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Short communication

Antagonistic effects of cadmium on lead accumulation in pregnant and non-pregnant mice

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ABSTRACT

People are frequently exposed to combinations of contaminants but there is a paucity of data on the effects of mixed contaminants at low doses. This study investigated the influence of cadmium (Cd) on lead (Pb) accumulation in pregnant and non-pregnant mice following exposure to contaminated soil. Exposure to Pb from contaminated soils increased Pb accumulation in both pregnant and non-pregnant mice compared to unexposed control animals (pregnant and non-pregnant). Lead accumulation in the liver and kidneys of exposure pregnant mice (40 ± 15 mg Pb kg^{-1}) was significantly higher ($P < 0.05$) than concentrations detected in control pregnant mice (<1 mg Pb kg^{-1}). The presence of Cd in contaminated soil had a major effect on the Pb and Fe accumulation in the kidneys and liver, respectively. This study shows that Pb uptake is mediated by the presence of Cd in the co-contaminated soil and demonstrates that further research is required to investigate the influence of co-contaminants on human exposure at sub-chronic concentrations.

1. Introduction

A major pathway for human exposure to contaminants is via the incidental ingestion or inhalation of contaminated soils or dusts [1]. However, when assessing human exposure to environmental contaminants, a major uncertainty in the assessment process is quantifying the dose that is absorbed into systemic circulation (i.e. the bioavailable fraction). Part of the variability in bioavailability is attributable to mineralogical or other soil factors controlling contaminant solubility following soil ingestion. In vivo and in vitro assays with metal contaminated soils have illustrated that contaminant relative bioavailability and bioaccessibility is extremely variable and may range from <10 to 100% [2–4]. In addition to mineralogical constraints in contaminated soil or dust, contaminant absorption may also be affected by age, sex and gastro-intestinal physiology. During times of elevated physiological stress, such as occurs during pregnancy, there is an increased metabolic requirement for nutrients [5]. These higher rates of nutrient uptake could potentially influence the absorption of inorganic contaminants such as Pb. Extensive studies have identified that diets low in calcium (Ca) or iron (Fe) have increased Pb absorption [6]. However, the increased nutrient demands during pregnancy imply that there is potential for the increased absorption of soil contaminants. To date, in vivo studies have generally investigated exposure of single soil contaminates [2–4]. However, co-contamination is common at many polluted sites and research has reported that several of these contaminants, such as lead (Pb) and cadmium (Cd), share similar metabolic transport pathways [7]. Metabolic studies have reported that the co-administration of Pb and Cd solution to animals mediates the accumulation of Pb in the liver and kidneys [8,9]. These limited co-administration studies focused on solution doses that are considerably higher than potential contaminant soil exposure and are therefore not representative of exposure via chronic soil ingestion. The objective of this research was to investigate the effect of co-administration of Cd and Pb in contaminated soil on Pb absorption in pregnant and non-pregnant mice.

2. Materials and methods

2.1. Contaminated soil

Lead contaminated soil (736 ± 71 mg kg^{-1}) was collected from a parkland area in a regional mining town located north of Adelaide, Australia. As cadmium concentrations in the soil were low (<0.1 mg kg^{-1}), $Cd(NO_3)_2$ solution was added to the soil to increase the final Cd concentration to 20 mg kg^{-1}. A control soil containing background concentrations of Pb (86 ± 3 mg kg^{-1}) was collected approximately 20 km from the contaminated site. Soils were air

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Table 1
Pertinent properties of contaminated and control soils used in this study.

Soil	Metal concentration (mg kg^{-1})			Fe_{total} (g kg^{-1})		pH	OC (%)
	Pb		Cd	<2 mm	<250 μm	<2 mm	<2 mm
	<2 mm	<250 μm	<250 μm				
Pb	736 ± 71	1020 ± 115	<0.1	13.8	23.9	8.9	3.0
Pb + Cd	736 ± 71	1020 ± 115	20 ± 0.5	13.8	23.9	8.9	3.0
Control	86 ± 3	73 ± 1	<0.1	9.7	13.2	9.0	3.5

dried then processed using stainless steel sieves to retrieve the <2 mm and <250 μm soil particle size fractions. The <2 mm fraction was used to determine the bulk soil properties, while the <250 μm soil fraction was retained for in vivo studies. The <250 μm soil fraction was utilised in this study as this is the particle size fraction that adheres to children's hands and is therefore available for incidental soil ingestion [2].

Pertinent properties of the soils used in the study are shown in Table 1. Soil physico-chemical properties were determined in duplicate for each soil. Soil pH was determined using 1:5 soil:water extracts and organic carbon content was determined by oxidation/combustion [10]. Total metal concentrations were determined using the USEPA 3015H aqua regia dissolution procedure [11] and a CEM MarsX microwave. Total metals in digest solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). A certified reference material (GBW 07411) was included in the analysis to ensure internal quality assurance/quality control (QA/QC) practices. The accuracy of the aqua regia digestion method was confirmed by a quantitative average Pb recovery of 2688 ± 84 mg kg^{-1} (n = 6) from GBW 07411 (2700 mg Pb kg^{-1}). During the determination of Pb concentration in soil digests and in vivo samples, duplicate sample analysis, spiked sample recoveries and check values were included. The average deviation between replicate samples (n = 40) was 16.9%, the average recovery from spiked samples (n = 4) was 94.9% whereas check value recoveries (n = 3) ranged from 89.2 to 108.4% (95.3% average recovery).

2.2. Determination of (in vivo) Pb accumulation in mouse tissues

In vivo studies were conducted with pregnant and non-pregnant female (Balb/c) mice with a body weight range between 20 and 25 g. Animals were housed in groups of 3 mice and received a 12/12 light/dark cycle and access to water ad libitum. Animal care was in compliance with the Standard Operating Procedures of the Veterinary Services Division, Institute of Medical and Veterinary Science, Adelaide, Australia and the Guide for Use and Care of Laboratory Animals [12]. Feeding of contaminated soil commenced after visual inspection indicated that the mice were pregnant and feeding continued for a period of 18 days. The animals received a daily dose (0.25 g) of soil, incorporated into a known mass of mouse chow at time of feeding. Group of pregnant and non-pregnant mice (n = 3) received the following treatments:

1. Pb contaminated soil.
2. Pb and Cd co-contaminated soil.
3. Control soil (i.e. background Pb concentrations).

After 18 days, prior to humane killing by cervical dislocation, animals were anaesthetised and blood was collected from all the animals in the study for analysis. Blood samples (0.5 mL) were stored in 7.5 mL EDTA collection tubes at −20 °C prior to Pb analysis. After euthanization, the liver and kidneys of 3 animals from each treatment were removed and analysed for total metal content.

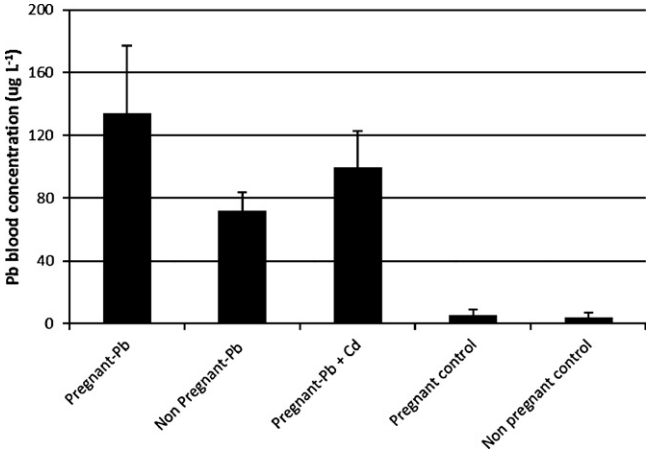

Fig. 1. Pb blood concentration in treated pregnant and non-pregnant mice after 18 days exposure to contaminated soil.

2.3. Determination of metals in tissues

Blood was diluted 10-fold in solution containing 1-butanol (2%, w/v), EDTA (0.05%, w/v), Triton X-100 (0.05%, w/v) and ammonium hydroxide (1%, w/v) in Milli-Q water [18] prior to analysis. Tissue samples were freeze dried prior to grinding and aqua regia digestion over 48 h. Solutions were dried to approximately 1 mL and then diluted to 20 mL with deionised H_2O. All samples were analysed by ICP-MS with the appropriate number of duplicate samples, duplicate analysis, spiked sample recoveries and check values included for quality assurance and quality control purposes.

3. Results and discussion

Lead accumulation in mice varied markedly depending on the metabolic state of the animals (pregnant versus non-pregnant), the tissues analysed and the presence or absence of Cd in the administered contaminated soil. Lead blood levels collected after 18 days of continuous soil exposure were elevated in the pregnant and non-pregnant mice exposed to Pb contaminated soil compared to the control animals which received soils containing background Pb concentrations (Fig. 1). Blood Pb concentrations ranged from less than 5 μg L^{-1} in control animals to 188 μg L^{-1} in the exposed animals. The mean Pb blood concentrations were markedly higher in the pregnant Pb and pregnant Pb and Cd exposed animals compared to the non-pregnant exposed and control animals. The elevated mean Pb blood concentration in the pregnant animals is attributed to the metabolic changes induced by pregnancy. This is consistent with the physiological changes associated with pregnancy that translates to not only increasing the absorption of micronutrients, but also the increased absorption of other non-essential metals such as Cd and Pb [13]. While there was no significant difference in Pb blood concentrations between pregnant mice exposed to Pb or Pb and Cd contaminated soil, the mean Pb blood concentration was markedly higher in the Pb treatment alone compared to the co-contaminant treatment. A similar influence of Cd on Pb

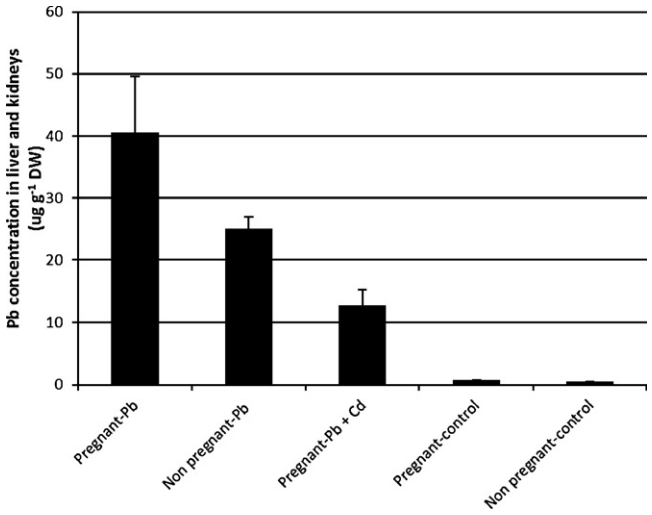

Fig. 2. Pb accumulation in soft tissues of liver and kidneys in treated pregnant and non-pregnant mice after 18 days exposure to contaminated soil.

accumulation was reported by Garcia and Corredor [9] following administration of Pb/Cd-acetate solutions to pregnant Wistar rats, although the Pb and Cd solution concentrations were markedly higher (300 mg Pb L^{-1} and 10 mg Cd L^{-1}) than the potential exposure from the contaminated soil feeding studies.

Metals which are absorbed into systemic circulation are distributed in various body tissues. Metal accumulation in liver and kidneys has been previously reported [14–17]. Liver and kidney analysis identified significant ($P > 0.05$) Pb accumulation in all animals exposed to Pb contaminated soils (Fig. 2) compared to control treatments. The mean Pb concentration in the liver and kidneys ranged from <1 mg kg^{-1} in the control animals to 40 mg kg^{-1} in the pregnant Pb exposed animals. Interestingly, Pb concentrations in the liver and kidneys were significantly higher in the pregnant Pb exposed animals compared to non-pregnant animals exposed to Pb and pregnant animals that received both Pb and Cd, implying a strong effect from the presence of Cd on Pb accumulation. The mean Pb concentration in the liver and kidneys was 40 mg kg^{-1} in the pregnant Pb exposed animals compared to 25 and 13 mg kg^{-1} in the non-pregnant Pb exposed and pregnant Pb and Cd exposed animals, respectively (Fig. 2). A considerable number of studies have investigated the accumulation of metals in the body tissues from a range of different sources [18–21]. Cadmium studies have reported that Cd accumulation in the kidney is mediated through the Cd-metallothionein complex which accrues in the proximal tubule of the renal cortex [22]. Similarly, Sprague–Dawley rat studies of Pb nephropathy reported Pb deposition in kidneys was also predominately localised in the proximal tubule of the real cortex [23]. The accumulation of Pb as such impacts on the transport processes in the renal tubules [23], such as inhibiting a range of ATPase enzymes associated with vectorial sodium transport [9]. The pathway of Pb accumulation within tissues has not been well characterised, but it is recognised that diets low in Fe result in an increase absorption of Pb and it appears there is a common molecular transport mechanism in many organs. To date research has focused on understanding the accumulation of Pb and Cd in nutrient deficient diets [24–28], however, there is paucity of information regarding the influence of co-contaminant divalent metals on contaminant relative bioavailability in soils. This is surprising considering that in the vast majority of cases, contaminants are present as mixtures in the environment and the cases where only a single contaminant is present are the exception rather than the norm. However, several clinical studies have investigated the interaction between Pb and Cd when co-administered [8,9]. These studies report that biochemical effects of Pb are mediated by Cd when Pb and Cd are co-administered together. Garcia and Correndor [9] reported that the independent administration of Pb or Cd decreased ATPase activity in the kidneys while co-administration of Pb and Cd reduced the effect of either metal separately. Similarly, Mahaffey et al. [29] reported that rats fed a diet containing both Cd (50 mg kg^{-1}) and Pb (200 mg kg^{-1}) accumulated less Pb in the kidneys than animals fed Pb alone, similar to the results found in this study. During pregnancy, an increase in the absorption of essential nutrients, such as iron (Fe), occurs. Foetal demand is primarily responsible for inducing changes in the absorption from the gastrointestinal tract for the increased production of divalent metal transporter 1 (DMT-1) which is responsible for Fe absorption. Divalent metal transporter 1 also mediates the absorption of Pb [30] and Cd [31] and may be up-regulated to enhance divalent metal absorption during pregnancy or when Fe is limiting in the diet [32,33]. Cannonne-Hergaux and Gros [34] identified that DMT1 was expressed in the proximal tubules of the renal cortex but not in the medulla region, accounting for the accumulation of Pb in the renal cortex region of the kidney [35]. Analysis of the elemental distribution in the liver and kidney indicated that there is a strong correlation ($R^2 = 0.94$) between Pb and Fe concentration in the liver and kidney, which is related to pregnancy state and exposure (Fig. 3). Fig. 3 shows that the accumulation of Pb is linearly correlated with the absorption of Fe indicating a similar mechanism of accumulation in these tissues. A higher Pb accumulation is observed in pregnant mice when exposed to the Pb only contaminated soil. The higher accumulation of Pb is correlated with the elevated accumulation of Fe in the

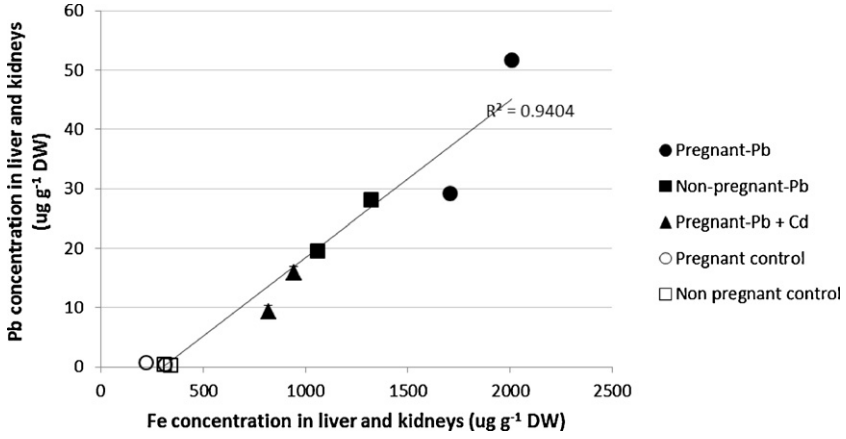

Fig. 3. Elemental relationship between Pb and Fe in liver and kidneys after 18 days exposure to Pb contaminated soil.

liver and kidneys, but the presence of Cd in the contaminated soil markedly decreased both the accumulation of Pb and Fe in the liver and kidneys to levels less than observed in the non-pregnant mice exposed to the Pb only contaminated soil (Fig. 3). This suggests that Cd reduces Fe and Pb accumulation in the liver and kidneys through a similar transport mechanism. Bannon et al. [31] reported similar effects of Cd on Fe transport in knockdown Caco-2 cell lines but did not observed an effect of Pb on Fe accumulation which suggests an independent transport mechanism.

Of interest was the effect that Pb may have on pregnancy outcome of the treated mice. No significant ($P < 0.05$) differences were found in pup number or weight between the pregnant treated (Pb or Pb + Cd soils) or the control group (no soil). The average number of pups born per treatment group was 6.67 ± 0.67, 6.67 ± 0.58 and 5.80 ± 0.91, for the Pb group, Pb + Cd group and the control group, respectively with the mean pup weight ranging from 0.97 ± 0.03 g (Pb + Cd pregnant group) to 1.01 ± 0.03 g (control group). Lead concentration in pups ($n = 6$) from the control group was low (0.023 ± 0.006 mg kg^{-1} per pup), however, exposure to Pb during pregnancy resulted in Pb concentrations in the treated groups of 0.060 ± 0.012 mg kg^{-1} per pup (Pb + Cd treatment) and 0.081 ± 0.005 mg kg^{-1} per pup (Pb treatment). Although higher Pb pup concentrations were observed in the exposed animals, those concentrations were significantly lower ($P < 0.05$) than those detected in other mouse tissues.

The findings in this study have important implications for human health exposure assessment at contaminated sites where co-contaminant divalent metals are present. The results indicate that the presence of divalent ions, such as Cd and Pb, are absorbed by similar mechanisms in the body and accumulate in the same body tissues. The presence of Cd has an antagonistic effect on the accumulation of Pb in the liver and kidneys and implies that the bioavailability of Pb is less than if Pb was the single contaminant present in the soil. Commonly utilised in vitro assays used for predicting the potential contaminant bioaccessible fraction do not replicate the dynamic physiology of the animal system and therefore may overestimate the metal bioaccessible fraction in contaminated soils.

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