Predicting the relative bioavailability of arsenic, cadmium and lead via the incidental soil ingestion pathway using in vitro techniques

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Abstract
In this study, the bioaccessibility and relative bioavailability of soil-borne contaminants were compared to determine whether a simple rapid, inexpensive in vitro assay may be used to predict *in-vivo* relative bioavailability for human health exposure assessment. Arsenic, cadmium and lead bioaccessibility in contaminated soil was assessed using a variety of *in-vitro* assays (SBRC, IVG, PBET and DIN) incorporating gastric (G) and intestinal phases (I) while *in-vivo* relative bioavailability was determined using mouse or swine assays. When linear regression models were developed in order to determine the suitability of *in-vitro* assays for predicting arsenic, cadmium and lead relative bioavailability, the correlation between bioaccessibility and relative bioavailability varied depending on the methodology used. While arsenic, cadmium and lead relative bioavailability could be accurately predicted using SBRC-G, PBET-I and Rel-SBRC-I respectively, a single *in-vitro* method was not suitable for predicting relative bioavailability for all three contaminants.

Key Words
Arsenic, Bioaccessibility, Bioavailability, Cadmium, Lead, Human Health Exposure Assessment.

Introduction

Incidental ingestion of contaminated soil is a major non-dietary exposure pathway for many inorganic contaminants. In order to more accurately quantify exposure to inorganic contaminants via soil ingestion, determination of contaminant bioavailability is required. It has been established that arsenic (As), cadmium (Cd) and lead (Pb) bioavailability may be less that 100% as a result of mineralogy, the influence of soil properties and contaminant-soil residence time (ageing) (Ruby *et al*. 1996; Rodriguez *et al*. 1999; Basta *et al*. 2001; Juhasz *et al*. 2007a; 2007b). As a result, exposure and therefore risk to human health may be overestimated if a conservative bioavailability approach is adopted (i.e. 100%).

In-vivo assays using a variety of animal models (e.g. primate, swine, dog, rabbit, rodent) have been used to quantify the relative bioavailability of contaminants in soil (Freeman *et al.* 1993; Groen *et al*. 1994; Ng *et al.* 1998; Roberts *et al.* 2002; Juhasz *et al.* 2007b). However, given the time and cost requirements, in addition to ethical issues, there is great demand for an appropriate *in-vitro* assay for estimating relative contaminant bioavailability. *In-vitro* assays are simple, rapid and inexpensive and numerous methods have been applied for the determination of contaminant bioaccessibility (Rodriguez *et al*. 1999; DIN 2000; Oomen *et al.* 2002; Kelley *et al.* 2002). However, before these assays can act as a surrogate measure for relative bioavailability, correlation between *in-vitro* bioaccessibility and *in-vivo* relative bioavailability is a mandatory prerequisite for regulatory as well as scientific acceptance. This paper discusses the development, assessment and validation of *in-vitro* assays for predicting the *in-vivo* relative bioavailability of soil contaminated with As, Cd and Pb.

Methods

Contaminated soils
Arsenic, Cd and Pb contaminated soils used in this study were collected from regional areas where the soil type, source and contaminant-soil residence time varied. Soils were air dried then sieved and the <250 µm particle size retained for chemical characterisation and bioaccessibility / relative bioavailability assessment.

Assessment of bioaccessibility
Arsenic, Cd and Pb bioaccessibility was determined using four different *in-vitro* methods incorporating both gastric and intestinal phases. In vitro methods included:

1. SBRC (Kelley *et al*. 2002)
2. IVG (Rodriguez *et al.* 1999)
3. PBET (Wragg *et al*. 2007)
4. DIN (DIN 2000)

Arsenic, Cd and Pb concentrations in soil digests or *in-vitro* solutions were determined using ICP-MS. Certified reference materials were included in all analysis to ensure internal quality assurance, quality control practices.

Assessment of relative bioavailability
In-vivo As and Pb relative bioavailability was determined using a swine model according to Rees *et al.* (2009). Bioavailability was calculated using pharmacokinetic analysis encompassing area under the blood-concentration time curve following zero correction and dose normalisation. When relative As and Pb bioavailability was determined, the area under the blood-concentration time curve for the respective reference dose (sodium arsenate or Pb acetate) oral treatment was used for comparison. *In-vivo* Cd bioavailability studies were conducted with mice. Cadmium acetate (reference dose) or Cd contaminated soil was incorporated into formulated mice pellets and 10 g of the respective feed mix supplied to individual animals once daily over a 15 day exposure period. At the end of the exposure period, animals were euthanized and the kidneys and liver collected for Cd determination. Following tissue digestion, samples were diluted to 20 ml with 0.1% HNO_3 then filtered (0.45 μm) for analysis by ICP-MS.

Comparison of bioaccessibility and bioavailability
Arsenic, Cd and Pb relative bioavailability, derived from in vivo mouse or swine assays, was compared to bioaccessibility data determined using SBRC, IVG, PBET and DIN methods. Bioaccessibility-relative bioavailability best fit models were determined using stepwise multiple regression (SPSS 16.0.1).

Results
For all four *in-vitro* methods, As, Cd and Pb bioaccessibility was greater when gastric phase values were compared to the intestinal phase. Due to the low pH environment of the gastric phase, release of As, Cd and Pb from the soil matrix occurred as a result of dissolution processes which are dependent on mineralogy in addition to the gastric phase pH of the in vitro method. Generally the gastric phase of the SBRC assay produced the highest bioaccessibility results presumably due to the differences in pH values of the four *in-vitro* methodologies (1.5 versus 1.8, 2.0 and 2.5). Increasing the pH from gastric to intestinal phase conditions resulted in a significant decrease in As, Cd and Pb bioaccessibility presumably due to co-precipitation with and/or sorption to iron via surface complexation or ligand exchange.

Relative bioavailability varied significantly between contaminated soils ranging from 6.9 ± 5.0% to 74.7 ± 11.2% for As, 10.1 ± 0.4% to 92.1 ± 7.3% for Cd and 10.1 ± 8.7% to 19.1 ± 14.9% for Pb. When linear regression models were developed in order to determine the suitability of *in-vitro* assays for predicting As, Cd and Pb relative bioavailability, the correlation between bioaccessibility and relative bioavailability varied depending on the methodology used. While As, Cd and Pb relative bioavailability could be accurately predicted using SBRC-G, PBET-I and Rel-SBRC-I respectively (Table 1), a single *in-vitro* method was not suitable for predicting relative bioavailability for all three contaminants.

Table 1. Best fit linear regression models for predicting in vivo As, Cd and Pb relative bioavailability using *in-vitro* assays.

Contaminant	In vitro assay/phase	*In-vivo* – *in-vitro* predictive model	Pearson correlation
As (n=12)	SBRC-G	RBA (%) = 0.992*SBRC-G (%) + 1.656, r^2 = 0.754	0.868
Cd (n=7)	PBET-I	RBA (%) = 1.091*PBET-I (%) – 5.140, r^2 = 0.835	0.914
Pb (n=5)	Rel-SBRC-I[a]	RBA (%) = 0.580*Rel-SBRC-I (%) + 1.980, r^2 = 0.530	0.730

[a] Relative Pb bioaccessibility in the intestinal phase of the SBRC assay was calculated by adjusting the dissolution of Pb from contaminated soil by the solubility of Pb acetate at the corresponding pH value (pH 6.5).

Conclusion

When *in-vivo* As, Cd and Pb relative bioavailability data was compared to bioaccessibility data, simple, rapid, inexpensive *in-vitro* assays could accurately predict relative bioavailability. However, relative bioavailability - bioaccessibility correlations demonstrated that the selection of an appropriate *in-vitro* assay for predicting relative bioavailability is contaminant specific and that one *in-vitro* methodology may not presently be suitable for all inorganic contaminants.

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