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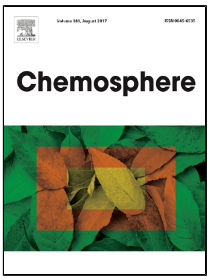

Measurement of soil lead bioavailability and influence of soil types and properties: a review

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Measurement of soil lead bioavailability and influence of soil types and properties: a review

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Abstract:

Lead (Pb) is a widespread heavy metal which is harmful to human health, especially to young children. To provide a human health risk assessment that is more relevant to real conditions, Pb bioavailability in soils is increasingly employed in the assessment procedure. Both *in vivo* and *in vitro* measurements for lead bioavailability are available. *In vivo* models are time-consuming and expensive, while *in vitro* models are rapid, economic, reproducible, and reliable while involving more uncertainties. Uncertainties in various measurements create difficulties in accurately predicting Pb bioavailability, resulting in the unnecessary remediation of sites. In this critical review, we utilised available data from *in vivo* and *in vitro* studies to identify the key parameters influencing the *in vitro* measurements, and presented uncertainties existing in Pb bioavailability measurements. Soil type, properties and metal content are reported to influence lead bioavailability; however, the differences in methods for assessing bioavailability and the differences in Pb source limit one's ability to conduct statistical analyses on influences of soil factors on Pb bioavailability. The information provided in the review is fundamentally useful for the measurement of bioavailability and risk assessment practices.

Key words: soil, *in vivo*, *in vitro*, lead, bioavailability, uncertainties.

Contents

61 Abbreviation

62

63 ABA----------absolute bioavailability

64 AUC----------Area under curve

65 BA---------- ----------bioavailability

66 BAc----------bioaccessibility

67 BW----------body weight

68 CEC----------cation exchange capacity

69 G-phase----------gastric phase

70 IEUBK----------Integrated Exposure Uptake Biokinetic model

71 I-phase----------intestinal phase

72 IV----------intravenous

73 IVG----------In Vitro Gastrointestinal (IVG) Method

74 IVIVC----------correlation between *in vivo* and *in vitro* methods

75 OM----------organic matter

76 PBET----------A Physiologically Based Extraction Test

77 RBA----------relative bioavailability

78 RBALP----------Relative Bioavailability Leaching Procedure

79 RIVM----------In Vitro Digestion Model (RIVM)

80 SBRC----------Solubility Bioaccessibility Research Consortium assay

81 S:L ratio----------solid:liquid ratio

82 TOC----------total organic carbon

83 UBM----------Unified BioAccessibility Research Group Europe (BARGE) method

84 US EPA----------U.S. Environmental Protection Agency

85

1 Introduction

Exposure to lead (Pb) is of increasing concern due to the global scale of its occurrence in the environment and adverse health effects (U.S. Environmental Protection Agency, 2014). Oral ingestion of Pb contaminated soil is a major pathway for exposure to humans especially children (U.S. Environmental Protection Agency, 2014). Ingestion of Pb contaminated soils by children is of particular concern due to their hand-to-mouth activities and higher metabolic rate (Gulson et al., 1995; Oomen et al., 2003; U.S. Environmental Protection Agency, 2007a), which will result in a permanent influence on children's development of neuronal systems, cell function and a decrease of children's intelligence quotient (Shannon, 1998). Even at a low blood lead level, a range of neurocognitive, behavioural and other specific issues have been reported as being linked to Pb exposure (Benetou-Marantidou et al., 1988; Dietrich et al., 1990). The U.S. Environmental Protection Agency (EPA) indicates that there is no safe threshold for children exposed to Pb (U.S. Environmental Protection Agency, 1994, 2007a).

Total Pb concentration in contaminated soils contributes to Pb exposure and influences blood lead level in children; however, an increasing number of investigations have indicated that using total Pb concentration may overestimate the risks from Pb exposure (C. R. Janssen et al., 2000; Oomen et al., 2006; U.S. Environmental Protection Agency, 2007a; Li et al., 2014; Wijayawardena et al., 2014), since only a fraction of Pb in ingested soil can cause adverse effects to human health due to the influence from soil properties and sources, Pb distribution and metabolism of Pb in organisms (Ruby et al., 1996; Oomen et al., 2006). Usage of the 'effective' fraction of total ingested Pb is recommended to assess risks and adverse effects from Pb exposure to humans especially children (Ruby et al., 1996; Oomen et al., 2006). Bioavailability (BA), as a linkage parameter between total concentration and the 'effective' fraction for exposure assessment, holds promise for determining a more realistic basis for

111 environmental risk assessment and remediation (Belfroid et al., 1996). The term BA in this
112 study is defined as the fraction of an ingested dose that crosses the gastrointestinal epithelium
113 and becomes available for distribution to internal target tissues and organs (U.S. Environmental
114 Protection Agency, 2007b).
115
116 Extensive research efforts have been made for Pb BA measurement; however, it continues as
117 a challenge due to the existence of a large number of uncertainties, inadequate information,
118 and lack of reliable predictive models (U.S. Environmental Protection Agency, 2014).
119 Although the U.S. EPA has established that relative bioavailability (RBA) of Pb in soil is 60%
120 in the Integrated Exposure Uptake Biokinetic (*IEUBK*) model, Pb RBA has been reported to
121 be wide-ranging. For example, Casteel et al. (2006) reported RBA of Pb using a swine model
122 ranging from 6% to 105%.
123
124 Numerous research attempts have been made on measuring Pb BA via *in vivo* models such as
125 in swine (*Sus scrofa*), rats (*Rattus*), mice (*Mus*), monkeys (*Cercopithecidae*), rabbits
126 (*Oryctolagus cuniculus*); however, limited data and information are available due to time-
127 consuming and cost-factors as well as ethical issues (Juhasz et al., 2007; U.S. Environmental
128 Protection Agency, 2007a). Moreover, challenges exists when extrapolating data from *in-vivo*
129 studies to human health due to the physiological differences and species diversity between
130 humans and the experimental animal models (Ruby et al. 1999). A potential alternative
131 approach to supersede *in vivo* studies is the use of *in vitro* tests to measure Pb bioaccessibility
132 (BAc) (i.e. the fraction that is soluble in the gastrointestinal environment and is available for
133 absorption), which are economic, rapid, and reproducible, but involves more uncertainties
134 (FaciesRuby et al., 1999; C. R. Janssen et al., 2000). At present there are various *in vitro*
135 models being developed to determine Pb BAc, such as the Relative Bioavailability Leaching

136 Procedure (RBALP), the unified BioAccessibility Research Group Europe (BARGE) method
137 (UBM), the Solubility Bioaccessibility Research Consortium assay (SBRC), a Physiologically
138 Based Extraction Test (PBET), the In Vitro Gastrointestinal (IVG) Method and the In Vitro
139 Digestion Model (RIVM). Although all these models were validated by various *in vivo* models
140 and correlations between *in vivo* and *in vitro* models (IVIVC) were found (Ruby et al., 1996;
141 Schroder et al., 2004; Oomen et al., 2006; Drexler and Brattin, 2007; Juhasz et al., 2009; Denys
142 et al., 2012), there are still many uncertainties due to varied soil properties and parameters of
143 each method. For example, for the soils from the same source, the IVIVC based on the same
144 *in vivo* model (swine) and different *in vitro* models (IVG and RIVM), the slopes and r^2 differ
145 from each other (Schroder et al., 2004; Oomen et al., 2006).
146
147 Lead in soil can be distributed in a range of discrete mineral phases, include co-precipitated or
148 sorbed Pb associated with soil minerals, clay and organic matter (OM), and dissolved Pb that
149 may be complexed with varied organic and inorganic ligands (Mortvedt, 1991). All these
150 phases are believed to control Pb dissolution properties and hence influence its BAc
151 (FaciesRuby et al., 1999). Oomen et al. (2006) stated that Pb BA can be affected by the soil
152 characteristics and Pb speciation. Moreover, soil properties like clay content, pH, OM, and
153 cation exchange capacity (CEC) are reported to be related to Pb BAc (Buchter et al., 1989; He
154 and Singh, 1993; Hornburg and Brümmer, 1993; Rieuwerts et al., 2006; Poggio et al., 2009;
155 Roussel et al., 2010). All this implies that it may therefore be possible to find a correlation
156 between Pb BA and soil properties.
157
158 In this critical review, a summary of current measurements of Pb RBA/BAc (*in vivo* and *in*
159 *vitro* models) is included, with an emphasis on the influence of soil type and properties on Pb
160 RBA/BAc, and uncertainties in measuring Pb RBA/BAc. An overall understanding is shown

in Figure 1, which illustrated the relationships between different concepts. The interaction of Pb contaminants with soil particles influence the Pb RBA/Bac which is to be incorporated in the risk assessment procedure. Detailed information on the measurement approaches, influence of soil properties and sources are included in the following sections. The information is important for understanding critical issues related to Pb RBA/BAc, including the mechanisms of soil properties in controlling Pb RBA/BAc. Indications on human health risk assessment and development of technologies for remediation of Pb contaminated soils can be also obtained.

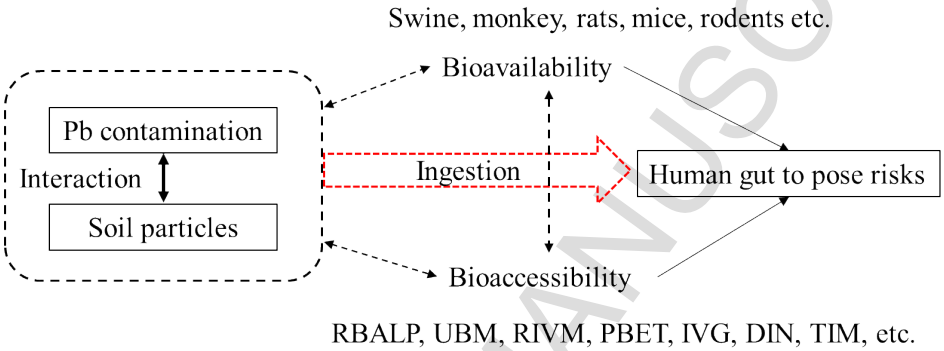

Figure 1 Illustration of concepts in Pb bioavailability research

(RBALP: the Relative Bioavailability Leaching Procedure; UBM: the unified BioAccessibility Research Group Europe (BARGE) method; SBRC: the Solubility Bioaccessibility Research Consortium assay; PBET: a Physiologically Based Extraction Test; IVG: the In Vitro Gastrointestinal (IVG) Method; RIVM: the In Vitro Digestion Model (RIVM)).

2 Measurement of Pb bioavailability/bioaccessibility

2.1 Pb bioavailability

2.1.1 Absolute bioavailability

As stated, BA data is essentially related to the amount of Pb in animal/human bloodstream and tissues (Wragg and Cave, 2003). The Pb BA is a fraction of a dose of Pb which is referred to as absolute bioavailability (ABA) (U.S. Environmental Protection Agency, 2007b). The calculation of ABA in blood is based on the area under curve (AUC) (**Error! Reference source not found.**), as defined in Equation 1 where Dose $_{IV}$ is the intravenous dose of reference material (Pb acetate, µg/L), AUC_{IV} is the area under the blood lead concentration curve after IV dosage (µg*h/L). These factors subscripted *ORAL* are equivalent values for oral dose of test soils/dust (R.Naidu, 2003).

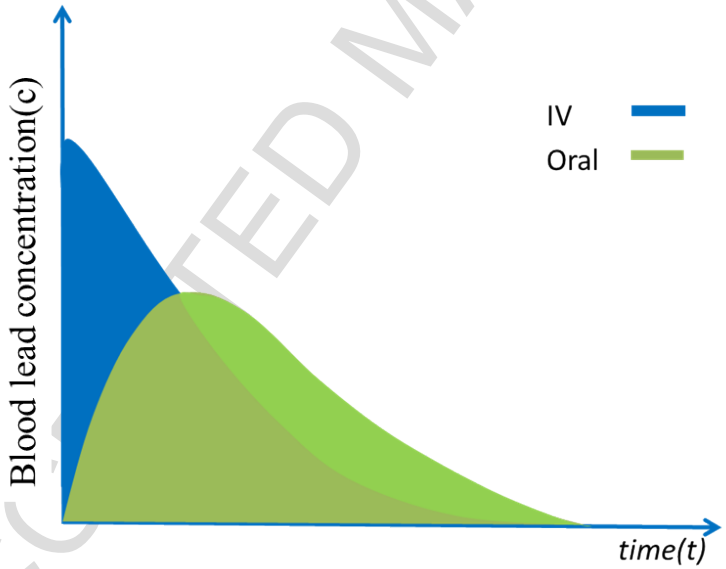

Figure 2 Bioavailability Plasma-concentration

$$ABA\ (\%) = (AUC_{oral})(Dose_{IV})/(AUC_{IV})(Dose_{oral}) * 100\% \quad (1)$$

2.1.2 *Relative bioavailability*

The Pb RBA is defined as the comparative bioavailability of different forms of Pb containing the substance (e.g., bioavailability of a metal from soil relative to its bioavailability from Pb acetate solution) (Ruby et al., 1999). In order to measure Pb RBA in a particular test material compared to Pb in a reference material (Pb acetate), the underlying principle is that equal absorbed doses of Pb will produce equal increases in Pb concentration in the tissues of exposed animals or human (U.S. Environmental Protection Agency, 2007c). This means RBA is the ratio of oral doses that contribute equal increases in the tissue burden of Pb. Lead RBA in soil has been measured either via blood or via tissues such as kidney, liver, femur and urine (Denys et al., 2012). The determination of Pb RBA in soil using blood is defined in Equation 2, where ABA_{soil} is the absolute bioavailability of soil, $Dose_{soil}$ is the Pb concentration of oral dose (μg/L) that is given, AUCsoil is the area under curve of blood concentration after soil being oral given (μg*h/L). These factors subscripted oral are equivalent values for oral dose of Pb acetate (Deshommes et al., 2012; Li et al., 2014).

$$\begin{aligned} RBA\ (\%) &= ABA_{soil}/ABA_{Pb\ acetate} * 100\% \\ &= (AUC_{soil})(Dose_{Pb\ acetate})/(AUC_{Pb\ acetate})(Dose_{soil}) * 100\% \end{aligned} \quad (2)$$

The ratio of the concentration of Pb in individual endpoints (kidney, liver, femur and urine) after oral giving soil compare to oral giving Pb acetate is used to determine Pb RBA in soil using tissues. As defined in Equation 3 where $Dose_{soil}$ is the Pb concentration of oral dose (μg/L) that is given, C_{soil} is the concentration of Pb in individual endpoints (kidney, liver, femur and urine) (μg/kg). These factors subscripted oral are equivalent values for oral dose of Pb acetate (Li et al., 2017).

$$RBA\ (\%) = (C_{soil})(Dose_{Pb\ acetate})/(C_{Pb\ acetate})(Dose_{soil}) * 100\% \quad (3)$$

211 The exponential model is recommended to describe a repeated dose of the dose-response AUC
212 curve for blood Pb, as shown in Equation 4 where *a, b,* and *c* are the terms of the mathematic
213 equation used to describe the shape of the AUC curve, and *Dose* is the total daily administered
214 dose of Pb (μg/kg BW /day) (U.S. Environmental Protection Agency, 2007c).

215 $$AUC = a + b \cdot [1\text{-}\exp(-c \cdot Dose] \quad (4)$$

216 ## 2.2 Measurement of Pb relative bioavailability (*in vivo*)

217 A basic approach to estimate Pb RBA is using the *in vivo* method which is performed in a
218 biological system and where the results can be extrapolated to humans (Weis and LaVelle,
219 1991). Rodents such as mice and rats are commonly employed to estimate Pb RBA, in addition
220 to swine, minipigs and monkeys. Previous *in vivo* studies of Pb bioavailability using various
221 source of contaminated soils are shown in Figure 3 and Table 1. Swine have been employed in
222 tests for assessing various sources of Pb contaminated soils, such as from mining, smelters,
223 small arm ranges, incinerators, residential, and spiking soils (Bannon et al., 2009; Juhasz et al.,
224 2009; Denys et al., 2012; Wijayawardena et al., 2014). For all source of soils, the swine model
225 shows both the highest (140% for small arm range) and lowest (0.75% for mining soils) Pb
226 RBA values among all animal models (Schroder et al., 2004; Bannon et al., 2009). Compared
227 to swine, small animals (rats and mice) are economic and also have been widely used in tests
228 for assessing soils from in mining, smelters, gasworks, shooting ranges, farmland, and house
229 dust (Ruby et al., 1996; Smith et al., 2011a; Li et al., 2014; Li et al., 2015). Lead RBA from
230 the rats and mice models ranged from 7% to 89% for all source soils and from 7% to 36% for
231 mining soils, which were smaller ranges compared to that from the swine model (Smith et al.,
232 2011a; Li et al., 2015).

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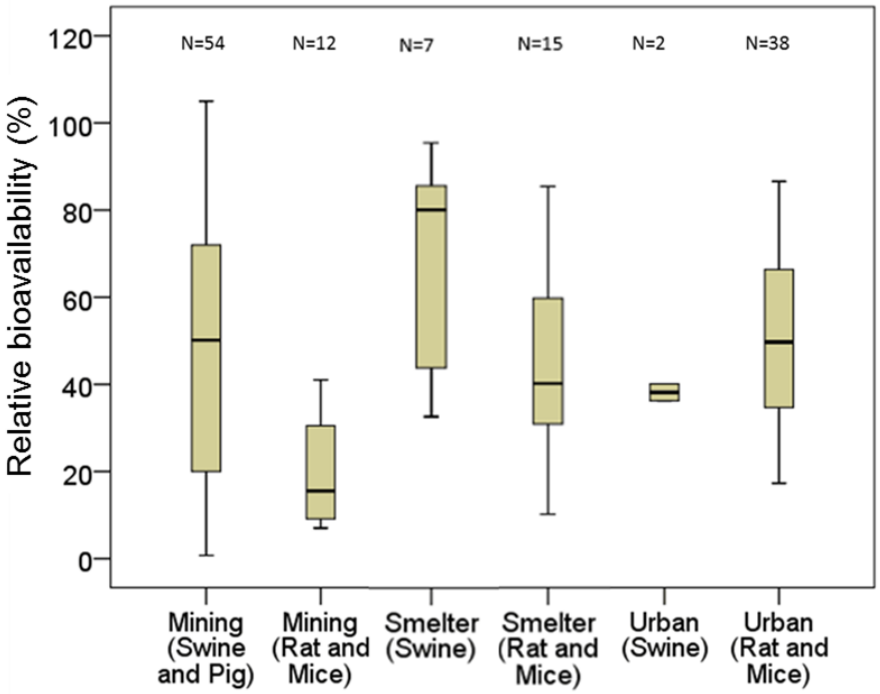

234

235 Figure 3 Lead bioavailability of various source soils in different animal studies. The central
236 mark on each boxis the median with the edges of the 25th and 75th percentiles. The whiskers
237 extend to the most extreme data points not considered outliers. Outliers were not plotted in our
238 study. The whiskers extend to the most extreme data points not considered outliers. Outliers
239 were not plotted in our study.

240 References: (Freeman et al., 1992; Ruby et al., 1996; Casteel et al., 1997; Hettiarachchi et al., 2003; Schroder et
241 al., 2004; Drexler and Brattin, 2007; Juhasz et al., 2009; Smith et al., 2011a; Denys et al., 2012; Li et al., 2015; Li
242 et al., 2016)

243

244 Various dosages of Pb were administrated to animals in different *in vivo* studies. Most of the
245 dosages of Pb given in *in vivo* studies are designed by the body weight and daily ingestion of
246 test animals (measured by the unit of μg Pb/kg BW day), and ranged from 50 μg Pb/kg BW
247 day for swine (Denys et al., 2012) to 10700 μg Pb/kg BW day for mice (Li et al., 2015). This
248 design is simulating the situation of both daily (repeat dosage) and accidental (single dosage)
249 exposure for young children to Pb contaminated soils. Both swine and rats studies are given
250 either repeat or a single dosage of Pb. For example, Pb dosages which ranged from 75 to 675

μg Pb/kg BW day were given to swine twice a day for 15 days to estimate Pb RBA (Casteel et al., 1997). Single dosages of Pb were given to mice (Smith et al., 2011a; Li et al., 2014) in most studies, this may be because mice have a relatively smaller body mass (BW = 20-25 g) and only limited blood samples are available. The only repeat dosage applied on mice (BW = 20-22g) is from (Li et al., 2016) where samples were collected from kidneys rather than blood. Both fasting and fed states are employed in previous studies, and the fasting state is more popular because this is equivalent to the situation where children and babies are prone to ingest soils when they feel hungry (U.S. Environmental Protection Agency, 2007a). For the biomarkers, swine offer more choices to estimate Pb RBA via blood, liver, kidney, bone, femur, and urine (Casteel et al., 2006; Bannon et al., 2009; Denys et al., 2012). Rats and rabbits can also offer various biomarkers such as blood, liver, kidney, and bone for estimating Pb RBA (Ruby et al., 1993; Hettiarachchi et al., 2003). Mice offer only limited blood, again, due to their small body mass (Smith et al., 2011a; Li et al., 2014).

Weis et al. (1995) initiated a juvenile swine model experimental procedure for assessing oral BA from soils, which was further developed by Casteel et al. (2006) and applied to various soils (U.S. Environmental Protection Agency, 2007a; Bannon et al., 2009). The swine model is recommended to estimate Pb RBA, because its accelerated metabolism offers better simulation of the process of an infant's and child's growth and development (Moughan et al., 1991; Casteel et al., 2006; U.S. Environmental Protection Agency, 2007a). Moreover, it obtains more biomarkers than other models.

A wide range of Pb RBA suggested a significant influence from the soil type and soil properties to Pb RBA, indicating that the IEUBK model may over- or under- estimate Pb RBA in some cases. For example, Casteel et al. (1997) tested Pb RBA using swine model on two soils from

mining sites, and Pb RBA was estimated from the biomarkers of kidney, liver, and bone after 15 days of experiments. Their results showed Pb RBA of the two tested soils are 63% and 64%, respectively, which were slightly higher than 60% (the value based on the IEUBK model from US EPA); However, in another study, Pb RBA tested by swine models on soils from mining sites showed a wider range from 0.75% to 105% (Schroder et al., 2004; Casteel et al., 2006; Denys et al., 2012). A similar performance was found from studies using rats and mice models either on soils from mining sites or from other sources (Hettiarachchi et al., 2003; Smith et al., 2011a; Li et al., 2015).

284 Table 1 *In vivo* studies on Pb contaminated soil/dust

Soil type	Pb concentration range (mg/kg)	Specimen and biomarker	Dose, period, state	RBA (%)	Reference
Mining	4482-40214	Swine (5 weeks age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50-4000 µg Pb/kg BW day, 14 days, fasting	8.25-58.67[b]	(Denys et al., 2012)
	1270-14200	Swine (5-6 weeks age, BW = 8 - 11 kg), blood/liver/kidney/femur	15 days, fasting	6-105	(Casteel et al., 2006)
	1270-14200	Swine (5-6 weeks age, BW = 10 ± 12 kg), blood/liver/kidney/bone	15 days, fasting	0.75-97.75	(Schroder et al., 2004)
	3900	Rabbits (BW = 2.1 kg), blood/liver/kidney/bone	2.0 ± 0.02 g Pb/kg BW, 36 hour, fasting	9	(Ruby et al., 1993)
	3908-10230	Rats	fed	8.7-36	(Ruby et al., 1996)
	200-6330	Minipigs (10 weeks age, BW = 4.8 kg), kidney/liver/bone/urine	500 µg Pb/kg BW day, 28 days, fasting	17-63	(Marschner et al., 2006)
	810, 3908	Rats (7-8 weeks age), blood/liver/ bone	30 days, fed	8.95, 13.57	(Freeman et al., 1992)
	2924	Human	Fast and fed	26.2(fast), 2.52 (fed)[a]	(Maddaloni et al., 1998)
	3870, 14200	Swine (BW = 8-9 kg), kidney/liver/bone	75, 225 and 675 µg Pb/kg BW day, 15 days, fasting	63, 64	(Casteel et al., 1997)
	516-4163	Mice (BW = 20-25 g), blood	2150 -10700 µg Pb/kg BW, 48 hour, fasting	7-26	(Li et al., 2015)
Smelter	1388, 2090	Rats		35, 41	(Ruby et al., 1996)
	1460-30155	Swine (5 weeks age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50-4000 µg Pb/kg BW day, 14 days, fasting	32.25-94.5[b]	(Denys et al., 2012)
	536-3200	Mice (BW = 20-25 g), blood	48 hour, fasting	10-63	(Smith et al., 2011a)

	2154	Rats blood/liver/kidney/bone	15 days, fed	35.5[c]	(Hettiarachchi et al., 2003)
	250-25329	Mice (BW = 20-25 g), blood	2150 -10700 μg Pb/kg BW, 48 hour, fasting	30.8-84.3	(Li et al., 2015)
	237-6330	Swine (6-8 weeks age, BW = 20-25 kg), blood	5 days, single dose, fasting	17-63[e]	(Juhasz et al., 2009)
Small arm range	4503-23409	Swine, blood/liver/kidney/femur	15 days	77-140[c]	(Bannon et al., 2009)
Gaswork	1343	Mice (BW = 20-25 g), blood	48 hour, fasting	43	(Smith et al., 2011a)
Shooting range	576, 1801	Mice (BW = 20-25 g), blood	48 hour, fasting	85, 89	(Smith et al., 2011a)
Dust	29-738	Mice (BW = 18-20 g), blood	340-6220 μg Pb/kg BW, 48 hour, fasting	29.1-60.1	(Li et al., 2014)
	1693-6799	Children		11.25-21.48[d]	(Oliver et al., 1999)
Incinerator and residential	646-3905	Swine (6-8 weeks age, BW = 20-25 kg), blood	5 days, single dose, fasting	10.1-19.1	(Juhasz et al., 2009)
Urban soil	12.6-1198	Female mice (BW = 20-22 g), kidney	10 days, repeat dose, fasting	17.3-86.6	(Li et al., 2016)
Farming	215-1543	Mice (BW = 20-25 g), blood	2150 -10700 μg Pb/kg BW, 48 hour, fasting	51.4-60.5	(Li et al., 2015)
Spiking and aging soils	1500	Swine (BW = 20-25 kg), blood	5 days, single dose, fasting	34-59	(Wijayawardena et al., 2014)

285 [a]: ABA; [b]: average of tissue point RBA (kidney, liver, bone, urine); [c]: average of blood RBA and tissue point RBA (kidney, liver, bone); [d]: blood Pb level of children; [e]: data from (Juhasz et al.,

286 2009); BW: body weight;

2.3 Uncertainties in measuring Pb relative bioavailability

A range of measurement uncertainties exists for Pb RBA determination. Early human experiments were conducted using traced Pb to identify absorption mechanisms for soluble Pb and interactions with food (James et al., 1985; Mushak, 1991). The only assay of Pb RBA performed on humans (adults) involved ingestion of Pb contaminated soils (Maddaloni et al., 1998). This is a significant assay as it was carried out directly on humans; however, there are still some uncertainties because the digestive adsorption system of adults' is different from that of children and babies, and children and babies are of particular concern.

More *in vivo* experiments have been conducted using young animals, including swine, rats, mice and rabbits, using various experimental designs. A major source of concern in *in vivo* models is the intra-species and inter-species uncertainties. The intra-species uncertainties, including animal age, development stage, feeding behaviour, absorption rate, and digestion processes, can influence the Pb RBA results. The inter-species uncertainties, including the difference between the digestive systems of animals and children/babies result in uncertainties when directly extrapolating measured Pb RBA to children/babies.

Several of these uncertainties relating to inter- and intra- species are reported. Compared to human stomachs, rodent stomachs have a smaller glandular region and less surface area for parietal cells to secreting acid (Weis and LaVelle, 1991). The gastrointestinal pH value of rabbits is significantly lower than that of humans (Merchant et al., 2011). The maturity of a rat's small intestine is weaning, which is different to a baby (Weis and LaVelle, 1991). Moreover, a rat has a relatively smaller surface area of small intestine than that of humans (about 1/5), which could decrease Pb RBA (Weis and LaVelle, 1991). Although it is reported that the juvenile swine could be a better alternative for predicting digestive and absorption

312 processes for infants, as there are many similarities between them, including gastric
313 hydrochloric acid (HCl) and protease secretion; small intestine configuration; limited digestive
314 capacity and gut maturity (Moughan et al., 1992; Heath et al., 2003), significant differences
315 also exists. For example, the capacity of a piglet's stomach is 2 times higher than that of infants
316 in the same body weight (5.75kg), which are 260 cm^3 and 130 cm^3, respectively (Moughan et
317 al., 1991). The above differences could lead to significant differences for the estimation of Pb
318 RBA and introduce uncertainties while extrapolating Pb RBA from an animal study to human
319 health.
320
321 In *in vivo* studies, the Pb RBA can be also affected by feeding state (fast or fed), dosage and
322 frequency of dose (single or repeat feeding) (Weis et al., 1995). A rat based study showed that
323 the uptake of PbAc reduced about 50% when Pb was fed with food, compared to the fasting
324 state (U.S. Environmental Protection Agency, 2007a). In another study, a higher stomach pH
325 of 3.9 was obtained for a mouse in the fasting state than 3.2 in a fed state (McConnell et al.,
326 2008). In another aspect, only rabbits present a significantly lower pH of 1.6 in a fed state
327 compared to humans (Merchant et al., 2011). The fasting state was employed in most of the
328 studies to simulate the situation of accidental oral ingestion for children (Casteel et al., 2006;
329 Denys et al., 2012; Li et al., 2014).
330
331 The daily ingested rate of soil and dust for infants and toddlers via normal hand-to-mouth
332 activities (no pica) is about 100 mg/day (Brunekreef et al., 1981; Mushak, 1991), and is 135
333 mg/day for late infants and toddlers based on the US EPA IEUBK model (P. Mushak, 1998).
334 Therefore, the dosages for *in vivo* testing should be considered to be representative of children's
335 exposure (Ruby et al., 1993). In past *in vivo* studies, various doses of Pb were given to test
336 animals. For example, for the swine with a similar age (5-6 weeks old), Casteel et al. (2006)

337 gave a dose of 75-675 μg Pb/kg bodyweight/day, while Denys et al. (2012) gave a dose of 50-
338 4000 μg Pb/kg bodyweight/day. The mice model was administered using a higher dose of Pb.
339 For example, Li et al. (2015) gave a dose of 2150-10700 μg Pb/kg bodyweight/day. In fact, the
340 design of the dosages for *in vivo* studies should consider not only being the representative of
341 children's exposure but also the detection limitation. Finally, some studies use Pb RBA
342 measured from blood (Li et al., 2014) while some of them using point estimation using samples
343 from bone, urine, liver, and kidney (Denys et al., 2012).
344
345 In conclusion, uncertainties in *in vivo* studies are mainly from the design of experiments, such
346 as dosages, fast or fed state, frequency of dose given, inter- and intra-species differences, and
347 extrapolation from test animals to humans, especially children. The swine model was
348 demonstrated to be the best model to estimate Pb RBA for the exposure of Pb to children;
349 however, it is more expensive than the other models such as rats, mice and monkeys.

2.4 Pb bioaccessibility (*in vitro*)

Although using *in vivo* models to estimate RBA has a number of potential benefits with less uncertainties, the application of *in vivo* methods is largely limited due to its expense- and time-consumption (U.S. Environmental Protection Agency, 2007a). On a large and wide scale, the *in vivo* methods are not therefore suitable to estimate site-specific Pb RBA (Li et al., 2015). The *in vitro* methods for determining the bioaccessible portion of Pb are proposed, although these methods may provide a conservative result (Paustenbach, 2000). The currently used *in vitro* methods are summarized in Table 2. Mainly two types of *in vitro* methods were developed to measure Pb BAc including physiological based and non- or partially physiological based. The physiological based tests simulate the biochemical conditions of a human's gastrointestinal environment to assess the leaching of Pb from soil/dust (Ruby et al., 1996; Oomen et al., 2002; Wragg and Cave, 2003; Oomen et al., 2006). Such trials were originally from the assessment of BA iron in food for nutrition studies (Miller et al., 1981). The non- or partially physiological based methods use various chemicals to extract bioaccessible Pb from soil/dust (Drexler and Brattin, 2007). Both of the two types of analysis can involve either a single extraction step or multiple extraction steps simulating different physiobiological phases.

366 Table 2 Summary of current *in vitro* models for estimating Pb bioaccessibility

Physiological based *in vitro* models	Non physiological based *in vitro* models
UBM: the unified BioAccessibility Research Group Europe (BARGE) method (Denys et al., 2012)	RBALP: the Relative Bioavailability Leaching Procedure (Drexler and Brattin, 2007)
PBET: a Physiologically Based Extraction Test (Ruby et al., 1996)	SBRC (Gastric): the Solubility Bioaccessibility Research Consortium assay (Juhasz et al., 2009)
RIVM: the In Vitro Digestion Model of RIVM (The Netherland) (Oomen et al., 2003)	
IVG: in-Vitro Gastrointestinal Method (Schroder et al., 2004)	
DIN: The German DIN model applied by the Ruhr-Universita¨t Bochum (RUB, Germany) (Oomen et al., 2002)	
TIM: The Gastrointestinal Model by TNO (The Netherlands) (Oomen et al., 2002)	
SHIME: Simulator of Human Intestinal Microbial Ecosystems of Infants (Oomen et al., 2002)	
SBRC (intestinal): the Solubility Bioaccessibility Research Consortium assay (Juhasz et al., 2009)	

367

368

369 After years of development and validation, six *in vitro* (PBET, UBM, RIVM, IVG, RBALP
370 and SBRC) models are widely used to measure Pb BAc. The six *in vitro* models vary in key
371 parameters (e.g. pH, reaction time, mixing mode, mixing speed, solid/liquid ratio) but not in
372 temperature (37°C) and soil particle size (< 250 μm). A summary of key parameters in these
373 six *in vitro* methods is shown in Table 3. The detailed procedure can be found elsewhere

374 (Hettiarachchi et al., 2003; Schroder et al., 2004; Oomen et al., 2006; Drexler and Brattin, 2007;
375 Juhasz et al., 2009; Denys et al., 2012).

376 Table 3 Key parameters in six *in vitro* methods

Model	Phase	Duration	pH	Mixing/speed	S/L ratio (g/ml)	pH monitor
RBALP (Drexler and Brattin, 2007)	G	1	1.5	Rotation, 30 rpm	1/100	Yes
UBM (Denys et al., 2012)	oral	10 s	6.5	Hand shake, 10s	1/15	No
	G	1 h	1.2	Rotation	1/37.5	Yes
	I	4 h	6.3		1/97.5	
PBET (Ruby et al., 1996)	G	1 h	2.5	Argon gas agitation	1/100	No
	I	4 h	7		1/100	
IVG (Schroder et al., 2004)	G	1 h	1.8	Stirring	1/150	No
	I	1 h	5.5		1/150	
SBRC (Juhasz et al., 2009)	G	1 h	1.5	Rotation, 40 rpm	1/100	Yes
	I	4 h	6.5		1/100	
RIVM (Oomen et al., 2006)	Oral	5 mins	6.5	Rotation, 55 rpm	1/15 or 1/150	No
	G	2 h	1-2		1/37.5 or 1/375	Yes
	I	2 h	5.5-6.5		1/96 or 1/958	Yes

377 G: gastric phase; I: intestinal phase; h: hour; s: second; S:L ratio: solid:liquid ratio. For full form of the
378 abbreviation please refer to Table 2.
379
380
381 Pb BAc varied depending on soil types and the different *in vitro* models employed. Van de
382 Wiele et al. (2007) compared the PBET, RIVM (0.6) and RIVM (0.06) models for the Bunker
383 Hill soil, and found Pb BAc values were 13%, 31.8% and 47.4% for the fasting state, and
384 21.8%, 23.9% and 38.8% for the fed state, respectively. In addition, the RBALP, UBM, PBET,

385 SBRC, IVG models were employed to estimate Pb BAc in peri-urban soils. Estimation using
386 the RBALP and IVG models were more conservative than that using the other models (Juhasz
387 et al., 2013b). Moreover, Li et al. (2014) estimated Pb BAc in house dusts using different *in*
388 *vitro* models (UBM, SBRC, IVG, PBET), which showed SBRC has the highest gastric BAc
389 value, followed by IVG, DIN and PBET, while PBET has a higher intestinal BAc value than
390 the other models.
391
392 A summary of available Pb BAc data is presented for different sources in Table 4. The Pb
393 concentration in smelter soils ranged from 5.2 to 150000 mg/kg, higher than that for mining
394 soils ranging from 59 to 77007 mg/kg. For all sources, the Pb BAc ranged from 0.49% to 105%
395 for gastric phase (G-phase) and from 0.03% to 73% for intestinal phase (I-phase), respectively
396 (note: relative BAc is not considered in this case). For the soil from mining and smelter sites,
397 the Pb BAc of G-phase ranged from 1.4% to 95% and 6.66% to 96%, respectively. Rieuwerts
398 et al. (2000) also reported that Pb concentration and Pb solubility in smelter soils and dust is
399 higher than that in mining and other soils and dusts.

400 Table 4 Lead bioaccessibility estimated using *in vitro* methods for different sources of soils.

Source	*In vitro* model	Pb concentration (%)	BAc (%)		Reference
			Gastric	Intestinal	
Mining	UBM	4482-40214	10.6-82[a]	9.2-90[a]	(Denys et al., 2012)
	RBALP	1270-14200	6-90	-	(Casteel et al., 2006)
	IVG	1270-14200	1.4-64.4	0.03-3.23	(Schroder et al., 2004)
	PBET (S/L=1:40)[b]	3900	4	NA	(Ruby et al., 1993)
	PBET (S/L=1:250)	3908-10230	9.5-49	1.1-14	(Ruby et al., 1996)
	IVG	237-6330	35-70.7	2.7-6.8	(Marschner et al., 2006)
	RIVM (0.06)[c]	1270-11700	3.7-82.6	1.1-65.8	(Oomen et al., 2006)
	RIVM (0.6)[d]	1270-11700	3.9-70.9	1.9-49.8	(Oomen et al., 2006)
	RIVM (0.6g)[d]	2141-77007	15-56	5-25	(Denys et al., 2007)
	RIVM (0.6g)[d]	623-5967	11-66	NA	(Oomen et al., 2002)
	RBALP		56-91	-	(Oomen et al., 2002)
	PBET (pH=1.3)	59-12100	4-54	NA	(Bruce et al., 2007)

	RIVM (0.6g)[d]	2924	70.9	31.8	(Van de Wiele et al., 2007)
	SBRC	86-6840	26.8-95	1.7-8.9	(Smith et al., 2011b)
	RBALP	24-56578	18.8-100	-	(Yang and Cattle, 2015)
Smelter	UBM	1460-30155	40.5-82.6[a]	33.4-90[a]	(Denys et al., 2012)
	SBRC	536-1489	34-96	1.6-16.3	(Smith et al., 2011a)
	PBET (pH=2.5)	1200-3500	25-43	7-12	(Berti and Cunningham, 1997)
	PBET (pH=2.5)	56.3-9585	6.66-22.43	0.77-9.78	(Finžgar et al., 2007)
	RBALP	390-150000	14.34-88.45	-	(Bosso and Enzweiler, 2008)
	PBET (Ph=1.7)	390-150000	10.36-78.88	NA	(Bosso and Enzweiler, 2008)
	UBM	984[e]	62[e]	32[e]	(Roussel et al., 2010)
	RBALP	5.2-6945	21.3-87.4	-	(Lamb et al., 2009)
Small arms range	RBALP	4503-23409	83-100	-	(Bannon et al., 2009)
Gasworks	SBRC	1343	45	8.8	(Smith et al., 2011a)
Shooting range	SBRC	576, 1801	94, 99	16.5, 17.3	(Smith et al., 2011a)

	SBRC	576-3026	50-105	2.2-11.1	(Smith et al., 2011b)
	RBALP	187-10403	46.1-70	-	(Sanderson et al., 2012)
Dust	SBRC	25-1173	47.6-93.3	1.4-10.4	(Li et al., 2014)
	IVG	25-1173	41.1-90.4	0.8-5.1	(Li et al., 2014)
	DIN	25-1173	22.5-63.0	0.3-5.7	(Li et al., 2014)
	PBET	25-1173	22.2-59.7	0.5-14.3	(Li et al., 2014)
	PBET (pH=2.5, S/L=1:200)	50.3-468	11.6-36.3	2-22	(Turner and Ip, 2007)
Pottery	RIVM (0.6g)[d]	50-11000	NA	0.3-73	(Oomen et al., 2003)
Paint	PBET (pH=2.5, S/L=1:100 to 1:143)	16-11110	0.49-18.24	0.49-5.78	(Turner et al., 2009)
Incinerator	RBALP	30.1-977	26.94-89.36	-	(Madrid et al., 2008)
	SBRC	2885-3905	60.9-64.1	1.2-2.3	(Juhasz et al., 2009)
Residential	SBRC	646, 765	35.7, 61	2.1, 2.7	(Juhasz et al., 2009)
	SBRC	105-954	35.2-85.1	0.6-2.8	(Smith et al., 2011b)
	UBM	71-441	45-92	NA	(Reis et al., 2014)
	SBRC	12.6-1198	19.7-91.2	NA	(Li et al., 2016)

[a]: relative bioaccessibility, PbAc as reference; [b]: S/L=solid liquid ratio; [c]: 0.06g soil per digestion tube; [d]: 0.6g soil per digestion tube; [e]: mean of 27 soils. NA: data not available; -: not applicable. For full form of the abbreviation please refer to Table 2.

2.5 Key parameters in *in vitro* models

The parameters used in *in vitro* methods could also influence the BAc results. The key parameters are listed in Table 5. Here we summarize and articulate the parameters during various *in vitro* methods to understand influencing factors for measurement of BAc.

2.5.1 pH

The pH value is more sensitive than other parameters as Pb solubility is highly dependent on pH — Pb BAc decreased with an increase in pH (Ellickson et al., 2001; U.S. Environmental Protection Agency, 2007c; Juhasz et al., 2009). The pH of human G-phase ranged from 1 to 4 for the fasting condition (Washington et al., 2000), and a range of 1.0 to 2.5 is employed to investigate Pb BAc (Ruby et al., 1993; Oomen et al., 2003; Bruce et al., 2007; Drexler and Brattin, 2007). It is critical to control the pH during the G-phase extraction (Wragg et al., 2011). Previous studies compared Pb BAc from extractions with or without pH control. For example, Oliver et al. (1999) reported that when the pH was monitored and maintained at 1.3, the measured Pb BAc for house dust was higher (26-46%) than that without pH control (20-30%). Furthermore, Ruby et al. (1996) measured the BAc of G-phase for 8 contaminated soils from various sources (mining, smelter, residential and tailing sites) and showed that the Pb BAc of G-phase at pH 1.3 is 2-4x higher than that at pH 2.5. A stable pH control during a G-phase test could provide more conservative results and it is critical to simulate acidic conditions.

2.5.2 Mixing mode

The mixing mode has a significant effect on measurement of Pb BAc since the dissolution of Pb bearing minerals/materials was controlled by the mixing mode through transport mechanisms, (FaciesRuby et al., 1999). Several mixing modes have been used in *in vitro* assays, including gas mixing, end-over-end rotation and shaking. The wrist-action shaker was initially applied by Ruby et al. (1993) on an *in vitro* assay. This assay was modified three years

later and is well known as the PBET model, where the argon (Ar) gas was used to mix Pb particles and the extraction solution (Ruby et al., 1996). This mixing mode is effective and aggressive which may overestimate the Pb BAc (Ruby et al., 1996). The shaking mode is effective while it may underestimate the Pb BAc as more particles may be adhered to the bottom and walls of the tube which reduces the effective contact surface between soil particles and solution (Drexler and Brattin, 2007; U.S. Environmental Protection Agency, 2007a). The end-over-end rotation is recommended as it maximizes the contact area of soil particles and digestive juices, and minimises contamination from interacting devices (Drexler and Brattin, 2007). A comparison study of shaking and end-over-end rotation modes using the RBALP method showed that the mean and median Pb BAcs of end-over-end rotation mode (66.8% and 77.1%, respectively) is higher than that of shaking mode (51.3% and 52.7%, respectively), and a significant difference was obtained between the two modes (p = 0.016, paired t-test) (Yan et al., 2016) .

2.5.3 Solid:liquid ratio

Numerous solid:liquid (S:L) ratios have been applied in various assays, and the S:L ratio can also significantly impact Pb BAc. A high S:L ratio could reduce Pb dissolution in the extractant and result in an increase in pH, therefore leading to an underestimate of Pb BAc (Oomen et al., 2006; Drexler and Brattin, 2007). Sorenson et al. (1971) found that the S:L ratio influenced dissolution of metals in extraction procedures in the range of 1:5 to 1:25, most likely due to diffusion-limited dissolution kinetics. Ruby et al. (1996) reported Pb BAc at a S:L ratio of 1:100 was higher than that at a S:L ratio of 1:10, which are 9.5% ~ 35% and under 6%, respectively. Yang et al. (2003) reported a 10% increase in BAc from S:L ratios of 1:40 to 1:100. Hamel et al. (1998) reported when the S:L ratio changed from 1:100 to 1:5000, Pb BAc increased obviously for the test soils. Meanwhile, Van de Wiele et al. (2007) have found a

453 significant difference in Pb BAc derived from the RIVM model (gastric phase) at S:L ratios of
454 1:100 and 1:1000; However a very low S:L ratio may add difficulty in analysis and lead to
455 poorer reproducibility and more uncertainties (Oomen et al., 2006). A S:L ratio of 1:100 was
456 recommended and care must be taken when selecting the S:L ratio for testing soils containing
457 high concentrations of Pb (Drexler and Brattin, 2007).
458
459 *2.5.4 Comparisons of in vitro models*
460 As discussed above, the pH and S:L ratio can significantly influence Pb BAc, and end-over-
461 end rotation is a better mixing mode (Table 5). Although the RBALP model is non-
462 physiologically based, has no I-phase, and may overestimate Pb BAc for some testing soils
463 (Juhasz et al., 2013b), it monitors pH during the G-phase, and is the most cost-effective,
464 simplest and fastest method with good validation using the swine model and statistical analysis.
465 The SBRC model has a similar procedure and the same components for G-phase as the RBALP
466 model, and has an extra I-phase which can be used to indicate Pb RBA (Juhasz et al., 2009).
467 The UBM method is fully physiologically based, validated using the swine model and
468 statistical analysis, and has pH control during G-phase, which are all favourable for Pb BAc
469 measurement. It has a relatively complicated procedure and may not be suitable for some soils
470 (Denys et al., 2012; Yan et al., 2016); however, it can provide a good estimation of Pb BAc.
471 The RIVM model is from the RIVM group in the Netherland, and has a very similar procedure
472 and components as the UBM model (Oomen et al., 2003). The PBET model offers a scientific
473 foundation for the other *in vitro* models; however, it has no pH monitor for the G-phase, and
474 was modified to several different procedures, including different pHs for the G-phase (1.5 to
475 2.5), different components for gastric fluids and different mixing modes (shaking, argon gas)
476 (Ruby et al., 1993; Ruby et al., 1996; Hettiarachchi et al., 2003; Li et al., 2015). In conclusion,
477 for a non-physiologically based method, the RBALP method is recommended and the UBM

478 method is recommended for a non-physiologically based method and fully physiologically
479 based method.

480 Table 5 Comparison of five commonly used *in vitro* methods

In vitro model	Mixing mode	pH monitor	Simple indexing	Time-consumption	Apply range
RBALP	R 30rpm	Yes	*	1 h	1-50000 mg/kg, only G-phase applied
UBM	R 40rpm	Yes	****	5 hours	Limitation: G-phase may not suit for some high Pb concentration soils which contain high proportion of bioaccessible Pb.
RIVM (0.6)	R 55rpm	Yes	****	4 hours	Limitation: G-phase may not suit for some high Pb concentration soils which contain high proportion of bioaccessible Pb.
RIVM (0.06)	R 55rpm	Yes	****	4 hours	Limitation: may bring poor reproducibility and more uncertainties.
SBRC	R 40rpm	No	**	5 hours	
PBET	Argon gas or shaking	No	***	2 or 5 hours	

481 *indicate simple and time-consuming level of the method. More * mean the method is more complex and longer. G-phase: gastric phase.

482

2.6 Correlations between *in vivo* and *in vitro* methods

Although *in vitro* methods have been proposed as the alternative method to *in vivo* RBA, strong and reliable correlations between the *in vivo* and the *in vitro* methods (IVIVC) are limited. Several mathematical models, such as linear, power and exponential models have been discussed and the linear regression model is recommended as it can take into account all measurement errors (U.S. Environmental Protection Agency, 2007a). Various studies to validate IVIVC have been conducted by researchers, which are summarised in Table 6. Ruby et al. (1996) measured Pb BAc using the PBET method for seven mining and residential soils and reported a correlation of Pb BAc based on G-phase and Pb RBA as determined using rats models (Pb RBA = 1.4*Pb BAc + 3.2, r^2 = 0.93). A later study of Pb IVIVC using the PBET method and Pb RBA (*in vivo* rats model) was carried out by Hettiarachchi et al. (2003), and both the G-phase and I-phase of PBET can predict Pb RBA. Schroder et al. (2004) measured Pb BAc using the IVG method and Pb RBA using the *in vivo* swine model, and found an IVIVC: Pb RBA = 0.39*Pb BAc (G-phase) + 2.97, r^2 = 0.86. Oomen et al. (2006) studied IVIVC using the RIVM method and the *in vivo* swine model, and found the IVIVC based on both G-phase and I-phase are similar. Drexler and Brattin (2007) reported that the RBALP model is simple, cost-effective, reliable and provides the best estimate of Pb RBA as determined using an *in vivo* swine model (Pb RBA = 0.878*Pb BAc - 0.028, r^2 = 0.924, $p <$ 0.001).

The IVIVC may vary (slope, r^2) depending on the *in vitro* and *in vivo* models applied, and the source of soil varying with soil properties, Pb concentration, and other heavy metals such as Fe and Ca which may have competitive adsorption to Pb in soil. As shown in Table 6, the RBALP, UBM, RIVM, PBET, SBRC and IVG were used to predict Pb RBA. For the same *in vitro* model used to predict Pb RBA in different sources of contaminated soils, various slope

508 and r^2 for IVIVC were obtained. For example, Drexler and Brattin (2007) and Smith et al.
509 (2011a) validated Pb BAc (RBALP) using swine and mice models, the slope and r^2 are 0.87,
510 0.69 and 0.924, 0.78, respectively. Even for the same *in vitro* and *in vivo* model applied on a
511 different source of Pb contaminated soils, different slope and r^2 for IVIVC were obtained. For
512 example, the SBRC model and the *in vivo* mice model were used for dust and
513 mining/smelter/farming soils, their IVIVC slope and r^2 are 0.61, 0.40 and 0.68, 0.43,
514 respectively (Li et al., 2014; Li et al., 2015). Moreover, for the same source soils, the IVIVC
515 based on the same *in vivo* model (swine) and different *in vitro* models (IVG and RIVM),
516 resulted in different slope and r^2 values (Schroder et al., 2004; Oomen et al., 2006). Wragg et
517 al. (2011) suggested that the IVIVC slope should between 0.8 and 1.2, y-intercept not
518 significantly different from 0 and r^2 should not below 0.6. Juhasz et al. (2013a) stated the same
519 requirements for the slope (0.8 to 1.2), and similar r (above 0.8). Although there are more than
520 30 IVIVCs based on both G-phase and I-phase using various models and soils/dusts (as shown
521 in Table 6), only a small fraction of IVIVCs meet the requirements proposed by Wragg (7 of
522 18 IVIVCs of G-phase and 3 of 15 IVIVCs of I-phase, respectively).
523
524 Although the intestine is the main place of Pb desorption, a detailed investigation of Pb
525 speciation in artificial human digestive fluid, Oomen et al. (2003) concluded that the amount
526 of free Pb^{2+} in I-phase is negligible, and most of the Pb in soil particles was in dynamic
527 equilibrium with soluble Pb presenting as Pb-phosphate and Pb-bile complexes. The
528 concentration of Pb in the aqueous phase is impacted by precipitation or adsorption onto non-
529 digestible and compatible particles (Deshommes et al. 2012), and consequently, elevated pH
530 in I-phase directly reduces Pb BAc. Studies by (Medlin, 1997) and (Drexler and Brattin, 2007)
531 have indicated that no small intestinal phase (pH~7) is required for the RBALP as the gastric
532 phase showed acceptable correlation with the *in vivo* results. As shown in Table 6, 11 of 13

533 studies using both gastric and intestinal phases to generate IVIVC showed that the slope of
534 IVIVC from gastric phase is better than that from intestinal phase. This indicated that the gastric
535 phase has on average a more reliable IVIVC than the intestinal phase.
536
537 Challenges still exist to predict Pb RBA using *in vitro* models due to various uncertainties
538 deriving from interspecies extrapolation, different soil types and *in vitro* methods. Thus reliable
539 *in vivo* and *in vitro* models are desired with minimised uncertainties and which provide an
540 accurate estimation of Pb RBA.

541 Table 6 Validation of *in vitro* methods using animal models (swine, rats, mice)

Soils source (sample number)	*In vivo* model/target	*In vitro* model	Key parameters used in *vitro models*				IVIVC	Reference
			Oral phase	S/L ratio in G-phase (g/ml)	G-phase	I-phase		
EPA region VIII (n=19)	Swine/blood	RBALP	No	1/100	1h, pH1.5	No	G: y = 0.87x - 0.028. r^2 = 0.924, p < 0.0001	(Drexler and Brattin, 2007)
Soils (n=12)	Mice/blood	RBALP	No	1/100	1h, pH1.5	No	G: y = 0.69x + 30.21. r^2 = 0.78	(Smith et al., 2011a)
Jasper Yard soils, residential soils, slag soils (n=12)	Swine/blood	UBM	10s, pH 6.5, hand shake	1/37.5	1h, pH1.2	4h, pH6.3	G*: y = 0.78x, r^2 = 0.61 I*: y = 0.76x, r^2 = 0.57	(Wragg et al., 2011)
Mining, smelting (n=14)	Swine/blood, kidney, liver, bone, urine	UBM	10s, pH 6.5, hand shake	1/37.5	1h, pH1.2	4h, pH6.3	G*: y = 1.86x + 1.10,r^2 = 0.93, p < 0.01 I*: y = 1.09x + 1.01,r^2 = 0.89, p < 0.01	(Denys et al., 2012)
Soils (n=12)	Mice/blood	SBRC	No	1/100	1h, pH1.5	4h, pH6.5	I*: y = 1.06x - 7.02, r^2 = 0.88	(Smith et al., 2011a)
Urban soils in China (n=38)	Mice/blood	SBRC	No	1/100	1h, pH1.5	-	G: y = 0.83x + 2.28, r^2 = 0.61	(Li et al., 2016)
Incinerator & urban soils (n=5)	Swine/blood	SBRC	No	1/100	1h, pH1.5	4h, pH6.5	I*: y = 0.58x + 1.98, r^2 = 0.53	(Juhasz et al., 2009)
EPA Region VIII (n=15)	Swine/blood	PBET	No	1/111	1h, pH1.5	No	G: y = 0.9x - 8.21. r^2 = 0.63. p < 0.001	(Medlin, 1997)
Mining&residential soils (n=7)	Rats/blood	PBET	No	1/100	1h, pH2.5	4h, pH7.0	G: y = 1.4x + 3.2. r^2 = 0.93	(Ruby et al., 1996)
Joplin soil (n=15)	Rats/blood, liver, kidney, bone	PBET	No	1/100	1h, pH2.0	4h, pH6.5	G: y = 0.82x + 11. r^2 = 0.95 I: y = 1.87x + 12. r^2 = 0.77	(Hettiarachchi et al., 2003)

EPA Region VIII (n=18)	Swine/blood	IVG	No	1/150	1h, pH1.8	4h, pH5.5	G: y = 0.39x + 2.97. r^2 = 0.86	(Schroder et al., 2004)
EPA Region VIII, Bunker hill (n=7)	Swine/blood	RIVM (0.6)	5 min, pH 6.5	1/37.5	2h, pH1-2	2h, pH5.5-6.5	G*: y = 0.79x, r^2 = 0.95 I*: y = 0.69x, r^2 = 0.81	(Oomen et al., 2006)
EPA Region VIII, Bunker hill (n=10)	Swine/blood	RIVM (0.06)	5 min, pH 6.5	1/375	2h, pH1-2	2h, pH5.5-6.5	G*: y = 1.08x, r^2 = 0.68 I*: y = 1.16x, r^2 = 0.66	(Oomen et al., 2006)
Dust in 15 cities in China (n=12)	Mice/blood	SBRC	No	1/100	1h, pH1.5	4h, pH7.0	G: y = 0.61x + 3.15. r^2 = 0.68 I: y = 1.72x + 42. r^2 = 0.15	(Li et al., 2014)
		IVG	No	1/150	1h, pH1.8	1h, pH5.5	G: y = 0.48x + 14.3. r^2 = 0.56 I: y= -0.57x + 51.6. r^2 = 0.01	
		DIN	No	1/50	2h, pH2.0	6h, pH7.0	G: y = 0.67x + 17.4. r^2 = 0.85 I: y = 6.9x + 36.9. r^2 = 0.38	
		PBET	No	1/100	1h, pH2.5	4h, pH7.0	G: y = 0.69x + 20.2. r^2 = 0.52 I: y = 1.60x + 35. r^2 = 0.35	
Farming, mining and smelter soils in China (n=12)	Mice/blood	UBM	10s, pH 6.5, hand shake	1/37.5 (G)	1h, pH1.2	4h, pH6.3	G: y = 0.80x + 9.99. r^2 = 0.67 I: y = 1.26x + 47.8. r^2 = 0.01	(Li et al., 2015)
		SBRC	No	1h, pH1.5	1/100	4h, pH7.0	G: y = 0.40x + 14.0. r^2 = 0.43 I: y=-2.54x + 26.3. r^2 = 0.21	
		IVG	No	1h, pH1.8	1/150	1h, pH5.5	G: y = 0.77x + 6.36. r^2 = 0.55 I: y = 4.17x + 22.7. r^2 = 0.24	
		PBET	No	1h, pH 2.5	1/100	4h, pH7.0	G: y = 0.87x + 18.9. r^2 = 0.38 I: y = 2.38x + 29.6. r^2 = 0.20	

*: the relative BAc was applied in the IVIVC. IVIVC: correlation between *in vivo* and *in vitro* methods. G-phase/G: gastric phase; I-phase/I: intestinal phase. S/L=solid liquid ratio. For full form of the abbreviation please refer to Table 2.

As shown in Table 6, although many studies have been conducted for validating the correlation between *in vivo* and *in vitro* models, there are still many uncertainties as the slope of IVIVC ranged from 0.39 to 1.86 for the gastric phase and 0.57 to 2.54 for the intestinal phase. A meta-analysis on the correlation showed a generic linear model based on the correlations from 5 commonly used *in vitro* models, which is (RBA (%) = (0.87 ± 0.16) × BAc + (4.70 ± 2.47)) (Dong et al., 2016; Yan et al., 2016). Even for the soils from the same source, the IVIVC based on the same *in vivo* model (swine) and different *in vitro* models (IVG and RIVM), results in different slope and r^2 values (Schroder et al., 2004; Oomen et al., 2006). Furthermore, most of the IVIVCs were validated by the Pb BAc value from the G-phase, some of the IVIVCs were also validated by Pb BAc both from the G-phase and I-phase, and some of the IVIVCs were only validated by relative Pb BAc values from the I-phase (Juhasz et al., 2009; Smith et al., 2011a). Moreover, Denys et al. (2012) use relative Pb BAc from both G-phase and I-phase to indicate Pb RBA and found significant correlations (G: y = 1.86x + 1.10, $r^2 = 0.93$, $p < 0.01$, I: y = 1.09x + 1.01, $r^2 = 0.89$, $p < 0.01$). All these uncertainties are largely because various soil properties and inter-species differences, as well as different *in vitro* methods. All uncertainties in the measurement of Pb RBA and Pb BAc are summarized in Table 7.

563 Table 7 Uncertainties in measurement of Pb RBA/BAc

Source of Uncertainty	Example
Intra-species	Variability using the same animals or human
Inter-species	Variability between different experimental animals or human
In vivo experiment design	Fast or fed state; single or repeat dose; dose of feeding; animal age and body weight difference; estimation Pb RBA by blood/kidney/bone/urine/liver
In vitro experiment design	Various key parameters influencing Pb BAc
Operation	Operation errors in experiment and analysis processes
Detection	Limitation of detection for Pb in soils or soil solution
Application of *in vitro* models	One *in vitro* model may not suit for measuring Pb BAc for all source of soils
Validation of IVIVC	Limited data on validation of IVIVC
Soil type	Soil types influence Pb concentration and soil properties, then affect Pb BA
Soil properties	Influence of soil properties on Pb BA or BAc
Modelling	Measurement and extrapolation errors

564 BA: bioavailability; BAc: bioaccessibility; IVIVC: correlation between *in vivo* and *in vitro*
565 methods; RBA: relative bioavailability.

3 Effect of soil properties on Pb bioavailability

Apart from the influence of measurement parameters on RBA/BAc, the soil properties can also have a significant influence on RBA/BAc. As discussed previously, the source of Pb contamination could result in different RBA/BAc, values and other soil properties, such as clay content, organic matter and oxides content can also cause different RBA/BAc. The following sections will focus on these aspects.

3.1 Source of Pb contaminated soil/dust

Nature of Pb released in the extract varied depending on different sources of contamination. Pure mineral phases of native Pb in natural soils may occur as Pb sulfide (PbS), Pb sulfate ($PbSO_4$), or Pb carbonate ($PbCO_3$) (FaciesRuby et al., 1999). In mining sites, Pb mineral may be encapsulated with other soil mineral grains, such as quartz. While in smelter sites, Pb minerals are often mixed with other pyrometallurgical waste materials and slags, and changed through various processes from different factories (FaciesRuby et al., 1999). All these changes are reported to influence Pb BA (Rieuwerts et al., 1998). Rieuwerts et al. (2000) reported that Pb concentration and solubility of soils from mining areas are lower compared to smelter urban areas. Moreover, the reactions of soil components like precipitation, adsorption, and degradation in the weathering process also changes Pb minerals phases in soils, and influences Pb BA in soils(Naidu et al., 2003).

588 Lead BA studies have been carried out on Pb contaminated soils from a great variety
589 of sources. As summarized in Table 8, the most popular spot is mining soils,
590 followed by smelter soils, small arms ranges, dust, shooting ranges, incinerators,
591 residential, and gasworks. All this data is obtained by *in vivo* models such as those
592 involving humans/swine/rats/mice/rabbits. As shown in Table 8 and Figure 4, soils
593 from mining have the widest range of Pb concentration (200 to 40214 mg/kg),
594 followed by smelter (536 to 30155 mg/kg), small arms ranges (4503 to 23409
595 mg/kg), and dust (29 to 6799 mg/kg). Small arm ranges show the highest mean Pb
596 concentration value, followed by mining soils, smelter soils, incinerator site,
597 gasworks, dust, shooting range, and residential, which are 16305 mg/kg, 7641
598 mg/kg, 3935 mg/kg, 3257 mg/kg, 2200 mg/kg, 1399 mg/kg, 1187 mg/kg and 706
599 mg/kg, respectively. As shown in Figure 4, around 90% of the total Pb
600 concentration values are in the range of 0-12500 mg/kg for all source of soils/dust,
601 except for small arms ranges which have most data out of the range.

602 Table 8 Pb concentration and relative bioavailability ranges sorted by different
603 sources

Source	Range of Pb concentration (mg/kg)	Range of Pb RBA (%)	Mean (%)	Median (%)
Mining	200-40214	0.75-105	42.23	40
Smelter	536-30155	10-94.5	49.3	42
Small arms ranges	4503-23409	77.3-139.9	108.9	109
Dust	29-6799	29.1-60.1	48.65	49.40
Shooting range	772-1602	85-89	87	87
Incinerator	2885-3905	13 -37.8	26.7	29.5
Residential/urban soils	12.6 -1198	17.3 – 86.6	48.2	48.7
Gasworks	2200	43	43	43
Farming	215-1543	51.4-60.5	57	57.8

604 RBA: relative bioavailability

605

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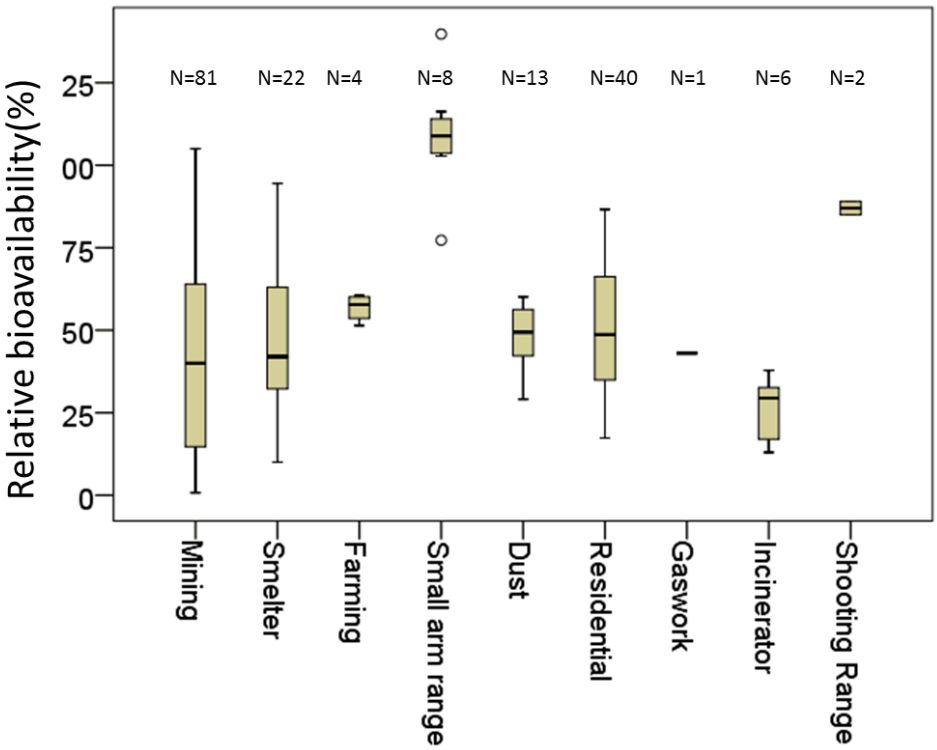

607

608 Figure 4 Distribution of Pb relative bioavailability from various sources. The
609 central mark on each boxis the median with the edges of the 25th and 75th
610 percentiles. The whiskers extend to the most extreme data points not considered
611 outliers. Outliers were not plotted in our study. The whiskers extend to the most
612 extreme data points not considered outliers. Outliers were not plotted in our study.

613 Rreferences: (Freeman et al., 1992; Ruby et al., 1992; Ruby et al., 1996; Casteel et al., 1997;
614 Maddaloni et al., 1998; Hettiarachchi et al., 2003; Schroder et al., 2004; Marschner et al., 2006;
615 Drexler and Brattin, 2007; Madrid et al., 2008; Bannon et al., 2009; Smith et al., 2011a; Denys et
616 al., 2012; Li et al., 2014; Li et al., 2015)

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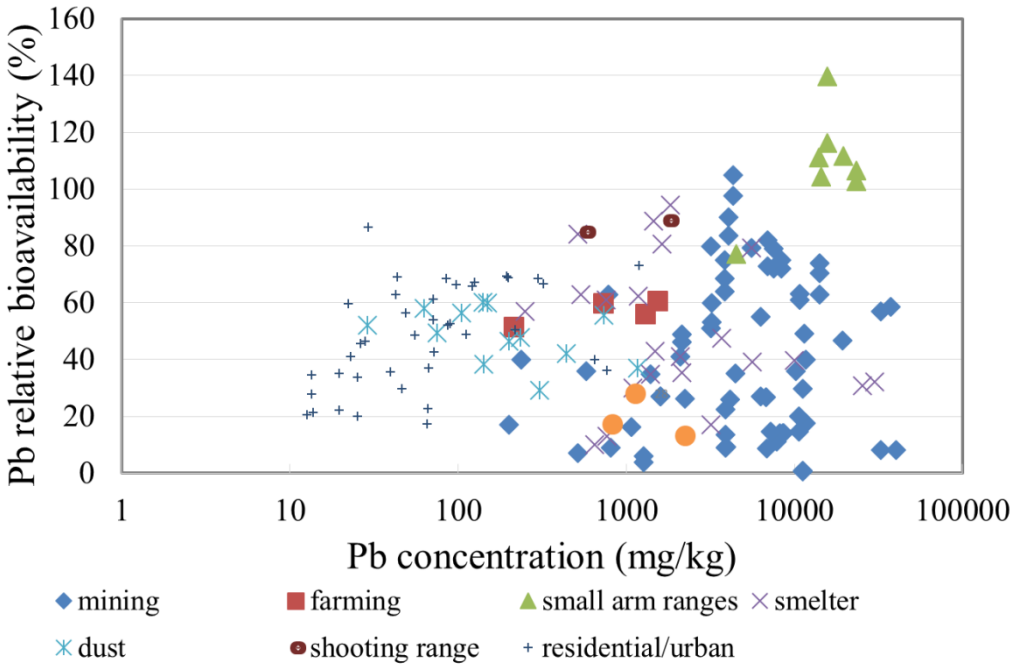

618

619 Figure 5 Distribution of Pb relative bioavailability values in various sources of
620 soils and dusts

621 References: (Freeman et al., 1992; Ruby et al., 1996; Casteel et al., 1997; Hettiarachchi et al.,
622 2003; Schroder et al., 2004; Drexler and Brattin, 2007; Juhasz et al., 2009; Smith et al., 2011a;
623 Denys et al., 2012; Li et al., 2015; Li et al., 2016)

624

625 All the Pb RBA data collected are shown in Figure 5. Soils from small arms ranges
626 showed the highest Pb RBA value than that from other sources, which ranged from
627 77.3% to 191%, with a median of 108.8% (Bannon et al., 2009). The mean Pb RBA
628 value for soils from mining, smelter, dust, incinerator sites, residential and
629 gasworks ranged from 33.8% to 44.5%. The median Pb RBA values for soils from
630 mining, smelter and house dusts are 38%, 42% and 49.4%, respectively. Both the
631 median and mean Pb RBA values of soils from mining and smelter sites are far
632 below the IEUBK default value of 60%. While the values for farming sites are very
633 close to 60%, the values for small arm ranges are far above the baseline 60%.

634

635 3.2 Influence of soil properties on Pb bioavailability

636 Different Pb minerals are present in natural weathered soils and anthropogenic
637 contaminated soils (e.g. smelter slags and other waste materials). Human activities
638 may alter BA by changing the original Pb mineral phases in soils. For example,
639 although Pb sulfide (PbS) occurs at mining, milling, smelting and ore-handing sites,
640 it can be encapsulated with other minerals to reduce its BA (Ruby et al., 1999).

641

642 The BA of Pb in soil is influenced by the physical and chemical properties of
643 various phases of Pb. Lead mineral phases, particle size, chemical reactions
644 including precipitation, adsorption, and degradation in the weathering process are
645 all believed to influence Pb BA (FaciesRuby et al., 1999; R.Naidu, 2003). As shown
646 in Figure 6, for the same form of Pb mineral phase, its RBA increases while the
647 particle size decreases. Lead RBA will be limited once Pb minerals are covered by
648 quartz and slag. The RBA of Pb mineral phase followed a sequence that $Pb(OH)^-$ =
649 $PbCl^-$= PbBrCl > PbO = Pb_3O_4 = $PbCO_3$ > Pb phosphate > PbS = $Pb_5(PO)_4Cl$ = Pb^o
650 (FaciesRuby et al., 1999). PbS shows the lowest Pb RBA while $Pb(OH)^-$ shows the
651 highest.

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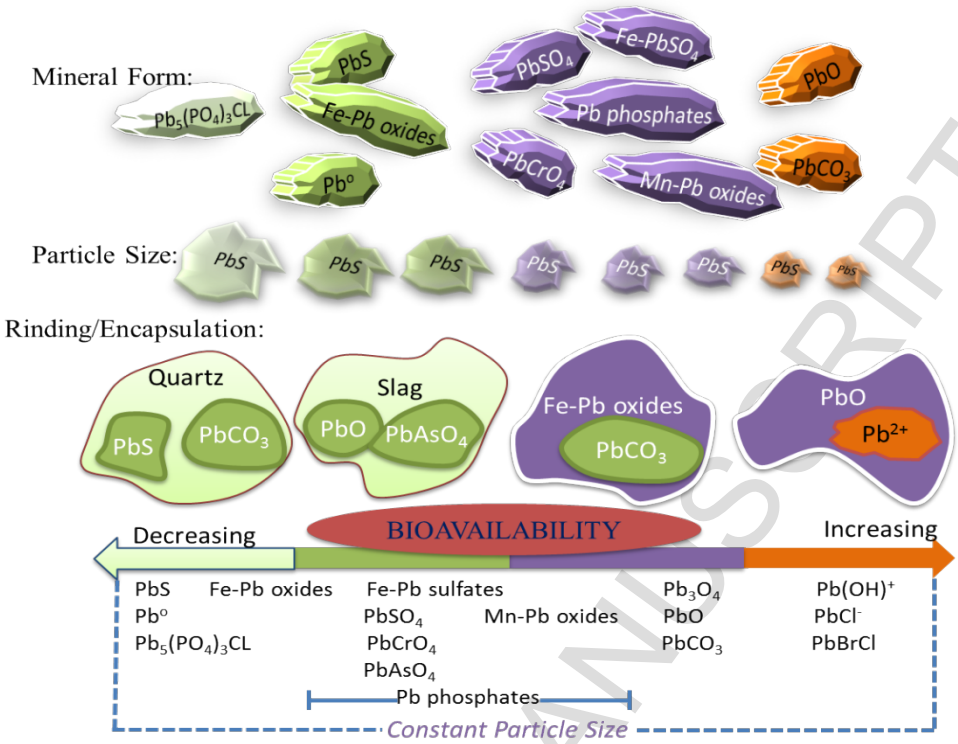


Figure 6 Lead mineral phases contribute to its bioavailability (FaciesRuby et al., 1999)

Moreover, the U.S. Environmental Protection Agency (2007a) reported a group-specific RBA values for various Pb minerals using swine and statistical analysis on 19 mining soils. As shown in Table 9, Pb RBA of various mineral morphologies are grouped into three categories, under 25%, 25% to 75%, and above 75%. It's worth noting that the group-specific results involve inherent uncertainties as they are only estimated using limited data sets and limited sources of soils, and many factors which can influence Pb RBA are not included (U.S. Environmental Protection Agency, 2007a). The US EPA also states that this is a semi-quantitative

665 rank-order classification of phase-specific RBA values (U.S. Environmental
666 Protection Agency, 2007a).
667
668 Table 9 A group-specific value of Pb relative bioavailability for various Pb mineral
669 morphologies (U.S. Environmental Protection Agency, 2007a)

Low Bioavailability (RBA <0.25)	Medium Bioavailability (RBA = 0.25-0.75)	High Bioavailability (RBA >0.75)
Fe(M) Sulfate Anglesite Galena Fe(M) Oxide Pb(M) Oxide	Lead Oxide Lead Phosphate	Cerussite Mn(M) Oxide

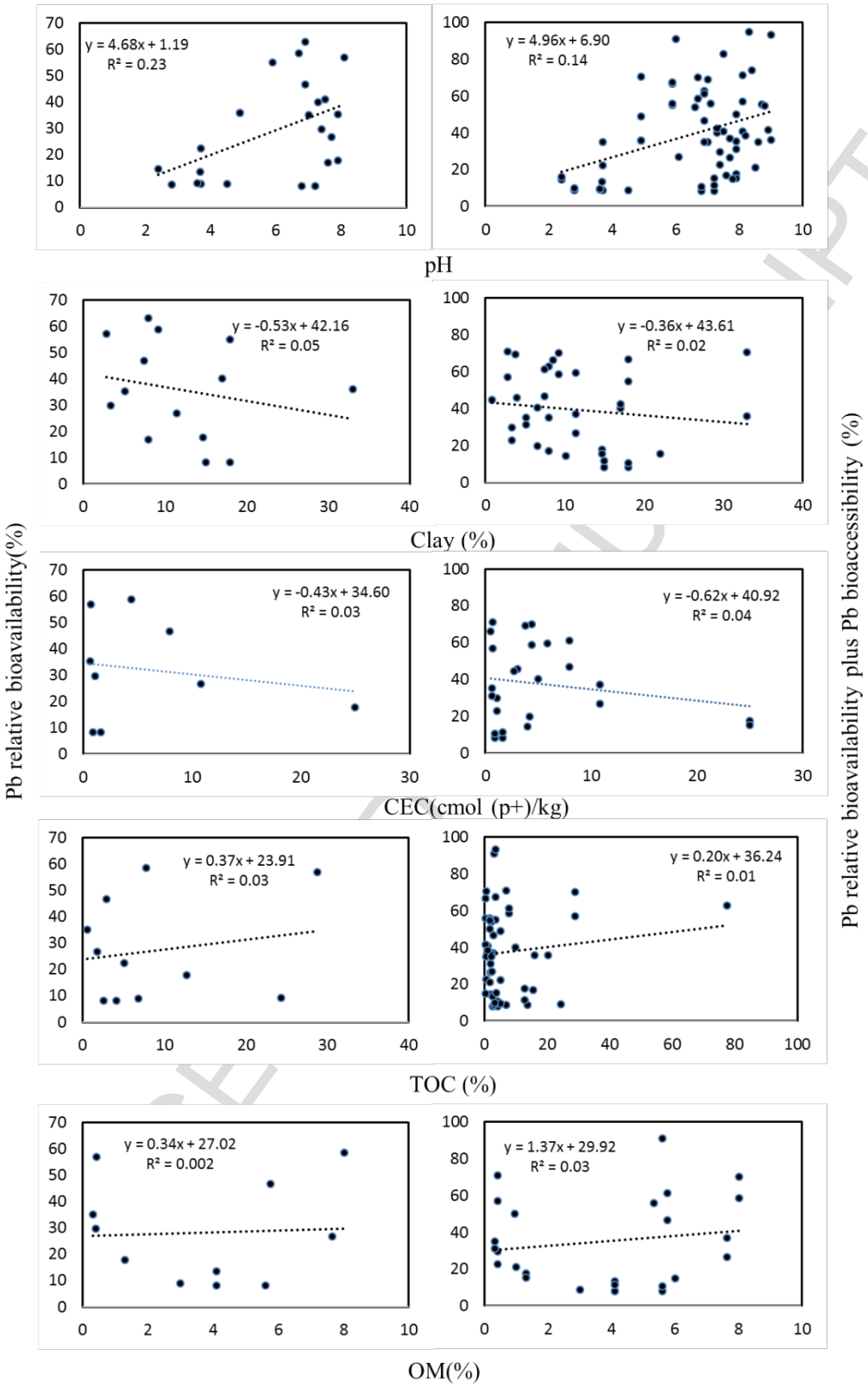
670 (M) = Metal; RBA: relative bioavailability.
671
672 Three main reactions which influence Pb RBA in soils include specific adsorption
673 to various solid phases, precipitation of sparingly soluble or highly stable
674 compounds, and formation of relatively stable complexes or chelates via interacting
675 with soil organic matter (Bradl, 2004). It has been reported that soil properties like
676 clay content, pH, OM, and CEC are related to Pb BAc (Buchter et al., 1989; He and
677 Singh, 1993; Hornburg and Brümmer, 1993; Rieuwerts et al., 2006; Poggio et al.,
678 2009; Roussel et al., 2010). For example, OM has an immobilisation effect on Pb
679 in soils via specific adsorption reactions (Pinheiro et al., 1999). The high CEC and
680 OM values enhance its metal retention ability by surface complexation, ion
681 exchange and surface precipitation (Kalbitz and Wennrich, 1998). Also it is
682 reported that clay can effectively remove heavy metals by specific adsorption and
683 cation exchanges (Crawford et al., 1993).
684

685 Efforts have been made to link soil properties and Pb RBA/BAc. For example,
686 Wijayawardena et al. (2015) investigated Pb RBA values of 11 Pb acetate spiked
687 soils (1 year aging, from Queensland and South Australia, Australia) by the swine
688 model. A strong correlation was found between soil properties (pH, clay, and CEC)
689 and Pb RBA, being RBA = 131.5 – 12.9 pH - 0.5 CEC + 0.9 clay, n = 11, r^2 = 0.88,
690 $p < 0.01$. Jin et al. (2015) reported that Pb BAc (PBET model) is related to soil
691 properties using spiked soils, a correlation being BAc (G-phase) = 106.8 +
692 0.627[Pb] + 19.1[Fe] + 11.3[OM], and BAc (I-phase) = 2.852 + 0.078[Pb], where
693 OM is organic matter; However, no relationship has been established between Pb
694 RBA value and soil properties from field contaminated soils. Moreover, Caboche
695 et al. (2010) and Morman et al. (2009) indicated that soil edaphic properties failed
696 to model Pb BAc as these properties could not be extrapolated from one site to
697 another. Hagens et al. (2009) measured Pb BAc using the RIVM model, as well as
698 soil properties of 90 Dutch soils, including pH, OM, clay, calcium carbonate, total
699 sulphur, and reactive iron. No relationships between Pb BAc and soil properties
700 were found, possibly because the soils appear to have uniform soil characteristics
701 (Hagens et al., 2009).
702
703 Although limited relationships were reported between Pb RBA/BAc and soil
704 properties, it was reported that Pb RBA/BAc of historically contaminated soils is
705 influenced by soil properties and Pb speciation (Oomen et al., 2006). This study
706 implied that Pb RBA in soils is site-specific, and it is possible to predict Pb RBA in
707 specific soils and/or Pb types using soil properties (Hagens et al., 2009). All the

data was clustered by source of soils based on end use, such as mining, smelter, small arms ranges, gas works, shooting ranges, farming, pottery and some other industry sites. Considering the effect of soil type on Pb RBA, and the availability of data number to model, the data of mining soils was used to investigate the relationship between soil properties and Pb RBA. Soil properties of mining soils, including pH, clay, CEC, total organic carbon (TOC) and OM, were used to correlate with Pb RBA by linear regression.

The linear correlation between soil properties and the soils Pb RBA/BAc of mining soils from all literature data is shown in Figure 7. No significant relationship was found between the single soil properties and Pb RBA (left set in Figure 7); however, results showed that soil properties can influence Pb RBA. Lead RBA decreases when clay content and CEC increase, this indicates that clay content and CEC may have a negative effect on Pb RBA. While for TOC and OM, a relatively weak positive trend was found for Pb RBA. For pH, most soils are neutral or even alkaline, the Pb RBA values showed a larger range compared to that for acidic soils. The literature data of Pb BAc was also collected and analysed for investigation of the relationship between soil properties and Pb RBA in addition to Pb BAc data (right set in Figure 7). Similar results were found despite the increasing amount of data. It is worth noting that the above findings are based on limited literature data, additional investigation and information will be useful to further investigate possible relationship between soil properties and Pb RBA/BAc. A key requirement of this investigation is the approach and methods used for the study unlike

731 information derived from the literature where methods adopted by researchers vary
732 considerably. This could be one reason for the weak relationship or no relationship
733 observed between soil properties and RBA.



734

735 Figure 7 Effect of soil properties on Pb bioavailability of mining soils

3.3 Influence of metal content on Pb bioavailability

Published data was collected in our study to investigate the relationship between Pb concentration and BA. The distribution of Pb concentration for all mining soil samples is shown in Figure 8. Most of the samples are within the range of 2500 to 12500 mg/kg (Figure 8a). More than 50% of the samples have a Pb concentration below 10000 mg/kg (Figure 8b).

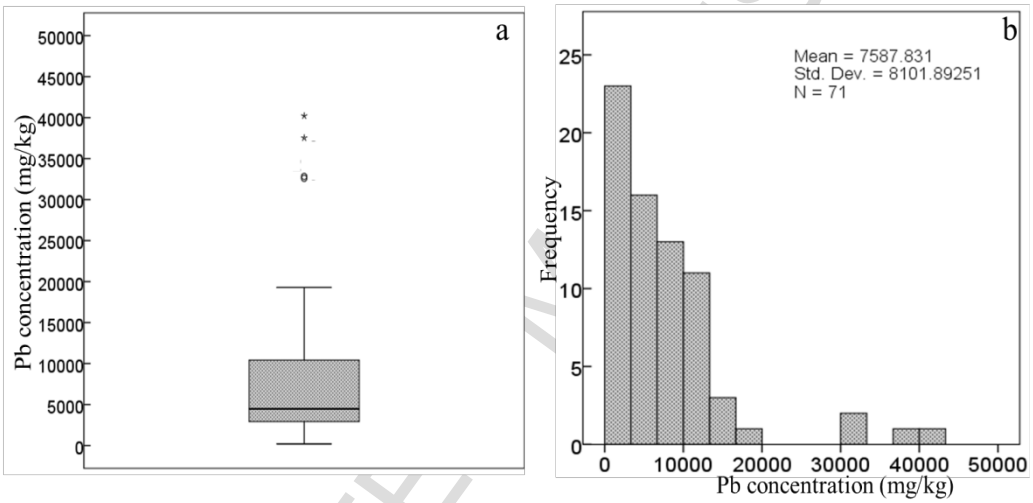


Figure 8 Distribution of Pb concentration for mining samples

References: (Freeman et al., 1992; Ruby et al., 1996; Casteel et al., 1997; Hettiarachchi et al., 2003; Schroder et al., 2004; Drexler and Brattin, 2007; Juhasz et al., 2009; Smith et al., 2011a; Denys et al., 2012; Li et al., 2015; Li et al., 2016)

Research efforts have been made to correlate total Pb concentration and Pb RBA/BAc. Roussel et al. (2010) found significant positive correlations between Pb BAc (UBM model) and total Pb concentration in 27 urban contaminated soils; However, Morman et al. (2009) reported that no correlations were found between

753 total metal content (Pb, As, Cd, Ni, Cr) and their BAc (RBALP model) in 20 soils
754 from various sources. Hagens et al. (2009) also stated that there was no relationship
755 between total Pb concentration and Pb BAc measured by RIVM model on 90 Dutch
756 soils. Moreover, Walraven et al. (2015) reported that Pb BAc does not necessarily
757 depend on the total Pb concentration. This was demonstrated by Casteel et al.
758 (1997), who estimated Pb RBA on two mining soils with Pb concentration of 3870
759 mg/kg and 14200 mg/kg, respectively. Their results showed that the Pb RBA for
760 these two soils was very close, 63% and 64%, respectively.
761
762 Literature data of Pb RBA/BAc and Pb/Ca/Fe concentration was collected and a
763 linear analysis was used to compare the influence of metal content on Pb RBA. As
764 shown in Figure 9, no relation was found between total Pb concentration and Pb
765 RBA/BAc. Other metals like Fe and Ca were reported to have competitive
766 adsorption effects on Pb BAc in the intestinal phase. For example, Bi et al. (2015)
767 found a significantly negative correlation between total Ca concentration and Pb
768 BAc (I-phase of PBET model), which is Pb BAc (I-phase) = 22.01* [Total Ca]$^{-1.16}$,
769 r^2 = 0.482. Li et al. (2014) demonstrated that Fe can co-precipitate with Pb during
770 the I-phase indicating that a high level of Fe resulted in a lower Pb RBA. In this
771 review, based on literature data, although no significant correlation is found
772 between Fe concentrations to Pb RBA, a weak negative influence can be observed
773 indicating Fe may have a competitive adsorption effect on Pb BAc in mining soils.
774 Calcium concentration showed no significant influence on Pb RBA/BAc in this
775 review.

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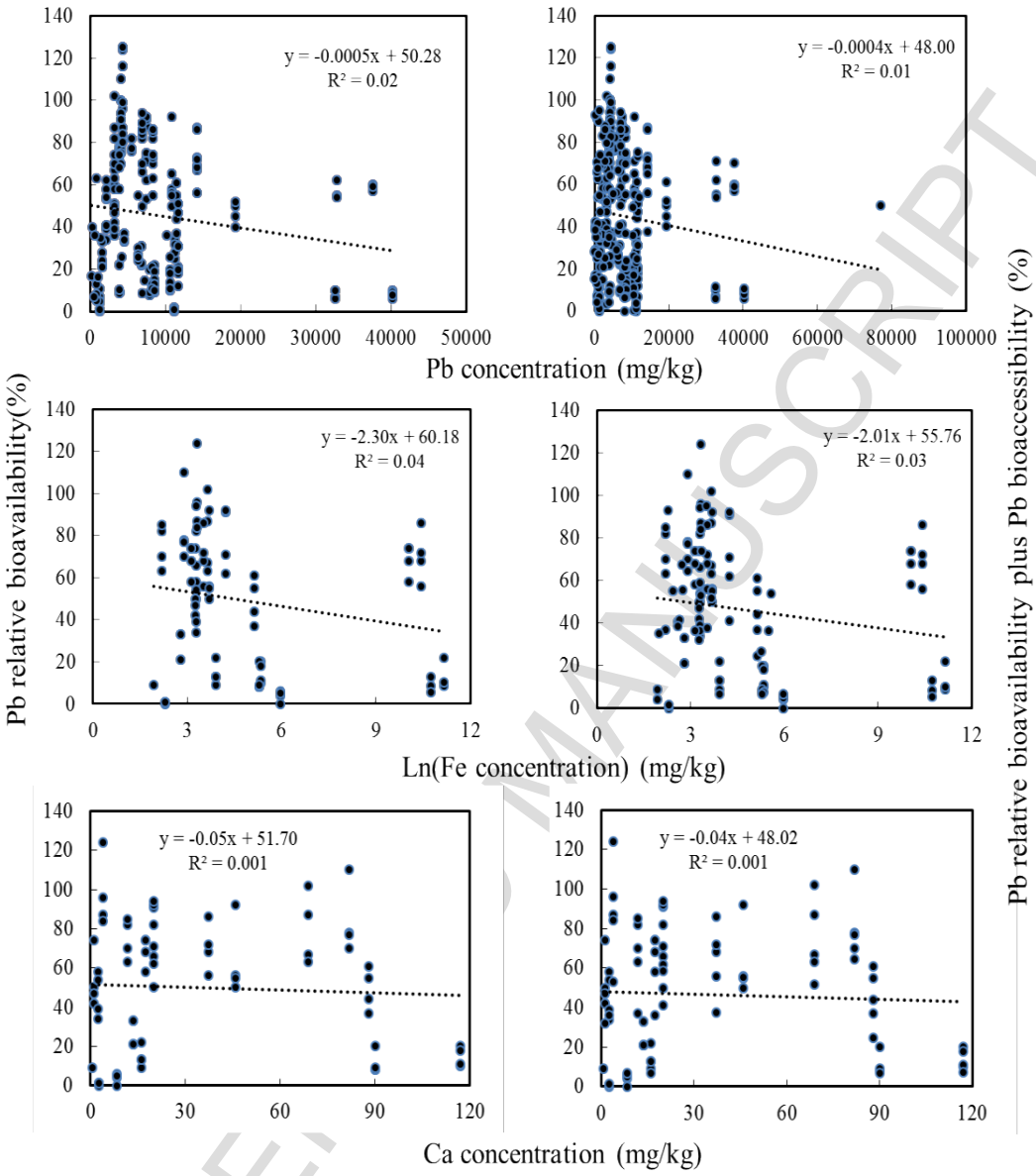

777

778 Figure 9 Comparison of metal content and Pb bioavailability in mining soil (Ln:

779 Napierian logarithm)

3.4 Future perspectives

Despite over three decades of research on bioaccessibility and bioavailability, it is still a challenge to estimate Pb RBA due to varying soil properties and many modelling uncertainties. More research efforts is expected to minimize uncertainties in measuring Pb RBA. Further research activities could include:

1) To address inter-species variability between different animal models, including swine, rats, and mice, to address uncertainties of measured Pb RBA.
2) Considering the advantage and benefits of using *in vitro* models to estimate Pb RBA/BAc, it is recommended that parameter uncertainties of commonly used *in vitro* models are investigated and addressed.
3) It is recommended that the best *in vitro* model to measure Pb BAc and then indicate Pb RBA is identified, and then further validated
4) More studies are required to research the influence of soil properties on Pb RBA/BAc, and to quantify the influence of soil properties, such as clay, CEC, OM, and TOC, to Pb RBA/BAc.
5) It is necessary to address the influence of competitive adsorption of metals onto soil components on Pb RBA/BAc.
6) To further investigate the adsorbtion/retention mechanism of Pb in soils, to offer fundamental information for the remediation of Pb contaminated soils.

4 Conclusion

In this review, we summarised the existing knowledge on the measurement of Pb RBA and BAc including their key influencing parameters, IVIVC correlations, the influence of soil type and properties on Pb BA, and existing uncertainties. Among the *in vitro* methods, we recommended the use of RBALP and UBM models to estimate Pb BAc on mining soils/dust as they are well validated using swine model, pH value was monitored in G-phase, and using end-over-end rotation for mixing,. Further studies can be devised for validating the correlation between *in vivo* and *in vitro* models by addressing uncertainties from various soil properties, inter-species differences of animal models, as well as difference *in vitro* models.

The influence of soils including soil type, soil properties and Pb concentration on Pb RBA/BAc are also discussed in this review. It is expected that significant correlations would be found between soil properties and Pb RBA/Bac for soils from the same type; However, limited information is available for using soil properties of field Pb contaminated soils to predict Pb RBA. The influence of soil properties on Pb RBA/BAc were analysed using existing literature information in this review, which showed a negative influence of clay and CEC content on Pb RBA/BAc. Although no significant correlation was found between metals content and Pb RBA, it is reported that metals content can influence Pb RBA. Fe concentration in mining soils is found to have a weak negative influence on Pb RBA indicating that metals may have a competitive adsorption effect on Pb in mining soils. Further investigation on the effect of soil on Pb RBA/BAc will provide help in addressing

822 the existing uncertainties in their measurement and provide indications on
823 development of remediation for Pb contaminated sites. The information provided
824 is critical and fundamental for future development of measurements for Pb
825 RBA/BAc and investigation of its influencing factors.

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Highlights

1) Key parameters influencing *in vitro* measurements of Pb bioaccessibility in soils and uncertainties are summarized.
2) Lead bioavailability varied with different soil type, soil properties and metal concentrations, indicating that those factors influence lead bioavailability.
3) Differences in *in vitro* methods and Pb source limit statistical analysis of the soil factors influencing Pb bioavailability.