

In Vivo Validation of the Unified BARGE Method to Assess the Bioaccessibility of Arsenic, Antimony, Cadmium, and Lead in Soils

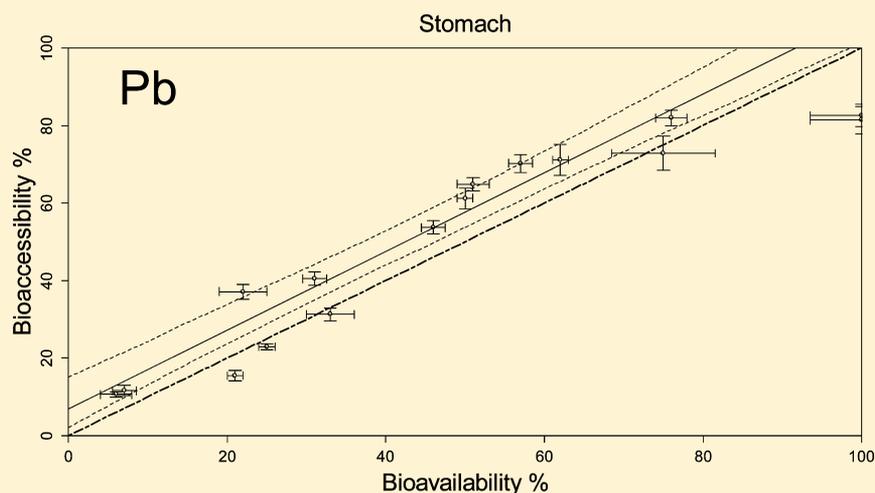
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S Supporting Information



ABSTRACT: The relative bioavailability of arsenic, antimony, cadmium, and lead for the ingestion pathway was measured in 16 soils contaminated by either smelting or mining activities using a juvenile swine model. The soils contained 18 to 25 000 mg kg⁻¹ As, 18 to 60 000 mg kg⁻¹ Sb, 20 to 184 mg kg⁻¹ Cd, and 1460 to 40 214 mg kg⁻¹ Pb. The bioavailability in the soils was measured in kidney, liver, bone, and urine relative to soluble salts of the four elements. The variety of soil types, the total concentrations of the elements, and the range of bioavailabilities found were considered to be suitable for calibrating the in vitro Unified BARGE bioaccessibility method. The bioaccessibility test has been developed by the BioAccessability Research Group of Europe (BARGE) and is known as the Unified BARGE Method (UBM). The study looked at four end points from the in vivo measurements and two compartments in the in vitro study (“stomach” and “stomach and intestine”). Using benchmark criteria for assessing the “fitness for purpose” of the UBM bioaccessibility data to act as an analogue for bioavailability in risk assessment, the study shows that the UBM met criteria on repeatability (median relative standard deviation value <10%) and the regression statistics (slope 0.8 to 1.2 and *r*-square > 0.6) for As, Cd, and Pb. The data suggest a small bias in the UBM relative bioaccessibility of As and Pb compared to the relative bioavailability measurements of 3% and 5% respectively. Sb did not meet the criteria due to the small range of bioaccessibility values found in the samples.

INTRODUCTION

Soils contaminated by potentially harmful elements (PHE), such as cadmium (Cd) and lead (Pb) constitute a potential risk to human health.^{1,2} Other important PHEs are the metalloids arsenic (As) and antimony (Sb). These elements are distributed through the environment as a result of both natural and anthropogenic activities such as mining or smelting.^{3,4} Once released into the environment, soils often serve as a sink for these PHEs and the question of human exposure to such elements must then be addressed. Indeed, As and Sb were

recognized as priority pollutants by the US-EPA in 1979 because of their contribution to cancer development, genotoxicity, and apoptosis in mammals.^{5,6} Ingestion is one of the major routes of soil exposure to these contaminants by children.^{7–9} Exposure is currently assessed using the total soil

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concentration of individual contaminants. However, several in vivo studies, using diverse animals such as monkeys, juvenile swine, rabbits, and rodents, have demonstrated that only a fraction of a contaminant, the bioavailable fraction, is absorbed following oral administration.^{10–16} In the literature, the juvenile swine model is considered to be a good physiological model for gastrointestinal (GI) absorption of contaminants in children.¹⁷ Recently, in the particular case of As, the swine model was described as being a particularly accurate representation of human physiology.¹⁸ Bioavailability is defined as the fraction of an ingested dose that crosses the GI epithelium and becomes available for distribution to internal target tissues and organs.^{19,20} Absolute bioavailability is directly determined in the blood plasma and consists in comparing the concentration in the plasma following an intravenous injection and an oral administration.^{13,19–21} However, this method is not easily achievable due to both experimental issues linked to blood sampling and to analytical limitations such as the generally low concentrations in the blood compared to quantification limits.²² Thus, in vivo protocols have been developed to estimate the relative bioavailability (RBA). This is measured as the uptake of the contaminant in the target organ from the soil matrix relative to the uptake from a readily soluble salt of the contaminant (reference matrix).^{16,19,23} Several studies have established that either absolute or relative bioavailability of soil metals were below 1 and are dependent on soil edaphic properties (e.g., pH, granulometry) and the soil metal speciation.^{10,15,16,20} Consequently, human exposure to soil bound contaminants can be overestimated when the bioavailability is not considered. The BioAccessibility Research Group of Europe (BARGE)²⁴ have developed an in vitro test, the Unified BARGE Method (UBM), to measure the bioaccessibility of soil contaminants. So far, a preliminary study²⁵ suggests that the UBM bioaccessibility data are correlated to in vivo bioavailability data. However, problems with the soils used in the study (they contained an unusually high content of mining slag) require that a more rigorous and robust validation of the UBM against in vivo data is essential before the UBM can be used as a routine tool in risk assessment. The aim of this study is to measure the relative bioavailability of As, Sb, Cd, and Pb in soil using a juvenile swine model, for 16 soils contaminated by either smelting or mining activities and to use the data from these soils to validate the UBM. So far, most other studies have focused on a single element and not on multicontaminated soil samples which are commonly found together on contaminated lands. Moreover, no study has been carried out on the human bioavailability of Sb.

The first part of this study is to measure the relative bioavailability (RBA) of As, Cd, Pb, and Sb from selected contaminated soils using a swine model. While this data gives some insight into the fraction of inorganic contaminants that is bioavailable, risk assessors need specific information about each site being studied. However, due to the high number of sites with soils contaminated with As, Cd, Pb, and/or Sb, it is not possible to determine the bioavailability in each case, as in vivo experiments are time-consuming, costly, and ethically problematic.¹⁹ To address this, numerous in vitro protocols have been designed to simulate the human digestive processes using artificial digestive fluids to determine the bioaccessible fraction of contaminants, i.e., the fraction of the PHE content of the soil released into solution within the GI system which is then potentially available for absorption, and have been comprehensively reviewed.^{26,27} The underlying hypothesis is that the

bioaccessibility reflects the bioavailability of a soil contaminant and allows for a more accurate estimation of the exposure concentration compared to the total soil concentration of the contaminant. However, from one in vitro test to another, the bioaccessibility can greatly vary for the same soil sample.^{28–31} Consequently, before such assays can act as a surrogate measurement for relative metals bioavailability, a correlation between in vitro bioaccessibility and in vivo bioavailability is necessary, for both regulatory and scientific acceptance. The objective of this research was to carry out a more robust validation study to demonstrate the physiological accuracy of the UBM for As, Cd, Pb, and Sb.

■ MATERIALS AND METHODS

Soil Collection Sample Preparation and Chemical Analysis. Full details of the soil collection, sample preparation, and chemical analysis of the soil and swine samples are given in the Supporting Information, SI.

Determination of in Vivo Relative Bioavailability. The RBA of As, Pb, Cd, and Sb were determined for each soil sample using readily soluble forms of the contaminants, sodium arsenate (NaH_2AsO_4), Pb-acetate ($(\text{CH}_3\text{COO})_2\text{Pb}$), Cd-chloride (CdCl_2), and potassium antimonate (KSbO_3). These reference matrices were chosen to estimate the RBA as they had been used in previous RBA studies for As and Sb^{14,21,32} and Pb and Cd.^{11,33}

The RBA of all elements studied were determined in four end points: urine; bone (metacarpal IV); liver; and kidney. The number of swine is 15 for the reference groups and 9 for the soil groups, leading to a total of 204 swine. Full details of the methodology are given in the SI.

Dose Response Curve and RBA Calculation. For a given contaminant, each soil and reference matrix, a dose–response curve was established by plotting the concentration in the target end point as a function of the administered dose. Before calculating the RBA, three conditions needed to be verified:³⁴

- That the response was linear for the soil and reference dose;
- That the intercepts for all of the lines were equal (i.e., had a common intercept);
- That the response at the zero level (called “blanks” i.e., the 3 g of moistened feed without any soil or reference dose; for details see the SI) was less than or equal to the common intercept value of the lines.

These assumptions were verified using SAS 9.1 (SAS Institute, Cary, NC, U.S.) using a standard methodology for animal bioavailability studies.³⁴

For each linear response, the slope value and the standard deviation were determined for each value. The RBA was calculated as the ratio of the soil to the reference matrix slope values, when the difference between the two slope values was significant ($P < 0.05$).³⁴ In the case of a nonsignificant difference between the two slope values, the RBA was assumed to be 100%.

Unified BARGE Method. Bioaccessibility measurements were performed on five replicates of each soil and reference matrix (Na-arsenate, K-antimonate, Pb-acetate and Cd-chloride) using the UBM. A full description of the method is given in the SI.

Bioaccessibility Calculation. The following equations are used to calculate bioaccessible concentration for the “stomach”

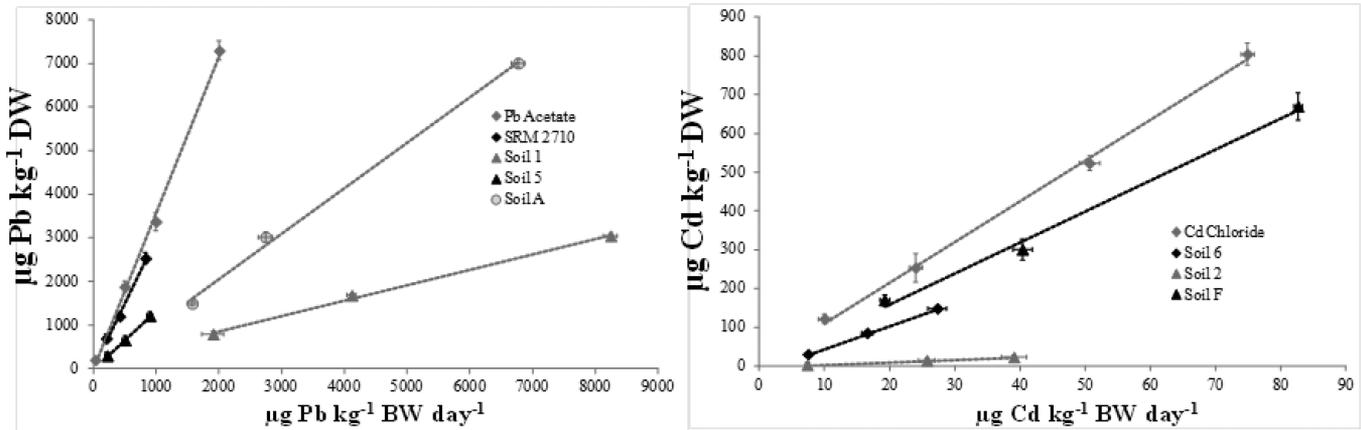


Figure 1. Examples of linear dose–response curves for Cd (kidney) and Pb (liver).

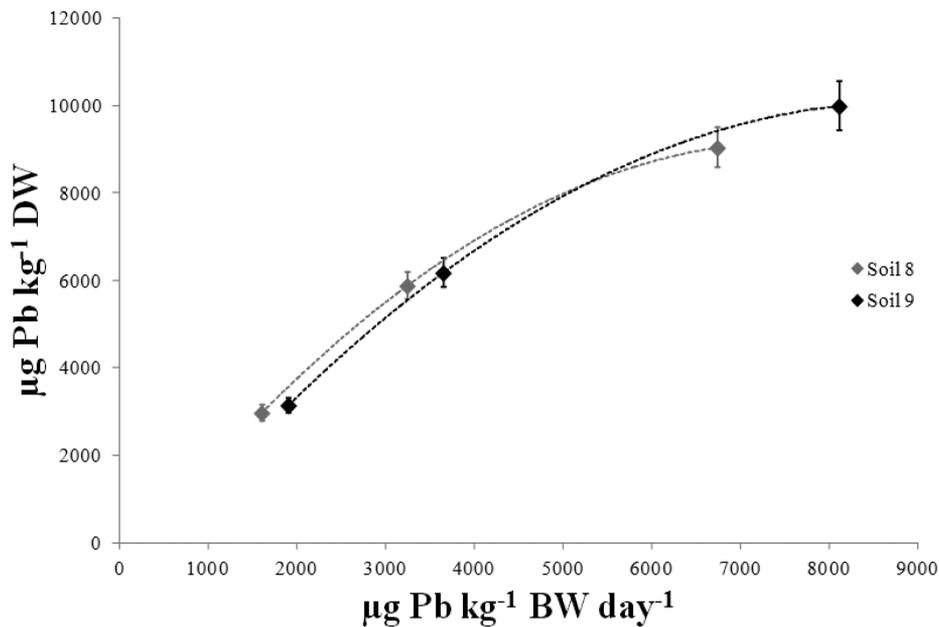


Figure 2. Nonlinear dose–response curve for Pb in liver.

and “stomach and intestine” phases and the bioaccessible fraction in the soil.

$$BA_{s\&int} = V_{s\&int} \times C_e \times d/m \tag{1}$$

$$BA_s = V_s \times C_e \times d/m \tag{2}$$

$$BAF_s = 100 \times BA_s/T_e \tag{3}$$

$$BAF_{s\&int} = 100 \times BA_{s\&int}/T_e \tag{4}$$

where:

BA_s = bioaccessible concentration for the “stomach” phase in the soil ($mg\ kg^{-1}$)

$BA_{s\&int}$ = bioaccessible concentration for the “stomach and intestine” phase in the soil ($mg\ kg^{-1}$)

V_s = volume of fluid used in the “stomach” phase extraction including any pH adjustments (mL)

$V_{s\&int}$ = volume of fluid used in the ‘stomach and intestine’ phase extraction including any pH adjustments (mL)

C_e = measured concentration of the contaminant e in the diluted extract solution ($mg\ L^{-1}$)

d = dilution applied to the extract solution prior to analysis

m = mass of soil used in the extraction (g)

T_e = total concentration of the contaminant e in the soil ($mg\ kg^{-1}$)

BAF_s = The “stomach” phase bioaccessible fraction (%)

$BAF_{s\&int}$ = The “stomach and intestine” phase bioaccessible fraction (%)

Statistical Analysis. The statistical analysis was carried out using the R programming language.³⁵ The regression analysis was carried out using Siegel’s repeated medians method³⁶ as implemented in the “mblm” R statistical analysis package.³⁷

RESULTS AND DISCUSSION

Animal Health over the Time Frame of the Experiment. During the 14 runs of the in vivo experiments, the animals exposed to As, Cd, Pb, and Sb contamination remained healthy, continued to consume their feed, grew normally, and none died. The mean BW of the swine at the beginning of experiment was $9.5 \pm 1.2\ kg$ ($n = 168$ swine) and, at the end $16.8 \pm 1.5\ kg$ ($n = 168$ swine). Moreover, there was no

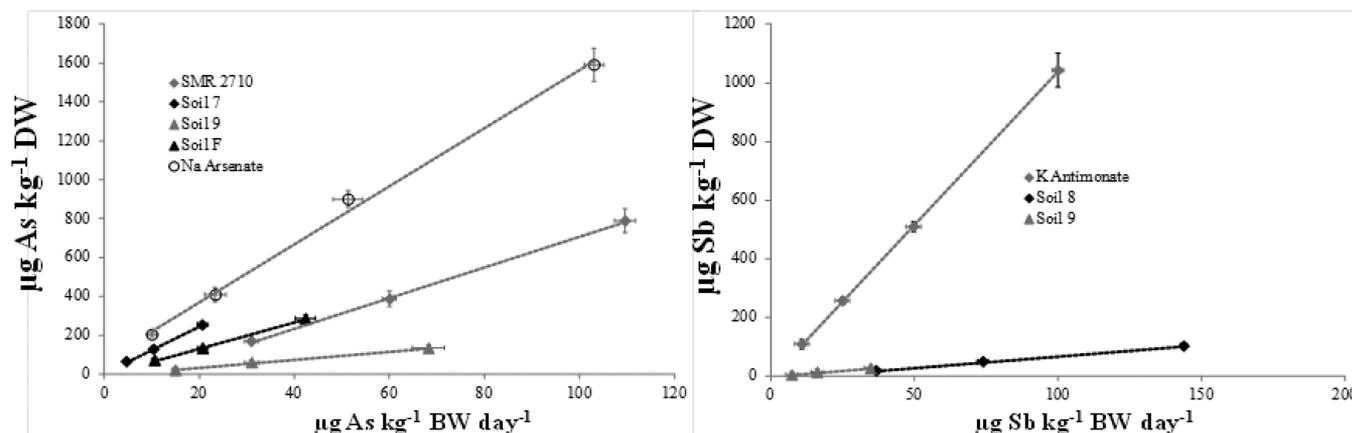


Figure 3. Examples of linear dose response curves for As and Sb in urine.

correlation between the several exposure doses for each contaminant and the final BW of each swine (r -square = 0.12, $p > 0.05$, $n = 168$). Similarly, for the different target end point (kidney, liver, and metacarpal IV), there was no impact of exposure doses on their final weight.

Dose–Response Curves. To ensure comparability between the dose response curves for the soluble salt and for the soils, the concentration of the soluble salt dose was designed to give a response which encompassed those obtained for each element in each soil for each end point (see Figures 1, 2, and 3).

Metals—Cd and Pb. The concentrations of Cd and Pb in the end points resulting from dosing with the reference matrix were all above quantification limits. For the soils, Pb concentrations were all above the quantification limits, whereas for Cd some concentrations were below. When the concentrations were measurable, the dose–response curves for both soils and reference matrix fitted to a linear model ($p < 0.05$) (example plots in Figure 1) except for soils 8 and 9 for the Pb response in the liver (Figure 2). A similar pattern in the dose–response curves (Figure 1) has been previously reported for both Pb and Cd.^{11,16,33}

The repeatability of each response was also evaluated by calculating the relative standard deviation (RSD) $n = 3$ data for each end point. Where RSD is calculated as the mean value divided by the standard deviation expressed as a percentage) For Pb, the RSD values were the lowest for Pb-acetate (less than 1%) and the highest for soils F and G (around 20%). For Cd, the RSD values were lowest for Cd-chloride (less than 1%) and highest for soil 2 (around 30%)

Metalloids—As and Sb. Arsenic was quantified in each end point, giving linear models ($p < 0.05$). For Sb, however, apart from soils 1 and 2 with high total Sb content (Table S1 of the SI), dose response data could only be obtained for urine. The dose–response curve for this end point fitted a linear model ($p < 0.05$) (Figure 3). Example dose response curves for both As and Sb are given in Figure 3.

The repeatability of the response was also evaluated by calculating its RSD for each end point. For As, the lowest RSD value was obtained for the reference matrix (around 0.6%) and the highest value was obtained for the soil 7 (70%). For Sb, the lowest RSD was obtained for urine on the reference material (less than 1%). The soils ranged from 15% to 50%. This reflects the difficulty of obtaining reproducible values of Sb concentrations due to the combined effect of relatively low

concentrations (apart from soils 1 and 2) and low bioavailability of this element.

Relative Bioavailability of Pb and Cd. The Cd and Pb RBA values and associated uncertainties are given in the SI (Table S4). Cd-RBA could not be calculated for soils 1, 4, 10, and A, C, E in any of the end points because the concentrations were below the quantification limits. The Pb-RBA could not be calculated in soils 8 and 9 from the liver results, as the dose–response curves for these end points were not linear. For soils E and F (kidney, bone and urine) and for soil D (bone and urine) there was no significant difference between the slopes obtained for the reference matrix and the contaminated soil for Pb. In these cases, the RBA was 100% meaning that Pb in these soils is as bioavailable as Pb-acetate for the purposes of oral exposure.

The RBA values were consistent among the end points (Table S2 of the SI) and were reproducible between the replicates. This confirms the robustness of the juvenile swine in vivo model to estimate the RBA of Pb and Cd in contaminated soils. The RBA values are within the range of other juvenile swine studies with the same soluble reference compounds.^{11,15,16,33}

Both Pb and the Cd showed a similar range of RBA values with a good coverage of the % RBA range with minimum to maximum values of 6–100% RBA for Pb and minimum to maximum values of 9–89% RBA for Cd. This is a fundamental prerequisite to use these data in correlation studies.^{22,38}

Relative Bioavailability of As and Sb. RBA values for As and Sb estimated from each end point and each soil samples are given in the SI (Table S3).

The RBA for As could not be calculated for any of the target compartments for soils 5, D and E and the RBA of Sb could not be calculated for soils 3, 5, D and E as the concentrations of the elements in the end points were below the quantification limits. This reflects a strong decrease of both As and Sb bioavailability compared to the reference matrix.

For Sb, the RBA could be calculated from kidney, liver, and bone only for soils 1 and 2, with the highest Sb content (Table S1). For these two soils, the RBA values of Sb were consistently low among the target end points, (<4%). For the other soils, the RBA was measured only in urine and did not exceed 11%. For As, the average minimum to maximum % RBA range was 3–74%.

The results obtained for Sb are critical for the overall objective of this study as a fundamental criterion of such a validation study is to have values of RBA evenly spread between

the minimum and maximum interval for the overall data set.^{22,38} This is probably due to a particularly low overall bioavailability of Sb irrespective of the soil properties. Unfortunately, no previous study on Sb has been published in the literature for comparison with the data produced here. The low average % RBA range for Sb (2–6%) is unlikely to be suitable for validation of in vitro bioaccessibility tests.

For As (Table S3 of the SI), however, the RBA values are evenly dispersed over the RBA range (3–100%). Moreover, the RBA values are similar to the range of values obtained by several authors on soils contaminated by both mining and smelting activities. For instance, Rodriguez et al.³⁹ reported values ranging between 3% and 43%, and Juhasz et al. reported¹³ 7–75%. A major factor that explains the variation observed among the soil samples is the solid phase distribution of As within the soil which differs according to soils type and physicochemical properties.¹³

Bioaccessibility of As, Sb, Cd, and Pb in the Reference

Matrix. The BAF of each PHE in the soluble salts used to measure the in vivo RBA were determined using the UBM procedure (As in Na-arsenate, Sb in K-antimonate, Pb in Pb-acetate, and Cd in Cd-chloride). These soluble salts were spiked to give a 1 mg kg⁻¹ concentration of each of the elements in the final “stomach” or “stomach and intestine” extract. This allowed the calculation of the relative bioaccessibility (RBAC), to allow a direct comparison with the RBA values. For the cations in the “stomach” phase, the BAF values were 99 ± 2% and 98 ± 3% for Pb-acetate and Cd-chloride, respectively. For the anions, the As BAF was 95 ± 3% and the Sb BAF was 93 ± 5%. This showed that all four elements were either indistinguishable or within 2% of being 100% bioaccessible for the reference compounds in this compartment. In contrast, in the “stomach and intestine” phase the cations had much reduced BAFs with Pb and Cd giving values of 66 ± 3% and 68 ± 3% with As and Sb BAFs of 92 ± 4% and 90 ± 2%, respectively. The lower recoveries of Pb and Cd can be explained by the fact that the behavior of these elements is strongly pH dependent. In the higher pH environment of the “stomach and intestine” phase these metals can precipitate from solution, be reabsorbed onto the soil, and complexed by pepsin.^{40,41} This is not observed in the case of elements (such as As and Sb) that form anions in solution and is consistent with previous studies.⁴²

Relative Bioaccessibilities of As, Cd, Pb, and Sb in the Contaminated Soils. The RBAC was estimated as the ratio of the soil bioaccessibility to the reference matrix bioaccessibility (%) for each phase and each element and are tabulated in the SI (Tables S4 and S5). When individual *t* statistic 95% confidence intervals were calculated for Cd and Pb the data indicated that, in general, the “stomach and intestine” bioaccessibility is not significantly different from the “stomach” phase bioaccessibility except for soils 5, 7, C and E for Pb and soil 5 for Cd where the “stomach phase” gives a significantly higher bioaccessibility. For Cd and Pb the “stomach phase” bioaccessibility is usually significantly higher than the GI bioaccessibility for these elements.²⁹ This is because of the behavior of Pb and Cd is strongly pH dependent with lower solubility in the higher pH environment of the GI compartment. In this instance, however, the bioaccessibility results have been calculated relative to the bioaccessibility of the soluble salts (Pb-acetate and Cd chloride) which also show reduced solubility at high pH. Taking measurement relative to the

soluble salts therefore corrects for the lower absolute Pb and Cd bioaccessibilities in the “stomach and intestine” phase.

For the mining soils, RBAC of Pb and Cd ranged from 9% to 75% and from 7% to 70%, respectively. For the smelting soils, the relative Pb bioaccessibility ranged from 40% to 90% and the relative Cd bioaccessibility ranged from 28% to 87%. These values are similar to values reported in the literature.^{16,42,43}

For As and Sb, no difference was observed between the two phases, apart from soil 2 for As. The values of As RBAC ranged between 3% and 11% for the mining soils and between 11% and 74% in the smelting soils. Thus, it seems that the bioaccessibility seems to be influenced by the source of contamination, being higher in the smelting contaminated soils. This might be due to the difference in the solid phase distribution of As within the soil constituents between the mining and smelting soils. In the mining soils As appears to be associated with iron oxides and sulphide minerals and consequently has a low bioaccessibility.^{44–46}

For Sb, RBAC was always 20% lower than RBA and no significant difference was observed between mining and smelting soils. This overall low bioaccessibility might be explained by the association of Sb and soil bearing phases like iron oxy-hydroxides, sulphides, and refractory soil constituents^{47–50} that are not easily dissolved by the artificial digestive solutions used during the UBM.

Correlation between Relative Bioavailabilities and Bioaccessibilities. For a given contaminant, the bioavailability theoretically results from three steps:

- the dissolution of the contaminant in the lumen that is determined as the bioaccessibility (BAC);
- the absorption of the contaminant through the GI membrane (ABS); and
- the metabolism of the contaminant within the internal media (this is assumed to be negligible for trace elements).⁴²

The RBA can be determined from the following formula:⁴²

$$RBA = RBAC \times ABSR \quad (5)$$

where:

- RBAC = the relative bioaccessibility of the contaminant, i.e., the soil:reference matrix bioaccessibility of the contaminant
- ABSR = the relative absorption of the contaminant.

If the RBA is properly reflected by the RBAC, then the bioaccessibility should be the limiting factor.^{38,42} As such, ABSR should be close to 1, meaning that the absorption step is independent from the initial form of the contaminant that is ingested. In this case, RBA should be equal to RBAC, i.e., the slope of the regression between RBA and RBAC should be equal to 1. The slopes of the regression between RBA and RBAC were calculated for each target compartment and for the two phases of the UBM.

Regression of the UBM Relative Bioaccessibility against In Vivo Relative Bioavailability. An earlier study comparing UBM data against in vivo bioavailability on test soils,²⁵ set out a series of benchmark criteria that should be met by the in vitro and in vivo data and any subsequent mathematical regression relationship in order for the in vitro methodology to supply “fit for purpose data” for risk assessments. The first criterion is that the median repeatability on the bioavailability data should be better than 20% RSD.

Figures S1 and S2 of the SI show boxplot summaries of the repeatability (RSD of the RBA replicate measurements) of the bioavailability the four end points of the sixteen soils for As, Cd, and Pb.

Sb has not been included since the bioavailability data were of insufficient quality to carry out a correlation with bioaccessibility data.

For both Cd and Pb, the median repeatability values are well within the benchmark (Figure S1 of the SI). The repeatability values for As values are higher for all end points with the kidney end point benchmark value of 20.6% and the liver end point only just above at 22.5% (Figure S1 of the SI). Although not strictly met, it is considered that a median value of 20.6% vs the ideal criteria of 20% for the two compartments was considered acceptable for the kidney end point and should not to compromise the use of the UBM for As in a soil risk assessment.

The second benchmark relates to the bioaccessibility repeatability (within-laboratory variability) and reproducibility (between-laboratory variability). The former should have a median value of 10% RSD and the latter a median value of 20% RSD.

Only within-laboratory data are available for this study so, only the repeatability can be tested. Figure S2 of the Supporting Information shows boxplots of the repeatability (RSD of the RBAC replicate measurements) for each of the elements studied.

In this case, Sb values have been included since robust results were obtained for this element from the UBM bioaccessibility test. Figure S2 of the SI shows that the median repeatability values for the UBM are all below the 10% benchmark for all elements in both the “stomach” and “stomach and intestine” compartments. The median reproducibility values are very similar for each compartment although the spread of values is consistently higher in the “stomach and intestine” compartment. Median repeatability values are all very similar at c. 5–7% RSD but As shows higher variability in values compared the other three elements. This is a similar pattern to the *in vivo* data shown in Figure S1 of the SI.

The next set of benchmark criteria relate to the statistical parameters associated with linear regression fits to the relationship between RBA and RBAC. Since there is significant error (median RSD of up to 30% for bioavailability and 8% for bioaccessibility, Figures S1 and S2 of the SI) on both the bioaccessibility and bioavailability data (Tables S2–S5 of the SI), ordinary linear regression is not appropriate as it assumes that there are errors only on the “y” coordinate. In this study, a repeated medians approach³⁶ is used, which makes no assumptions about errors and is robust to outliers. The method has been applied using a Monte Carlo approach varying each point over a normal distribution described by its mean value and standard deviation. The advantage of this is that it produces a distribution of values for the descriptive statistics for the regression (intercept, slope, and *r*-square) so that 95% confidence intervals can be calculated and can then be judged against a benchmark value. Wragg et al²⁵ suggested that the benchmark criteria should be as follows:

- (i) The intercept is not significantly different from 0;
- (ii) The slope should be between 0.8 and 1.2; and
- (iii) The *r*-square value (measure of the scatter around the line) should be greater than 0.6.

Using this methodology, the linear regressions of relative bioaccessibility against relative bioavailability were calculated using the data from the SI (Tables S2–S5). All data were included in the calculation apart from the RBA values which could not be calculated because the absolute concentration of the elements in the target organ was below detection limit or because the dose response curves were not linear. Summary statistics, in the form of a mean value of the intercept, slope, or *r*-square value and their associated 95% confidence intervals for each element regression for each end point and each stomach compartment are shown in Figures 4, 5, and 6.

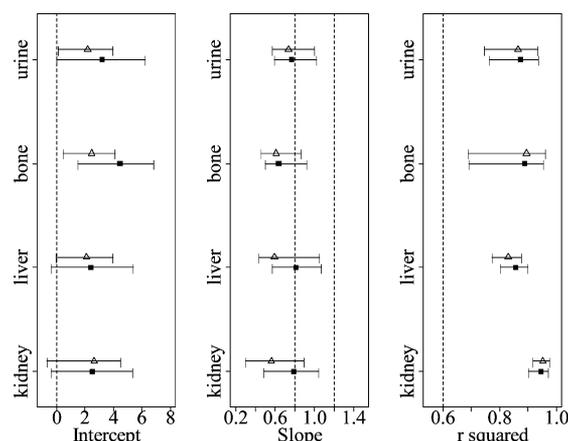


Figure 4. Summary of the RBA vs RBAC regression statistics for the four end points for As. Black squares show data for the “stomach” phase and white triangles for the “stomach and intestine” phase. Error bars represent 95% confidence limits dotted lines show benchmark values.

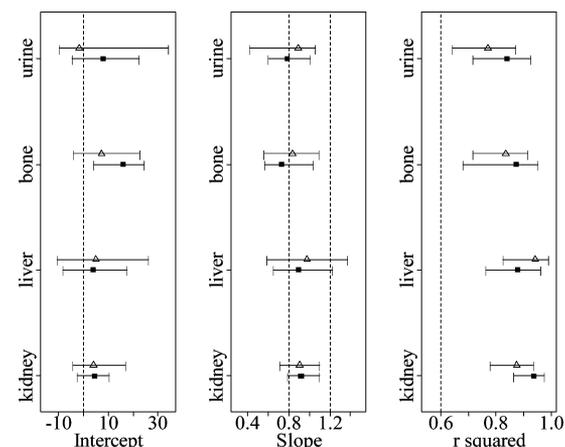


Figure 5. Summary of the RBA vs RBAC regression statistics for the four end points for Cd. Black squares show data for the “stomach” phase and white triangles for the “stomach and intestine” phase. Error bars represent 95% confidence limits, dotted lines show benchmark values.

Examination of Figures 4–6 shows that, for all elements in all end points the slope and the *r*-square values all meet the benchmark criteria. For Cd (Figure 5) the intercepts only shows one incidence out of eight where the intercept is positive (bone in the stomach compartment). For both As and Pb (Figures 4 and 6), however, there are five incidences out of eight where the intercepts are shown to be >0. This suggests there is a small bias in the RBAC measurement for these

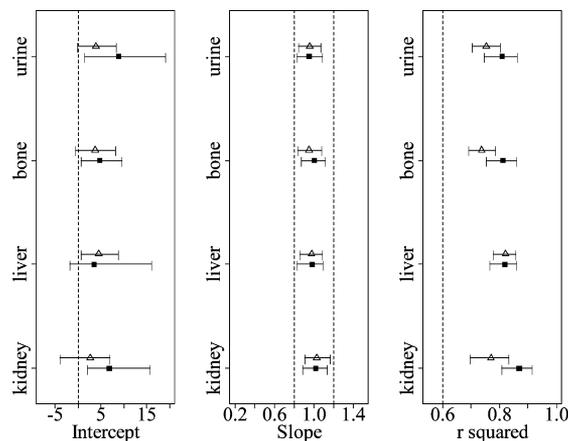


Figure 6. Summary of the RBA vs RBAC regression statistics for the four end points for Pb. Black squares show data for the “stomach” phase and white triangles for the “stomach and intestine” phase. Error bars represent 95% confidence limits, dotted lines show benchmark values.

elements compared to the RBA (3% RBAC for As and 5% RBAC for Pb averaged over all end points and compartments). The plots also confirm that there is no significant difference between the “stomach” and “stomach and intestine” compartments for all three elements and all four end points.

For the four target end points selected for this study (kidney, urine, bone, and liver), the *r*-square value for the RBAC and RBA regressions were all significantly different from 0 both for the “stomach” and the “stomach and intestine” phases. Since the slopes of regressions are all close to 1, it appears that the RBAC is actually the limiting factor of the RBA. This confirms the ability of the UBM test to assess the bioaccessibilities of As, Cd, and Pb in the contaminated soils studied. The bioaccessibility as measured by UBM better reflects the external exposure to soil contaminants following an oral ingestion than the total concentration.

Figures 7 and 8 show example RBAC plots versus RBA showing how the fitted regressions are very close to the ideal 1:1 relationship for As (Figure 8, parts a and b), Cd and Pb (Figure 7) but with evidence for small positive intercepts in As in the “stomach” phase (Figure 8a) and Pb in the “stomach” phase (Figure 7a). For Sb, however, the low bioavailabilities and bioaccessibilities that were measured for the soils sampled in this work meant that the correlations could only be studied in the 0–20% area. Under these conditions, the UBM test could not be validated for Sb due to a lack of statistical significance, which is clearly illustrated in Figure 8, parts c and d. The 95% confidence interval in the line of best fit is far too wide to provide a useful relationship between RBAC and RBA, which could be used by a risk assessor.

The juvenile swine model has been shown to produce RBA values that are consistent within the target end points for As, Cd, and Pb for the 16 soils studied. The variety of soil types and the range of total element values are representative of the total concentrations of these elements that would normally be considered for bioaccessibility testing.²⁵ The RBA values for all three of these elements covered at least 70% of the RBA range making them highly suitable for calibrating *in vitro* testing protocols.

For Sb, however, the RBA values were approximately 10% or less for all soils and it was difficult to measure the amount of Sb absorbed into the target end points, apart from urine, for all but

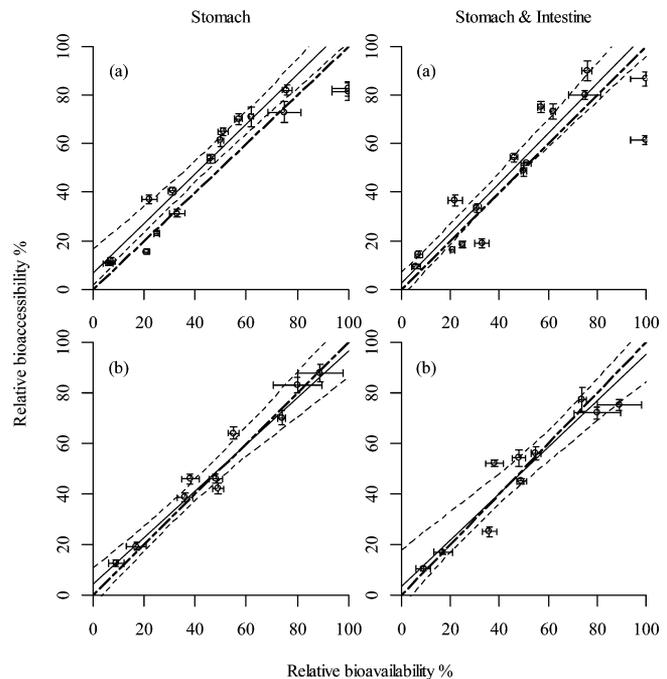


Figure 7. Correlation plots for RBAC against RBA for (a) Pb and (b) Cd for the “stomach” and “stomach and intestine” phases for the kidney end point. Bold dashed dotted line is the line of equivalence, dashed lines are the 95% confidence intervals, and the solid lines is the best line of fit.

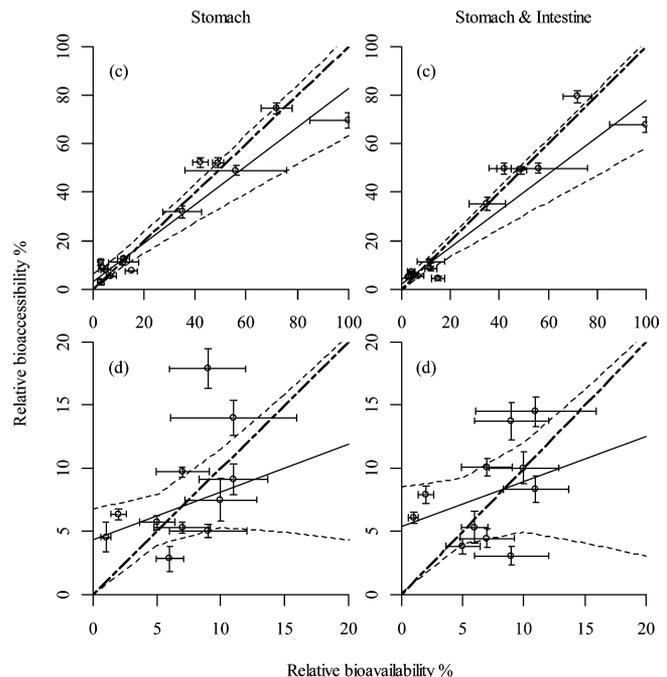


Figure 8. Correlation plots for RBAC against RBA for (c) As and (d) Sb for the “stomach” and “stomach and intestine” phases for the urine end point. Bold dashed dotted line is the line of equivalence, dashed lines are the 95% confidence intervals, and the solid line is the best line of fit.

soils 1 and 2, which are grossly contaminated with Sb (>50 000 mg kg⁻¹, Table S1 of the SI). The small RBA range covered will not make this data set suitable for calibrating Sb bioaccessibility measurements from *in vitro* testing methods.

While it would be impossible to show that the UBM has been validated for all soil types, this study has concentrated on soils with anthropogenic contamination (combined with their natural PHE content) which are likely candidates for human health risk assessment. The study has used soils from a variety of spatial locations with a range of physicochemical properties and which exhibit a good range of PHE bioaccessibilities. These results provide strong evidence that, through a pragmatic choice of soils, the UBM provides a robust tool for use in risk assessment of As, Cd, and Pb. The study suggests the “stomach” compartment alone is a good analogue of in vivo bioaccessibility but this need to be confirmed by use of the method on a wider variety of soils.

This study has addressed many of the issues arising from a preliminary interlaboratory trial of the UBM²⁵ showing that a specifically designed in vivo study with soils relevant to European conditions along with better control on pH in the “stomach” phase that the UBM produces bioaccessibility data that is a very good analogue of juvenile swine bioavailability measurements for As, Cd, and Pb. The one point that this study has not yet addressed is the interlaboratory reproducibility that was problematic in the study of Wragg et al.²⁵ A further follow up study on interlaboratory performance is required to provide the last piece of evidence that the method can be used as a routine test in risk assessment studies.

■ ASSOCIATED CONTENT

● Supporting Information

Details of the procedures used to determine the bioavailability and bioaccessibility of the PHEs and methods used for the preparation, analysis, and quality control of the PHEs in the soil and swine samples. Tabulations of the soil properties and the bioavailability of the bioaccessibility of the PHE in each of the soils tested, along with box and whisker plots of the repeatability of the bioavailability and bioaccessibility measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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