicate that solid phase speciation influences biological uptake of Zn and that in vitro tests can be used to predict Zn bioavailability in exposure assessment and effective remediation design.

Keywords
zinc; metal speciation; bioaccessibility; bioavailability; sequential extractions; physiologically-based extraction tests

1. Introduction

Remediation of contaminated sites requires accurate assessments of risk, yet total contaminant concentrations alone are often poor predictors of uptake and toxicity. Human health risks from contaminants are determined by inherent chemical toxicity, extent of exposure and contaminant bioavailability (fraction of a contaminant in exposure media that can be absorbed by organisms). Thus, evaluating bioavailability can improve dose estimation from exposure assessment (Kobayashi and Okamura, 2005; Milton and Johnson, ...
1999; Ruby et al., 1996). Metals are common contaminants at mining and other contaminated locations. Children are especially vulnerable to metal-contaminated soils and indoor dust. Hand-to-mouth and inhalation exposures in playgrounds and home environments are common.

In addition to bioavailability, metal mobility in the environment and biological uptake are influenced by two other related parameters: geoavailability and bioaccessibility. Geoavailability describes the release of metals from solid phases under environmentally relevant conditions. It is the fraction of total metals that can be released to the biosphere through mechanical, chemical and biological processes (Smith, 2007). Bioaccessibility describes the ability of a metal to be solubilized in a biological fluid after ingestion, such as gastric fluid (following oral ingestion) or phagolysosomal fluid (following macrophage uptake after inhalation).

Multiple mechanisms control absorption of particle-bound metals. Oral bioavailability is influenced by physiological factors such as fasted/fed state, associated motility, gastric emptying, intestinal transit time, variability in gastrointestinal (GI) contents and nutritional status (Amidon et al., 1995). For instance, iron (Fe) status regulates expression of divalent metal transporter 1 (DMT1), which mediates absorption of Fe and many other divalent metals such as manganese (Mn), zinc (Zn), cadmium (Cd), and lead (Pb) (Au et al., 2008). In the respiratory tract, inhaled airborne particles may be deposited depending on their aerodynamic size. The majority (92%) of larger particles (>8 μm) deposit in nasal passages and upper airways, while inhaled smaller particles (<2 μm) deposit in the deep lung (93–97%) where they are phagocytized by macrophages (Brain and Valberg, 1979; Heyder and Svarten, 2002).

Bioavailability also varies widely depending on properties of the metal, metal speciation (distribution among various chemical forms), particle size, morphology, and solubility. Metal sulfides and some other primary metal ores tend to have low bioavailability (Ruby, 2004). However, metal ores undergo temporal shifts in speciation that can affect bioavailability. For example, weathering of sulfide minerals in the presence of oxygen can result in speciation changes and the formation of secondary mineral phases that often have greater bioavailability than the parent ore. Metals can also decrease in bioavailability as they become more strongly incorporated into the soil matrix. Temporal variability in metal bioavailability highlights the value of repeated sampling over time.

In vivo determination of metal bioavailability in animal models is time-consuming and expensive. Moreover, the results must be extrapolated to humans. Thus, simpler in vitro bioaccessibility tests to estimate bioavailability would be valuable. Several studies have estimated oral bioavailability of metals in contaminated soils and mine wastes using in vitro tests with a single extraction or series of extractions designed to mimic the GI system (Bradham et al., 2011; Casteel et al., 2006; Juhasz et al., 2009a; Juhasz et al., 2009b; Navarro et al., 2006; Ruby et al., 1996; Schroder et al., 2004). These studies concluded that the characteristics of each metal (Navarro et al., 2006), pH of the extraction solution (Juhasz et al., 2009a; Juhasz et al., 2009b; Ruby et al., 1996), mineralogical composition of samples (Bradham et al., 2011; Navarro et al., 2006), matrix properties and presence of other organic or inorganic components (Casteel et al., 2006) significantly influenced bioaccessibility.

Validation of in vitro tests as predictors of bioavailability requires evaluating both bioavailability and bioaccessibility on the same materials. Correlations between bioaccessibility and bioavailability have been established for Cd (Juhasz et al., 2010; Schilderman et al., 1997), Pb (Casteel et al., 2006; Kelley et al., 2002; OSWER, 2004; Ruby et al., 1996) and As (Bradham et al., 2011; Juhasz et al., 2009b). Studies of Pb- and As-
contaminated soils showed that in vitro extraction results successfully predicted Pb bioavailability in rats, but over-predicted As bioavailability in rabbits and primates (Ruby et al., 1996). Bioaccessibility of Cd in contaminated soils extracted in simulated gastric or intestinal fluids was significantly correlated with relative bioavailable Cd in swine (Schroder et al., 2004).

Less is known about relationships between bioaccessibility and bioavailability for micronutrient metals such as Zn, Cu and Mn. While the consequences of Zn deficiency are well known (Otten et al., 2006), Zn is also among the top 100 hazardous substances on the U.S. Agency for Toxic Substances and Disease Registry’s Substance Priority List (ATSDR, 2012). Toxicity from excessive intake of Zn is increasingly recognized, including inhalation of zinc oxide fumes in workplaces or polluted environments (El Safty et al., 2008). Consequences include Cu deficiency, immune dysfunction, anemia and other hematological abnormalities (Fosmire, 1990). To our knowledge, there have been no systematic comparisons of Zn bioaccessibility and bioavailability across a range of chemical forms and routes of exposure.

The goals of this study were to determine whether Zn bioavailability and bioaccessibility are influenced by mineralogy and particle characteristics and the extent to which these in vitro bioaccessibility tests and geochemically-based sequential extractions may predict in vivo bioavailability. We determined Zn geoavailability, in vitro bioaccessibility, and in vivo bioavailability following gavage and intranasal administration of five Zn minerals and a Zn-rich mine waste sample. These exposure pathways were selected since larger airborne particles from piles of mine wastes are more likely deposited in the nose than in the lungs and are likely to be ingested via hand-to-mouth transfer, especially by children playing in contaminated sites. We compared common pure-phase Zn minerals with Zn-rich mine waste containing a complex mixtures of different phases. Studying pure phase Zn minerals allows us to directly evaluate the effect of mineralogy on bioavailability and bioaccessibility, and other factors that may affect metal mobility such as organic matter, iron oxides, and cation exchange capacity.

2. Materials and methods

2.1 Particle characterization

We obtained samples of smithsonite [ZnCO$_3$], hydrozincite [Zn$_5$(CO$_3$)$_2$(OH)$_6$], hemimorphite [Zn$_4$Si$_2$O$_7$(OH)$_2$H$_2$O], sphalerite [(Zn,Fe)$_2$S] and zincite [(Zn,Mn)O] (Table 1) from the Harvard University Mineralogical Museum (Cambridge, MA). Samples were ground with a mortar and pestle and then sieved through a 38-μm pore-sized brass sieve with stainless steel wire. In addition, a sample of weathered mine waste collected from a pile at the Tar Creek Superfund Site (Oklahoma, U.S.A.) was air-dried at room temperature and sieved to <38 μm without grinding. This site was a former Pb and Zn mine where the major ores produced were sphalerite and galena (PbS) (McKnight and Fischer, 1970). The high Zn concentrations (up to 22% Zn by mass in <1 μm particles) in mine waste (Schaider et al., 2007) raise concerns about elevated Zn exposures for residents. Previous analyses of the <38 μm mine waste samples using X-ray diffraction showed that 20–35% of Zn was present as hemimorphite and sphalerite and the majority in poorly crystalline secondary mineral forms (Schaider et al., 2007). These particles are small enough to be transported by wind (Duggan et al., 1985) and deposited in the nose and oral pharynx (Brain and Valberg, 1979).

All samples were divided into three aliquots. One was analyzed for specific surface area (SSA) by the Brunauer-Emmett-Teller (BET) method of N$_2$ adsorption at the National Institute for Occupational Safety and Health (Morgantown, WV) (Brunauer et al., 1938). The second set of aliquots was analyzed for size, morphology and elemental composition.
Aqueous suspensions of particles were analyzed for size distribution in a Beckman-Coulter LS 13 320 Laser Diffraction Particle Size Analyzer (LDPSA) (Beckman Coulter, Inc., Brea, CA). Similar suspensions of particles were spread onto carbon adhesive tabs on aluminum scanning electron microscopy (SEM) specimen mount stubs (Electron Microscopy Sciences, PA). After drying under vacuum, morphology and elemental composition were assessed using a Leo 1450 VP scanning electron microscope with energy-dispersive X-ray spectroscopy (SEM-EDX) at 15 kV (Carl Zeiss SMT, Inc., Thornwood, NY). Dry samples of minerals were also analyzed with X-ray diffraction for phase verification.

The third set of aliquots was neutron-activated at the MIT Nuclear Reactor Laboratory (Cambridge, MA) with a thermal neutron flux of $5 \times 10^{13}$ n/cm$^2$/s for 120 hours (mine waste) or 6 hours (pure-phase Zn minerals). Longer neutron activation of mine waste was necessary because the Zn concentration was lower. Neutron irradiation generated $^{65}$Zn, which decays with a half-life of 244.3 days and emits gamma energies of 345, 770 and 1115 keV. These radioactive particles were used in in vitro extractions and geochemically-based extractions and in pharmacokinetic studies in rats. Specific activities for $^{65}$Zn in each particle are listed in Table 1 and reflect their respective Zn concentration. Major and trace elemental composition of the mine waste was determined with instrumental neutron activation analysis.

### 2.2 In vitro extraction tests

We used a range of pH and chemical conditions to measure in vitro bioaccessibility. Simulated gastric fluid extraction was performed in a pH 1.5 fluid (1 hour, 37°C) (OSWER, 2007). A pH 4.5 weak salt solution (5 days, 37°C) was used to simulate some biological environments such as phagolysosomal fluid in phagocytic cells (Stefaniak et al., 2005). A pH 7.4 solution (1 hour, 37°C) was used to simulate the conditions in the nasal epithelium and oropharynx. All extractions were performed with constant mixing using an Adams nutator Model 1105 (Clay Adams, Parsippany, NJ) at 12 rpm. Extraction solutions were filtered through a 0.2 μm syringe tip filter to remove particle-bound $^{65}$Zn. Since some particles may be smaller than 0.2 μm, the filtrate was then centrifuged at 317,000 x g and the supernatant was carefully removed. This extra step reduced the probability of measuring $^{65}$Zn in very small particles that may pass through the filter. For each extraction, bioaccessibility was calculated as the percent of total $^{65}$Zn that was dissolved and present in the filtered and ultracentrifuged solution.

Simulated gastric fluid extractions followed a protocol developed by the U.S. Environmental Protection Agency (USEPA) for Pb and validated with in vivo results (OSWER, 2007). The solid:liquid ratio was modified to 1:1000. While the method recommends a solid:liquid ratio of 1:100, it also notes the potential for precipitation of dissolved metal when extractions are performed on solid phases with concentrations above 50,000 ppm (OSWER, 2004, 2007). The pure phase Zn minerals used in this study had 52–73% Zn by mass. To maintain the same limit on Zn concentration in solution implies that a solid:liquid ratio of 1:1000 is appropriate. Although Zn is more soluble than Pb (used for validation), a similar limit on metal concentration in solution was selected to minimize potential precipitation of other secondary mineral phases.

### 2.3 Geochemically-based sequential extractions

Geochemically-based sequential extractions were performed on the six particle samples. This protocol uses a series of increasingly stringent solutions selected to extract metals in 5 operationally defined phases: (I) ion-exchangeable (1 M MgCl$_2$, pH 7, 1 h); (II) carbonate (1 M Na acetate, pH 5, 5 h); (III) reactive sulfide (1 M HCl, 12 h); (IV) crystalline sulfide (concentrated HNO$_3$, 2 h); and (V) residual (remainder after previous extraction steps). The
The protocol was adapted from published methods (Bostick et al., 2001; Huerta-Diaz and Morse, 1992; Keon et al., 2001; Tessier et al., 1979). The solid:liquid ratio was similarly modified to 1:1000. The first two steps encompass parameters that may realistically be found under environmental conditions. Thus, the sum of metals extracted in these two steps can be considered geoavailable.

### 2.4 In vivo bioavailability studies

The protocols were approved by the Harvard Medical Area Animal Care and Use Committee. Weanling Sprague-Dawley male rats were obtained from Taconic Farms (Germantown, NY) and housed at the Harvard Center for Comparative Medicine. They had access to commercial chow (PicoLab Rodent Diet 5053, Framingham, MA) and water ad libitum. All animals were acclimatized to our facilities for a week before starting the experiments. Neutron-activated mine waste and Zn mineral particles were suspended in phosphate-buffered saline solution (PBS) at 25 mg/ml for intranasal (IN) (0.2 ml/kg body weight) or at 3.33 mg/ml for gavage administration (1.5 ml/kg). Particles in suspension were dispersed using a bath sonicator for five minutes before dosing. Aliquots of each suspension were measured in a Packard gamma counter (Cobra Quantum, Packard Instrument, Downers Grove, IL), and specific activities were calculated (Table 1). The particle dose was 5 mg/kg for both routes of administration. Based on particle Zn concentrations, the estimated Zn dose ranged from 0.5 to 3.65 mg/kg body weight. Soluble $^{65}$ZnCl$_2$ (specific activity = 2.39 mCi/mg) was obtained from Perkin Elmer (Billerica, MA) and administered at the same volume doses and at a dose of 0.006 mg Zn/kg and 16.1 μCi $^{65}$Zn/kg body weight.

Before IN instillation or gavage, rats were anesthetized with isoflurane (Halocarbons Lab, Inc., North Augusta, SC). The particle suspension or soluble $^{65}$ZnCl$_2$ was instilled into the nasal cavity. Gavage administration was performed in similarly anesthetized rats, placed on a slanted board where they were supported by an elastic band under the upper incisors. Each dose was instilled into the stomach using a gavage needle inserted transorally through the esophagus to the stomach. All rats (six per group) were dosed twice weekly for three weeks to simulate continuous exposure likely to occur in exposed populations. Rats were sacrificed and tissue $^{65}$Zn levels were analyzed four days after the last dosing. The whole brain, spleen, kidneys, heart, liver, lungs, GI tract, testes and multiple samples of blood, bone marrow, skeletal muscle, and skin were collected, weighed, and analyzed in a gamma counter. We did not collect and analyze urine and feces so the amount of $^{65}$Zn that was first absorbed and then excreted cannot be estimated. Absolute bioavailability (ABA) was calculated as the percentage of the total $^{65}$Zn dose retained in each organ. The sum of all analyzed organs and tissues was considered as the total ABA. Relative bioavailability (RBA) was calculated as ABA of each particle sample relative to $^{65}$ZnCl$_2$.

### 2.5 Statistical analyses

Differences in the $^{65}$Zn tissue distribution among the particles were analyzed using multivariate analysis of variance (MANOVA) with Bonferroni (Dunn) post hoc tests using SAS Statistical Analysis software (SAS Institute, Cary, NC). In vitro extraction assays, sequential extractions and particle characteristics were tested as predictors of ABA with multivariate linear regression analyses using Stata/SE statistical software (StataCorp LP, College Station, TX). Bioaccessibility, sequential extraction and bioavailability data were logit transformed to meet assumptions of normality.

### 3. Results and discussion

We compared in vitro extractions and geochemically-based extractions with in vivo bioavailability of Zn in five pure-phase Zn minerals and a Zn-rich mine waste in rats.
also examined the influence of particle characteristics on bioavailability. By measuring Zn
dissolution from radioactive neutron-activated particles, we utilized a highly sensitive
method that measured only $^{65}$Zn released from the particles and excluded background Zn
from dietary sources. The sensitivity of $^{65}$Zn detection also avoided the use of high Zn doses
that may alter Zn homeostasis or saturate Zn transport mechanisms.

3.1 Particle morphology and elemental composition

Particles were examined by SEM (Figure 1), which revealed differences in size, shape and
morphology. The micrographs showed a high degree of heterogeneity in shapes and particle
sizes within and among samples. Elemental mapping using SEM-EDX in randomly selected
micrographs showed that Zn was uniformly distributed in these minerals, regardless of size
and shape. By comparison, mine waste particles had more heterogeneous elemental
distributions (data not shown). Approximately 35% of randomly selected mine waste
particles contained no detectable Zn. Elements associated with host rock minerals (chert,
dolomite) or other metal sulfides (FeS$_2$, PbS) were also detected, including calcium,
magnesium, silicon, arsenic, lead, iron, aluminum, and sulfur. Other metals were also
detected in the Zn mineral particles. Zincite contained manganese, which typically co-occurs
with Zn in this mineral. Smithsonite contained some silicon, calcium and cadmium. XRD
analyses showed that hydromagnesite hemimorphite, sphalerite, and zincite samples were pure
while smithsonite contained 13.1% quartz. Previous XRD analyses of the <38 μm mine
waste samples showed that 20–35% of Zn was present as hemimorphite and sphalerite and
the majority in poorly crystalline secondary mineral forms (Schaider et al., 2007).

3.2 Surface area and size distribution

Higher surface area of particles increases their dissolution rates (Kreyling et al., 1990) and
thus metal bioavailability. Our BET analyses showed that specific surface areas (SSA)
varied significantly (Table 1), with hydromagnesite having 10 to 100 times greater SSA than the
other samples. Based on LDPSA analyses of particle size distributions (Table 1), mine waste
had the smallest geometric mean diameter while hemimorphite had the largest. Although
hydromagnesite had the largest SSA according to BET analyses, its mean and mode particle
diameters were among the largest, suggesting that particle diameter, as measured by
LDPSA, does not necessarily reflect available surface area. As seen in Figure 1F, variable
particle shapes may account for these discrepancies.

3.3 In vitro bioaccessibility tests

Zn bioaccessibility varied widely depending on the mineral form and extraction conditions
(Table 2). Low Zn bioaccessibility was observed at pH 7.4; ≤0.1% of $^{65}$Zn was dissolved
from smithsonite, hydromagnesite, zincite, and sphalerite and <1% of $^{65}$Zn was dissolved from
hemimorphite and mine waste. At pH 4.5, mine waste showed the highest bioaccessibility,
with 92% $^{65}$Zn dissolved into the extraction fluid. Smithsonite, hemimorphite, zincite and
hydromagnesite showed moderate bioaccessibility (35–57%) and sphalerite showed minimal
bioaccessibility (1.1%). Zn bioaccessibility was consistently higher at pH 1.5 than at pH 4.5
or 7.4. Bioaccessibility at pH 1.5 was 98% for mine waste, and 96–97% for hemimorphite,
zincite, and hydromagnesite. Smithsonite showed slightly lower bioaccessibility (72%), and
sphalerite again showed minimal bioaccessibility (1.7%).

Enhanced Zn bioaccessibility under more acidic conditions is consistent with previous
studies showing increased Zn mobility in soils at lower pH (Sauvé et al., 2000) and
increased bioaccessibility of As, Cd and Pb. Juhasz et al. (2009b) compared As
bioaccessibility in contaminated soils using four in vitro gastric fluid extraction solutions
(pH 1.5–2.5) and four in vitro intestinal extractions (pH 5.5–7.5). Similarly, Cd
bioaccessibility was higher in gastric fluid extractions than in intestinal fluid extractions, and
among gastric fluid extractions, the pH 1.5 solution (same composition as in our study) generally showed the highest bioaccessibility 1.5 (Juhasz et al., 2010). Arsenic bioaccessibility was generally higher in gastric extractions than in intestinal extractions, although the differences were less pronounced (Juhasz et al., 2009a).

3.4 Geochemically-based sequential extractions

Mine waste, hemimorphite, zincite and hydrozincite contained Zn in relatively labile phases (Table 3). In these samples, 81–89% of $^{65}\text{Zn}$ was present in extraction stage I and II, representing geoavailable Zn. The majority of remaining Zn (10–16%) was present in stage V (residual fraction), with ≤2% present in stages III and IV. Consistent with in vitro extraction results, sphalerite was the least labile mineral, with >90% of $^{65}\text{Zn}$ extracted in stages IV and V. Surprisingly, only 28% of $^{65}\text{Zn}$ in smithsonite was dissolved in stage II, with 57% of $^{65}\text{Zn}$ in the stage III fraction. This may be due to presence of recalcitrant Si- and Al-containing mineral impurities as well as kinetic constraints that may have led to low dissolution of the carbonate mineral during the extraction period. In addition, for each type of mineral, there may be a range of bioaccessibility depending on the properties of each specific mineral sample. Results from geochemically-based sequential extractions should be viewed as a guide since there are inherent limitations to sequential extractions, such as lack of specificity and the potential sorption or re-precipitation of solubilized metals back onto the solid sample surface (Kheboian and Bauer, 1987; Ostergren et al., 1999).

3.5 In vivo bioavailability of Zn after intranasal and gavage administration in rats

Absolute bioavailability results (Tables 4 and 5) showed that the overall organ distributions for both IN and gavage administration were statistically different among particles and compared to $^{65}\text{ZnCl}_2$ (MANOVA, P<0.05). We considered the total percentage of $^{65}\text{Zn}$ dose in blood and all tissues examined as total ABA, an indicator of its retention in the body. In general, total ABA after IN administration decreased in the following order: mine waste > $^{65}\text{ZnCl}_2$ > hydrozincite > hemimorphite > zincite > smithsonite > sphalerite (Table 4). Interestingly, Zn ABA was higher for mine waste than dissolved $^{65}\text{ZnCl}_2$. We hypothesize that Zn ions from ZnCl$_2$ may quickly form conjugates with proteins in nasal or gastrointestinal mucosa that attenuate their absorption, whereas interactions with Zn from particles may be different due to slow dissolution. The highest amounts of $^{65}\text{Zn}$ were found in the skeletal muscle and liver, mainly due to their large weights. The 5 tissues and organs with highest concentrations of $^{65}\text{Zn}$ were hair, nails, teeth, bone marrow, and liver (data not shown). The low levels of $^{65}\text{Zn}$ in the lungs confirmed that very few IN administered particles were aspirated into the lungs. Therefore, $^{65}\text{Zn}$ in peripheral tissues primarily reflects absorption through the nasal or intestinal epithelium.

A similar distribution of $^{65}\text{Zn}$ among tissues was observed after gavage administration (Table 5). Significant differences among the particles in Zn total ABA were observed in the same decreasing order measured after IN administration. This suggests that tissue uptake of $^{65}\text{Zn}$ after IN dosing likely resulted from swallowed particles. Overall, ABA for $^{65}\text{Zn}$ was 1.2 to 1.6 times higher following gavage compared to IN administration, suggesting greater Zn bioavailability from ingested particles compared to particles deposited in the nasal passage. These relative differences may be due to loss of a portion of the particles deposited in the nasal cavity before being swallowed. ABA post IN and gavage ranged from 0.6 to 13.7% depending on particle type and route of exposure. Estimates of ABA may be slightly underestimated due to the exclusion of urine (Johnson et al., 1988) and body clearance of Zn over the study period. Other factors such as phytic acids in the chow (Davies and Nightingale, 1975), iron and zinc status (Johnson et al., 1988; Miller et al., 1971), dietary proteins (Mahalko et al., 1983), and ingestion of other metals also influence absorption and excretion of Zn (Gooneratne et al., 2011). However, in our experiment, relative differences
among particles are most likely due to the mineralogy and morphology of the particles themselves, since these other factors did not vary in our experiment.

Few studies have compared bioaccessibility and bioavailability of Zn in animals. Zn bioavailability from different grades of ZnO, and Zn metals relative to ZnSO₄, were significantly different confirming that chemical forms of Zn influence bioavailability (Edwards and Baker, 1999). Another study compared Zn and Cu bioaccessibility with bioavailability in lugworms (Arenicola marina) from contaminated marine sediments and found that Zn bioaccessibility in fluids containing serum albumin (a surrogate for the gut fluids of sediment feeders) did not correlate with bioaccumulation (Turner et al., 2008). Low Zn bioavailability from inhaled zinc-cadmium sulfide was reported in rats (Bergmann et al., 2000). Although previous studies have shown that Zn speciation influences bioaccessibility and bioavailability, our study also compares these parameters across a broader range of chemical forms as well as routes of exposure.

3.6 Correlation of mineralogy and particle characteristics with bioavailability and bioaccessibility

Our results show that mineralogy influences bioavailability and bioaccessibility of Zn. Mine waste particles had the highest bioavailability and bioaccessibility at pH 1.5 and 4.5, consistent with our previous findings that the surface of these particles contained Zn in secondary minerals that are poorly crystalline and relatively bioaccessible (Schaider et al., 2007). Mine waste from the Tar Creek site has undergone weathering for at least 35 years. Simulated aging of contaminated soil using EDTA leaching has been shown to increase Pb and decrease Cd mobility and bioaccessibility, as determined by in vitro extractions, whereas Zn bioaccessibility was not affected (Udovic and Lestan, 2009). Zn bioavailability and bioaccessibility in sulfide form (sphalerite) were lowest, consistent with previous studies (Bergmann et al., 2000; Klimisch, 1993). Surprisingly, zinc carbonate (smithsonite) yielded lower bioavailability than hemimorphite. As noted in Section 3.4, a range of factors may have limited the dissolution of smithsonite.

Other studies have shown that particle surface area influences bioavailability. We found that SSA alone was not correlated with Zn ABA, although to some extent, particles with higher SSA tended to have greater bioavailability. The highest SSA was measured for hydrozincite (93.4 m²/g), which may have contributed to the relatively high tissue concentrations of Zn after both IN and gavage administration of hydrozincite. This is followed by hemimorphite (3.9 m²/g) and smithsonite (2.7 m²/g), particles with similar SSA and corresponding ABA. Lower tissue ⁶⁵Zn from zincte and sphalerite corresponded with their lower SSA. However, mine waste had higher Zn bioavailability than hydrozincite despite having 10 times lower SSA. Smithsonite and zincte showed similar Zn ABA, although smithsonite had 3 times higher SSA. Although influenced by SSA, our results demonstrated that the mineralogical form is a major determinant of Zn bioavailability.

3.7 Regression analyses of zinc in vitro bioaccessibility, in vivo bioavailability, and geoavailability

While in vitro tests have been developed for assessing bioaccessibility in a risk assessment context, geochemists have used sequential extractions as one method to assess metal lability and to characterize the speciation of metals in solid phases (Tessier et al., 1979). Depending on the extraction solutions used, sequential extractions may provide a measure of geoavailability. Thus, we conducted regression analyses to examine whether in vitro measures of zinc bioaccessibility were correlated with geoavailability as measured by sequential extractions and with in vivo bioavailability, and whether geoavailability was correlated with in vivo bioavailability (Table 6).
A significant relationship was observed between bioavailability and in vitro bioaccessibility. In vivo bioavailability post-IN installation was significantly correlated with bioaccessibility at pH 4.5 ($R^2=0.86$, $p=0.005$) and at pH 1.5 ($R^2=0.79$, $p=0.01$). In vivo bioavailability post-gavage was significantly correlated with bioaccessibility at pH 4.5 ($R^2=0.83$, $p=0.008$) and at pH 1.5 ($R^2=0.72$, $p=0.02$). Finally, correlation coefficients between bioavailability post-IN and post-gavage with geoavailability were 0.67 ($p=0.03$) and 0.60 ($p=0.04$), respectively. Both in vitro bioaccessibility tests appeared to overpredict absolute bioavailability estimated as the total retained dose. One reason for this apparent overprediction is that part of the bioavailable Zn absorbed by the animals was excreted over the course of three-week dosing experiment. When comparing in vitro bioaccessibility with IN absolute bioavailability, the slopes of the relationship were $y=0.062x – 0.0014$ (pH 1.5) and $y = 0.114 – 0.0031$ (pH 4.5). For gavage ABA, the slopes were $y=0.083x – 0.0003$ (pH 1.5) and $y=0.151x – 0.002$ (pH 4.5). Figure 2 shows correlation of bioaccessibility at pH 4.5 on in vivo RBA of $^{65}$Zn (relative to ZnCl$_2$) post-gavage (Fig 2A) or post-IN instillation (Fig 2B). The strengths of the correlations are shown by the high $R^2$. In both instances, variability in in vitro bioaccessibility accounted for 88% of the variability observed in in vivo bioavailability.

Zinc geoavailability (sequential extraction fractions I + II) was also correlated with bioaccessibility when particle size (mean diameter in LDPSA analyses) was included in the regression models (pH 4.5, $R^2=0.98$, $p=0.002$ and pH 1.5, $R^2=0.99$, $p<0.0001$). The particle size term in the regression models had negative coefficients (Table 6), indicating that larger particles had lower bioaccessibility.

Thus, our data showed that in vitro bioaccessibility tests with simulated gastric fluid extraction or phagolysosomal environment correlate strongly with in vivo bioavailability of Zn from particles of different mineralogy either after being ingested or deposited into the nasal passages.

4. Conclusions

Our data show that in vivo bioavailability of Zn from pure-phase minerals and a complex mixture of Zn phases (mine waste) depends on the chemical form of Zn, particle size, and route of exposure. The trends observed in in vitro extractions and geochemically-based sequential extractions were consistent with the in vivo results. The significance of these correlations confirms that solid phase speciation and geochemical alteration of speciation during weathering can have significant impacts on the biological uptake of zinc. Our study shows that in vitro tests can predict relative bioavailability of micronutrient metals such as Zn and can be useful in exposure assessment and for determining the efficacy of remediation strategies.

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References


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Highlights

- Zinc particle mineralogy influences bioaccessibility and bioavailability.
- Zn bioavailability via gavage was 1.2–1.6 times higher than via intranasal route.
- Zn particle geoavailability correlates with bioaccessibility.
- In vitro bioaccessibility tests can predict in vivo Zn bioavailability.
- Metal speciation and geochemical alterations can impact Zn bioavailability.
Figure 1.
Scanning electron micrographs of particles. Representative images from mine waste (A), hemimorphite (B), zincite (C), smithsonite (D), sphalerite (E) and hydrozincite (F) obtained using the backscatter mode. Micron bar = 10 μm. Brighter areas have higher average atomic number and indicate areas enriched in zinc and other metals.
Figure 2.
Regression analysis of bioaccessibility at pH 4.5 on in vivo RBA of $^{65}$Zn (relative to ZnCl$_2$) after IN (2A) or gavage (2B) administration. The in vitro extraction results significantly correlated with both post-intranasal ($r^2=0.88$) and post-gavage ($r^2=0.88$) Zn relative in vivo bioavailability. n=6 rats per group.
Table 1

Characterization of pure-phase Zn minerals and Zn-rich mine waste samples

<table>
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<tr>
<th>Particle</th>
<th>Source</th>
<th>Zn (%)</th>
<th>Specific Activity $^{65}$Zn (μCi/mg$^0$)</th>
<th>BET SSA (m$^2$/g)$^c$</th>
<th>Mean Dia. (μm)</th>
<th>Mode Dia. (μm)</th>
<th>90th % Dia. (μm)</th>
<th>&lt; 2.5 μm (%)</th>
<th>&lt; 10 μm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mine waste</td>
<td>Picher Mining Field, Oklahoma, U.S.A.</td>
<td>10.4</td>
<td>3.58</td>
<td>7.1±0.2</td>
<td>9.91</td>
<td>13.6</td>
<td>24.2</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>Hydrozincite</td>
<td>Numa Mine, Cantabria, Spain</td>
<td>59.5</td>
<td>1.58</td>
<td>93±0.3</td>
<td>18</td>
<td>28.7</td>
<td>34.3</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Hemimorphite</td>
<td>Unknown source, 1916</td>
<td>54.3</td>
<td>1.24</td>
<td>3.9±0.2</td>
<td>19.8</td>
<td>26.1</td>
<td>48.6</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>Zincite</td>
<td>Franklin Mine, New Jersey, U.S.A.</td>
<td>73.2</td>
<td>2.89</td>
<td>0.8±0.1</td>
<td>18.7</td>
<td>28.7</td>
<td>37.6</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>Smithsonite</td>
<td>Carroll County, Arkansas, U.S.A.</td>
<td>52.1</td>
<td>1.34</td>
<td>2.7±0.2</td>
<td>13.9</td>
<td>19.8</td>
<td>33.2</td>
<td>24</td>
<td>55</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>Las Manforas Mine, Cantabria, Spain</td>
<td>64.1</td>
<td>1.48</td>
<td>1.5±0.0</td>
<td>12.5</td>
<td>21.7</td>
<td>29.4</td>
<td>27</td>
<td>51</td>
</tr>
</tbody>
</table>

$^a$Zn concentrations in Zn mineral particles were calculated based on chemical formula.

$^b$Specific activity of $^{65}$Zn varied depending on length of irradiation and Zn concentration. Neutron activation analysis of mine waste particles (data not shown) revealed the presence of multiple metals including Zn (10.36%), Fe (1.93%), Mg (1.39%), Cd (0.027%) and Mn (0.024%). Despite lower concentration (10.4%) of Zn in mine waste particles, specific activity was higher due to longer (20-fold) irradiation than Zn mineral particles.

$^c$BET SSA – Specific surface area from BET analysis.
Table 2

Results of in vitro extraction tests. Results are presented as percentage of total $^{65}$Zn measured in dissolved extraction solution after each specified time of incubation.

<table>
<thead>
<tr>
<th></th>
<th>pH 7.4$^a$</th>
<th>pH 4.5$^b$</th>
<th>pH 1.5$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>5 d</td>
<td>1 h</td>
</tr>
<tr>
<td>Mine waste</td>
<td>0.22</td>
<td>91.7</td>
<td>98.2 ± 5.1</td>
</tr>
<tr>
<td>Hydrozincite</td>
<td>0.09</td>
<td>56.8</td>
<td>97.4 ± 3.7</td>
</tr>
<tr>
<td>Hemimorphite</td>
<td>0.48</td>
<td>39.1</td>
<td>95.8 ± 1.4</td>
</tr>
<tr>
<td>Zincite</td>
<td>0.06</td>
<td>35.0</td>
<td>96.5 ± 1.8</td>
</tr>
<tr>
<td>Smithsonite</td>
<td>0.09</td>
<td>36.4</td>
<td>72.1 ± 9.4</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>0.03</td>
<td>1.07</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$ pH 7.4: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na$_2$HPO$_4$·2 H$_2$O, 1.76 mM KH$_2$PO$_4$

$^b$ pH 4.5: 20 mM KHC$_8$H$_4$O$_4$, 110 mM NaCl, 6.0 mM glycine, 1.0 mM Na$_2$HPO$_4$, 0.5 mM Na$_2$SO$_4$, 0.20 mM CaCl$_2$, 50 ppm benzalkonium chloride

$^c$ pH 1.5: 0.4 M glycine. Results expressed as mean ± SE (n=2).
Table 3

Results of geochemically-based sequential extractions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TARGET</td>
<td>Ionically bound-displaced by cationic exchange</td>
<td>Carbonate-bound and other phases mobilized by slightly acidic conditions</td>
<td>Reactive sulfides or amorphous Fe (hydr)oxides and sorbed or coprecipitated metals</td>
<td>Coprecipitated with pyrite or other crystalline sulfides</td>
<td>Residual, including silicates and other minerals not extracted by previous solutions</td>
</tr>
<tr>
<td>EXTRACTION CONDITIONS</td>
<td>1 M MgCl₂, pH 7, 1 h, 20°C</td>
<td>1 M NaAcetate, pH 5, 5h, 20°C</td>
<td>1 M HCl 12 h, 20°C</td>
<td>concentrated HNO₃, 2 h, 20°C</td>
<td>total – sum of extracted (I-IV)</td>
</tr>
<tr>
<td>PARTICLES</td>
<td>PERCENT EXTRACTED</td>
<td>PERCENT EXTRACTED</td>
<td>PERCENT EXTRACTED</td>
<td>PERCENT EXTRACTED</td>
<td>PERCENT EXTRACTED</td>
</tr>
<tr>
<td>Mine Waste</td>
<td>1.08 ± 0.01</td>
<td>79.7 ± 0.15</td>
<td>2.08 ± 0.08</td>
<td>0.49 ± 0.004</td>
<td>16.6 ± 0.22</td>
</tr>
<tr>
<td>Hydrozincite</td>
<td>0.43 ± 0.04</td>
<td>89.0 ± 0.63</td>
<td>0.26 ± 0.01</td>
<td>0.08 ± 0.05</td>
<td>10.2 ± 0.62</td>
</tr>
<tr>
<td>Hemimorphite</td>
<td>0.40 ± 0.02</td>
<td>87.9 ± 0.37</td>
<td>0.26 ± 0.04</td>
<td>0.04 ± 0.01</td>
<td>11.4 ± 0.31</td>
</tr>
<tr>
<td>Zincite</td>
<td>0.49 ± 0.002</td>
<td>88.8 ± 1.50</td>
<td>0.45 ± 0.05</td>
<td>0.04 ± 0.04</td>
<td>10.3 ± 1.45</td>
</tr>
<tr>
<td>Smithsonite</td>
<td>1.17 ± 0.05</td>
<td>28.5 ± 0.57</td>
<td>56.7 ± 0.24</td>
<td>0.24 ± 0.09</td>
<td>13.3 ± 0.28</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>0.12 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>8.71 ± 0.43</td>
<td>59.3 ± 6.45</td>
<td>31.7 ± 6.89</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n=2) for percentage of total ⁶⁵Zn measured in dissolved extraction solution after each stage.
Table 4

Absolute bioavailability of $^{65}$Zn after 6 intranasal administrations of $^{65}$ZnCl$_2$ or neutron-activated particles in rats$^a$.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$^{65}$ZnCl$_2$</th>
<th>Mine Waste</th>
<th>Hydrozincite</th>
<th>Hemimorphite</th>
<th>Zincite</th>
<th>Smithsonite</th>
<th>Sphalerite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.16±0.01</td>
<td>0.17±0.01</td>
<td>0.14±0.01</td>
<td>0.12±0.01$^*$</td>
<td>0.05±0.01$^*$</td>
<td>0.04±0.01$^*$</td>
<td>0.002±0.001$^*$</td>
</tr>
<tr>
<td>Brain</td>
<td>0.10±0.01</td>
<td>0.07±0.004$^*$</td>
<td>0.04±0.002$^*$</td>
<td>0.05±0.002$^*$</td>
<td>0.02±0.002$^*$</td>
<td>0.01±0.001$^*$</td>
<td>0.001±0.001$^*$</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.03±0.004</td>
<td>0.04±0.004$^*$</td>
<td>0.02±0.002</td>
<td>0.03±0.003</td>
<td>0.01±0.001$^*$</td>
<td>0.01±0.001$^*$</td>
<td>0.001±0.0002$^*$</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.08±0.01</td>
<td>0.13±0.01$^*$</td>
<td>0.08±0.01</td>
<td>0.06±0.005</td>
<td>0.03±0.004$^*$</td>
<td>0.02±0.002$^*$</td>
<td>0.001±0.0002$^*$</td>
</tr>
<tr>
<td>Heart</td>
<td>0.03±0.003</td>
<td>0.04±0.003$^*$</td>
<td>0.03±0.002</td>
<td>0.02±0.002</td>
<td>0.01±0.001$^*$</td>
<td>0.01±0.001$^*$</td>
<td>0.0002±0.0001$^*$</td>
</tr>
<tr>
<td>BM</td>
<td>0.49±0.06</td>
<td>0.64±0.06</td>
<td>0.39±0.03</td>
<td>0.33±0.02</td>
<td>0.14±0.02$^*$</td>
<td>0.09±0.01$^*$</td>
<td>0.004±0.002$^*$</td>
</tr>
<tr>
<td>SkM</td>
<td>3.02±0.25</td>
<td>4.18±0.26$^*$</td>
<td>2.35±0.17</td>
<td>2.20±0.19$^*$</td>
<td>0.84±0.09$^*$</td>
<td>0.71±0.08$^*$</td>
<td>0.03±0.01$^*$</td>
</tr>
<tr>
<td>Liver</td>
<td>0.62±0.07</td>
<td>0.96±0.05$^*$</td>
<td>0.57±0.04</td>
<td>0.45±0.02</td>
<td>0.20±0.02$^*$</td>
<td>0.16±0.02$^*$</td>
<td>0.01±0.004$^*$</td>
</tr>
<tr>
<td>Lung</td>
<td>0.06±0.01</td>
<td>0.08±0.00$^*$</td>
<td>0.05±0.004</td>
<td>0.05±0.004</td>
<td>0.02±0.002$^*$</td>
<td>0.01±0.00$^*$</td>
<td>0.002±0.001$^*$</td>
</tr>
<tr>
<td>GIT</td>
<td>0.86±0.09</td>
<td>1.07±0.06</td>
<td>0.70±0.07</td>
<td>0.61±0.05</td>
<td>0.27±0.05$^*$</td>
<td>0.38±0.20$^*$</td>
<td>0.28±0.11$^*$</td>
</tr>
<tr>
<td>Testes</td>
<td>0.10±0.01</td>
<td>0.15±0.01$^*$</td>
<td>0.10±0.01</td>
<td>0.07±0.004$^*$</td>
<td>0.03±0.003$^*$</td>
<td>0.03±0.003$^*$</td>
<td>0.002±0.0003$^*$</td>
</tr>
<tr>
<td>Skin</td>
<td>1.61±0.19</td>
<td>2.41±0.18$^*$</td>
<td>1.12±0.04</td>
<td>1.29±0.07</td>
<td>0.54±0.06$^*$</td>
<td>0.35±0.03$^*$</td>
<td>0.03±0.01$^*$</td>
</tr>
<tr>
<td>Total</td>
<td>8.15±0.62</td>
<td>10.64±0.60$^*$</td>
<td>6.06±0.33$^*$</td>
<td>5.80±0.33$^*$</td>
<td>2.59±0.30$^*$</td>
<td>2.15±0.27$^*$</td>
<td>0.61±0.12$^*$</td>
</tr>
</tbody>
</table>

Abbreviations: BM = bone marrow; SkM = skeletal muscle; GIT = gastrointestinal tract;

$^a$ P < 0.05 compared to $^{65}$ZnCl$_2$.

$^a$ Results expressed as percentage of total dose ± SE, n=6.
Table 5

Absolute bioavailability of $^{65}$Zn after 6 gavage administrations of $^{65}$ZnCl$_2$ or neutron-activated particles in rats$^d$.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$^{65}$ZnCl$_2$</th>
<th>Mine Waste</th>
<th>Hydrozincite</th>
<th>Hemimorphite</th>
<th>Zincite</th>
<th>Smithsonite</th>
<th>Sphalerite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.24±0.01</td>
<td>0.25±0.02</td>
<td>0.18±0.03</td>
<td>0.14±0.01$^*$</td>
<td>0.06±0.01$^*$</td>
<td>0.07±0.005$^*$</td>
<td>0.02±0.01$^*$</td>
</tr>
<tr>
<td>Brain</td>
<td>0.08±0.004</td>
<td>0.08±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.004$^*$</td>
<td>0.02±0.003$^*$</td>
<td>0.02±0.002$^*$</td>
<td>0.003±0.0005$^*$</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.04±0.003</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
<td>0.03±0.002</td>
<td>0.01±0.002$^*$</td>
<td>0.02±0.001$^*$</td>
<td>0.002±0.0004$^*$</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.13±0.01</td>
<td>0.17±0.02</td>
<td>0.11±0.01</td>
<td>0.08±0.005$^*$</td>
<td>0.04±0.005$^*$</td>
<td>0.04±0.004$^*$</td>
<td>0.003±0.00$^*$</td>
</tr>
<tr>
<td>Heart</td>
<td>0.05±0.002</td>
<td>0.06±0.01</td>
<td>0.04±0.01</td>
<td>0.03±0.002</td>
<td>0.01±0.002$^*$</td>
<td>0.01±0.001$^*$</td>
<td>0.002±0.0001$^*$</td>
</tr>
<tr>
<td>BM</td>
<td>0.74±0.07</td>
<td>0.85±0.07</td>
<td>0.56±0.08</td>
<td>0.37±0.04$^*$</td>
<td>0.17±0.02$^*$</td>
<td>0.13±0.01$^*$</td>
<td>0.03±0.01$^*$</td>
</tr>
<tr>
<td>SkM</td>
<td>4.31±0.20</td>
<td>5.53±0.33$^*$</td>
<td>3.27±0.31</td>
<td>2.53±0.19$^*$</td>
<td>1.09±0.13$^*$</td>
<td>1.04±0.09$^*$</td>
<td>0.08±0.02$^*$</td>
</tr>
<tr>
<td>Liver</td>
<td>0.94±0.05</td>
<td>1.24±0.12$^*$</td>
<td>0.80±0.09</td>
<td>0.55±0.04$^*$</td>
<td>0.25±0.04$^*$</td>
<td>0.28±0.03$^*$</td>
<td>0.02±0.002$^*$</td>
</tr>
<tr>
<td>Lung</td>
<td>0.11±0.02</td>
<td>0.27±0.14</td>
<td>0.90±0.34</td>
<td>0.98±0.55</td>
<td>0.06±0.01</td>
<td>0.57±0.29</td>
<td>0.27±0.16</td>
</tr>
<tr>
<td>GIT</td>
<td>1.36±0.06</td>
<td>1.44±0.13</td>
<td>1.47±0.41</td>
<td>0.81±0.06</td>
<td>0.47±0.11$^*$</td>
<td>0.28±0.02$^*$</td>
<td>0.46±0.21$^*$</td>
</tr>
<tr>
<td>Testes</td>
<td>0.15±0.01</td>
<td>0.18±0.02</td>
<td>0.15±0.02</td>
<td>0.09±0.01$^*$</td>
<td>0.04±0.01$^*$</td>
<td>0.04±0.004$^*$</td>
<td>0.003±0.001$^*$</td>
</tr>
<tr>
<td>Skin</td>
<td>2.33±0.08</td>
<td>2.96±0.25$^*$</td>
<td>1.55±0.15</td>
<td>1.50±0.12$^*$</td>
<td>0.70±0.09</td>
<td>0.49±0.05$^*$</td>
<td>0.05±0.005$^*$</td>
</tr>
<tr>
<td>Total</td>
<td>11.0±0.50</td>
<td>13.7±0.97</td>
<td>9.6±1.24</td>
<td>7.50±0.97$^*$</td>
<td>3.08±0.44$^*$</td>
<td>3.14±0.42$^*$</td>
<td>0.95±0.20$^*$</td>
</tr>
</tbody>
</table>

Abbreviations: BM = bone marrow; SkM = skeletal muscle; GIT = gastrointestinal tract.

$^*$P < 0.05 compared to $^{65}$ZnCl$_2$.

$^d$Results expressed as percentage of total dose ± SE, n=6.
### Table 6

Regression analyses of in vivo absolute bioavailability with in vitro bioaccessibility and geoavailability tests.

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept ($\beta_0$)</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
<th>Adj. $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability = $f$(Bioaccessibility)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{\text{intranasal}}(\text{pH}=1.5)$</td>
<td>$-3.9 \pm 0.23$ (p&lt; 0.0001)</td>
<td>$0.31 \pm 0.07$ (p= 0.01)</td>
<td>0.79 (p= 0.01)</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{gavage}}(\text{pH}=1.5)$</td>
<td>$-3.55 \pm 0.26$ (p&lt; 0.0001)</td>
<td>$0.30 \pm 0.08$ (p= 0.02)</td>
<td>0.72 (p= 0.02)</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{intranasal}}(\text{pH}=4.5)$</td>
<td>$-3.11 \pm 0.16$ (p&lt; 0.0001)</td>
<td>$0.44 \pm 0.08$ (p= 0.005)</td>
<td>0.86 (p= 0.005)</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{gavage}}(\text{pH}=4.5)$</td>
<td>$-2.77 \pm 0.18$ (p&lt; 0.0001)</td>
<td>$0.42 \pm 0.08$ (p= 0.008)</td>
<td>0.83 (p= 0.008)</td>
<td></td>
</tr>
<tr>
<td>Bioavailability = $f$(Geoavailability)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{\text{intranasal}}(\text{pH}=1.5)$</td>
<td>$-3.41 \pm 0.25$ (p&lt; 0.0001)</td>
<td>$0.28 \pm 0.08$ (p= 0.03)</td>
<td>0.67 (p= 0.03)</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{gavage}}(\text{pH}=1.5)$</td>
<td>$-3.06 \pm 0.26$ (p&lt; 0.0001)</td>
<td>$0.26 \pm 0.09$ (p= 0.04)</td>
<td>0.60 (p= 0.04)</td>
<td></td>
</tr>
<tr>
<td>Bioaccessibility = $f$(Geoavailability)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{\text{pH}=1.5}$</td>
<td>$3.95 \pm 0.19$ (p&lt; 0.0001)</td>
<td>$1.04\pm0.02$ (p&lt;0.0001)</td>
<td>$-0.14 \pm 0.01$ (p =0.001)</td>
<td>0.99 (p&lt; 0.0001)</td>
</tr>
<tr>
<td>$F_{\text{pH}=4.5}$</td>
<td>$4.89 \pm 0.72$ (p= 0.006)</td>
<td>$0.81 \pm 0.06$ (p= 0.001)</td>
<td>$-0.35 \pm 0.05$ (p =0.021)</td>
<td>0.98 (p= 0.002)</td>
</tr>
</tbody>
</table>

In vivo ABA was based on the total percentage of $^{65}$Zn dose recovered in all tissues.

LDPSA Mean = particle mean diameter.

SeqExt(I+II) = sum of fractions I and II.

$\beta$ values represent coefficient estimates $\pm$ standard error with the p value in parentheses.