Science of the Total Environment 595 (2017) 660–665

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Influence of sample matrix on the bioavailability of arsenic, cadmium and lead during co-contaminant exposure

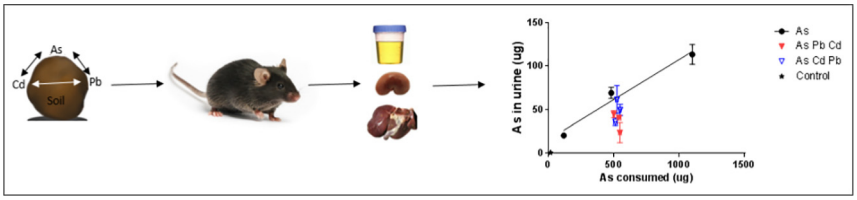
Cameron J. Ollson [a,b,*], Euan Smith [a], Paul Herde [c], Albert L. Juhasz [a]

[a] Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] Cooperative Research Centre for Contamination Assessment and Remediation of Environment (CRC CARE), eUniversity of Newcastle, Callagham, NSW 2308, Australia
[c] South Australian Health and Medical Research Institute, Gilles Plains, SA 5086, Australia

HIGHLIGHTS

- Spiked soil was assessed using an in vivo mouse model to determine co-contaminant interaction.
- Cd co-exposure with As decreased the relative bioavailability of As in urine.
- Cd co-exposure with Pb decreased the relative bioavailability of Pb in the liver.
- As and Pb co-exposure with Cd decreased the relative bioavailability of Cd in the kidneys.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 February 2017
Received in revised form 27 March 2017
Accepted 5 April 2017
Available online xxxx

Editor: F.M. Tack

Keywords:
Bioavailability
Arsenic
Lead
Cadmium
Spiked-soil
Co-exposure

ABSTRACT

In this study, the influence of sample matrix on the relative bioavailability of arsenic (As), cadmium (Cd) and lead (Pb) was assessed following exposure of C57BL/6 mice to spiked aged (12 years) soils. AIN93G mouse chow was amended with individual and tertiary As, Cd and Pb soil combinations which were administered to mice over a 9 day exposure period. Contaminant relative bioavailability was calculated by comparing As urinary excretion and Cd-kidney/Pb-liver accumulation to corresponding values for compounds used to derive the respective toxicity reference value. Strong linear dose-responses were observed for mice exposed to AIN93G mouse chow augmented with individually spiked soil with As, Cd and Pb. When mice were exposed to co-contaminants, As relative bioavailability (RBA) decreased similar to results from previous co-contaminant salt experiments presumably due to the influence of Cd on phosphate transport proteins, which are utilized for As absorption. However, a decrease in Cd-kidney and Pb-liver accumulation was also observed following co-co-exposure. It was postulated that this resulted from interactions with other (essential) metals (e.g. iron, aluminium, manganese, magnesium) within the soil matrix and their influence on absorption via divalent metal transporters.

1. Introduction

Determining the bioavailability of contaminants in soil is a key variable in exposure assessment calculations, when conducting human health risk assessments (HHRA). A large body of research performed over the past 25 years has enabled HHRA practitioners to refine the conservative assumption that contaminants are 100% bioavailable, and instead, use more accurate estimates derived from bioavailability (in vivo) or bioaccessibility (in vitro) models (Bradham et al., 2011; Diamond et al., 2016; Drexler and Brattin, 2007; Juhasz et al., 2007; Ollson et al., 2016; Rees et al., 2009; Rodriguez and Basta, 1999; Ruby et al., 1996; Wragg et al., 2011). This is particularly true for inorganic contaminants (arsenic [As], cadmium [Cd], lead [Pb]) which have been the focus of this field of research due to their known toxicological properties and prevalence at contaminated sites (ATSDR, 2013). Previous bioavailability research has typically evaluated these contaminants

* Corresponding author at: Future Industries Institute, University of South Australia, Building X, Mawson Lakes, SA 5095, Australia.
E-mail address: cameron.ollson@mymail.unisa.edu.au (C.J. Ollson).

http://dx.doi.org/10.1016/j.scitotenv.2017.04.036
0048-9697/

individually, with little attention to co-contaminant exposure and the potential influence that each element may have on one another's bioavailability.

Limited studies have been undertaken to assess co-contaminant bioavailability. Those that have been conducted have used soluble forms (e.g. sodium arsenate for As) of each contaminant and have utilized different experimental methodologies, with respect to animal model, dosing strategy and bioavailability endpoint (Diacomanolis et al., 2013; Diacomanolis et al., 2014; Ollson et al., 2017). Although Diacomanolis et al. (2014) and Ollson et al. (2017) utilized rat and mouse models, respectively, both studies determined that Cd co-exposure decreased As bioavailability. While similar outcomes were observed, the proposed mechanisms responsible for this effect were different (i.e. formation of Cd arsenate in the stomach versus disruption of phosphate transporters, respectively). Diacomanolis et al. (2013) also determined that As co-exposure with Pb decreased the bioavailability of Pb (via the formation of Pb arsenates), however, a similar result was not observed by Ollson et al. (2017), who determined no influence of As-Pb co-exposure on As urinary excretion or Pb-liver accumulation. In addition, Ollson et al. (2017), determined that Cd-Pb co-exposure increased the accumulation of Pb in the liver of C57 BL/6 mice presumably due to changes to in divalent metal compartmentalization or transport protein overexpression (Andrade et al., 2010; Spurgeon et al., 2010).

To date, the influence of co-exposure on contaminant absorption has been limited to the assessment of soluble contaminant forms (e.g. sodium arsenate, cadmium chloride, lead acetate) without considering more complex matrices, such as soil. Based on this knowledge gap, this study built on previous research by Ollson et al. (2017) to determine whether co-contaminant exposure effects influence As, Cd and Pb relative bioavailability (RBA) in spiked aged soil matrices. Experiments utilized an in vivo mouse model whereby C57 BL/6 mice were exposed to AIN93G mouse chow amended with As, Cd and Pb spiked aged soil (individual and tertiary combinations). Arsenic urinary excretion, Cd-kidney accumulation and Pb-liver accumulation were utilized as endpoints for bioavailability assessment, while contaminant RBA was calculated using soluble salts (sodium arsenate, cadmium chloride, lead acetate) data from Ollson et al. (2017). It was hypothesised that soil matrix effects will modulate contaminant release in the gastrointestinal tract which in turn will influence co-contaminant interactions and RBA outcomes.

2. Materials and methods

2.1. Test materials

2.1.1. Spiked aged soil

Previously (2004), multiple aliquots (15 kg) of a red ferrosol (see Table 1 for physico-chemical properties) was spiked with As, Cd or Pb individually at concentrations ranging from 100 to 2500, 10–1000 and 300–10,000 mg kg^{-1} respectively. After spiking, the soil was stored at 40–60% water holding capacity at ambient temperature. After 12 years the soils were processed in order to produce co-contaminant mixtures with varying contaminant concentrations. Soils were leached using rainwater (5 flushes of 70% water holding capacity), dried in an oven at 40 °C, homogenised in an end-over-end shaker for 12 h, and sieved to a particle size of <250 μm. Soils were then blended to produce single and tertiary contaminant combinations with As, Cd and Pb concentrations representative of concentrations equivalent to, five-fold and ten-fold the National Environmental Protection Measure for Assessment of Site Contamination Health Investigation Level ((NEPM), 2013) (Table S1). Total soil elemental concentration was quantified by ICP-MS following aqua-regia digestion using USEPA method 3051 in a Mars6 microwave (CEM) (USEPA, 1998).

Table 1
Concentration of major and minor elements in AIN93G mouse chow and the red ferrosol prior to the addition of As, Cd and Pb.

Constituent	AIN93G	Red ferrosol
Major elements (g kg^{-1})		
Al	–	9.2
Fe	0.04	10
Mg	0.05	1.4
Minor elements (mg kg^{-1})		
As	0.16	9
Cd	0.01	1
Cu	6	75
Mn	11	1900
P	3600	1600
Pb	0.35	35
Zn	35	70

2.1.2. Mouse chow preparation

Spiked aged soil was amended into AIN93G mouse chow prior to in vivo assessment of As, Cd and Pb RBA. Mouse chow was oven dried at 70 °C then mixed with spiked aged soil (<250 μm particle size fraction) to achieve concentrations of 1, 5, 10 mg As kg^{-1}, 0.2, 1, 2 mg Cd kg^{-1}, 3, 15, 30 mg Pb kg^{-1} or combinations thereof (Table S1). These concentrations were chosen as they represent diet concentrations equivalent to, five-fold and ten-fold the National Environmental Protection Measure for the Assessment of Site Contamination ((NEPM), 2013) health investigation (HIL A) level ((NEPM), 2013) when spiked aged soil was amended to mouse chow at a 1% (w/w soil:diet) loading. To confirm the concentration of As, Cd and Pb in amended AIN93G mouse chow, triplicate samples were digested using 70% HNO_3 (10 ml; Univar analytical reagent), on a block digester ramped to 140 °C (A.I. Scientific AIM500). Once the volume was reduced to 1–2 ml, samples were cooled to room temperature, diluted with Milli-Q water (10 ml), filtered through 0.45 μm cellulose acetate filters (Millipore Millex-HV) and stored at 4 °C until analysed by ICP-MS.

2.2. In vivo assessment of As, Cd and Pb exposure

In vivo studies utilized female C57 BL/6 mice, aged between 4 and 6 weeks. Ethical and experimental approval was given by the SA Pathology/South Australian Health and Medical Research Institute Animal Ethics Committees (application number SAM73). Animal care was compliant with the Standard Operating Procedures of the South Australian Health and Medical Research Institute, and the Guidelines for the Care and Use of Laboratory Animals ((NRC), 1996). Arsenic, Cd and Pb RBA was assessed according to Ollson et al. (2017). Briefly, mice were housed in metabolic cages (3 per cage; 12 per treatment) and supplied spiked aged soil amended AIN93G mouse chow for a period of 9 days. Food consumption was monitored daily by determining the weight difference of the food hopper after filling and before replenishment while cumulative food consumption was the sum of the daily food consumption. At the end of the exposure period, mice were maintained on unamended AIN93G mouse chow for an additional 24 h after which they were humanely euthanized. Targeted tissues were collected for the determination of Cd (kidney) (Juhasz et al., 2010; Liu et al., 2000) and Pb (liver) (Marschner et al., 2006) RBA while urine (cumulative excretion) (Bradham et al., 2013) was utilized as the endpoint for As RBA assessment. A control group of mice was also included which consumed unamended AIN93G mouse chow. Following collection, target tissues were frozen (−20 °C) then freeze dried (Modulyod Freeze Dryer) prior to digestion while urine remained frozen (−20 °C) prior to digestion. Whole kidneys and livers and duplicate aliquots of urine (5 ml) were digested with 70% HNO_3 using the block digestion method that was utilized for the amended mouse chow. Following digestion, samples were diluted with Milli-Q water (10 ml), filtered through 0.45 μm cellulose acetate filters (Millipore Millex-HV) and stored at 4 °C until analysed by ICP-MS.

Quality assurance/quality control measures were used during digestion and analysis. Experimental blanks ($n = 26$) were below the level of