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## Use of simulated epithelial lung fluid in assessing the human health risk of Pb in urban street dust

John R. Dean<sup>a,\*</sup>, Nwabueze I. Elom<sup>a</sup>, Jane A. Entwistle<sup>b</sup>

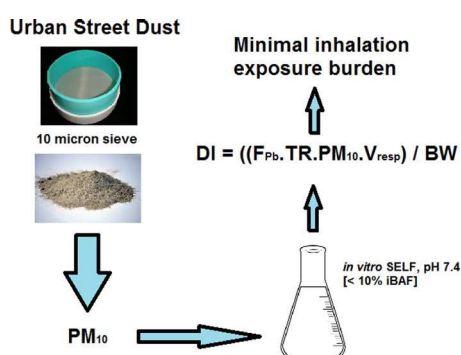
<sup>a</sup> Department of Applied Sciences, Northumbria University, Ellison Building, Newcastle Upon Tyne NE1 8ST, UK

<sup>b</sup> Department of Geography, Northumbria University, Ellison Building, Newcastle Upon Tyne NE1 8ST, UK.

### HIGHLIGHTS

- Exposure assessment of inhalation pathway
- High pseudo-total Pb in PM<sub>10</sub> fraction of urban dusts
- Extraction using simulated epithelial lung fluid
- Inhalation bioaccessibility of Pb in PM<sub>10</sub> < 10%
- Exposure burden from the inhalation pathway observed to be minimal

### GRAPHICAL ABSTRACT



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### ABSTRACT

In many urban contexts, non-dietary Pb exposure from street dusts may add to the overall exposure burden, and the presence of high total Pb content is well documented in urban street dust from across the globe. Given the increasing recognition of the potential adverse health effects from both the quantity and the chemical and physical composition of the inhaled fraction, and the recognition that it is the soluble fraction rather than the total element content that has more direct links to health effects, attention has focused in this study on the human health risks via this exposure pathway. In order to investigate the environmental exposure to Pb from the inhalation of urban street dusts, a newly developed *in vitro* simulated epithelium lung fluid (SELF) has been applied to the < 10 μm fraction of urban street dusts. In this context, 21 urban street dust samples, across five UK cities, were selected based on their high pseudo-total Pb content. The work revealed that inhalation bioaccessibility, and hence inhalation dose, varied across the cities but was generally found to be low (< 10%). Indeed, the lung bioaccessibility was far lower (% lung bioaccessibility ranged from 1.2 to 8.8) than is currently applied in two of the most commonly employed risk assessment models i.e. the Integrated Exposure Uptake Biokinetic model (IEUBK, USA) and the Contaminated Land Exposure Assessment model (CLEA, UK). The estimated inhalation dose (for adults) calculated from the PM<sub>10</sub> bioaccessibility ranged from 7 ng kg<sup>-1</sup> BW day<sup>-1</sup> (Edinburgh) to 1.3 ng kg<sup>-1</sup> BW day<sup>-1</sup> (Liverpool). The results indicate a low potential inhalation bioaccessibility for Pb in these urban street dust samples when modelled using the neutral pH conditions of the SELF.

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\* Corresponding author.

E-mail address: [John.Dean@northumbria.ac.uk](mailto:John.Dean@northumbria.ac.uk) (J.R. Dean).

## 1. Introduction

Particulate matter (PM) is ubiquitous in the atmosphere and is comprised of a wide range of materials sourced from both natural processes and human activity: sea-salt, combustion-derived carbonaceous particles, sulfates, silicates, oxides, carbonates, alloys, glass and biogenic material (Kelly and Fussell, 2012). Lead is one of the most enriched metals in urban particulate matter (De Silva et al., 2016; Goix et al., 2016; Laidlaw et al., 2012; Lei et al., 2016; Sharma and Pervez, 2003; Xu et al., 2012; Zereini et al., 2005) and despite the phasing out of Pb in fuel in many countries around the world it continues to be a potentially harmful element (PHE) of concern in the urban environment. Lead is a well-known neurotoxin, with exposure leading to neurobehavioural effects in children (e.g. Lanphear et al., 2005), and cardiovascular (hypertension) and renal toxicity effects in adults (EFSA, 2010). Indeed, a growing number of studies suggest that the threshold for clinical concern should be reduced to a blood Pb level below  $5 \mu\text{g dL}^{-1}$  (Budtz-Jorgensen et al., 2013; Navas-Acien et al., 2009; Zahran et al., 2013). Whilst there are strict regulations in many developed countries on the use and release of lead into the environment, particularly, on the former use of lead in petrol as an anti-knock additive (Kaysi et al., 2000; Kummer et al., 2009), this is not the case with most developing countries where leaded petrol is still in use (Gwilliam, 2003). Over the years the emphasis has been on leaded fuel (Oudijk, 2010; Nriagu et al., 1996; Romieu et al., 1992), but there are many other activities that release Pb into the environment particularly in the urban/industrial setting: activities such as metal mining, smelting and processing, the use of Pb in lead-acid batteries, pigments, alloys, lead wool, chemical manufacturing, cables, solders, plumbing components, food cans, coal combustion, lead based paint (including that in road markings), and industrial waste (Ajmore-Marsan and Biasioli, 2010; Brown and Longoria, 2010; De Silva et al., 2016; Laidlaw and Taylor, 2011; Mielke et al., 2010; Shen et al., 2002). Studies have shown that the Pb retained in soil/dust because of anthropogenic activity typically occurs in highly bioavailable, exchangeable and carbonate forms, whereas, Pb retained because of natural occurrence is often found in residual or less-bioavailable forms (Chlopecka et al., 1997; Cox et al., 2013; Palumbo-Roe et al., 2013; Pelfrène et al., 2012; Laidlaw and Filippelli, 2008; Reis et al., 2014; Ruby et al., 1994). Direct exposure to urban dust through inhalation is expected thus the respiratory tract is a potentially significant pathway through which urban dusts can enter the human body.

Urban dust represents a significant health risk to humans due to its small size and ubiquitous nature. Though the human respiratory system is naturally equipped with coordinated mechanisms to provide protection against inhaled particulates not all inhaled dusts are expelled; in addition, there is a significant possibility that the soluble fraction would be dissolved by interstitial lung fluids. In assessing risk to humans, particle size is a very important parameter. The particle size (and shape) of urban dusts and associated chemical composition determine their behaviour in the human respiratory system and ultimately their pathogenic mechanism. The particle size fraction considered potentially significant in this study is the  $<10 \mu\text{m}$  as it represents the easily inhalable fraction. This particle size is of growing concern because it can easily be carried and re-mobilised by air flows generated by wind, traffic or human movement. Moreover, it has been shown that toxic element concentrations in urban dusts increase with decreasing particle size fraction (Duong and Lee, 2009; Duong and Lee, 2011). Smaller particle size fractions have an increased surface to volume ratio and as result this provides an increased surface area for the deposition of potentially harmful elements (PHEs), relative to a similar mass of larger particles. Therefore, the  $<10 \mu\text{m}$  potentially represents a higher risk to human health.

Lead remains of concern in the urban environment, particularly those associated with legacy Pb from fossil-fuel and paint-derived sources, such as in cities with a rich industrial heritage and in smelting

and mining communities (e.g. Mielke et al., 2001; Mielke et al., 2010; Mielke et al., 2011; Taylor et al., 2013; Taylor et al., 2014). A survey (Thornton et al., 1990) of metals in UK dusts has shown that most of the dusts collected from urban environments had widespread Pb contamination ranging from 172 to 9600 mg/kg; other studies have reported (Wang et al., 2006) that for example in China, 80% of children exposed to urban dust had an excessive blood lead level. Similarly, the current authors had previously investigated the human health risk from Pb in urban street dust in five UK cities (Elom et al., 2014) using an oral bioaccessibility protocol. The study (Elom et al., 2014), which covered 75 dust samples from five cities, revealed that both pseudo-total Pb content and the oral bioaccessible Pb fractions were significantly elevated in nearly a third of the dust samples analysed. In vitro experiments use simulated fluids that are used to represent the natural physiological fluids in assessing the human health risks from contaminants. Exposure from environmental contaminants are normally considered against three exposure pathways, specifically, ingestion, inhalation and dermal contact. The oral (ingestion) pathway, considered the critical exposure route when modelling public open space, residential, and commercial settings (DEFRA, 2014a), has been the focus of much research to assess the human risk assessment from exposure to environmental contaminants predominantly via hand-to-mouth (deliberate) or unintentional ingestion of soils and related materials (Boisa et al., 2013; Cai et al., 2016; Intawongse and Dean, 2006; Li et al., 2014; Lorenzi et al., 2012; Okorie et al., 2012; Oomen et al., 2002; Smith et al., 2011; Wragg et al., 2009; Wragg et al., 2011). However, given the increasing acknowledgement of the association of PM concentrations (the chemical composition, as well as the physical presence) with both short-term and long-term health consequences (Kelly and Fussell, 2015), and the recognition that it is the soluble fraction rather than the total element content that has more direct links to health effects (Adamson et al., 2000; Ghio and Devlin, 2001; Heal et al., 2005), the inhalation exposure pathway was the focus of this current study. Indeed, in many urban and high dust generating contexts it is now timely, given increasing evidence of the link between PM10s and a range of human disease pathologies (Kelly and Fussell, 2012; Kelly and Fussell, 2015; Uzu et al., 2011), to consider the potential inhalation burden and bioaccessibility of PHEs in airborne PM and other environmental samples with a particle size fraction  $<10 \mu\text{m}$ . The probability of inhalation depends on the particle size fraction, air movement within the exposure routes, and breathing rate. Inhalable particulate matter could be inhaled through the nose and thus the fate of inhaled particulates depends on the nature of the physiological fluids and physiochemical properties of the particulates. The inhalable particle size fraction (aerodynamic diameters  $<10 \mu\text{m}$ ) penetrates, deposits and is retained in different compartments of the human respiratory tract with the larger components commonly found in the nasopharynx and tracheobronchial region whilst the finer ( $<1-2 \mu\text{m}$ ) particles are deposited in the deepest region (alveolar) (Lippmann and Albert, 1969; Gokhale and Patil, 2004; Plumlee et al., 2006). An understanding of the respiratory compartments and their functions, as well as chemical composition is fundamental in formulating fluids that truly represent the respiratory system. Simplistically, the respiratory system is comprised of three compartments; the nasopharynx, tracheobronchial, and the pulmonary (Task Group on Lung Dynamics, 1966; U.S. EPA, 2008). Classification of the respiratory system into different compartments gives a clearer understanding of the processes involved in particle inhalation, deposition and removal. Neutral synthetic lung fluids, largely based on modifications of the original 'Gamble's solution', are widely used for the exposure assessment of humans to inhalable pollutants (Wragg and Klinck, 2007; Caboche et al., 2011; Lima et al., 2013). More recently, the crucial (though often unclear) role of high molecular mass proteins, antioxidants and surfactant lipids in determining bioaccessibility has been recognised, along with the development and application of a new generation of in vitro lung fluid formulations (Boisa et al., 2014; Gray et al., 2010; Li et al., 2014; Stebounova et al., 2011). The development

of specialised fluids to mimic other parts of the human respiratory system is also required, as particles in the lung can also be phagocytized, exposing the PM to lower pH environments. Indeed, a growing number of studies have observed higher Pb bioaccessibility following exposure to an artificial lysosomal fluid (ALF, pH 4.5–5.0) than observed for a more neutral Gamble's-type solution (pH 7.2–7.4) (Colombo et al., 2008; Potgieter-Vermaak et al., 2012; Zereini et al., 2012). Whilst inhaled particles would have to be phagocytized before significant exposure to a more acidic environment occurs, all PM will be exposed to the neutral lung fluid. Coupled with the fact that the actual extent of phagocytosis remains unclear, in this study we utilised the neutral synthetic epithelial lung fluid (or SELF) proposed by Boisa et al. (2014). Furthermore, recent testing by Li et al. (2016) indicated the important role played by some of the key constituents included in the SELF in influencing bioaccessibility and highlighted the greater bioaccessibility of Pb in the SELF when compared to the standard Gamble's-type fluid. As such we considered the SELF provided a sufficiently conservative (health protective) approach to assess the inhalation bioaccessibility of Pb in 21 urban street dust samples collected from five northern UK cities, several of which have a long industrial history.

## 2. Material and methods

### 2.1. Sample collection and preparation

Twenty-one (21) urban street dust samples from across five northern UK cities (Durham, Edinburgh, Liverpool, Newcastle upon Tyne and Sunderland) were selected for this work. In each city, samples sites were selected from areas with the highest pedestrian flows or 'footfalls' created by large volumes of people going about their daily urban routines e.g. to/from work, shopping and leisure pursuits. The urban street dust samples were collected using a plastic dustpan and brush; to avoid cross-contamination separate dustpans and brushes were used at each site (Elom et al., 2014). Each sample (up to 5 g) was placed in a labelled self-sealing bag (kraft bags). They were then transported back to the laboratory where they were dried in a drying cabinet at a temperature of 35 °C for 48 h, manually disaggregated and all extraneous material removed before sieving into the <125 µm particle size fraction. The <125 µm dust samples were then manually sieved through a < 10 µm nylon sieve and the collected fraction was stored in Sarstedt plastic tubes prior to extraction (and subsequent analysis).

### 2.2. Instrument and reagents

All chemicals used in the analysis were certified analytical grade. Concentrated nitric acid (HNO<sub>3</sub>) and concentrated hydrochloric acid (HCl) were supplied by Fisher Scientific UK Ltd. (Loughborough, Leicestershire). Sodium hydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>) and potassium hydrogen phosphate (NaHPO<sub>4</sub>) were purchased from Sigma-Aldrich Co. (Gillingham, UK). Sodium chloride (NaCl), anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>), potassium chloride (KCl), calcium chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O), sodium bicarbonate (NaHCO<sub>3</sub>), magnesium chloride (MgCl<sub>2</sub>·6H<sub>2</sub>O), sodium hydroxide (NaOH), uric acid, bovine serum albumin (BSA) and concentrated nitric acid (69% HNO<sub>3</sub>) were all obtained from Merck (Poole, England), Mucin (pig) was obtained from Carl Roth, GmbH (Karlsruhe, Germany). Ascorbic acid, glutathione, cysteine, dipalmitoylphosphatidylcholine (DPPC), and glycine were obtained from Sigma-Aldrich Co. (Gillingham, UK). A multi-element standard containing Pb and other trace elements and an internal standard solution containing terbium (Tb) were obtained from SPEXCertiPrep (Middlesex, UK). Ultra-pure water of conductivity 18.2 MΩ-cm was produced by a direct Q™ Millipore system (Molsheim, France). A range of certified reference materials (CRMs) of varying composition and of related particle size (ranging from <10 µm up to <105 µm), and potentially characteristic of urban street dust were obtained: BCR 038

(Fly ash from pulverised coal, <10 µm particle size), BCR 143R (Sewage sludge amended soil, <90 µm particle size), BCR 176R (Fly ash, <105 µm particle size), and BCR 723 (Road dust, <90 µm particle size) were obtained from LGC-Promotech (London, UK), whilst BGS Guidance Material 102 (a naturally contaminated soil from North Lincolnshire, <40 µm particle size) was obtained from the British Geological Survey (Keyworth, UK). Sample digestions were carried out using a Start D multiprep 42 high throughput rotor microwave system (Milestone Microwave Laboratory Systems) supplied by Analytix Ltd. (Peterlee, UK). Measurement of PHE extracts and digests of urban street dust samples was carried out by using an ICP-MS X series II (Thermo Electron Corporation, Cheshire, UK).

### 2.3. Preparation of simulated epithelial lung fluid (SELF) for in vitro extraction

Chemical components needed to prepare 1000 mL of simulated epithelial lung fluid were prepared in two phases (inorganic and organic). To prepare the inorganic phase (500 mL): 6020 mg NaCl, 256 mg CaCl<sub>2</sub>, 150 mg NaHPO<sub>4</sub>, 2700 mg NaHCO<sub>3</sub>, 298 mg KCl, 200 mg MgCl<sub>2</sub> and 72 mg Na<sub>2</sub>SO<sub>4</sub> were accurately weighed into 500 mL HDPE screw top bottle and made up to the set volume with ultra-pure water and then thoroughly mixed. To prepare the organic phase (500 mL): 18 mg ascorbic acid, 16 mg uric acid and 30 mg glutathione were also accurately weighed into 500 mL HDPE screw top bottle and made up to the set volume with ultra-pure water; the resulting solution was then thoroughly mixed. The inorganic and organic components were simultaneously poured into a 2 L HDPE screw top bottle containing additional constituents; 260 mg albumin, 122 mg cysteine, 100 mg DPPC, 376 mg glycine and 500 mg mucin, this was thoroughly mixed until all the components dissolved. The pH was measured and adjusted to 7.4 ± 0.2 by adding 0.2 mL HCl.

### 2.4. Sample preparation and bioaccessibility extraction protocol

An approximate 0.3 g of five certified reference/guidance materials (in triplicate) and the 21 urban street dust samples (in duplicate) were accurately weighed into labelled 50 mL screw-cap Sarstedt tubes (extraction tubes) to which 20 mL of the SELF was added (Boisa et al., 2014). The mixture was then shaken, on an end-over-end rotator, maintained at 37 °C ± 0.2 for 96 h (Boisa et al., 2014). The resulting solution was then centrifuged at 3000 rpm for 10 min. Then, 1 mL of the supernatant was pipetted into a 10 mL Sarstedt tube previously holding 9 mL of 0.1 M HNO<sub>3</sub> and 30 µL of internal standard (<sup>159</sup>Tb, 10 µg mL<sup>-1</sup>). The sample was stored at <4 °C prior to ICP-MS analysis.

### 2.5. Protocol for total Pb determination via microwave digestion system

An approximate 0.5 g of each sample and the certified reference/guidance materials were accurately weighed into a 65 mL PFA (a perfluoroalkoxy resin) microwave vessel. Then, 13 mL of aqua regia (HCl: HNO<sub>3</sub>, 3: 1 v/v) was added into the PFA vessels prior to digestion in a microwave oven (Okorie et al., 2010). The microwave oven was operated at a temperature of 160 °C and a power of 750 watts. The temperature programme was operated for a total time of 40 min; this was followed by a 30 min cooling time. After cooling, the digested samples were filtered, diluted to 50 mL with ultrapure water and quantitatively transferred into Sarstedt tubes and stored in the refrigerator (<4 °C) prior to analysis by ICP-MS.

### 2.6. ICP-MS protocol/quality control

The robustness of the analytical procedure (i.e. digestion protocol using microwave oven and sample analysis via ICP-MS) was tested using five certified reference/guidance materials. Pb concentrations in both the total digestion blanks and the SELF blanks were not detected.

The ICP-MS was operated under standard operating conditions, as previously reported (Boisa et al., 2014).

### 3. Results and discussion

A calibration curve for Pb, based on a concentration range of 0–400 ng mL<sup>-1</sup>, with 7 calibration data points, was done; a regression coefficient (R<sup>2</sup>) of 0.999 was obtained. Accuracy was assessed by analysis of the CRMs/guidance material. The results in all cases were excellent (i.e. within one standard deviation of the certificate certified value), with precision also <5% in all cases (Table 1). Analysis of the urban street dusts (<10 µm fraction) showed both intra- and inter-sampling location Pb concentration variation. Typical intra- and inter-city pseudo-total Pb variation was noted as follows (Table 2): Durham ranged from 534 mg/kg to 2435 mg/kg; Edinburgh ranged from 472 mg/kg to 1248 mg/kg; Liverpool ranged from 452 mg/kg–1408 mg/kg; Newcastle upon Tyne ranged from 772 mg/kg to 1778 mg/kg; whilst Sunderland ranged from 535 mg/kg to 2357 mg/kg. It is noted that 12 of the sampled sites (Table 2) exceed the 'normal background concentration' (95th percentile) for Pb in urban soil of 820 mg/kg (in England) (DEFRA, 2012). The presence of high Pb content in urban street dust could pose an important exposure route through involuntary dust ingestion, often dependent upon climatic conditions (Laidlaw et al., 2005). In addition, the transfer of urban street dust into the workplace and domestic situation is also likely to be prevalent and totally involuntary via direct contact with footwear as well as through the deposition of airborne material such as on outer clothing and window sills (Campbell et al., 2003; Rich et al., 2002).

Whilst no guideline concentrations exist for Pb in urban street dusts, it is possible to consider the potential human health risk through various exposure pathways. Previous work from this group (Elom et al., 2014) has considered the ingestion (oral) bioaccessibility pathway from these sampling sites and concluded that a child would only need to ingest <73 mg dust/day to exceed an oral Tolerable Daily Intake (TDI<sub>oral</sub>) of 3.6 µg·kg<sup>-1</sup><sub>bw</sub>·day<sup>-1</sup> (Elom et al., 2014). The calculations were based on a daily soil/dust ingestion rate of 100 mg/day. Whilst this rate is consistent with recommended central tendency child ingestion rates (U.S. EPA, 2008; U.S. EPA, 2011), given time spent outdoors is a key predictor of child soil/dust ingestion rates (Van Wijnen et al., 1990), this rate is likely to be an overestimate in the context of urban street dust. In terms of urban street dusts, the quantity of inhaled material is typically modelled by the particle emission factor (PEF) or equivalent, such as the total suspended solids of the requisite size (U.S. EPA, 2011; Boisa et al., 2014; Brown et al., 2015). In the urban context where a range of weathering, industrial, combustion, construction and

demolition processes generate fine particles, that are then available for further pulverisation and disturbance, an approach to assess the human health risk from inhalation of urban dusts is necessary, one that also considers the inhalation bioaccessible fraction (iBAF).

No certified reference material is available for assessing the iBAF; however, five certified reference/guidance materials were used to assess the robustness and variability of the iBAF analytical protocol as applied to a range of environmental matrices and particle sizes (Table 1). The % iBAF is consistently low, irrespective of the different sample types and source location of the samples, and determined as follows: BCR 038 (0.33%), BCR 143R (8.8%), BCR 176R (4.3%), BCR 723 (4.0%), and BGS 102 (5.7%).

The SELF was then applied to assess the Pb iBAF from the 21 urban street dust samples (Table 2). The urban street dust samples showed similarly low % iBAF values as follows: Durham ranging from 3.2 to 6.8; Edinburgh ranging from 3.3 to 7.7; Liverpool ranging from 3.0 to 8.8; Newcastle upon Tyne ranging from 1.2 to 3.2; and, Sunderland ranging from 1.4 to 4.2. The overall mean % iBAF across all sampling locations was 4.2 ± 2.2. Considering all individual % iBAF values, across all sampling locations, the maximum inhalation bioaccessibility remained <10%. This is perhaps not unexpected considering the pH (7.4 ± 0.2) of the epithelial linings of the tracheobronchial region as mimicked via the SELF. This pH represents a neutral environment, representative of the extracellular environment of the lung (Zoitos et al., 1997), which would normally release less Pb into solution when compared to the more acidic bioaccessibility fluids (with pHs down to 4.5) as used by some researchers modelling lysosomal fluids (Denys et al., 2007; Wiseman, 2015). Similarly, it was found (Potgieter-Vermaak et al., 2012) that the Pb iBAF was substantially higher using an artificial lysosomal fluid (pH 4.55) on roadside dusts than that of their Gamble's solution (pH 7.35). However, metal speciation is also of relevance here. For example, Li et al. (2016) observed that PbSO<sub>4</sub> had lower bioaccessibilities compared to PbO in ALF whilst the opposite was observed for the Gamble's fluid; PbSO<sub>4</sub> being more soluble in the neutral Gamble's environment.

Comparing lung inhalation bioaccessibility data is complicated due to the wide variation in experimental parameters and conditions employed by different workers, specifically, the chemical composition of the SELF (Boisa et al., 2014), fluid pH and extraction time. The simulated fluids originally used to assess pollutant bioaccessibility, via the inhalation route, has been referred to as Gamble's solution (Diem and Lenter, 1970; Moss, 1979; Anosborlo et al., 1999). The absence of an accepted standard in vitro method for assessing the bioaccessibility of pollutants particularly PHEs in the human lung has resulted in the development of different inhalation bioaccessibility methods (for

**Table 1**  
Lead in certified/guidance materials: pseudo-total, inhalation bioaccessible fraction and residual.

Sample	Source material (particle size fraction)	Certified reference/guidance material values (mg/kg)	Measured pseudo-total Mean ± SD (mg/kg) (n = 3)	Stage I (Inhalation bioaccessible Pb)		Stage II (Residual digest) Mean ± SD (mg/kg) (n = 3)	Total Pb content (Stage I + II)	
				Mean ± SD (mg/kg) (n = 3)	% iBAF		Mean	% Recovery (n = 3)
BCR 038	Fly ash from pulverised coal (<10 µm)	262 ± 11	258 ± 2	0.85 ± 0.01	0.33	254 ± 10	255	98.8
BCR 143R	Sewage sludge amended soil (<90 µm)	174 ± 5	173 ± 6	15.3 ± 2.2	8.84	158 ± 2	173	96.6
BCR 176R	Fly ash (<105 µm)	5000 ± 500	5009 ± 17	206 ± 26	4.31	4860 ± 31	5066	101
BCR 723	Road dust (<90 µm)	866 ± 16	860 ± 12	34.7 ± 2	4.03	820 ± 10	855	98.7
BGS 102	Naturally contaminated soil from North Lincolnshire (<40 µm)	79.4 ± 1.4	76.1 ± 1.6	4.3 ± 0.5	5.65	70.1 ± 0.2	74.4	93.7
Mean					4.63 ± 2.75			

% iBAF: stage related inhalation bioaccessibility, calculated as a fraction of the total content.

**Table 2**

Lead in urban dust samples (&lt;10 µm): pseudo-total, inhalation bioaccessible fraction, residual and inhalable dose.

City	Sample location	Total pseudo-Pb content <sup>a</sup> , Mean ± SD (mg/kg) (n = 3)	Stage I (inhalation bioaccessible)		Stage II (Residual digest)		Total Pb content (Stage I + II)		Inhalable dose (DI) (ng·kg <sup>-1</sup> <sub>bw</sub> ·day <sup>-1</sup> )	
			Mean ± SD (mg/kg) (n = 2)	% iBAF	Mean ± SD (mg/kg) (n = 2)	Mean (mg/kg) (n = 2)	% Total recovery	Adult (generic-site PM <sub>10</sub> )	Adult (site-specific PM <sub>10</sub> )	
Durham	D1	1279 ± 19	40.4 ± 0.3	3.2	1152 ± 6	1192	93.2	5.1	NA	
	D2	846 ± 3	57.2 ± 0.6	6.8	684 ± 11	741	87.6	5.1	NA	
	D3	2435 ± 13	112 ± 1	4.6	2218 ± 7	2330	95.7	2.2	NA	
	D4	534 ± 8	31.7 ± 0.2	5.9	472 ± 4	504	94.3	4.7	NA	
Edinburgh	E1	472 ± 4	36.1 ± 0.1	7.7	421 ± 3	457	96.8	3.7	0.6	
	E2	586 ± 5	19.2 ± 1	3.3	537 ± 5	556	94.9	2.4	0.8	
	E3	1082 ± 14	39.4 ± 0.8	3.6	1028 ± 17	1067	98.7	7.0	1.4	
	E4	486 ± 15	27.3 ± 1	5.6	435 ± 3	462	95.1	1.5	0.6	
	E5	1248 ± 45	60.8 ± 2	4.9	1174 ± 29	1235	98.9	2.4	1.6	
Liverpool	L1	837 ± 6	24.7 ± 0.1	3.0	719 ± 3	744	88.9	1.8	4.5	
	L2	646 ± 4	56.5 ± 0.2	8.8	533 ± 9	590	91.3	4.0	3.5	
	L3	1408 ± 9	65.9 ± 0.4	4.7	1291 ± 12	1357	96.4	1.4	7.7	
	L4	495 ± 5	16.1 ± 0.8	3.3	451 ± 2	467	94.4	1.3	2.7	
	L5	452 ± 5	38.2 ± 0.4	8.5	398 ± 6	436	96.5	1.4	2.5	
Newcastle	N1	1778 ± 22	20.9 ± 0.5	1.2	1690 ± 10	1711	96.2	1.7	2.0	
	N2	1766 ± 17	28.7 ± 1	1.6	1702 ± 22	1731	98.0	3.1	2.0	
	N3	772 ± 1	25.1 ± 0.1	3.2	685 ± 9	710	92.0	1.4	0.9	
	N4	1627 ± 16	37.4 ± 0.2	2.3	1584 ± 16	1621	99.7	3.6	1.9	
Sunderland	S1	2357 ± 22	37.1 ± 0.6	1.6	2309 ± 19	2346	99.5	6.7	NA	
	S2	535 ± 20	22.7 ± 0.1	4.2	487 ± 3	510	95.3	1.5	NA	
	S3	2073 ± 42	28.7 ± 0.4	1.4	1984 ± 6	2013	97.1	5.9	NA	
Mean ± SD				4.2 ± 2.2				3.2 ± 1.8		

% iBAF: stage related inhalation bioaccessibility, calculated as a fraction of the total Pb content.

Notes: The sample distribution was as follows: Durham (4 samples): Durham University (D1), Durham Cathedral (D2), Saddler Street (D3), and Penny Ferry Bridge (D4); Edinburgh (5 samples): George Street (E1), Princess Street (E2), Leith Walk Street (E3), Nicolson Street (E4) and Saint John's Street (E5); Liverpool (5 samples): London Road (L1), Pembroke Place (L2), Brown Low Hill Road (L3), Royal Hospital (L4) and Prescott Street (L5); Newcastle upon Tyne (4 samples): Gray Street (N1), Cathedral Church (N2), Central Station (under the arch) (N3) and Clayton Street (N4); and, Sunderland (3 samples): High Street West (S1), Royal Theatre (S2) and Bridges Shopping Complex (S3).

<sup>a</sup> Total pseudo-Pb content, as determined by microwave digestion using aqua regia.

example, Wragg and Klinck, 2007; Caboche et al., 2011; Boisa et al., 2014). The simulated fluids used (Wragg and Klinck, 2007; Caboche et al., 2011; Boisa et al., 2014) were all modifications of the original Gamble's solution; however, each research group has modified the simulated fluid in terms of the chemical composition, pH, operating conditions (and especially the sample/solution ratio) and the extraction procedure. However, it is important to note that despite these differences, these methods have been used to assess the bioaccessibility of Pb and other PHEs in many environmental matrices. For example, the bioaccessibility of Pb from urban road dust was investigated (Potgieter-Vermaak et al., 2012), and it was observed that the %iBAF of Pb was 0.3 (at pH 7.35 using Gamble's solution) and 46.5 (at pH 4.55 using an Artificial Lysosomal Fluid) at 24 h. In another study, Caboche et al., 2011 examined the iBAF of a range of elements, including Pb, from four certified reference materials using water and Gamble solution for extraction and revealed that simulated lung fluids released a higher %iBAF than water; the typical %iBAF Pb, from the Gamble solution, was found to range from 1.3% to 24.6% with a calculated mass balance of between 73 and 117% across the certified reference materials. Furthermore, the iBAF of Pb in Pb contaminated soil, tailing and smelter samples using SELF was also investigated (Boisa et al., 2014). The results showed that the % iBAF in the samples ranged from 0.02 to 11.0% with a mass balance of 100% to 103%. These data ranges accord well the iBAF of Pb in our urban street dusts, where %iBAF ranged from 1.2 to 8.8%. The differences in these studies are expected considering that different methods were employed and samples sourced from different environmental matrices.

Literature on the bioavailability and bioaccessibility of Pb in humans is predominantly focussed on the oral ingestion pathway, however this literature base provides context for our iBAF data. Relative bioavailability has variously been defined as the ratio of the bioavailability (or bioaccessibility) of the contaminant in the environmental media to the

bioavailability of the contaminant in a standardized reference material (such as soluble lead acetate; U.S. EPA, 2007) or the critical study used to derive the health criteria (DEFRA, 2014b). With respect to Pb, the health criteria are based on dietary intakes modelled to produce the adopted blood Pb action value (DEFRA, 2014b). The IEUBK model assumes default bioavailability figures of 50% for dietary intake and 30% for ingestion of soil and dust (U.S. EPA, 2007) and a relative bioavailability (based on soil to dietary exposure) of 60% (DEFRA, 2014b). The Dutch soil Pb intervention values are modelled on a similar relative bioavailability of 0.74 (SoBRA, 2012). In the UK, the current Pb guideline values (referred to as category 4 screening levels; C4SLs) are modelled on a relative bioavailability of 0.60 (DEFRA, 2014b). This is based, in part, on the body of literature evidencing Pb oral bioaccessibilities with a mean of approximately 60% and an assumed oral bioaccessibility of dietary exposure of 100%. Whilst there is no consistent literature approach to relate bioaccessibility data to relative bioavailability (e.g. U.S. EPA, 2009; Smith et al., 2011), it is clear our data for iBAF are far lower than those observed for the oral ingestion pathway. The IEUBK model also assumes that 32% of the inhaled Pb is retained within the lung and that 100% of this is bioavailable, i.e. 32% of Pb intake via inhalation is absorbed (U.S. EPA, 2007; DEFRA, 2014b). This equates to an inhalation relative bioavailability of 64% (i.e. 32% divided by 50% for dietary intake as this is the critical comparison study) and this factor has been adopted in the UK for derivation of the C4SLs (DEFRA, 2014b). Based on our iBAF data (maximum <10%), a much lower inhalation relative bioavailability (such as <0.10, i.e. 32% retained in the lung, but of this only 10% is bioavailable, so 3.2% divided by 50% for dietary intake) would seem appropriate. Far lower than is currently employed in the risk assessment models.

In addition, and based on the data available, it is possible to estimate human exposure to airborne contaminants by determination of the inhalable dose in the PM<sub>10</sub> fraction. The theoretical inhalation dose

(DI) ( $\text{ng} \cdot \text{kg}^{-1}_{\text{bw}} \cdot \text{day}^{-1}$ ) is defined as follows (SFT, 1999; Chen et al., 2011):

$$\text{DI} = ((F_{\text{pb}} \times \text{TR} \times \text{PM}_{10} \times V_{\text{resp}}) / \text{BW}) \times 10^3 \quad (1)$$

where  $F_{\text{pb}}$  is the mass fraction (pseudo-total concentration) of Pb in the  $\text{PM}_{10}$  fraction; TR is the tracheobronchial retention, expressed as a fraction;  $\text{PM}_{10}$  is the concentration of particles with a diameter  $< 10 \mu\text{m}$  ( $\mu\text{g} \text{m}^{-3}$ );  $V_{\text{resp}}$  is the inhalation rate ( $\text{m}^3 \text{day}^{-1}$ ); and, BW is the body weight (kg). A  $10^3$  factor allows the unit to be expressed as  $\text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ .

A variety of literature values are available for the terms: TR,  $\text{PM}_{10}$ ,  $V_{\text{resp}}$  and BW from both regulatory agencies and others (Table 3). It can be seen (Table 3) that values for the tracheobronchial retention appear to be limited in terms of occurrence and range. An experimentally derived value, from human volunteers, identified that the TR was  $47 \pm 8\%$  (Svartengren et al., 1996). Unfortunately, this was only done on  $< 6 \mu\text{m}$  particles. Nevertheless, it does provide an estimate of the number of particles that can be retained by the lungs. This value for TR has therefore been used in subsequent calculations (as a fraction i.e. 0.47). Values for the concentration of  $\text{PM}_{10}$  particles in ambient air are abundant in the literature (e.g. Cho et al., 2011; Tittarelli et al., 2008). It is interesting to note that the annualised maximum daily rate target value is  $20 \mu\text{g} \text{m}^{-3}$  (WHO, 2014; WHO, 2016) and we adopted this value for the calculation of both the generic-site and site-specific inhalation (daily) dose (DI) values. In addition, for three of the cities investigated the actual  $\text{PM}_{10}$  concentration was obtained (DEFRA, 2010), from a fixed location, on the day(s) sampling took place. It was therefore possible to calculate a site-specific DI for Edinburgh, Liverpool and Newcastle upon Tyne. The inhalation rate ( $V_{\text{resp}}$ ) also had various values (in  $\text{m}^3 \text{d}^{-1}$ ) based on

studies done by regulatory agencies and other government bodies (ECHA, 2008a; ECHA, 2008b; EFSA, 2010; U.S. EPA., 2011; NCM, 2011; SFT, 1999; DEFRA, 2014c; WHO/IPCS, 1994; WHO/IPCS, 1999). An inhalation rate of  $21.3 \text{m}^3 \text{d}^{-1}$  was chosen as it represented an upper value which is linked with a higher risk. Finally, body weight expressed in units of kg; whilst various body weights have been used (60, 65, 70 and 80 kg) the most common value chosen is currently 70 kg.

Based on the selected values (Table 3) the daily generic-site inhalation dose (DI) was then estimated, using Eq. (1) and the values, per location, are shown in Table 2. The overall mean generic-site inhalation dose, based on  $\text{PM}_{10}$  values available from the literature, was  $3.2 \pm 1.8 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  (range:  $1.3\text{--}7 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ ). Site-specific inhalation dose values varied; for Edinburgh the mean was  $1.0 \pm 0.4 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  (range:  $0.6\text{--}1.6 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ ); for Liverpool the mean was  $4.2 \pm 1.9 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  (range:  $2.5\text{--}7.7 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ ); and, for Newcastle upon Tyne the mean was  $1.7 \pm 0.5 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  (range:  $0.9\text{--}2.0 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ ). The slightly elevated site-specific value for Liverpool is due to the higher  $\text{PM}_{10}$  value ( $38 \mu\text{g} \text{m}^{-3}$ ) as compared to the other locations (Table 3). This range equates well to that reported by EFSA (2010) where the DI for Pb for an adult from outdoor arranged from  $0.7\text{--}2.4 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ , based on a mean and high Pb content of  $0.003 \mu\text{g} \text{m}^{-3}$  and  $0.010 \mu\text{g} \text{m}^{-3}$ , respectively, an assumed body weight of 70 kg and a respiration rate of  $17 \text{m}^3 \text{d}^{-1}$ . The DI increased to a maximum of  $15 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  and  $32 \text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$  based on smoking (20 cigarettes) or from environmental tobacco smoke, respectively (re-calculated for 70  $\text{kg}_{\text{BW}}$ ).

To set this within a health context it is necessary to consider how this equates to limits of concern for human health. In 2010, the WHO JECFA committee withdrew the provisional tolerable weekly intake (PTWI) of Pb stating that the blood Pb concentration of  $10 \mu\text{g} \text{dL}^{-1}$  on

**Table 3**  
Terms and literature values used in the calculation of the inhalable dose ( $\text{ng} \text{kg}^{-1}_{\text{BW}} \text{day}^{-1}$ ).

Term	Symbol	Units	Value	Reference
Total concentration of Pb in the $\text{PM}_{10}$ fraction	–	mg/kg	Experimentally determined	This work
mass fraction of Pb in the $\text{PM}_{10}$ fraction	$F_{\text{pb}}$	mg $\text{mg}^{-1}$	Calculated from the total concentration	This work
Tracheobronchial retention (expressed as a fraction)	TR	–	0.70 (theoretical calculation) 0.47 (range: 0.39–0.55, n = 10; measured on $6 \mu\text{m}$ particles) 0.75	Sturm, 2007 Svartengren et al., 1996 SFT, 1999
Concentration of particles with a diameter $< 10 \mu\text{m}$	$\text{PM}_{10}$	$\mu\text{g} \text{m}^{-3}$	9 <sup>a</sup> 38 <sup>b</sup> 8 <sup>c</sup> 20 (annual mean) 50 (24 h mean) 50 (daily average, 24 h) 25 (annual average standard) 41	DEFRA, 2010 DEFRA, 2010 DEFRA, 2010 WHO, 2014; WHO, 2016 WHO, 2014 EU, 2016 NEPC, 2016 SFT, 1999
Inhalation (respiration) rate	$V_{\text{resp}}$	$\text{m}^3 \text{d}^{-1}$	17 15.7 (recommended mean); 21.3 (recommended 95th percentile value). Based on an adult 16 to $<65$ years old); 20 20 18 (Adult aged 20–75 years old; body weight 70 kg) 16.1 (daily inhalation rate – long term exposure; based on adult body weight of 70 kg) 22 (Adult: male and female combined)	EFSA, 2010 U.S. EPA., 2011; DEFRA, 2014c ECHA, 2008a; NCM, 2011 SFT, 1999 ECHA, 2008b; NCM, 2011 EFSA, 2010; NCM, 2011
Body weight	BW	kg	60 65 70 80	WHO/IPCS, 1994; WHO/IPCS, 1999; NCM, 2011 EFSA, 2012; CEC, 2001; NCM, 2011. ECHA, 2008a; ECHA, 2008b; NCM, 2011 EFSA, 2012; NCM, 2011 U.S. EPA, 2009; NCM, 2011

<sup>a</sup> Air quality data (air particulate matter,  $\text{PM}_{10}$ ) measured on the collection dates (12 June 2010) in Edinburgh (<http://uk-air.defra.gov.uk/data>). © Crown 2016 copyright Defra via [uk-air.defra.gov.uk](http://uk-air.defra.gov.uk), licenced under the Open Government Licence (OGL).

<sup>b</sup> Air quality data (air particulate matter,  $\text{PM}_{10}$ ) measured on the collection dates (5 June 2010) in Liverpool (<http://uk-air.defra.gov.uk/data>). © Crown 2016 copyright Defra via [uk-air.defra.gov.uk](http://uk-air.defra.gov.uk), licenced under the Open Government Licence (OGL).

<sup>c</sup> Air quality data (air particulate matter,  $\text{PM}_{10}$ ) measured on the collection dates (27 and 28 May 2010) in Newcastle upon Tyne (<http://uk-air.defra.gov.uk/data>). © Crown 2016 copyright Defra via [uk-air.defra.gov.uk](http://uk-air.defra.gov.uk), licenced under the Open Government Licence (OGL).

which it was based could no longer be considered suitably protective of human health (WHO/JECFA, 2010). In the same year, EFSA published a review of the key toxicology data and set out a context to establish a Pb PTWI based on modelling data for neurobehavioural effects in children and renal and cardiovascular effects in adults (EFSA, 2010). Subsequently, the US Centres for Disease Control and Prevention (CDC) set a blood lead action level of  $5 \mu\text{g dL}^{-1}$  (CDC, 2012), whilst the UK moved to define a Low Level of Toxicological Concern (LLTC) for Pb and a blood Pb level of  $3.5 \mu\text{g dL}^{-1}$  was recommended (DEFRA, 2014a). DEFRA (2014b) have converted this LLTC to an estimate of intake dose giving a Pb LLTC for a child of  $1.4 \mu\text{g kg}^{-1}\text{BW day}^{-1}$  (using the Integrated Exposure Uptake Biokinetic (IEUBK) model; U.S. EPA, 2007) and an adult of  $1.3 \mu\text{g kg}^{-1}\text{BW day}^{-1}$  (based on the Carlisle and Wade model; Carlisle and Wade, 1992), (DEFRA, 2014b). Based on our mean generic-site inhalation dose of  $3.2 \text{ ng kg}^{-1}\text{BW day}^{-1}$  these LLTC values are of the order of  $450\times$  larger. Of course, it is recognised that Pb intake via the inhalation pathway is only one exposure pathway, and for the general European population human exposure to Pb is mainly via the diet with an average adult Pb dietary exposure ranging from 0.36 to  $2.43 \mu\text{g kg}^{-1}\text{BW day}^{-1}$  in high consumers in Europe (EFSA, 2010). Indeed, a dietary intake of  $0.50 \mu\text{g kg}^{-1}\text{BW day}^{-1}$  has been linked to neurodevelopmental effects in children (EFSA, 2010). Clearly in many urban contexts, non-dietary Pb exposure from urban street dusts may add to the overall exposure burden. Although Pb exposure in urban environments has declined over the last two decades, concomitant with the phasing out of lead in fuel, particular activities, and living in the towns and cities in which these activities take place, Pb has been shown to pose a risk to human health. Dust generating activities, such as, bulk mineral transport (Taylor, 2015; Kristensen et al., 2015), lead smelting and mining activity (Taylor et al., 2013; Taylor et al., 2014) and Pb recycling plants (Uzu et al., 2011), have all been shown to create contemporary Pb exposure through airborne dust generation and subsequent deposition. Whilst the principal exposure pathway is typically oral ingestion, particularly in children because of the increased hand-to-mouth activity, in contexts where the concentration of Pb in the PM10 fraction of urban street dust/airborne dusts exceeds the adopted reference concentration, then further quantification of the additional metal burden from the inhalation exposure pathway is recommended. However, in this study, the exposure burden from the inhalation pathway is minimal; an approximate 1000-fold reduction in exposure compared to the ingestion rate (Elom et al., 2014). The maximum generic-site inhalable dose in this study was  $7 \text{ ng}\cdot\text{kg}^{-1}\text{bw}\cdot\text{day}^{-1}$  as compared to a maximum daily ingestion rate of  $12.0 \mu\text{g}\cdot\text{kg}^{-1}\text{bw}\cdot\text{day}^{-1}$  (Elom et al., 2014). It is therefore evident that the inhalation dose would therefore not significantly contribute to the overall risk assessment in our sampled locations. However, given the exposure to PHEs such as Pb is just one component of the overall health burden associated with PM10's in urban environments then we still recommend suitable pollution controls are in place and in some urban contexts specific management strategies, such as regular washing down of playgrounds, in addition to regular street sweeping, may also be desirable.

#### 4. Conclusions

Despite the high pseudo-total Pb concentration in the PM10 fraction observed for many of the urban street dusts analysed as part of this study (ranging from 452 to 2435 mg/kg), when compared to the current UK LLTC (DEFRA, 2014b) and considered within the context of an inherently low inhalation bioaccessibility (<10%), the human health risk posed by Pb in PM10s through the inhalation pathway is considered negligible in the urban cities studied. However, given the long-term persistence and toxicity of Pb in ionic and particulate form (Uzu et al., 2011) there is a need for further quantification of the PM10 fraction in urban street environments.

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