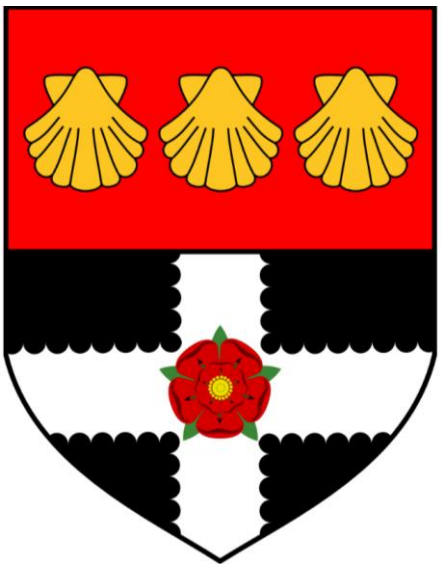

The Impact of Land Contamination on Human Health

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A thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Engineering (EngD)

UNIVERSITY OF READING

Technologies for Sustainable Built Environments Centre

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DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Eric Dede
June 2017

To God be the glory for great things He has done for me.

Abstract

Land contamination is an issue of concern in land regeneration and the built environment. To ensure the sustainability of the built environment, it is important that the risk to human health due to land contamination is addressed adequately. Current generic assessment criteria (GAC) values used in the assessment of contaminated land in the United Kingdom (UK) are very conservative. Although this is protective of human health, it may lead to un-necessary and costly remediation of land or result in land being left un-used. This highlights the need for improved understanding of human exposure to soil contaminants, which this work sought to promote.

This thesis presents findings from our assessment of human exposure to five toxic elements; arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and nickel (Ni), carried out using individuals who grow and consume their allotment produce. The primary exposure pathway investigated was oral ingestion through the consumption of produce. Concentrations of these elements were measured in samples of soil and produce. Site-specific risk assessment carried out using element concentrations and participants' produce consumption data indicated no significant health risk to the participants. During the risk assessment process, it is necessary that element bioaccessibility values are determined and considered in the assessment to ensure that the risk is not over-estimated.

To improve our understanding of actual human exposure to these elements though the oral ingestion pathway, we carried out biomonitoring and produced human physiologically-based kinetic models to assess internal exposure to these elements. Measured concentrations of blood Pb and urinary As, Cd, Cr and Ni were similar to the corresponding levels in the general (non-occupationally exposed) populations in the UK; indicating that the participants were not exposed to these elements at levels importantly higher than other adults in the UK. In addition, this indicates that participants' consumption of allotment produce did not result in them having significant additional exposure to the elements. The models, implemented in MATLAB, predicted the literature data and our biomonitoring data well. Because these models are capable of predicting internal exposure to these elements, they improve our understanding of exposure to the elements, which is important in the sustainable management of land contamination. To our knowledge, it is the first time combined biomonitoring and physiologically-based models for the five toxic elements have been used to assess exposure among allotment users.

Acknowledgments

First and foremost, I thank God for His grace that carried me throughout this project.

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I would like to appreciate a number of people who *made-the-difference* during this project. At the TSBE centre, I thank Jenny and Emma for their support during the course of my study. Many thanks to Mr. David Todd (IOM) for training me on sample preparation and analysis, and for his skilful technical assistance in the lab. Thumbs-up Dave! I thank Mr. Joseph Cueto for assisting in venous blood sample collection, Dr. Laura MacCalman, formerly of IOM, for her support with modelling and statistics. To the study participants, without whom the success of the research may not have been realised, a massive *thank you* to you all.

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To those who I have not mentioned, but supported me in one way or the other, or just made me smile during tough times, I appreciate you (you know yourselves!).

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Dissemination of Research Findings

Research findings were disseminated through academic papers and conference presentations. A summary of publications and key conference presentations (excluding posters) is given below.

Title of Publication	Conference / Journal	Relevant Chapter	Externally peer-reviewed (Y/N)
Developing a methodology for measuring selected metals in human blood using ICP-AES.	Proceedings of the 5th Annual TSBE EngD Conference 2014.	3	N
Preliminary bioaccessibility assessment of selected metals in urban allotment soils in the UK.	Proceedings of the 6th Annual TSBE EngD Conference 2015.	3	N
Investigating the human health impact of contaminated land – results from a pilot study.	Proceedings of the 7th International Conference on Sustainable Development in Building and Environment (SuDBE) 2015.	3	Y
Estimating human exposure to selected soil-bound metals using PBPK modelling and biomonitoring.	Proceedings of the 7th Annual TSBE EngD Conference 2016.	6	N
Quantifying human exposure to selected soil-bound metals using human biomonitoring and physiologically-based pharmacokinetic modelling.	Proceedings of the 3rd International Symposium on Environment and Health (ISEH) and 10th International Symposium on Environmental Geochemistry (ISEG) 2016.	6	Y
Quantifying human exposure to five toxic elements in selected allotments in Scotland through oral ingestion.	Proceedings of the 5th UK and Ireland Exposure Science meeting, University of Birmingham, 29 March 2017.	4/6	Y
Quantifying human exposure to lead (Pb) in selected allotments in Scotland through oral ingestion.	Proceedings of the 8th Annual TSBE EngD Conference 2017.	4/6	N
Physiologically-based pharmacokinetic and toxicokinetic models for estimating human exposure to five toxic elements through oral ingestion.	Paper has been submitted for journal publication.	2/6	Y
Assessing potential health risk from the consumption of food grown in soil contaminated with toxic elements.	Journal paper in preparation.	3/4/5/7	Y

Abbreviations

ADE	Average Daily Exposure
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centre for Disease Control and Prevention (USA)
CIEH	Chartered Institute of Environmental Health
C4SL	Category 4 Screening Level
CAS No.	Chemical Abstracts Service Registry Number
CL:AIRE	Contaminated Land: Applications in Real Environments
CLEA	Contaminated Land Exposure Assessment
CSM	Conceptual Site Model
DCLG	Department for Communities and Local Government
DEFRA	Department for Environment, Food & Rural Affairs
DMA	Dimethylarsinic Acid
DQRA	Detailed Quantitative Risk Assessment
EDTA	Ethylenediamine Tetraacetic Acid
EFSA	European Food Safety Authority
EngD	Engineering Doctorate
EPSRC	Engineering and Physical Sciences Research Council (UK)
GAC	Generic Assessment Criteria
HCV	Health Criteria Value
HSE	Health and Safety Executive (UK)
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IOM	Institute of Occupational Medicine
IPCS	The International Program of Chemical Safety
IR	Intake Rate (daily) of an element
LQM	Land Quality Management Ltd
MMA	Monomethylarsenic Acid
NIOSH	National Institute for Occupational Safety and Health (USA)
NHANES	The National Health and Nutritional Examination Survey (USA)
OSHA	Occupational Safety and Health Administration (USA)
PBPK/TK	Physiologically-Based Pharmacokinetic / Toxicokinetic (model)
QA	Quality Assurance
RBC	Red Blood Cells
SAGS	Scottish Allotments & Gardens Society
S4UL	Suitable 4 Use Level
SGV	Soil Guideline Value
SPOSH	Significant possibility of significant harm
SSAC	Site-Specific Assessment Criteria
TSBE	Technologies for Sustainable Built Environments
TSO	The Stationery Office
UK	United Kingdom
UREC	University Research Ethics Committee (University of Reading)
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Glossary

Terms that may not be familiar to the reader or that have specific connotations in this thesis are defined in this glossary.

Allotment	A householder's small horticultural plot of land.
Bioaccessibility	*Oral bioaccessibility*, which is the fraction of a substance that is soluble in the gastrointestinal environment and therefore is potentially available for absorption.
Bioavailability	*Oral bioavailability*, which refers to the fraction of the ingested contaminant that can enter the systematic circulation of the human body from the gastrointestinal tract.
Biomarker	A substance or its metabolite in a human biological specimen (e.g., urine, blood, body tissue), which reflect exposure to that substance.
Biomonitoring	The assessment of an individual's exposure to a substance through the measurement of a biomarker.
Brownfield	Previously developed land that may have been contaminated as a result of its past usage.
Creatinine	A chemical compound generated from muscle metabolism of creatine (an important energy store for muscles). The production of creatinine is relatively constant within an individual, thus creatinine is commonly used to adjustment element concentrations in urine samples to account for variability in urine volume.
Exposure frequency	The number of days per year in which a daily exposure event is considered to occur.
Hazard quotient	A ratio of the potential exposure to a chemical substance to the reference level at which no adverse effects are expected to occur in an individual exposed to that substance.

1. INTRODUCTION

1.1 Project background

Land contamination is a common problem associated with land regeneration and the built environment. Sources of land contamination include natural processes and anthropogenic activities such as past or present land uses. In the United Kingdom (UK), the re-use of so-called 'brownfield' sites is important to the nation's sustainable development goals (Dixon, Otsuka & Abe, 2011). Remarkably, brownfield sites are increasingly being regenerated with new development (Adams, Sousa & Tiesdell, 2010; Dixon, 2007). The increased re-use of brownfield sites could lead to human exposure to soil contaminants.

The assessment of risk to contaminated land involves identifying the link between a source of contamination, exposure pathway(s) and receptor(s) (Environment Agency, 2004). Therefore, a clear understanding of exposure is crucial in carrying out a comprehensive risk assessment. However, current models used in contaminated land exposure assessments, such as the '*Contaminated Land Exposure Assessment (CLEA)*' model used in the UK, are highly conservative because many of the model input parameters are based on conservative measurements (Environment Agency, 2009e). This could lead to over-estimation of human exposure to soil contaminants, which may subsequently result in negative financial implications through un-necessary costly remediation (Gbefa, Entwistle & Dean, 2011) or restrictions on land-use. This highlights the need for improved understanding of human exposure to soil contaminants; this research was conceived in response to this need.

1.1.1 Project partners and funding

This Engineering Doctorate (EngD) research project arose from the collaboration between the University of Reading and the Institute of Occupational Medicine (IOM). IOM is one of the leading providers of workplace health research and consultancy services, with expertise extending across a very wide range of scientific disciplines. The main focus of IOM's work is associated with understanding and minimising the risks to human health from hazards in the workplace and in the wider environment. As part of their work, IOM advises their clients on human health risks associated with contaminated land and brownfield redevelopment according to *Part IIA of the Environmental Protection Act (1990)*.

This research was funded by the UK Engineering and Physical Sciences Research Council (EPSRC), through the Technologies for Sustainable Built Environments (TSBE) Centre at the University of Reading. Additional financial support was provided by the IOM.

1.1.2 Project setting

The occurrence of toxic elements is common in the urban environment, including urban allotments and gardens (Bechet et al., 2016; Alloway, 2004). In the UK, there is an increasing demand for allotments to meet the needs of urban dwellers wanting to grow their own food. Fruits and vegetables grown in contaminated soil may result in human exposure to toxic elements through the consumption of home-grown produce. Allotment land-use was selected as a case study for this project. The study was limited to sites located in Scotland, UK.

Although a wide range of toxic elements can be present in soil, this project focused on arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni). These elements were selected because they are common in soil, and due to IOM's interest in studying human exposure to these elements. Exposure to these elements can cause various adverse impacts on human health, including cancer, damage to liver, kidney and other organs (Kumar et al., 2016; Zheng et al., 2007; Jarup, 2003). The International Agency for Research on Cancer (IARC) classifies As, Cd, Cr and Ni as *Group 1* human carcinogens (IARC, 2012).

1.2 Aim and objectives

Using biomonitoring and physiologically-based pharmacokinetic (PBPK) models, the aim of this research was *"to investigate the level of human exposure to the selected elements in soil, with a view of improving our understanding of actual exposure to these elements"*. This is linked to IOM's need to build their capacity in biomonitoring of these elements to support their exposure assessment work.

The objectives were:

1. To carry out a literature review to establish current knowledge relating to human exposure to As, Cd, Cr, Pb and Ni in soil (focusing on allotment land-use), bioaccessibility of these elements in humans, application of biomonitoring and PBPK models in exposure studies, and identify knowledge gap to be addressed.
2. To identify allotment sites for the study and recruit site users to participate in the study.
3. To determine the elements' concentrations at the study sites, through allotment sampling and subsequent samples analysis.
4. To carry out biomonitoring (collection of biological samples and determination of element biomarker concentrations).
5. To produce human physiologically-based kinetic models for the elements using existing models published in the literature, evaluate the predictive performance of these models, and subsequently apply the models in estimating exposure to the elements through the oral ingestion pathway.

These objectives are mapped against the thesis chapter structure in section 1.5.

1.3 Hypotheses

The following hypotheses were tested in various chapters of this thesis.

- *(h1)* There is a good correlation between element concentrations in allotment soil and the corresponding element concentrations in produce (chapter 4).
- *(h2)* The levels of the elements in blood and urine samples obtained from the participants are similar to the local background levels (chapter 5).
- *(h3)* PBPK model predicted element concentrations in blood and urine are good indicators of the corresponding measured biomarker concentrations (chapters 6 and 7).

1.4 Ethical approval

The University of Reading '*Code of Good Practice in Research'* requires every research involving human participants, human material and personal data to be subject to ethical approval before the research can proceed. Therefore, in compliance with the university requirements and the *'Human Tissue (Scotland) Act 2006'*, a comprehensive study protocol was prepared and submitted to the University Research Ethics Committee (UREC) as part of the ethical approval process. UREC granted ethical approval for both the pilot study phase (Reference: UREC 14/24) and the main study phase (Reference: UREC 15/21). Copies of these approval letters are given in Appendix A.

1.5 Thesis structure

This thesis consists of 8 chapters. This section provides information on the organisation and structure of the chapters, and shows how the chapters relate to the research objectives and hypotheses, as presented in Table 1.1.

Table 1.1: Thesis structure

Thesis Chapter	Purpose of the Chapter
Chapter 1 Introduction	To provide the project background, aim and objectives, hypotheses, and introduce the structure of the thesis. Ethical requirements and approval obtained for the research are highlighted in this chapter.
Chapter 2 Literature Review	This chapter establishes current knowledge and identify knowledge gap relevant to the project. Information reviewed in this chapter include the current UK contaminated land policy, occurrence of the selected elements in the soil environment and their toxicity, conceptual exposure model for allotment land-use, bioaccessibility of the elements in humans, biomonitoring and PBPK models of the elements. This chapter addresses objective 1.
Chapter 3 Materials and Methods	This chapter provides information on the materials and methods used in the research. It includes sampling, elemental analyses, urinary creatinine determination, computer software and statistics, estimation of sample size and identification of study sites. A summary of the pilot study conducted to trial methods and procedures is also presented in this chapter. This chapter addresses objectives 2, 3 and 4.
Chapter 4 Results and Discussion: Soil and Produce	In this chapter, analytical test results for soil and produce samples are presented and discussed (including statistical evaluation of the data). An evaluation of the potential risks to health of site users the soil and produce element concentrations is presented. This chapter addresses objective 3 and hypothesis '*h1*'.
Chapter 5 Results and Discussion: Blood and Urine	Analytical test results for blood and urine samples are presented and discussed (including statistical evaluation of the data) in this chapter. Comparisons of biomarker concentrations with the corresponding health guideline values and UK background levels are included. This chapter addresses objective 4 and hypothesis '*h2*'.
Chapter 6 Physiologically-based Kinetic Models	This chapter provides details of the modified models, evaluation of the models using literature data, parameter sensitivity analysis carried out, and the application of the models in the planning of biomonitoring. Objective 5 and hypothesis '*h3*'are addressed in this chapter.
Chapter 7 Application of Modified Models in Exposure Estimation	This chapter provides information on the evaluation of the models using biomonitoring data. The potential for application of these models in contaminated land exposure assessment is discussed. This chapter addresses objective 5 and hypothesis '*h3*'.
Chapter 8 Further Discussion, Conclusions and Recommendations	Finally, chapter 8 draws together the research findings, highlights the contribution to knowledge arising from this research and the importance of the findings to the industry. It presents the conclusions, and makes suggestions for further research.

2. LITERATURE REVIEW

2.1 Introduction

The purpose of this chapter is to review literature pertaining to human exposure to contaminated soil, with emphasis on the five selected toxic elements (As, Cd, Cr, Pb, Ni) and allotment land use. It establishes the relevant background information useful in achieving the aim and objectives of the research (chapter 1). Firstly, the chapter looks at the contaminated land management framework in the UK, followed by an overview of the occurrence of these elements in soil and their toxicity. The chapter further reviews the soil-to-plant uptake of these elements and conceptual exposure model for allotment land use. A review of oral bioaccessibility test methods relevant to these elements is then presented, along with justification for selecting the bioaccessibility test method used in this research. Towards the end of the chapter, biomonitoring is defined, and a review of biomarkers of exposure and PBPK models for these elements is presented. From this chapter, the knowledge gap addressed by this thesis is identified, leading to the methods and contents presented in subsequent chapters.

2.2 Contaminated land management framework in the UK

Contaminated land is often a legacy of bad industrial and waste management practices (Gay & Korre, 2006). The UK's rich industrial heritage has resulted in a legacy of land contamination. Consequently, the UK has established a framework for assessing and managing the risk posed by contaminated land, through regulatory bodies such as the Environment Agency and the Department for Environment, Food & Rural Affairs (DEFRA).

In the UK, the contaminated land regime is regulated mainly by *Part IIA of the Environmental Protection Act (1990)*, which provides a means of dealing with unacceptable risks posed by land contamination to human health and the environment. This Act operates alongside other legislative instruments, supported by the *National Planning Policy Framework* (for England) produced by the Department for Communities and Local Government (DCLG). Within the framework, land contamination is a material consideration in the planning process (DCLG, 2012). A similar framework exists for Scotland (The Scottish Government, 2014).

The Act defines contaminated land as *"any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that – significant harm is being caused or there is a significant possibility of such harm being caused; or pollution of controlled waters is being, or is likely to be, caused"* (DEFRA, 2012; TSO, 1990). The Act (which came into force in Scotland in 2000) places a duty on local authorities to identify and secure the remediation of contaminated land in their respective areas (Scottish Executive, 2006). In

order for land to be declared 'contaminated', a valid contaminant *source – pathway – receptor* linkage must be present (Environment Agency, 2004).

Although land affected by contamination may pose a risk to several receptors including humans, water resources and ecological receptors (O'Halloran, 2006; Bone et al., 2010), this research focuses on risk to humans only, since other receptors were outside the scope of the research. Therefore, the remainder of this section focuses on human exposure.

A tiered approach to assessing risk to human health is used in the UK to manage land contamination. It involves the development of a Conceptual Site Model (CSM) using the *source – pathway – receptor* contaminant linkage, as illustrated in Fig. 2.1.

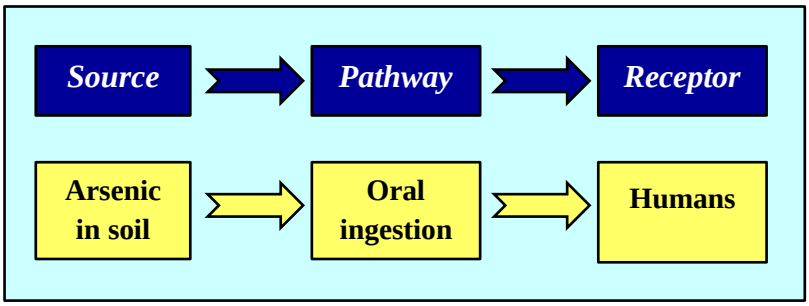

Fig. 2.1: An illustration of the source-pathway-receptor contaminant linkage

A contaminant linkage exists if all three (source, receptor and pathway) are present. One thing to note is that, the contaminant level must be sufficient to pose harm. As shown in Fig. 2.1, arsenic in soil (contaminant source) can reach humans (receptor) through oral ingestion (pathway) of arsenic in food grown on the soil. There may be more than one pathway of exposure to a contaminant, and therefore a CSM should consider all relevant exposure pathways.

Having established the presence (or the likely presence) of a contaminant linkage, risk assessment is then required. This may be either qualitative (based on the CSM), or quantitative using generic (and site-specific, if required) assessment criteria values. Further information on the assessment criteria values is presented in the following section.

2.2.1 The CLEA model

DEFRA and the Environment Agency have produced a human toxicological risk assessment method to guide the human health risk assessment for contaminated land, which is built into the CLEA model (Environment Agency, 2009a; Environment Agency, 2009b). The CLEA model is used to derive generic assessment criteria (GAC) values, such as soil guideline values (SGVs) (Environment Agency, 2009f), and site-specific assessment criteria (SSAC) values. SGVs are derived using the same procedures and algorithms used to derive SSAC, but applied to 'standard' land use classes characterised by generic exposure assumptions. The standard land use classes are

'residential', *'allotment'* and *'commercial'* (Environment Agency, 2009e). SGVs are contaminant-specific 'trigger values' used in assessing the risk to human health from chronic exposure (excluding waterborne pathways) to soil contamination. They indicate that soil concentrations above a given SGV could pose a risk to human health, and that further investigation or remediation should be considered. Similar risk assessment approaches have been adopted by other European countries (Carlon, 2007).

In addition to the SGVs, DEFRA's research project 'SP1010' led to the development of provisional *'Category 4 Screening Levels (C4SLs)'* for a number of soil contaminants, including As, Cd, Cr and Pb (CL:AIRE, 2014). The C4SLs were produced using the CLEA model following the introduction of a new four-category system for identifying and managing contaminated land, ranging from Category 4 to Category 1 (DEFRA, 2012). Category 4 refers to where there is no risk that land poses a significant possibility of significant harm (SPOSH) or where the level of risk is low, while Category 1 refers to where the risk that land poses a SPOSH is unacceptably high. The C4SLs are used to determine if a site falls within Category 4, and hence no need for further risk assessment of the site. They are used in a similar manner to SGVs. However, C4SLs are less stringent than the SGVs (CL:AIRE, 2014).

In the absence of SGVs and C4SLs from the regulators, other GACs have been developed by other authoritative bodies such as the Land Quality Management (LQM) Ltd. and the Chartered Institute of Environmental Health (CIEH) (Nathanail et al., 2015).

2.2.2 Differences between Part IIA in Scotland and England and Wales

Although the regulations and statutory guidance documents in Scotland and England and Wales are derived from the same primary legislation (Part IIA of the Environmental Protection Act 1990), there are some differences in the implementation of Part IIA between Scotland and England and Wales. The key differences[1] are:

- The provisions came into force in England in April 2000 and in July 2000 in Scotland.
- In Scotland, the Scottish Environment Protection Agency (SEPA) provides advice to local authorities in relation to potential special sites, whereas in England, the Environment Agency (EA) undertakes the inspection on behalf of local authorities.
- Notifications of identification of contaminated land appear in the public registers in Scotland, whereas in England they do not.
- The 2000 Scottish regulations specify the form of the remediation notice, while the English regulations just specify the content.
- In 2012, the statutory guidance in England was revised, and now provides a four-category test (i.e., the C4SLs discussed in the preceding section) to assist local authority risk-based

[1] Available at: https://www.sepa.org.uk/regulations/land/contaminated-land/faqs/

decisions on whether land is to be identified as contaminated land; the approach in Scotland is also risk-based, but does not provide this categorisation process for the local authority decisions.

2.2.3 Limitation of GACs and similar assessment criteria values

Many of the model input parameters used in the CLEA model are based on conservative measurements (Environment Agency, 2009e). Consequently, the SGVs, C4SLs and similar GACs derived using CLEA model are prone to over-estimate the actual human exposure to soil contamination. In addition, the SGVs are derived assuming that ingested contaminants are 100% bioaccessible in the body (Gbefa, Entwistle & Dean, 2011). However, this is not necessarily correct because where the contaminant exists in an insoluble form or strongly sequestered in soil, then its bioaccessibility is less than 100% (Hough et al., 2004; Ruby et al., 1999; Davis et al., 1996). It has been suggested that, in many cases, bioaccessibility of these elements is less than 50% (Oomen et al., 2002). This indicates that if any of these elements in soil were ingested, more than half of the element would not be soluble in the gastrointestinal (GI) tract and thus not potentially available for absorption in the body. However, one thing to note is that the bioaccessibility of these elements in soil is site-specific, given that soil properties (such as mineralogy, pH, organic matter and clay content) could influence element bioaccessibility (Pelfrêne et al., 2011; Roussel et al., 2010; Fairbrother et al., 2007). Therefore bioaccessibility of these elements could vary considerably from site to site.

The assumption of 100% bioaccessibility when deriving GACs could result in over-estimation of exposure. Based on bioaccessibility alone, the extent of over-estimation of exposure would be the reciprocal of the site-specific bioaccessibility values. From the perspective of a regulator, there is an advantage in a conservative approach because it is protective of public health. However, over-estimation of human exposure to soil contaminants may result in unnecessary and costly remediation being carried out (i.e., negative financial implications) (Gbefa, Entwistle & Dean, 2011; Nathanail & McCaffrey, 2003). It may also lead to restrictions on land-use, and cause brownfield sites to remain derelict where developers are unable to fund the required level of remediation. This emphasises the need for a more robust risk assessment approach to facilitate the sustainable management of contaminated land, which this research sought to promote.

In addition, the bioaccessible fraction of an element in the GI tract needs to be absorbed in the body in order to cause toxicity in organs and tissues (i.e., the element needs to be bioavailable) (Oomen et al., 2003; Environment Agency, 2009). It is the bioavailable proportion that determines actual exposure. The difference between bioaccessibility and bioavailability is explained further in section 2.6.

2.3 Elements of interest, their occurrence in soil and human toxicity

2.3.1 Arsenic

Arsenic (As) (CAS No. 7440-38-2) is frequently referred to as a metal, but it is chemically classified as a metalloid, having both properties of a metal and a non-metal (ATSDR, 2007a). Arsenic occurs in both organic and inorganic forms (Jara & Winter, 2014). Organic arsenic results from the combination of arsenic with carbon and hydrogen, and are found mainly in seafood (Hughes, 2006; ATSDR, 2007a). Meanwhile, inorganic arsenic is formed when arsenic combines with other elements such as oxygen, chlorine and sulphur (ATSDR, 2007a). Inorganic arsenic in the environment comes from both natural and anthropogenic sources. It occurs naturally in soils due to weathering of the parent rock, especially argillaceous sedimentary rocks (e.g., shales and mudstones) where the greatest concentrations of arsenic tend to be found, and in heavily sulphidic mineralised areas (Environment Agency, 2009d). Apart from weathering reactions, arsenic can also be mobilised naturally through biological activity and volcanic emissions (Smedley & Kinniburgh, 2002). Major anthropogenic sources of inorganic arsenic include non-ferrous metal smelters and coal combustion plants (Hughes, 2006). In addition, agricultural practices such as historical use of arsenic-based pesticides, application of fertilisers, sludge and manure containing arsenic are potential anthropogenic sources of inorganic arsenic (Environment Agency, 2009d; ATSDR, 2007a). Arsenite (As(III)) and arsenate (As(V)) are the two biologically important inorganic arsenic valence states (Stamatelos et al., 2011). As(V) predominates in aerobic soils while As(III) predominates in slightly reduced soils (e.g., temporarily flooded or sediment soil (ATSDR, 2007a). This indicates that As(V) would be the dominant arsenic species in shallow allotment soils.

Arsenic is highly toxic in its inorganic form, while organic arsenic is less relevant in toxicological studies (Keil et al., 2011; Bornhorst & McMillin, 2006). Inorganic arsenic is a Group 1 human carcinogen (IARC, 2012). In human populations orally exposed to As, it could cause cancers of the bladder, skin (ATSDR, 2007a; Marshall et al., 2007; Haque et al., 2003), and potentially linked to liver, prostrate and kidney cancer (Tokar et al., 2011). The toxicity of arsenic depends on the chemical form in which it is present, its solubility and rate of absorption and elimination (Keil et al., 2011).

2.3.2 Cadmium

Cadmium (Cd) (CAS No. 7440-43-9) is a metal that exists in the environment in one oxidation state (+2) and does not undergo oxidation-reduction reactions (ATSDR, 2012a). Cadmium can be emitted to the environment from natural phenomena such as volcanic eruptions, forest fires, and generation of sea salt aerosols (ATSDR, 2012a). Anthropogenic sources of Cd into the environment include metal mining and refining, waste incineration and disposal, batteries, smelting and electro-plating industries, use of phosphate fertilisers, tobacco use, and ash from fossil fuel

combustion (Keil et al., 2011; Pan et al., 2010). Apart from inhalation of cigarette smoke, dietary ingestion of foods with high Cd content is a major pathway through which the general population is exposed to Cd (ATSDR, 2012a). The high content of Cd in edible crops is because Cd is readily absorbed by plants in contaminated soil (Swartjes, Versluijs & Otte, 2013). Cd is classified as a Group 1 carcinogen (IARC, 2012) and kidney is the primary target of organ of Cd toxicity following oral exposure (ATSDR, 2012a). Other toxicological effects of ingested Cd that have been reported in humans include liver damage, cardiovascular effects, prostrate and breast cancer (Waalkes, 2000; Joseph, 2009; Waalkes, 2003).

2.3.3 Chromium

Elemental chromium (Cr) (CAS No. 7440-47-3) does not occur naturally; instead chromium is present in nature primarily as chromite ore with chromium in the trivalent form (Cr(III)), which is the most stable oxidation state. Chromium also occurs in divalent (II) and hexavalent (VI) oxidation states, and the very unstable IV and V oxidation states (ATSDR, 2012b). Hexavalent chromium is the second most stable oxidation state, and therefore, Cr(III) and Cr(VI) are the species that are of interest in relation to human exposure (ATSDR, 2012b; Langard & Costa, 2007).

Cr(III) occurs naturally in environmental media such as rocks, soil, plants, animals, volcanic dust and gases. It is also found in a wide range of foods and is an essential element for human (Cefalu & Hu, 2004). However, Cr(VI) compounds primary arise from anthropogenic sources (Shanker et al., 2005). As a nutritional element, Cr(III) has very low toxicity, while Cr(VI) is highly toxic (Cefalu & Hu, 2004; Kerger et al., 1996). Therefore, the reduction of Cr(VI) to Cr(III) is significant in the toxicity of Cr(VI) compounds. For example, human consumption of plants and animals that have been exposed to Cr(VI) is considered safe because of the reduction of Cr(VI) to Cr(III) in these organisms (Langard & Costa, 2007). Cr(VI) is a Group 1 human carcinogen (IARC, 2012), which has been associated with several toxicological effects in humans following oral ingestion, including DNA damage (IARC, 2012). Inhaled Cr(VI) has been known to cause lung cancer (ATSDR, 2012b).

2.3.4 Lead

Lead (Pb) (CAS No. 7439-92-1) is a heavy metal that occurs naturally in the earth's crust (ATSDR, 2007b). Lead has many different uses. For example, it is used in the production of batteries, ammunition, paints, dyes, cable sheeting, solders, alloys, gasoline and medical equipment (Skerfving & Bergdahl, 2007; Keil et al., 2011). The uses of lead can result in anthropogenic sources of lead. Human activities provide major sources of lead found in the environment compared to lead releases from natural events such as volcanoes, windblown dust and erosion (ATSDR, 2007b). Lead is known to be persistent in soils and sediments because it binds strongly to various soil minerals (Lark & Scheib, 2013; Intawongse, 2007). This indicates that historical land

use(s) may have a pronounced influence on the contemporary soil lead content. Lead exists in both organic and inorganic forms. Organic lead compounds are dominated by tetramethyl and tetraethyl lead, which have been used mainly as additives to gasoline (ATSDR, 2007b). Inorganic lead is the most common form of lead in the environment, and includes the lead found in soil, paint and various other products.

In the general un-occupationally exposed population, exposed to lead may occur through ingestion of contaminated food and water, and inhalation of cigarette smoke (ATSDR, 2007b). Oral exposure to lead can cause toxic effects on various body organs and tissues. These include kidney dysfunction, cardiovascular effects (e.g., hypertension), gastrointestinal effects, haematological effects, musculoskeletal effects, respiratory effects and brain damage (Skerfving & Bergdahl, 2007; Hu et al., 2006).

Lead has four stable isotopes (^{204}Pb at 1.4%, ^{206}Pb at 24.1%, ^{207}Pb at 22.1% and ^{208}Pb at 52.3%), with their ratios being variable in environmental medium, such that Pb of different sources generally has its own specific isotopic compositions (Li et al., 2012). Therefore, Pb isotope ratios can be used for tracing source of Pb, especially, for distinguishing between anthropogenic and natural sources of Pb (Saint-Laurent et al., 2010). In this research, we used Pb isotope ratios of ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb that are commonly used as tracers (Farmer et al., 2011; MacKinnon et al., 2011).

2.3.5 Nickel

Nickel (Ni) (CAS Number 7440-02-0) is found naturally in soil, and is also emitted from volcanoes. In the environment, nickel is mainly found combined with oxygen or sulphur as oxides or sulphides. Nickel can exist in various oxidation states, but Ni(II) is the prevalent oxidation state under normal environmental conditions (ATSDR, 2005). Nickel releases to the atmosphere occur from natural discharges (e.g., windblown dust, volcanic emissions, forest fires and vegetation) and anthropogenic activities (e.g., nickel mining, industrial use of nickel compounds, power plants burning oil and coal, and waste incinerators) (Cempel & Nikel, 2006; ATSDR, 2005).

According to IARC (2012), oral ingestion of nickel in food, and to a lesser extent water, is the primary route of exposure to the non-smoking general population. Nickel is a Group 1 carcinogen. Inhaled nickel is known to cause lung cancer (IARC, 2012). It has been reported that the carcinogenic risk is limited to conditions of occupational exposure (Cempel & Nikel, 2006). Oral exposure to Ni may cause cardiovascular and kidney diseases, skin allergies, and genotoxicity (Cempel & Nikel, 2006; Klein & Costa, 2007).

2.4 Soil-to-plant uptake of the elements

Plants can take-up metal contaminants from soil through the roots, and also through the shoots (via stomata) following atmospheric deposition of pollutants (Bermudez et al., 2012; Environment Agency, 2006). However, the most important uptake process is via the roots, and therefore this section presents a review of the uptake of the metals of interest from soil via the plant roots, and indicates the forms in which these metals are found in plants. A summary of soil-plant-transfer factors for the five elements obtained from the literature are presented at the end of this section.

Plants absorb essential and non-essential elements from soil in response to concentration gradients induced by selective uptake of ions by plant roots, or by diffusion of the elements in the soil (Peralta-Videa et al., 2009). For example, nickel is known to be a micronutrient because it is required by plants in a minute quantity, whereas arsenic, cadmium, chromium and lead are not considered essential elements for plant growth (Peralta-Videa et al., 2009).

Growing edible plants in contaminated soils is known to contribute to food contamination (through plant uptake of the soil contaminants). For example, approximately two-thirds of cadmium dietary intake is known to be attributed to plant products (Nasreddine & Parent-Massin, 2002). In addition, it has been estimated that approximately half of human lead intake is through food, with around half originating from plants (Nasreddine & Parent-Massin, 2002). Both cadmium and lead are known to have the potential to accumulate in plants (Wolnik et al., 1983). This indicates that vegetables and fruits grown in contaminated soils have the potential to provide a source of human exposure to toxic elements in soil.

2.4.1 Arsenic

The most common and stable form of arsenic found in aerobic soils is the As(V), and is therefore more available for plant uptake (Meharg & Hartley-Whitaker, 2002; FAO, 2006[2]). The soil pH controls the transport and availability of arsenic in soil, such that at low pH values (pH 4), arsenic is found complexed with iron, whereas at high pH values (pH 6 – 8) arsenic is mostly bound to calcium (Fayiga, Ma & Zhou, 2007). It has been reported that the presence of iron and manganese oxides in soil increase arsenic mobility and availability (Zavala & Duxbury, 2008).

Plants generally take up and mobilise arsenate (As(V)) through the phosphate transport channels, thus causing competition between As(V) and phosphate for root uptake (Tripathi et al., 2007). Once inorganic arsenate (As(V)) has been absorbed into the plant tissues, the As(V) is reduced to arsenite (As(III)) and/or bio-transformed to organic compounds such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) or as inorganic As(III) complexed with thiol groups, all of which are commonly found in plants (Peralta-Videa et al., 2009; Meharg & Hartley-Whitaker,

[2] Available at: ftp://ftp.fao.org/docrep/fao/009/ag105e/ag105e00.pdf

2002; Ruiz-Chancho et al., 2008). It has also been reported that the majority of plants are able to synthesise arsenate reductase, which reduces most of the As(V) to As(III) (Dhankher et al., 2006). Arsenic accumulation and resistance in plants varies between plant species due to genetic differences and diversity in detoxification processes (Meharg & Hartley-Whitaker, 2002).

2.4.2 Cadmium

Soil-to-plant transfer of cadmium is the major pathway though which humans are exposed to cadmium in contaminated soil (Liang et al., 2013), since cadmium is easily taken up by vegetables due to its high mobility in soil and plant system (Sarwar et al., 2010; Swartjes et al., 2013). Consumption of vegetable foods can contribute approximately 70% (Sarwar et al., 2010) to 80% (Olsson et al., 2002) of total cadmium intake by humans.

Three processes (speciation in soil, plant root uptake from the pore water, and transport within the plant) control the cadmium concentration in different parts of plants due to cadmium uptake through the plant roots (Swartjes et al., 2013). It has been reported that the electrochemical potential gradient of the plasma membrane in the plant root cells drives cadmium and other cations into the root cells (Peralta-Videa et al., 2009). However, increased concentration of iron in soil has been linked to the reduction of the uptake of cadmium (Sharma et al., 2004). It has also been identified that cadmium uptake by plants increases with decreasing soil pH, whereas it decreases with increasing soil organic carbon content (Liang et al., 2013). In addition, root and leafy vegetables are reported to have a greater soil cadmium uptake efficacy than fruit and seed vegetables (Alexander, Alloway & Dourado, 2006).

2.4.3 Chromium

In plants, chromium at low concentrations promotes growth and increases yield, but it is not considered essential to plants (Peralta-Videa et al., 2009). The entry of chromium into plants is controlled by chromium reduction and/or complexation with root exudates (e.g., organic acids), which increase chromium solubility and mobility through the root xylem (Peralta-Videa et al., 2009). It has been reported that both Cr(VI) and Cr(III) cross the endodermis (via symplast pathway) where Cr(VI) in cells is reduced to Cr(III) and accumulated in the root cortex cells (Shanker et al., 2005).

A study by Mandiwana et al. (2007) on the solubility of Cr(III) and Cr(VI) compounds is soil and their availability to plants found out that the concentrations of Cr(VI) in plants correlated with the soluble fraction of Cr(VI) in soil, while Cr(III) concentration in plants was limited by the concentration of low molecular weight organic acids in soil. This indicates that soils rich in organic acids facilitate higher plant absorption of Cr(III). According to Shanker et al. (2005), chromium is largely retained in the roots, with Cr(III) being the predominant species in roots.

2.4.4 Lead

Lead is strongly adsorbed onto soil particles reducing their availability to plants (Intawongse, 2007). The uptake of lead is regulated by pH, soil particle size, soil cation exchange capacity, root surface area, root exudation and other physico-chemical parameters (Sharma & Dubey, 2005). At the root surface, lead is bound to carboxylic groups of mucilage uronic acids, and some of the bound lead is released when mucilage is biodegraded (Sharma & Dubey, 2005). Lead transported from the soil to the root cells crosses the root-cell plasma membrane. The transport pathway for lead across the plasma membrane occurs through plasma membrane cation channels (e.g., calcium channels) (Peralta-Videa et al., 2009; Sharma & Dubey, 2005). Once lead has been taken up by the roots, most lead is bound to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of lead carbonate deposited (Peralta-Videa et al., 2009; Sharma & Dubey, 2005). Various plant species have the ability to absorb lead by roots and translocate it from the roots to the shoots (Huang & Cunningham, 1996). However, it has been reported that most of the absorbed lead remains in the roots, since the endodermis acts as a partial barrier to the movement of lead between the root and shoot (Sharma & Dubey, 2005).

2.4.5 Nickel

Nickel is an essential nutrient for plants, but the amount of nickel required for normal plant growth is very low (Chen et al., 2009). The uptake of nickel in plants occurs mainly through the root system by means of passive diffusion and active transport (Seregin & Kozhevnikova, 2006). The overall uptake of nickel by plants depends on the concentration of Ni(II) ions, plant metabolism, soil acidity, the presence of other metals and soil organic matter composition (Chen et al., 2009). However, nickel accessibility is reduced at higher pH values of the soil solution due to the formation of less soluble complexes (Yusuf et al., 2011). It has been estimated that over 50% of the nickel absorbed by plants is retained in the roots (Chen et al., 2009).

The transport pathway for nickel is from the roots to shoots and leaves through the transpiration stream via the xylem (Chen et al., 2009). According to Yusuf et al. (2011), nickel is freely translocated in the stellar tissues and can easily reach the upper (above ground) parts of the accumulator plants. It is supplied to meristematic parts of the plants by re-translocation from old to young leaves, and to buds, fruits and seeds through the phloem (Chen et al., 2009; Yusuf et al., 2011).

2.4.6 Soil-to-plant transfer factors

Soil-to-plant transfer can occur though plant uptake mechanisms, by soil or dust deposition on the plant, and by soil particles adhering to plant parts (Environment Agency, 2006). Soil-to-plant factors quantity the potential plant uptake of elements present in the soil, and they are not constant for either a specific element or a specific vegetable (Swartjes, Versluijs & Otte, 2013). For

example, higher soil-to-plant factors for arsenic have been reported in tree fruit compared to green and root vegetables (Environment Agency, 2009d). However for cadmium, higher factors have been reported in green and root vegetables than in tree fruit (Environment Agency, 2009c). Table 2.1 presents soil-plant-transfer factors for the five elements obtained from selected literature.

Table 2.1: Summary of soil-to-plant transfer factors from selected literature

Element	Soil-to-plant transfer factors		
	Min	Max	Reference
As	0.00043	0.0011	*a*
	0.001		*b*
Cd	0.0014	0.052	*c*
	0.192	0.778	*b*
Cr	0.008	0.029	*b*
	0.003	0.22	*d*
Ni	0.037	0.039	*b*
	0.03	0.89	*d*
Pb	0.008	0.065	*b*
	0.001	0.432	*d*

[a]Environment Agency (2009d)
[b]Jolly et al. (2013)
[c]Environment Agency (2009c)
[d]Intawongse (2007)

2.5 Conceptual exposure model for allotment land use

A conceptual site model (CSM) is a depiction of a site's exposure conditions that has the potential to connect contamination source(s) to possible receptor(s) (i.e., *source – pathway – receptor* linkage). The CSM discussed in this section defines potential exposure pathways relevant to this study, which subsequently informed the methods, materials and sample requirements for the study.

2.5.1 Source of potential contamination

For allotments land use, the source of contamination refers to the occurrence of the five toxic elements in allotment soil and in allotment produce samples.

2.5.2 Pathways

In consideration of the allotment land use, the elements of interest and the receptors, potential exposure pathways investigated include consumption of allotment produce (and the adhering soil), inadvertent soil ingestion, and inhalation of outdoor allotment dust. However, exposure through dermal uptake was considered insignificant. According to the Environment Agency (2009e), the estimated dermal absorption fraction for arsenic is 0.03, while that for cadmium is 0.001. Based on these low values and the general lack of literature data, a default value of zero is often adopted for

inorganic chemicals (Environment Agency, 2009e). In addition, vapour inhalation pathway was not investigated because the elements of interest are not particularly volatile. Another potential pathway that was not considered relevant is the 'tracking back' from site to indoor environment. This is because tracking back predominantly occurs over a short distance (7 to 8m of building entrance) (Hunt, Johnson & Griffith, 2006) but allotments were located away from participants' dwellings. This is consistent with the approach adopted in the CLEA model, where tracking back into a building is only considered where a building is located on a contaminated site (Environment Agency, 2009e).

2.5.2.1 Consumption of allotment produce

Oral ingestion pathway (through the consumption of allotment produce and adhering soil particles) is regarded as the dominant route through which human get exposed to contaminants present in allotments (Environment Agency, 2009e), which is why oral bioaccessibility testing was carried out as part of this study. For example, it has been estimated that vegetable consumption contributes a major proportion (up to 80%) of the total cadmium intake by humans (Nabulo, Young & Black, 2010; Liang et al., 2013). The calculation of daily exposure through vegetable consumption is as follows (Swartjes et al., 2013):

$$Exp_{veg} = \frac{\sum\left(Q_{veg\ i} \times C_{veg\ i} \times f_{hm-grown} \times F_{bioavailability}\right)}{BW} \quad (2.1)$$

Where:

Exp_{veg} is the exposure due to vegetable consumption (mg kg^{-1} $_{body\ weight}$ day^{-1}).
$Q_{veg\ i}$ is the consumption rate of vegetable *i* (kg $_{dry\ weight}$ day^{-1}).
$C_{veg\ i}$ is the contaminant concentration in vegetable *i* (mg kg^{-1} $_{dry\ weight}$).
$f_{hm-grown}$ is the fraction of vegetables that is home-grown. In this study, this is equivalent to 1 because only allotment produce was considered.
$F_{bioavailability}$ is the correction for relative bioavailability in the human body.
BW is the body weight (kg).

Soil loading onto allotment produce samples can be reduced by washing edible plant parts, which mimics conventional food preparation in the kitchen (Prasad & Nazareth, 2000):

Oral ingestion through direct intake of soil may also occur. Daily exposure through soil ingestion is calculated as follows (Swartjes et al., 2013):

$$Exp_{soil} = \frac{Q_{soil} \times C_{soil} \times F_{bioavailability}}{BW} \quad (2.2)$$

Where:

Exp_{soil} is the exposure due to soil ingestion (mg kg^{-1} $_{body\ weight}$ day^{-1}).
Q_{soil} is the soil ingestion rate (kg $_{dry\ weight}$ day^{-1}).
C_{soil} is the contaminant concentration in soil (mg kg^{-1} $_{dry\ weight}$).
$F_{bioavailability}$ is the correction for relative bioavailability in the human body.
BW is the body weight (kg).

However, direct soil intake is more relevant to children than adults, due to children's 'mouthing' (hand-to-mouth exploration) behaviours (Abrahams, 2012; Ljung et al., 2006; Davis et al., 1996), which result in intentional soil ingestion. A study by Davis & Mirick (2006) identified that soil ingestion in children was associated with eating of dirt.

2.5.2.2 Inadvertent soil ingestion

Exposure by inadvertent ingestion arises from contact between the mouth or peri-oral region and contaminated hands or objects, which results in ingestion, of which the individual may be oblivious (Ng et al., 2012). This exposure pathway is known to occur among children, but adults may also be exposed (Irvine et al., 2014; Ng et al., 2012). The general form for equations used in estimating inadvertent ingestion is as follows (Ng et al., 2012).

$$E_{ii} = Ld \times SA \times TE \times N \tag{2.3}$$

Where:

E_{ii} is the exposure by inadvertent ingestion (mg).
Ld is the loading of substance on hand or object (mg cm^{-2}).
SA is the surface area of hand or object that comes into contact with the mouth (cm^2).
TE is the transfer efficiency of substance from hands or object to the mouth (proportion).
N is the number of hand or object-to-mouth contacts.

Although substance transfer is influenced by a number of factors including individual behavioural tendencies (such as nail biting, smoking, licking lips, finger sucking, personal hygiene, risk perception) and flow of sweat, hands play a central role in the exposure process (Cherrie et al., 2006; Ng et al., 2012). We collected a number of moist-wipe samples from participants' hands to estimate potential exposure through this pathway; even though this pathway was not considered significant for the adult participants.

2.5.2.3 Inhalation of allotment dust

Dust can be generated from soil by a variety of activities. The amount of soil dust inhaled by humans usually dependents on the grain size of soil particles, with finer particles being susceptible to inhalation into human lung (Bi, Liang & Li, 2013). In addition, finer soil particles are expected to contain higher metal concentrations than larger particles, due to the higher surface-to-mass ratio of finer particles (Duong & Lee, 2011). The average daily exposure through inhalation of allotment dust is calculated as follows (Gay & Korre, 2006).

$$ADI_{inh} = \frac{IR_{inh} \times EF_{inh} \times ED_{inh}}{BW \times AT} \quad (2.4)$$

Where:

ADI_{inh} is the average daily intake of chemical from soil through inhalation (mg kg^{-1} $_{body\ weight}$ day^{-1}).
IR_{inh} is the chemical intake/uptake rate (mg day^{-1}).
EF_{inh} is the exposure frequency (days $year^{-1}$).
ED_{inh} is the exposure duration (year).
BW is the body weight (kg $_{body\ weight}$).
AT is the averaging time (days).

Inhaled dust can also be ingested and swallowed as part of mucus clearance (Knowles & Boucher, 2002); in which case exposure can be calculated using (Eq. 2.2).

The contribution of outdoor allotment dust inhalation to total exposure from allotment soil is estimated to be 0.1% (for arsenic) and less than 0.1% (for cadmium) (Environment Agency, 2009d; Environment Agency, 2009c). These values indicate that exposure through inhalation of outdoor allotment dust is not particularly significant for the selected elements.

2.5.3 Receptor

The critical receptor for an allotment land use is a young female child (aged 0 to 6 years old) (Environment Agency, 2009e). However in this study, adult receptors (aged over 18 years) were targeted because of biomonitoring requirements. It was anticipated that using children in biomonitoring would be ethically complicated (i.e., obtaining ethical approval to use children in biomonitoring would be difficult), and the chances of obtaining parental consents would be minimal.

2.6 Oral bioaccessibility of the elements

This section provides an overview of procedures currently used for evaluating the bioaccessibility of selected metals, in order to determine the most appropriate bioaccessibility test procedure to adopt in this research. The procedures reviewed relate to human and animal oral bioaccessibility studies. Various definitions and interpretations of *bioaccessibility* and *bioavailability* concepts have been proposed (Ruby et al., 1996; Semple et al., 2004; Fernández-García et al., 2009). However, there is a general agreement that these concepts address the potential for a chemical substance to interact with an organism. For clarity, the difference between '*oral bioaccessibility'* and '*oral bioavailability'* is explained further in the following section.

2.6.1 Differentiating between bioaccessibility and bioavailability

Soil ingestion can be a major route of human exposure to immobile soil contaminants (Oomen et al., 2002; Oomen et al., 2003). Therefore, estimating the amount of metal that is available for absorption following soil ingestion is a key variable in estimating the potential human exposure to

soil contamination (Hamel, Ellickson & Lioy, 1999). *Oral bioaccessibility* is the fraction of the contaminant that is soluble in the gastrointestinal environment and is available for absorption (Oomen et al., 2002). However, *oral bioavailability* refers to the fraction of the ingested contaminant that can enter the systematic circulation of the human body (i.e., the fraction that reaches the blood compartment) from the GI tract (Ruby et al., 1999). It is the product of contaminant fraction that is bioaccessible, fraction transported across the intestinal epithelium and the fraction passing the liver without being metabolised (Oomen et al., 2003). Therefore, to become *bioavailable*, contaminants must first become *bioaccessible*. A schematic diagram showing the difference between oral *bioaccessibility* and *bioavailability* is given in Fig. 2.2. Following element uptake, bioaccumulation may occur in the target organs.

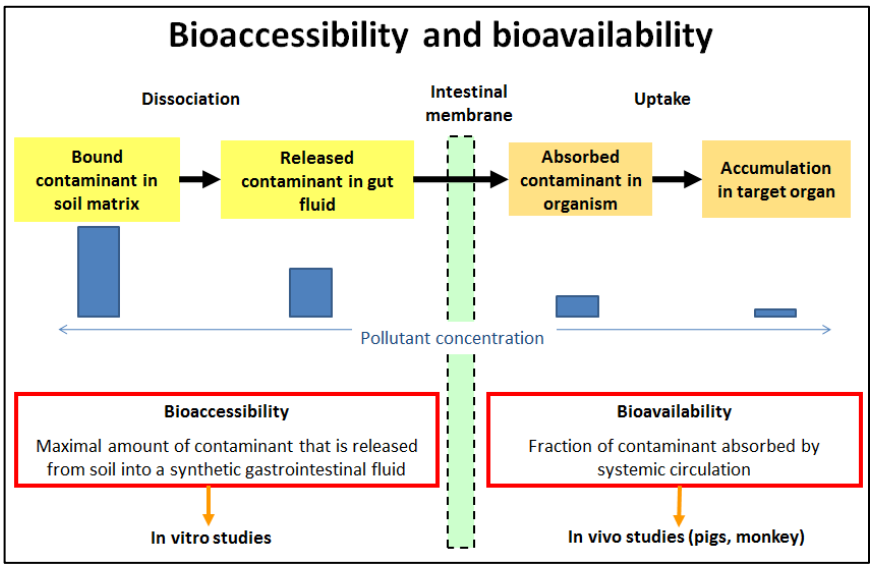

Fig. 2.2: A sketch diagram showing the difference between oral bioaccessibility and bioavailability

The CLEA model allows users to apply site-specific bioavailability values (expressed as '*relative bioavailability*') when deriving SSAC values. Relative bioavailability refers to the relationship between the bioavailability of a contaminant in soil and the bioavailability of the same contaminant measured in the media used for the toxicological study in deriving the health criteria of that contaminant (Environment Agency, 2009a).

Relative bioavailability can be derived using the following expression (Environment Agency, 2009a):

$$RBA_{soil,tox} = \frac{ABA_{soil}}{ABA_{tox}} = \frac{F_B \times F_A \times F_H}{ABA_{tox}} \quad (2.5)$$

Where:

$RBA_{soil,tox}$ is the relative bioavailability between soil sample and the media used in the toxicological studies.
ABA_{soil} is the absolute element bioavailability in soil (dimensionless).
ABA_{tox} is the absolute element bioavailability in the media used in the toxicological studies (dimensionless).
F_B is the oral bioaccessible fraction of an element in soil.
F_A is the fraction of a solubilised element transported across the gastrointestinal wall into systematic circulation.
F_H is the fraction absorbed that does not undergo first pass metabolism in the intestinal epithelium and/or the liver.

Absolute bioavailability values (ABA_{soil} and ABA_{tox}) can be obtained from *in-vivo* studies (Denys et al., 2012; Casteel et al., 2006), but *in-vivo* studies would be impractical to carry out on a site-by-site basis, due to time, cost and ethical constraints. This is a limitation that makes it difficult to calculate oral bioavailability values. In the absence of bioavailability data, it is common practice to assume a conservative bioavailability value of 100% (Suedel et al., 2006; Kang et al., 2016). The product of parameters F_A x F_H in Eq. (2.5) can be estimated using physiologically-based kinetic models as demonstrated in section 7.4.

2.6.2 Role of element bioaccessibility in contaminated land risk assessment

Knowledge of the bioaccessible fraction of a contaminant presents a better estimate of human exposure to soil contamination than using the total soil contaminant concentration, since not all ingested contamination is taken-up by the human body. The United States Environmental Protection Agency (USEPA) reported that in most cases, the toxicity of an ingested chemical depends, in part, on the degree to which it is absorbed from the GI tract into the body (USEPA, 2007). Several studies have been carried out on the bioaccessibility of soil-bound metal contaminants. A study by Broadway et al. (2010) showed that the bioaccessibility of chromium in Glasgow soils was considerably less than 100% (i.e., 1 to 31%, with an average of 5%). A mean soil lead bioaccessibility value of 22% was obtained following a study using urban soils from Glasgow in Scotland (Farmer et al., 2011). A study on oral bioaccessibility of selected metals in an urban catchment in Newcastle upon Tyne (Gbefa et al., 2011) determined bioaccessibility values of <10% (Cr, Ni, Pb, Zn, Cu) and 34% (Cd). Element bioaccessibility is site-specific and therefore the difference in bioaccessibility values obtained at various sites across the UK is not surprising. These bioaccessibility values demonstrate that the risk associated with potential ingestion of soil-bound metal contaminants could be exaggerated if total soil concentrations (assuming 100% bioaccessibility) are used in the risk assessment models. Therefore, contaminant bioaccessibility determination plays a crucial role in avoiding overly conservative human health risk assessment resulting from over-estimation of exposure to soil contaminants. An overly conservative risk

assessment could lead to site remediation that is costly, time-consuming and unnecessary (Pelfrêne, Waterlot & Douay, 2011).

2.6.3 Techniques for assessing oral bioaccessibility

There are several *in-vitro* techniques for assessing the bioaccessibility of soil-bound toxic elements, ranging from single-stage to multi-stage extraction methods. The common oral bioaccessibility assessment techniques are reviewed in this section, thus providing information used in selecting the most suitable bioaccessibility test procedure for this investigation.

2.6.3.1 The Physiologically Based Extraction Test (PBET)

The PBET method was developed following a study by Ruby et al. (1993), which mimics the leaching of soil matrix in the human GI tract, and determines the bioaccessibility of an element. It was originally developed for evaluating the bioaccessibility of ingested Pb. The method was designed around the GI tract for a child, given that children are believed to be at greatest risk from accidental soil ingestion (Ruby et al., 1993). In addition, children can absorb higher percentage of a contaminant through the digestive system than adults, and therefore children are more susceptible to adverse health effects (i.e., critical receptors) (Hamel et al., 1998). This explains why bioaccessibility extraction tests are generally based around the gastrointestinal parameters for young children.

PBET method is a two-stage sequential extraction method simulating both the gastric and the small intestine compartments, with extraction carried out at 37^0C (the standard human body temperature). The pH value for the gastric phase is set at 2.5, while the pH for the small intestine phase is set at 7.0. Samples are introduced into the simulated gastric solution to solubilise any bioaccessible metal present. After a gastric sample (stage 1 extraction) has been collected, the conditions are then modified to mimic the small intestine (stage 2). This method has been used in a number of studies. For example, a study carried out by Ruby et al. (1996) using the PBET method, established that arsenic data obtained by this method were found to be over-predictive (by between 2 and 11%) of bioavailability when compared to rabbit and primate models. However, they found out that lead results from the PBET method were linearly correlated with results from a Sprague-Dawley rat model (Ruby et al., 1996). The method was used by Gbefa et al. (2011) to analyse the oral bioaccessibility of selected elements (including Cr, Ni, Cd, Pb) in an urban catchment. Using a certified reference soil material to test the recovery of the extraction method, they reported element recovery values within ±2 % of the certified reference values (Gbefa et al., 2011). No information regarding the evaluation of this method using *in-vivo* experiments was obtained.

2.6.3.2 Potentially Bioavailable Sequential Extraction (PBASE)

This method has four sequential extraction stages to evaluate the relationship between metal fractionation and the bioaccessibility of metal contaminants in soil (Basta & Gradwohl, 2000). A schematic representation of the PBASE method is shown in Fig. 2.3.

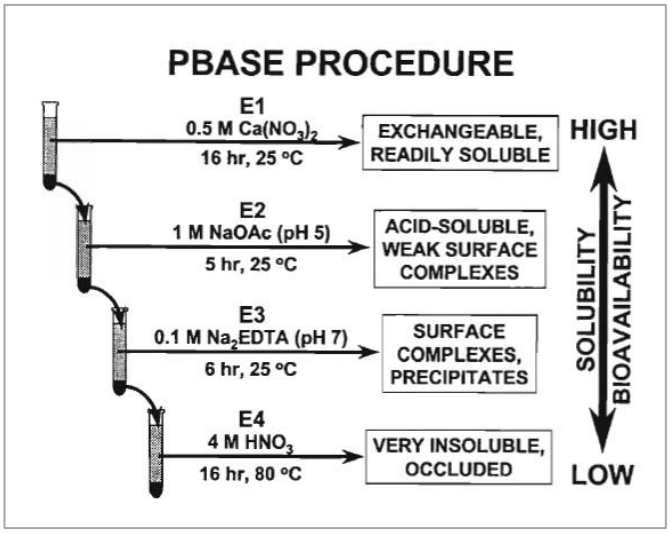

Fig. 2.3: A schematic representation of the PBASE method
Adapted from Basta & Gradwohl (2000)

The first stage (E1) uses calcium nitrate to extract readily soluble and exchangeable elements, and lasts 16 hours. The second extraction stage (E2) (5 hours duration) uses sodium acetate to extract acid-soluble weak surface complexes, while the third stage (E3) uses disodium adetate to extract surface complexes and precipitates for a period of 6 hours. The final stage (E4) employs nitric acid to extract the very insoluble elements, and runs for 16 hours. Stages E1 to E3 are carried out at 25^0C, while the stage E4 temperature is 80^0C. Given that this procedure takes approximately 43 hours to complete, it is not considered practical for use in analysing large batches of samples. In addition, the extraction sequence used in this method does not represent the actions of the human GI tract.

2.6.3.3 In-Vitro Gastrointestinal Method (IVG)

This method was developed to simulate the human GI tract environment and estimate the bioaccessibility of arsenic in soil. IVG involves the extraction of arsenic using simulated gastric and small intestine fluids at 37 ^{0}C (Rodriguez et al., 1999). It was developed to address the limitations of the PBET test, given that PBET method was found to be over-predictive of arsenic bioavailability when compared to rabbit and primate models (Ruby et al., 1996). The key difference between IVG and PBET is that IVG uses lower pH values (1.8 and 5.5, for the gastric and intestinal phases, respectively) compared to PBET (gastric pH of 2.5 and intestinal pH of 7.0).

No subsequent studies were found where this method has been used, and consequently, no data was obtained as to its performance on other elements.

2.6.3.4 RIVM In-Vitro Digestion Method (RIVM)

This is an *in-vitro* digestion model used by the National Institute of Public Health and the Environment (RIVM) in the Netherlands. The description of this method given by Oomen et al. (2002) indicate that this test is a three stage extraction method using a five minute saliva phase at pH of 6.5, followed by a 2 hours stomach extraction (using gastric juice) at pH of approximately 1.1, and another 2 hours small intestine extraction phase (using a mixture of duodenal and bile juices) at pH of at least 5.5. The test is carried out at 37^0C. Although this method simulates the conditions of the human GI tract, no data validating this method against *in-vivo* studies were obtained.

2.6.3.5 Unified BARGE Method (UBM)

This method was developed by the Bioaccessibility Research Group of Europe (BARGE) for the measurement of inorganic contaminant bioaccessibility from soil. It is a modified version of the RIVM *in-vitro* digestion method. BARGE undertook the joint decision to progress research in the field of *in-vitro* bioaccessibility method development in order to harmonise the use of bioaccessibility in human health risk assessments for contaminated soils in Europe.

UBM simulates the human gastrointestinal tract through three different compartments (mouth, stomach and small intestine), and employs synthetic digestive fluids comprising saliva, gastric fluid, duodenal fluid and bile. The gastric phase is a digestive extract collected after 1 hour agitation with saliva and gastric fluids at 37 ^{0}C. This is followed by the gastrointestinal phase involving 4 hours of agitation with duodenal fluid and bile, where samples are mixed by end-over-end agitation at 37^0C.

This method has been validated *in-vivo* for assessing the bioaccessibility of As, Sb, Cd, and Pb in soil (Denys et al., 2012). UBM has been used in a number of studies to analyse the bioaccessibility of trace elements (Wragg & Cave, 2012; Wragg et al., 2011; Broadway et al., 2010). Research institutions which are BARGE members have also been applying the UBM to contaminated land issues in their respective countries. In addition, the British Geological Survey (BGS) have prepared a bioaccessibility reference material (BGS Guidance Material 102) with bioaccessibility values for As and Pb, determined using UBM as part of the international inter-laboratory study with seven participating laboratories (Wragg et al., 2009). The BGS reference material was obtained and used in in quality control checks during laboratory analysis.

2.6.3.6 Summary of the reviewed bioaccessibility test methods

The *in vitro* bioaccessibility test methods discussed in the preceding sections are summarised in Table 2.2.

Table 2.2: Summary of in-vitro bioaccessibility test methods

Test Method	Stages of Digestion / Extraction	Method Validation (*In-Vivo*)	Duration of Sample Digestion (approximate)	Reference Materials Availability
PBET	Two stages (gastric and small intestine compartments).	No	5 hours, excluding sample pre-drying	No
PBASE	Four stages evaluating the relationship between metal fractionation and the bioaccessibility of metal contaminants in soil.	No	43 hours	No
IVG	Two stages (gastric and small intestine compartments). The method was developed for arsenic bioaccessibility measurements.	No	5.5 hours	No
RIVM	Three stages (mouth, stomach and small intestine compartments)	No	4 hours	No
UBM	Three stages (mouth, stomach and small intestine compartments)	Yes (for As, Sb, Cd & Pb)	5 hours	Yes (for As & Pb)

2.6.4 Selection of a bioaccessibility test method for use in this research

The UBM test method was selected and used in this study, based on the following considerations:

a) UBM is a well-established methodology, which has been used by various researchers in Europe;
b) the BGS have developed bioaccessibility reference material with bioaccessibility values for arsenic and lead using the UBM test. Therefore, it is possible to do quality control checks on the performance of the method using a material of known arsenic and lead concentrations;
c) the method has been validated *in-vivo* for assessing the bioaccessibility of selected metals (As, Sb, Cd and Pb) in soil; and
d) the method is not complex to carry out and takes approximately 5 hours to digest each sample batch. UBM is therefore suitable for testing large batches of samples.

2.7 Biomonitoring and biomarkers of exposure

Human biological monitoring (biomonitoring) refers to monitoring activities using biomarkers in human body fluids and tissues that focus on environmental occupational exposures to hazardous

substances (Joas et al., 2012). This requires targeted biological sampling depending on the chemicals/pollutants under investigation. Biomonitoring is a recognised tool in assessing exposure to environmental pollutants (Zhang et al., 2002; Gil et al., 2011; Rodrigues et al., 2009), and has many potential uses that include the assessment of exposure and the potential health effects of individuals (Clewell et al., 2008). However, the interpretation of the health implications of human biomonitoring data requires understanding of exposure scenarios to relate the observed concentrations in the biomonitoring study to exposures associated with adverse health effects in toxicity studies (Clewell et al., 2008). This is because biomonitoring data reflects total exposure, yet not all routes of exposure may be under investigation in a biomonitoring study. The biomarkers used in this study are presented in the following section.

2.7.1 Biomarkers of exposure

According to Jakubowski & Trzcinka-Ochocka (2005), *biomarker* is a general term for the specific measurements of an interaction between a biological system and an environmental agent (e.g., metals). Usually, the biomarker is the agent or its metabolite in a biological specimen derived from the individual, such as urine, blood, hair, body tissue or nail. The International Program of Chemical Safety (IPCS) identifies three types of biomarkers, namely; *biomarker of exposure*, *biomarker of effects* and *biomarker of susceptibility* (IPCS, 1993)[3]. Biomarker of exposure, in this case, refers to the measurement of selected toxic elements in urine and blood reflecting exposure to the elements (Nordberg, 2010). Human blood and urine are the most widely used matrices for biological monitoring of exposure to toxic elements in occupational and environmental toxicology (Angerer, Ewers & Wilhelm, 2007; Kakkar & Jaffery, 2005).

Reliable analytical measurements are at the core of any biological monitoring programme. However, even when the analytical methods are adequate, additional factors should be considered to ensure the quality of biological monitoring data (Calafat & Needham, 2008). Some of the factors that require consideration in biomonitoring are presented in the following sections.

2.7.1.1 Biomarker selection

Selection of the most relevant biomarkers of exposure is influenced by the knowledge of the toxicokinetics of the chemical being investigated. Biomarkers (the parent chemical or its metabolite) can be present in various human tissues such as blood and urine. For example, the biotransformation of arsenic absorbed in the body consists of oxidation/reduction and methylation reactions (Mann, Droz & Vahter, 1996). Methylation of inorganic arsenic takes place primarily in the liver and specifically in hepatocytes, producing both mono-methylated and di-methylated arsenicals, which are excreted in urine (Stamatelos et al., 2011). Therefore, measured biomarker concentrations should account for the toxicologically relevant metabolites as well as the parent

[3] Available at: http://apps.who.int/iris/bitstream/10665/39037/1/9241571551-eng.pdf

chemical. In addition, where there are a number of metabolite pathways, it is important to know the proportion of external exposure that goes with each metabolite. The biomarkers of exposure used in this study are presented in Table 2.3.

Table 2.3: Summary of biomarkers of exposure used in this study

Element	Biomarkers of exposure	References
As	Inorganic arsenic is the most relevant in toxicological studies due its high toxicity compared to organic arsenic. Therefore, we used urinary inorganic arsenic concentration (i.e., sum of all inorganic species) as an indicator of exposure to As.	a, b, c
Cd	Both blood and urine have been reported as useful biomarkers in studying Cd exposure. In this study, urine was used as the biomarker. Urine has been indicated to reflect Cd levels in the kidney, which is a target organ for Cd.	b, d, e
Cr	Chromium in blood and urine are considered the most reliable indicators of exposure to chromium. In this study, urine was used because absorbed Cr is predominantly excreted through urine.	f, g, h
Pb	Total lead concentration in blood is the preferred biomarker of recent lead exposure.	b, i, j
Ni	Serum or urine nickel concentrations are the most useful biomarkers of nickel exposure. Urine was used as the biomarker because urinary excretion is the main clearance route for absorbed nickel.	k, l

[a](Hughes, 2006); [b](Bornhorst & McMillin, 2006); [c](ATSDR, 2007a); [d](Järup & Åkesson, 2009); [e](ATSDR, 2012a); [f](Caglieri et al., 2005); [g](ATSDR, 2012b); [h](Paustenbach et al., 1997); [i](ATSDR, 2007b); [j](Keil et al., 2011); [k](Kakkar & Jaffery, 2005); [l](ATSDR, 2005).

2.7.1.2 Effect of half-life on biomarker concentration

The kinetics and storage of an element in the body is important in the interpretation of biomonitoring results, and determines the timing and frequency of sample collection (Aitio et al., 2007). Biomarker concentrations change over time in relation to the half-life of the chemical involved. Some chemicals with short half-lives (e.g., a few days) are rapidly eliminated from the body, while those with longer half-lives are eliminated over a longer time period. For example, the elimination half-life of lead in blood is about 30 days (ATSDR, 2007b), while absorbed cadmium is excreted very slowly with a range of half-lives of several years (4 to 19 years in human liver; 6 to 38 years in human kidney) (ATSDR, 2012a). Therefore, the half-lives of the chemicals under investigation should be considered during biological monitoring, in order to ensure the biological samples are obtained before the chemical or its metabolite has been eliminated from the body.

2.7.1.3 Biological monitoring data provide a measure of all exposures

Quantification of exposure relies on knowledge of the relationship between the exposure and the biomarker concentration. Biological monitoring measures the total environmental exposures from

all sources and exposure routes (Friberg & Elinder, 1993). The contribution of the main pollution sources and pathways to exposure are key components for risk assessment. Without information on exposure pathway, it is difficult to relate biomonitoring data to sources and routes of exposure and to develop effective risk management strategies (Doerrer, 2007; Albertini et al., 2006).

2.7.1.4 Ethical issues

The use of biological monitoring data in risk assessment involves a variety of ethical issues. Some methods for obtaining biological samples (e.g., blood and urine) are invasive, which some participants may find uncomfortable. Invasive methods of collecting biological samples also pose some risk to the person. Biomonitoring produces data on the volunteering individual and, thus, information that is confidential. Biological monitoring studies should be undertaken in accordance with the fundamental and widely accepted ethical principles, namely; *beneficence* ('do positive good'), *non-maleficence* ('do no harm'), *informed consent* and *privacy and dignity* (Sepai et al., 2008).

2.7.2 Creatinine adjustment of biomonitoring results

Creatinine is the metabolite of creatine (an important energy store for muscles), and therefore the production of creatinine in an individual reflects muscle mass (Cocker et al., 2011). The production of creatinine is relatively constant within an individual (Cocker et al., 2011). The variability in the volume of urine from void to void is a major disadvantage of spot urine sampling. Common methods for adjusting dilution and for determining whether a spot urine sample is valid for assessing chemical exposures include urinary creatinine concentrations, specific gravity and osmolality. The most widely used method is creatinine adjustment, which involves dividing the analyte concentration by the creatinine concentration. (Barr et al., 2005, Falco et al., 2001). The World Health Organization (WHO) recommends that if a sample is too dilute (creatinine concentration < 0.3 g L^{-1}) or too concentrated (creatinine concentration > 3 g L^{-1}), another urine void should be collected (WHO, 1996) and analysed for creatinine and the target chemical. We used these WHO guideline values to assess the validity of urine samples, such that urine samples that were either too dilute or too concentrated were discarded. This is a standard practice in biomonitoring studies.

2.8 PBPK models and model selection

PBPK modelling is recognised as a technology for simulating and predicting the fate of substances in the body (Schmitt & Willmann, 2005). PBPK models are based on compartments (e.g., body organs, tissues) and the interconnections among the compartments. The level of model detail relates to the compartments and elements (including associated chemical forms such as metabolites) that are tracked within the model (Krishnan et al., 2010). The models describe

(quantitatively) the absorption, distribution, metabolism and excretion of chemicals in the body; and thus can provide a scientific basis for quantitatively estimating risk to human health (Yu, 1999).

The absorption, distribution, metabolism (relevant for As and Cr) and excretion of the five elements are summarised in this section. Previously published kinetic models for these elements are also presented, in order to select models to apply in this study. Schematic representations of the original models adopted are given in Appendix B.

Models were selected based on the following preferences:

a) whether the model was reproducible (based on model details presented),
b) relevance of the model to adult humans,
c) relevance of model to the ingestion pathway, and
d) most recent and/or most used models.

Some of these models are quire complex in terms of their structure and formulation because of the differences in physicochemical properties of the elements, organs involved in metabolism and exposure pathways simulated by the authors. To construct models suitable for our purpose (i.e., simulation of oral ingestion pathway in adults), some of the published models required modification by reducing or removing some compartments whilst still maintaining their predictive ability. Evaluation of the predictive performance of the constructed models is presented in chapter 6.

2.8.1 Arsenic

Following oral ingestion of inorganic As (in the form of either As(III) or As(V), the two biologically important valence states), absorbed As from the GI tract is transported by the blood to other parts of the body (Fowler et al., 2007). The distribution of As to the tissues depends on their blood perfusion (the permeability of the capillary membranes) and the affinity of the tissues for the arsenic metabolites (Mann et al., 1996). The biotransformation / metabolism of absorbed As in the body consists of oxidation/reduction and methylation reactions (Mann et al., 1996; El-Masri & Kenyon, 2008). The reduction facilitates the uptake of As(III) by cells for further methylation reactions since As(V) does not undergo methylation (Stamatelos et al., 2011; Yu, 1999; El-Masri & Kenyon, 2008). Methylation takes place primarily in the liver and kidney, producing both monomethylarsenic acid (MMA) and dimethylarsinic acid (DMA). Generally, whole body clearance of As is fairly rapid, with half-lives of 40 to 60 hours in humans (Fowler et al., 2007; ATSDR, 2007a). Bornhorst & McMillin (2006) indicate that ingested inorganic forms of As are excreted over the course of 1 to 3 weeks; therefore making urine the preferred sample for biomonitoring since arsenic can be observed for up to 3 weeks following exposure. Unabsorbed fraction is excreted in the faeces.

A number of published human PBPK models for inorganic As were identified in the literature (Mann et al., 1996; Yu, 1999; Liao et al., 2008; El-Masri & Kenyon, 2008). These models are largely similar in structure, and they account for oxidation of As(III) to As(V), reduction of As(V) to As(III), and methylation of As(III) to MMA and DMA in the body. One key difference between these models is that the model by Mann et al. (1996) included both oral and inhalation exposure pathways, whereas other models only included the ingestion pathway. Ingestion is the primary route of exposure studied, because exposure to As from allotment land use occurs mainly through oral intake (CL:AIRE, 2014). In addition, a pilot study we carried out identified air As concentrations at the allotment that were not significantly elevated enough above background air concentrations to warrant inclusion in our model.

The model proposed by Liao et al. (2008) for children was not evaluated using experimental data, whereas the other models were tested using data from human studies. The model published by El-Masri & Kenyon (2008), which is the most recent out of the tested models, was adopted in this study. This model comprises 9 compartments, namely: GI tract, liver, kidney, blood, muscles, brain, skin, heart and lung. The choice of tissues was based on physiochemical properties of As, oral exposure route, target tissues, and sites for As metabolism (El-Masri & Kenyon, 2008). Although the model does not include the inhalation exposure pathway, the lung compartment was included in the model because it receives total blood flow, thus mathematically accounting for As reduction that may occur in other body tissues (El-Masri & Kenyon, 2008). Model parameters were determined from data derived using human cells and tissues (El-Masri & Kenyon, 2008). The authors evaluated the model using urinary excretion data. A sketch of this model is given in Appendix B (Fig. B1).

2.8.2 Cadmium

Absorbed Cd is widely distributed throughout the body via the blood, with the highest concentrations found in the liver and kidney (ATSDR, 2012a). Cd has a range of half-lives of several years (4 to 19 years in human liver; 6 to 38 years in human kidney) (ATSDR, 2012a). The concentration of cadmium in the kidney is reflected in urinary cadmium levels (Keil et al., 2011; Järup & Åkesson, 2009). According to ATSDR (2012), Cd is not known to undergo direct metabolic conversion (e.g., oxidation, reduction, alkylation). The major cadmium excretion routes are through urine (absorbed fraction) and faeces (unabsorbed fraction) (Kjellstrom & Nordberg, 1978).

There are a number of PBTK models for Cd published in the literature (Choudhury et al., 2001; Amzal et al., 2009; Ju et al., 2012; Fransson et al., 2014), based on the original physiologically-based toxicokinetic (PBTK) model published by Kjellstrom & Nordberg (1978) (KN model). The KN model consists of eight compartments, describing Cd uptake from the gastrointestinal (GI) tract and the lungs, distribution of absorbed Cd to three blood compartments (B1 to B3, representing Cd

bound to albumin and other proteins, Cd in erythrocytes, and Cd bound to metallothionein in plasma, respectively), liver, kidney and a compartment for 'other tissues', and Cd elimination through urine and faeces. The KN model was formulated for oral and inhalation exposure pathways, based on animal and human tissue distribution data (Kjellstrom & Nordberg, 1978). The distribution coefficients of the KN were estimated by fitting the calculated Cd concentrations in 5 compartments (kidney, liver, urine, blood, and other tissues) to the observed concentrations for humans with different smoking habits, and humans with and without occupational Cd exposure (Kjellstrom & Nordberg, 1978).. Further testing of the model with data on Cd metabolism in humans was carried out by Nordberg & Kjellstrom (1979). Different authors have used the K&N model to simulate Cd exposure through oral ingestion (Ruiz et al., 2010; Ju et al., 2012; Fransson et al., 2014). The KN model was adopted in this study because it was the most used model and it is also the basis for other published PBTK models for Cd. A schematic representation of the original KN model is given in Appendix B (Fig. B2).

2.8.3 Chromium

It has been estimated that the absorbed fraction of Cr in the GI tract in humans is less than 10% of the ingested dose (ATSDR, 2012b). The uptake is much quicker for Cr(VI) than Cr(III) because Cr(III) is poorly absorbed from the GI tract (Langard & Costa, 2007). Once in the blood, Cr(VI) is readily taken up into the red blood cells (RBCs) where it is rapidly reduced to a more stable Cr(III) (Kerger et al., 1996; De Flora, 2000; O'Flaherty et al., 2001). The Cr(III) can either be trapped within the RBCs or exit the cells into the plasma (Kerger et al., 1996; O'Flaherty, 1996). The absorbed chromium is distributed to nearly all tissues, but the highest concentrations are found in the kidney and liver (ATSDR, 2012b). After absorption, any Cr(VI) that is not reduced to Cr(III) in the portal vein would reach the liver, which also has a capacity to reduce Cr(VI) to Cr(III) (De Flora et al., 1997; O'Flaherty et al., 2001). Given the reducing capacities of Cr(VI) in the tissues, Cr is present in the body mainly in the form of Cr(III) (Paustenbach et al., 1997).

The half-life of Cr in the human RBCs *in vivo* is estimated to be about 30 days (O'Flaherty et al., 2001). This suggests that Cr does not remain in the RBCs for the entire lifespan of the cell, which is approximately 120 days (Paustenbach et al., 1997). However, Kerger et al. (1996) indicated that haemoglobin-bound Cr complexes remain part of the RBC for the entire lifespan of the cell. On the contrary, the urinary half-life is much shorter. According to ATSDR (2012b), Cr absorbed following Cr(VI) ingestion has a half-life of approximately 40 hours, the half-life is about 10 hours when absorbed as Cr(III). This seems consistent with the half-life of less than 2 days indicated by Paustenbach et al. (1997). Absorbed Cr is excreted predominantly through urine (ATSDR, 2012b), while unabsorbed fraction (nearly 90% of ingested dose) is excreted in the faeces (Kirman et al., 2013). Two key models for Cr were identified in the literature. A physiologically based model for the ingestion of Cr(III) and Cr(VI) by humans was developed by O'Flaherty et al. (2001). The

compartments included in the model are the GI tract, blood (plasma and red blood cells), liver, kidney, bone (trabecular and cortical), well- and poorly-perfused tissues with pulmonary absorption of inhaled Cr, and excretion pathways via faeces and urine. The model was calibrated using blood and urine data from controlled studies in which human volunteers drank solutions containing Cr(III) and Cr(VI). Another PBPK model for humans orally exposed to Cr was developed by (Kirman et al., 2013). This model includes compartments for GI tract (stomach and intestines), blood (systemic and portal plasma and red blood cells), liver, kidney, bone and other tissues. It models absorbed Cr(III) in systemic circulation as belonging to two general pools: (i) a distribution pool, which describes the distribution of Cr(III) from the GI tract to tissues through the plasma, and (ii) a storage/excretion pool, which describes the release of Cr(III) from tissues to its ultimate excretion in urine (Kirman et al., 2013). Model performance was evaluated using toxicokinetic data for Cr in human tissues and excreta obtained from the literature. The authors reported that the model provides a good description of Cr toxicokinetics in humans. One key difference between these two models is that Kirman et al. (2013) included detailed Cr toxicokinetics in various GI tract compartments in their model, while O'Flaherty et al. (2001) modelled GI tract as a single compartment. The model by Kirman et al. (2013), which is the most recent model, was adopted in this study; a sketch of this model is given in Appendix B (Fig. B3).

2.8.4 Lead

The extent and rate of absorption of ingested inorganic Pb in the gastrointestinal tract are influenced by physiological properties of the exposed individual, including age, fasting status, nutritional calcium and iron status, and pregnancy (ATSDR, 2007b). Once absorbed, the concentration of Pb in the plasma governs the rate of Pb transfer out of the blood, and thus influences Pb transport throughout the body (Leggett, 1993; O'Flaherty, 1993). Pb is circulated through the vascular system to soft tissues (e.g., liver, kidney, muscles) and the bone (Fleming et al., 1999; White et al., 1998). In adult humans, over 90% of the Pb entering the body throughout a person's life is stored in the bones (ATSDR, 2007b; Keil et al., 2011). According to Skerfving & Bergdahl (2007), the skeleton contains approximately 20% trabecular bone and approximately 80% cortical bone, providing two different skeletal Pb pools. It has been suggested that Pb is mobilised from the skeleton during bone mineralisation, and therefore Pb in the bones can be transferred into the blood long after exposure has ended (Smith et al., 1996; Fleming et al., 1997).

The half-life of Pb in blood is about 30 days (ATSDR, 2007b). However, Pb has a longer half-life in the bone (about 1 year in the trabecular bone, and 10 to 32 years in the cortical bone) (Skerfving & Bergdahl, 2007; Keil et al., 2011; Bornhorst & McMillin, 2006). Blood Pb is the preferred indicator of relatively recent exposure to lead (Keil et al., 2011), while the lead concentration in the bone indicates long-term exposure to lead (Skerfving & Bergdahl, 2007). Absorbed Pb is primarily excreted in urine and biliary secretion, while minor routes of excretion include sweat, saliva, hair

and nails (ATSDR, 2007b; Bornhorst & McMillin, 2006; White et al., 1998). Unabsorbed Pb is excreted in the faeces.

There are a number of Pb kinetic models for humans in the literature. The most cited in the literature reviewed are the Leggett model (Leggett, 1993), the Integrated Exposure Uptake Biokinetic (IEUBK) model (White et al., 1998) and the O'Flaherty model (Fleming et al., 1999; O'Flaherty, 1991; O'Flaherty, 1993). The IEUBK model deals with Pb kinetics in children only, yet our study involved adults. Another observation is that the Leggett model does not account for physiological factors in detail, it gives the model 'transfer rates' that are age-specific and regards ≥ 25years as one age-class. Therefore, the Leggett model does not account for physiological variabilities that can be associated with varying body weights (Prakash et al., 2013; Bailey et al., 2004). In view of the above, both the IEUBK and the Leggett models were not considered suitable for this study. The O'Flaherty model was considered suitable for this study because it is physiologically-based and it is for adults. Full details of the construction of this model are provided by O'Flaherty (1991), O'Flaherty (1993) and Fleming et al. (1999). Briefly, this model uses 9 body compartments, namely: GI tract, blood, liver, kidney, bone (cortical and trabecular), other tissues (well- and poorly-perfused) and the lung. This model has been evaluated against data from human subjects exposed to Pb through oral and inhalation pathways (O'Flaherty, 1993). A sketch of this model is given in Appendix B (Fig. B4).

2.8.5 Nickel

Following exposure to Ni, part of Ni load is absorbed into the blood stream. A study conducted by Sunderman et al. (1989) on human volunteers indicated that following oral exposure, about 27% of the Ni given to the volunteers in drinking water was absorbed, while only about 1% was absorbed when it was given in food. It has also been suggested that only 5% of ingested Ni is absorbed (Rojas et al., 1999). According to Klein & Costa (2007), Ni does not generally accumulate in tissues due to its efficient excretion. The urinary excretion half-life for absorbed Ni has been reported to vary between approximately 20 and 27 hours (Nielsen et al., 1999). This correlates well with the half-life of 28±9 hours previously reported by Sunderman et al. (1989). Urinary excretion is the main clearance route of absorbed Ni (Kakkar & Jaffery, 2005; Klein & Costa, 2007), hence urine can be used as a biomarker for Ni exposure. The fraction that remains unabsorbed in the GI tract is excreted in the faeces.

There are limited PBPK models for Ni compounds in the literature relevant to humans. Models describing the deposition, retention and clearance of inhaled Ni (in the lung) have been developed by others (Hsieh et al., 1999; Yu et al., 2001). However, given that inhalation route is not the dominant pathway for allotments land use, these models were not reviewed here.

The only relevant Ni PBTK model for humans found in the literature is that of Sunderman et al. (1989), following oral intake of Ni in water and food by human volunteers. They developed the model based on two experiments, in which Ni levels in serum, urinary and faecal excretions were monitored after eight subjects were given an oral dose of Ni (as $NiSO_4$) in either water (experiment 1) or food (experiment 2). The model comprises four compartments (gut, serum, urine and tissues) with the parameters based on model-data-fitting to the two experiments. This allowed rate constants for alimentary Ni absorption from the gut, Ni transfer rate constants from serum to tissues and urine, and from tissues to serum to be determined. Given that our study involves exposure through oral ingestion of food, the model parameters provided by Sunderman et al. (1989) from experiment 2 were considered more relevant to our study than parameters from experiment 1. However, Sunderman et al. (1989) did not determine the rate transfer from tissues to serum in experiment 2, which they indicated was due to the small mass of Ni absorbed from the gut into subsequent compartments. Therefore, we used the Ni rate transfer from tissues to serum from their experiment 1 in our simulations. A sketch of this model is given in Appendix B (Fig. B5).

2.9 Chapter summary

This chapter provides useful background information that highlights the context of the research. Using a CSM for allotment land use, it has been established that the primary route through which humans could be exposed to As, Cd, Cr, Pb and Ni present in allotment soil is oral ingestion. The CSM has informed the sampling and material requirements necessary in characterising the '*source – pathway – receptor*' linkage. Although the conservative GACs currently used in contaminated land risk assessment are protective of public health, they may result in over-estimation of human exposure to soil contaminants. Subsequently, this could lead to un-necessary remediation or restrictions on land use. To promote sustainable reuse of land, there is a need for improved understanding of the actual human exposure to soil contaminants. This highlights the knowledge gap, which this research sought to address in relation to the five elements selected.

3. MATERIALS AND METHODS

3.1 Introduction

The materials and methods used in this study ranged from IOM's internal standard procedures to methods obtained from the literature, and were determined based on information gathered from the literature review (Chapter 2). In order to trial the materials and methods and test the logistical requirements for biomonitoring, we carried out a pilot study. A summary of the pilot study is given in the following section.

3.2 Pilot study

The preliminary investigation was carried out between July 2014 and January 2015 to test the methods and procedures, and logistical requirements for biomonitoring. No statistical emphasis was placed on sample size requirements and site selection in this trial phase. Five allotments were identified and their respective users were recruited to participate in the pilot study. Findings of the pilot study (presented in Appendix C) were used in the planning of the main study phase.

Changes and adjustments made to the methods as a result of the pilot study include exclusion of the inhalation (of allotment dust) pathway from the main study phase. This was because air concentrations of the elements at the allotments were determined to be similar to background air concentrations, which indicate negligible exposure to the elements investigated through inhalation of allotment dust. This is consistent with the CLEA model parameters which show that this pathway contributes to $\leq$ 0.1% of total human exposure to these elements (CL:AIRE, 2014; Environment Agency, 2009d; Environment Agency, 2009c). Following the completion of the pilot study, the main study phase was carried out using the materials and methods presented in the following sections.

3.3 Estimation of sample size

Sample size determination is important in statistical studies for economic and ethical reasons (Lenth, 2001). Therefore, in order to estimate the number of participants required for the study, a range of *a priori* power calculations were carried out using *G*Power* statistical software (version 3.1.9.2) (Faul et al., 2007). The following parameters were used in the calculations (Schulz & Grimes, 2005):

- Type I error (α) – which is the probability of detecting a false-positive result (conventionally set at 0.05, indicating a desire for less than 5% chance of making a false-positive conclusion).

- Type II error (β) – is the probability of detecting a false-negative result (conventionally set at 0.20, indicating a desire for less than 20% chance of making a false-negative conclusion).
- Power (1-β) – represents the probability of avoiding a false-negative conclusion. The power of 0.8 usually suffices (Schulz & Grimes, 2005).
- Effect size (expressed as a ration greater that 0 but less than 1) – due to scant data on the effect of exposure to the selected elements from allotment land-use, an effect size of 0.5 was assumed (Galea et al., 2011). This indicates the ability to detect a doubling of background levels.

Various statistical tests (built into *G*Power*) were used in calculations. These tests predicted sample sizes of approximately 26 subjects (at power of 0.8), as illustrated in Fig. 3.1.

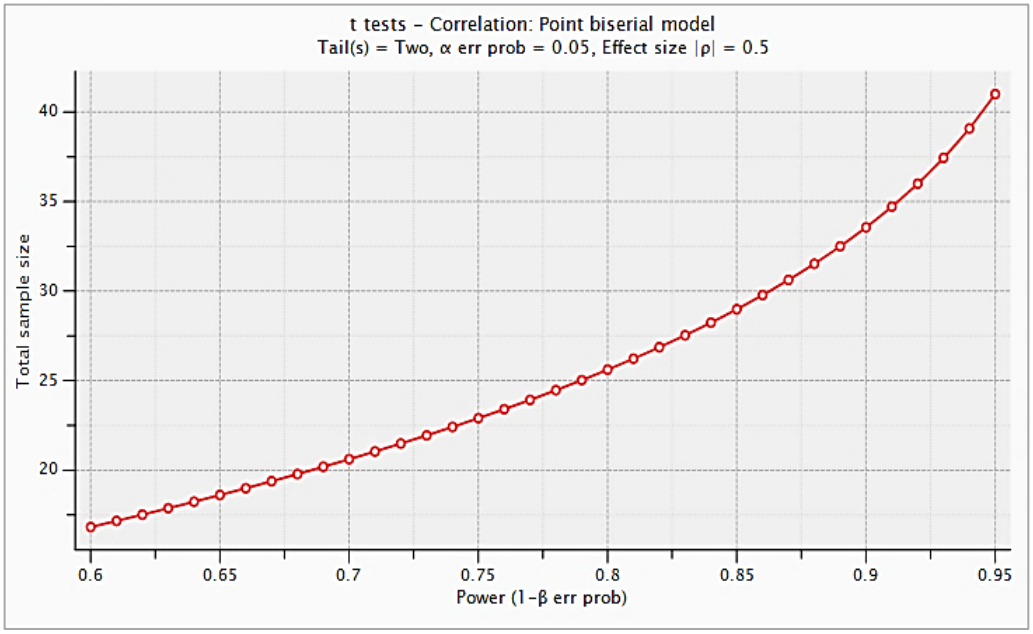

Fig. 3.1: Estimation of sample size

A sample size of 25 to 30 subjects was considered reasonable and achievable, given the intrusive nature of the study (e.g. biomonitoring requirements) and the resources (time and funding) available for this study.

3.4 Identification of study sites and participant recruitment

Reference was made to the full list of allotment sites in Scotland published by SAGS (2007). Thereafter, online inspection of historical maps held by the National Library of Scotland was carried out to identify potential study sites in the central part of Scotland. We identified a number of allotment sites of interest based on the historical features recorded at these sites, and their potential for soil contamination with the elements of interest. Reference was made to the Department of Environment Industry profiles[4] in identifying potential contamination from historical features. In addition, some sites were randomly selected. Thirty allotment plots (from 16 separate sites) were chosen.

Each plot was assigned a unique identification number preceded by letter 'A' (A01, A02, etc.). The geographical locations of these sites are shown in Fig. 3.2 (produced using QGIS version 2.14).

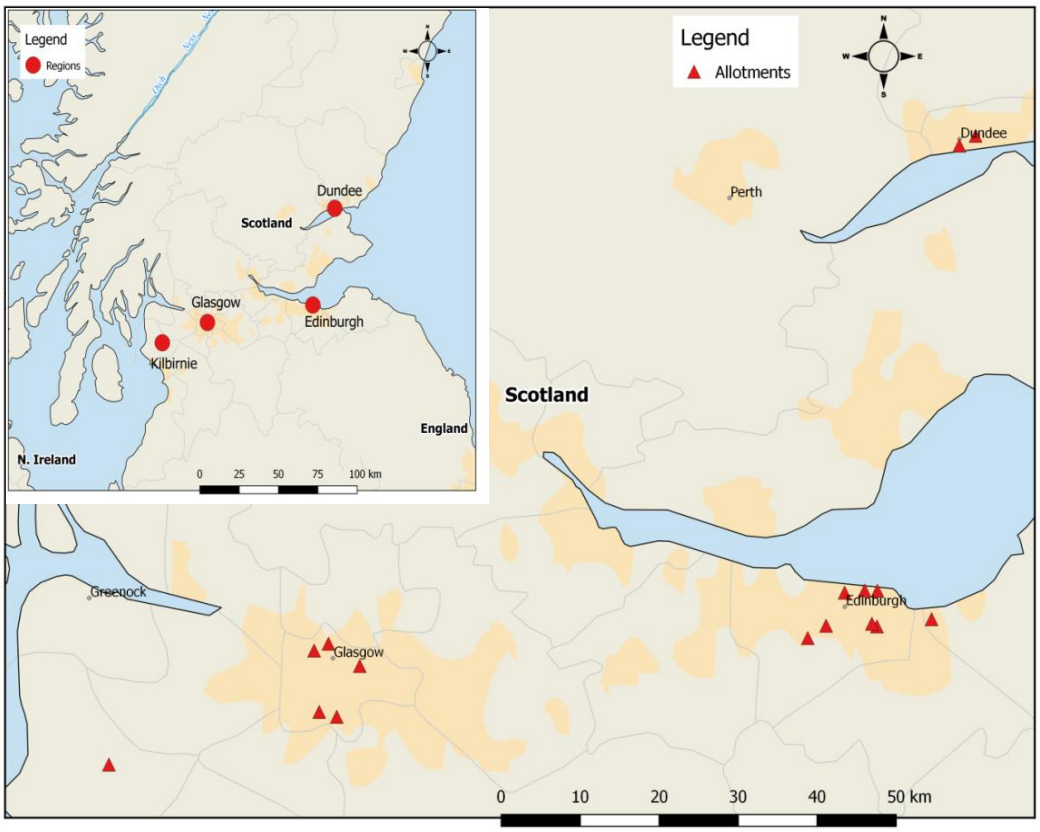

Fig. 3.2: Map showing the geographical locations of the chosen allotment sites in Scotland
(made with Natural Earth. Free vector and raster map data at naturalearthdata.com).

After obtaining ethical approval from UREC, initial contacts were made with representatives of the various allotment associations, who were requested to distribute the recruitment poster to members of their associations. Other participants were also reached through personal contacts. We recruited 37 allotment users (consisting of 20 males and 17 females) from the chosen sites to participate in

[4] Available at: http://www.claire.co.uk/useful-government-legislation-and-guidance-by-country/76-key-documents/198-doe-industry-profiles

the study. This included one participant per plot from 23 plots and two participants (couples) per plot from 7 plots. The age range of the participants was between 30 and 80 years old (with mean and standard deviation of 59 ± 11 years). Within the participants, there were 23 non-smokers (never smoked), 11 ex-smokers, 2 current smokers, 1 unknown smoking status (not declared). Participants were assigned unique identification numbers (P01, P02, etc.).

Available historical information for the allotments and the durations the participants have used their allotment plots are presented in the following Table 3.1.

Table 3.1: Introductory information for allotment plots

Allotment plot ID	Regional location of allotment	Available historical information for allotment sites where plots used in this study were located	Duration of allotment use (years)
A01	Edinburgh	Site shown as vacant from 1880s until allotments were shown on site by 1950s.[#]	20
A03	Edinburgh	The plot is located on the same allotment site as plot A01.	1
A04	Edinburgh	No information was obtained.	2.5
A05	Edinburgh	Historical maps show allotment at the site by 1940s.[#]	20
A06	Edinburgh	No information was obtained.	5
A07	Edinburgh	There was a railway line on site from 1900s until the 1940s when allotments were shown on site.[#]	2
A08	Dundee	The site was occupied by buildings from 1890s to 1960s.[#]	3
A09	Edinburgh	Site was used for allotments by 1944.[#] It was understood that the site was part of a Roman Settlement.[$] Plots A09, A10 and A12 were located within the same allotment site.	3.5
A10	Edinburgh		17
A12	Edinburgh		20
A15	Edinburgh	The plot is located on the same allotment site as plot A05.	8
A16	Glasgow	The site had been used for allotments since the 1920s.[$]	4
A17	Kilbirnie	Site was formerly used for disposal of building waste / rubble.[$]	4
A18	Kilbirnie	The plot is located on the same allotment site as plot A17.	2
A19	Glasgow	Allotment gardens were set up on site by 1917.[#]	20
A20	Glasgow	From 1950s to 1980s, site was used for allotments, then as a playground until 2013 when the current allotment was started.[$]	1.5
A21	Edinburgh	The site was an open park until the allotment started in 1920s.[$]	20
A23	Glasgow	The site has been used for allotments since at least 1935.[$]	30
A25	Edinburgh	The plot is located on the same allotment site as plot A21.	19
A26	Edinburgh	The plot is located on the same allotment site as plot A21.	19
A27	Glasgow	The plot is located on the same allotment site as plot A20.	1.5
A28	Glasgow	Site was used for gardens (1945 to 1960s), then was vacant until 2010 when current allotment was started.[$]	0.2
A29	Kilbirnie	The plot is located on the same allotment site as plot A17.	5
A31	Edinburgh	The plot is located on the same allotment site as plot A05.	1
A32	Edinburgh	The plot is located on the same allotment site as plot A05.	6
A33	Edinburgh	Historical maps show the site as vacant until the 1940s when allotments were recorded on site.[#]	7
A34	Glasgow	The site has been used for allotments since the 1940s.[$]	17
A36	Dundee	Site was located within a historically industrial area in Dundee. Historical maps show allotment on site by early 1900s.[#]	0.2
A37	Dundee	The plot is located on the same allotment site as plot A36.	20.5
A38	Glasgow	The plot is located on the same allotment site as plot A20.	1.5

[#]Information obtained from historical maps available on the National Library of Scotland online portal (maps.nls.uk).

[$]Information provided by the participant (allotment plot-holder).

Written consent was obtained from each participant. Questionnaires and diaries were used to gather information from the participants. Copies of the recruitment poster, questionnaires and diaries are presented in Appendix D. Information gathered from the participants including participants' details, frequency and duration of allotment visits, and produce consumption rates are summarised in Appendix E.

3.5 Sample collection and storage

Soil samples (n=258) were collected from the allotment plots in May 2015. Between 6 and 14 samples were collected from each plot. Samples were obtained from hand dug pits at approximately 0.3 m depth (corresponding to 'single dig' cultivation method reported by most participants) using a trowel. To minimise potential cross-contamination of samples, the trowel was cleaned (using wet wipes) between successive sampling locations. Sampling locations were randomly selected, avoiding heavily planted areas to minimise damage to plants. Samples were placed in plastic tubs and labelled with a unique identifying number. A collection of 270 allotment produce samples was carried out in August 2015. Between 3 and 18 produce samples were collected from each plot. Fibrous roots and non-edible plant parts were removed and discarded on site. Samples were placed in sealable plastic bags and labelled with a unique identifying number. In addition, hand-moist-wipes (n = 40) were obtained from participants while doing allotment work with bare hands. Samples were collected using '*Ghost*' wipes (i.e., sturdy wiping materials moistened with deionised water). Wipes were collected to investigate potential exposure to the elements through inadvertent ingestion, which mainly rises from hand-to-mouth contact (Ng et al., 2012). During sampling, it was observed that participants stored their gardening gear in allotment sheds away from home, which supports our consideration in the conceptual site model (section 2.5.2) that 'tracking back' of allotment soil to participants' homes was not a significant pathway of exposure.

Collection of venous blood samples from 32 consenting participants were scheduled on four different occasions over a period of 12 months. Blood samples (n=108, 5 mL each) were collected by venepuncture in tubes containing ethylenediamine tetraacetic acid (EDTA). Between 2 and 4 venous blood samples were obtained from each participant; some of the participants were not available during some of the scheduled visits. Five participants who did not consent to giving blood were excluded from blood sampling. A qualified nurse was recruited to collect venous blood samples. In addition, collection of dried blood spot samples from finger-prick (from 32 consenting participants) was scheduled on a monthly basis over a period of 12 months, to investigate if finger-prick samples (i.e., capillary blood) could be used as a surrogate for venous blood (as Pb biomarker). We collected 315 dried blood spot samples on filter paper, of which, 108 were paired to the venous blood samples because both sample types were collected at the site time. Each filter (in a cassette) was weighed (average of 3 weights) before and after sampling to determine the

weight of blood sampled. Subsequently, the volume of blood collected on the filter was estimated using the density of blood (at 37 ^{0}C). First-void urine samples (n = 748) were collected in polystyrene bottles throughout the 12 months period of biomonitoring. Collection of urine samples was scheduled on a fortnightly-basis. In addition, we obtained 113 spot urine samples (collected over 2 to 3 consecutive days) from 13 participants, who also provided a record of allotment produce consumed during the same period (see sampling dairy in Appendix D). These additional urine samples (and produce consumption data) were collected to aid the evaluation of model performance, especially for As, Cr and Ni that have short biological half-lives. All samples were transported to the laboratory for storage and subsequent analysis. Blood and urine samples were stored at –20 ^{0}C.

3.6 Laboratory test methods

The laboratory tests carried out internally by the researcher at IOM laboratory are outlined below. Detailed descriptions of the laboratory test procedures used and the associated Quality Assurance (QA) measures are given in Appendix F (parts F1 to F5).

- Determination of the total concentrations of the elements (As, Cd, Cr, Ni, Pb) in samples of soil, produce, hand moist-wipes and allotment dust was carried out according to IOM's internal Standard Operating Procedure (ICP-SOP2). This procedure is based on method 7300 provided by the National Institute of Occupational Safety and Health (NIOSH). Details of this procedure are given in Appendix F, part F1.
- Bioaccessibility extraction test on soil and produce samples was carried out using the UBM procedure described in Appendix F, part F2, to estimate the element fraction that would be soluble in the human GI tract and hence potentially available for absorption. Reasons for selecting this method are outlined in chapter 2 (section 2.6).
- Determination of the total elemental concentrations (As, Cd, Cr, Ni) in urine samples. Urine samples were prepared for analysis by sample dilution (10 fold) using nitric acid solution (2 to 4% HNO_3) (Goulle et al., 2005; Auray-blais et al., 2011; Castano et al., 2012). A detailed description of the procedure is presented in Appendix F, part F3.
- Determination of the total Pb concentrations in blood samples. The procedure used in this study was obtained from Goulle et al. (2005), Ikeda et al. (2011) and method ID-121 by the Occupational Safety and Health Administration (OSHA, 2002), modified and subsequently trialled and evaluated using pigs blood and a certified reference material (CRM) for human blood (BCR-636). The procedure is described in Appendix F, part F4.
- Speciated Cr testing, to determine Cr(VI) in soil and produce samples was determined using OSHA method ID-121, Table 4 Alternative Procedures AP2 and AP5.
- Pb isotope ratios were determined in soil, produce and blood samples. The procedure used in isotopic analysis is described in Appendix F, part F5.

3.6.1 Assessment of precision of chemical analyses

To ensure the test results were reliable, the following measures and assessments were carried out:

- Instrument calibration included 5 points, with correlation coefficients ($r^2 > 0.99$) for each sample batch analysed.
- Repeated sample analyses (after every 10 consecutive samples) were carried out to check for instrument drift, and variations from the original results were within 10%.
- Recoveries of the elements in in spiked samples were within ±20% of the known/specified concentrations (Li et al., 2015).
- Sample blanks (i.e., the acid solution used in sample dilution) were analysed in duplicates while spiked samples were analysed in triplicates, for correction of instrument readings.
- Measured sample concentrations were restricted within the calibration range (up to 25% above the maximum calibration standard); any sample concentration above this limit would require dilution and re-analysis.
- Standard / certified reference materials (CRM) were used for quality control by including CRM with each sample batch analysed. For bioaccessibility tests, a standard soil material (BGS Guidance Material 102) was used. For urine samples, a commercially available CRM for human urine (*ClinChek-Control*) was used. For blood samples, a commercially available CRM for human blood (BCR-636) was used. Recovered element concentrations were within ±20% of the specified CRM concentrations.
- A high-purity Pb metal (NIST SRM 981) was used as a Pb isotope standard for quality control of Pb isotope analyses.

3.7 External testing

Testing of urinary creatinine and inorganic As (iAs) in urine were carried out by Trace Laboratories Ltd. (Tracelabs), a UK External Quality Assurance Scheme (UKEQAS) accredited laboratory. Creatinine was tested to enable the adjustment of urinary element concentrations for hydration status (Fillol et al., 2010). Urinary inorganic As (and not total As) was measured because this is the toxicologically relevant species when assessing environmental exposure (Fillol et al., 2010). To determine inorganic As (sum of inorganic As species) in urine, samples were ‘made acidic and taken through two extraction stages into a final aqueous solution’. The solution was then analysed using ICP-MS. Similar tests have been conducted by others (Guo, Baasner & Tsalev, 1997; Heitland & Koster, 2008) to determine inorganic As content in urine. Creatinine was determined using the Jaffe's reaction technique (picric acid added to alkaline urine) and measured using a spectrophotometer (Randviir & Banks, 2013).

As part of QA, some samples (3 samples per batch) were sent to Tracelabs in duplicates and the reported results were consistent (within 10%). Samples had unique labels and no participant identifying information was passed on to the lab.

3.8 Computer software and statistical analysis

Database: Microsoft Office Access 2010 was used to create a database for the project.

Statistical analysis of sample concentrations: Measured element concentrations in samples were subjected to statistical analysis performed using R statistical software (version 3.3.2)[5] and Microsoft Excel 2010.

Left-censored data (non-detects) were subjected to multiple imputation (using R) to substitute non-detects with randomly calculated values between 0 and the LOD. It has been reported that distribution-based imputation procedures may generate accurate estimates of population parameters when there are non-detects in a dataset (Baccarelli et al., 2005). Imputed values were used in subsequent data analyses.

The probability of data distribution was checked using the Shapiro–Wilk test (Razali & Wah, 2011; Yap & Sim, 2011). In addition, normal quantile-quantile (Q-Q) plots were used to show the probability distribution of element concentrations in blood and urine. The relationship between studied variables was investigated using Spearman's rank correlation test (and Pearson's linear regression). The differences between medians of datasets for soil and produce were examined using Kruskal-Wallis test (non-normally distributed data). The differences between the medians of unmatched groups of datasets (blood and urine concentrations) representing the various participants' subgroups were examined using the Mann-Whitney *U* test (Castano et al., 2012). However, since the finger-prick blood Pb samples were paired with the corresponding venous blood samples, the difference between the median blood Pb levels in finger-prick and the corresponding venous samples was examined using the Wilcoxon matched pairs test (Whitley & Ball, 2002). The probability value of $p < 0.05$ was set as the level of statistical significance. Data were described using median, geometric mean, 95% confidence interval (CI) values and percentiles (5^{th} and 95^{th}). Box and whisker plots were produced for soil results and blood Pb results using R.

Mixed-effects modelling: A linear mixed model (LMM) quantifies the relationships between a continuous dependent variable and various predictor variables, which may include *fixed-effect* parameters associated with one or more continuous or categorical covariates and *random effects* associated with one or more random factors (West, Welch & Galecki, 2007). In addition to the classical linear models (i.e., linear regression tests), we carried out mixed-effects modelling (also

[5] Available at: https://www.R-project.org/ Full citation for R programme
R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

known as 'repeated measures regression' test) to quantify the effects of selected variables (e.g., participant's age, gender, smoking status, consumption rate of allotment produce) on elemental concentrations in biological samples (the response variable). Mixed-effects modelling was carried out using the *lme* function in the *lme4* package for R (Bates et al., 2015).

Model solving and analysis: The models were coded in the *SimBiology* application of MATLAB (version R2016a) (MathWorks®). We used the stiff solver *ode15s* in the numerical simulations. This solver passes though 'stiff' parts of simulations with fewer 'time-steps' without compromising the simulation output – which speeds up the simulation and avoids generating un-necessarily large data. Relative tolerance was set at the default value (0.001) during simulations. The relationship between predicted data and the corresponding literature data were examined using linear regression (Pearson's correlation test) at statistical level of significance $p < 0.05$. Statistical analyses and processing of data were carried out using R and Microsoft Excel 2010.

3.9 Evaluation of model performance

We sought to evaluate the predictive performance of the modified models by comparing model simulations with data presented in the literature. The predictive accuracy of the models were assessed using the root mean square error (RMSE), calculated as (Ju et al., 2012; Walther & Moore, 2005):

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(C_{lit} - C_s)^2} \qquad (3.1)$$

Here, C_s and C_{lit} refer to the simulated data and literature data, respectively for a time point *i*, and *n* is the total number of data points. We expressed maximum C_s as a percentage of C_{lit}, to determine the magnitude of over- or under-prediction of the models. The correlations between the predicted and measured element concentrations in blood and urine were examined using linear regression.

3.10 Sensitivity analysis

Parametric sensitivity analysis was performed using sensitivity coefficients (SC) to determine which parameters were more sensitive to change. Values of SC were calculated using the following expression (Choudhury et al., 2001; Evans & Andersen, 2000).

$$SC = \left(\frac{\delta m}{\delta p}\right) \times \frac{p}{m} \qquad (3.2)$$

In equation (3.2), δm is the change in model output (m) resulting from the change (δp) in an input parameter value (p). SC of zero implies that there is no change in model output regardless of the parameter value used. Positive and negative SC values indicate an increase in model output with a

given increase in parameter value, and a decrease in model output with a given increase in parameter value, respectively. High SC values indicate high sensitivity of the model to that input parameter (Choudhury et al., 2001). Sensitivity analysis was performed in MATLAB (using full normalisation option); input values were varied by up to ±50%.

3.11 Chapter summary

The research methods commonly applied in exposure studies were utilised in this research, in order to generate the data needed to address the knowledge gap identified from the literature review. A pilot study was carried out to trial-out the methods and procedures. Before participants were recruited, suitable sample size for the study was estimated and potential study sites were identified within Scotland. Sample collection and subsequent laboratory analyses were carried out using conventional methods. Computer software and statistical methods used in processing data are described.

4. RESULTS AND DISCUSSION OF ENVIRONMENTAL SAMPLES

4.1 Introduction

The measured element concentrations in environmental samples are presented and discussed in this chapter. In reference to the conceptual exposure model described in chapter 2, soil results represent the concentrations at 'source', and produce results represent concentrations both at 'source' and 'pathway'. To put the recorded concentrations in human health context, results were compared to the commonly used criteria values (where available) used in assessing potential risk to human health. In addition, risk assessment was carried out using hazard quotient and the CLEA model.

4.2 Element concentrations in soil

The preferential adherence of soil and dust particles to hands and fingers occurs in the particle size range 0.5 – 65 μm (Juhasz, Weber & Smith, 2011). Therefore, testing of <63 μm soil particles was considered appropriate for investigating the soil ingestion exposure pathway. This is consistent with other studies (Loh et al., 2016; Zia et al., 2011). The laboratory soil test results are presented in Appendix G (part G1). Soil concentrations, expressed as mg kg^{-1} dry weight (dw), from the 16 sites were grouped according to the regional locations of the samples. The grouped results are presented using box and whisker plots in Fig. 4.1. For comparison, generic values used in assessing potential risk to human health in the UK are also indicated on the plots.

Concentrations of As ranged from 9 to 21 mg kg^{-1} (Dundee), 2 to 82 mg kg^{-1} (Edinburgh), 2 to 25 mg kg^{-1} (Glasgow) and 2 to 10 mg kg^{-1} (Kilbirnie). Soil samples from one site within Edinburgh (site A04) recorded As concentrations varying between 43 and 82 mg kg^{-1}, corresponding to the outliers above 40 mg kg^{-1} indicated in Fig. 4.1(a). Six samples from this site recorded As concentrations above the provisional Category 4 Screening Level (pC4SL) of 49 mg kg^{-1} for allotments land use (CL:AIRE, 2014). A number of <LOD concentrations of Cd were recorded in soil samples; these were subjected to data imputation (except for plots in Kilbirnie which recorded Cd values <LOD in all soil samples). The imputed values (between 0 and the LOD) were used in subsequent data analysis. Log-normal data distribution was used in imputation calculations, since the normality test showed that the soil results did not follow a normal distribution ($p < 0.05$). This is consistent with other studies involving environmental datasets (Baccarelli et al., 2005; Caudill et al., 2007; Huybrechts et al., 2002). Cd concentrations were generally low, with median concentrations across the four regions ranging from 0.5 to 1.2 mg kg^{-1}. One sample from a site in Edinburgh recorded Cd concentration of 5.2 mg kg^{-1} (an outlier), which is above the corresponding pC4SL value of 3.9 mg kg^{-1} (Fig. 4.1(b)).

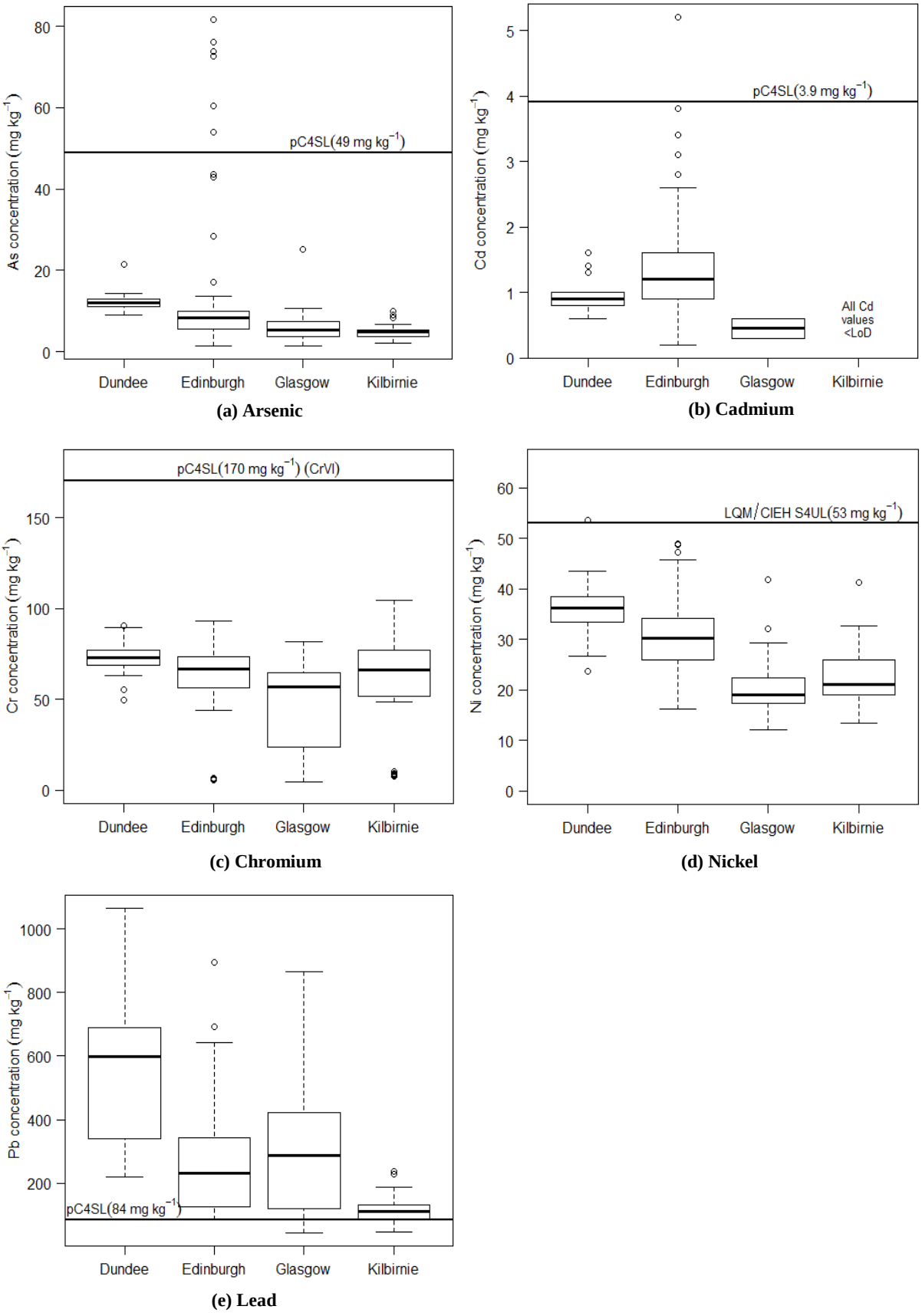

Fig. 4.1: Box and whisker plots of element concentrations in soil samples from the four sampling regions. (Box indicates median and interquartile range (IQR), whiskers indicate 1.5 x IQR and points are outliers).

Concentrations (mg kg^{-1}) of Ni ranged from 24 to 54 (Dundee), 16 to 49 (Edinburgh), 12 to 42 (Glasgow) and 13 to 41 (Kilbirnie). With the exception of a single sample from a plot in Dundee (site A36), all Ni concentrations were below the LQM/CIEH 'Suitable 4 Use Level' (S4UL) of 53 mg kg^{-1} (Nathanail et al., 2015). The recorded Cr (total) concentrations across the four regions varied between 4 and 105 mg kg^{-1}. Cr species of interest are trivalent Cr (Cr(III)) and hexavalent Cr (Cr(VI)) (Langard & Costa, 2007). The toxicity of Cr is usually assessed based on Cr(VI), because of the carcinogenic potential of the hexavalent species (Schlosser & Sasso, 2014). However, the recorded Cr (total) concentrations were well below 170 mg kg^{-1}, the pC4SL for Cr(VI). The recorded Pb concentrations (mg kg^{-1}) in soil samples ranged from 46 to 237 (Kilbirnie), 44 to 865 (Glasgow), 84 to 896 (Edinburgh) and 220 to 1065 (Dundee). Limited samples from Edinburgh and Kilbirnie recorded outlier Pb concentrations (Fig. 4.1(e)). Majority (95 %) of the soil Pb concentrations exceeded the corresponding pC4SL of 84 mg kg^{-1}, making Pb the element with the most values above its guideline value. The recorded Pb concentrations are within the range of those found in a previous survey of soils in the UK (Prasad & Nazareth, 2000), which recorded a maximum concentration of 1676 (the 90^{th} percentile) for UK urban soils.

A Kruskal-Wallis test indicated that the differences between the medians of As, Cr, Ni and Pb were not statistically significant ($p > 0.05$) in the four sampling regions, while medians of Cd were identical for sites in Dundee, Edinburgh and Glasgow. The similarity in the median concentrations suggests the common presence of these elements in the allotments, which may be indicative of the similarity of soil additives used by plot holders over the years.

Bioaccessibility fractions (as %) in soil samples varied from 33 to 92 (As), 18 to 93 (Cd), 6 to 30 (Cr), 17 to 90 (Ni) and 29 to 94 (Pb). The bioaccessibility of each element in the gastric phase was higher than that in the intestinal phase. This can be attributed to the higher solubility of these elements in an acid environment (gastric phase) than in a near-neutral environment (intestinal phase) (Li et al., 2015; Poggio et al., 2009). The bioaccessibility values of As in soil samples from site A04 were below 60%. A sample from site A05 in Edinburgh which had a Cd concentration in soil above the corresponding pC4SL recorded a bioaccessibility fraction of 27 %. By multiplying bioaccessibility fractions with total element concentrations recorded in soil samples, it was determined that the bioaccessible concentrations of As and Cd (the potentially soluble fractions in the gastrointestinal tract which could be absorbed) were below their respective pC4SLs (Fig. 4.1). This shows that the risk associated with potential ingestion of soil could be exaggerated if total concentrations (i.e., assuming 100% bioaccessibility) are used in risk assessment models. However, bioaccessible concentrations of Pb that exceed the pC4SL of 84 mg kg^{-1} were recorded in majority (90 %) of the plots. This indicates that Pb concentrations in soil at these plots provide a potential source of contamination.

Spearman's rank correlation tests indicated that the total concentrations of As, Cd and Pb in soil were strongly correlated ($\rho > 0.8$, $p < 0.05$) with their corresponding bioaccessible concentrations.

However, weak positive correlations ($\rho < 0.5$) were determined for Cr and Ni. This suggests that site-specific bioaccessible concentrations of As, Cd and Pb could be predicted from their soil concentrations. Similar observations have been made in other studies. For example, the study by Barsby et al. (2012) identified strong linear correlations ($r > 0.9$) between measured total and the respective bioaccessible concentrations for a number of potentially toxic elements including As, Cd and Pb. A positive linear correlation ($r > 0.7$) between total Pb in soil and bioaccessible Pb was also observed by Farmer et al. (2011) in their study of Pb in urban soils from Glasgow.

4.3 Element concentrations in produce

The concentrations of the elements varied between the sites investigated and the type of allotment produce. Table 4.1 shows the range of element concentrations, expressed as mg kg^{-1} fresh weight (fw), recorded in allotment produce samples, grouped according to produce categories used in the CLEA model (Environment Agency, 2009e). Detailed element concentrations in produce samples are given in Appendix G (part G2).

Table 4.1: Summary of element concentrations according to produce category

CLEA produce category	Range of element concentrations (mg kg^{-1} fw)				
	As	Cd	Cr	Ni	Pb
Green vegetables[1]	<0.01-1.62	<0.01-0.70	<0.01-11.81	<0.01-5.89	<0.01-15.77
Root vegetables[2]	0.03-2.14	<0.01-1.87	<0.01-9.84	<0.01-7.44	<0.01-3.81
Tuber vegetables[3]	<0.01-0.91	<0.01-0.06	<0.01-0.17	<0.01-0.72	<0.01-2.49
Herbaceous fruit[4]	0.16-0.91	<0.01-0.30	<0.01-4.84	<0.01-2.20	0.13-1.78
Shrub fruit[5]	0.11-1.65	<0.01-0.39	<0.01-0.31	<0.01-1.69	<0.01-5.15
Tree fruit[6]	0.30-0.97	<0.01	<0.01-0.27	<0.01-0.31	<0.01-0.87

[1]Includes beans, cabbage, kale, cauliflower, lettuce, spinach, peas, herbs, broccoli, oriental green, yam leaves, chives, fennel, pak choi, rocket, chard and corn
[2]Includes carrot, turnip, swede, onion, shallot, garlic, leek, rhubarb, beetroot, artichoke, kohlrabi, parsnip and radish
[3]Refers to potato
[4]Includes cucumber, courgette and tomato
[5]Includes berries and currants
[6]Includes apple, pear, plum and green gauge

The recorded maximum element concentrations (mg kg^{-1} fw) were 2.1 (As), 1.9 (Cd), 11.8 (Cr), 7.4 (Ni) and 15.8 (Pb), measured in the green and root vegetables. Out of the samples that recorded detectable Cr (total) concentrations, randomly selected samples ($n = 25$) were subjected to Cr(VI) testing. All Cr(VI) concentrations were <LOD, indicating negligible levels of Cr(VI) in samples investigated. This was expected because Cr(VI) does not occur naturally but arises mainly from anthropogenic sources, and it has also been reported by Langard & Costa (2007) that chemical reduction of Cr(VI) to Cr(III) occurs in plants, which reduces the potential of exposure to Cr(VI) through plant consumption.

For purposes of statistical analysis, the concentrations of each element in a plot were pooled together (irrespective of the type of allotment produce). For values >LOD, plot-specific median concentrations (mg kg^{-1} fw) of the elements varied from 0.13 to 0.99 (As), 0.04 to 1.06 (Cd), 0.06 to 3.37 (Cr), 0.04 to 1.52 (Ni) and 0.17 to 3.45 (Pb). The median concentrations (mg kg^{-1} dw) of Pb in allotment produce and soil were found to be weakly correlated (Spearman's $\rho = 0.38$, $p < 0.05$), while As, Cd, Cr and Ni were not correlated ($p > 0.05$). This is contrary to hypothesis '*h1*' that '*there is a good correlation between element concentrations in allotment soil and the corresponding concentrations in produce*.' The weak / lack of correlation between concentrations in soil and produce could be attributed to the plant uptake of these elements through other pathways, such as aerial deposition which could provide a source of element accumulation in aerial parts of vegetables (Li et al., 2015).

The bioaccessible fractions (%) of the elements in allotment produce samples ranged from 13 to 94 (As), 10 to 89 (Cd), 14 to 80 (Cr), 14 to 82 (Ni) and 30 to 81 (Pb). The medians of the bioaccessibility values were 40% (As), 17% (Cd), 35% (Cr), 35% (Ni) and 45% (Pb). These bioaccessibility values indicate that not all quantities of the elements ingested through consumption of allotment produce would be available for potential uptake in the body, thus reducing the potential for exposure to these elements. Similar Cr and Pb results were found by Intawongse & Dean (2008); however their results for Cd (61-90%) and Ni (43-91%) were generally higher that our results. These variabilities suggest that bioaccessibility may be influenced by the plant species and the element concentrations in samples (Pan et al., 2016).

4.3.1 Soil-to-plant transfer factors

Where soil and allotment produce samples were collected from the same spot, soil-to-plant transfer factor (TF) of each element was estimated as the ratio of that element concentration in allotment produce to its concentration in soil. The estimated TF values (regardless of plant type) are presented in Table 4.2, along with selected values obtained from the literature.

Table 4.2: Summary of soil-to-plant TF values

Element	Range of estimated TF values	TF values from the literature
As	0.0036 – 0.4675	0.00043 – 0.0011[a] 0.001[c]
Cd	0.004 – 0.791	0.0014 – 0.052[b] 0.192 – 0.778[c]
Cr	0.0001 – 0.2647	0.008 – 0.029[c] 0.003 – 0.22[d]
Ni	0.0002 – 0.4907	0.037 – 0.039[c] 0.03 – 0.89[d]
Pb	0.0001 – 0.0889	0.008 – 0.065[c] 0.001 – 0.432[d]

[a]Environment Agency (2009d), [b]Environment Agency (2009c)
[c]Jolly et al. (2013), [d]Intawongse (2007)

The higher the TF value the higher the potential element accumulation by plants, suggesting a potential for increased exposure through human diet. However, a comparison of the estimated TF values with those in the literature suggests that TF values could vary considerably (Table 4.2), and are not constant for a specific element in a specific vegetable (Swartjes, Versluijs & Otte, 2013).

A number of studies have used sequential extraction tests to relate soil to plant element concentrations. For example, a study by Chojnacka et al. (2005) reported that there was a good correlation between the concentrations of a number of elements (including As, Cd, Cr and Pb) in plants and the corresponding concentrations obtained from soil extraction test using 2 % ammonium citrate solution. In another study, Wang et al. (2004) demonstrated that the phytoavailability of selected trace elements strongly correlated with the soil-extracted fractions of the elements using calcium chloride. These sequential extraction methods provide alternative means of estimating soil to plant transfer of elements present in soil.

4.3.2 Potential health risk assessment from consumption of produce

The potential risk to human health resulting from consumption of allotment produce was estimated using the hazard quotient (HQ) expressed as (Nabulo, Young & Black, 2010; Jolly et al., 2013):

$$HQ = \frac{\left((DI \times C_{Fveg})/W_B\right)}{RfD}, \qquad (4.1)$$

where HQ represents the hazard quotient resulting from ingestion of an element through consumption of allotment produce, DI denotes the daily intake of allotment produce (kg day^{-1}), C_{Fveg} represents the element concentration in allotment produce (mg kg^{-1} fw), W_B is the individual body weight (bw) (kg), and RfD is the reference dose (mg kg^{-1} bw day^{-1}) defined as the maximum tolerable daily intake of an element that has no adverse effect. In Eq. (4.1), the numerator represents the average daily intake (ADI) (mg kg^{-1} bw day^{-1}). If HQ > 1, then the ADI of a particular element exceeds its RfD, indicating that there is a potential risk associated with that element.

We used information provided by the participants (allotment produce consumed and their fresh weight) covering up to 12 consecutive months to calculate their DI. Values of W_B were obtained from participants' records and C_{Fveg} were adjusted to take account of the bioaccessible fractions. Where the type of allotment produce consumed was not sampled or tested, plot-specific median element concentrations and bioaccessibility values were used in the calculations. However, where a median bioaccessibility value of an element was not calculated, we assumed 100% bioaccessibility of the total element concentration. The ADI for each participant was calculated for each month. Subsequently, monthly ADI values were separated into 'summer' months (April to September) and 'winter' months (October to March) to reflect seasonal variation in allotment-related activities and consumption of allotment produce (as summarised in Appendix E, part E4). This is consistent with the approach taken by Environment Agency (2009e). Using RfD values obtained from the

literature, we calculated element HQ for each participant. A summary of the ADI, RfD and HQ is given in Table 4.3.

Table 4.3: Summary of ADI, RfD and HQ

Element	ADI (maximum) (mg kg^{-1} bw day^{-1})		RfD (mg kg^{-1} bw day^{-1})	HQ (maximum)	
	Summer	Winter		Summer	Winter
As	$2.9x10^{-4}$	$2.9x10^{-4}$	$3.0x10^{-4}$ [a]	0.97	0.96
Cd	$1.2x10^{-4}$	$1.3x10^{-4}$	$1.0x10^{-3}$ [a]	0.12	0.13
Cr	$8.7x10^{-4}$	$8.7x10^{-4}$	1.5 [a,b]	$5.8x10^{-4}$	$5.8x10^{-4}$
Ni	$1.7x10^{-3}$	$1.4x10^{-3}$	$2.0x10^{-2}$ [a]	$8.5x10^{-2}$	$6.9x10^{-2}$
Pb	$3.2x10^{-3}$	$3.1x10^{-3}$	$3.6x10^{-3}$ [c]	0.88	0.86

[a]RfD obtained from the Integrated Risk Information Systems (IRIS) database provided by USEPA (www.epa.gov/iris); [b]RfD for Cr-III; [c]Song et al. (2009)

The similarity in the ADI values in summer and winter seasons could be because some participants stored frozen produce for consumption in the winter, especially those who had large amount harvest during the 'growing season'. This suggests overall similarities in consumption patterns during both seasons, even though the consumption patterns for a given individual may vary between the seasons.

Values of HQ for individual elements were generally greatest for As, followed sequentially by Pb, Cd, Ni and Cr. However, all elements recorded HQ <1. The HQ values in Table 3 indicate that the potential risk is higher for As and Pb, but not significant enough to warrant concern. Although Pb was identified as a potential source of contamination in soil in most plots, calculated HQ values did not identify potential risk from the consumption of allotment produce. This indicates that based on the participants' consumption of allotment produce records, no potential risk to the elements was identified from this source.

Given the potentially higher risk identified from HQ for As and Pb, the calculated ADI values for As and Pb (Table 4.3) were compared with the values for average dietary exposure for adult consumers in Europe, as provided by the European Food Safety Authority (EFSA). The maximum ADI of 3.2 (µg kg^{-1} bw day^{-1}) for Pb exceeds the average Pb dietary exposure for an adult consumer in Europe of 2.43 (µg kg^{-1} bw day^{-1}) by approximately 30% (EFSA, 2010). However, the maximum ADI for As represents approximately 50% of the EFSA value of 0.56 (µg kg^{-1} bw day^{-1}) for an average adult consumer (EFSA, 2009). This suggest that participants would need to consume at least twice the current amount of produce in order to exceed the EFSA value for As.

4.3.3 Site-specific risk assessment using the CLEA model

The potential risk to As and Pb was evaluated further using the CLEA model (version 1.071).[6] Site-specific health risk assessment was carried out using the maximum recorded element concentrations in soil and produce. In addition, participant specific produce consumption data (average values) were used in the model, in lieu of the default produce consumption rates built into the CLEA model. Simulations were carried out for CLEA age classes 17 and 18 only (corresponding to ages 16-65 and 65-75 years, respectively) (Environment Agency, 2009a), because study participants were aged 30 years old and above.

Model predicted oral average daily exposure (ADE) to health criteria value (HCV) ratios were less than 0.1, indicating very low levels of exposure to the elements (i.e., very low hazard indices). The soil assessment criteria values (oral HCV) calculated by the model for As and Pb were up to 3 and 4 orders of magnitude, respectively, which are well above the recorded soil concentrations. This indicates that the soil concentrations (As, Pb) do not present significant contamination source.

4.4 Soil and produce Pb isotope ratios

Pb isotope ratio analysis is a useful means of identifying sources of Pb exposure in routine investigations (Oulhote et al., 2011). The site-specific mean isotopic ratios for soil and produce samples are plotted in Fig. 4.2. The soil values ranged from 1.103 to 1.161 ($^{206}Pb/^{207}Pb$) and 2.315 to 2.498 ($^{208}Pb/^{207}Pb$). Values for produce samples ranged from 1.081 to 1.134 ($^{206}Pb/^{207}Pb$) and 2.355 to 2.422 ($^{208}Pb/^{207}Pb$). Mean isotopic ratios for petrol Pb (indicating an anthropogenic source), Scottish coal and Leadhills Pb ore (indicating geogenic sources) (MacKinnon et al., 2011) are also plotted. Error bars associated with the isotopic ratios were expressed as ± *standard deviation* (Farmer et al., 2011). To enhance legibility, only the error bars for petrol Pb, Scottish Coal and Leadhills Pb ore (Farmer et al., 2011) were included in Fig. 4.2. Although not shown in Fig. 4.2, the errors (expressed as relative standard deviation (RSD)) for isotopic ratios for soil and allotment produce samples were up to 1.5 % (i.e., RSD of 0.015).

[6] Available at: https://www.gov.uk/government/publications/contaminated-land-exposure-assessment-clea-tool

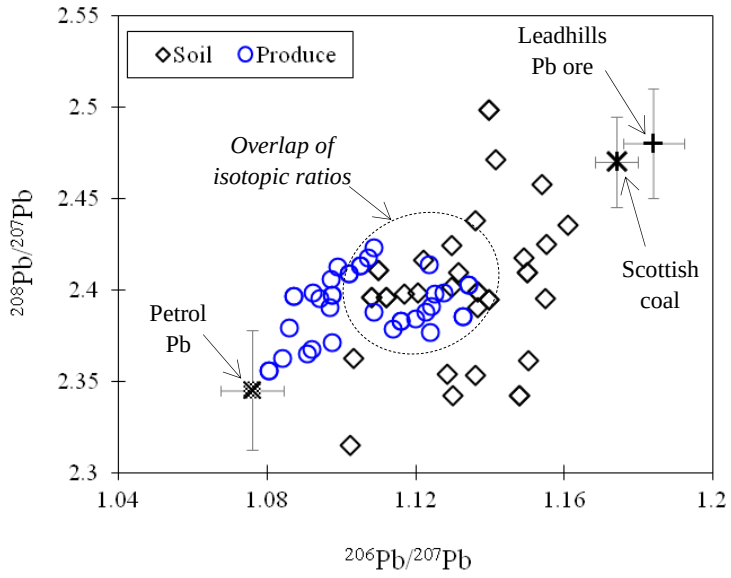

Fig. 4.2: Plot of $^{206}Pb/^{207}Pb$ ratio against $^{208}Pb/^{207}Pb$ ratio for soil and produce samples.
Also indicated are the mean Pb isotopic ratios for petrol Pb, Scottish coal and Leadhills Pb ore. Error bars are ± *standard deviation*. Error bars for soil and produce data were excluded to enhance legibility of the figure.

The Pb isotope ratios (Fig. 4.2) extend between the values reported for leaded petrol and indigenous geological Pb ores (Scottish coal and Landhills Pb ore), which suggests that Pb in soil and produce are linked to multiple sources (rather than a single source). In particular, soil Pb appears to originate from a wide range of sources, although geological Pb seems to be the dominant source of soil Pb. Some of the values for produce samples lie close to the value for leaded petrol, which points to potential anthropogenic source of Pb in those produce samples. Atmospheric deposition can contribute to the deposition of anthropogenic Pb in vegetables (Li et al., 2012). Although leaded petrol is no longer in use in the UK, it has been reported that exhaust emissions present a minor, but finite, contemporary source of Pb (MacKinnon et al., 2011). Resuspension of soil near industrial facilities and highways have also been reported to contribute to Pb in aerial particulate matter (Young et al., 2002). The lack of a single Pb source in soil and produce supports our finding that Pb in produce and soil were weakly correlated (section 4.3). The soil and produce isotopic ratios overlap in the central area between both extremes of the spread of data points (Fig. 4.2). Only 3 allotment plots had their soil and produce ratios lying within the area of overlap; none of the remaining data points for soil and produce came from the same site. This further demonstrates the varying sources of Pb in soil and produce.

4.4.1 Binary mixing model of Pb isotope ratios

The relative contribution of natural and anthropogenic Pb in soil and produce samples was calculated using a binary mixing model (Li et al., 2012; Monna et al., 1997), using the following expressions:

$$X_{nat}\% = \frac{(206Pb/207Pb)_{anth} - (206Pb/207Pb)_{sample}}{(206Pb/207Pb)_{anth} - (206Pb/207Pb)_{nat}} \times 100, \quad (4.2)$$

$$X_{anth}\% = \frac{(206Pb/207Pb)_{sample} - (206Pb/207Pb)_{nat}}{(206Pb/207Pb)_{anth} - (206Pb/207Pb)_{nat}} \times 100, \quad (4.3)$$

where X_{nat} and X_{anth} represent the contributions (%) of natural (or geogenic) and anthropogenic sources, respectively; $(206Pb/207Pb)_{nat}$, $(206Pb/207Pb)_{anth}$, and $(206Pb/207Pb)_{sample}$ are the Pb isotopic ratios ($^{206}Pb/^{207}Pb$) for natural (geogenic), anthropogenic and samples, respectively. The $^{206}Pb/^{207}Pb$ isotopic ratios for Leadhills Pb ore (1.184) and petrol Pb ore (1.076) were used for geogenic and anthropogenic sources, respectively.

The percentage contributions of Pb from geogenic and anthropogenic sources calculated using the biliary mixed model are presented in Fig. 4.3, which shows that soil Pb originates from both geogenic and anthropogenic sources at nearly equal proportions of 25-79% and 21-75%, respectively. However for produce samples, anthropogenic source of Pb (46-96%) is more dominant than geogenic source (4-54%). The calculated dominance of anthropogenic source of Pb in produce samples is consistent with Fig. 4.2, which shows data points for produce extending towards petrol Pb ratio plot.

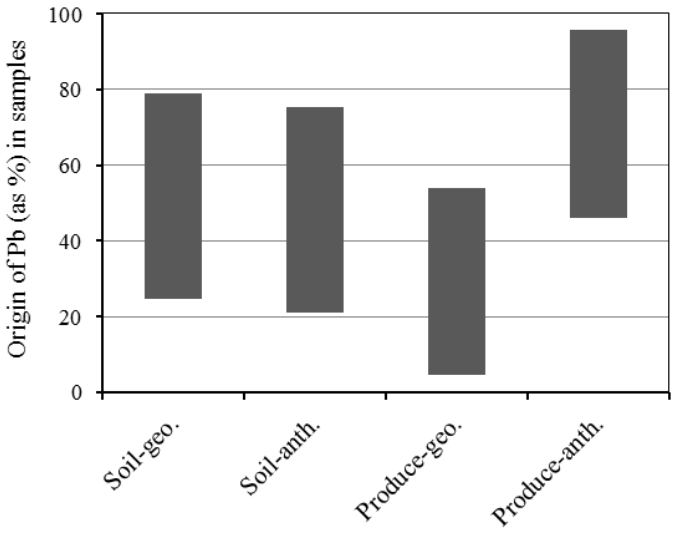

Fig. 4.3: Calculated percentage contribution of Pb derived from geogenic (geo.) and anthropogenic (anth.) sources in soil and produce samples

4.5 Hand moist-wipes

In addition to the soil and produce, element concentrations were determined in the hand-moist wipe samples, which were collected when participants were working at their allotments. Where detected, element weights (µg) in hand moist-wipes varied between 0.3 – 6.7 (As), 0.4 – 1.9 (Cd), 0.5 – 34 (Cr), 0.9 – 125 (Pb) and 0.8 – 19 (Ni). These results suggest that hand-to-mouth contact could add to human exposure in adults, although the inadvertent exposure pathway is more prevalent among children. For adults, the major pathway of exposure is the consumption of allotment produce (as discussed in chapter 2, section 2.5). Therefore, no efforts were made to investigate this potential 'pathway' further.

4.6 Chapter summary

Widespread elevated Pb concentrations were recorded in soil, making Pb the element with the most values above its guideline value. A weak correlation between Pb in soil and produce was determined, while other elements were not correlated. These findings contradict hypothesis '*h1'* that *"there is a good correlation between element concentrations in allotment soil and the corresponding element concentrations in produce"*. It has been established that risk associated with potential ingestion of soil could be exaggerated if total element concentrations (i.e., assuming 100% bioaccessibility) are used in risk assessment models. Hazard quotients calculated from produce consumption records indicated potential higher risk to As and Pb, but overall, no significant risk was identified to warrant concern. Although the maximum ADI values for Pb exceed the corresponding EFSA value by approximately 30%, further site-specific risk assessment using the CLEA model has not identified a significant concern to human health.

5. RESULTS AND DISCUSSION OF BIOLOGICAL SAMPLES

5.1 Introduction

The element concentrations in the biological samples (blood and urine) are presented and discussed in this chapter. These results were compared with commonly used biomonitoring reference values used in assessing potential risk to human health. Associations between the elemental concentrations in biological samples and various participants' characteristics were explored statistically and the results from the Pb isotopic analysis are also presented and discussed.

5.2 Blood Pb concentrations

The laboratory test results of Pb concentrations in venous blood samples (n = 108) are summarised in Appendix G (part G3). Pb concentrations varied between 3.12 and 30.60 µg dL^{-1}. Approximately 56% of the concentrations were below 24 µg dL^{-1}, while 89% of the concentrations were below 20 µg dL^{-1}. Apart from one sample, from a male participant with a recorded concentration of 30.6 µg dL^{-1}, the remaining concentrations were below 30 µg dL^{-1}. Seven concentrations which were above 24 µg dL^{-1} were outliers. The Shapiro-Wilk test indicated that the results did not follow a normal distribution ($p < 0.05$). In addition, Fig. 5.1 (A) shows that the data did not fit a normal distribution since the data points do not plot evenly along the straight Q-Q line. The data were skewed towards the lower concentrations as shown in Fig. 5.1 (B). However when the results were log-transformed, they were found to be log-normally distributed ($p > 0.05$). It is common for biomonitoring data to be log-normally distributed (Morton et al., 2014).

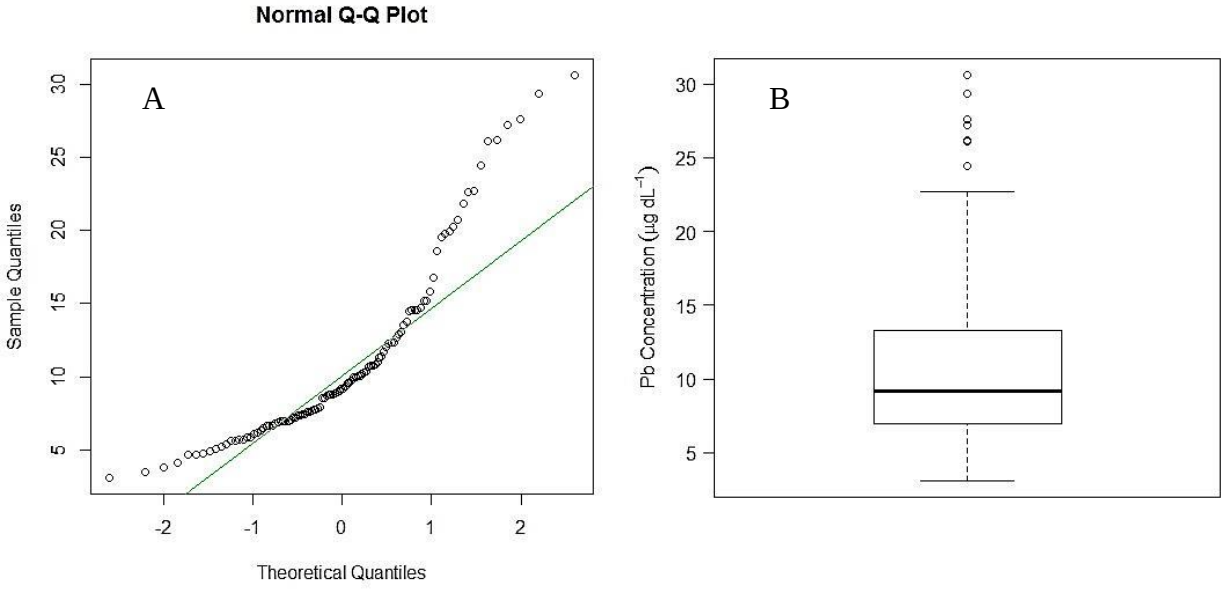

Fig. 5.1: The probability distribution and asymmetry of venous blood Pb concentrations.
(A) normality test using Q-Q plot, (B) box and whisker plot shows that the data is asymmetric and skewed towards the lower concentrations, the dots represent outliers.

Since the blood Pb concentrations were not normally distributed, the spread of blood Pb data were described using median and geometric mean. In addition, the 95% confidence intervals (CI) and percentiles (5^{th} and 95^{th}) were estimated. The arithmetic mean was also reported. Table 5.1 presents a summary of the statistics of the blood Pb data. In addition, the data were sub-grouped according to four participants' characteristics (age, gender, smoking status and produce consumption rate); factors that can influence blood Pb content (Bocca et al., 2011).

Table 5.1: Summary statistics for blood Pb concentrations (µg dL^{-1})

	N	Min	Max	Median	GM (GSD)	Mean (CI at 95%)	P5	P95
All participants	108	3.12	30.60	9.19	9.73 (1.65)	11.1 (9.91-12.22)	4.72	24.17
Age (years)								
30-50	14	4.89	23.61	9.13	9.34 (1.58)	10.3 (7.40-13.25)	4.99	18.15
51-60	33	3.50	26.19	9.18	8.86 (1.54)	9.8 (8.04-11.52)	4.68	18.60
61-70	43	3.12	27.19	9.99	10.20 (1.71)	11.7 (9.77-13.62)	4.20	22.54
71-80	18	5.65	30.60	8.69	10.65 (1.74)	12.5 (8.51-16.44)	5.81	29.54
Gender								
Males	60	3.12	30.60	8.89	9.80 (1.75)	11.5 (9.68-13.37)	5.19	26.24
Females	48	3.79	23.61	10.03	9.64 (1.52)	10.5 (9.21-11.77)	4.72	20.15
Smoking status								
Smokers	5	5.7	14.69	8.94	9.72 (1.50)	10.4 (5.32-15.41)	6.15	14.67
Ex-smokers	32	3.79	30.60	8.77	10.10 (1.76)	11.9 (9.14-14.72)	4.92	28.16
Non-smokers (and 1 unknown smoking status)	71	3.12	26.19	9.76	9.57 (1.61)	10.7 (9.46-11.98)	4.68	22.24
Average produce consumption rate (g fw kg^{-1} bw day^{-1})								
2 – 3	11	6.96	24.47	9.18	10.04 (1.53)	11.0 (7.12-14.97)	6.97	22.36
1 – 2	17	4.78	27.19	9.19	10.08 (1.68)	11.6 (8.0-15.17)	5.24	26.33
<1	80	3.12	30.60	9.20	9.61 (1.66)	11.0 (9.63-12.29)	4.63	22.72

N (number of samples), GM (geometric mean), GSD (geometric standard deviation), CI (confidence interval of means at 95%), P5 (5^{th} percentile), P95 (95^{th} percentile), fw (fresh weight of produce), bw (body weight of participant).

The data summarised in Table 5.1 shows that median blood Pb levels were similar across the various participants' subgroups. A statistical examination of the data using the Mann-Whitney *U* test confirmed this observation. The differences between the medians of blood Pb concentrations for the various categories of age, gender, smoking status and produce consumption rate were not statistically significant ($p > 0.05$). This indicates that blood Pb levels were not significantly dependent on age, gender, smoking status or rate of produce consumption. Our findings are consistent with those published by Moffat (1989), who found no evidence of association of locally grown vegetables with raised blood Pb levels.

We carried out further evaluation of the blood Pb data using mixed-effects modelling, where we treated a participant's age, gender, smoking status and produce consumption rate as 'fixed' effects, while participants were treated as 'random' effects. Mixed-effects models did not indicate

statistically significant associations between a participant's age, gender, smoking status and produce consumption rate to their blood Pb levels ($p > 0.05$), which confirms the findings obtained from the Mann-Whitney *U* tests. R codes used for the mixed-effects models and the model results are included in Appendix H (part H1).

In terms of human toxicity, Pb is a 'non-threshold' substance, and therefore there is no known safe exposure level for Pb (Oulhote et al., 2011). However for comparison with UK guidelines for occupational exposure, the recorded Pb concentrations are below the UK Health and Safety Executive (HSE) occupational exposure action levels of 50 and 25 µg dL^{-1}, for males and females, respectively.[7] The upper 95% confidence intervals of means were 13.4 µg dL^{-1} (male) and 11.8 µg dL^{-1} (female), which are approximately 27% and 47% of the respective HSE actions levels.

In the United States of America (USA), the Centre for Disease Control and Prevention (CDC) set an 'action level' of blood Pb at 5 µg dL^{-1} (CDC, 2012). The basis for choosing this action level was that it represents 97.5th percentile of the National Health and Nutritional Examination Survey (NHANES) generated blood lead level distribution in children 1-5 years old; but the action level was not related to a defined risk. In addition, this action level is relevant to children who are more sensitive than adults. The provisional Category 4 Screening Level (pC4SL) of 84 mg kg^{-1} for allotment land use (i.e. generic assessment criterial value for Pb used in the UK) was derived using the 5 µg dL^{-1} CDC action level for blood Pb (CL:AIRE, 2014). The median blood Pb concentration recorded from our study (9.2 µg dL^{-1}) is approximately twice the CDC action level.

In the UK, biomonitoring of blood Pb is routinely carried out in occupationally exposed populations. Occupational exposure to Pb is common among those who work in the manufacture and recycling of batteries, construction industries, glass and pottery making and chemical related industries (Morton et al., 2009). None of the study participants worked in any of these occupations. However, there is no routine public screening for Pb toxicity in the non-occupationally exposed UK population (Kar-Purkayastha et al., 2011). Therefore data on blood Pb levels in the general non-occupationally exposed UK public is scarce, and we found no recently published data on blood Pb levels in un-occupationally exposed UK population. The 1995 health survey of the general population in England recorded mean blood Pb levels of 3.5 µg dL^{-1} for adult males and 2.7 µg dL^{-1} for adult females (Morton et al., 2009). In another study (Watt et al., 1996), the blood Pb levels recorded for 150 mothers from Glasgow (Scotland) ranged from 1.04 to 24.24 µg dL^{-1}, and the mean blood Pb levels (divided according to exposure to water Pb) varied from 3.64 to 6.63 µg dL^{-1} among those who had Pb in water. The subjects that were not significantly exposed to Pb from tap water (i.e., water Pb concentration < 2 µg L^{-1}) had blood Pb levels ranging from 1.04 to 21.13 µg dL^{-1}, with a mean of 3.64 µg dL^{-1} (Watt et al., 1996). These blood Pb levels from Glasgow are higher than those recorded in adult females during the 1995 health survey in England.

[7] Available at: http://www.hse.gov.uk/lead/exposure.htm

Changes in blood Pb levels in young people (aged 14 to 17 years) living in Edinburgh (Scotland) were studied by Macintyre et al. (1998) using blood data dated approximately 8 years apart. The range of blood Pb levels recorded in 1983-1985 ranged from 4.1 to 28.4 µg dL^{-1} (mean of 11.0 µg dL^{-1}); the corresponding values were 1.4 to 15.6 µg dL^{-1} (mean of 4.0 µg dL^{-1}) in 1992-1993 (Macintyre et al., 1998). These values indicated a declining trend in blood Pb levels in the young population studied, which was attributed to the reduction in household water Pb levels because of improved water treatment and removal of lead water pipes (Macintyre et al., 1998).

A study by Moffat (1989) determined blood Pb concentrations in a population living in a former lead mining area in Southern Scotland (Leadhills and Wanlockhead). As part of their study, blood Pb levels in a control population (living in Moniaive) where there was no obvious Pb pollution was also measured. The mean and standard deviation were 11±4.6 µg dL^{-1} (males, n = 43) and 8.3±3.1 µg dL^{-1} (females, n = 41) for the control adult population. These concentrations were slightly lower than blood Pb levels from the population that was considered exposed to Pb in the former mining area, whose blood Pb levels were reported to be 15.9±5.4 µg dL^{-1} (males, n = 55) and 12.4±5.2 µg dL^{-1} (females, n = 71) (Moffat, 1989). The findings by Moffat (1989) appear to suggest high background levels of Pb in the Scottish population studied. In addition, the mean blood Pb levels recorded by Moffat (1989) are consistent with the mean blood Pb level (11.0 µg dL^{-1}) reported by Macintyre et al. (1998) in young people living in Edinburgh during 1983-1985.

In our study, levels of Pb in drinking water samples were not determined, mainly because water Pb levels is now strictly regulated. In Scotland, the regulatory limit for Pb levels in drinking water, called the Prescribed Concentration or Value (PCV), was reduced from 2.5 µg dL^{-1} to 1.0 µg dL^{-1} in December 2013 (Health Protection Scotland, 2016)[8]. In addition, none of the participants indicated in the questionnaire that they had lead paint in their homes. It is possible that background levels of Pb in central Scotland have been higher than other parts of the UK. However, more data are needed to confirm this. The blood Pb results from our study presents an important contribution to the literature due to the lack of recent comparable blood Pb data in the UK, particularly in relation to a population that consume produce from their allotments. Our results are consistent with the findings by Moffat (1989) and Macintyre et al. (1998), which would support our hypothesis *'h2'* should additional recent data confirm similar blood Pb levels in the local population.

The concentrations of Pb in finger-prick dried blood spot (DBS) samples (for the 108 samples paired with venous samples) varied between <LOD and 28.9 µg dL^{-1}. Out of the paired samples, 38 DBS samples recorded concentrations below the LOD (minimum 0.003 µg L^{-1}). The non-detect concentrations were recorded mainly in the filters with the lowest amount of dried blood spots. A summary of blood Pb concentrations for venous and finger-prick samples is given in Table 5.2.

[8] Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=5678

Table 5.2: Results of blood Pb (µg dL^{-1}) by sample collection method

Method	N	N < LOD	Range	Median	GM ± GSD
Venous	108	0	3.12 – 30.60	9.19	9.74 ± 1.65
Finger-prick	108	41	2.31 – 28.87[#]	12.14[#]	11.15 ± 1.79[#]

N (number of paired samples), LOD (limit of detection), GM (geometric mean), and GSD (geometric standard deviation).

[#]Relate to concentrations >LOD.

The correlations between Pb in venous blood and the corresponding DBS samples were observed to vary among the participants. High correlations (r^2 = 0.71) were noted for 2 participants, moderate correlations (r^2 between 0.44 and 0.62) were noted for 5 participants, and low / weak correlations (r^2 between 0.12 and 0.34) were noted for 3 participants. Poor or no correlations ($r^2 \leq 0.07$) were observed for 22 participants. When the concentrations for venous blood samples from all participants were plotted against the corresponding concentrations for DBS samples, a scatter gram presented as Fig. 5.2 was obtained, which shows no clear correlation ($r^2 < 0.01$, $p > 0.05$) between the two datasets. The data points do not plot consistently along (or equally scatter above and below) the 45-degree line included in Fig. 5.2. Furthermore, the Wilcoxon matched pairs test indicated that the difference between the medians of the two paired datasets was statistically significant (p = 0.02), further confirming the lack of correlation between the two datasets. Therefore, we did not use DBS samples to predict Pb levels in venous blood.

Some studies have found good correlations between blood Pb levels in samples collected by finger-prick and venous methods, particularly in children (e.g., Shen et al., 2003; Funk et al., 2015). However, venous blood Pb is still the most useful screening and diagnostic test for Pb exposure (ATSDR, 2007b).

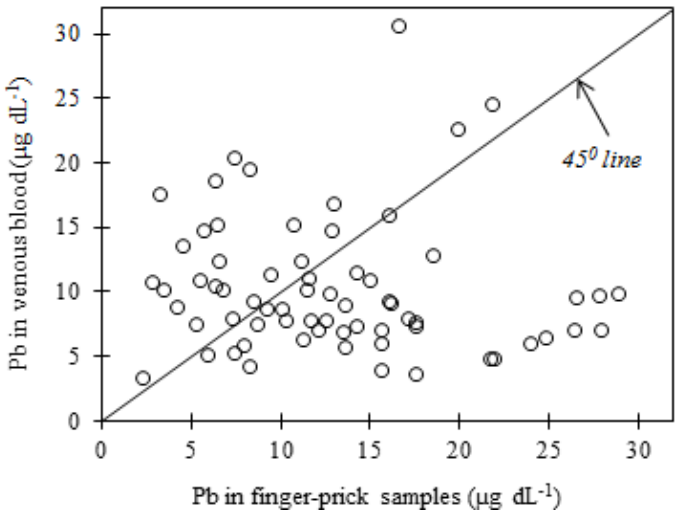

Fig. 5.2: Comparison of Pb concentrations in samples of venous blood and finger-prick

5.2.1 Pb isotope ratios

The comparative abundance of Pb isotope ratios in any give Pb source provides a 'fingerprint' of the Pb from that source, because the isotope ratios remain constant over time (Patel et al., 2008). The Pb isotopic ratios measured in soil, produce and blood samples were plotted for each plot and the corresponding participant; examples of these plots are given in Appendix G (part G3). The participant-specific mean isotopic ratios for blood samples ranged from 1.123 to 1.145 ($^{206}Pb/^{207}Pb$) and 2.390 to 2.438 ($^{208}Pb/^{207}Pb$). Fig. 5.3 shows the values for blood samples aggregate where mean isotopic ratios for soil and produce overlap, which may be indicative of the association between blood Pb and Pb in either soil or produce where the isotopic overlap occurs. The close aggregation of blood Pb isotopic ratios also suggest that soil and produce were unlikely to be the only sources of blood Pb. The errors (expressed as RSD) for isotopic ratios for blood samples were up to 1%.

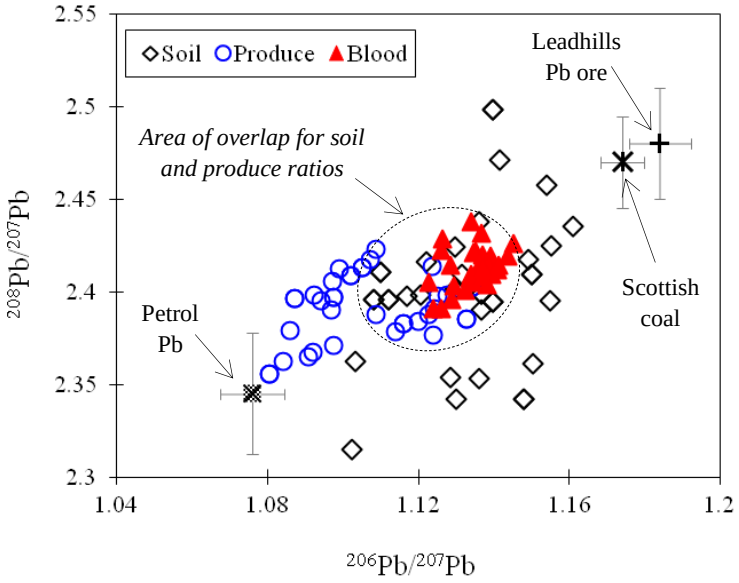

Fig. 5.3: Plot showing Pb isotope ratios for soil, produce and blood.
Also indicated are the mean Pb isotopic ratios for petrol Pb, Scottish coal and Landhills Pb ore. Error bars are ± *standard deviation*. Error bars for soil, produce and blood data were excluded to enhance legibility of the figure.

Using the binary mixed model equations (4.2) and (4.3), the calculated contributions (%) of geogenic and anthropogenic sources of Pb to blood Pb are shown in Fig. 5.4, which suggests that on average, geogenic Pb contributes a higher proportion (43-64%) of blood Pb than anthropogenic Pb (36-57%).

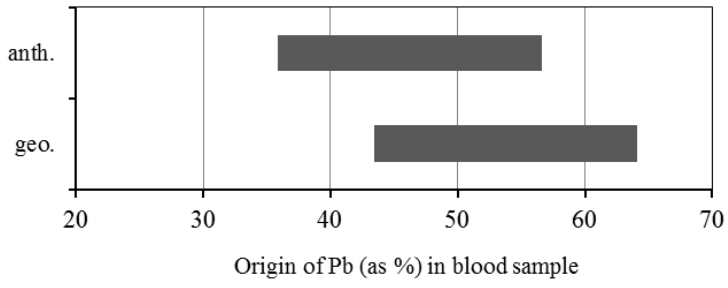

Fig. 5.4: Calculated percentage contribution of Pb derived from geogenic (geo.) and anthropogenic (anth.) sources in blood samples.

5.3 Element concentrations in urine

A summary of the recorded urinary concentrations of inorganic arsenic (iAs), Cd, Cr and Ni is presented in Appendix G (part G4). The limits of detection varied between 0.005 and 0.01 µg L^{-1}. The concentrations were expressed with respect to urinary creatinine. The concentrations of urine samples that had creatinine values below 0.3 g L^{-1} (n = 27) were discarded because the samples were considered too dilute (WHO, 1996). The ranges of the detectable (≥ LOD) elemental concentrations were: 0.01 to 25 µg L^{-1} (0.02 to 19.39 µg g^{-1} creatinine) for inorganic As, 0.01 to 2.38 µg L^{-1} (0.01 to 4.25 µg g^{-1} creatinine) for Cd, 0.01 to 15.83 µg L^{-1} (0.01 to 28.24 µg g^{-1} creatinine) for Cr, and 0.05 to 26.91 µg L^{-1} (0.05 to 22.42 µg g^{-1} creatinine) for Ni. The probability distribution of the urinary concentrations did not follow a normal distribution ($p < 0.05$), as shown in the normal Q-Q plots presented in Appendix G (part G4). Data imputation was carried out to replace the concentrations that were <LOD (as previously discussed in section 3.8). An example of R code used for the imputation of non-detectable iAs concentrations is given in Appendix H (part H2). The imputed values were used in subsequent data analyses. The medians and 95th percentiles of the elemental concentrations are presented in Table 5.3, which also include the biological limit values for occupational exposure published by WHO.

Table 5.3: The medians and 95th percentiles of elemental concentrations in urine and WHO guidelines

Element	% <LOD[a]	Concentrations (µg L^{-1})		Concentrations (µg g^{-1} creatinine)		Biological limit values (WHO, 1996)[d] (µg g^{-1} creatinine)
		Median	95th Percentile	Median	95th Percentile	
As (inorganic)	27	1.39[b] (0.92)[c]	8.00[b] (7.00)[c]	1.59[b] (1.17)[c]	7.33[b] (6.42)[c]	50
Cd	39	0.06[b] (0.02)[c]	0.53[b] (0.40)[c]	0.07[b] (0.03)[c]	0.65[b] (0.50)[c]	5
Cr	31	0.59[b] (0.20)[c]	6.02[b] (5.42)[c]	0.64[b] (0.25)[c]	6.68[b] (5.64)[c]	30
Ni	29	1.65[b] (0.98)[c]	7.17[b] (6.81)[c]	1.78[b] (1.22)[c]	8.99[b] (7.66)[c]	30

[a]Limits of detection (LOD) varied between 0.005 and 0.01 µg L^{-1}.

[b]Calculated using concentrations that were ≥LOD.

[c]Includes values that were imputed to replace concentrations that were <LOD.

[d]The WHO limit values relate to biological monitoring of chemical exposure in the workplace.

Although the study participants were not occupationally exposed to the elements, the test results were compared with the WHO biological limit values (Table 5.3) to show that the recorded concentrations did not exceed the WHO guideline values, which indicate the permissible concentrations for occupationally exposed workers (WHO, 1996). The ratios of the WHO guideline values to the 95th percentiles of urinary concentrations (µg g^{-1} creatinine) were 8 (iAs), 10 (Cd), 5 (Cr) and 4 (Ni). These ratios indicate the estimated multiples of current exposure levels that would result in participants' urinary levels of the elements above the WHO guidelines.

Table 5.4: Comparison of the elemental concentrations in urine from this study with other published data

Element	Concentrations µg L^{-1} (µg g^{-1} creatinine)		Range (median) *per mmol creatinine*		Concentrations µg L^{-1} (µmol mol^{-1} creatinine)				Concentrations µg L^{-1} (µg g^{-1} creatinine)	
	This study		UK data[1] (Sieniawska et al., 2012)		UK data[2] (Morton et al., 2014)		UK data[3] (White & Sabbioni, 1998)		USA data[4] NHANES survey 2013-14	
	Median	95th Percentile	Men	Women	Median	95th Percentile	Mean or Median	Experimental range	Geometric Mean	95th Percentile
As (inorganic)	0.92 (1.17)	7.00 (6.42)	N/A		N/A		N/A		4.75 (5.51)	14.8 (17.7)
As (total)	N/A		0.01 – 0.67 (0.04), *µmol*	0.01 – 0.78 (0.11), *µmol*	10.48 (19.07)	152.40 (254.43)	3.65 (7.7)	<0.5 – 48.2 (1 – 60.6)	6.49 (7.58)	48.0 (54.0)
Cd	0.02 (0.03)	0.40 (0.50)	0.31 – 2.3 (0.63), *nmol*	0.39 – 1.3 (0.72), *nmol*	0.13 (0.15)	0.52 (0.57)	3.38 (0.48)	0.06 – 1.64 (0.05 – 3.4)	0.156 (0.182)	0.971 (0.868)
Cr	0.20 (0.25)	5.42 (5.64)	0.16 – 1.4 (0.4), *nmol*	0.21 – 1.1 (0.36), *nmol*	0.35 (0.91)	0.79 (2.85)	0.13 (0.28)	0.04 – 0.96	N/A	N/A
Ni	0.98 (1.22)	6.81 (7.66)	ND – 21.6 (4.4), *nmol*	ND – 18.6 (8.4), *nmol*	1.99 (5.01)	6.35 (10.66)	0.84 (1.7)	<0.3 – 59.0	N/A	N/A

[1]24 hour urinary concentrations for healthy UK adults comprising 77 men and 34 women; ND (non-detect), units reported as published.

[2]Data for non-occupationally exposed UK adult population (data for 132 individuals).

[3]Trace element concentrations in urine of healthy UK citizens (data for 224 individuals).

[4]Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017, Volume One (CDC, 2017).

N/A (not applicable / not reported).

For comparison, concentrations in *µg g^{-1} creatinine* can be expressed in *µmol mol^{-1} creatinine* by multiplying the concentrations by 1.5. The value 1.5 is the ratio of creatinine molecular weight (113 g mol^{-1}) to arsenic molecular weight (75 g mol^{-1}).

The results were also compared with data from previous studies in the UK and with data from NHANES 2013-2014 (CDC, 2017), as presented in Table 5.4. The UK data presented in Table 5.4 are for non-occupationally exposed populations, which makes the data relevant for comparing with test results from this study. The median concentrations of Cd and Cr recorded by this study were similar to the corresponding median values published by Sieniawska et al. (2012), who measured urinary concentrations of healthy UK adults comprising 77 men and 34 women. The median Ni concentration from this study was below those reported by Sieniawska et al. (2012). Data from another UK based study, where elemental urinary concentrations were measured in urine samples from 132 non-occupationally exposed individuals (Morton et al., 2014), have also been compared to our results. The median and 95th percentile concentrations recorded in this study were either similar or below the corresponding values published by Morton et al. (2014), apart from the 95th percentile concentration for Cr which exceeded the corresponding value. In addition, Cd concentrations from this study were lower than those published by White & Sabbioni (1998); but Cr and Ni concentrations were similar. The previous UK studies in Table 5.4 did not report the urinary concentrations of iAs (i.e., total As concentrations were reported), therefore it was not possible to compare iAs concentrations from this study with total As from these previous studies. When compared with the NHANES data, this study recorded lower concentrations iAs and Cd. It should be noted that the NHANES data do not include urinary levels of Cr and Ni.

In the same manner as with the blood Pb data, urinary concentrations were sub-grouped according to participants' age, gender, smoking status and produce consumption rate, and summarised in Table 5.5 using range (minimum and maximum) median, geometric mean, confidence interval (at 95%) and percentiles (5th and 95th). The arithmetic mean was also reported. The differences between medians of urinary concentrations for the various sub-groups were examined using the Mann-Whitney *U* test, as illustrated using the R code example in Appendix H (part H3). There were no significant differences between the medians of iAs and Cr in males and females ($p > 0.05$), which indicate that urinary levels of iAs and Cr were not dependent on gender. However, we found significant differences ($p < 0.05$) between urinary Cd and Ni levels in males and females; with females recording higher levels of both elements than males. This observation agrees with previous studies (e.g., Ruiz et al., 2010; Choudhury et al., 2001) that have reported higher urinary Cd levels in females than males, due to a higher rate of Cd absorption in females (Choudhury et al., 2001). With respect to participants' age groups, there were significant differences between the medians of Cd and Cr with age. The highest 95th percentiles for Cd and Cr were recorded in ages above 61, and between 30-50 years, respectively. Higher levels of iAs were found in those aged 71-80 years old than in other age groups, but the participant's age had no significant effect on urinary Ni levels. There was a significant difference ($p = 0.04$) between urinary iAs levels for non-smokers and ex-smokers; higher iAs were recorded for ex-smokers. Similarly, a significant difference was identified between Ni levels in non-smokers and ex-smokers ($p < 0.05$).

Table 5.5: Statistical summary of urinary concentrations (µg g^{-1} creatinine) according to sub-groups

	Element	Min	Max	Median	GM	Mean (CI, 95%)	P5	P95
All participants	iAs	0.002	19.39	1.17	0.50	1.93 (1.76 – 2.11)	0.005	6.42
	Cd	0.002	4.25	0.03	0.03	0.12 (0.10 – 0.14)	0.005	0.50
	Cr	0.002	28.24	0.25	0.18	1.23 (1.06 – 1.39)	0.005	5.64
	Ni	0.002	22.42	1.22	0.49	2.15 (1.95 – 2.36)	0.005	7.66
Age (years)								
30-50	iAs	0.002	19.39	1.03	0.43	1.85 (1.41 – 2.29)	0.005	6.23
	Cd	0.002	0.88	0.01	0.02	0.06 (0.04 – 0.08)	0.003	0.29
	Cr	0.002	19.48	0.35	0.20	1.48 (0.98 – 1.97)	0.005	7.52
	Ni	0.002	21.99	1.53	0.39	2.64(2.04 – 3.25)	0.005	9.12
51-60	iAs	0.003	17.65	0.96	0.45	1.67 (1.41 – 1.94)	0.005	5.43
	Cd	0.003	1.92	0.03	0.03	0.12 (0.09 – 0.15)	0.005	0.47
	Cr	0.003	12.00	0.18	0.16	1.07 (0.83 – 1.31)	0.005	5.21
	Ni	0.003	22.42	1.09	0.44	1.84 (1.53 – 2.16)	0.005	7.00
61-70	iAs	0.003	15.33	1.36	0.52	1.96 (1.68 – 2.24)	0.005	6.27
	Cd	0.003	4.25	0.04	0.04	0.16 (0.11 – 0.20)	0.005	0.52
	Cr	0.002	28.24	0.19	0.17	1.22 (0.93 – 1.52)	0.005	5.37
	Ni	0.002	19.51	1.18	0.58	2.17 (1.83 – 2.52)	0.005	8.00
71-80	iAs	0.003	13.04	1.92	0.90	2.82 (2.19 – 3.46)	0.005	9.60
	Cd	0.003	1.00	0.03	0.04	0.11 (0.07 – 0.16)	0.005	0.53
	Cr	0.003	7.71	0.58	0.31	1.29 (0.90 – 1.67)	0.005	5.59
	Ni	0.003	10.71	1.73	0.52	2.20 (1.70 – 2.70)	0.005	6.83
Gender								
Males	iAs	0.003	13.04	1.03	0.44	1.82 (1.60 – 2.03)	0.005	6.34
	Cd	0.003	1.92	0.02	0.03	0.10 (0.08 – 0.12)	0.005	0.45
	Cr	0.002	19.48	0.27	0.17	1.10 (0.91 – 1.29)	0.005	5.04
	Ni	0.002	12.02	1.04	0.39	1.87 (1.57 – 1.99)	0.005	6.82
Females	iAs	0.002	19.39	1.27	0.59	2.08 (1.79 – 2.34)	0.005	6.71
	Cd	0.002	4.25	0.03	0.04	0.14 (0.11 – 0.18)	0.005	0.52
	Cr	0.002	28.24	0.24	0.20	1.38 (1.09 – 1.66)	0.005	6.49
	Ni	0.002	22.42	1.50	0.62	2.60 (2.23 – 2.98)	0.005	10.33
Smoking status								
Smokers	iAs	0.007	7.54	1.03	0.49	1.91 (1.19 – 2.64)	0.008	6.37
	Cd	0.004	1.92	0.03	0.04	0.14 (0.02 – 0.26)	0.006	0.50
	Cr	0.005	8.46	0.37	0.21	1.21 (0.54 – 1.89)	0.006	5.32
	Ni	0.005	12.02	1.15	0.28	1.62 (0.84 – 2.40)	0.006	5.84
Ex-smokers	iAs	0.003	19.39	1.43	0.61	2.13 (1.80 – 2.46)	0.005	7.21
	Cd	0.003	4.25	0.02	0.03	0.13 (0.08 – 0.17)	0.005	0.45
	Cr	0.002	14.63	0.19	0.14	1.09 (0.85 – 1.34)	0.005	5.31
	Ni	0.002	19.51	0.82	0.33	1.83 (1.46 – 2.19)	0.005	6.00
Non-smokers (and 1 unknown smoking status)	iAs	0.002	17.65	1.04	0.46	1.84 (1.63 – 2.05)	0.005	6.24
	Cd	0.002	2.73	0.03	0.04	0.12 (0.10 – 0.14)	0.004	0.51
	Cr	0.002	28.24	0.26	0.20	1.29 (1.06 – 1.52)	0.005	5.66
	Ni	0.002	22.42	1.44	0.61	2.35 (2.10 – 2.62)	0.005	8.18
Average produce consumption rate (g fw kg^{-1} bw day^{-1})								
<1	iAs	0.003	19.39	1.03	0.47	1.86 (1.66 – 2.06)	0.005	6.35
	Cd	0.003	4.25	0.02	0.03	0.11 (0.09 – 0.13)	0.005	0.42
	Cr	0.003	15.83	0.29	0.19	1.14 (0.98 – 1.30)	0.005	5.33
	Ni	0.003	22.42	1.28	0.48	2.12 (1.89 – 2.36)	0.005	7.28
1 – 2	iAs	0.002	10.00	1.54	0.60	2.04 (1.67 – 2.40)	0.005	6.38
	Cd	0.002	1.97	0.02	0.03	0.11 (0.07 – 0.15)	0.003	0.51
	Cr	0.002	19.48	0.27	0.17	1.41 (0.91 – 1.92)	0.004	6.19
	Ni	0.002	19.51	0.87	0.42	2.31 (1.70 – 2.93)	0.005	8.81
2 – 3	iAs	0.003	15.33	1.50	0.58	2.25 (1.61 – 2.89)	0.005	9.21
	Cd	0.003	2.73	0.10	0.08	0.23 (0.14 – 0.31)	0.006	0.78
	Cr	0.005	28.24	0.11	0.16	1.47 (0.72 – 2.23)	0.007	7.05
	Ni	0.003	12.15	1.21	0.65	2.10 (1.56 – 2.65)	0.006	7.73

GM (geometric mean), CI (confidence interval of means at 95%), P5 (5^{th} percentile), P95 (95^{th} percentile),
fw (fresh weight of produce), bw (body weight of participant).
Imputed values (for <LOD concentrations) were included in the statistical calculations.

Although smoking contributes markedly to the amount of Cd excreted in urine (Castano et al., 2012), no significant differences in urinary Cd levels were identified for the different smoking categories. Smoking also had no significant relationship with urinary Cr levels.

There was a tendency towards higher urinary concentrations of iAs and Cr with increasing rate of consumption of produce; however, the associations between the concentrations and the rate of consumption of produce were not statistically significant ($p > 0.05$). The rate of produce consumption had a significant effect on urinary Cd levels, but not on Ni levels.

Further examination of the urinary concentrations was carried out using mixed-effects modelling, where participant's age, gender, smoking status and produce consumption rate were treated as 'fixed' effects, while participants were treated as 'random' effects. Significant associations ($p < 0.05$) were found between iAs and age, Ni and gender, and between Cd and both age and produce consumption. These associations are consistent with the findings obtained from the Mann-Whitney *U* tests.

Although we found a number of significant associations between urinary elemental concentrations and the various participants' characteristics, the recorded urinary levels were consistent with published urinary levels for non-occupationally exposed populations in the UK (Table 5.4). This shows that the participants' consumption of allotment produce did not result in them being exposed to these elements at higher levels than other adults in the UK, which supports hypothesis '*h2*'.

5.4 Chapter summary

The blood Pb levels recorded in this study were consistent with data published in historic studies carried out in central Scotland, but were higher than the levels previously reported in other parts of the UK. Due to the scarcity of published data on blood Pb levels in the un-occupationally exposed UK population, the blood Pb results from the present study presents an important contribution to the literature, particularly in relation to a population that consume produce from their allotments. Using Pb isotope analysis, it was determined that both geogenic and anthropogenic sources of Pb contributed to Pb in blood, with geogenic Pb sources contributing higher higher proportions (up to 64%) than anthropogenic Pb sources. Measured element concentrations in urine were similar to the corresponding levels in the general (non-occupationally exposed) populations in the UK; indicating that the participants were not exposed to these elements at levels importantly higher than other adults in the UK. Urine concentrations were between 0.1 and 0.25 times the WHO guideline values used in assessing potential health risk for occupationally exposed workers. Overall, the measured elemental concentrations in biological samples support hypothesis *'h2'* that *"the levels of the elements in blood and urine samples obtained from the participants are similar to the local background levels"*.

6. PHYSIOLOGICALLY-BASED KINETIC MODELS

6.1 Introduction

A review of the physiologically-based kinetic models is presented in chapter 2 (section 2.8), which also indicates the models adopted in this study. Some of these models are quite complex in terms of their structure and formulation because of the differences in physicochemical properties of the elements, organs involved in metabolism and exposure pathways simulated by the authors. To construct models suitable for our purpose (i.e., simulation of oral ingestion pathway in adults), some of the published models required modification by reducing or removing some compartments whilst still maintaining their predictive ability. This chapter presents the modified models, evaluation of the predictive performance of the modified models using data published in the literature, parametric sensitivity analysis, and a demonstration of the use of the models in informing and optimising the design of the biomonitoring study.

The physiological and chemical-specific parameters describing the kinetics of these elements in humans were obtained from the literature. In addition, we obtained mathematical equations describing these models from the literature and re-wrote the equations to fit the modified models. Mathematical equations and parameters of the constructed models are presented in Appendix I.

6.2 Modified models

6.2.1 Arsenic

The model published by El-Masri & Kenyon (2008) was adopted in this study, and was modified as follows: (i) the original model includes oxidation and reduction in the lung, liver and kidney only, however it was assumed that oxidation and reduction occurs in all perfused tissues as previously reported by Mann et al. (1996) and Yu (1999), (ii) the oxidation and reduction reactions between MMA(III) and MMA(V), DMA(III) and DMA(V) were ignored, thus MMA and DMA were treated as single species, because in our laboratory analysis we tested for total inorganic arsenic (the sum of all inorganic arsenic species), and (iii) biliary excretion of As from the liver was included in the model, as reported by Yu (1999) and Liao et al. (2008). Fig. 6.1 shows the schematic representation of the modified PBPK model for As.

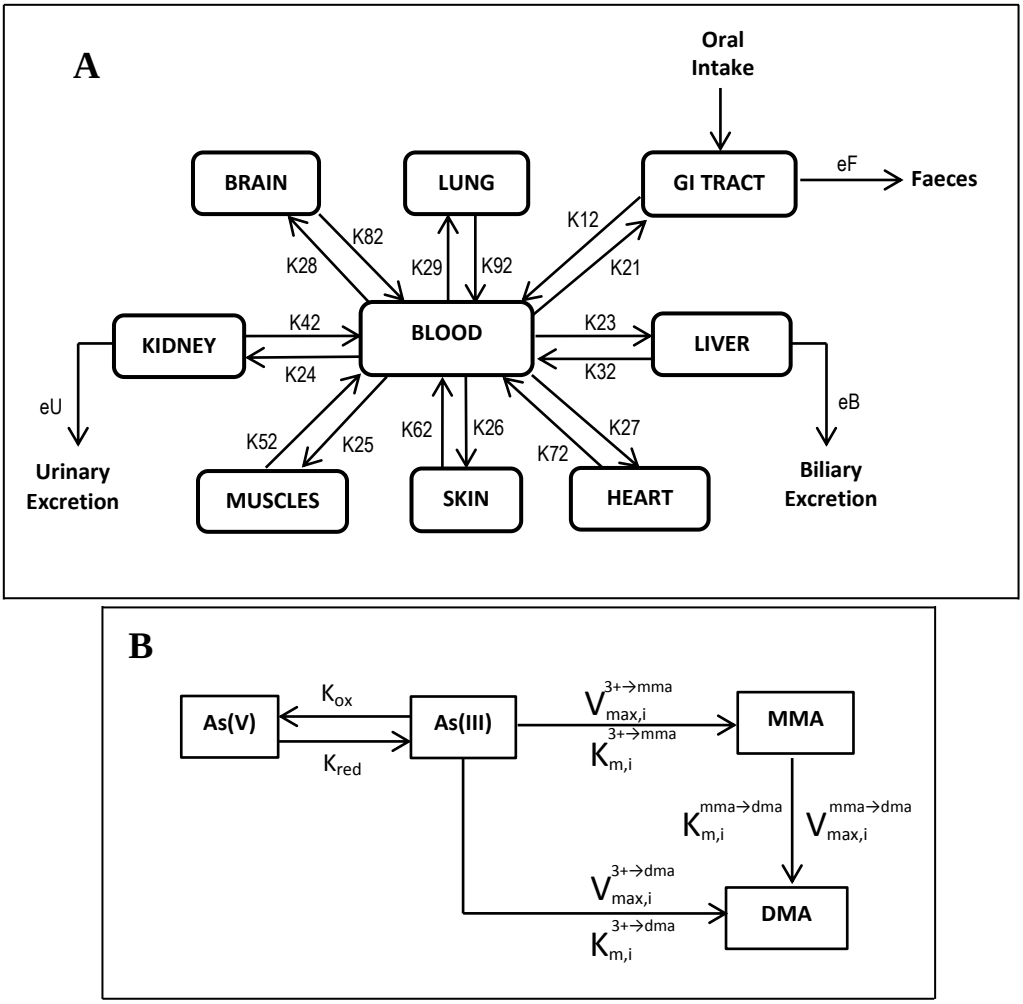

Fig. 6.1: A modified version of the PBPK model for As published by El-Masri & Kenyon (2008). (Part A) Oral absorption of As(III), As(V), MMA, DMA was accounted for in the GI tract, eF is faecal excretion rate of As species (day^{-1}), eB is biliary excretion rate of As species (day^{-1}), eU is urinary excretion rate of As species (day^{-1}). K refers to the transfer rates (day^{-1}) we calculated for As species between compartments. (Part B) Shows the oxidation/reduction of inorganic arsenic in all tissues and methylation of As(III) in kidney and liver (Liao et al. 2008). K_{ox} and K_{red} are metabolic constants (day^{-1}) for oxidation and reduction, respectively, V_{max} (µmol day^{-1}) and K_m (µmol L^{-1}) are metabolic constants for methylation of As(III), and *i* refers to compartment (liver, kidney). MMA (monomethylarsenic acid) DMA (dimethylarsinic acid).

Given the modifications made to the As model, the model's ability to predict literature data was tested. The model was used to simulate cumulative urinary As metabolites based on single and multiple oral doses of As(III) and As(V) reported in the literature by (Mann et al., 1996; El-Masri & Kenyon, 2008; Buchet et al., 1981). Selected results from the simulations are plotted in Fig. 6.2, which shows that the simulated results are consistent with the corresponding literature data.

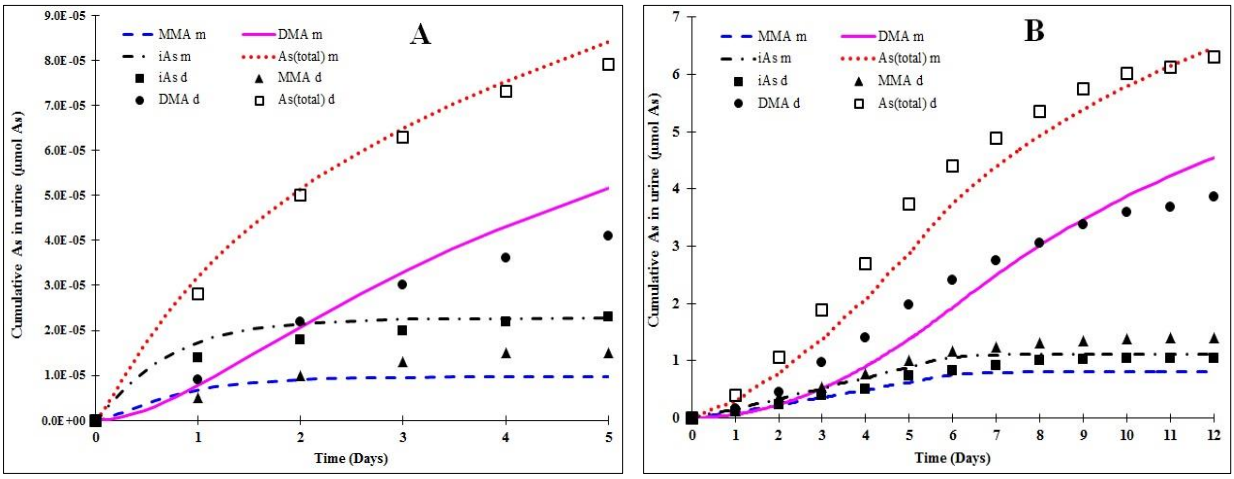

Fig. 6.2: Comparison of predicted urinary As metabolites with experimental data in the literature.
(**A**) following single oral ingestion of arsenic acid ($1.3x10^{-4}$ µmol As) (Mann et al. 1996), (**B**) following repeated daily oral ingestion of 1.67 µmol As for 5 days (Buchet et al. 1981). [iAs is the sum of As(III) and As(V); m, model; d, experimental data].

Further evaluation of the model was carried out using oral ingestion of 6.67 µmol (500 µg) As (Buchet et al., 1981) and 1.33 µmol (100 µg) As (El-Masri & Kenyon, 2008). Model predictions of cumulative urinary As (total) were within 8% of the reported data in the literature.

Ingested inorganic As undergoes oxidation and reduction in body tissues and methylation to MMA and DMA in the liver and kidney (Liao et al., 2008; El-Masri & Kenyon, 2008). Generally, studies have found DMA to be the major metabolite in urine following exposure to inorganic As (Yu, 1999; Buchet et al., 1981; Hughes, 2006; Hwang et al., 2002), as biotransformation of inorganic As to MMA and DMA occurs rapidly making DMA the dominant metabolic species after approximately 2 days. Our model simulations (Fig. 6.2) agree with this observation with the modified As model able to reproduce the literature data well ($r > 0.9$ for total As in urine), with low RMSE values for total inorganic As ranging from $4.7x10^{-6}$ to 0.44 (µmol). Analytical experimental procedures for determining As species in urine are often complex and costly. Therefore, the PBPK model can be used to estimate internal doses and urinary concentrations of speciated As, thus providing a proxy for the analysis of speciated As in urine.

6.2.2 Cadmium

We adopted the KN model as described in section 2.8 because it is the most used model and it is also the basis for other published PBTK models for Cd. Given that our study involves exposure through oral ingestion of food, we excluded the inhalation pathway from the model. In addition, the direct transfer of unabsorbed Cd to faeces was added to the modified model to account for unabsorbed Cd. The schematic representation of the modified PBTK model for Cd is given in Fig. 6.3.

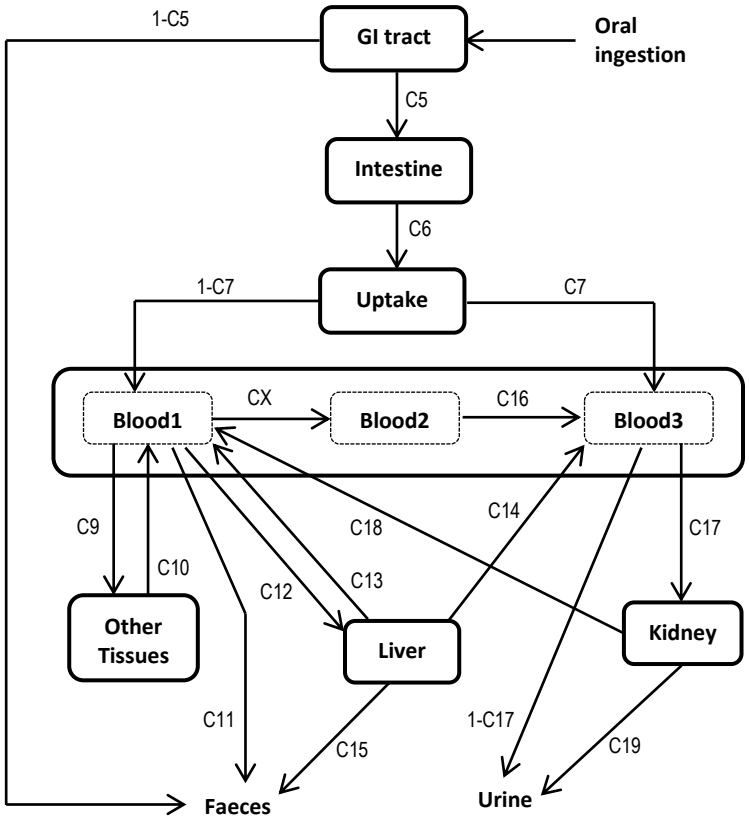

Fig. 6.3: A modified version of the Cd PBTK model published by Kjellström & Nordberg (1978). Blood1 to Blood3 refer to '*plasma other*', '*red blood cells*' and '*plasma metallothionein*', respectively; C5 to C19 and CX refer to the parameters describing the transfer of Cd between compartments as defined in the original model.

Based on Cd ingestion data published by Ju et al. (2012), the modified Cd model was used to predict Cd concentrations in blood and urine. Simulations were performed with all parameters fixed to the values given in the original KN model and only the bioaccessibility values were varied to correspond to values used by Ju et al. (2012). The model-predicted results and literature data are presented in Fig. 6.4, which shows that predicted concentrations closely match the literature data ($r = 0.99$).

We also used the dietary intake of Cd reported by Berglund et al. (1994) in their study investigating intestinal absorption of dietary Cd in women subjects (20 to 50 years of age) to predict Cd concentrations in urine and blood following daily dietary exposure. Our simulations mimicked five long-term exposure scenarios lasting between 10 and 50 years which are compared in Table 6.1, against the results of Cd measured in urine and blood by Berglund et al. (1994). Although no corresponding data (with respect to duration after exposure) was given by the authors (this would allow direct comparison with our simulated results) the model predictions fit within the reported data ranges.

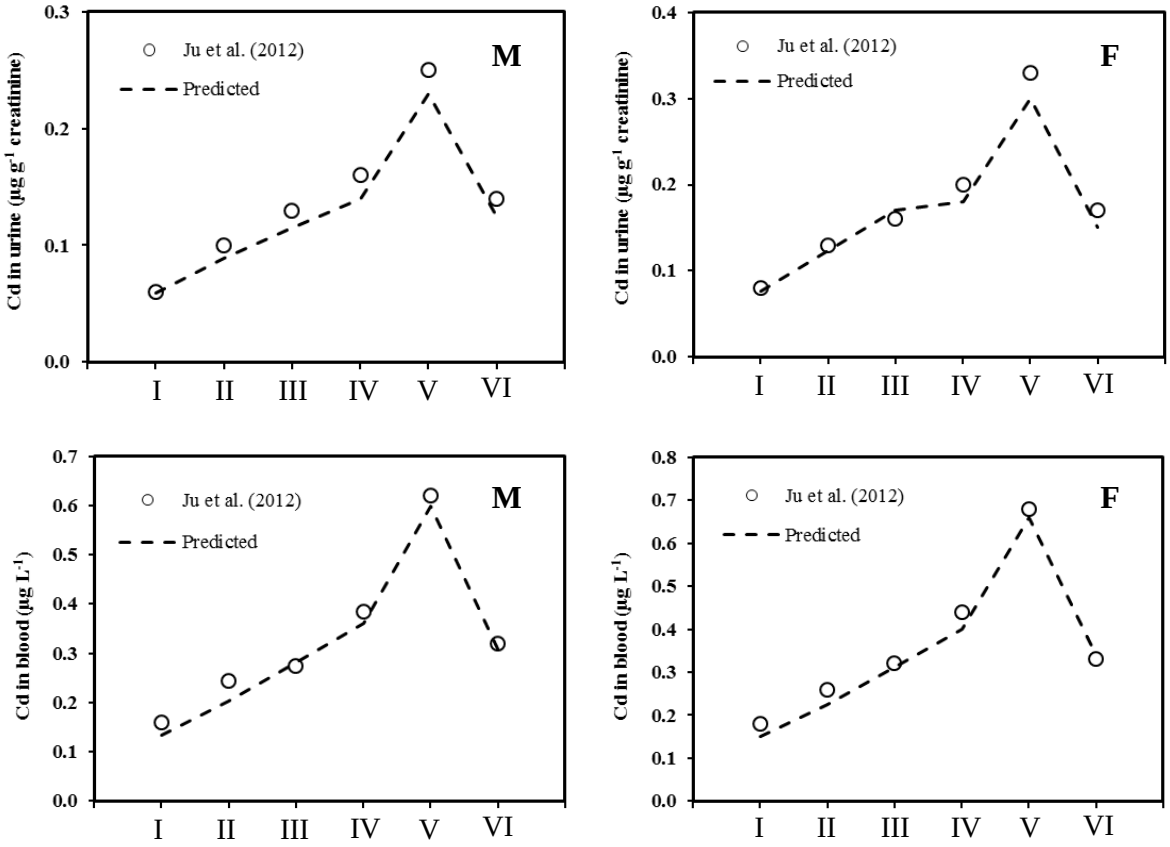

Fig. 6.4: Comparison of predicted Cd levels in urine and blood with data (median) reported by Ju et al. (2012).
M and F refer to male and female non-smoking human subjects, respectively. Six scenarios of Cd bioaccessibility as used by Ju et al. (2012) were simulated. Creatinine values used in expressing predicted urinary Cd were obtained from Ju et al. (2012). I-V represent Cd bioaccessibility values (dimensionless) of 0.021, 0.032, 0.044, 0.057 and 0.094, respectively. VI represents the scenario of using the original fraction of Cd absorbed to gastrointestinal tract and systematic circulation (0.048) adopted by Kjellstrom & Nordberg (1978).

Table 6.1: Predicted urine and blood Cd concentrations and data reported by Berglund et al. (1994)

	Mixed diet		**High fibre diet**	
	Urine	Blood	Urine	Blood
Measured concentrations[a]				
Median	0.10	0.23	0.09	0.25
Range	0.02 - 0.32	≤0.09 - 0.68	≤0.02 - 0.41	≤0.09 - 0.96
Predicted Cd concentrations after simulating 10 to 50 years of daily dietary exposure to Cd				
10 years	0.04	0.29	0.05	0.38
20 years	0.08	0.33	0.08	0.43
30 years	0.10	0.38	0.11	0.48
40 years	0.12	0.39	0.13	0.51
50 years	0.13	0.41	0.14	0.53

[a]Berglund et al. (1994).
All concentrations are expressed in µg L^{-1}. The median daily dietary intakes of Cd reported by Berglund et al. (1994) (10 µg day^{-1} for mixed diet and 13 µg day^{-1} for high fibre diet) were used in the simulations. Predicted Cd masses in urine were converted into concentrations using the median daily urine volumes reported by Berglund et al. (1994). Likewise, Cd loads in blood were converted to concentrations using blood volume of 5.2 L (Ju et al., 2012).

Cd model predictions were consistent with the literature data (*r = 0.99*), with low RMSE values ranging from 0.01 to 0.02 (μg g^{-1} creatinine). The creatinine values reported by Ju et al. (2012) were used in the conversion of the predicted urinary Cd concentrations. The KN model for Cd has been used in similar studies. It was modified by Ruiz et al. (2010), re-coded and used to sufficiently predict the urinary Cd concentrations using the National Health and Nutrition Examination Survey (NHANES) data from Cd exposure by oral ingestion. Using a modified version of the KN model in their study, Choudhury et al. (2001) also predicted urinary Cd concentrations consistent with the NHANES data.

6.2.3 Chromium

The model published by Kirman et al. (2013) was adopted and simplified by ignoring the detailed competing toxicokinetic processes of Cr in the stomach and intestines, because there are published absorption rates for Cr in the GI tract (Kirman et al., 2013; Sasso & Schlosser, 2015). Therefore, the 'stomach', 'small intestines' and 'large intestines' were lumped into a single compartment (GI tract), which is consistent with the approach used by O'Flaherty et al. (2001), who treated the GI tract as a single compartment in their model. Fig. 6.5 shows the structure of the modified PBPK model for Cr.

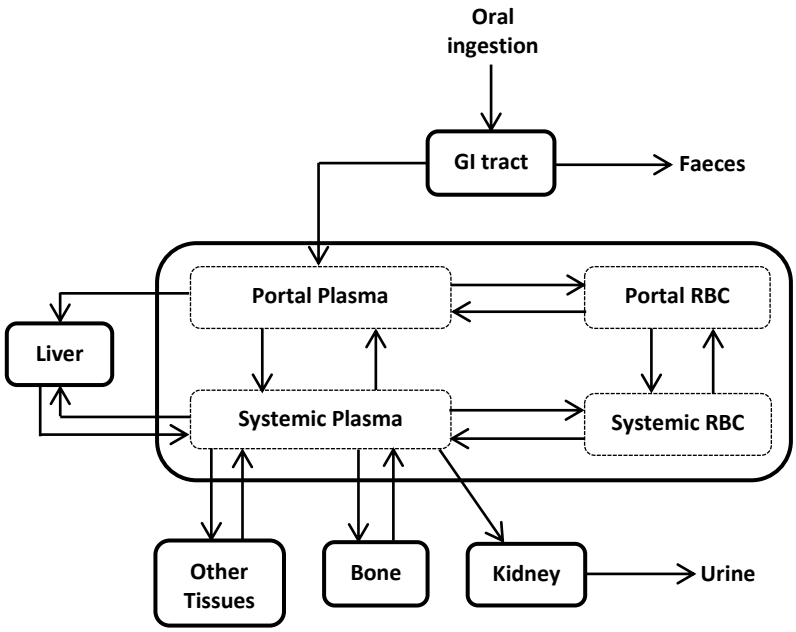

Fig. 6.5: A modified version of the PBPK model for Cr published by Kirman et al. (2013). RBC refers to red blood cells. All compartments contain Cr(III) and Cr(VI). Reduction of Cr(VI) to Cr(III) occurs in GI tract, blood, liver, kidney, and other tissues. Cr is excreted in urine mainly as Cr(III) due to rapid reduction of Cr(VI) to Cr(III) in the body (O'Flaherty et al., 2001).

The predictive performance of the Cr modified model was tested using data published by Paustenbach et al. (1996), Kirman et al. (2013) and Kerger et al. (1996). The results are plotted in Fig. 6.6.

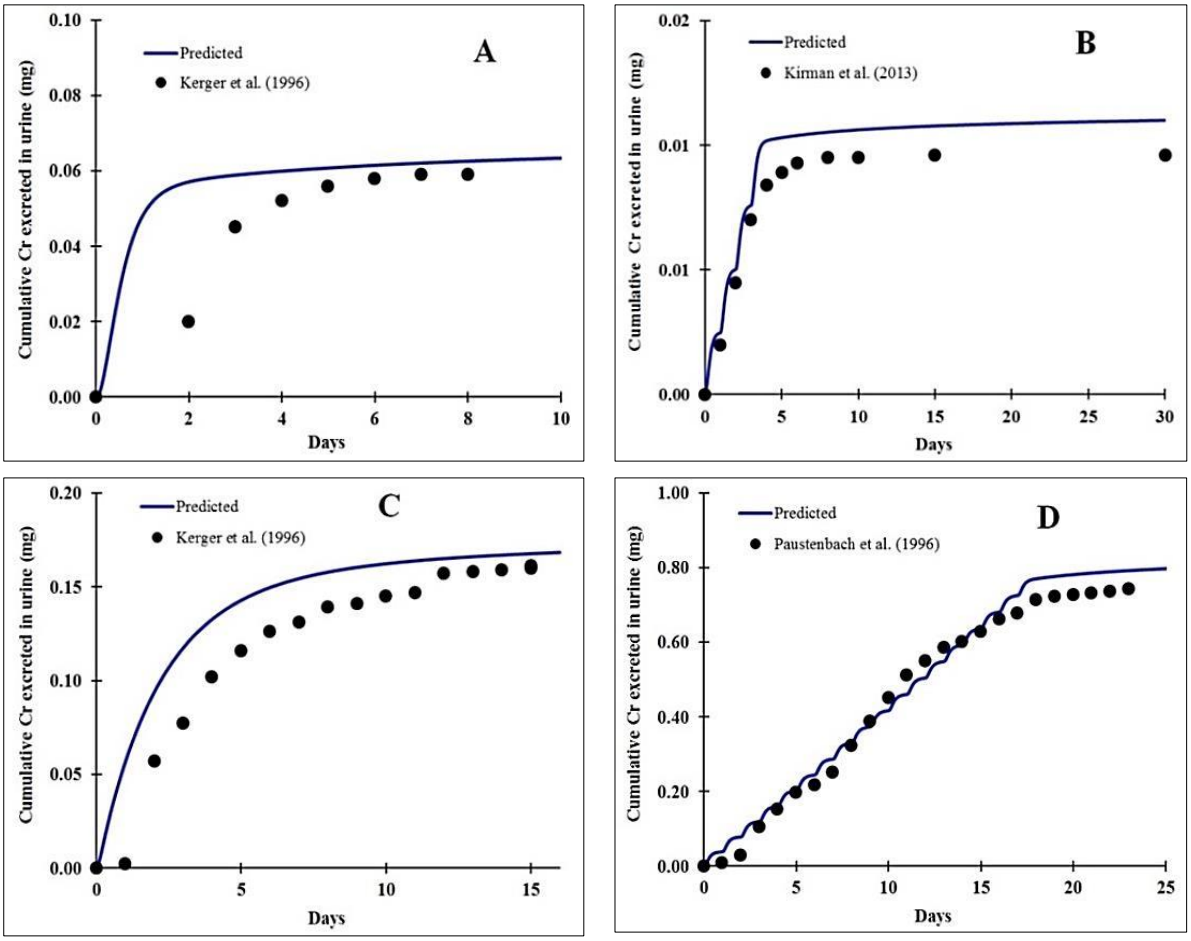

Fig. 6.6: Comparison of predicted and measured cumulative urinary excretion of Cr in human subjects. (**A**) for humans exposed to a single dose of 5 mg of Cr(III) as reported by Kerger et al. (1996), (**B**) for humans exposed to 0.4mg of Cr(III) per day for 3 days (Kirman et al. 2013), (**C**) for humans exposed to a single dose of 5mg of Cr(VI) as reported by Kerger et al. (1996), and (**D**) for a human volunteer exposed to 4mg Cr(VI) per day for 17 days (Paustenbach et al. 1996).

In Fig. 6.6 (A), the model predicts a faster urinary excretion of Cr than the measured data in the literature. However, the cumulative urinary Cr is consistent with the literature data. The predicted results show reasonable fit with the literature data following oral ingestion of Cr(III) and Cr(VI) in single and multiple doses.

It has been observed that human absorption of Cr(VI) in the GI tract can vary between individuals and also in the same individual at different times, with suggestions that physiological fluids in the GI tract such as gastric juice and diet constituents like orange juice lead to poor intestinal absorption of Cr(VI), because of their capacities to reduce Cr(VI) to Cr(III) (De Flora et al., 1997; Kerger et al., 1996; Sasso & Schlosser, 2015). Intra-individual variability in Cr(VI) absorption due

to differences in this reduction capacity was noted in studies involving human subjects (Finley et al., 1997; Paustenbach et al., 1996; O'Flaherty et al., 2001). Absorption values of 0.25 day^{-1} for Cr(III) and 2.5 day^{-1} for Cr(VI) were specified by O'Flaherty et al. (2001). In addition, we used the mean rate constants for absorption of Cr(III) ($4.6x10^{-6}$ L hr-cm^{-1}) and Cr(VI) ($3.2x10^{-4}$ L hr-cm^{-1}) in the small intestines given by Kirman et al. (2013) to estimate corresponding intestinal absorption rates of 0.05 day^{-1} for Cr(III) and 3.5 day^{-1} for Cr(VI). In our simulations, we used a range of GI tract absorption rates, 0.05 – 0.25 day^{-1} for Cr(III) and 1 – 2.5 day^{-1} for Cr(VI) to fit model predictions with the literature data ($r > 0.8$, $RMSE \leq 0.01$ *mg*). This suggests that oral absorption values for Cr should be carefully selected when fitting a model using experimental data.

6.2.4 Lead

The O'Flaherty model for Pb was adopted for this study. The model was modified by: (i) ignoring the inhalation component in our simulations because oral ingestion is the primary route of exposure investigated in this study. Furthermore, pilot study results indicated that air Pb concentrations at the allotments were not elevated above background air concentrations; and (ii) the model was simplified by neglecting the detailed Pb kinetics in the bone associated with bone growth, since human skeletal growth is considered to be complete by the age of about 25 years (O'Flaherty, 1993) and all of our study participants were aged 30 years old and above. Fig. 6.7 provides schematic representation of the modified PBPK model for Pb.

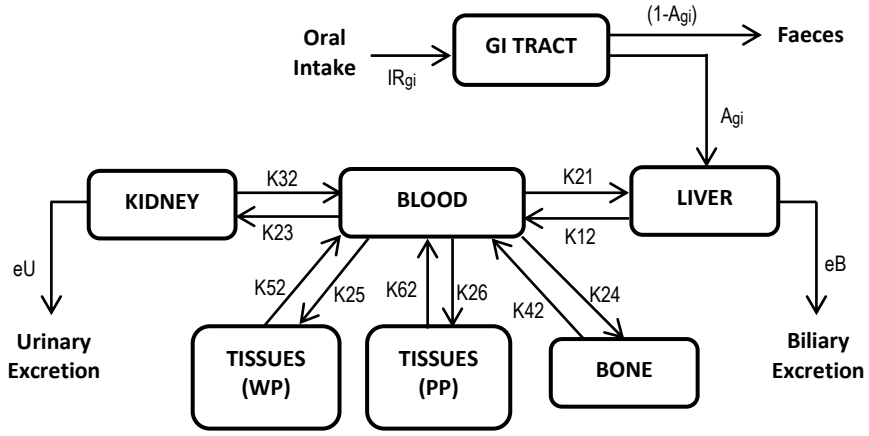

Fig. 6.7: A modified version of the O'Flaherty PBPK model for Pb.
WP is well-perfused tissues, PP is poorly-perfused tissues, IR_{gi} is the oral intake rate of Pb (mg day^{-1}), A_{gi} is the Pb absorption coefficient from gastrointestinal tract (unitless), the unabsorbed fraction is represented by $(1-A_{gi})$, eB is the biliary excretion rate of Pb (day^{-1}), eU is the urinary excretion rate of Pb (day^{-1}), K12 to K62 refer to the transfer rates of Pb (day^{-1}) between compartments which we calculated.

Evaluation of the modified Pb model was carried out using of Rabinowitz et al. (1976), who studied the steady-state kinetics of Pb in five healthy men (subjects A to E) with stable isotope tracers. Since the transport of Pb throughout the body is governed by its concentration in the plasma (O'Flaherty, 1993; Fleming et al., 1999; Leggett, 1993), Pb concentration in whole blood

was calculated from the model-predicted plasma Pb concentrations using the expression (MacMillan et al., 2015).

$$CB = ((1 - HCT) \times CPLASMA) + \left(HCT \times CPLASMA \times \left(G + \left(\frac{BIND}{KBIND + CPLASMA} \right) \right) \right) \quad (6.1)$$

Here, *CB* is Pb concentration in whole blood, *HCT* is the haematocrit fraction of whole blood (0.45), *CPLASMA* is Pb concentration in blood plasma, *G* is the ratio of unbound erythrocyte Pb concentration to plasma Pb concentration (1.2), BIND is the Pb binding capacity of erythrocytes (0.437 mg Pb L^{-1} cell), and KBIND is the binding constant of erythrocytes (3.72×10^{-4} mg Pb L^{-1} cell). This allowed for comparison to be made between model-predicted plasma concentrations and Pb measurements in whole blood. Simulated results for the five subjects are presented in Fig. 6.8, which show peak Pb concentrations similar to the observations made by Rabinowitz et al. (1976).

The predicted Pb concentrations in blood were highly correlated to the literature data ($r > 0.9$ for subjects A, B, D, E, and $r = 0.7$ for subject C). Notably, there were fewer data points for subject C, which could be the reason for the reduced correlation. In addition, the literature data for subject C show constant blood concentration beyond day 2, while the model simulated declining concentrations (Fig. 6.8). Individual variabilities (such as differences in absorption rates and stomach clearance rates) cannot be ruled out as potential contributing factors to this variation. Despite the reduced correlation for subject C, the predicted peak Pb in blood (which is relevant in understanding exposure) was consisted with experimental data. Predicted peak Pb concentrations were within 10% of the literature data, apart from subject E which recorded 38% above the literature data. Similar observations were made by O'Flaherty (1993) and Morisawa et al. (2001) who tested their Pb models using the same data by Rabinowitz et al. (1976); although data for subject C was not reported in both publications.

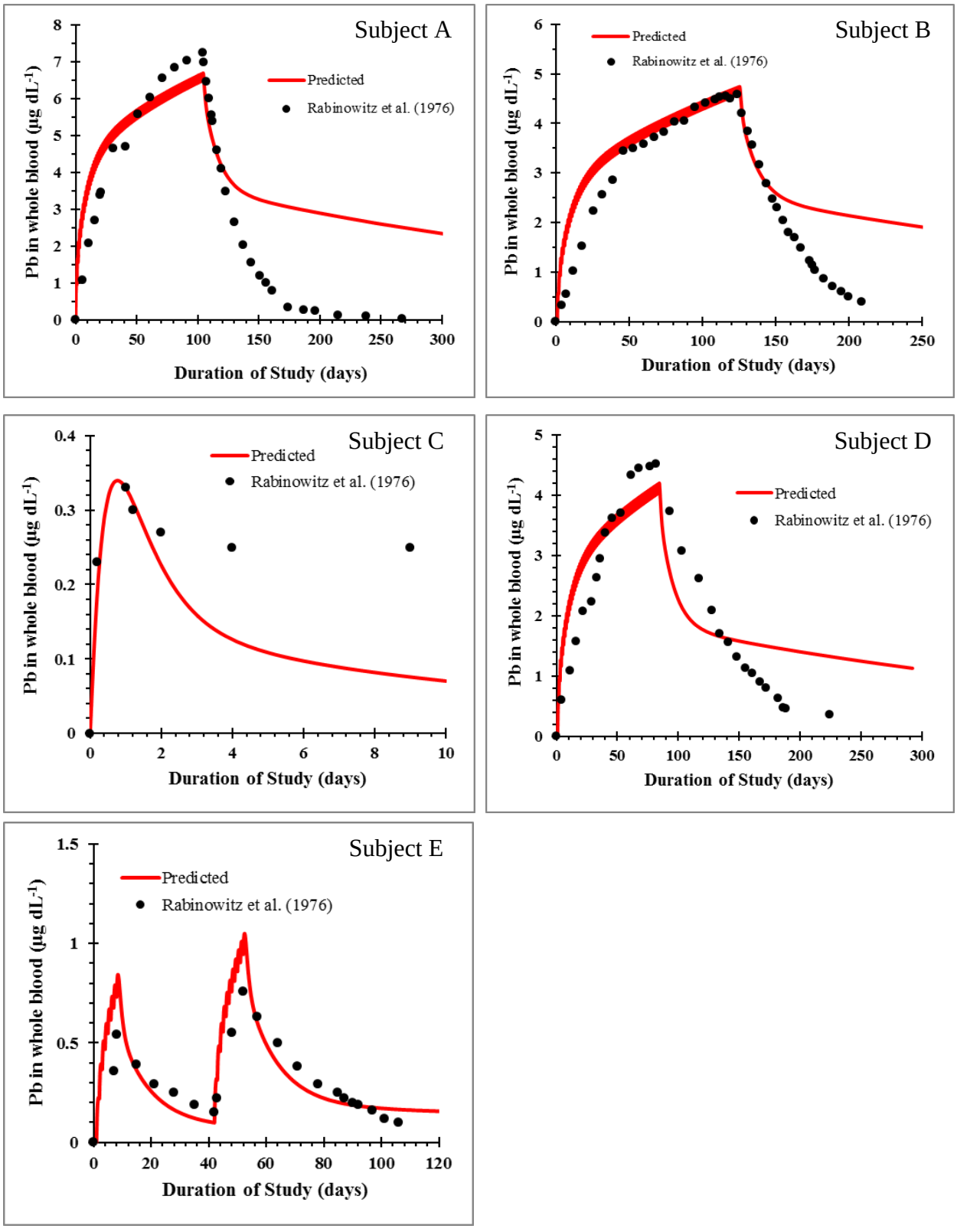

Fig. 6.8: Comparison of model predicted versus measured Pb concentrations in blood from the Rabinowitz et al. (1976) study.

Duration of study refers to time after the beginning of controlled ingestion of Pb isotope. Ingestion periods for Subjects A (204 µg day^{-1}), B (185 µg day^{-1}) and D (105 µg day^{-1}) were 104, 124 and 82 days, respectively. Subject C ingested 68 µg day^{-1} for 1 day, while subject E ingested 99 µg day^{-1} for the first 8 days and again from days 42-52.

6.2.5 Nickel

The Ni model published by Sunderman et al. (1989) was slightly modified by the addition of a 'faeces' compartment to account for faecal excretion of un-absorbed Ni. The rate of transfer to faeces was determined based on the absorption rate applied in the model. The schematic representation of the modified toxicokinetic model for Ni is given in Fig. 6.9.

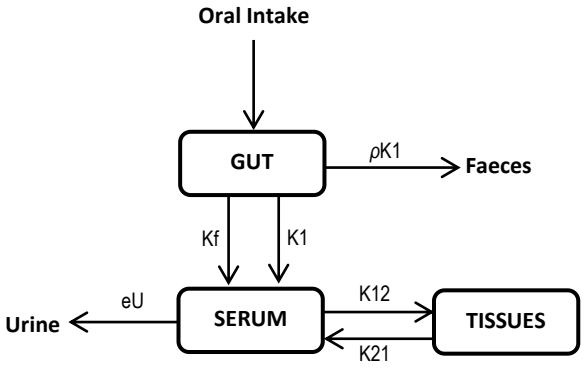

Fig. 6.9: A modified version of the Ni PBTK model by Sunderman et al. (1989).
K1, Kf, K12 and K21 refer to the transfer rates of Ni between respective compartments and eU is the rate constant for urinary elimination as defined in the original model. The absorbed fraction of Ni dose in the gut (A_{gut}) was 0.011, as determined by Sunderman et al. (1989). We expressed the daily rate constant for faecal excretion of unabsorbed Ni in dose as ρK1, where ρ was calculated as $(1-A_{gut})/A_{gut}$.

The predictive performance of the Ni modified model was tested using data obtained from Sunderman et al. (1989) and Nielsen et al. (1999). The model results and the corresponding literature data are presented in Fig. 6.10, which indicates that the predicted urinary excretion of Ni match closely with the literature data; thus showing the capability of the model to reproduce literature data.

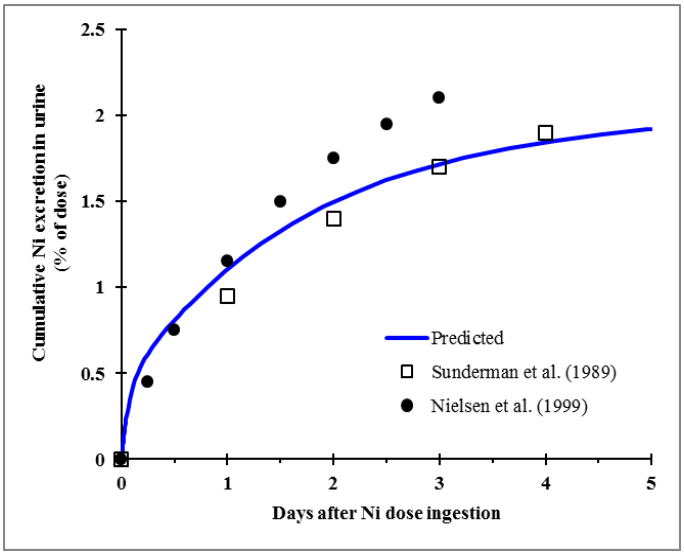

Fig. 6.10: Comparison of predicted urinary Ni excretion with literature data

The mass fraction (mean) of Ni dose absorbed from the gut in the experiment by Sunderman et al. (1989) was 0.7±0.4 % for Ni dose ingested in food. In a similar study involving controlled ingestion of Ni dose in food, Nielsen et al. (1999) reported a median value of Ni oral absorption of 2.95±1.32 % (mean 2.5%), which is notably higher than oral absorption values reported by Sunderman et al. (1989). The mean oral absorption values reported by these authors were used in the simulations (Fig. 6.10). The model predicted literature data well ($r > 0.9$), with low RMSE values ranging from 0.02 to 0.9 µg.

Varied oral absorption values of Ni in the gut were also noted in the literature. Studies by Horak & Sunderman (1973), McNeely et al. (1972) and McNeely et al. (1971) reported Ni absorption values between 1 and 1.6 %. Overall, these oral absorption values indicate that faecal excretion is a major route for elimination of Ni from the human body.

6.3 Sensitivity analysis

The sensitivity coefficients (SC) of all model input parameters were determined as described in chapter 3 (section 3.10), to determine the influence of parameter variation to the model output (urinary As, Cd, Cr, Ni concentrations and blood Pb). Input values were varied by up to ±50%. The SC values show the relative magnitude of change in model output for a given change in the model input, such that high values of SC indicate high sensitivity of the model to that variable. The parameters with SC > 0.1 are shown in Fig. 6.11.

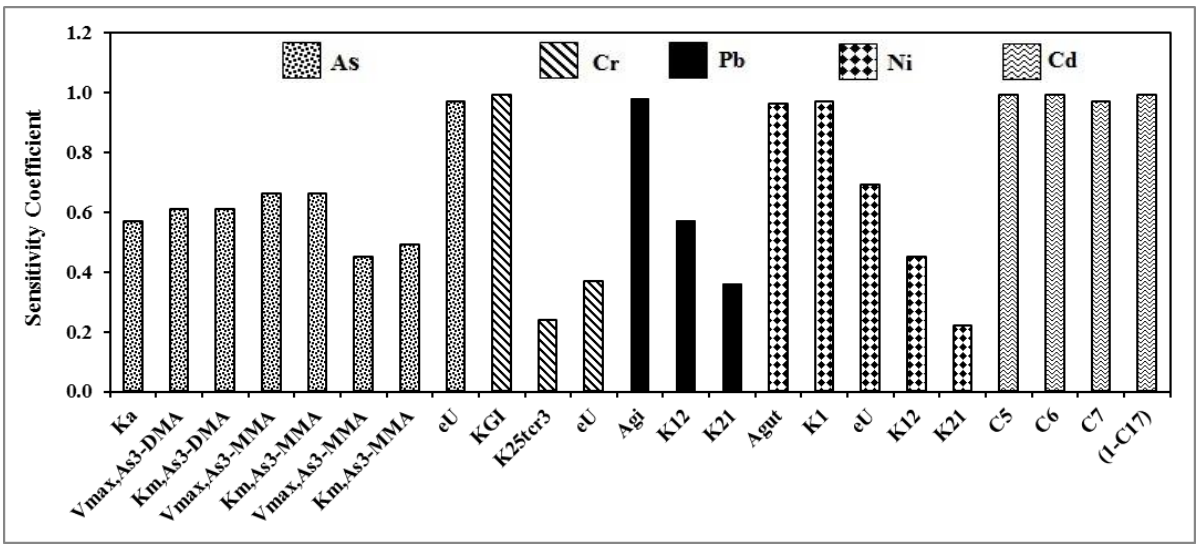

Fig. 6.11: Sensitivity analysis of urinary As, Cr, Ni, Cd concentrations and blood Pb for various model parameters.

eU is urinary elimination rate. Other parameters: (As) K_a is oral absorption in the GI tract, V_{max} and K_m are methylation constants in the kidney. (Cr) KGI is rate constant for absorption of Cr from the GI tract, K25tcr3 is transfer rate of Cr(III) to the kidney from the distribution pool in systemic plasma. (Pb) A_{gi} is absorption coefficient from the GI tract, K12 and K21 are transfer rates from liver to blood and the reverse, respectively. (Ni) A_{gut} is fractional absorption in the gut, K1 is alimentary absorption rate, K12 and K21 are transfer rates from serum to tissues and the reverse, respectively. (Cd) C5 is fractional absorption to GI tract, C6 is rate constant for systematic absorption from GI tract, C7 transfer from systematic circulation to blood compartment 3, (1-C17) is transfer from blood compartment 3 to urine.

Fig. 6.11 shows that As model is most sensitive to *eU* (SC = 0.97), and moderately sensitive to methylation constants in the kidney (V_{max}, K_m) and oral absorption constant (K_a). The results also indicate that models for Cd, Cr, Ni and Pb were highly sensitive to adjustment of their oral absorption constants (SC > 0.96). The model for Cd recorded high SC values for parameters describing systemic absorption of Cd and the transfer of Cd from blood to urine. Overall, the determined SC values suggest that the most important parameters controlling model outputs relate to urinary elimination (As, Cd), oral absorption (Cd, Cr, Ni, Pb) and systemic absorption (Cd). Similar studies have also recorded high SC for a number of these parameters. For example, the sensitivity analysis performed by Yu (1999) for their PBPK model for inorganic As determined that V_{max} coefficients, urine constants and reduction constant were the input parameters that affected model output significantly. Using a modified version of the K&N model for Cd, Ruiz et al. (2010) determined a high SC value of 0.99 for fractional absorption of Cd in the GI tract. The study by Kirman et al. (2013) also identified a number of sensitive parameters to their Cr model, including parameters associated with Cr absorption in the GI tract and urinary excretion rate. Notably, the literature gives various oral absorption values for Cr due to inter- and intra- individual differences linked to physiological fluid contents of the GI tract that influence the reduction of Cr(VI) to Cr(III). The determined SC values point us to the model parameters that require close attention during the modelling process.

6.4 Model application in planning biomonitoring

These modified models for As, Cd, Cr, Ni and Pb were used to predict optimal times for the collection of biological samples during our biomonitoring study. The aim was to identify the best time points, following oral ingestion of allotment produce, at which to collect urine and blood samples (the biomarkers of exposure) given their expected low element concentrations. Model results would thus maximise the potential of detecting these elements in biological samples.

The simulated element doses (mg day^{-1}) were calculated based on the following:

a) Average produce concentrations recorded during our pilot study. However for Cd, we assumed a value equivalent to the limit of detection (0.03 mg kg^{-1}) because all samples recorded Cd concentrations below the limit of detection.
b) Produce consumption rate of 3.34 (g^{-1} fw kg^{-1} bw day^{-1}) for 'high end' consumer, derived from the data used in the CLEA model (as illustrated in Table 6.2). This consumption rate relates to CLEA age classes 17 and 18, which correspond to adults aged between 16 and 65 (age class 17) and 65 to 75 (age class 18). Our study participants are aged over 30 years old.
c) Average adult body weight of 70 kg (Brown et al., 1997).

d) Short durations of exposure (1, 3 and 7 days) were simulated to mimic hypothetical minimal (worst-case) exposure scenarios.

Table 6.2: Calculation of allotment-related produce consumption rates from CLEA data

CLEA Produce Category[3]	Produce Consumption for CLEA Age Class 17–18 (Adults)[1,2]				
	Consumption Rate[4]	Homegrown fraction		Allotment related Consumption Rate[4,5]	
		(average)	(high end)	(average)	(high end)
Green	2.94	0.05	0.33	0.15	0.97
Root	1.40	0.06	0.40	0.08	0.56
Tuber	1.79	0.02	0.13	0.04	0.23
Herbaceous	1.61	0.06	0.40	0.10	0.64
Shrub	0.22	0.09	0.60	0.02	0.13
Tree	2.97	0.04	0.27	0.12	0.80
			Total	***0.50***	***3.34***

[1]Environment Agency (2009e).
[2]We assumed individuals consuming all CLEA produce categories.
[3]Vegetables (Green, Root, Tuber) and Fruits (Herbaceous, Shrub, Tree).
[4]Units (g fw kg^{-1} bw day^{-1}), fw (fresh weight, produce), bw (body weight).
[5]Allotment-related consumption rate is the product of 'consumption rate' and 'homegrown fraction'.

Table 6.3: Planning of biomonitoring using model predictions

Element	Oral dose (mg day^{-1})	Exposure Duration (days)	Optimal sampling time after exposure (range)	Corresponding concentration (ppb) in urine[1] (As, Cd, Cr, Ni) and blood[2] (Pb) at optimal time
As	0.44 (*5.9 µmol*)	1	7 – 14 days	2.6 – 9.0
		3	7 – 21 days	9 – 24
		7	10 – 30 days	18 – 43
Cd	0.004	1	N/A	<0.01
		3	N/A	<0.01
		7	N/A	<0.01
Cr	0.1	1	N/A	< 0.01
		3	N/A	< 0.01
		7	10 – 20 days	~ 0.01
Ni	0.19	1	3 – 9 days	1.2 – 2.3
		3	10 – 20 days	1.4 – 3.8
		7	14 – 30 days	1.4 – 4.8
Pb	0.47	1	15 – 90 days	≥ 0.3
		3	15 – 90 days	≥ 1.0
		7	15 – 90 days	≥ 2.3

[1]Urinary concentrations were calculated using urine volume of 1.4 L day^{-1} (Ju et al. 2012)
[2]Blood concentrations were calculated using blood volume of 5.2 L day^{-1} (Ju et al. 2012)
N/A implies 'not applicable' (very low concentrations predicted).

The predicted optimal sample collections times are presented in Table 6.3. Model predictions indicate that at very low levels of exposure, detection of Cd and Cr in urine might not be

achievable, unless the participants get exposed to higher doses than simulated. We relied on a detection limit of 0.01 µg L^{-1} for ICP-MS. Higher intake would increase the 'window' of optimal sampling times. On the contrary, detection of urinary As (total) and Ni, and blood Pb would not prove difficult. These optimal times informed the sampling frequencies adopted in the biomonitoring.

6.5 Chapter Summary

Using existing models and published data in the literature, modified kinetic models were produced for As, Cd, Cr, Pb and Ni, for subsequent use in this study. The predictive performances of the modified models were tested using data published in the literature. Simulations carried out using the modified models showed high correlations between model-predicted data and the literature data (section 6.2), with majority of the data recording $r > 0.9$. A summary of the performance of the models discussed in section 6.2 is given in Table 6.4.

Table 6.4: Summary of calculated values (range) of predictive performance of the modified models

Element (range of doses simulated)	**RMSE**	***Correlation coefficient (r) (p<0.05)***	**Maximum C_s (as % of C_{lit})**
As ($1.3x10^{-4}$ – 6.67 µmol)	$4.7x10^{-6}$ – 0.44 (µmol)[a]	0.98 – 0.99[a]	89 – 109[a]
Cd (6.9 – 7.6 µg)[b]	0.01 – 0.02 (µg g^{-1} creatinine)	0.99	83 – 106
Cr (0.4 – 5 mg)	0.001 – 0.01mg	0.84 – 0.99	105 – 114
Ni (50 – 900 µg)	0.02 – 0.9 µg	0.98 – 0.99	100 – 102
Pb (68 – 204 µg)	0.11 – 1.4 µg dL^{-1}	0.74 – 0.95	93 – 138

C_s is the simulated data, C_{lit} is the corresponding literature data.
[a]Results summarised are for As (total).
[b]Excludes Cd simulations in Table 6.1, no corresponding data was given in the literature to allow direct comparison.

Majority of the predicted maximum results were within 17% of the literature data, apart from one dataset from Pb simulations with 38% over-prediction (see Fig. 6.8, subject E) . Ni results recorded the closest match to the literature data. RMSE values were low in respect to the oral doses simulated, indicating the closeness of data points to the regression line, which demonstrates the ability of the models to reproduce literature data well. These findings support hypothesis '*h3*' that *"PBPK model predicted element concentrations in blood and urine are good indicators of the corresponding measured biomarker concentrations"*. Therefore, these models are useful tools in the analysis of human exposure to the selected elements through the oral ingestion pathway. One advantage of these models is that they are adaptable, because model input parameters can be adjusted to reflect the actual exposure characteristics being investigated.

7. EVALUATION OF MODELS USING BIOMONITORING DATA AND MODELLING OF EXPOSURE SCENARIOS

7.1 Introduction

In this chapter, the models presented in the preceding chapter are evaluated using our biomonitoring data. Numerous model simulations were carried out, based on three broad exposure scenarios (i.e. using participants' data from our study, using data published in CLEA documents, and using hypothetical scenarios). Both short-term and long term exposure frequencies were simulated. The relationships between model outputs and the recorded element concentrations in biological samples have been examined. Selected figures prepared from the simulations are included in this chapter, and in addition, outputs of selected simulations are given in Appendix J.

7.2 Simulation of blood Pb concentrations

7.2.1 Exposure modelling using data from our study

The PBPK model for Pb (Fig. 6.7) was used to predict blood Pb concentrations for each of the participants where blood Pb was determined. The daily intake rate (IR) of Pb (μg day^{-1}) was calculated by multiplying the participant body weight (kg) by the corresponding average daily intake (ADI) values of Pb. ADI values are summarised in Appendix E (part E4). IR values were calculated for each month, and subsequently used to schedule Pb 'doses' in the model. In our simulations, values of the Pb absorption coefficient from the GI tract (A_{gi}) were selected at random from the range of 0.06 to 0.12 (Rabinowitz et al., 1976) in order to improve the data fit between the predicted concentrations with biomonitoring data. The oral absorption coefficient was the most sensitive parameter for the Pb PBPK model (Fig. 6.11), thus, it was necessary to vary the parameter during the simulations to cater for individual variabilities of oral absorption among the participants.

The model predicts the mass (μg) of Pb in the plasma, which were converted to plasma concentrations (μg L^{-1}) by dividing the predicted mass with plasma volume of 2.8 L (O'Flaherty, 1991). To enable comparison with measured Pb concentrations in whole-blood, the predicted plasma Pb concentrations were converted to whole-blood Pb concentrations using Eq. (6.1). Examples of model output are presented in the time series plots in Fig. 7.1, which show that the predicted Pb concentrations in blood vary on a monthly basis, depending on the IR used for each month.

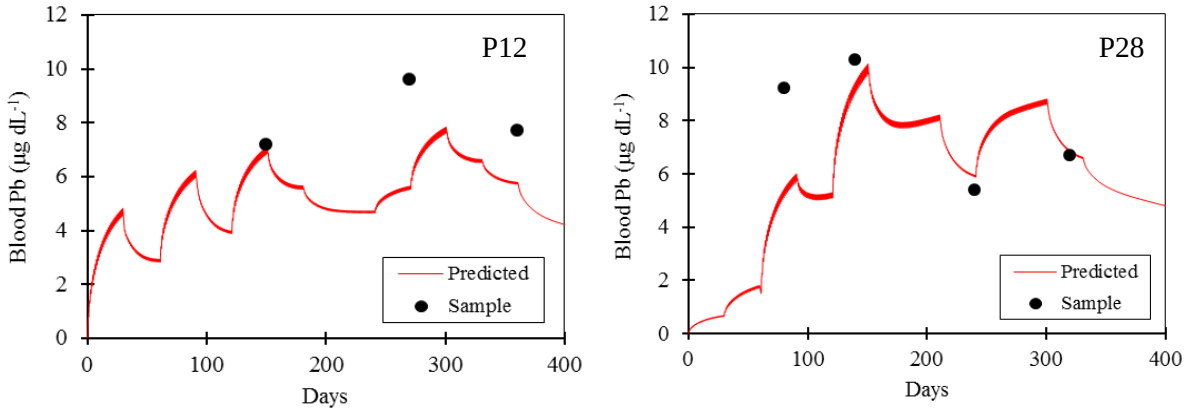

Fig. 7.1: Predicted and measured blood Pb concentrations for participants P12 and P28.
The calculated daily intake (IR) of Pb varied between 23 and 86 µg day^{-1} (P12) and 10 to 155 µg day^{-1} (P28) were used in the simulations, for an exposure frequency of 365 days. Concentrations of Pb recorded in blood samples are also indicated.

We carried out simulations similar to Fig. 7.1 for all the 32 participants who gave blood samples (selected plots from these simulations are presented in Appendix J, part J1). From each simulation, we identified the predicted blood Pb concentrations along the time series that corresponds to the time-points when blood samples were collected during the 365 days simulated. For example in Fig. 7.1, 3 blood samples (represented by dots) were collected from participant P12 on day 150, 270 and 360, respectively. Therefore, predicted blood Pb concentrations at day 150, 270 and 360 were obtained from the predicted time series plot. Subsequently, we combined all the predicted blood Pb levels and plotted them against the corresponding measured concentrations of Pb in participants' blood samples. The relationship between the measured and the predicted blood Pb concentrations is presented as Fig. 7.2, which shows a good correlation between the two data sets ($r^2 = 0.7$, $p < 0.05$).

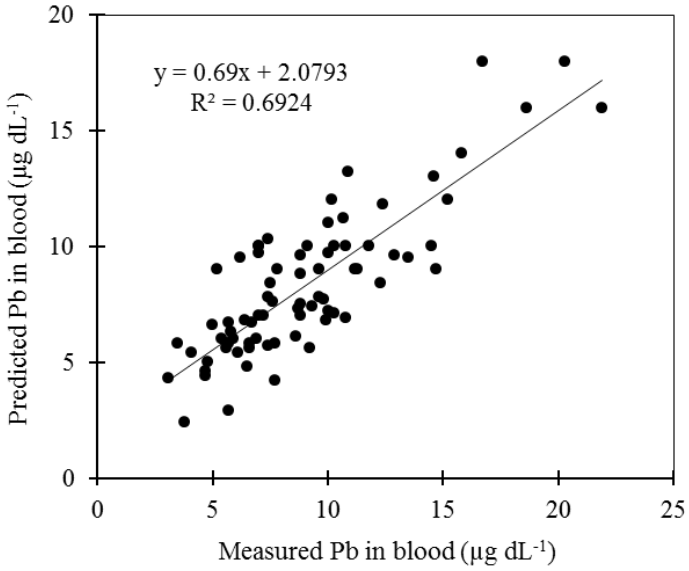

Fig. 7.2: Relationship between the predicted blood Pb concentrations and the corresponding measured Pb concentrations in blood samples.
This plot was prepared using 88% of the data points; the remaining 12% were excluded from the plot to improve data correlation. Excluded data points relate to the blood concentrations previously identified as outliers (n=7) and six other data points. A weak correlation ($r^2 < 0.3$) was determined when all data points were plotted.

However, the regression line indicates that the model under-predicts the measured concentrations. This under-prediction was considered to be indicative of exposure to additional sources of Pb apart from soil Pb. This consideration is consistent with the findings from Pb isotopic ratio analysis, which indicated that the origins of Pb in blood samples were associated with both geogenic (43 to 64 %) and anthropogenic (36 to 56 %) sources (Fig. 5.4). In addition, we used averaged dose values in the simulations, which further explain why not all model predictions match the measured data perfectly.

Again, it should be noted that evaluation of the performance of the PBPK model for Pb using literature data (where human volunteers were subjected to controlled ingestion of Pb isotope) had indicated that the model performs well (Fig. 6.8), and is capable of simulating scenarios of oral Pb exposure though oral ingestion. Therefore, the differences between the measured and predicted blood Pb concentrations should not be viewed as an under-performance of the model.

Information gathered from the study participants indicated that the majority of them (60 %) had used their plots for at least 5 years (up to 30 years was reported), while 40 % had used their plots for durations of less than 5 years. This information suggests possible chronic exposure to the elements during the previous years of allotment use. Therefore, we carried out simulations using a long-term exposure frequency of 3650 days (10 years) for all the participants. A participant's monthly IRs for Pb were averaged to obtain a single IR value for chronic exposure simulations. Selected outputs from these simulations are presented as Fig. 7.3.

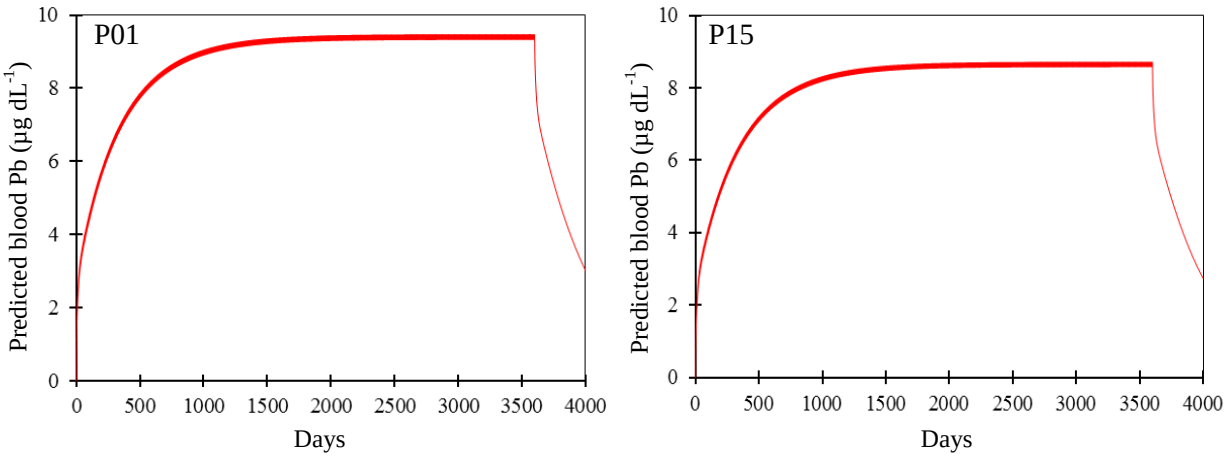

Fig. 7.3: Predicted blood Pb concentrations for participants P01 and P15.
The average IR values used were 50 µg day^{-1} (P01) and 45 µg day^{-1} (P15). Predicted blood Pb concentrations became steady at 9.4 µg dL^{-1} (P01) and at 8.6 µg dL^{-1} (P15) after approximately 1500 days (4 years) of continuous daily intake of PB at the specified IR values.

The model predicted steady-state blood Pb levels after continuous consumption of allotment produce for between 3 to 4 years. The predicted blood concentrations were within the range of measured blood Pb concentrations.

7.2.2 Exposure modelling using produce consumption rates in CLEA model

The CLEA model provides consumption rates for allotment produce based on CLEA produce categories and age classes. Data for CLEA age class 17 – 18 (the relevant adult age group) were used to derive allotment produce consumption rates (g fw kg^{-1} bw day^{-1}) of 0.5 and 3.34 for 'average' and 'high end' produce consumers, respectively (Table 6.2). These produce consumption rates were multiplied by both the median bioaccessible Pb concentration (mg kg^{-1}) recorded in produce at a given site and the participant body weight (kg bw) to determine the daily intake rate (µg day^{-1}) of Pb. The CLEA default exposure frequency for allotment land use (365 days $year^{-1}$) was adopted. The predicted blood Pb levels simulated using produce consumption rates for CLEA and for individual participants were compared, as illustrated in Fig. 7.4.

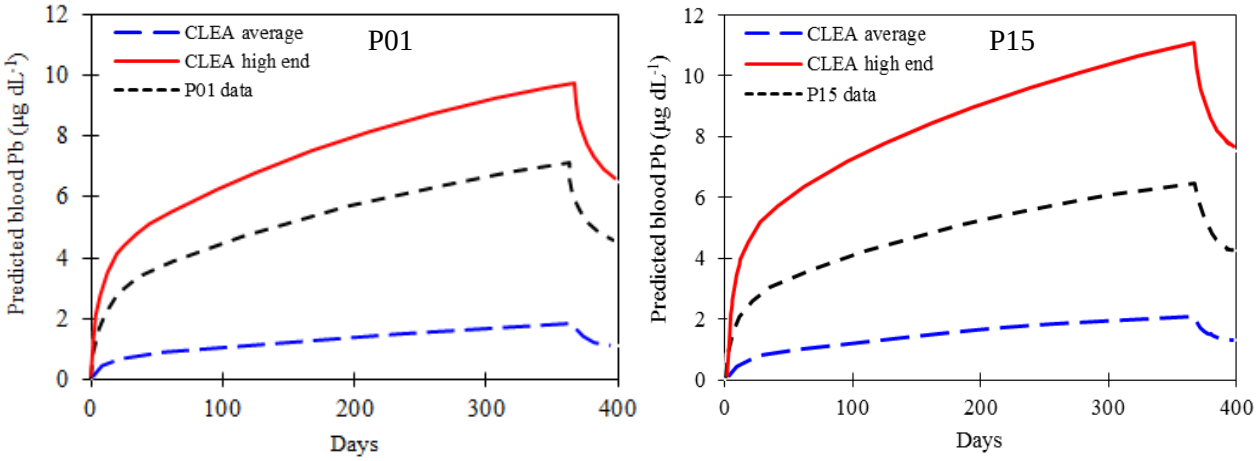

Fig. 7.4: Plots showing predicted blood Pb concentrations for participants P01 and P15 using produce consumption rates used in the CLEA model.
The calculated daily Pb intake rates (µg day^{-1}) for P01 were 11 (average) and 73 (high end), while those for P15 were 13 (average) and 87 (high end). Exposure frequency of 365 days was simulated. Predicted blood Pb levels based on participant-specific produce consumption rates are also indicated.

In this example (Fig. 7.4), daily Pb intake rates of 50 µg day^{-1} (P01) and 45 µg day^{-1} (P15) for 1 year would result in maximum blood Pb levels of 7.0 µg dL^{-1} and 6.5 µg dL^{-1}, respectively. Predicted blood Pb levels using participants' produce consumption rates were below the blood Pb levels predicted using CLEA 'high end' consumer's produce consumption rate. This is consistent with the calculated participants' produce consumption rates summarised in Appendix E (part E3), which indicates none of the participants' averaged produce consumption rates exceeding the calculated consumption rate for a CLEA 'high end' consumer of 3.34 (g fw kg^{-1} bw day^{-1}).

7.2.3 Exposure modelling of hypothetical scenarios

To demonstrate the application of the PBPK model for Pb in estimating blood Pb levels following oral ingestion of Pb, model simulations were carried out using hypothetical exposure scenarios as presented in Table 7.1.

Table 7.1: Simulated blood Pb concentrations using hypothetical exposure scenarios

Scenario No.	Pb dietary exposure (µg kg^{-1} bw day^{-1})[a]	Pb intake rate (µg day^{-1})[b]	Hypothetical Exposure Frequency (days)	Predicted maximum blood Pb concentration (µg dL^{-1})
1	0.36	25	7	0.9
2	2.43	170	7	4.4
3	0.36	25	30	1.7
4	2.43	170	30	7.0
5	0.36	25	180	2.9
6	2.43	170	180	10.8
7	0.36	25	365	3.6
8	2.43	170	365	12.6
9	0.36	25	3650	4.7
10	2.43	170	3650	16.8

[a]We used minimum and maximum values given by EFSA (2010) for adult consumers in European countries.
[b]Obtained by multiplying the dietary exposure with adult body weight of 70kg (assumed for all simulations).

From Table 7.1, it is unlikely that an adult (of 70 kg body weight) with a Pb dietary exposure of 0.36 µg kg^{-1} bw day^{-1} would have corresponding blood Pb levels above the 5 µg dL^{-1} CDC action level used in the generation of pC4SL for Pb (CL:AIRE, 2014), even after nearly 10 years of exposure. However, blood Pb levels for adults with higher Pb dietary exposure (2.43 µg kg^{-1} bw day^{-1}) are likely to exceed the CDC action level within 1 month of continuous exposure to Pb. It is important to note here that the 5 µg dL^{-1} CDC action level relates to children who are more sensitive than adults. In addition, actual dietary exposures are likely to be irregular and/or sporadic, especially for chronic exposures. Consequently, blood Pb levels could vary from those in Table 7.1.

7.3 Simulation of urinary concentrations of As, Cd, Cr and Ni

7.3.1 Exposure modelling using data from our study

The PBPK model for As (Fig. 6.1) was used to predict the quantity of inorganic arsenic (iAs) in urine samples. Given the short half-life of As in humans (2 to 3 days) (ATSDR, 2007a), simulations were carried out using data collected over the 2 to 3 consecutive days from selected participants (as previously described in section 3.5). The quantities of iAs ingested by the participants were calculated using the records of produce they consumed and the recorded As concentrations in produce samples (adjusted with bioaccessibility fractions) (Appendix E, part E5). The calculated quantities of iAs ingested by each participant varied with time.

Previously, we identified that the most sensitive parameters of the PBPK model for As include the rate of oral absorption in the GI tract (K_a), methylation constants in the kidney (K_m and V_{max}) for the various As species, and the urinary elimination rate (eU) (Fig. 6.11). During the simulations, these parameters were maintained at the default values (Appendix I, Table I1).

The model predicts urinary iAs (sum of inorganic As, MMA and DMA) in µmol. Therefore, to enable comparison of predicted urinary iAs with the measured iAs in urine samples, we converted the measured concentrations of iAs in urine samples to µmol iAs (on a daily basis) in the following manner:

a) Urine concentrations of iAs were converted from µg g^{-1} creatinine to µmol mol^{-1} creatinine by multiplying the concentrations by 1.5. The value 1.5 is the ratio of creatinine molecular weight (113 g mol^{-1}) to arsenic molecular weight (75 g mol^{-1}).
b) Measured creatinine concentrations were converted from g L^{-1} to mol L^{-1} by dividing the concentrations by the molecular weight of creatinine.
c) The urine concentrations in µmol mol^{-1} creatinine were multiplied by creatinine concentrations in mol L^{-1} to obtain iAs concentrations in µmol L^{-1}.
d) The quantity of iAs (µmol day^{-1}) in urine samples were determined by multiplying urine concentrations (µmol L^{-1}) by the average daily urine excretion rate for an adult of 1 L day^{-1} (Ruiz et al., 2010).

The predicted daily urinary iAs and the corresponding measured urinary iAs (≥ LOD) were compared as illustrated using the time series plots in Fig. 7.5, which shows the change of iAs in urine with time. We carried out similar simulations for the 13 participants who provided urine samples and produce consumption data over the period of 2-3 days; selected graphical representations of these simulations are included in Appendix J (part J2).

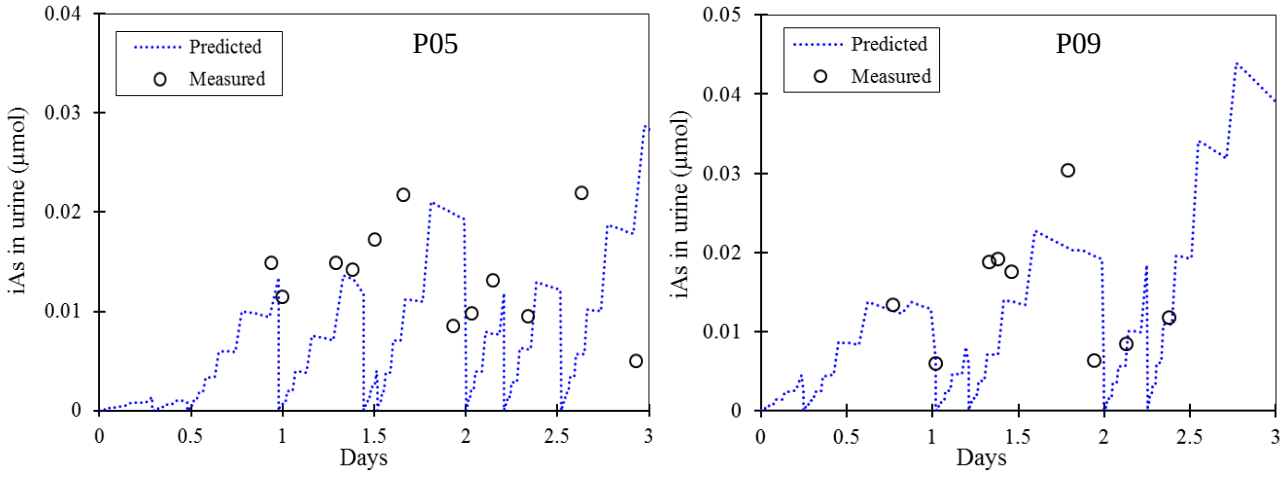

Fig. 7.5: Comparison of measured and predicted iAs in urine of participants P05 and P09 with time. Simulated doses of iAs varied with time and ranged from 0.09 to 1.25 µmol (P05) and from 0.6 to 1.73 µmol (P09).

From each simulation, values of predicted urinary iAs corresponding to the time-points of urine sampling were obtained. A linear regression analysis of all the predicted iAs in urine and the corresponding measured iAs in urine samples (Fig. 7.6) indicated a good correlation ($r^2 > 0.8$) between the two datasets, with a low RMSE value of 0.003 µmol. These results indicate that the PBPK model for As is capable of simulating human exposure to As through oral ingestion, and predicts the biomonitoring data reasonably well.

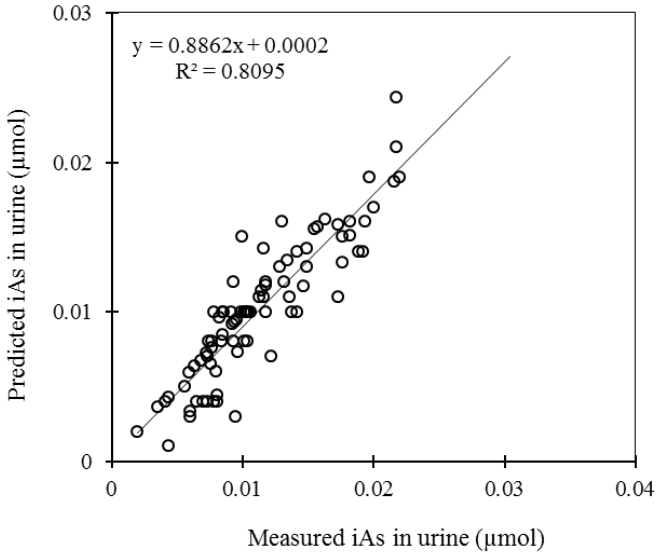

Fig. 7.6: Relationship between the predicted iAs in urine and the corresponding measured iAs in urine samples.

Predicted values were obtained from time points (in the time series plots) that match the urine sampling time-points. This plot was generated using 90% of the data points. The remaining data points (which include dilute urine samples that recorded creatinine concentrations below 0.3 g L^{-1}) were excluded from the plot to improve the correlation.

Simulations of oral exposure to Cd were carried out using the PBTK model for Cd (Fig. 6.3), based on the participants' data on the consumption of allotment produce and the Cd concentrations recorded in produce samples. A participant's daily IR values of Cd were calculated in the same manner as previously described for Pb in section 7.2.1. Subsequently, IR values were averaged to obtain a representative annual IR value, which was entered into the model as 'dose' to simulate chronic exposure to Cd based on the durations the participants have been using their plots (Appendix E, part E1). We simulated chronic exposure to Cd because of the long biological half-life of Cd (e.g., 6 to 38 years in the kidney) (ATSDR, 2012a). In addition, the highest concentrations of absorbed Cd are reported to occur in the liver and kidney (ATSDR, 2012a); and Cd in the kidney is reflected in urinary levels (Keil et al., 2011). Our approach of simulating chronic exposure to Cd is consistent with similar studies by others (Fransson et al., 2014; Choudhury et al., 2001; Ruiz et al., 2010), where exposures to Cd were simulated for periods lasting decades. The most sensitive parameters for the Cd PBTK model (i.e., parameters denoted C5, C6, C7 and 1-C7 in Fig. 6.11) were maintained at the default values (Table I2 in Appendix I)

when simulating exposure to male participants. However, for female participants, an oral absorption rate (C6) of 10 % (i.e., twice the male absorption rate) was assumed. This assumption is consistent with other studies (Ruiz et al., 2010; Choudhury et al., 2001; Diamond, Thayer & Choudhury, 2003), where it has been reported that females may absorb up to twice the amount absorbed by males, resulting in higher urinary Cd levels in females than males. The difference in Cd absorption between males and females has been linked to lower iron body stores in females compared to males; low iron stores have been associated with increased absorption of Cd in the GI tract (Choudhury et al., 2001; Berglund et al., 1994). The average daily urine excretion rate for an adult human of 1 L day^{-1} (Ruiz et al., 2010) was used to convert simulated Cd (µg) released daily in urine to Cd concentrations (µg L^{-1}). The simulated urinary Cd concentrations were compared with the measured Cd concentrations (medians) in the corresponding participants' urine samples. Exposure doses, exposure frequencies, the predicted urinary Cd and the medians of measured Cd concentrations in urine are summarised in Table 7.2 (detailed results from the simulations are included in Appendix J, part J3).

Table 7.2: Ranges of doses, exposure frequencies and urinary Cd concentrations

Calculated Cd doses (µg day^{-1})[a]	Simulated exposure frequencies (years)[b]	Predicted Cd in urine (µg L^{-1})	Medians of measured Cd in urine (µg L^{-1})[c]
1.4 – 10.4	0.2 – 30	0.002 – 0.12	0.02 – 0.17

[a]Doses were calculated for 34 participants where Cd concentrations in produce were available.
[b]Based on the reported number of years a participant has been using their allotment.
[c]For urinary concentrations ≥LOD.

Linear regression analysis (Fig. 7.7) indicated a reasonable fit ($r^2 > 0.8$) between the predicted and measured urinary Cd, with a RMSE value of 0.02 µg L^{-1}. Thus, the PBTK model for Cd has performed well in predicting our biomonitoring data.

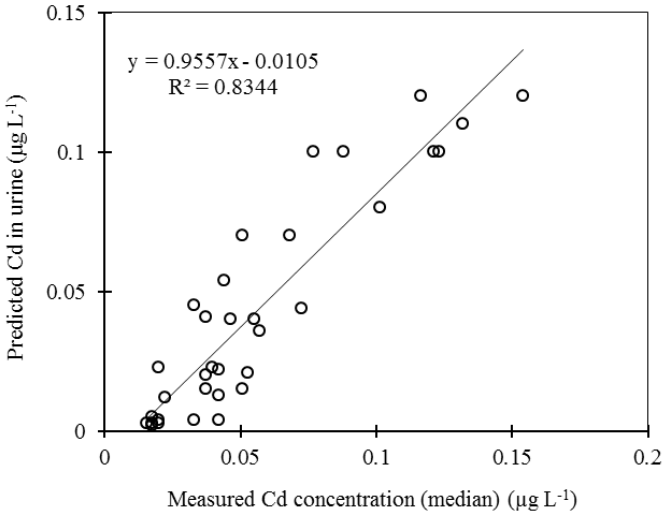

Fig. 7.7: Relationship between predicted Cd in urine and medians of measured Cd concentrations in urine for 34 participants.

Our findings are similar to those observed by Ju et al. (2012), who used a modified version of the KN model for Cd to predict urinary levels of Cd following oral ingestion of Cd from seafood consumption by male and female non-smoking subjects, for a period of 45 years. Using average daily Cd intake rates of about 7.5 µg day^{-1} (male) and 6.9 µg day^{-1} (female), they predicted mean urinary Cd levels (expressed in *µg g^{-1} creatinine*) of 0.09 – 0.34 (female) and 0.07 – 0.26 (male), when they adopted the oral bioaccessibility value (C5) for the original model (Ju et al., 2012). A study by Ruiz et al. (2010), which also used a modified version of the KN model for Cd to interpret NHANES biomonitoring data, indicated similar urinary Cd levels when they simulated low Cd intake rates between 13.5 and 22.4 µg day^{-1}, depending on the age group of the subjects. The consistency of our simulation results with other published data supports our observation that our results are similar to those reported elsewhere.

Oral exposures to Cr(III) were simulated using the PBPK model for Cr (Fig. 6.5). Given that Cr absorbed following Cr(III) ingestion has a half-life of less than 2 days (ATSDR, 2012b; Paustenbach et al., 1997), simulations were carried out using data collected during the 2 to 3 days sampling period (as described in section 3.5). The quantities of Cr(III) ingested by the 13 participants were calculated using the record of produce they consumed during the 2 to 3 days, and the recorded Cr concentrations in produce samples (adjusted with bioaccessibility fractions) (Appendix E, part E5). The calculated quantities of Cr(III) ingested by each participant varied with time. In our simulations, we used a GI tract absorption rate (KGI3) of 0.25 day^{-1} for Cr(III) (O'Flaherty et al., 2001).

The predicted daily urinary concentrations of Cr(III) were compared with the measured Cr concentrations (≥ LOD) in urine samples, as illustrated using time series plots in Fig. 7.8, which shows the change of Cr in urine with time. Selected plots of additional simulations are included in Appendix J (part J4).

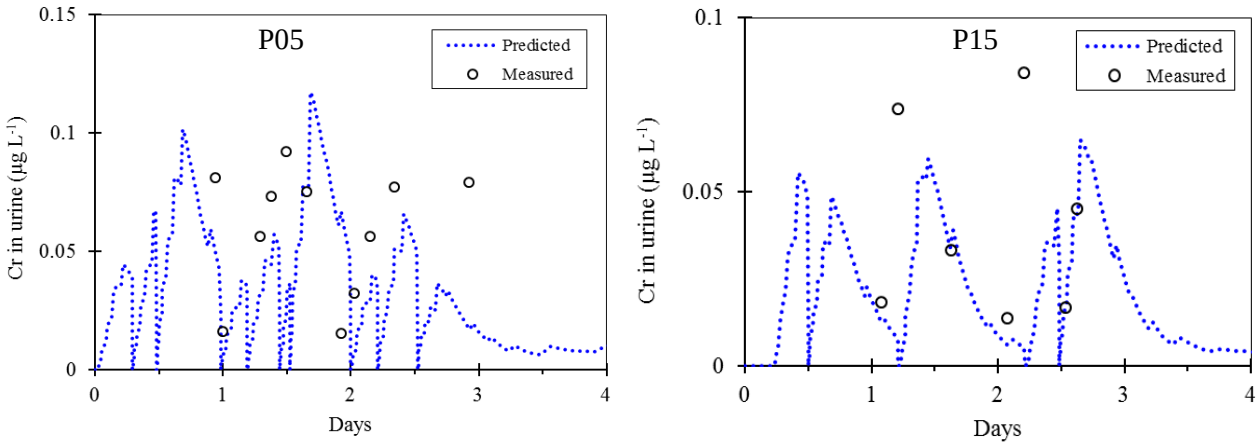

Fig. 7.8: The predicted and measured Cr(III) concentrations in urine for participants P05 and P15.
The simulated doses of Cr(III) varied with time and ranged from 22 to 86 µg (P05) and from 45 to 89 µg (P15).

Simulations were carried out for each of the 13 participants who provided urine samples and produce consumption data over the period of 2-3 days. From each simulation, values of predicted urinary Cr(III) concentrations corresponding to the time-points of urine sampling were obtained. Subsequently, all the predicted urinary Cr(III) data were plotted against the corresponding measured urinary concentrations (Fig. 7.9). Generally, both data sets correlated well ($r^2 = 0.7$, $p < 0.05$) with a low RMSE of 0.01 µg L^{-1} indicating the ability of the model to predict the biomonitoring data well.

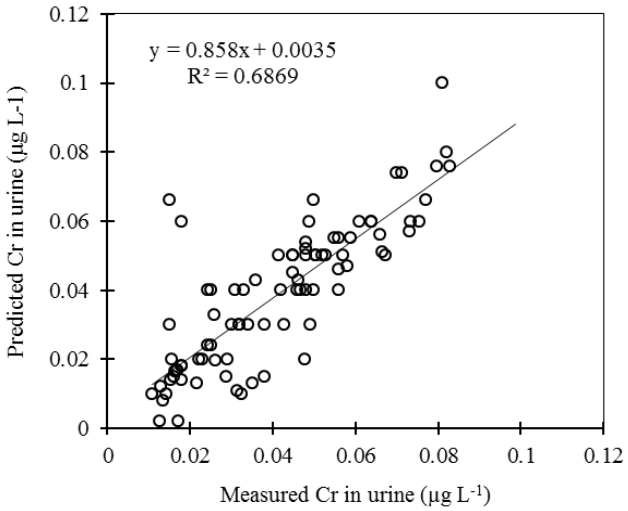

Fig. 7.9: Relationship between the predicted Cr(III) concentrations in urine and the corresponding measured Cr(III) in urine samples.

The oral intake of Ni was simulated using the Ni model (Fig. 6.9) and the data collected during the 2 to 3 days period. Doses used in the simulations were calculated in the same manner as previously discussed for As and Cr (Appendix E, part E5). The calculated quantities of Ni ingested by each participant varied with time. Simulations were carried out for all the participants. Subsequently, the predicted daily urinary Ni concentrations were compared with the measured Ni concentrations (≥ LOD) in urine using time series plots as shown in Fig. 7.10. Selected plots of additional simulations are included in Appendix J (part J5).

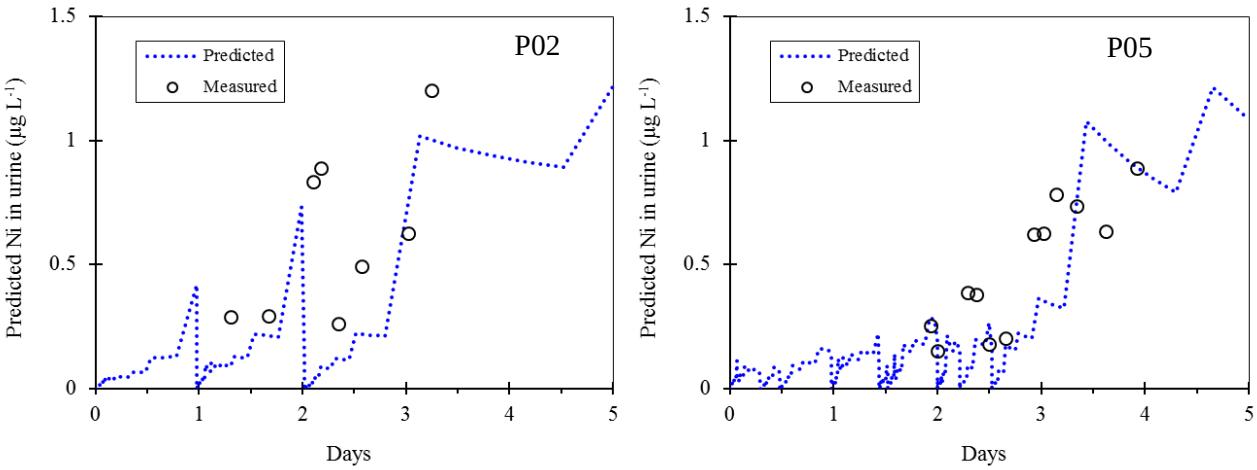

Fig. 7.10: Plots showing the predicted and measured Ni concentrations in urine for participants P02 and P05.
Simulated doses of Ni varied with time and ranged from 16 to 94 µg (P02) and from 21 to 108 µg (P05).

Values of predicted urinary Ni concentrations corresponding to the time-points of urine sampling were obtained from each simulation. The predicted urinary Ni data were plotted against the corresponding measured urinary concentrations (Fig. 7.11). Both data sets correlated well (r^2 = 0.63, $p < 0.05$) with a low RMSE of 0.1 µg L^{-1} indicating the ability of the model to predict the biomonitoring data well.

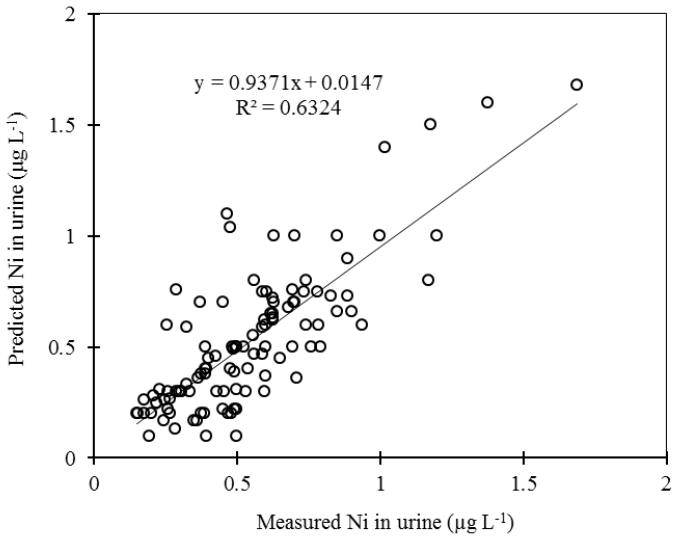

Fig. 7.11: Relationship between the predicted Ni concentrations in urine and the corresponding measured Ni concentrations in urine samples.

During the simulations of Ni concentrations, we used various values for the three most sensitive model parameters with SC > 0.5 (i.e., A_{gut}, K1 and eU) (Fig. 6.11). The ranges used for these to fit our biomonitoring data are presented in Table 7.3. Other model parameters were kept at their default values (Appendix I, Table I5).

Table 7.3: Range values for sensitive parameters with SC>0.5 used in Ni model simulations

Model parameter (*symbol, units*)	Range of values used in to fit model predictions with biomonitoring data	Parameter value given in the literature
Mass fraction of Ni absorbed from the gut (A_{gut}, *%*)	2 – 4	0.7±0.4[a] 2.95±1.32[b]
Alimentary absorption of Ni from ingested dose (*K1*, day^{-1})	8 – 12	7.92±5.76[a]
Urinary elimination rate of Ni (*eU*, day^{-1})	4 – 6	3.6±2.64[a]

[a]Values used in the original Ni PBTK model published by Sunderman et al. (1989).

[b]Nielsen et al. (1999).

7.3.2 Exposure modelling using produce consumption rates in CLEA model

The models for As, Cd, Cr and Ni were used to estimate urinary levels of these elements based on the calculated 'high end' (maximum) produce consumption rate obtained from the CLEA model

documentation (Table 6.2). Daily intake rates for the elements were calculated in the same manner as previously described in section 7.2.2. The CLEA default exposure frequency for allotment land use (365 days year^{-1}) was adopted. The predicted cumulative elemental levels in urine were plotted against time as illustrated in Fig. 7.12. The gradient of the slope (0.56 µmol) indicates the amount of iAs released to urine on a daily basis, after approximately 17 days.

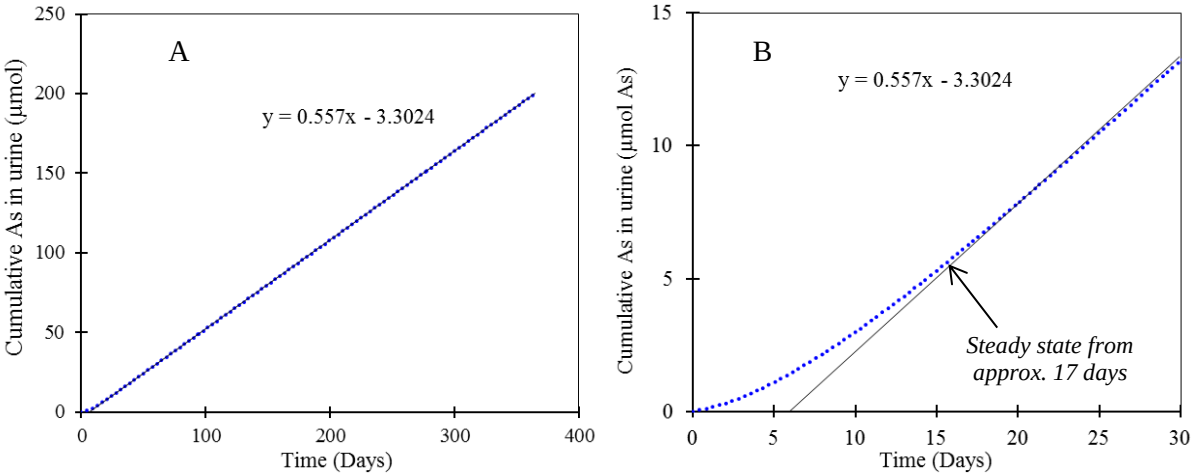

Fig. 7.12: Predicted cumulative iAs in urine for participant P16, based on 'high-end' produce consumption rate for the CLEA model.
(A) Showing cumulative urinary iAs for 365 days. (B) Shows a gradual build-up of iAs released to urine until a steady state is reached after approximately 17 days.

Model outputs from selected simulations are presented in Table 7.4, which shows Cd, Cr and Ni concentrations similar to the measured concentrations in this study. However, predicted urinary iAs exceeded iAs levels from our biomonitoring study. This was the case with the simulations performed for other participants.

Table 7.4: Selected model outputs from exposure simulations based on CLEA model data

Element	Participant ID	Dose simulated[a] (µg day^{-1})	Predicted element levels released in urine (daily, steady state)
iAs	P16	47 (*0.63 µmol*)	42 µg (*0.56 µmol*)
	P32	58 (*0.77 µmol*)	51 µg (*0.68 µmol*)
Cd	P01	17	0.02 (µg L^{-1})[b]
	P07	33	0.04 (µg L^{-1})[b]
Cr	P04	108	1.9 (µg L^{-1})[b]
	P20	17	0.3 (µg L^{-1})[b]
Ni	P06	28	3.5 (µg L^{-1})[b]
	P38	57	4.7 (µg L^{-1})[b]

[a]Doses were calculated based on the '*high end*' produce consumption rate from CLEA model, and not participant-specific produce consumption rates. Exposure frequency of 365 days per year.
[b]A daily urine excretion rate of 1 L day^{-1} (average for an adult) was used in calculating urinary concentrations.

Further predictions of urinary elemental levels were carried out using the maximum ADI values calculated for the elements based on the findings of this study (Table 4.3), for a 'standard' adult with a body weight of 70 kg and exposure frequency of 365 year^{-1} used in the CLEA model. The results are presented in Table 7.5.

Table 7.5: Predicted element levels in urine based on maximum ADI from this study, for a 'standard' adult and exposure frequency under the CLEA model

Element	Maximum ADI[a] (from Table 4.3) (µg kg^{-1} bw day^{-1})	Dose simulated[b] (µg day^{-1})	Predicted element levels released in urine daily (exposure for 365 days)
iAs	0.29	20 (*0.27 µmol*)	18 µg (*0.24 µmol*)
Cd	0.13	9	0.01 (µg L^{-1})[b]
Cr	0.87	61	1.1 (µg L^{-1})[b]
Ni	1.7	120	7.2 (µg L^{-1})[b]

[a]ADI (average daily intake).

[b]Doses were calculated by multiplying ADI by the average adult weight of 70 kg.

[c]A daily urine excretion rate of 1 L day^{-1} (average for an adult) was used in calculating urinary concentrations.

The predicted urinary levels for iAs, Cd, Cr and Ni (Table 7.5) are well below the WHO guideline values in Table 5.3. Although the simulations have considered a constant daily rate of exposure to the elements, any variations in the rates of exposure throughout the year would result in variable urinary levels different from those given in Table 7.5.

7.3.3 Exposure modelling to estimate conservativeness of GAC values

The conservativeness of the current GAC values used in contaminated land risk assessment was identified earlier in Chapter 2 as a knowledge gap, which this study sought to address. Therefore, it was necessary to quantify the magnitude of over-estimation of exposure that would result from the use of the GAC values. In order to do this, the modified models were used to predict biomarker (urine) concentrations of the elements following oral exposure at the intake rates and exposure frequencies used in the generation of the GAC values. A 365 days year^{-1} exposure frequency was used for iAs, Cr and Ni, while Cd was simulated based on 50 years exposure (Nathanail et al., 2015). Subsequently, the predicted urine concentrations were compared with WHO biological limit values which indicate the permissible concentrations for occupationally exposed workers (WHO, 1996). In addition, the predicted urine concentrations were compared with reference values for a non-occupationally exposed population in the UK. Exposure to environmental chemicals in the general population can vary over time, therefore recent biomonitoring data were used as reference values. In addition, NHANES data for iAs was used a reference value since no data was obtained for iAs from studies carried out in the non-occupationally exposed populations in the UK.

Note that reference values are not limit values, but instead they indicate element levels in urine of the general population studied. To allow comparison of the predicted urine concentrations with WHO biological limit values and the reference values, a urinary creatinine concentration of 1 g L^{-1} was assumed (this is similar to the median of urinary creatinine recorded in this study). Table 7.6 shows the element intake rates used in the simulations, the predicted urinary levels and the calculated ratios between the predicted urine levels, WHO limit values and reference values.

Table 7.6: Estimates of conservativeness of GAC values

Element	Oral TDI / ID (µg kg^{-1}bw day^{-1})	Oral MDI, calculated for a 70kg adult or published value (µg day^{-1})	Predicted urinary levels[a,e] (A)	Reference Values[f] (95th percentile) (B)	Ratio of B/A	WHO biological limit values[b] (C)	Ratio of C/A
iAs	0.30[c]	21 (*0.28 µmol*)	19 µg (*0.25 µmol*)	14.8 (17.7)[g]	N/A	50	N/A
Cd	0.54[c]	38	0.8	0.52 (0.6)[h]	0.75	5	6
Cr(III)	150[d]	60.2 (published value)[d]	1.1	0.79 (2.9)[h]	2.6	30	27
Cr(VI)[i]	0.44[c]	31	1.4	0.79 (2.9)[h]	2.1	30	21
Ni	12[d]	134 (published value)[d]	7.7	6.35 (10.7)[h]	1.4	30	4

TDI (tolerable daily intake), MDI (mean daily intake), ID (index dose, used for iAs which is a non-threshold substance), N/A (not calculated because iAs is a non-threshold substance).

[a]For creatinine correction of urinary concentrations, we assumed a creatinine concentration of 1 *g* L^{-1}.

[b]Units in *ug* g^{-1} *creatinine*.

[c](CL:AIRE, 2014).

[d](Nathanail et al., 2015).

[e]Units in *ug* L^{-1} (same values in *ug* g^{-1} *creatinine* since a creatinine concentration of 1 *g* L^{-1} was assumed). A daily urine excretion rate of 1 *L day-1* was also assumed.

[f]Units in *ug* L^{-1} (*ug* g^{-1} *creatinine*). Creatinine corrected values were used in ratio calculations.

[g]NHANES data from the Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017, Volume One (CDC, 2017).

[h]A recent UK study on a non-occupationally exposed population (Morton et al., 2014).

[i]Cr(VI) is transformed in the body and excreted in urine as Cr(III).

Looking at the ratios of the reference values to the predicted urinary levels (ratio of B/A in Table 7.6), the predicted urine concentrations are generally consistent with the reference values for a non-occupationally exposed population in the UK; even though the model suggests that an individual would need to ingest approximately twice the simulated Cr intake to result in Cr urine concentrations at the same level as the reference value used. The predicted iAs in urine is similar to the NHANES data used as a reference value. Given that iAs is a non-threshold substance (Environment Agency, 2009d), the ratios between the predicted iAs urine level, WHO limit value and the reference value were not calculated.

The ratios of the WHO biological limit values to the predicted urine concentrations (ratio of C/A in in Table 7.6), indicate the extent of conservativeness of the GAC values, assuming that other sources of exposure (e.g., inhalation) are negligible. Therefore, an individual who ingests the elements at similar intake rates used in deriving the GAC values would need to ingest additional multiples of the intake (6 for Cd, 27 for Cr(III), 21 for (Cr(VI) and 4 for Ni) to result in urinary levels similar to the WHO limit values. It is important to reiterate that the calculated ratios (hence the estimated conservativeness of GACs) are based on the assumptions used in the calculation of predicted urine concentrations and the subsequent creatinine correction of the urine concentrations.

7.4 Estimation of relative bioavailability values

As previously identified in Chapter 2, bioaccessibility and bioavailability values influence the absorption of ingested substances in the human body. Bioaccessibility values of an element in the ingested medium can be estimated using *in-vitro* measurements. Although bioavailability values can be derived from *in-vivo* studies, PBPK / PBTK models can also be used to estimate absorption / uptake of the elements, which is the product of parameters F_A x F_H in Eq. (2.5). Therefore in this section, we illustrate how the models can be used to derive relative bioavailability values that can be entered into the CLEA model during the risk assessment process.

The median of bioaccessibility values reported in produce samples were used to represent parameter F_B in Eq. (2.5). Values of absolute bioavailability (ABA_{tox}) of the elements in the media (i.e., drinking water) used in the derivation of the toxicological criteria for the elements were either obtained from the literature or assumed. For each element, a hypothetical dose of 10 µg (10 µmol for iAs) was simulated. The estimated relative bioavailability (RBA) values are presented in Table 7.7. It should be noted that RBA values are site-specific because their calculation involves bioaccessibility values that are measured on a site-specific basis. The calculated RBA values relate to adults because of the adult models used in the simulations. Children often have higher oral absorption values which would result in higher RBA values.

It can be seen in Table 7.7 that the estimated RBA for iAs is approximately equal to the bioaccessibility value for iAs, because both the uptake and the absolute bioavailability of iAs are approximately the same. The absolute bioavailability of Pb in water assumed by USEPA (i.e., 50 %) was used in calculating the relative bioavailability of Pb. However, it is important to note that Pb bioavailability is dependent on the physical and chemical form of Pd in diet, the quantity of ingested Pb, and therefore the value assumed by USEPA may not be appropriate for all cases (Juhasz et al., 2009; ATSDR, 2007b).

Table 7.7: Estimated relative bioavailability values

Element	Median of bioaccessibility values in produce (F_B)	Uptake (fraction of dose) (F_A*F_H)	Absolute bioavailability in media used to derive toxicological limit (ABA_{tox})	Relative bioavailability (RBA) (F_B*F_A*F_H) / ABA_{tox}
iAs	0.4	0.9	0.95[a]	0.38
Cd	0.17	0.025	0.42[b]	0.01
Cr	0.35	0.017	0.03[c]	0.2
Ni	0.35	0.04	0.44[d]	0.03
Pb	0.45	0.1	0.5[e]	0.1

[a]Absorption of soluble As in drinking water is approximately 95% (ATSDR, 2007a). Index dose for arsenic is based on drinking water guideline.

[b]Assumed value, for fraction of Cd dose in porridge retained in the body (ATSDR, 2012a). No data was obtained for absorption of soluble Cd in water.

[c]Absorption fraction of soluble Cr(III) (ATSDR, 2012b).

[d]Reported maximum absorption value for Ni ingested in water (Sunderman et al., 1989). The tolerable daily intake for nickel is based on deriving drinking water guidelines (Nathanail et al., 2015).

[e]Value assumed by USEPA was adopted (Casteel et al., 2006). Low level of toxicological concern is consistent with EU drinking water standard.

F_B is the oral bioaccessible fraction of an element in ingested produce.
F_A is the fraction of a solubilised element transported across the GI wall into systematic circulation.
F_H is the fraction absorbed that does not undergo first pass metabolism in the intestinal epithelium and/or the liver.
ABA_{tox} is the absolute element bioavailability in the media used in the toxicological studies (dimensionless).

7.5 Chapter Summary

Evaluation of the modified PBPK/PBTK models for Pb, Cd, Cd and Ni using biomonitoring data gathered as part of this study has indicated that the models are capable of predicting the biomonitoring data reasonably well. This supports our hypothesis '*h3*' that *"PBPK model predicted element concentrations in blood and urine are good indicators of the corresponding measured biomarker concentrations"*. Therefore, revision of the model parameters was not considered necessary. However, during the evaluation process, attention was paid to the most sensitive model parameters and where appropriate, a range of parameter values (which fit with the range of data given in the literature) were used to improve the fit between the predicted and the corresponding measured data. The models were also used to predict elemental levels in biological samples, based on exposure scenarios depicting the CLEA model and hypothetical conditions. By simulating oral MDI values used in deriving GAC values for the elements, the conservativeness of GAC values (for Cd, Cr and Ni) were estimated to range from multiples of 4 to 27, based on the WHO biological limit values. Estimates of relative bioavailability of the elements have also been derived using data from model simulations.

8. FURTHER DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 Introduction

This concluding chapter draws together the research findings, highlights the contribution to knowledge arising from this research and the importance of the findings to the contaminated land industry. Key conclusions are given and recommendations for further work are outlined.

8.2 Further discussion

8.2.1 Key findings

This study identified widespread elevated Pb concentrations in allotment soil, consistent with soil concentrations previously recorded in UK soils (Prasad & Nazareth, 2000). Apart from As which had elevated concentrations at one allotment site, Cd, Cr and Ni did not record soil concentrations above their GAC values. Similarly, the concentrations of Pb were generally highest in allotment produce samples, compared to other elements. The results of a physiologically-based extraction test indicated that the bioaccessibility values of these elements in soil and produce were considerably less than 100%, indicating that the risk associated with potential ingestion of these elements through soil or allotment produce could be exaggerated if total element concentrations (that assumes 100% bioaccessibility) are used in risk assessment models. For the allotment land use, the dominant exposure pathway through which humans get exposed to soil contaminants is the consumption of allotment produce. It has been reported that some individuals adopt gardens/allotments as a way to provide fresh produce and save on food costs (Sipter et al., 2008). A site-specific risk assessment carried out using participants' produce consumption data did not identify a significant concern to human health. This finding is beneficial to the use of allotments by encouraging the use of allotments (and urban gardens in general), considering that occurrences of elevated Pb and As concentrations above their corresponding GAC values were recorded in some allotments. In other words, sites that would have potentially been 'condemned' as 'contaminated' did not result in significant concern to human health. This highlights the importance of carrying out site-specific risk assessment where possible site contamination has been identified.

Regarding the biomonitoring results, blood Pb levels recorded in this study were higher than previously reported in a health survey conducted in England (Morton et al., 2009). (i.e., recorded median concentration of 9.2 μg dL^{-1} is approximately 3 times the values recorded in the English health survey of 1995). Although published studies reporting blood Pb levels in Scottish populations indicated higher values than those from the English health survey, one Scottish study (Macintyre et al., 1998) indicated a declining trend of blood Pb levels between 1983 and 1993 in

the population studied. Blood Pb levels from our study were consistent with those recorded in a control population for a study carried out in southern Scotland in 1989 (Moffat, 1989). No recently published data on blood Pb levels in non-occupationally exposed UK population was available, because in the UK, biomonitoring of blood Pb is not routinely carried out in the non-occupationally exposed populations. Due to the scarcity of published data on blood Pb levels in non-occupationally exposed UK population, blood Pb levels from this study contributes to literature data that can be used for comparison with other similar studies. From the review of the limited published data on blood Pb levels in non-occupationally exposed populations in the UK, it is possible that background levels of Pb in central Scotland have been higher than other parts of the UK. Further survey of blood Pb levels would be beneficial in giving a clearer picture of blood Pb levels in non-occupationally exposed population in Scotland.

Statistical analysis did not indicate significant associations between blood Pb concentrations and participant's age, gender, smoking status and produce consumption rate; which indicate that the participants were not exposed to Pb at statistically significant levels above the local background levels. With regards to the produce consumption pathway, the consistency of recorded blood Pb levels with previously published blood Pb data in Scotland suggests that consumption of allotment produce did not result in blood Pb levels that were significantly different from the local background levels. Pb isotope analysis indicated that both geogenic and anthropogenic sources of Pb contributed to Pb in blood, with a higher proportion from geogenic sources. The recorded element concentrations in urine were similar to the published urine concentrations for the general (non-occupationally exposed UK populations). Again, this indicates that participants' consumption of allotment produce did not result in them having significant additional exposure to the elements.

Physiologically-based kinetic models were produced using existing models and the accompanying model parameters published in the literature by modifying and/or simplifying the existing models while maintaining their predictive ability. The predictive ability of the models was evaluated using data published in the literature. The models reproduced the literature data well (within ±17%, apart from one dataset from Pb simulations with +38%). We have demonstrated how the models were used to inform and optimise the design of our biomonitoring study, by simulating low oral doses of the elements and predicting optimal sampling times that would allow element concentrations in biological samples to be detected during laboratory analysis. This indicates that the models could be used in the planning of other similar longitudinal studies. Evaluation of the models using biomonitoring data gathered from this study indicated that the models are capable of predicting the biomonitoring data reasonably well ($r^2 > 0.6$ with low RMSE values). Therefore, these evaluated models are useful tools in predicting uptake of the elements through oral ingestion, thus, improving our understanding of actual exposure to the elements. The models are adaptable since model parameters can be adjusted to reflect the exposure characteristics under consideration. The models were used to simulate oral ingestion of the elements under different exposure scenarios and to

estimate the conservativeness of the GAC values (for Cd, Cr and Ni) – given that the potential for the GAC values to over-estimate health risk was identified as a knowledge gap for this study. By comparing the predicted urinary concentrations with the WHO biological limit values, the conservativeness of GAC values were estimated to range from multiples of 4 (Ni) to 27 (Cr). How conservative GAC values are depends on the number of exposure sources used in their derivation. In most cases the health criteria values used in deriving GAC values take account of ingestion though drinking water and inhalation pathway. Here, the estimated extents of conservativeness of GAC values assume oral ingestion (e.g., through food intake) only. Both Pb and iAs are non-threshold substances, and therefore the conservativeness of their GAC values was not estimated from model simulations. However, no significant concerns to human health were identified from sites that had elevated concentrations of As and Pb (up to approximately 2 times, and 13 times the corresponding pC4SL, respectively). We have demonstrated how the models can be used to estimate relative bioavailability values that can be entered into the CLEA model when carrying out a risk assessment.

8.2.2 Industrial relevance of research findings and contribution to knowledge

This study has produced and evaluated physiologically-based kinetic models that are capable of predicting uptake of the elements through oral ingestion, which increases our understanding of actual exposure to the elements and meets the aim of the study. Because of improved understanding of actual exposure to these elements, the models provide a platform for a more robust risk assessment, and thus, promote sustainable reuse of brownfield sites and the creation of sustainable built environments. This work opens a new chapter in detailed quantitative risk assessment (DQRA) of human health, where the models can be incorporated into site-specific risk assessment of contaminated land to predict internal exposure. In addition, there could be instances in exposure studies where biomonitoring may not be practical, for example due to ethical restrictions. In such instances the models can be used in lieu of biomonitoring to estimate exposure to the elements, thus, enabling such studies to be accomplished.

For the IOM, the biomonitoring techniques used in this project provide in-house capability to conduct similar exposure studies. This project has also promoted the collaboration between the IOM and the University of Reading, creating an opportunity for future joint research.

The use of physiologically-based kinetic models is not new in exposure studies. However based on the available literature, their application in simulating oral exposure to toxic elements in soil is limited. For example, the Integrated Exposure Uptake Biokinetic (IEUBK) model has been used to simulate exposure to Pb in children from a number of sources including soil (White et al., 1998). During the process of developing the pC4SL for Pb, the IEUBK model was used to investigate the relationship between Pb dose and the predicted blood Pb concentration in children (CL:AIRE, 2014). Apart from the IEUBK model, no information was obtained describing the use of

physiologically-based models for As, Cd, Cr and Ni in the context of contaminated land exposure (e.g., in the generation of GAC values). Therefore, the models used in this study present a new extension of the existing use of physiologically-based kinetic models from other forms of exposure studies to contaminated land risk assessment.

The study provides useful data that can be used for comparison with similar studies. In particular, due to the scarcity of published data on blood Pb levels in the non-occupationally exposed UK population, the blood Pb results from the present study presents an important contribution to the literature, especially in relation to a population that consume produce from their allotments.

8.2.3 Limitations of this research

Children are the critical receptors when conducting health risk assessment for allotment land use. Therefore, the use of children in this study would have been more appropriate than using adults. However children were not recruited to the study because of ethical reasons (i.e., complexity of obtaining ethical approval for using children in biomonitoring, collection of biological samples from children for a prolonged period could potentially expose children to harm), and because it would have been difficult to obtain parental consents. Due to the above limitation, physiologically-based models for adults were used in this study. The models are adaptable and the model parameters can be changed. However, where the models are modified to simulate exposure to children, evaluation of the modified models should be done using data relevant to children to test the suitability of the models in predicting exposure to children.

8.3 Conclusions

Land contamination is a common problem associated with land regeneration and the built environment. Sources of land contamination include natural processes and anthropogenic activities such as past or present land uses. The generic assessment criteria (GAC) values currently used in contaminated land risk assessment are conservative – which although are protective of public health, they may result in over-estimation of human exposure to soil contaminants. Subsequently, this could lead to un-necessary remediation or restrictions on land use. This work sought to improve our understanding of the actual human exposure to selected toxic elements in soil in order to promote sustainable reuse of land. The occurrence of toxic elements is common in the urban environment, including urban allotments and gardens. Consequently, fruits and vegetables in contaminated allotment soil may result in human exposure to toxic elements through the consumption of home-grown produce.

We investigated the potential risk to human health associated five common toxic elements (As, Cd, Cr, Pb, Ni) in selected allotments in Scotland. The concentrations of these elements were measured

in allotment soil and produce samples. 95% of soil Pb concentrations were elevated above the GAC value and locally elevated soil concentrations were observed for As and Cd.

However, the bioaccessible concentrations of As and Cd were not elevated, but 60% of bioaccessible Pb concentrations were still elevated above the GAC (indicating a potential source of soil Pb exposure in most plots). Pb recorded the maximum concentration in allotment produce samples, followed sequentially by Cr, Ni, As, and Cd. There was no significant correlation between element concentrations in soil and produce samples, contrary to our hypothesis *'h1'* that *"there is a good correlation between element concentrations in allotment soil and the corresponding element concentrations in produce"*. The bioaccessibility of the elements in allotment produce were considerably below 100%. The use of bioaccessibility concentrations in exposure estimations can give more realistic indications of the risk to human health, since they are estimates of the potentially soluble fractions in the human gastrointestinal tract. Based on the participants' consumption of allotment produce records, no potential risk to the elements was identified (HQ<1 for all elements). This emphasises the need for site-specific risk assessments rather than relying on generic assessments based solely on soil concentrations.

To improve our understanding of actual exposure to these toxic elements, we used biomonitoring and human physiologically-based kinetic models to assess the levels of these elements in participants' biological samples (biomarkers). Measured blood Pb levels were consistent with data published by previous studies carried out in central Scotland, but were higher than the levels previously reported in other parts of the UK. Urine concentrations were similar to the reference values obtained from studies carried out in non-occupationally exposed populations in the UK. Statistical analyses indicated that the participants were not exposed to these elements at significant levels compared to the published background levels, which supported out hypothesis *'h2'* that *"the levels of the elements in blood and urine samples obtained from the participants are similar to the local background levels"*. Physiologically-based kinetic models were produced using existing models published in the literature. The models predicted well both the literature data and our biomonitoring data, thus supporting our hypothesis '*h3*' that *"PBPK model predicted element concentrations in blood and urine are good indicators of the corresponding measured biomarker concentrations"*. The models were used to estimate the conservativeness of GAC values and relative bioavailability values for the elements.

In conclusion, no significant health risk to the participants was identified from their use of allotment and consumption produce. Given the increasing demand for urban allotments in the UK, our findings will benefit the allotment community by encouraging the use of allotments. The models produced from this work promote robust risk assessment of contaminated land because of their capability to predict internal exposures well. By improving our understanding to human exposure, these models are important in the sustainable management of land contamination. To our

knowledge, it is the first time combined biomonitoring and physiologically-based modelling for the five toxic elements have been used to assess exposure to these elements among allotment users.

8.4 Recommendations

The following recommendations arose from the findings of this study:

- The lack of recent published data on blood Pb levels for the general UK population points to the need for further research to obtain data on blood Pb levels among non-occupationally population, especially in Scotland where higher blood Pb levels have been recorded, compared to other parts of the UK.
- The physiologically-based kinetic models used in this study present a foundation upon which further extension of the models can be based. Future work could extend the models to cover other land uses or soil contaminants.
- Further work is recommended to use the models to create user-friendly modelling tools for the contaminated land industry, to be used as part of the DQRA process.

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APPENDICES

Appendix A: UREC Ethical Approval

University of Reading

Coordinator for Quality Assurance in Research
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Dr Chris Collins
School of Construction Management and Engineering
University of Reading
RG6 6AW

12 May 2014

Dear Chris

UREC 14/24: The human health impact of contaminated land.
Favourable opinion

Thank you for helpful response (email from Eric Dede, dated 12 May 2014 and including attachments refers) to the Committee's report on your recent application. I can confirm that the Chair is pleased to confirm a favourable ethical opinion on the basis of this information and documentation.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here: http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely

Mike Proven

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Professor Stuart Green (Head of School); Eric Dede (PhD student); Stephanie Weller, Research Manager

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12 March 2015

Dear Chris

UREC 15/21: The human health impact of contaminated land.
Favourable opinion

Thank you for the application (email from Eric Dede, dated 11 February 2015 and including attachments refers) which was considered by a UREC sub-committee at its March meeting. I can report that the Chair is pleased to confirm a favourable ethical opinion on the basis of this documentation that was reviewed by the sub-committee.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here: http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely

Mike Proven

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Professor Stuart Green (Head of School); Eric Dede (PhD student)

Appendix B: Sketches of the Original PBPK/PBTK Models

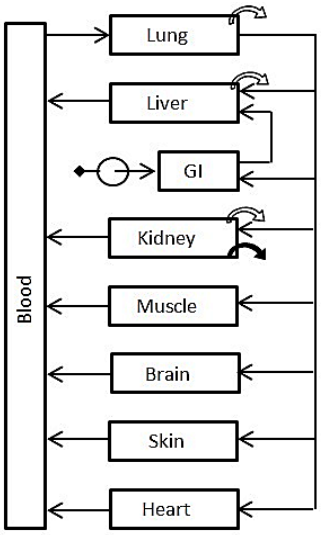

Fig. B1: A sketch of the PBPK model for As published by El-Masri & Kenyon (2008).
The sketch is for one As species, El-Masri & Kenyon (2008) published four similar sketches that are interlinked, one for each As species. The model accommodates oral exposure to As(III), As(V), MMA(V) and DMA(V) as represented by block arrow to the gastrointestinal (GI) tract lumen (circle). Excretions of As species are shown by dark curved arrow for As(III), As(V), MMA(V) and DMA(V), and light curved arrows for MMA(III) and DMA(III).

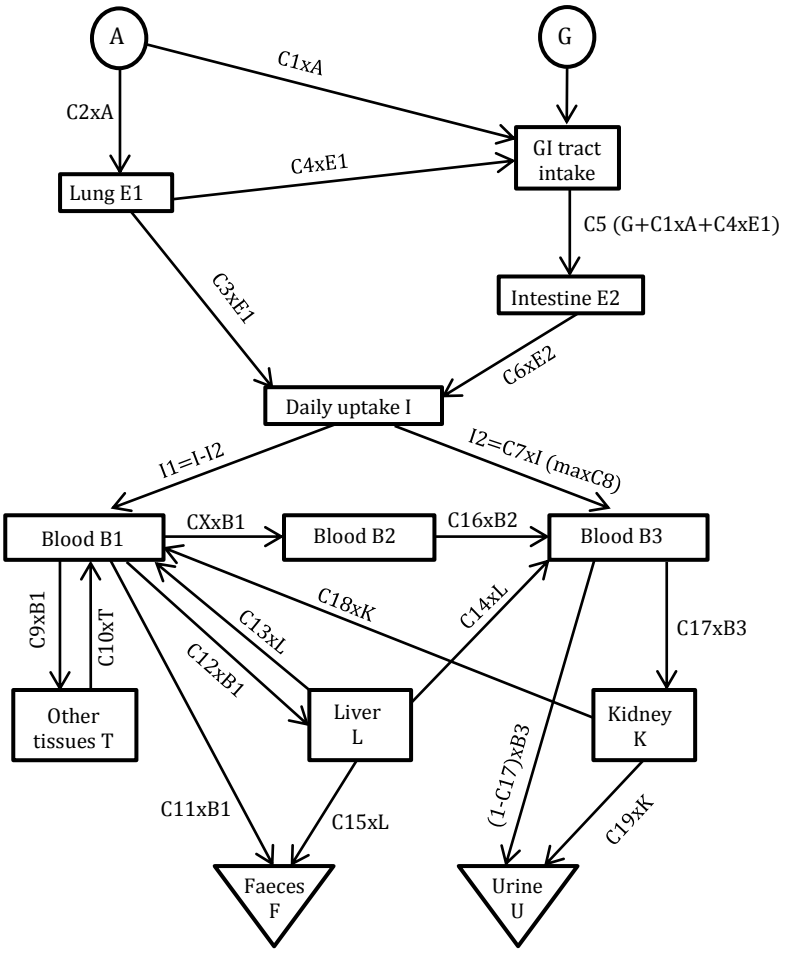

Fig. B2: A sketch of the kinetic model for Cd metabolism in human published by Kjellsröm & Nordberg (1978).
'A' refers to inhalation pathway, 'G' refers to oral ingestion pathway, Cd transfer coefficients between compartments are shown next to the arrows connecting the compartments.

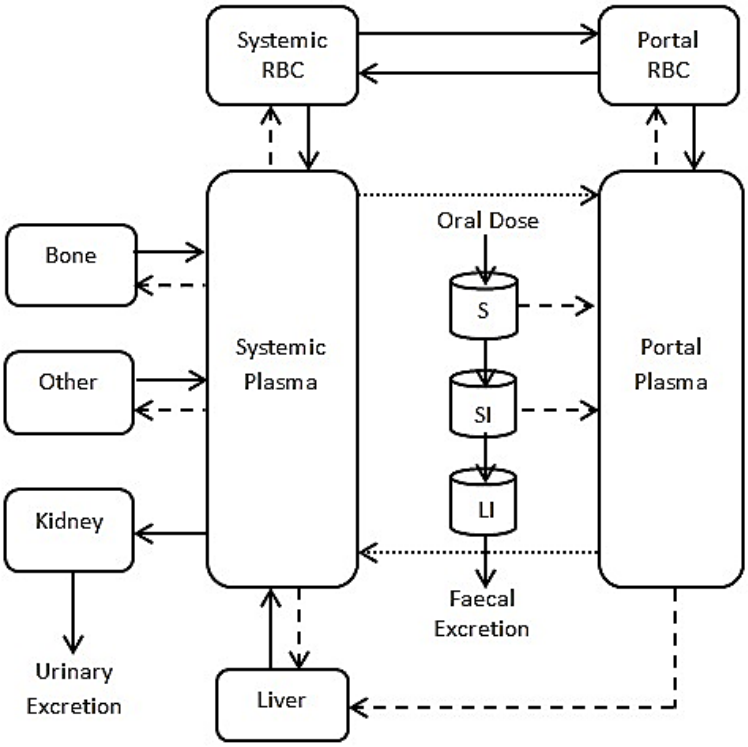

Fig. B3: Schematic representation of the PBPK model structure for Cr published by Kirman et al. (2013). RBC is red blood cells, S is stomach, SI is small intestines, and LI is large intestines. All compartments contain Cr(VI), dotted arrows and the plasma compartments contain Cr(III) pools . S, SI, LI and dashed arrows depict Cr(III) in the distribution pool, the remaining compartments depict Cr(VI) in the storage/excretion pool.

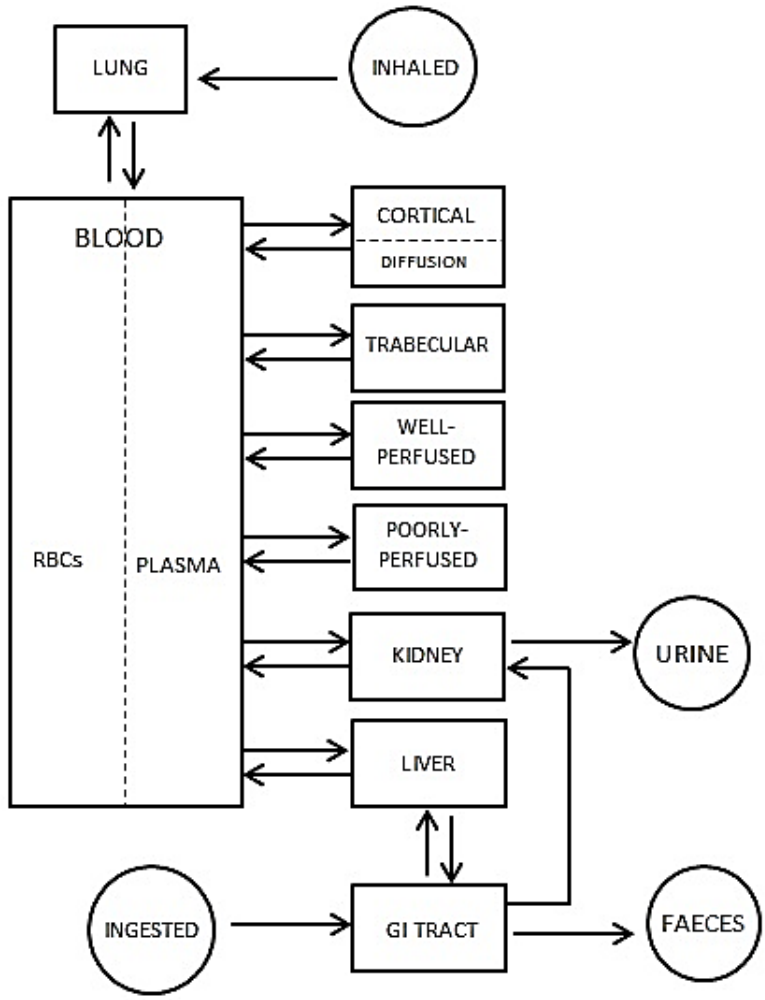

Fig. B4: Schematic representation of the O'Flaherty PBPK model for Pb published by Fleming et al. (1999). RBCs refer to red blood cells.

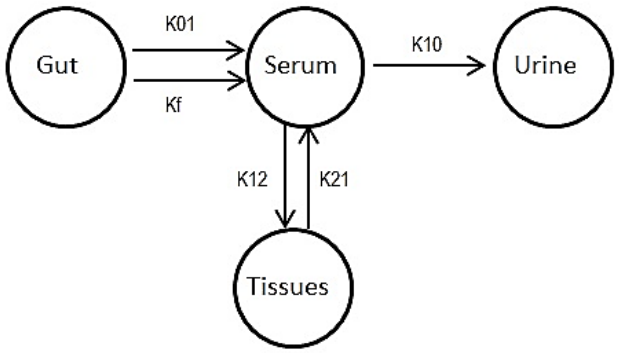

Fig. B5: A schematic diagram of the compartmental model for Ni metabolism published by Sunderman et al. (1989). Kf is a pseudo zero-order rate constant for fractional absorption of dietary Ni; K01 is first-order rate constant for intestinal absorption of Ni from the oral dose of $NiSO_4$; K12 is first-order rate constant for Ni transfer from serum to tissues; K21 is first-order rate constant for Ni transfer from tissues to serum; K10 is first-order rate constant for Ni excretion in urine.

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Appendix C: Pilot Study Findings (SuDBE Conference Paper)

Topic: T1.4 Resource (energy, water and land) and Waste Management Reference number: 1060

Investigating the human health impact of contaminated land – results from a pilot study

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Abstract: The human health risks from land contamination must be adequately addressed to ensure the sustainability of the built environment. This needs a clear understating of the potential human exposure pathways to soil contaminants. This paper presents the findings of the pilot phase of a study to investigate the potential magnitude of human exposure to selected toxic elements (As, Cd, Cr, Ni, Pb) from soils within urban allotments in the UK. Exposure pathways investigated include consumption of allotment produce (including contaminants in adhering soil), along with inadvertent ingestion and inhalation of outdoor allotment dust. Samples collected and analysed include allotment soil and plant produce, airborne allotment dust, hand moist-wipes from allotment workers, and biological samples (urine and blood) from allotment users. Ethical approval for the study was granted by the University of Reading Research Ethics Committee.

The recorded mean soil concentrations (mg kg^{-1}) ranged between 8–94 (As), 1–2 (Cd), 81–93 (Cr), 222–760 (Pb) and 45–69 (Ni). The corresponding bioaccessibility values were 53%, 51%, 8%, 22% and 57%, respectively; which indicate that the risk associated with potential soil ingestion could be exaggerated if bioaccessibility values are not factored in risk assessment models. Arsenic was found to have the highest soil-to-plant transfer factor, indicating a greater potential for exposure through human diet. It was also observed that washing of allotment produce prior to preparation and consumption lowered the potential for human exposure to the metals in soil particles adhering to produce surfaces. In addition, inadvertent ingestion (hand-to-mouth contact) could increase human exposure to soil contaminants. However, exposure via allotment dust inhalation was found to be negligible. No detectable metals were recorded in urine and blood samples, indicating negligible background/exposure levels for the participants monitored.

Key words: contaminated land, allotment, human health, biological monitoring

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1 Introduction

Land contamination is a common problem associated with land regeneration. Risk assessment of contaminated land involves identifying a source of contamination, exposure pathway and a receptor; land is considered contaminated where these three elements are linked [1]. A clear understanding of potential human exposure pathways to soil contaminants is crucial for a comprehensive risk assessment. However, current exposure models used in contaminated land risk assessment are highly conservative (precautionary) [2], making them prone to over-estimating exposure to soil contamination. This can have negative financial implications due to unnecessary remediation requirements or restrictions on land-use [3]. We are carrying out a study that seeks to improve our understanding of actual human exposure to soil contamination, which should help improve contaminated land risk assessment.

Metal contamination is a common phenomenon in urban allotments and gardens, due to pollution from roads, general urban/industrial activities and actions of allotment tenants [4, 5, 6]. Exposure to metal contaminants such as lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr) and nickel (Ni) can cause deleterious health effects in humans [7, 8]. For this reason, our study focuses on these toxic elements in allotment soils. This paper presents data obtained from a pilot study conducted between June 2014 and January 2015, which was aimed at testing the investigation's methods and procedures.

Five urban allotments in Edinburgh were identified for the pilot study. Adult (>18 years old) allotment users were recruited to participate in biological monitoring of potential exposure to these elements. Exposure pathways investigated included consumption of allotment produce (including contaminants in adhering soil), along with inadvertent ingestion and inhalation of outdoor allotment dust. Samples collected and analysed comprised allotment soil, allotment produce (fruits and vegetables), airborne allotment dust, hand moist-wipes from allotment workers and biological (urine and blood) samples from consenting allotment users. Ethical approval for the study was obtained from the University of Reading Research Ethics Committee.

Increased understanding of human exposure to these elements will promote sustainable management of contaminated land, thus supporting sustainability of the built environment.

2 Materials and Methods

2.1 Site identification and recruitment of study participants

Given that this pilot study was aimed at testing the investigation's methods and procedures, no statistical emphasis was placed on sample size requirements and site selection. Between three and five sites were deemed adequate for the pilot study.

Initial contact with allotment users was made through representatives of local allotment associations who were requested to distribute the study flyer to the members of the associations. Individuals who responded to the advert were contacted. Five allotment plots were identified and six adult users of these plots were successfully recruited to participate in the pilot study.

2.2 Collection of samples and laboratory analyses

Thirty soil samples were collected from the five allotments from hand-dug pits (maximum depth of 0.3 m) in June 2014. A portion of each sample (about 100 g) was oven dried overnight at about 100 ^{0}C. The dry samples were gently disintegrated by hand using a porcelain pestle and mortar to break-up aggregates, and then sieved to obtain fine (<63 µm) particles. Sample digestion of the fine particles was carried out in accordance with method 7300 provided by the National Institute of Occupational Safety and Health (NIOSH) [9]. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine total

concentrations of As, Cd, Cr, Ni and Pb in the digests. Selected sieved samples (3 per site) were subjected to bioaccessibility testing using the Unified BARGE Method (UBM) [10, 11].

Allotment produce (38 samples) were collected in July 2014. Sample portions were washed using tap water to mimic the washing of fresh fruits and vegetables that would normally be carried out in the home. In addition, selected samples were divided into two parts prior to washing, to allow one part to be processed unwashed in order to investigate the metal content in soil particles adhering to produce surfaces. The samples were oven dried at approximately 60 ^{0}C for 48 hours. This was followed by grinding and homogenisation of the samples into fine particles. Samples were digested using NIOSH method 7300 and metal contents determined using ICP-AES.

In September 2014, moist-wipe samples were collected from three participants using Ghost wipes (i.e., a sturdy wiping material moistened with deionised water) while doing allotment work. Hand moist-wipes were collected to investigate potential exposure to the metals through inadvertent ingestion, which mainly rises from hand-to-mouth contact [12].

Airborne allotment dust samples were collected using a body mounted dust sampler (an additional sampler was mounted on site concurrently). Air pumps were operated at the recommended sampling rate of 2 L min^{-1} [13] and left to run for 1 to 2 hours (duration of work). Moist wipes and dust samples were analysed as per the allotment soil and produce procedure.

The participants provided four rounds of urine samples between July 2014 and January 2015 (total of 24 urine samples). Aliquots (1 mL) of urine samples were diluted using 5 mL concentrated nitric acid (HNO_3) and subsequently analysed using ICP-AES. Venous blood samples (5 mL each) were collected from five consenting participants in January 2015. Aliquots (0.5 mL) of blood samples were digested in concentrated nitric and hydrochloric (HCl) acids and analysed using ICP-AES. The method employed in digesting blood samples was derived from the literature [13, 14, 15], modified and subsequently trialled and validated at the IOM laboratory using pig's blood.

2.3 Quality assurance

To ensure the reliability of the test results, appropriate quality assurance procedures and precautions were taken. Samples were carefully handled to avoid cross-contamination. Reagents used were of analytical grades. Blank determinations were used to apply corrections to the instrument readings. Repeat sample analysis and analysis of samples spiked with known concentrations were used to validate the analytical procedure. Validation of the UBM test procedure was undertaken using BGS guidance material 102 [16]. In addition, commercially certified reference materials (*ClinChek Urine Control* and *BCR-636 Human Blood*) were used to validate analytical procedures for urine and blood, respectively. Instrument readings were within ±20 % of specified reference values and spiked sample concentrations [17]. ICP-AES was calibrated using known standards prior to sample analyses ($r > 0.99$).

3 Results and discussion

3.1 Soil total concentrations and bioaccessibility fractions

The preferential adherence of soil and dust particles to hands and fingers occurs in the particle size range 0.5–65 µm [18]. Therefore, <63 µm soil particles were considered the most relevant for investigating the soil ingestion exposure pathway.

The mean soil total concentrations (mg kg^{-1}) ranged between 8–94 (As), 1–2 (Cd), 81–93 (Cr), 222–760 (Pb) and 45–69 (Ni). Table 1 compares the soil concentrations of As, Cd and Ni with the current UK soil guideline values (SGVs) provided by the Environment Agency [19, 20, 21]. SGVs are used as initial screening criteria to identify sites with the potential to cause risk to humans.

Table 1: A comparison of the recorded soil concentrations with SGVs for allotment land use

Metals	Mean soil concentrations (mg kg^{-1})	SGV (mg kg^{-1})	Remarks
As	8 – 94	43	SGV exceeded in site 3
Cd	1 – 2	1.8	SGV exceeded marginally in sites 1, 2 and 4
Ni	45 – 69	230	SGV not exceeded
Note: SGVs are not presently available for Pb and Cr.			

Bioaccessibility test results gave mean bioaccessibility fractions of 53% (As), 51% (Cd), 8% (Cr), 22% (Pb) and 57% (Ni), which is a surrogate for the fractions of the metals that are soluble in the human gastrointestinal tract and potentially available for absorption [22, 23]. This indicates that the risk associated with potential soil ingestion could be exaggerated if soil total concentrations are adopted in risk assessment models. For example, all samples from one allotment (site 3) recorded total arsenic concentrations between 80 and 122 mg kg^{-1}; however only approximately 48% of the arsenic was found to be bioaccessible. This indicates that if the arsenic in soil were ingested, nearly half of the arsenic would not be soluble in the gastrointestinal tract and thus not potentially available for absorption.

Following regression analysis, a positive correlation was observed between the soil total concentrations of As and Pb and the corresponding bioaccessible concentrations (p <0.001), as shown in Fig. 1 and 2. These linear correlations indicate that the bioaccessible As and Pb concentrations could be predicted from the measured total soil concentration. A study by Barsby et al. [24] also identified linear correlations ($r > 0.7$) between measured individual pseudo-total and the respective bioaccessible concentrations of a number of potentially toxic elements (PTEs) including As, Cd and Pb.

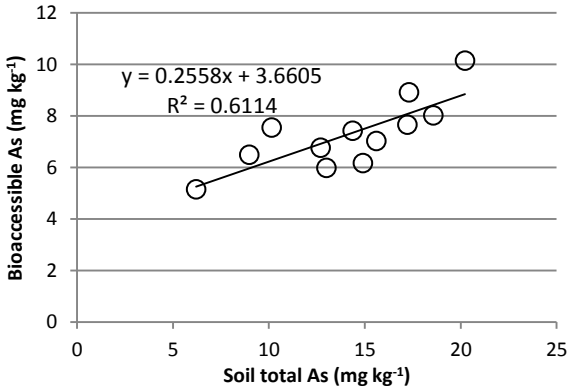

Fig.1: Relationship between soil total and bioaccessible As concentrations

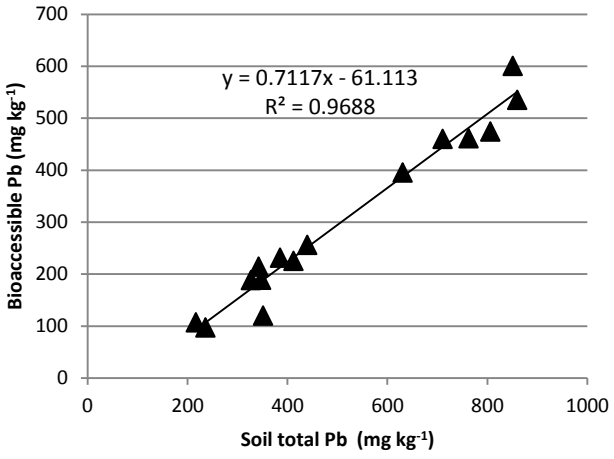

Fig.2: Relationship between soil total and bioaccessible Pb concentrations

3.2 Metal concentrations recorded in allotment produce

Table 2: Metal concentrations in allotment fruits and vegetables (mg kg^{-1})

Metals	Site 1 (n=5)	Site 2 (n=4)	Site 3 (n=11)	Site 4 (n=12)	Site 5 (n=6)	TF values (site averages)
As	2.4 - 6.2 (3.8)	2.5 - 3.6 (3.2)	n.d. - 4.3	n.d. - 1.2	n.d. - 1.1	0.027 - 0.266
Cd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cr	n.d. - 1.5	n.d.	n.d. - 0.6	n.d. - 1.0	n.d. - 0.5	0.006 - 0.01
Pb	0.4 - 2.7 (1.5)	4.2 - 7.9 (6.8)	0.4 - 8.2 (3.2)	0.3 - 8.1 (4.0)	0.7 - 9.2 (3.1)	0.004 - 0.017
Ni	0.3 - 1.1 (0.6)	n.d. - 1.4	0.7 - 5.1 (2.6)	0.6 - 3.4 (1.4)	0.4 - 2.1 (1.2)	0.012 - 0.038
'n.d.' refers to non-detectable mean values (where calculated) are given in brackets						

The metal concentrations (mg kg^{-1}) in allotment produce are in Table 2, along with the estimated soil-to-plant transfer factors (TF). The TF values were calculated as follows [25]:

$$TF = \frac{C_{plant}}{C_{total-soil}} \quad (1)$$

where C_{plant} is the metal concentration in the plant material (dry weight basis) and $C_{total\text{-}soil}$ is the total concentration of the same metal in soil (dry weight basis) where the plant was grown. The higher the TF value the higher the potential accumulation by plants [22], suggesting a potential for increased exposure through human diet. According to USEPA [26], TF value is a major parameter in determining the risk of human exposure to metals in soils. A comparison of the estimated TF values with those in literature suggests that TF values could vary considerably. For instance, a study by Intawongse [27] recorded TF values varying between 0.14–0.92 (Cd), 0.003–0.05 (Cr), 0.001–0.133 (Pb) and 0.03–0.22 (Ni) in lettuce, spinach, carrot and radish grown on soils spiked with inorganic salt solutions in a greenhouse. According to Wang et al. [28], soil conditions that affect phytoavailability of trace elements include soil pH, organic matter, cation exchange capacity and total soil concentration.

Metal concentrations found in unwashed samples were compared to washed samples. Washing of allotment fruits and vegetables decreased the metal concentrations in the produce by between 3–32 % (As), 16–63 % (Cr), 15–77 % (Ni) and 16–87 % (Pb). This indicates that metal contents associated with adhering soil and dust (from aerial depositions) provides a key exposure pathway. Therefore, washing allotment produce prior to preparation and consumption lowers the potential for human exposure to the metals in adhering soil/dust. For example, Fig. 3 shows a comparison of Pb concentrations measured in washed and unwashed samples from this study.

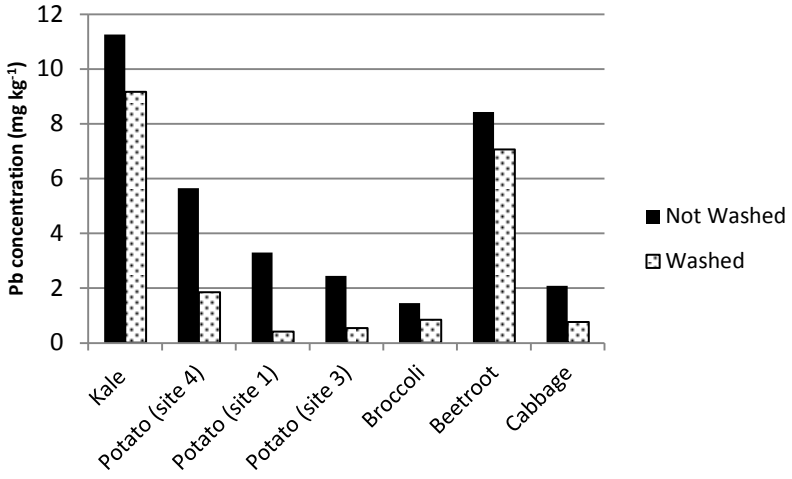

Fig.3: Comparison of Pb concentrations in washed and unwashed vegetable samples

The washed and unwashed Pb concentrations in edible shoots (kale, broccoli and cabbage) demonstrate the effect of washing in removing aerial depositions. Likewise, washing edible roots (potato and beetroot) remove adhering soil particles, leading to a reduction in metal content of the produce. Similar observations have been reported in other studies [29, 30].

3.3 Metal in hand moist-wipes and airborne allotment dust

Detected metal weights (µg) in hand moist-wipes varied between 0.5–39.8 (As), 0.1–1.7 (Cd), 4.3–31.2 (Cr), 9.7–141 (Pb) and 2.1–21.4 (Ni). These results indicate that hand-to-mouth contact could add to human exposure.

In addition, airborne allotment dust samples were collected to investigate the dust inhalation exposure pathway. The dust samples recorded negligible metal concentrations (<0.001mg m^{-3}). Based on approximate air inhalation rate of 0.84 m^{-3} per hour (for an adult aged 60–70 years old doing a light intensity activity) [31], this translates to an estimated metal inhalation rate of <0.00084 mg per hour of allotment activity. This suggests that exposure via inhalation of outdoor allotment dust is a negligible pathway for allotment land use.

3.4 Urine and blood test results

Non-detectable metal concentrations were recorded in urine and blood samples, which suggest negligible background / exposure levels for the participants monitored. Extensive biomonitoring for the second phase of the study, lasting 12 months, has begun.

4 Conclusions

A study is being carried out to improve our understanding of the actual human exposure to selected toxic elements (As, Cd, Cr, Pb and Ni) from allotment soils. This pilot phase of the study has indicated that the risk associated with potential soil ingestion could be exaggerated if soil total concentrations are adopted in risk assessment models without taking into account the corresponding bioaccessibility values. Arsenic was found to have the highest soil-to-plant transfer factor, indicating a greater potential for exposure through human diet. It was found that metals contained in soil/dust particles adhering to allotment produce could increase human exposure, since washing of allotment produce decreased the metal concentrations in the produce. In addition, it was found that inadvertent ingestion (hand-to-mouth contact) could increase human exposure. However, exposure via inhalation of outdoor allotment dust was negligible. Non-detectable metal concentrations recorded in urine and blood samples suggest negligible background / exposure levels for the participants monitored.

The second phase of the study has begun. Thirty five volunteers have been recruited to take part in the study, scheduled to last for 12 months.

Acknowledgements

This work was completed under EPSRC Grant No. EP/G037787/1 as part of the TSBE Centre. Additional financial support from IOM is also gratefully acknowledged.

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Appendix D: Recruitment Poster, Questionnaires and Diary

RECRUITING NOW

Project: *The Human Health Impact of Contaminated Land*

Study Volunteers Required – can you HELP?

I am an EngD research student undertaking a study through the University of Reading and the Institute of Occupational Medicine (IOM), investigating whether or not there is an effect of low level soil-bound contaminants on human health resulting from working on allotments sites and consumption of allotment produce. The study is being funded by the UK Engineering and Physical Sciences Research Council and IOM.

There is an increasing demand for allotment plots in the UK, which increases the pressure to re-use urban or brownfield land as allotments. Therefore, it is important that the actual risks posed to human health from allotment land use is clearly understood. The information obtained from this academic study will improve our understanding of human health risks associated with contaminated land. Consequently, this will promote sustainable management of contaminated land.

We would like to recruit adult volunteers (aged 18 years and above) to participate in the study. We require:

- Those who have allotments and consume produce from their allotments;
- Adults in a household who consume produce from allotments owned by a member of their household.

Benefits of participating in the study

- National Garden gift vouchers will be issued on completion of the study.
- Increased understanding of the human health risks associated with allotment land use would benefit the society in general.

If you decide you would like to help or would like additional information, please contact me using the details given below. Your participation is highly appreciated.

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Research Project: The impact of contaminated land on human health

Brief Initial Questionnaire *(please complete as appropriate)*

Participant ID:
Site ID:

1. How many allotments do you have? ☐

2. Please provide below the location of the allotment(s) you have, and how long you have been using the allotment(s).

Allotment location	Duration of allotment use	
	No of Years	*No. of Months*

3. Do you grow fruit/vegetables in the allotment(s)? YES ☐ NO ☐

4. Do you eat the fruit/vegetables grown in the allotment(s)? YES ☐ NO ☐

5. How do you cultivate the allotment(s)?

Single digging	
Double digging	
No dig policy	
Other (*indicate*)	

6. Do you add fertilizer or imported materials to improve soil quality?

Fertilizer type	
Imported material (*details*)	
Other (*indicate*)	

7. Do you know past usage of the site prior to the allotments (i.e., historical use)?

8. Do you know the historical uses of the surrounding land?

9. Is there any other information on how the allotment is used (*please indicate*)?

10. Are there any adults in your household who would like to participate in the study?

YES ☐ *if YES, please* ☐ NO ☐

END – Thank you

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Participant Background Questionnaire

INSTRUCTIONS

1. For some questions there is a list of possible answers. Please choose your answer and put a tick in the box beside it.
2. Some questions will ask you for a number, please enter this in the box.
3. There are spaces in some questions for you to write your answer.
4. Some questions are followed by instructions. Please follow these carefully.

All information collected will be strictly confidential and no names or identifying information will be published in any report.

Participant ID No:

Question 1: Personal Details & General Health (*for identification purposes & provide data for modelling etc.*)

Date questionnaire is completed:

a). Your name:

b). Year of birth (*e.g., 1960*):

c). Sex (*please tick*): Male Female

d). How tall are you? ft, inches or cm

e). What weight are you? st, lbs or kg

f). Do you usually bite your nails? (*please tick*) YES NO

g). Do you suffer from osteoporosis? (*please tick*) YES NO

h). Do you have any illness for which you are taking medication? (*please give details below*)

Illness	Medication

Participant ID No:

Question 2: Employment Details (*to assess occupational exposure*)

a). Are you employed (*full-time or part time*)?

☐ YES (*please go to question 2b)* ☐ NO (*please go to question 3*)

b). Please provide employment details below.

Occupation:

Place or address where you typically work:

Question 3: Allotment use (*to assess exposure from allotment use*)

a). In the table below, please tell us the allotment produce you consume.

Fruits and vegetables you consume at least once in a month	AVERAGE consumption (number of portions / servings) (*please tick*) [**1 adult portion is about 80grams**[#]]							
	1-3 per month	Once per week	2-4 per week	5-6 per week	Once per day	2-3 per day	Over 3 per day	Proportion from allotment (*e.g. all, half, 25%*)
Potatoes								
Onions								
Leeks								
Broad beans								
Courgettes								
Peas								
Strawberries								
Raspberries								
Black/red/white currants								
Rhubarb								
Tomatoes								
Cabbage, Kale								
Lettuce								
Carrots								
(others, please list)								

[#]*Food Statistics Pocketbook 2013 – in year update (DEFRA, 2013)*

Participant ID No:

c). Please provide the details of any soil improvers, pesticides or other chemicals that have been used at your allotment(s).

d). Please indicate (*tick*) which month/months you typically work on allotment and also which month/months you typically eat allotment produce.

Months you typically work on allotment						**Months you typically eat allotment produce**					
January		May		September		January		May		September	
February		June		October		February		June		October	
March		July		November		March		July		November	
April		August		December		April		August		December	

e). Please indicate (*tick*) the typical duration and frequency of allotment visits you make in a month during summer (April – September) and winter (October – March) periods.

Frequency per month (Summer)		**Duration per visit (Summer)**	
Every day		Less than 30 minutes	
Several times a week		Between 30 minutes and 1 hour	
Once a week		Between 1 hour and 3 hours	
Two to three times a month		Between 3 to 6 hours	
Once a month or less		More than 6 hours	
Frequency per month (Winter)		**Duration per visit (Winter)**	
Every day		Less than 30 minutes	
Several times a week		Between 30 minutes and 1 hour	
Once a week		Between 1 hour and 3 hours	
Two to three times a month		Between 3 to 6 hours	
Once a month or less		More than 6 hours	

Participant ID No:

Question 4: Personal Protective Equipment (PPE) and General Hygiene

a). Please indicate (*tick*) the PPE you typically wear when working at the allotment(s).

Personal Protective Equipment	***Please tick***
Welly boots (foot protection)	
Hand gloves (hand protection)	
Apron/protective clothing	
Goggles (eye protection)	
Other (*please specify*)	

b). If you use PPE, where do you keep your gardening footwear and gloves? (*please tick*)

At home ☐ In allotment shed ☐

c). Do you wash your hands before eating on site (e.g. snacks/sandwich)? (*please tick*)

Always ☐ Sometimes ☐ Never ☐ When I remember to ☐

d). Do you wash your hands before leaving the allotment? (*please tick*)

Always ☐ Sometimes ☐ Never ☐ When I remember to ☐

e). Do you peel root and tuber vegetables before use? (*please tick*)

Always ☐ Sometimes ☐ Never ☐ When I remember to ☐

f). Do you wash ALL fruits and vegetables before use? (*please tick*)

Always ☐ Sometimes ☐ Never ☐ When I remember to ☐

Question 5: Lead Paint (*exposure from domestic lead paint*)

a). Do you have lead paint in your home? YES ☐ NO ☐ I don't know ☐

Question 6: Tobacco Smoking (*smoking related exposure*)

a). Have you ever been a smoker? (*please tick*) YES ☐ NO ☐

b). Do you currently smoke? (*please tick*) YES ☐ NO ☐

c). If YES to 'a'/'b' above, please state the regular brand(s) smoked ☐

d). Do you currently live with a smoker? (*please tick*) YES ☐ NO ☐

e). If YES to 'd' above, does this person smoke inside the house? YES ☐ NO ☐

End of Questionnaire – Thank you for filling this questionnaire.

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Diary / Log of allotment use

INSTRUCTIONS:

- *Each log is divided into parts A to D.*
- *Please complete a log for EACH DAY **only when** any of the parts A to D is applicable.*

Your Name:
Participant No:

Today's Date:		**PART B – Allotment work**	**PART C – Other activities**		**PART D – Urine sampling**
PART A – Allotment produce consumed & approx. weight		Duration spent at the allotment (*Hr/Min*)	If relevant, please indicate duration spent on these tasks		*If you have collected urine today, please give time of sampling*
Fruit/Veg. Eaten	*Weight (e.g., 50g)*		*Activity*	*Duration (Hr/Min)*	Time:
		Did you touch allotment soil? YES ☐ NO ☐	Lead smelting		Within the last 3 days before providing your urine sample, did you eat seafood or rice?
			Auto repair		
			Radiator repair		
		Did you wear gloves? YES ☐ NO ☐	Furniture refinishing		
			Construction (DIY, painting)		Seafood YES ☐ NO ☐
		Did you wash your hands before leaving allotment? YES ☐ NO ☐	Art restoration		
			Burning lead painted wood		White rice YES ☐ NO ☐
			Precious metal refining		
			Making pottery, ceramics		Brown rice YES ☐ NO ☐

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2-3 Days Continuous Urine Sampling

Your Name:
Participant No:

Please start recording allotment produce consumed 1 day before urine collection starts – and continue recording produce consumption throughout the sampling period.

Allotment produce consumed & approx. weight				**Allotment work**
Date	*Time*	*Fruit/Veg. Eaten*	*Weight (e.g., 50g)*	Duration spent at the allotment in the **past 24 Hours** before start of sampling (or during sampling)
				Did you touch allotment soil? YES ☐ NO ☐
				Did you wear gloves? YES ☐ NO ☐
				Did you wash your hands before leaving allotment? YES ☐ NO ☐

Urine Samples			**Seafood & Rice**
Sample No.	***Date** of Sampling*	***Time** of Sampling*	Within the last **3 days** before providing your urine sample (or during sampling), did you eat seafood or rice?
1			
2			
3			
4			Seafood YES ☐ NO ☐
5			
6			White rice YES ☐ NO ☐
7			
8			
9			Brown rice YES ☐ NO ☐
10			

Appendix E: Summary of Information Gathered from Participants

Part E1: Participants' details

Participant ID	Gender (Male / Female)	Age (years)	Body Weight (Kg)	Declared Smoking status	ID of allotment used by participant	Duration participant has been using the allotment (years)
P01	M	63	76	Non-smoker	A01	20
P02	F	59	67	Non-smoker	A01	20
P03	M	64	95	Non-smoker	A03, A04	1
P04	F	63	68	Non-smoker	A03, A04	2.5
P05	F	64	54	Non-smoker	A05	20
P06	M	64	83	Non-smoker	A06	5
P07	F	44	55	Non-smoker	A07	2
P08	F	30	65	Non-smoker	A08	3
P09	F	46	62	Ex-smoker	A09	3.5
P10	M	59	108	Ex-smoker	A10	17
P11	F	59	114	Non-smoker	A10	17
P12	M	67	85	Ex-smoker	A12	20
P13	F	68	65	Ex-smoker	A12	20
P15	M	59	98	Non-smoker	A15	8
P16	M	56	73	Smoker	A16	4
P17	M	55	90	*Not declared*	A17	4
P18	F	45	76	Non-smoker	A18	2
P19	M	80	100	Ex-smoker	A19	20
P20	M	63	80	Non-smoker	A20	1.5
P21	M	63	84	Ex-smoker	A21	20
P22	F	61	63	Ex-smoker	A21	20
P23	M	71	99	Ex-smoker	A23	30
P24	F	71	79	Non-smoker	A23	30
P25	M	55	89	Non-smoker	A25, A26	19
P26	F	66	68	Non-smoker	A25, A26	19
P27	M	58	77	Non-smoker	A27	1.5
P28	M	34	73	Non-smoker	A28	0.2
P29	M	56	89	Smoker	A29	5
P31	F	49	70[#]	Non-smoker	A31	1
P32	M	73	73	Non-smoker	A32	6
P33	F	54	53	Non-smoker	A33	7
P34	M	67	70	Non-smoker	A34	17
P35	F	73	43	Ex-smoker	A34	17
P36	M	55	82	Non-smoker	A36	0.2
P37	F	63	71	Ex-smoker	A37	20.5
P38	F	44	71	Ex-smoker	A38	1.5
P39	M	76	70[#]	Non-smoker	A32	6

Two participants (P14 and P30) dropped out of the study within the first month.

[#]Body weight not declared by participant, average adult weight of 70kg was assumed.

Part E2: Frequency and the duration of visits to allotments

	Summer months[1]		Winter months[1]	
	No. of Respondents	**(% of Participants)**	**No. of Respondents**	**(% of Participants)**
Frequency[2]				
Virtually everyday	5	14	Nil	Nil
Several times a week	26	70	6	16
Once a week	1	3	14	38
Two or three times a month	1	3	7	19
Once a month or less	1	3	6	16
Duration				
Less than 30 minutes	Nil	Nil	3	8
Between 30 minutes and 1 hour	3	8	11	30
Between 1 hour and 3 hours	24	65	18	49
Between 3 hours and 6 hours	7	19	1	3
More than 6 hours	Nil	Nil	Nil	Nil

[1]Summer (April to September), Winter (October to March).

[2]Based on terminology used in 'Environment Agency (2009). *Updated technical background to the CLEA model - Science Report: SC050021/SR3*. Bristol: Environment Agency'.

Part E3: Participants' consumption rates for allotment produce

Calculation of consumption rate: The reported weights of all produce consumed in a given month were aggregated, and then divided by both the participant body weight (kg bw) and 30 days.

Participant ID	Consumption rate of produce (g fw kg^{-1} bw day^{-1})							
	Summer months[1]				Winter months[1]			
	Min	Max	Average	Average (as % of CLEA data)[2]	Min	Max	Average	Average (as % of CLEA data)[2]
P01	0.01	1.67	0.60	18	0.35	0.89	0.67	20
P02	0.01	1.22	0.67	20	0.35	1.01	0.66	20
P03	0.28	0.64	0.40	12	0.14	0.49	0.29	9
P04	0.01	0.56	0.31	9	0.15	0.60	0.38	11
P05	0.62	2.20	1.29	39	2.19	4.52	3.01	90
P06	0.44	1.10	0.74	22	0.04	1.53	0.58	17
P07	0.40	1.41	0.83	25	0.24	0.67	0.56	17
P08	1.03	2.72	1.51	45	0.21	3.28	1.24	37
P09	0.04	0.89	0.43	13	0.19	0.89	0.35	10
P10	0.18	0.26	0.22	7	0.05	0.09	0.07	2
P11	0.01	0.28	0.14	4	0.01	0.05	0.03	1
P12	0.03	2.23	0.87	26	0.33	1.63	1.09	33
P13	0.08	2.56	0.82	25	0.42	1.15	0.88	26
P15	0.21	1.56	0.88	26	0.15	0.90	0.43	13
P16	0.39	1.10	0.71	21	0.37	1.14	0.75	22
P17	0.03	0.40	0.22	7	0.01	0.19	0.09	3
P18	0.01	0.72	0.22	7	0.01	0.75	0.16	5
P19	0.02	0.16	0.07	2	0.05	0.11	0.08	2
P20	0.45	0.54	0.50	15	0.06	0.89	0.38	11
P21	0.02	1.37	0.68	20	0.67	1.79	1.08	32
P22	0.08	1.80	1.12	34	0.37	2.20	1.18	35
P23	0.06	0.66	0.36	11	0.15	1.32	0.54	16
P24	0.25	0.91	0.68	20	0.15	1.27	0.58	17
P25	0.46	4.89	2.50	75	0.70	2.28	1.33	40
P26	0.51	6.52	2.98	89	0.68	2.62	1.51	45
P27	N/A	0.13[3]	N/A		N/A	N/A	N/A	
P28	0.05	1.32	0.61	18	0.61	2.79	1.87	56
P29	0.23	1.68	0.84	25	0.05	0.62	0.30	9
P31	0.19	0.81	0.37	11	0.01	0.23	0.07	2
P32	0.32	2.01	0.94	28	0.25	1.03	0.63	19
P33	0.47	1.63	0.94	28	0.04	1.81	0.58	17
P34	0.11	3.91	1.39	42	N/A	N/A	N/A	
P35	0.06	1.52	0.69	21	N/A	N/A	N/A	
P36	0.24	1.22	0.77	23	0.28	0.30	0.29	9
P37	N/A	N/A	N/A		0.02	0.21	0.09	3
P38	0.07	1.27	0.42	13	0.07	0.56	0.23	7
P39	N/A	N/A	N/A		0.21	1.01	0.53	16

fw (fresh weight), bw (body weight), N/A (not applicable).

[1]Summer (April to September), Winter (October to March).

[2]Produce consumption rate of 3.34 (g fw kg^{-1} bw day^{-1}) for 'high end' consumer, calculated using CLEA data (see Table 6.2 in section 6.4 for details).

[3]P27 reported consumption data for one 'summer' month only.

Part E4: Summary of participants' average daily intake (ADI) values of the elements

Element	Range of calculated average daily intake (ADI) values (mg kg^{-1} bw day^{-1})[$,#]	
	Summer months[+]	Winter months[+]
As	$8.7x10^{-7}$ – $2.9x10^{-4}$	$6.6x10^{-8}$ – $2.9x10^{-4}$
Cd	$1.2x10^{-8}$ – $1.2x10^{-4}$	$1.2x10^{-8}$ – $1.3x10^{-4}$
Cr	$6.9x10^{-8}$ – $8.7x10^{-4}$	$9.8x10^{-9}$ – $8.7x10^{-4}$
Ni	$5.3x10^{-7}$ – $1.7x10^{-3}$	$7.6x10^{-8}$ – $1.4x10^{-3}$
Pb	$7.0x10^{-8}$ – $3.2x10^{-3}$	$7.0x10^{-8}$ – $3.1x10^{-3}$

[$]For each moth, a constant daily ADI value was determined. ADI values were calculated using the expression: *ADI = (Σ(Wp*Cp*BAF))/kg bw/30*; where *Wp* is the aggregated weight (kg) of each produce type consumed in a month, *Cp* is the element concentration (mg kg^{-1}) recorded in the produce samples and *BAF* is the corresponding bioaccessibility fraction. A sum was obtained for all produce types and divided by the participant body weight (kg) and 30 days in a month. Excludes extra data collected over the 2-3 consecutive days.

[#]Where the type of allotment produce consumed was not sampled or tested, plot-specific median element concentrations and bioaccessibility values were used in the calculations. However, where a median bioaccessibility value of an element was not calculated, we assumed 100% bioaccessibility values.

[+]Summer months (April to September), Winter months (October to March).

Part E5: Calculated elemental intake rates for participants during 2-3 days

Participant ID	Range of calculated intake rates[$,#]				
	iAs (*µmol*)[+]	Cd (µg)	Cr (µg)	Pb (µg)	Ni (µg)
P01	0.34 – 0.77	9 – 18	52 – 94	58 – 106	50 – 95
P02	0.35 – 0.77	1 – 17	26 – 69	60 – 108	16 – 94
P05	0.09 – 1.25	1 – 5	22 – 86	6 – 107	21 – 108
P09	0.60 – 1.73	3 – 12	42 – 72	32 – 110	15 – 102
P10	0.10 – 0.44	4 – 8	72 – 80	9 – 12	83 – 100
P11	0.10 – 0.44	3 – 7	70 – 85	8 – 12	80 – 100
P12	0.09 – 1.52	3 – 10	45 – 86	22 – 95	32 – 155
P13	0.35 – 1.20	2 – 11	28 – 58	12 – 66	17 – 105
P15	0.17 – 2.50	8 – 15	45 – 89	70 – 96	49 – 176
P21	0.10 – 0.98	8 – 23	25 – 92	40 – 80	22 – 157
P22	0.10 – 0.88	6 – 23	25 – 86	40 – 86	21 – 148
P25	0.01 – 1.64	4 – 11	11 – 83	3 – 56	23 – 193
P26	0.05 – 1.46	3 – 19	48 – 68	59 – 61	24 – 180

[$]Doses were calculated using the expression: *Dose = Wp*Cp*BAF*; where *Wp* is the weight (g) of produce consumed at any time during the 2-3 days, *Cp* is the element concentration (µg/g) recorded in produce samples and *BAF* is the corresponding bioaccessibility fraction.

[#]Where the type of allotment produce consumed was not sampled or tested, plot-specific median element concentrations and bioaccessibility values were used in the calculations. However, where a median bioaccessibility value of an element was not calculated, we assumed 100% bioaccessibility values.

[+]Doses of iAs (µg) were converted to µmol by dividing the doses with the molecular weight of As (75 g mol^{-1}).

Appendix F: Laboratory Analytical Methods

Part F1: Total concentrations of As, Cd, Cr, Ni and Pb in samples (soil, produce, hand moist-wipes, allotment dust)

Soil samples:

A portion of each soil sample (about 100 g) was oven dried overnight at about 100 ^{0}C. The dry samples were gently disintegrated by hand using a porcelain pestle and mortar to break-up aggregates, and then sieved to obtain fine (<63 µm) particles, which were kept until analysis was carried out.

On the day of analysis, small portions (weighing < 1 g) of the <63 µm soil particles were placed in glass beakers (25 mL), and were digested according to IOM's internal Standard Operating Procedure (ICP-SOP2). This procedure is based on method 7300 provided by the National Institute of Occupational Safety and Health (NIOSH, 2003). In this method, ashing reagents comprise concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$), combined at a ratio of 4:1. We obtained acids of super purity quality from ROMIL®, UK.

Outline of procedure:

- Calibration standards (covering 'blank' to 20 mg/L analytical range) were prepared from stock standards. Dilution acid was made up using distilled water 4% HNO_3 and 1% $HClO_4$.
- 3 samples were spiked with known concentrations and included in each analysis batch to check recoveries. 2 blanks were also included with each analysis batch.
- Ashing acid of (4:1) $cHNO_3$:$cHClO_4$ was prepared.
- Samples were placed in beakers and 5 mL of ashing acid was added to samples, blanks and spikes. Beakers were covered with watch glasses and left at room temperature for 30 minutes.
- Samples were heated on hotplate at 120 ^{0}C until approximately 0.5 mL remained in the beaker.
- Watch glasses were removed and rinsed into the beaker with distilled water.
- Temperature was increased to 150 ^{0}C until approximately 0.5 mL remained in the beaker.
- Sample solutions were transferred to 25 mL volumetric flasks and solutions made up to the 25 mL mark using distilled water. Then, solutions were transferred to sterile tubes.

Subsequently, sample solutions were analysed using inductively coupled plasma – atomic emission spectroscopy (ICP-AES) (Thermo Fisher Scientific®, UK).

For each element, the minimum limit of detection (LOD) was 0.03 mg/kg. LOD was calculated as *Mean of concentrations of blanks + 3*Standard Deviation of blanks* (Armbruster & Pry, 2008).

The ranges of LODs (mg/kg) recorded for each element were: As (0.03 to 0.08), Cd (0.03 to 0.05), Cr (0.03 to 0.07), Ni (0.03 to 0.06) and Pb (0.03 to 0.09).

Allotment produce samples:

Randomly selected portions of allotment produce samples were processed for analysis, as outlined below:

- Samples (especially leaves and tubers) were washed under running tap water to remove all visible traces of soil, which mimics normal food preparation in the kitchen.
- Washed samples were allowed to drain off water before each sample was placed in aluminium sample dish.
- Sample weight was recorded before being oven-dried at approximately 60 ^{0}C for at least 48 hours (this temperature was sufficient to prevent burning of samples).
- Dried samples were weighed to allow for moisture content determination.
- Dried samples were ground and homogenised into fine particles using a pestle and mortar.

Subsequently, processed samples were digested and analysed using ICP-AES in the same manner as soil samples. For each element, the minimum LOD was 0.01 mg/kg. Individual element LOD (mg/kg) ranges were: As (0.01 to 0.04), Cd (0.01 to 0.019), Cr (0.01 to 0.015), Ni (0.01 to 0.02) and Pb (0.01 to 0.05)

Hand-moist wipes and allotment dust samples:

The samples were digested and analysed using ICP-AES in the same manner as soil samples. For these pilot study samples, the minimum LODs for each element were 0.03µg (wipes) and 0.001 mg/m^3 (dust).

Quality Assurance (QA)

To ensure the results were reliable, the following QA measures were implemented:

- Instrument calibration included 5 points, with correlation coefficients ($r^2 > 0.99$) for each sample batch analysed.
- Repeated sample analyses (after every 10 consecutive samples) were carried out to check for instrument drift, and variations from the original results were within 10%.
- Recoveries of the elements in in spiked samples were within ±20% of the known/specified concentrations (Li et al., 2015).
- Blanks and spikes were used to correct instrument readings.
- Measured sample concentrations were restricted within the calibration range (up to 25% above the maximum calibration standard); samples above this limit would require dilution and re-analysis.

Part F2: Bioaccessibility extraction test on soil and produce samples

Soil and allotment produce samples were prepared for bioaccessibility testing using the Unified BARGE Method (UBM), to estimate the contaminant fraction that would be soluble in the human gastrointestinal tract and hence potentially available for absorption. The test procedure is illustrated in Figure F2.

Fig. F2: Schematic flow diagram of the UBM test procedure
Sources: Wragg et al. (2009a); https://www.bgs.ac.uk/barge/ubm.html

Digestive fluids (saliva, gastric fluid, duodenal fluid and bile) were prepared in the laboratory using synthetic enzymes combined with a range of inorganic and organic solutions, as described in the UBM procedure (Wragg et al., 2009a). The pH values were adjusted using NaOH (1 M) and HCl

(37%). The LODs were similar to total element determination in soil and produce samples. For each element, bioaccessibility fractions (BAF %) were calculated as follows:

$$BAF(\%) = \frac{Bioaccessible\ concentration\ in\ sample}{Total\ concentration\ in\ sample} \times 100$$

Quality Assurance (QA)

To ensure the results were reliable, the following QA measures were implemented:

- Standard soil material (BGS Guidance Material 102) (Wragg et al., 2009b) was used for quality control. Recovered element concentrations were within ±20% of the specified concentrations.
- Blanks for saliva (S) and gastric fluid (G) were included in each sample batch. Blank concentrations were used to correct instrument readings.
- Repeated sample analyses (after every 10 consecutive samples) were carried out to check for instrument drift, and variations from the original results were within 10%.
- ICP-AES was calibrated prior to analysis of each sample batch.

Part F3: Determination of total element concentrations in urine samples

Element concentrations were determined in urine samples using the procedure outlined below. During the pilot study, the minimum LOD for each element was 0.03 mg/L (ICP-AES). During the main study, the LODs for each element varied from 0.005 to 0.01 µg/L (ICP-MS); individual element LOD (µg/L) ranges were: As (0.005 to 0.01), Cd (0.005 to 0.007), Cr (0.005 to 0.008) and Ni (0.005 to 0.01).

- Instrument calibration standards were prepared from stock standards.
- Dilution acid solution (2 to 4 % HNO_3) was made up using distilled water.
- 3 samples were spiked with known concentrations and included in each analysis batch to check recoveries.
- Following homogenisation, aliquots of urine samples (1 mL) were pipetted into sterile tubes and diluted 10 fold using the dilution acid solution (except for the pilot study samples where samples were diluted using 5 mL of concentrated HNO_3).
- Samples were analysed using ICP-MS, but samples from the pilot study were analysed using ICP-AES.

Quality Assurance (QA)

To ensure the results were reliable, the following QA measures were implemented:

- A commercially certified reference material (CRM) for human urine (*ClinChek-Control*) was used for quality control. Recovered element concentrations were within ±20% of the specified CRM concentrations.
- Blanks (dilution acid solution) were analysed in duplicates while spikes were analysed in triplicates, for correction of instrument readings.
- Repeated sample analyses (after every 10 consecutive samples) were carried out to check for instrument drift, and variations from the original results were within 10%.
- Rhodium (Rh) was used as an internal standard to check for instrument drift.
- ICP-MS was calibrated prior to analysis of each sample batch.

Part F4: Determination of total Pb concentrations in blood samples

There are a number of methods documented in the literature describing how metal concentrations can be determined in human blood (e.g., Heitland & Köster, 2006; Iarmarcovai et al., 2005; Olmedo et al., 2010). A number of methods were trialled and abandoned due to their poor performance. The procedure used in this study was derived and modified from a combination of methods in Goullé et al. (2005); Ikeda et al. (2011) and OSHA (2002). Factors considered in method selection comprised their reported performance (% recovery), ease of reproducibility, analytical instrument used and duration of sample preparation. Using ICP-AES, the procedure summarised below was trialled and evaluated using pig blood, which was obtained from a local abattoir. The sample was kept in a freezer at a temperature of approximately -20 ^{0}C. Portions of the frozen blood were removed and allowed to liquefy at room temperature, and aliquots (0.5 mL) of the blood were used in the analyses.

- Sample aliquots (0.5 mL) were digested in 4 mL of concentrated nitric acid ($cHNO_3$) and heated for about two hours on a hotplate until approximately 1mL of solution remained.
- Samples were allowed to cool, and then 4 mL of concentrated hydrochloric acid (cHCl) was added.
- The solution was warmed gently and swirled for 30 minutes, filtered and made up to desired volume (10 mL) in a conical flask using distilled water, and subjected to ICP-AES analysis.
- Blank samples were analysed in duplicates while spiked samples were analysed in triplicates.
- Calibration standards and quality control solutions were prepared using commercial standard solutions of known metal concentrations dissolved in a weak acid background solution comprising 4% HNO3, 16% HCl and distilled water (80%).

For each sample batch, instrument calibration was carried out using standard solutions of known concentrations (between 0.001 and 5.0 mg/L). A positive linear correlation ($r^2 > 0.99$) was obtained from each calibration, indicating a positive fit between the specified standard concentrations and instrument readings.

Thirty-six pig blood samples were analysed in six separate batches. Samples spiked with known metal concentrations recorded recovery percentages ranging from 80% and 107%, which indicated that the procedure was consistent, reproducible, and fit within the generally accepted recovery of ±20% of known concentrations.

Further evaluation of this procedure was carried out using a commercially certified reference material (CRM) BCR-636 for human blood. Nine CRM samples were analysed and the recovered Pb concentrations were within ±20% of the certified CRM concentration. Generally, ICP-AES has higher detection limits than ICP-MS, and therefore to improve element detection, blood samples from the main study phase were analysed using ICP-MS.

For ICP-MS analysis, final sample solutions were made up in 2% HNO_3 to conform to instrument conditions. Similarly, calibration standards (2.5 to 50 µg/L) and quality control solutions were made up in 2% HNO_3 dilution acid solution. The LODs for Pb varied from 0.01 to 0.03 µg/L (ICP-MS).

Blood spot samples (from finger pricks) were also analysed using ICP-MS in the same manner as venous blood samples. EDTA solution (~5 mmol/L) was added to the filters to increase the recovery of Pb from the filters (Di Martino et al., 2004). The LODs varied from 0.003 to 0.006 µg/L.

Quality Assurance (QA)

To ensure the results were reliable, the following QA measures were implemented:

- A commercially certified reference material (CRM) for human blood (BCR-636) was used for quality control. Recovered element concentrations were within ±20% of the specified CRM concentrations.
- Blanks were analysed in duplicates while spikes were analysed in triplicates, for correction of instrument readings.
- Repeated sample analyses (after every 10 consecutive samples) were carried out to check for instrument drift, and variations from the original results were within 10%.
- Rhodium (Rh) was used as an internal standard to check for instrument drift.
- ICP-MS was calibrated prior to analysis of each sample batch.

Part F5: Isotopic analysis (Pb) in soil, produce and blood samples

Samples were prepared according to the procedures already described in the preceding part F1 (soil and produce) and part F4 (blood). The final sample solutions were made-up in 2% HNO_3 solution in order to conform to ICP-MS operating conditions.

Pb isotopes (206, 207, 208) were measured simultaneously using ICP-MS. For quality control, a high-purity Pb metal (NIST SRM 981) was used as a Pb isotope standard (Tanimizu & Ishikawa,

2006; Farmer et al., 2011). A prepared Pb isotope standard solution was obtained from the Department of Archaeology, University of Reading. The solution was prepared by dissolving approximately 0.1 g of the Pb metal in 1 M HNO_3 to obtain a 100 mg/L stock solution, which was further diluted (in portions) with 2% HNO_3 for regular use. The reagents were of analytical-grade quality.

As part of the QA, blanks were analysed in duplicates. Isotope intensity readings from the blanks and standard solutions were used for correction of instrument readings. Repeated sample analyses were carried out to check for instrument drift, and variations from the original results were within 10%.

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Appendix G: Laboratory Test Results

Part G1: Summary of soil test results

Allotment plot ID	N	Range of total element concentrations (mg kg^{-1})				
		As	Cd	Cr	Ni	Pb
A01	7	8.1 – 10.3	<0.03 – 1.3	62.4 – 81.3	27.1 – 38.3	286.6 – 552.8
A03	6	9.3 – 11.3	1.2 – 1.4	69.1 – 79.2	28.9 – 35.8	478.0 – 895.7
A04	8	42.9 – 81.6	1.8 – 3.8	66.1 – 78.8	33.7 – 47.2	232.4 – 325.6
A05	11	4.2 – 12.0	0.8 – 5.2	35.0 – 76.4	13.7 – 48.9	250.0 – 693.2
A06	6	3.2 – 7.7	<0.03	63.2 – 74.2	20.7 – 25.9	100.5 – 296.8
A07	8	7.9 – 11.4	<0.03	50.1 – 68.1	26.7 – 38.1	214.0 – 403.7
A08	9	9.5 – 14.4	<0.03 – 0.7	63.7 – 84.7	30.2 – 43.6	219.7 – 340.0
A09	8	10.7 – 17.1	0.9 – 1.7	54.2 – 84.4	21.3 – 39.0	83.5 – 123.6
A10	9	8.1 – 10.3	1.4 – 2.8	46.6 – 57.0	28.0 – 33.7	141.1 – 164.9
A12	8	8.1 – 10.5	0.9 – 1.3	49.3 – 60.1	22.2 – 29.8	98.9 – 125.6
A15	8	6.8 – 10.2	<0.03 – 1.0	53.4 – 88.7	24.3 – 42.7	251.1 – 452.7
A16	7	5.9 – 9.7	<0.03	64.5 – 79.1	16.2 – 20.9	234.8 – 518.3
A17	14	2.2 – 10.1	<0.03 – 1.4	48.7 – 99.4	13.3 – 27.7	46.4 – 237.4
A18	7	3.2 – 5.3	<0.03	75.6 – 102.5	15.6 – 23.0	65.1 – 119.8
A19	10	6.6 – 10.7	0.3 – 0.6	54.6 – 81.5	15.4 – 24.6	279.0 – 429.5
A20	9	3.5 – 6.3	<0.03	57.9 – 72.9	13.5 – 19.9	95.5 – 165.4
A21	6	2.1 – 3.7	<0.03	44.1 – 73.5	16.2 – 28.1	151.2 – 196.4
A23	7	4.3 – 6.1	<0.03	50.0 – 68.1	22.4 – 32.1	411.1 – 865.1
A25	6	1.9 – 6.9	<0.03	55.6 – 64.8	18.1 – 20.2	93.8 – 111.8
A26	6	1.5 – 2.4	<0.03	60.1 – 66.8	19.0 – 22.9	85.2 – 159.7
A27	8	2.4 – 3.8	<0.03	43.1 – 68.4	17.3 – 25.6	44.5 – 123.0
A28	9	5.7 – 25.1	<0.03 – 1.1	47.1 – 71.6	16.9 – 41.9	251.8 – 802.9
A29	10	3.1 – 6.7	<0.03	60.3 – 104.7	18.8 -41.3	95.5 – 152.7
A31	8	5.8 – 7.1	0.2 – 0.4	61.4 – 75.1	28.5 – 35.3	237.6 – 535.7
A32	9	4.8 – 28.4	0.2 – 1.2	47.0 – 93.0	21.0 – 40.3	182.7 – 396.5
A33	9	4.2 – 6.1	<0.03	62.2 – 75.8	26.8 – 34.3	118.3 – 155.0
A34	10	4.0 – 6.2	<0.03	39.5 – 72.7	12.1 – 25.5	148.3 – 612.6
A36	15	9.1 – 13.5	0.6 – 1.6	49.3 – 77.4	23.6 – 53.6	483.1 – 1064.9
A37	10	10.7 – 21.4	0.8 – 1.4	67.0 – 90.7	33.1 – 43.5	485.3 – 705.5
A38	8	1.5 – 2.9	<0.03	52.8 – 68.8	16.6 – 21.7	93.6 – 147.3

N (number of soil samples).

Allotment plot ID	N	Range of element bioaccessibility fractions (%) in soil				
		As	Cd	Cr	Ni	Pb
A01	5	76.7 – 91.3	N/A	11.7 – 18.9	53.0 – 89.1	57.5 – 73.2
A03	5	77.5 – 89.6	N/A	14.2 – 14.9	55.0 – 67.2	64.6 – 73.2
A04	5	69.6 – 85.1	72.5 – 93.1	9.3 – 13.1	39.3 – 54.9	59.7 – 80.6
A05	5	85.4 – 91.9	26.5 – 77.9	14.5 – 20.4	44.6 – 68.7	68.0 – 93.6
A06	5	44.0 – 65.0	18.1 – 29.9	10.2 – 17.6	33.1 – 73.2	47.9 – 68.9
A07	5	39.1 – 59.2	N/A	7.9 – 15.5	23.8 – 45.6	46.5 – 53.4
A08	5	32.9 – 38.7	69.8 – 79.0	6.5 – 11.1	18.2 – 25.6	47.5 – 56.9
A09	5	45.9 – 57.8	59.5 – 81.3	8.0 – 15.3	17.9 – 41.8	41.1 – 47.5
A10	5	43.7 – 50.4	63.1 – 77.5	8.7 – 12.8	24.4 – 31.5	36.1 – 48.6
A12	5	33.5 – 48.8	54.5 – 83.8	6.3 – 13.0	19.0 – 34.7	28.5 – 45.8
A15	5	62.5 – 77.7	N/A	19.7 – 26.0	24.4 – 35.2	69.5 – 79.6
A16	5	56.7 – 69.2	29.2 – 69.8	21.5 – 25.2	29.6 – 34.6	65.1 – 72.0
A17	5	47.7 – 80.8	26.8 – 64.7	20.6 – 29.4	31.6 – 41.3	48.6 – 71.9
A18	5	41.5 – 72.5	18.6 – 70.3	13.3 – 16.0	21.4 – 27.0	54.2 – 63.4
A19	5	63.7 – 72.4	N/A	25.7 – 29.5	26.7 – 32.0	66.5 – 70.3
A20	5	58.5 – 78.7	18.1 – 38.8	9.9 – 22.0	18.8 – 29.9	34.6 – 70.1
A21	5	59.2 – 67.5	N/A	10.0 – 12.6	29.8 – 32.3	40.5 – 45.6
A23	5	63.8 – 73.6	N/A	11.1 – 15.1	37.1 – 41.4	43.6 – 53.1
A25	5	55.9 – 68.4	N/A	10.7 – 15.3	24.6 – 36.8	38.9 – 48.8
A26	5	59.4 – 71.8	N/A	8.9 – 14.1	22.4 – 37.5	38.1 – 48.9
A27	5	58.2 – 67.3	N/A	9.0 – 10.5	24.3 – 30.6	33.6 – 44.3
A28	5	74.3 – 90.6	N/A	13.4 – 18.9	28.1 – 40.4	39.9 – 44.4
A29	5	79.5 – 85.2	N/A	10.2 – 11.5	28.6 – 35.7	33.3 – 40.8
A31	5	47.9 – 56.0	25.2 – 36.3	9.4 – 10.6	27.0 – 31.2	53.7 – 57.4
A32	5	39.6 – 74.6	43.7 – 55.2	8.0 – 14.1	22.7 – 32.7	45.5 – 56.9
A33	5	36.8 – 45.5	N/A	9.0 – 11.1	25.9 – 30.2	47.8 – 58.2
A34	5	42.1 – 45.5	N/A	10.3 – 13.1	29.0 – 35.2	52.5 – 62.5
A36	5	44.9 – 71.0	56.9 – 62.4	9.4 – 13.6	17.0 – 33.5	55.4 – 65.7
A37	5	52.8 – 69.8	48.9 – 58.2	9.4 – 13.4	24.7 – 28.3	50.5 – 58.5
A38	5	41.1 – 75.3	N/A	7.5 – 10.9	24.6 – 30.4	44.6 – 53.5

N (number of soil samples subjected to bioaccessibility test).

N/A implies not calculated (because either the total or bioaccessibility concentrations were below the LOD).

Part G2: Summary of elemental concentrations in allotment produce samples

Allotment produce	N	Total element concentration (mg kg^{-1} fw) Range (average, for values ≥ LOD)				
		As	Cd	Cr	Ni	Pb
Apple	6	0.32-0.73 (0.52)	<0.01	<0.01-0.27 (0.18)	<0.01-0.27 (0.18)	<0.01-0.87 (0.35)
Artichoke	2	0.28-0.51	<0.01-0.04	<0.01-9.84	0.79-7.44	0.22-0.58
Beetroot	20	0.03-1.37 (0.43)	<0.01-0.45 (0.15)	<0.01-0.76 (0.2)	<0.01-5.07 (0.73)	0.12-2.94 (1.17)
Beans	27	<0.01-0.99 (0.49)	<0.01-0.04 (0.03)	<0.01-11.8 (1.75)	0.01-5.89 (1.35)	<0.01-2.92 (0.74)
Broccoli	8	0.18-1.52 (0.68)	<0.01-0.11 (0.07)	<0.01-0.2 (0.17)	<0.01-1.48 (0.73)	0.15-0.77 (0.42)
Cabbage	4	0.34-0.79 (0.49)	<0.01-0.11 (0.1)	<0.01-0.8 (0.48)	<0.01-1.17 (0.98)	0.37-4.51 (1.6)
Carrot	4	0.24-0.45 (0.34)	<0.01-0.16 (0.13)	<0.01-0.32	<0.01-0.55 (0.29)	0.18-1.57 (0.71)
Cauliflower	1	0.36	<0.01	<0.01	0.28	0.69
Chard	2	0.33-0.44	0.56-0.66	<0.01-0.55	1.36-1.86	0.49-4.71
Chives	2	<0.01-0.83	<0.01-0.11	<0.01	<0.02-0.82	0.57-0.77
Corn	2	0.33-0.43	<0.01	<0.01	<0.01-0.33	0.17-1.38
Courgette	10	0.2-0.88 (0.47)	<0.01-0.43 (0.19)	<0.01-3.35 (0.82)	<0.01-2.2 (0.97)	0.19-1.78 (0.75)
Cucumber	2	0.42-0.47	<0.01-0.06	<0.01-0.13	<0.01	0.36-0.4
Currants	7	0.18-1.65 (0.64)	<0.01-0.64 (0.15)	<0.01-0.05 (0.04)	<0.01-1.46 (0.66)	<0.01-1.33 (0.53)
Fennel	3	0.31-0.45 (0.39)	<0.01	<0.01-0.16	<0.01	0.13-2.35 (0.95)
Garlic	2	0.32-0.39	<0.01-0.04	<0.01	<0.01-0.04	<0.01-0.05
Green gauge	1	0.97	<0.01	<0.01	0.31	<0.01
Herbs	11	0.17-1.01 (0.59)	<0.01-0.12 (0.08)	<0.01-3.44 (0.92)	<0.01-2.43 (1.1)	0.41-5.39 (2.42)
Kale	8	0.21-0.97 (0.56)	<0.01-0.12 (0.09)	<0.01-0.25 (0.14)	<0.01-1.09 (0.65)	0.13-4.57 (0.9)
Kohlrabi	1	0.28	0.05	0.36	0.14	2.68
Leek	1	0.41	0.06	0.2	0.95	1.81
Lettuce	10	0.2-0.98 (0.5)	0.13-0.53 (0.26)	<0.01-3.33 (1.29)	<0.01-1.7 (0.89)	0.44-15.29 (5.13)
Onion/Shallot	30	0.01-1.09 (0.36)	<0.01-0.62 (0.13)	<0.01-1.57 (0.3)	<0.01-1.41 (0.35)	<0.01-3.31 (0.71)
Oriental green	1	0.48	<0.01	0.34	<0.01	1.27
Pak choi	1	0.56	0.26	0.47	0.45	2.38
Parsnip	5	0.25-1.3 (0.57)	<0.01-0.08 (0.07)	<0.01-0.29 (0.24)	0.26-2.51 (0.96)	0.67-3.81 (2.12)
Pear	2	0.3-0.86	<0.01	<0.01-0.1	0.24-0.26	0.35-0.7
Peas	8	0.16-1.62 (0.48)	<0.01-0.36 (0.2)	<0.01-0.28 (0.19)	<0.01-0.74 (0.53)	0.1-4.33 (1.1)
Plum	1	0.9	<0.01	<0.01	0.27	0.87
Potato	30	<0.01-0.91 (0.37)	<0.01-0.06 (0.04)	<0.01-0.17 (0.07)	<0.01-0.72 (0.2)	<0.01-2.49 (0.82)
Radish	1	0.47	<0.01	0.17	0.91	2.26
Berries	21	0.11-1.1 (0.4)	<0.01-0.39 (0.13)	<0.01-0.31 (0.14)	<0.01-1.69 (0.53)	<0.01-5.15 (0.77)
Rhubarb	16	0.15-2.14 (0.51)	<0.01-1.87 (0.24)	<0.01-2.04 (0.49)	<0.01-2.84 (0.96)	<0.01-2.98 (0.99)
Rocket	1	0.21	0.11	0.32	0.41	0.79
Spinach	10	0.16-1.13 (0.48)	<0.01-0.7 (0.38)	<0.01-2.49 (0.98)	<0.01-1.87 (1.0)	<0.01-15.8 (4.12)
Tomato	4	0.16-0.91 (0.49)	<0.01-0.07 (0.05)	<0.01-4.84 (1.72)	<0.01-2.04 (0.86)	0.13-0.62 (0.26)
Turnip/Swede	4	0.22-1.0 (0.52)	<0.01-0.05	<0.01-0.24 (0.16)	<0.01-0.79 (0.56)	0.11-1.7 (0.89)
Yam leaves	1	<0.01	<0.01	<0.01	3.65	<0.01

N (number of samples).

Allotment plot ID	N	Range of element bioaccessibility fractions (%) in allotment produce				
		As	Cd	Cr	Ni	Pb
A01	5	13.1 – 37.2	13.7 – 24.0	14.5 – 18.4	15.6 – 64.1	32.0 – 61.0
A03	3	19.2 – 61.3	12.2 – 58.4	16.8 – 43.7	14.4 – 18.6	36.8 – 42.7
A04	5	21.5 – 44.0	13.0 – 25.9	16.0 – 29.6	14.7 – 49.1	32.0 – 61.0
A05	6	23.4 – 76.6	13.9 – 17.8	16.2 – 48.7	16.1 – 27.4	30.0 – 61.0
A06	5	19.3 – 77.0	14.0 – 59.1	N/A	15.6 – 19.4	31.0 – 78.6
A07	5	26.0 – 50.5	16.2 – 26.5	17.7 – 53.3	14.8 – 21.6	39.0 – 42.8
A08	5	31.9 – 61.1	12.7 – 30.2	34.5 – 75.1	17.5 – 26.9	45.1 – 47.5
A09	5	13.9 – 64.5	15.2 – 31.7	N/A	18.4 – 82.3	44.2 – 52.8
A10	3	25.6 – 57.3	12.2 – 23.6	15.5 – 17.3	18.1 – 22.4	35.2 – 48.8
A12	5	25.5 – 55.9	10.5 – 11.9	24.6 – 27.3	19.6 – 25.8	48.5 – 52.0
A15	5	25.0 – 43.8	12.1 – 14.1	15.0 – 63.7	21.2 – 29.5	41.9 – 57.9
A16	5	22.6 – 49.6	10.9 – 12.6	36.8 – 45.1	19.1 – 28.4	42.0 – 55.9
A17	5	37.8 – 77.6	10.1 – 12.9	N/A	49.8 – 68.3	39.7 – 52.1
A18	4	45.8 – 79.0	9.8 – 17.2	N/A	N/A	31.7 – 32.0
A19	5	45.2 – 77.3	9.6 – 11.5	33.1 – 80.0	17.0 – 33.8	41.8 – 52.0
A20	3	51.5 – 62.6	9.5 – 18.6	14.3 – 16.1	21.9 – 27.2	31.0 – 43.3
A21	6	53.8 – 61.7	27.5 – 77.1	22.7 – 38.2	38.4 – 71.2	31.3 – 80.0
A23	6	44.7 – 58.9	27.1 – 67.0	16.7 – 28.2	31.2 – 62.4	43.8 – 77.3
A25	5	31.1 – 60.9	47.5 – 86.1	21.9 – 35.6	58.3 – 78.7	38.5 – 54.9
A26	3	N/A	52.6 – 88.5	25.5 – 38.1	47.3 – 63.8	41.0 – 81.0
A27	3	30.2 – 47.3	N/A	N/A	36.8 – 56.5	44.2 – 59.3
A28	5	26.4 – 48.9	31.1 – 42.5	22.7 – 39.3	18.7 – 59.6	30.0 – 32.0
A29	4	22.4 – 65.6	24.1 – 67.6	32.7 – 41.8	24.9 – 74.5	33.4 – 42.0
A31	5	40.3 – 41.0	16.7 – 73.7	31.6 – 62.0	54.7 – 75.3	35.8 – 59.5
A32	5	21.9 – 42.3	10.2 – 33.8	15.0 – 62.3	17.4 – 17.7	32.0 – 67.3
A33	5	24.5 – 45.6	10.9 – 89.3	18.0 – 76.8	14.3 – 20.3	66.8 – 69.7
A34	3	76.3 – 93.8	13.9 – 24.8	16.2 – 22.3	22.8 – 36.5	37.9 – 53.9
A36	5	45.6 – 56.1	12.0 – 20.0	29.1 – 75.5	27.3 – 74.9	31.0 – 79.0
A37	2	34.8 – 46.9	10.3 – 25.9	58.5 – 80.0	19.2 – 28.8	46.0 – 54.8
A38	4	36.9 – 48.9	9.7 – 14.3	N/A	15.8 – 20.5	34.1 – 42.1

N (number of produce samples subjected to bioaccessibility test).

N/A implies not calculated (because either the total or bioaccessibility concentrations were below the LOD). N/A was used only if all samples from a give site had concentrations <LOD; otherwise the results for concentrations ≥LOD were reported.

Part G3: Summary of venous blood Pb test results

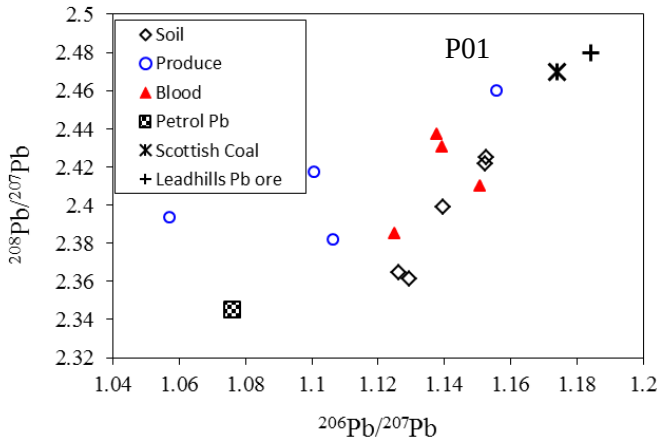
Participant ID	N	Pb concentration (µg dL^{-1})	
		Min	Max
P01	4	5.72	22.61
P02	3	8.80	13.08
P03	4	3.12	14.59
P04	4	4.13	16.74
P05	3	6.96	20.25
P09	4	5.04	12.31
P10	4	3.50	8.76
P11	4	10.07	12.38
P12	4	7.17	27.19
P13	4	3.79	19.97
P15	4	6.10	9.76
P16	2	5.70	14.69
P17	3	4.66	9.59
P18	4	4.89	15.21
P19	2	6.66	29.35
P20	4	5.22	18.58

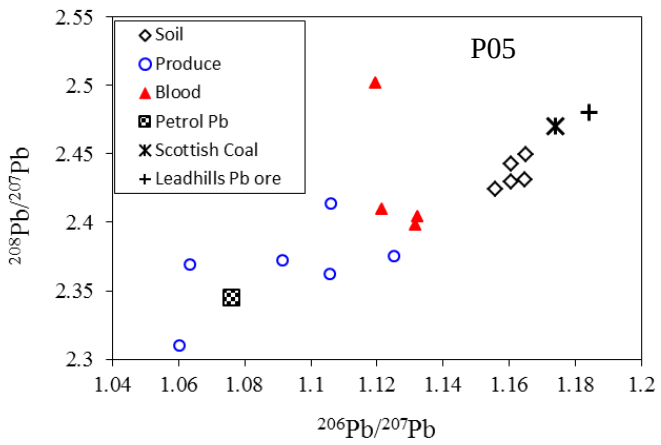
Participant ID	N	Pb concentration (µg dL^{-1})	
		Min	Max
P21	3	6.47	26.12
P22	3	4.78	12.93
P23	4	5.84	30.60
P24	3	7.58	13.75
P25	4	7.00	24.47
P26	4	6.97	10.88
P28	4	5.36	10.34
P29	3	7.94	14.57
P32	4	6.81	22.67
P33	4	4.69	11.75
P34	3	11.00	21.87
P35	3	8.55	11.42
P36	2	7.02	26.19
P37	3	7.59	20.70
P38	2	8.82	23.61
P39	2	6.92	15.19

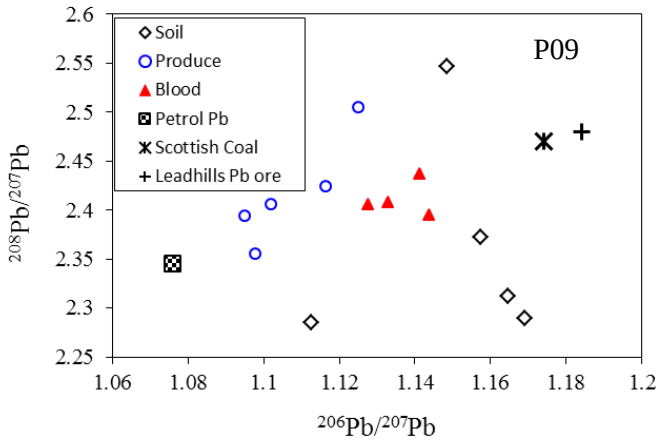
N (number of samples).

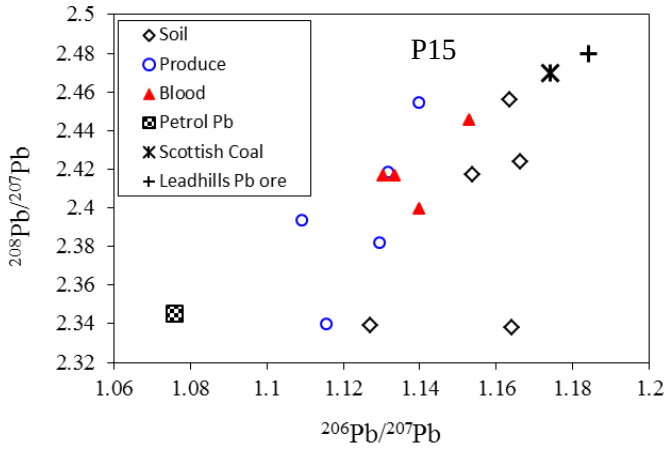
Examples of Pb isotopic plots for soil, produce and blood samples relating to participants P01, P05, P09, P15, P23 and P32

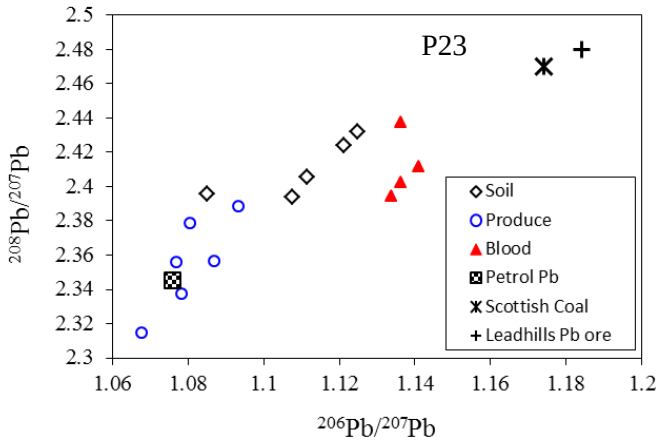
Note: *error bars were excluded from these plots to enhance legibility*.

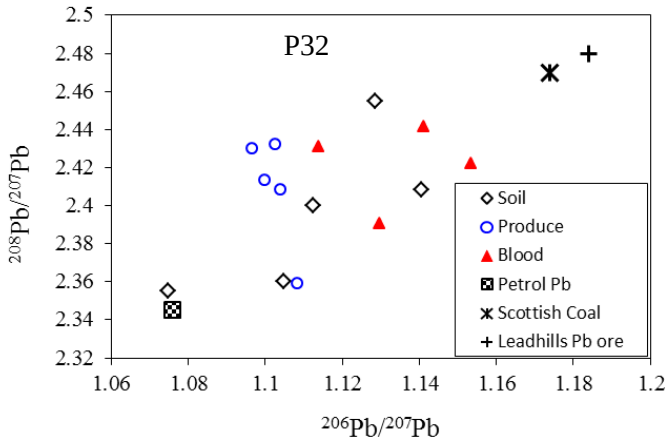












Part G4: Summary of urinary elemental concentrations

Summary of elemental urine concentrations (expressed in *ug L^{-1}*)

Participant ID	N	Urinary concentrations (µg L^{-1})$^{\$}$							
		As (inorganic)		Cd		Cr		Ni	
		Median	95 PC	Median	95 PC	Median	95 PC	Median	95 PC
P01	34	0.73	2.87	0.12	0.35	0.48	3.28	0.94	4.84
P02	32	1.14	5.95	0.10	0.37	0.19	4.06	1.54	5.45
P03	18	3.00	6.00	0.02	0.07	0.60	5.86	3.47	7.21
P04	18	2.10	8.50	0.02	0.09	0.53	4.02	1.45	7.13
P05	34	0.88	1.65	0.12	0.30	0.10	1.53	0.78	3.77
P06	24	1.00	8.80	0.12	0.56	0.79	3.15	1.66	5.75
P07	24	1.20	4.50	0.02	0.09	1.31	11.26	1.91	12.28
P08	24	2.40	6.15	0.04	0.68	1.00	7.16	3.54	9.53
P09	32	1.37	9.80	0.05	0.16	0.34	3.43	0.82	3.21
P10	27	1.41	3.81	0.12	0.78	0.43	2.32	0.83	3.31
P11	29	0.60	6.40	0.13	0.50	0.24	7.42	1.51	15.25
P12	33	1.35	4.17	0.15	0.58	0.50	3.07	1.31	3.89
P13	31	0.91	3.17	0.07	0.55	0.82	7.16	2.17	6.47
P15	33	1.36	7.40	0.04	0.46	0.53	4.96	1.73	8.41
P16	19	2.10	7.06	0.04	0.49	1.56	6.15	2.00	7.07
P17	22	2.00	6.80	0.02	0.29	0.89	6.17	1.44	6.57
P18	22	1.85	8.25	0.04	0.54	0.82	6.81	2.30	14.65
P19	19	2.56	14.36	0.04	0.44	1.55	4.06	2.60	5.47
P20	19	1.98	12.58	0.04	0.34	0.61	8.11	2.57	9.18
P21	33	2.23	5.16	0.07	0.51	0.14	4.64	0.63	6.46
P22	28	1.02	4.80	0.03	0.50	0.44	5.05	0.74	8.64
P23	21	2.00	4.04	0.05	0.51	0.43	2.27	1.64	3.44
P24	18	1.85	5.89	0.09	0.62	0.45	5.89	2.02	5.92
P25	33	0.88	6.90	0.04	0.31	0.32	6.73	0.88	6.99
P26	30	1.18	5.58	0.08	0.79	0.16	5.32	0.98	3.40
P27	25	1.40	7.66	0.12	0.80	0.76	5.36	2.68	7.34
P28	23	0.80	2.50	0.02	0.26	0.25	4.02	3.04	4.99
P29	21	1.15	5.45	0.06	0.53	0.37	3.00	1.13	3.58
P31	23	0.78	3.30	0.02	0.25	1.47	5.56	4.01	7.40
P32	13	3.00	6.622	0.06	0.77	0.85	2.71	1.57	5.29
P33	18	1.34	7.05	0.05	0.30	0.41	1.51	2.80	3.98
P34	13	1.25	7.90	0.05	0.27	1.31	7.82	2.87	5.73
P35	11	2.00	2.08	0.04	0.26	1.66	4.61	2.35	5.89
P36	19	0.93	7.50	0.03	0.07	0.31	3.62	1.73	4.10
P37	17	0.91	4.35	0.09	1.16	0.65	4.87	1.23	2.69
P38	14	1.72	19.80	0.02	0.13	0.71	4.72	3.05	5.26
P39	7	2.53	7.05	0.02	0.05	0.18	0.78	1.41	1.78

N (the total number of urine samples collected from participant).

95 PC (95^{th} percentile).

$^{\$}$For concentrations ≥ LOD.

Summary of elemental urine concentrations (expressed in *ug g^{-1} creatinine*)

Participant ID	N	Creatinine[#] (g L^{-1})		Urinary concentrations (µg g^{-1} creatinine)[$]							
				As (inorganic)		Cd		Cr		Ni	
		Min	Max	Median	95 PC	Median	95 PC	Median	95 PC	Median	95 PC
P01	34	0.3	2.2	1.12	2.65	0.20	0.50	0.51	3.53	1.75	4.46
P02	32	0.4	1.8	1.62	5.24	0.12	0.43	0.23	4.06	1.79	5.00
P03	18	0.4	1.8	2.82	7.39	0.03	0.05	0.61	7.78	3.00	9.20
P04	18	0.5	1.5	1.84	6.99	0.03	0.07	0.77	6.61	1.59	11.06
P05	34	0.3	1.1	1.55	2.77	0.21	0.75	0.23	4.76	1.42	9.83
P06	24	0.7	1.9	1.04	4.97	0.13	0.45	0.81	3.58	1.45	6.17
P07	24	0.5	1.9	1.10	4.78	0.02	0.09	1.35	11.26	1.91	13.08
P08	24	0.4	2.6	2.14	5.50	0.04	0.82	1.15	12.48	2.79	8.73
P09	32	0.3	2.2	1.10	6.61	0.06	0.27	0.34	4.96	1.48	3.13
P10	27	0.4	1.0	2.00	5.18	0.95	1.33	0.50	3.48	1.18	4.41
P11	29	0.3	3.0	1.05	6.33	0.15	0.49	0.22	6.18	1.40	12.33
P12	33	0.6	1.9	1.25	5.08	0.15	0.54	0.28	2.55	1.09	3.32
P13	31	0.4	2.2	1.19	3.69	0.07	0.63	0.79	4.04	1.84	6.00
P15	33	0.5	2.3	0.97	6.02	0.08	0.43	0.36	6.80	1.67	8.37
P16	19	0.3	2.0	2.86	6.95	0.03	0.63	1.60	5.27	2.37	6.20
P17	22	0.9	2.1	2.00	4.72	0.03	0.26	0.48	4.63	1.04	4.20
P18	22	0.6	2.0	1.66	5.81	0.03	0.46	0.58	4.88	2.04	18.51
P19	19	0.4	1.9	2.94	8.97	0.05	0.37	1.19	6.05	2.20	5.01
P20	19	1.0	2.3	1.85	10.33	0.03	0.26	0.30	3.67	1.77	6.18
P21	33	0.3	2.6	2.00	6.14	0.14	0.43	0.27	4.60	0.75	7.28
P22	28	0.3	1.0	2.35	9.61	0.04	1.28	1.22	10.71	1.50	18.35
P23	21	0.5	1.8	1.99	5.01	0.04	0.48	0.50	3.70	1.71	5.32
P24	18	0.4	1.7	1.37	6.40	0.08	0.60	0.52	3.99	1.93	8.06
P25	33	0.4	2.0	1.43	7.87	0.05	0.63	0.31	7.08	1.19	7.18
P26	30	0.3	0.9	2.70	13.57	0.12	1.96	0.27	11.69	2.01	7.03
P27	25	1.0	2.9	1.00	4.10	0.08	0.42	0.32	4.58	1.79	6.43
P28	23	0.3	0.6	1.43	5.57	0.04	0.47	0.75	12.42	6.42	8.92
P29	21	0.3	1.6	1.27	5.47	0.07	0.91	0.46	6.04	1.25	6.32
P31	23	0.5	1.2	1.20	4.12	0.03	0.32	1.86	5.44	5.98	8.25
P32	13	0.4	2.1	3.43	8.92	0.06	0.79	1.18	3.72	2.40	4.52
P33	18	0.4	2.3	1.54	6.24	0.04	0.52	0.29	3.25	3.07	6.14
P34	13	0.8	2.1	0.77	5.21	0.03	0.30	1.27	5.38	2.12	4.02
P35	11	0.3	0.7	3.51	5.95	0.13	0.39	2.84	7.08	5.72	10.31
P36	19	0.8	3.0	0.68	4.64	0.02	0.06	0.22	2.94	1.34	2.18
P37	17	0.3	1.4	1.43	6.51	0.17	1.90	0.94	6.95	1.32	4.62
P38	14	0.8	2.3	1.37	13.28	0.02	0.12	0.63	4.51	2.32	4.87
P39	7	0.3	1.1	7.41	12.48	0.04	0.06	0.36	0.79	2.05	3.24

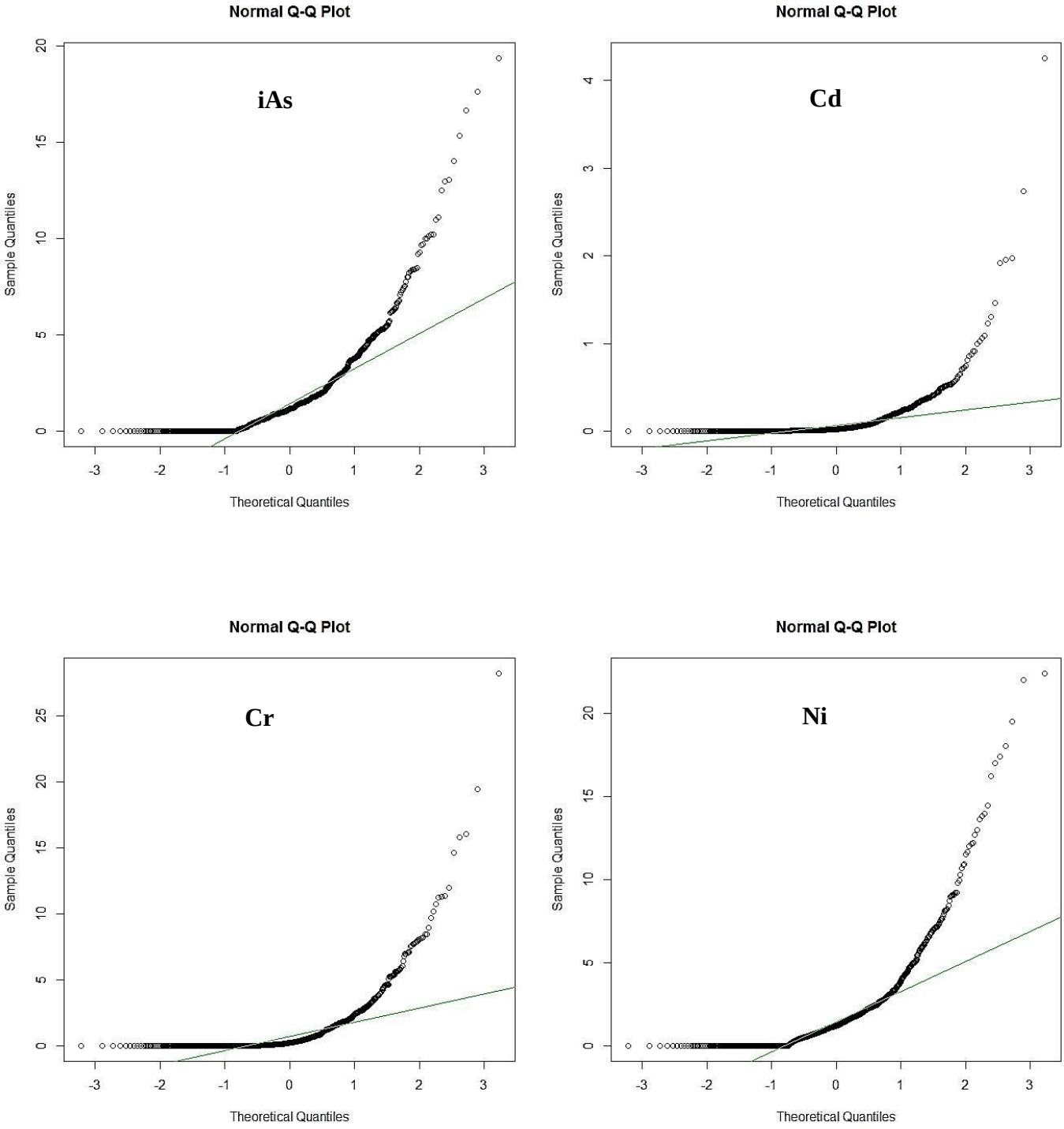
N (the total number of urine samples collected from participant).

95 PC (95th percentile).

[#]Excludes urine samples with creatinine values < 0.3 (g L^{-1}) which were discarded (as explained in the text, section 2.7.2).

[$]For concentrations ≥ LOD.

Probability distribution of elemental urine concentrations – normal Q-Q plots



The normal Q-Q plots show that the urinary concentrations (µg g^{-1} creatinine) of iAs, Cd, Cr and Ni do not follow a normal distribution, because data points do not plot evenly along the straight Q-Q line.

Appendix H: Examples of R Codes used in Statistical Calculations

Part H1: R Code for mixed-effects modelling for blood Pb concentrations

```
# R Codes

install.packages("lme4")
library(lme4)

Pb<-read.csv("BloodPb-lmer.csv",header=T)
Pb
attach(Pb)
summary(Pb)
str(Pb)
head(Pb)
plot(Pb)

nofixedlmm<-lmer(BloodPb~1+(1|Participant),data=Pb,REML=FALSE)
summary(nofixedlmm)

genderlmm<-lmer(BloodPb~Gender+(1|Participant),data=Pb,REML=FALSE)
summary(genderlmm)

agelmm<-lmer(BloodPb~Age+(1|Participant),data=Pb,REML=FALSE)
summary(agelmm)

smokinglmm<-lmer(BloodPb~SmokingStatus+(1|Participant),data=Pb,REML=FALSE)
summary(smokinglmm)

producelmm<-lmer(BloodPb~ProduceConsRate+(1|Participant),data=Pb,REML=FALSE)
summary(producelmm)

install.packages("car")
library(car)
anova(nofixedlmm,genderlmm,agelmm,smokinglmm,producelmm)
```

```
# Results of Statistical Significance Test

> anova(nofixedlmm,genderlmm,agelmm,smokinglmm,producelmm)
Data: Pb
Models:
nofixedlmm: BloodPb ~ 1 + (1 | Participant)
agelmm: BloodPb ~ Age + (1 | Participant)
producelmm: BloodPb ~ ProduceConsRate + (1 | Participant)
genderlmm: BloodPb ~ Gender + (1 | Participant)
smokinglmm: BloodPb ~ SmokingStatus + (1 | Participant)
           Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
nofixedlmm  3 704.28 712.32 -349.14   698.28
agelmm      4 705.53 716.26 -348.77   697.53 0.7435      1     0.3885
producelmm  4 705.90 716.63 -348.95   697.90 0.0000      0     1.0000
genderlmm   5 707.61 721.02 -348.80   697.61 0.2958      1     0.5866
smokinglmm  5 708.12 721.53 -349.06   698.12 0.0000      0     1.0000
```

Interpretation of Results: The *p* values indicate that there are no statistically important differences between the models, and that there are no significant associations ($p > 0.05$) between the modelled variables and blood Pb levels.

Part H2: R Code for data imputation of <LOD urine concentrations – example for iAs

```
library(EnvStats)
Data <- read.csv("iAs.csv", header=TRUE)
Arsenic <- Data$iAs
NR = length(Arsenic)
Data$Arsenic_REP = Arsenic
for (i in 1:NR)
{
      if (Data$Non.detects[i]==TRUE)
{
      RANDU = runif(1,0,1)
      a = 0
      b = Arsenic[i]
      c = b/2
      F = (c-a)/(b-a)
      if (0 < RANDU & RANDU < F)
      {
            trival = a +sqrt(RANDU*(b-a)*(c-a))
      }
      if (F < RANDU & RANDU < 1)
      {
            trival = b-sqrt((1-RANDU)*(b-a)*(b-c))
      }
Data$Arsenic_REP[i] = trival
}
}
write.table(Data, file='iAs - imputed data.txt')
```

Part H3: R Codes for Mann-Whitney *U* test – example for urine concentrations sub-grouped according to participant gender

```
gender<-read.csv("Gender.csv",header=T)
gender
attach(gender)
names(gender)
summary(gender)

# Wilcoxon rank test (Mann-Whitney U test) for unmatched pairs, Results

>
wilcox.test(iAsMale,iAsFemale,data=gender,mu=0,alt="two.sided",paired=F,c
onf.int=T,conf.level=0.95)

        Wilcoxon rank sum test with continuity correction

data:  iAsMale and iAsFemale
W = 71390, p-value = 0.08706
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -0.281742888  0.001099057
sample estimates:
difference in location
           -0.07368445

>
wilcox.test(CdMale,CdFemale,data=gender,mu=0,alt="two.sided",paired=F,con
f.int=T,conf.level=0.95)

        Wilcoxon rank sum test with continuity correction

data:  CdMale and CdFemale
W = 68586, p-value = 0.009474
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -0.0081679420 -0.0008116486
sample estimates:
difference in location
          -0.004240437

>
wilcox.test(CrMale,CrFemale,data=gender,mu=0,alt="two.sided",paired=F,con
f.int=T,conf.level=0.95)

        Wilcoxon rank sum test with continuity correction

data:  CrMale and CrFemale
W = 73128, p-value = 0.2444
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -0.035807673  0.003261563
sample estimates:
difference in location
          -0.004984143
```

```
>
wilcox.test(NiMale,NiFemale,data=gender,mu=0,alt="two.sided",paired=F,con
f.int=T,conf.level=0.95)

        Wilcoxon rank sum test with continuity correction

data:  NiMale and NiFemale
W = 66718, p-value = 0.001461
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -0.501438188 -0.008956675
sample estimates:
difference in location
            -0.2670566
```

Interpretation of Results: At 5% significance level, the difference between the medians of datasets examined is statistically significant when $p < 0.05$, and not significant when $p > 0.05$.

Appendix I: Mathematical Equations and Parameters of the Modified Models

Part I1: Arsenic PBPK model equations and parameters

GI tract:

$$\frac{dAMT_{gi}^{3}}{dt} = Q_{gi} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{gi}^{3}}{V_{gi} \times P_{gi}^{3}}\right) + \left(K_{red} \times AMT_{gi}^{5}\right) - \left(K_{ox} \times AMT_{gi}^{3}\right) - \left(eF \times AMT_{gi}^{3}\right)$$

$$\frac{dAMT_{gi}^{5}}{dt} = Q_{gi} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{gi}^{5}}{V_{gi} \times P_{gi}^{5}}\right) - \left(K_{red} \times AMT_{gi}^{5}\right) + \left(K_{ox} \times AMT_{gi}^{3}\right) - \left(eF \times AMT_{gi}^{5}\right)$$

$$\frac{dAMT_{gi}^{mma}}{dt} = Q_{gi} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{gi}^{mma}}{V_{gi} \times P_{gi}^{mma}}\right) - \left(eF \times AMT_{gi}^{mma}\right)$$

$$\frac{dAMT_{gi}^{dma}}{dt} = Q_{gi} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{gi}^{dma}}{V_{gi} \times P_{gi}^{dma}}\right) - \left(eF \times AMT_{gi}^{dma}\right)$$

Liver:

$$\frac{dAMT_{liv}^{3}}{dt} = Q_{liv} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{liv}^{3}}{V_{liv} \times P_{liv}^{3}}\right) - \left(\frac{V_{max,liv}^{3\to mma} \times C_{liv}^{3}}{K_{m,liv}^{3\to mma} + C_{liv}^{3}}\right) - \left(\frac{V_{max,liv}^{3\to dma} \times C_{liv}^{3}}{K_{m,liv}^{3\to dma} + C_{liv}^{3}}\right) + \left(K_{red} \times AMT_{liv}^{5}\right) - \left(K_{ox} \times AMT_{liv}^{3}\right) - \left(eB \times AMT_{liv}^{3}\right)$$

$$\frac{dAMT_{liv}^{5}}{dt} = Q_{liv} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{liv}^{5}}{V_{liv} \times P_{liv}^{5}}\right) - \left(K_{red} \times AMT_{liv}^{5}\right) + \left(K_{ox} \times AMT_{liv}^{3}\right) - \left(eB \times AMT_{liv}^{5}\right)$$

$$\frac{dAMT_{liv}^{mma}}{dt} = Q_{liv} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{liv}^{mma}}{V_{liv} \times P_{liv}^{mma}}\right) + \left(\frac{V_{max,liv}^{3\to mma} \times C_{liv}^{3}}{K_{m,liv}^{3\to mma} + C_{liv}^{3}}\right) - \left(\frac{V_{max,liv}^{mma\to dma} \times C_{liv}^{mma}}{K_{m,liv}^{mma\to dma} + C_{liv}^{mma}}\right) - \left(eB \times AMT_{liv}^{mma}\right)$$

$$\frac{dAMT_{liv}^{dma}}{dt} = Q_{liv} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{liv}^{dma}}{V_{liv} \times P_{liv}^{dma}}\right) + \left(\frac{V_{max,liv}^{3\to dma} \times C_{liv}^{3}}{K_{m,liv}^{3\to dma} + C_{liv}^{3}}\right) + \left(\frac{V_{max,liv}^{mma\to dma} \times C_{liv}^{mma}}{K_{m,liv}^{mma\to dma} + C_{liv}^{mma}}\right) - \left(eB \times AMT_{liv}^{dma}\right)$$

Kidney:

$$\frac{dAMT_{kid}^{3}}{dt} = Q_{kid} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{kid}^{3}}{V_{kid} \times P_{kid}^{3}}\right) - \left(\frac{V_{max,kid}^{3\to mma} \times C_{kid}^{3}}{K_{m,kid}^{3\to mma} + C_{kid}^{3}}\right) - \left(\frac{V_{max,kid}^{3\to dma} \times C_{kid}^{3}}{K_{m,kid}^{3\to dma} + C_{kid}^{3}}\right) + \left(K_{red} \times AMT_{kid}^{5}\right) - \left(K_{ox} \times AMT_{kid}^{3}\right) - \left(eU \times AMT_{kid}^{3}\right)$$

$$\frac{dAMT_{kid}^{5}}{dt} = Q_{kid} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{kid}^{5}}{V_{kid} \times P_{kid}^{5}}\right) - \left(K_{red} \times AMT_{kid}^{5}\right) + \left(K_{ox} \times AMT_{kid}^{3}\right) - \left(eU \times AMT_{kid}^{5}\right)$$

$$\frac{dAMT_{kid}^{mma}}{dt} = Q_{kid} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{kid}^{mma}}{V_{kid} \times P_{kid}^{mma}}\right) + \left(\frac{V_{max,kid}^{3\to mma} \times C_{kid}^{3}}{K_{m,kid}^{3\to mma} + C_{kid}^{3}}\right) - \left(\frac{V_{max,kid}^{mma\to dma} \times C_{kid}^{mma}}{K_{m,kid}^{mma\to dma} + C_{kid}^{mma}}\right) - (eU \times AMT_{kid}^{mma})$$

$$\frac{dAMT_{kid}^{dma}}{dt} = Q_{kid} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{kid}^{dma}}{V_{kid} \times P_{kid}^{dma}}\right) + \left(\frac{V_{max,kid}^{3\to dma} \times C_{kid}^{3}}{K_{m,kid}^{3\to dma} + C_{kid}^{3}}\right) + \left(\frac{V_{max,kid}^{mma\to dma} \times C_{kid}^{mma}}{K_{m,kid}^{mma\to dma} + C_{kid}^{mma}}\right) - \left(eU \times AMT_{kid}^{dma}\right)$$

Muscles:

$$\frac{dAMT_{muscle}^{3}}{dt} = Q_{muscle} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{muscle}^{3}}{V_{muscle} \times P_{muscle}^{3}}\right) + \left(K_{red} \times AMT_{muscle}^{5}\right) - \left(K_{ox} \times AMT_{muscle}^{3}\right)$$

$$\frac{dAMT_{muscle}^{5}}{dt} = Q_{muscle} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{muscle}^{5}}{V_{muscle} \times P_{muscle}^{5}}\right) - \left(K_{red} \times AMT_{muscle}^{5}\right) + \left(K_{ox} \times AMT_{muscle}^{3}\right)$$

$$\frac{dAMT_{muscle}^{mma}}{dt} = Q_{muscle} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{muscle}^{mma}}{V_{muscle} \times P_{muscle}^{mma}}\right)$$

$$\frac{dAMT_{muscle}^{dma}}{dt} = Q_{muscle} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{muscle}^{dma}}{V_{muscle} \times P_{muscle}^{dma}}\right)$$

Skin:

$$\frac{dAMT_{skin}^{3}}{dt} = Q_{skin} \times \left(\frac{AMT_{blood}^{3}}{V_{blod}} - \frac{AMT_{skin}^{3}}{V_{skin} \times P_{skin}^{3}}\right) + \left(K_{red} \times AMT_{skin}^{5}\right) - \left(K_{ox} \times AMT_{skin}^{3}\right)$$

$$\frac{dAMT_{skin}^{5}}{dt} = Q_{skin} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{skin}^{5}}{V_{skin} \times P_{skin}^{5}}\right) - \left(K_{red} \times AMT_{skin}^{5}\right) + \left(K_{ox} \times AMT_{skin}^{3}\right)$$

$$\frac{dAMT_{skin}^{mma}}{dt} = Q_{skin} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{skin}^{mma}}{V_{skin} \times P_{skin}^{mma}}\right)$$

$$\frac{dAMT_{skin}^{dma}}{dt} = Q_{skin} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{skin}^{dma}}{V_{skin} \times P_{skin}^{dma}}\right)$$

Heart:

$$\frac{dAMT_{heart}^{3}}{dt} = Q_{heart} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{heart}^{3}}{V_{heart} \times P_{heart}^{3}}\right) + \left(K_{red} \times AMT_{heart}^{5}\right) - \left(K_{ox} \times AMT_{heart}^{3}\right)$$

$$\frac{dAMT_{heart}^{5}}{dt} = Q_{heart} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{heart}^{5}}{V_{heart} \times P_{heart}^{5}}\right) - \left(K_{red} \times AMT_{heart}^{5}\right) + \left(K_{ox} \times AMT_{heart}^{3}\right)$$

$$\frac{dAMT_{heart}^{mma}}{dt} = Q_{heart} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{heart}^{mma}}{V_{heart} \times P_{heart}^{mma}}\right)$$

$$\frac{dAMT_{heart}^{dma}}{dt} = Q_{heart} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{heart}^{dma}}{V_{heart} \times P_{heart}^{dma}}\right)$$

Brain:

$$\frac{dAMT_{brain}^{3}}{dt} = Q_{brain} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{brain}^{3}}{V_{brain} \times P_{brain}^{3}}\right) + \left(K_{red} \times AMT_{brain}^{5}\right) - \left(K_{ox} \times AMT_{brain}^{3}\right)$$

$$\frac{dAMT_{brain}^{5}}{dt} = Q_{brain} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{brain}^{5}}{V_{brain} \times P_{brain}^{5}}\right) - \left(K_{red} \times AMT_{brain}^{5}\right) + \left(K_{ox} \times AMT_{brain}^{3}\right)$$

$$\frac{dAMT_{brain}^{mma}}{dt} = Q_{brain} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{brain}^{mma}}{V_{brain} \times P_{brain}^{mma}}\right)$$

$$\frac{dAMT_{brain}^{dma}}{dt} = Q_{brain} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{brain}^{dma}}{V_{brain} \times P_{brain}^{dma}}\right)$$

Lung:

$$\frac{dAMT_{lung}^{3}}{dt} = Q_{lung} \times \left(\frac{AMT_{blood}^{3}}{V_{blood}} - \frac{AMT_{lung}^{3}}{V_{lung} \times P_{lung}^{3}}\right) + \left(K_{red} \times AMT_{lung}^{5}\right) - \left(K_{ox} \times AMT_{lung}^{3}\right)$$

$$\frac{dAMT_{lung}^{5}}{dt} = Q_{lung} \times \left(\frac{AMT_{blood}^{5}}{V_{blood}} - \frac{AMT_{lung}^{5}}{V_{lung} \times P_{lung}^{5}}\right) - \left(K_{red} \times AMT_{lung}^{5}\right) + \left(K_{ox} \times AMT_{lung}^{3}\right)$$

$$\frac{dAMT_{lung}^{mma}}{dt} = Q_{lung} \times \left(\frac{AMT_{blood}^{mma}}{V_{blood}} - \frac{AMT_{lung}^{mma}}{V_{lung} \times P_{lung}^{mma}}\right)$$

$$\frac{dAMT_{lung}^{dma}}{dt} = Q_{lung} \times \left(\frac{AMT_{blood}^{dma}}{V_{blood}} - \frac{AMT_{lung}^{dma}}{V_{lung} \times P_{lung}^{dma}}\right)$$

Blood:

$$\frac{dAMT_{blood}^{3}}{dt} = \left(\sum_{i=1}^{n} Q_i \times \frac{AMT_i^3}{V_i \times P_i^3} - \sum_{i=1}^{n} Q_i \times \frac{AMT_{blood}^{3}}{V_{blood}}\right) + \left(K_{red} \times AMT_{blood}^{5}\right) - \left(K_{ox} \times AMT_{blood}^{3}\right)$$

$$\frac{dAMT_{blood}^{5}}{dt} = \left(\sum_{i=1}^{n} Q_i \times \frac{AMT_i^5}{V_i \times P_i^5} - \sum_{i=1}^{n} Q_i \times \frac{AMT_{blood}^{5}}{V_{blood}}\right) - \left(K_{red} \times AMT_{blood}^{5}\right) + \left(K_{ox} \times AMT_{blood}^{3}\right)$$

$$\frac{dAMT_{blood}^{mma}}{dt} = \left(\sum_{i=1}^{n} Q_i \times \frac{AMT_i^{mma}}{V_i \times P_i^{mma}} - \sum_{i=1}^{n} Q_i \times \frac{AMT_{blood}^{mma}}{V_{blood}}\right)$$

$$\frac{dAMT_{blood}^{dma}}{dt} = \left(\sum_{i=1}^{n} Q_i \times \frac{AMT_i^{dma}}{V_i \times P_i^{dma}} - \sum_{i=1}^{n} Q_i \times \frac{AMT_{blood}^{dma}}{V_{blood}}\right)$$

Urine:

$\frac{dAMT_{urine}^{3}}{dt} = \left(eU \times AMT_{kid}^{3}\right);$ $\frac{dAMT_{urine}^{5}}{dt} = \left(eU \times AMT_{kid}^{5}\right)$

$\frac{dAMT_{urine}^{mma}}{dt} = (eU \times AMT_{kid}^{mma});$ $\frac{dAMT_{urine}^{dma}}{dt} = \left(eU \times AMT_{kid}^{dma}\right)$

Biliary Excretion:

$\frac{dAMT_{bile}^{3}}{dt} = \left(eB \times AMT_{liv}^{3}\right)$ $\frac{dAMT_{bile}^{5}}{dt} = \left(eB \times AMT_{liv}^{5}\right)$

$\frac{dAMT_{bile}^{mma}}{dt} = (eB \times AMT_{liv}^{mma})$ $\frac{dAMT_{bile}^{dma}}{dt} = \left(eB \times AMT_{liv}^{dma}\right)$

Faecal Excretion:

$\frac{dAMT_{faeces}^{3}}{dt} = \left(eF \times AMT_{gi}^{3}\right)$ $\frac{dAMT_{faeces}^{5}}{dt} = \left(eF \times AMT_{gi}^{5}\right)$

$\frac{dAMT_{faeces}^{mma}}{dt} = \left(eF \times AMT_{gi}^{mma}\right)$ $\frac{dAMT_{faeces}^{dma}}{dt} = \left(eF \times AMT_{gi}^{dma}\right)$

Where:
AMT^{3}, AMT^{5}, AMT^{mma} and AMT^{dma} refer to the amount (µmol) of As(III), As(V), MMA and DMA, respectively.
C is the concentration (µmol/L) of As species.
Q is the blood flow rate into a compartment (L/day).
V is the volume of compartment (L).
P is the partitioning coefficient of As species between compartments and the blood (unitless).
eU is the urinary excretion rate of As species (/day).
eB is biliary excretion rate of As species (/day).
eF is faecal excretion rate of As species (/day).
Abbreviations used: gastrointestinal tract (gi), kidney (kid) and liver (liv).
i = organ/compartment.
n = total number of organs/compartments that exchange arsenic with blood.
K_{red} and K_{ox} are metabolic constants (/day) for reduction and oxidation, respectively.
V_{max} and K_m are metabolic constants (µmol/day and µmol/L, respectively) for methylation of As(III) to MMA and DMA, and MMA to DMA.

Table I1: Physiologically based parameters used in the PBPK model for As

Arsenic			
Parameter Symbol	**Parameter Value**	**Reference**	**Description**
BW	70 kg	a	Average adult body weight
V_{blood}	5.53[$] L	b	Volume of blood of adult (70kg)
	5.11 L	c	
V_{gi}	1.2 L	b	Volume of GI tract
V_{liv}	1.82[$] L	b	Volume of liver
	1.75 L	c	
V_{kid}	0.28[$] L	b	Volume of kidney
	0.29 L	c	
V_{muscle}	55.5 L	b	Volume of muscles
V_{skin}	2.6 L	b	Volume of skin
V_{heart}	0.35 L	b	Volume of heart
V_{brain}	1.4 L	b	Volume of brain
V_{lung}	0.56 L	b	Volume of lung
QCC	340 L/day/kgBW	c, d	Cardiac blood flow
QC	7886 L/day	c, d	Cardiac blood output = QCC*(BW^0.74)
	7488[$] L/day	b	Total cardiac blood output
QLC	0.25	d	Fraction of QC going to liver
QKC	0.17[$]	d	Fraction of QC going to kidney
	0.19	e	Fraction of QC going to kidney
QS	0.058	a	Fraction of QC going to skin
QM	0.19	a	Fraction of QC going to muscles
QH	0.04	a	Fraction of QC going to heart
QB	0.114	a	Fraction of QC going to brain
Q_{gi}	1440[$] L/day	b	Blood flow to GI tract
	1273 L/day	d	Blood flow to GI tract (same as kidney)
Q_{kid}	1440[$] L/day	b	Blood flow to kidney
	1273 L/day	d	Blood flow to kidney = QKC*QC
Q_{liv}	446.4[$] L/day	b	Blood flow to liver
	1872 L/day	d	Blood flow to liver = QLC*QC
Q_{muscle}	2592[$] L/day	b	Blood flow to muscles
	1423 L/day	a	Blood flow to muscles = QM*QC
Q_{skin}	374.4[$] L/day	b	Blood flow to skin
	434 L/day	a	Blood flow to skin = QS*QC
Q_{heart}	288[$] L/day	b	Blood flow to heart
	299 L/day	a	Blood flow to heart = QH*QC
Q_{brain}	907.2[$] L/day	b	Blood flow to brain
	854 L/day	a	Blood flow to brain = QB*QC
Q_{lung}	7488 L/day	b	Blood flow to lung (QC)
Reduction / Oxidation of inorganic arsenic			
K_{red}	32.88 /day	f	As(V) reduction in tissues (first order rate)
	42 /day	f	As(V) reduction in kidney (first order rate)
K_{ox}	43.92 /day	f	As(III) oxidation in tissues (first order rate)
Metabolism constants for methylation of arsenic in the Liver			
V_{max}, As(III)→MMA	748.8	g	µmol/day
	763[$]	b	
V_{max}, As(III)→DMA	1500	g	µmol/day
	2880[$]	b	
V_{max}, MMA→DMA	1067	g	µmol/day
	950.4[$]	b	
$K_{m,}$ As(III)→MMA	100	g	µmol/L
	3[$]	b	
$K_{m,}$ As(III)→DMA	100	g	µmol/L
	3[$]	b	
$K_{m,}$ MMA→DMA	100	g	µmol/L
	3[$]	b	

Metabolism constants for methylation of arsenic in the Kidney			
V_{max}, As(III)→MMA	499.7	g	µmol/day
	305.2[$]	b	
V_{max}, As(III)→DMA	667	g	µmol/day
	1152[$]	b	
V_{max}, MMA→DMA	333	g	µmol/day
	380.16[$]	b	
$K_{m,}$ As(III)→MMA	100	g	µmol/L
	3[$]	b	
K_m, As(III)→DMA	100	g	µmol/L
	3[$]	b	
K_m, MMA→DMA	100	g	µmol/L
	3[$]	b	
Tissue/blood partition coefficients (unitless)			
$P_{gi\text{-}As3}$	8.3[b]		GI tract/blood partition coefficient for As(III)
$P_{gi\text{-}As5}$	2.7[b]		GI tract/blood partition coefficient for As(V)
$P_{gi\text{-}MMA}$	2.2[b]		GI tract/blood partition coefficient for MMA
$P_{gi\text{-}DMA}$	2.1[b]		GI tract/blood partition coefficient for DMA
$P_{kid\text{-}As3}$	11.7[b], 20[f$], 4.15[g]		Kidney/blood partition coefficient for As(III)
$P_{kid\text{-}As5}$	8.3[b$], 40[f], 4.15[g]		Kidney/blood partition coefficient for As(V)
$P_{kid\text{-}MMA}$	4.4[b], 100[f$], 1.8[g]		Kidney/blood partition coefficient for MMA
$P_{kid\text{-}DMA}$	3.8[b], 5[f#], 2.08[g]		Kidney/blood partition coefficient for DMA
$P_{liv\text{-}As3}$	16.5[b$], 200[f], 5.3[g]		Liver/blood partition coefficient for As(III)
$P_{liv\text{-}As5}$	15.8[b$], 1[f], 5.3[g]		Liver/blood partition coefficient for As(V)
$P_{liv\text{-}MMA}$	3.3[b$], 10[f], 2.35[g]		Liver/blood partition coefficient for MMA
$P_{liv\text{-}DMA}$	3.3[b$], 1[f], 2.35[g]		Liver/blood partition coefficient for DMA
$P_{muscle\text{-}As3}$	7.4[b$], 2.6[g]		Muscle/blood partition coefficient for As(III)
$P_{muscle\text{-}As5}$	7.9[b$], 2.6[g]		Muscle/blood partition coefficient for As(V)
$P_{muscle\text{-}MMA}$	2.61[b$], 1.8[g]		Muscle/blood partition coefficient for MMA
$P_{muscle\text{-}DMA}$	2.4[b$], 2.8[g]		Muscle/blood partition coefficient for MMA
$P_{skin\text{-}As3}$	7.4[b$], 60[f], 2.5[g]		Skin/blood partition coefficient for As(III)
$P_{skin\text{-}As5}$	7.9[b$], 1[f], 2.5[g]		Skin/blood partition coefficient for As(V)
$P_{skin\text{-}MMA}$	2.61[b$], 50[f], 1.25[g]		Skin/blood partition coefficient for MMA
$P_{skin\text{-}DMA}$	2.4[b$], 1[f], 1.25[g]		Skin/blood partition coefficient for MMA
$P_{heart\text{-}As3}$	7.4[b]		Heart/blood partition coefficient for As(III)
$P_{heart\text{-}As5}$	7.9[b]		Heart/blood partition coefficient for As(V)
$P_{heart\text{-}MMA}$	2.61[b]		Heart/blood partition coefficient for MMA
$P_{heart\text{-}DMA}$	2.4[b]		Heart/blood partition coefficient for DMA
$P_{brain\text{-}As3}$	2.4[b]		Brain/blood partition coefficient for As(III)
$P_{brain\text{-}As5}$	2.4[b]		Brain/blood partition coefficient for As(V)
$P_{brain\text{-}MMA}$	2.2[b]		Brain/blood partition coefficient for MMA
$P_{brain\text{-}DMA}$	3.3[b]		Brain/blood partition coefficient for DMA
$P_{lung\text{-}As3}$	6.7[b$], 1[f], 4.15[g]		Lung/blood partition coefficient for As(III)
$P_{lung\text{-}As5}$	2.1[b$], 1[f], 4.15[g]		Lung/blood partition coefficient for As(V)
$P_{lung\text{-}MMA}$	1.3[b$], 1[f], 1.8[g]		Lung/blood partition coefficient for MMA
$P_{lung\text{-}DMA}$	1.3[b$], 20[f], 2.08[g]		Lung/blood partition coefficient for DMA

Dimensional parameters (/day) calculated / obtained from the literature		
Rate	*Calculation / value*	
$K12_{As3}$	$Q_{gi}/(V_{gi}*_{Pgi\text{-}As3})$	
$K12_{As5}$	$Q_{gi}/(V_{gi}*P_{gi\text{-}As5})$	
$K12_{MMA}$	$Q_{gi}/(V_{gi}*P_{gi\text{-}MMA})$	
$K12_{DMA}$	$Q_{gi}/(V_{gi}*P_{gi\text{-}DMA})$	
K21	Q_{gi}/V_{blood}	
$K32_{As3}$	$Q_{liv}/(V_{liv}*P_{liv\text{-}As3})$	
$K32_{As5}$	$Q_{liv}/(V_{liv}*P_{liv\text{-}As5})$	
$K32_{MMA}$	$Q_{liv}/(V_{liv}*P_{liv\text{-}MMA})$	
$K32_{DMA}$	$Q_{liv}/(V_{liv}*P_{liv\text{-}DMA})$	
K23	Q_{liv}/V_{blood}	
$K42_{As3}$	$Q_{kid}/(V_{kid}*P_{kid\text{-}As3})$	
$K42_{As5}$	$Q_{kid}/(V_{kid}*P_{kid\text{-}As5})$	
$K42_{MMA}$	$Q_{kid}/(V_{kid}*P_{kid\text{-}MMA})$	
$K42_{DMA}$	$Q_{kid}/(V_{kid}*P_{kid\text{-}DMA})$	
K24	Q_{kid}/V_{blood}	
$K52_{As3}$	$Q_{muscle}/(V_{muscle}*P_{muscle\text{-}As3})$	
$K52_{As5}$	$Q_{muscle}/(V_{muscle}*P_{muscle\text{-}As5})$	
$K52_{MMA}$	$Q_{muscle}/(V_{muscle}*P_{muscle\text{-}MMA})$	
$K52_{DMA}$	$Q_{muscle}/(V_{muscle}*P_{muscle\text{-}DMA})$	
K25	Q_{muscle}/V_{blood}	
$K62_{As3}$	$Q_{skin}/(V_{skin}*P_{skin\text{-}As3})$	
$K62_{As5}$	$Q_{skin}/(V_{skin}*P_{skin\text{-}As5})$	
$K62_{MMA}$	$Q_{skin}/(V_{skin}*P_{skin\text{-}MMA})$	
$K62_{DMA}$	$Q_{skin}/(V_{skin}*P_{skin\text{-}DMA})$	
K26	Q_{skin}/V_{blood}	
$K72_{As3}$	$Q_{heart}/(V_{heart}*P_{heart\text{-}As3})$	
$K72_{As5}$	$Q_{heart}/(V_{heart}*P_{heart\text{-}As5})$	
$K72_{MMA}$	$Q_{heart}/(V_{heart}*P_{heart\text{-}MMA})$	
$K72_{DMA}$	$Q_{heart}/(V_{heart}*P_{heart\text{-}DMA})$	
K27	Q_{heart}/V_{blood}	
$K82_{As3}$	$Q_{brain}/(V_{brain}*P_{brain\text{-}As3})$	
$K82_{As5}$	$Q_{brain}/(V_{brain}*P_{brain\text{-}As5})$	
$K82_{MMA}$	$Q_{brain}/(V_{brain}*P_{brain\text{-}MMA})$	
$K82_{DMA}$	$Q_{brain}/(V_{brain}*P_{brain\text{-}DMA})$	
K28	Q_{brain}/V_{blood}	
$K92_{As3}$	$Q_{lung}/(V_{lung}*P_{lung\text{-}As3})$	
$K92_{As5}$	$Q_{lung}/(V_{lung}*P_{lung\text{-}As5})$	
$K92_{MMA}$	$Q_{lung}/(V_{lung}*P_{lung\text{-}MMA})$	
$K92_{DMA}$	$Q_{lung}/(V_{lung}*P_{lung\text{-}DMA})$	
K29	Q_{lung}/V_{blood}	
eU^{+}	100.8[b]	Urinary excretion rate for As(III) and As(V) (/day)
	432[b]	Urinary excretion rate for MMA (/day)
	187.2[b]	Urinary excretion rate for DMA (/day)
	1.2[g]	Urinary elimination constant for As(III) (/day)
	1.8[g]	Urinary elimination constant for As(V) (/day)
	100.8[g]	Urinary elimination constant for MMA (/day)
	57.6[g]	Urinary elimination constant for DMA (/day)
eB	0.43[g]	Biliary excretion rate for As(V), (assumed for all species) (/day)
eF	0.03[g]	Faecal excretion rate for As(V), (assumed for all species) (/day)
Ka	5.76[b]	Oral absorption in the GI tract for As(III) (/day)
	4.32[b]	Oral absorption in the GI tract for As(V) (/day)
	10.08[b]	Oral absorption in the GI tract for MMA and DMA (/day)

[a]Brown et al. (1997), [b]El-Masri & Kenyon (2008), [c]Kirman et al. (2013), [d]O'Flaherty et al. (2001), [e]Liao et al. (2008), [f]Mann et al. (1996), [g]Yu (1999).

[$]Value used in simulations (to fit literature data).

[#]Partition coefficient of 1 provided a better fit with literature data. Mann et al. (1996) reported a DMA partitioning coefficient of 1 for liver, skin and 'other' tissues.

[+]We observed that the following eU values fitted well with the literature data: 57.6 day^{-1} for As(III) and As(V), 100.8 day^{-1} for DMA and 432 day^{-1} for MMA.

Part I2: Cadmium PBTK model equations and parameters

GI tract: $\frac{dAMT_{gi\ tract}}{dt} = -\left(C5 \times AMT_{gi\ tract}\right) - \left((1 - C5) \times AMT_{gi\ tract}\right)$

Intestine: $\frac{dAMT_{intestine}}{dt} = \left(C5 \times AMT_{gi\ tract}\right) - (C6 \times AMT_{intestine})$

Uptake: $\frac{dAMT_{uptake}}{dt} = (C6 \times AMT_{intestine}) - \left(C7 \times AMT_{uptake}\right) - \left((1 - C7) \times AMT_{uptake}\right)$

Blood1 (Plasma other): $\frac{dAMT_{Blood1}}{dt} = (C10 \times AMT_{other\ tissues}) + \left(C18 \times AMT_{kidney}\right) + \left((1 - C7) \times AMT_{uptake}\right) - (C9 \times AMT_{Blood1}) - (C11 \times AMT_{Blood1}) - (C12 \times AMT_{Blood1}) - (Cx \times AMT_{Blood1}) + (C13 \times AMT_{liver})$

Blood2 (Red blood cells): $\frac{dAMT_{Blood2}}{dt} = (Cx \times AMT_{Blood1}) - (C16 \times AMT_{Blood2})$

Blood3 (Plasma metallothionein): $\frac{dAMT_{Blood3}}{dt} = (C16 \times AMT_{Blood2}) + \left(C7 \times AMT_{uptake}\right) + (C14 \times AMT_{liver}) - (C17 \times AMT_{Blood3}) - \left((1 - C17) \times AMT_{Blood3}\right)$

Liver: $\frac{dAMT_{liver}}{dt} = (C12 \times AMT_{Blood1}) - (C13 \times AMT_{liver}) - (C14 \times AMT_{liver}) - (C15 \times AMT_{liver})$

Kidney: $\frac{dAMT_{kidney}}{dt} = (C17 \times AMT_{Blood3}) - (C18 \times AMT_{kidney}) - (C19 \times AMT_{kidney})$

Other Tissues: $\frac{dAMT_{other\ tissues}}{dt} = (C9 \times AMT_{Blood1}) - (C10 \times AMT_{other\ tissues})$

Urine: $\frac{dAMT_{urine}}{dt} = (C19 \times AMT_{kidney}) + \left((1 - C17) \times AMT_{Blood3}\right)$

Faeces: $\frac{dAMT_{faeces}}{dt} = \left((1 - C5) \times AMT_{gi\ tract}\right) + (C11 \times AMT_{Blood1}) + (C15 \times AMT_{liver})$

Where,

AMT is the amount (mass) of Cd in compartments (µg).

Terms beginning with letter 'C' refer to transfer coefficients, as described in Table S2.

Table I2: Physiologically based parameters used in the Cd PBTK model

Cadmium		
Parameter Symbol	**Parameter Value[a]**	**Description**
C5	0.048	Fraction absorbed to GI tract and systemic circulation
C6	0.05/day	Rate constant for Cd absorption to system from GI tract
C7	0.25	Fraction of absorbed Cd in system that is taken up to blood compartment 3 (B3)
C8	1 μg/day	Maximum rate that can be up taken to B3
C9	0.44	Fraction of Cd transferred from blood compartment 1 (B1) to other tissues
C10	1.4×10^{-4} /day	Rate constant for Cd transfer from other tissues to B1
C11	0.27	Fraction of Cd transferred from B1 to feces
C12	0.25	Fraction of Cd transferred from B1 to liver
C13	3.0×10^{-5} /day	Rate constant for Cd transfer from liver to B1
C14	1.6×10^{-4} /day	Rate constant for Cd transfer from liver to B3
C15	5.0×10^{-5} /day	Rate constant for Cd transfer from liver to feces
C16	0.012/day	Rate constant for Cd transfer from blood compartment 2 (B2) to B3
C17	0.95	Fraction of Cd transferred from B3 to kidney
C18	1.0×10^{-5} /day	Rate constant for Cd transfer from kidney to B1
C19	1.4×10^{-4} /day	Rate constant for transfer of Cd from kidney to urine
C20	0.1	Fraction of Cd in B1 and B3 contributing to Cd in whole blood
Cx	0.04	Fraction of CD transfer from B1 to B2

[a]Kjellström & Nordberg (1978)

Part I3: Chromium PBPK model equations and parameters

Gastrointestinal tract:

$$\frac{dAMT_{gi}^{6}}{dt} = -\left(KGI6 \times AMT_{gi}^{6}\right) - \left(KRED \times AMT_{gi}^{6}\right) - \left(KFX \times AMT_{gi}^{6}\right)$$

$$\frac{dAMT_{gi}^{3}}{dt} = -\left(KGI3 \times AMT_{gi}^{3}\right) + \left(KRED \times AMT_{gi}^{6}\right) - \left(KFX \times AMT_{gi}^{3}\right)$$

Bone:

$$\frac{dAMT_{bone}^{6}}{dt} = \left(KIN6 \times \frac{AMT_{sysP}^{6}}{V_{sysP}} \times Q_{bone}\right)$$

$$\frac{dAMT_{bone}^{3}}{dt} = \left(KINTCRB \times \frac{AMT_{sysP}^{3}}{V_{sysP}} \times Q_{bone}\right) - \left(KOUTCCRB \times \frac{AMT_{bone}^{3}}{V_{bone}} \times Q_{bone}\right)$$

Other Tissues:

$$\frac{dAMT_{OT}^{6}}{dt} = \left(KIN6 \times \frac{AMT_{sysP}^{6}}{V_{sysP}} \times Q_{OT}\right) - \left(KRED \times AMT_{OT}^{6}\right)$$

$$\frac{dAMT_{OT}^{3}}{dt} = \left(KINTCROT \times \frac{AMT_{sysP}^{3}}{V_{sysP}} \times Q_{OT}\right) - \left(KOUTCCROT \times \frac{AMT_{OT}^{3}}{V_{OT}} \times Q_{OT}\right) + \left(KRED \times AMT_{OT}^{6}\right)$$

Kidney:

$$\frac{dAMT_{kid}^{6}}{dt} = \left(KIN6 \times \frac{AMT_{sysP}^{6}}{V_{sysP}} \times Q_{kid}\right) - \left(KRED \times AMT_{kid}^{6}\right)$$

$$\frac{dAMT_{kid}^{3}}{dt} = \left(KINTCRK \times \frac{AMT_{sysP}^{3}}{V_{sysP}} \times Q_{kid}\right) + \left(KINCCR \times \frac{AMT_{sysP}^{3}}{V_{sysP}} \times Q_{kid}\right) + \left(KRED \times AMT_{kid}^{6}\right) - \left(KURC \times \frac{AMT_{kid}^{3}}{V_{kid}}\right)$$

Urine: $\frac{dAMT_{urine}^{3}}{dt} = \left(KURC \times \frac{AMT_{kid}^{3}}{V_{kid}}\right)$

Liver:

$$\frac{dAMT_{liv}^{6}}{dt} = \left(KIN6 \times \frac{AMT_{sysP}^{6}}{V_{sysP}} \times Q_{liv}\right) + \left(KIN6 \times \frac{AMT_{portalP}^{6}}{V_{portalP}} \times Q_{portalP}\right) - \left(KRED \times AMT_{liv}^{6}\right)$$

$$\frac{dAMT_{liv}^{3}}{dt} = \left(KINTCRL \times \frac{AMT_{sysP}^{3}}{V_{sysP}} \times Q_{liv}\right) + \left(KINTCRL \times \frac{AMT_{portalP}^{3}}{V_{portalP}} \times Q_{portalP}\right) + \left(KRED \times AMT_{liv}^{6}\right) - \left(KOUTCCRL \times \frac{AMT_{liv}^{3}}{V_{liv}} \times \left(Q_{liv} + Q_{portalP}\right)\right)$$

Blood (Portal Plasma):

$$\frac{dAMT^6_{portalP}}{dt} = \left(Q_{portalP} \times \frac{AMT^6_{sysP}}{V_{sysP}}\right) - \left(Q_{portalP} \times \frac{AMT^6_{portalP}}{V_{portalP}}\right) + \left(KGI6 \times AMT^6_{gi}\right) - \left(KRBCIN6 \times \frac{AMT^6_{portalP}}{V_{portalP}}\right) - \left(KIN6 \times \frac{AMT^6_{portalP}}{V_{portalP}} \times Q_{portalP}\right) - \left(KRED \times AMT^6_{portalP}\right)$$

Cr(III) storage/excretion pool

$$\frac{dAMT^3_{portalP}}{dt} = \left(Q_{portalP} \times \frac{AMT^3_{sysP}}{V_{sysP}}\right) - \left(Q_{portalP} \times \frac{AMT^3_{portalP}}{V_{portalP}}\right) + \left(KGI3 \times AMT^3_{gi}\right) - \left(KRBCIN3 \times \frac{AMT^3_{portalP}}{V_{portalP}}\right) - \left(KINTCRL \times \frac{AMT^3_{portalP}}{V_{portalP}} \times Q_{portalP}\right) + \left(KRED \times AMT^6_{portalP}\right)$$

Cr(III) distribution pool

$$\frac{dAMT^3_{portalP}}{dt} = \left(KRBCOUT3 \times \frac{AMT^3_{portalP}}{V_{portalP}}\right) - \left(Q_{portalP} \times \left(\frac{AMT^3_{portalP}}{V_{portalP}} - \frac{AMT^3_{sysP}}{V_{sysP}}\right)\right)$$

Blood (Portal Red Blood Cells):

$$\frac{dAMT^6_{portalRBC}}{dt} = \left(Q_{portalRBC} \times \left(\frac{AMT^6_{sysRBC}}{V_{sysRBC}} - \frac{AMT^6_{portalRBC}}{V_{portalRBC}}\right)\right) + \left(KRBCIN6 \times \frac{AMT^6_{portalP}}{V_{portalP}}\right) - \left(KREDRC \times AMT^6_{portalRBC}\right)$$

$$\frac{dAMT^3_{portalRBC}}{dt} = \left(Q_{portalRBC} \times \left(\frac{AMT^3_{sysRBC}}{V_{sysRBC}} - \frac{AMT^3_{portalRBC}}{V_{portalRBC}}\right)\right) + \left(KRBCIN3 \times \frac{AMT^3_{portalP}}{V_{portalP}}\right) + \left(KREDRC \times AMT^6_{portalRBC}\right) - \left(KRBCOUT3 \times \frac{AMT^3_{portalRBC}}{V_{portalRBC}}\right)$$

Blood (Systemic Plasma):

$$\frac{dAMT^6_{sysP}}{dt} = \left(Q_{portalP} \times \frac{AMT^6_{portalP}}{V_{portalP}}\right) - \left(Q_{portalP} \times \frac{AMT^6_{sysP}}{V_{sysP}}\right) - \left(KIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}} \times Q_{liv}\right) - \left(KIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}} \times Q_{kid}\right) - \left(KIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}} \times Q_{bone}\right) - \left(KIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}} \times Q_{OT}\right) - \left(KRBCIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}}\right) - \left(KRED \times AMT^6_{sysP}\right)$$

Cr(III) distribution pool

$$\frac{dAMT^3_{sysP}}{dt} = \left(Q_{portalP} \times \frac{AMT^3_{portalP}}{V_{portalP}}\right) - \left(Q_{portalP} \times \frac{AMT^3_{sysP}}{V_{sysP}}\right) - \left(KINTCRL \times \frac{AMT^3_{sysP}}{V_{sysP}} \times Q_{liv}\right) - \left(KINTCRK \times \frac{AMT^3_{sysP}}{V_{sysP}} \times Q_{kid}\right) - \left(KINTCRB \times \frac{AMT^3_{sysP}}{V_{sysP}} \times Q_{bone}\right) - \left(KINTCROT \times \frac{AMT^3_{sysP}}{V_{sysP}} \times Q_{OT}\right) - \left(KRBCIN3 \times \frac{AMT^3_{sysP}}{V_{sysP}}\right) + \left(KRED \times AMT^6_{sysP}\right)$$

Cr(III) storage/excretion pool

$$\frac{dAMT^3_{sysP}}{dt} = \left(KOUTCCRL \times \frac{AMT^3_{liv}}{V_{liv}} \times \left(Q_{liv} + Q_{portalP}\right)\right) + \left(KOUTCCROT \times \frac{AMT^3_{OT}}{V_{OT}} \times Q_{OT}\right) + \left(KOUTCCRB \times \frac{AMT^3_{bone}}{bone} \times Q_{bone}\right) + \left(KRBCOUT3 \times \frac{AMT^3_{sysRBC}}{V_{sysRBC}}\right) - \left(KINCCR \times \frac{AMT^3_{sysP}}{V_{sysP}} \times Q_{kid}\right) + \left(Q_{portalP} \times \left(\frac{AMT^3_{portalP}}{V_{portalP}} - \frac{AMT^3_{sysP}}{V_{sysP}}\right)\right)$$

Blood (Systemic Red Blood Cells):

$$\frac{dAMT^6_{sysRBC}}{dt} = \left(Q_{portalRBC} \times \left(\frac{AMT^6_{portalRBC}}{V_{portalRBC}} - \frac{AMT^6_{sysRBC}}{V_{sysRBC}}\right)\right) + \left(KRBCIN6 \times \frac{AMT^6_{sysP}}{V_{sysP}}\right) - \left(KREDRC \times AMT^6_{sysRBC}\right)$$

$$\frac{dAMT^3_{sysRBC}}{dt} = \left(Q_{portalRBC} \times \left(\frac{AMT^3_{portalRBC}}{V_{portalRBC}} - \frac{AMT^3_{sysRBC}}{V_{sysRBC}}\right)\right) + \left(KRBCIN3 \times \frac{AMT^3_{sysP}}{V_{sysP}}\right) - \left(KRBCOUT3 \times \frac{AMT^3_{sysRBC}}{V_{sysRBC}}\right) + \left(KREDRC \times AMT^6_{sysRBC}\right)$$

Faeces:

$$\frac{dAMT^6_{faeces}}{dt} = \left(KFX \times AMT^6_{gi}\right); \qquad \frac{dAMT^3_{faeces}}{dt} = \left(KFX \times AMT^3_{gi}\right)$$

Where,
AMT^3 and AMT^6 refer to the mass of Cr(III) and Cr(VI) in compartments, respectively.
Abbreviations used for compartments: gastrointestinal tract (gi), systemic plasma (sysP), portal plasma (portal), other tissues (OT), kidney (kid), liver (liv), systemic red blood cells (sysRBC), portal red blood cells (portalRBC). Transfer parameters beginning with letter 'K' are described in Table S3.
Q is the blood flow into a compartment (L/day), V is the volume of compartment (L).

Table I3: Physiologically based parameters for the Cr PBPK model

Chromium			
Parameter Symbol	**Parameter Value**	**Reference**	**Description**
BW	70 kg		Average adult body weight
HCT	0.45[$]	a	Blood has approx. 45% hematocrit
	0.43	b	
VLC	0.025 L/kgBW	a, b	Liver volume fraction in adult
VKC	0.0042 L/kgBW	b	Kidney volume fraction in adult
VBC	0.14 L/kgBW	b	Bone volume fraction in adult
VBLC	0.073 L/kgBW	b	Blood volume fraction in adult
V_{blood}	5.53 L	c	Volume of blood of adult (70kg) male
	5.11[$] L	b	Volume of blood = VBLC*BW
V_{rbc}	2.3 L	b	Red Blood Cell volume = (V_{blood}*HCT)
V_{plasma}	2.8 L	b	Blood Plasma volume = (V_{blood}*(1-HCT))
FPT	0.205	b	Fraction of blood volume in portal system
V_{liv}	1.82 L	c	Volume of liver of adult (70kg) male
	1.75[$] L	b	Volume of liver = VLC*BW
V_{kid}	0.28 L	c	Volume of kidney of adult (70kg) male
	0.29[$] L	b	Volume of kidney = VKC*BW
V_{bone}	9.8 L	b, d	Volume of bone = VBC*BW
V_{OT}	53 L	b	Volume of other tissues = BW-(V_{bone} + V_{liv} + V_{kid} + V_{blood})
V_{sysRBC}	1.83 L	b	Volume of Red Blood Cell in systemic blood = V_{rbc}*(1-FPT)
$V_{portalRBC}$	0.47 L	b	Volume of Red Blood Cell in portal blood = V_{rbc}*FPT
V_{sysP}	2.23 L	b	Volume of Plasma in systemic blood = V_{plasma}*(1-FPT)
$V_{portalP}$	0.57 L	b	Volume of Plasma in portal blood = V_{plasma}*FPT
QCC	340 L/day/kgBW	a, b	Cardiac blood flow
QC	7885.9 L/day	a, b	Cardiac blood output = QCC*(BW^0.74)
QCG	4337.2 L/day	b	Cardiac plasma output = QC*(1-HCT)
QBC	0.03[$]	a	Fraction of QC going to bone
	0.05	b	
QKC	0.17[$]	a	Fraction of QC going to kidney
	0.19	b	
QLC	0.25	a	Fraction of QC going to liver
QPTC	0.19	b	Fraction of QC going to portal plasma
Q_{liv}	1084.3 L/day	a	Blood plasma flow to liver = QLC*QCG
Q_{kid}	737.3 L/day	a	Blood plasma flow to kidney = QKC*QCG
	824 L/day	b	
$Q_{portalP}$	824 L/day	b	Blood plasma flow in portal system = QPTC*QCG
$Q_{portalRBC}$	674.2 L/day	b	Red Blood Cell flow in portal system = $Q_{portalP}$ *HCT/(1-HCT)
Q_{bone}	130.1 L/day	a	Blood plasma flow to bone = QBC*QCG
	216.9 L/day	b	
Q_{OT}	1561.4 L/day	b	Blood plasma flow to other tissues = QCG-(Q_{liv}+ Q_{kid}+Q_{bone}+$Q_{portalP}$)
		Kinetic Rate Constants	
KGI3	0.25[#] /day	a	Absorption of Cr(III) from the GI tract
KGI6	2.5[#] /day	a	Absorption of Cr(VI) from the GI tract
KFX	14 /day	a	Loss of Cr from GI tract to faeces
KIN6	2	b, e	Transfer of Cr(VI) from plasma to tissues
KINTCRB	0.0625	b	Cr(III) transfer from distribution pool in plasma to bone
KOUTCCRB	0.00228	b	Cr(III) transfer from bone to plasma
KREDGI	100 /day	a	Reduction of Cr(VI) to Cr(III) in GI tract
KREDRC	7 /day	a	Reduction of Cr(VI) to Cr(III) in the red blood cells
KREDBP	0.2 /day	a	Reduction of Cr(VI) to Cr(III) in blood plasma
KREDKL	500 /day	a	Reduction of Cr(VI) to Cr(III) in kidney and liver
KRED	5 /day	a	Reduction of Cr(VI) to Cr(III) in all other tissues
KINTCROT	0.004	b	Cr(III) transfer from distribution pool in plasma to other tissues

KOUTCCROT	0.008	b	Cr(III) transfer from other tissues to plasma
KINTCRK	0.08	b	Cr(III) transfer from distribution pool in plasma to kidney
KINCCR	0.04	b	Cr(III) transfer from storage/excretion pool in plasma to kidney
KURC	1.74 L/day	b	Urinary excretion rate for Cr(III) in storage/excretion pool
KINTCRL	0.0203	b	Cr(III) transfer from distribution pool in plasma to liver
KOUTCCRL	0.016	b	Cr(III) transfer from storage/excretion pool in liver to plasma
KRBCIN6	192 L/day	b	Cr(VI) transfer from plasma to red blood cells
KRBCIN3	0.0192 L/day	b	Cr(III) transfer from plasma to red blood cells
KRBCOUT3	0.036 L/day	b	Cr(III) transfer from red blood cells to plasma

Transfer rates (/day) calculated from physiological parameters and rate constants

Compartment	*Calculation*
Bone	$(KIN6*Q_{bone})/V_{sysP}$
	$(KINTCRB*Q_{bone})/V_{sysP}$
	$(KOUTCCRB*Q_{bone})/V_{bone}$
Other Tissues	$(KIN6*Q_{OT})/V_{sysP}$
	$(KINTCROT*Q_{OT})/V_{sysP}$
	$(KOUTCCROT*Q_{OT})/V_{OT}$
Kidney	$(KIN6*Q_{kid})/V_{sysP}$
	$(KINTCRK*Q_{kid})/V_{sysP}$
	$(KINCCR*Q_{kid})/V_{sysP}$
	$KURC/V_{kid}$
Liver	$(KIN6*Q_{liv})/V_{sysP}$
	$(KIN6*Q_{portalP})/V_{portalP}$
	$(KINTCRL*Q_{liv})/V_{sysP}$
	$(KINTCRL*Q_{portalP})/V_{portalP}$
	$(KOUTCCRL*(Q_{liv}+Q_{portalP}))/V_{liv}$
Blood	$Q_{portalP}/V_{sysP}$
	$Q_{portalP}/V_{portalP}$
	$KRBCIN6/ V_{portalP}$
	$KRBCIN3/ V_{portalP}$
	$KRBCOUT3/ V_{portalRBC}$
	$Q_{portalRBC}/V_{sysRBC}$
	$Q_{portalRBC}/V_{portalRBC}$
	$KRBCIN6/ V_{sysP}$
	$KRBCIN3/ V_{sysP}$
	$KRBCOUT3/ V_{sysRBC}$

[a]O'Flaherty et al. (2001), [b]Kirman et al. (2013), [c]El-Masri & Kenyon (2008), [d]O'Flaherty (1993) , [e]Kirman et al. (2012)
[$]Value used in simulations.
[#]Because of the inter- and intra-individual variability of gastrointestinal absorption of Cr, we used KGI3 values ranging from 0.05 to 0.25 day^{-1} and KGI6 values ranging from 1 to 2.5 day^{-1} to fit model simulations to the literature data (see discussion in report section 6.2.3).

Part I4: Lead PBPK model equations and parameters

GI tract: $\frac{dAMT_{gi}}{dt} = -\left(A_{gi} \times IR_{gi}\right) - \left(\left(1 - A_{gi}\right) \times IR_{gi}\right)$

Blood-plasma: $\frac{dAMT_{Bp}}{dt} = \left(Q_{liver} \times \left(\frac{AMT_{liver}}{V_{liver} \times P_{liver}} - \frac{AMT_{plasma}}{V_{plasma}}\right)\right) + \left(Q_{kidney} \times \left(\frac{AMT_{kidney}}{V_{kidney} \times P_{kidney}} - \frac{AMT_{plasma}}{V_{plasma}}\right)\right) + \left(Q_{bone} \times \left(\frac{AMT_{bone}}{V_{bone} \times P_{bone}} - \frac{AMT_{plasma}}{V_{plasma}}\right)\right) + \left(Q_{wp} \times \left(\frac{AMT_{wp}}{V_{wp} \times P_{wp}} - \frac{AMT_{plasma}}{V_{plasma}}\right)\right) + \left(Q_{pp} \times \left(\frac{AMT_{pp}}{V_{pp} \times P_{pp}} - \frac{AMT_{plasma}}{V_{plasma}}\right)\right)$

Liver: $\frac{dAMT_{liver}}{dt} = \left(A_{gi} \times IR_{gi}\right) + \left(Q_{liver} \times \left(\frac{AMT_{plasma}}{V_{plasma}} - \frac{AMT_{liver}}{V_{liver} \times P_{liver}}\right)\right) - eB \times AMT_{liver}$

Kidney: $\frac{dAMT_{kidney}}{dt} = Q_{kidney} \times \left(\frac{AMT_{plasma}}{V_{plasma}} - \frac{AMT_{kidney}}{V_{kidney} \times P_{kidney}}\right) - eU \times AMT_{kidney}$

Bone: $\frac{dAMT_{bone}}{dt} = Q_{bone} \times \left(\frac{AMT_{plasma}}{V_{plasma}} - \frac{AMT_{bone}}{V_{bone} \times P_{bone}}\right)$

Tissues (wp): $\frac{dAMT_{wp}}{dt} = Q_{wp} \times \left(\frac{AMT_{plasma}}{V_{plasma}} - \frac{AMT_{wp}}{V_{wp} \times P_{wp}}\right)$

Tissues (pp): $\frac{dAMT_{pp}}{dt} = Q_{pp} \times \left(\frac{AMT_{plasma}}{V_{plasma}} - \frac{AMT_{pp}}{V_{pp} \times P_{pp}}\right)$

Urinary Excretion: $\frac{dAMT_{urine}}{dt} = eU \times AMT_{kidney}$

Biliary Excretion: $\frac{dAMT_{bile}}{dt} = eB \times AMT_{liver}$

Faecal Excretion: $\frac{dAMT_{faeces}}{dt} = \left(\left(1 - A_{gi}\right) \times IR_{gi}\right)$

Where,
AMT is the mass of Pb in compartments,
Q is the blood flow into a compartment (L/day),
V is the volume of compartment (L),
IR_{gi} is the oral intake rate of Pb (mg/day),
A_{gi} is the Pb absorption coefficient from gastrointestinal tract (unitless),
eB is the biliary excretion rate of Pb (/day),
eU is the urinary excretion rate of Pb (/day),
GI tract refers to the gastrointestinal tract,
WP is well-perfused tissues, and
PP is poorly-perfused tissues.

Table I4: Physiologically based parameters for the Pb PBPK model

Lead			
Parameter Symbol	**Parameter Value**	**Reference**	**Description**
BW	70 kg	a, c	Average adult body weight
QCC	340 L/day/kgBW	a, b	Cardiac blood flow
QC	7885.9 L/day	a, b	Cardiac blood output = QCC*(BW^0.74)
HCT	0.45	a, b	Blood has approx. 45% hematocrit
QLC	0.25	a, b	Fraction of QC going to liver
QKC	0.17	a, b	Fraction of QC going to kidney
QBC	0.03	a, b	Fraction of QC going to bone
QWC	0.44	a, b	Fraction of QC going to tissues (wp)
QPC	0.11	a, b	Fraction of QC going to tissues (pp)
QCG	4337.2 L/day	a, b	Cardiac plasma output = QC*(1-HCT)
Q_{liver}	1084.3 L/day	a, b	Blood plasma flow to liver = QLC*QCG
Q_{kidney}	737.3 L/day	a, b	Blood plasma flow to kidney = QKC*QCG
Q_{bone}	130.1 L/day	a, b	Blood plasma flow to bone = QBC*QCG
Q_{wp}	86.74 L/day	a, b	Blood plasma flow to tissues (wp) =(QWC*QCG)-Q_{liver}-Q_{kidney}
Q_{pp}	346.98 L/day	a, b	Blood plasma flow to tissues (pp) =(QPC*QCG)-Q_{bone}
VLC	0.025 L/kgBW	a, b	Constant for liver volume
VKC	0.0042 L/kgBW	a, b	Constant for kidney volume
VBC	0.14 L/kgBW	a, b	Constant for bone volume
VBLC	0.073 L/kgBW	a, b	Constant for blood volume
V_{liver}	1.75 L	a, b	Volume of liver = VLC*BW
V_{kidney}	0.29 L	a, b	Volume of kidney = VKC*BW
V_{bone}	9.8 L	a, b	Volume of bone = VBC*BW
V_{blood}	5.11 L	a, b	Volume of blood = VBLC*BW
V_{plasma}	2.81 L	a, b	Volume of plasma (55% of V_{blood})
V_{wp}	1.96 L	a	Volume of well-perfused tissues (wp) = (BW^0.7)*0.1
V_{pp}	58.24 L	a	Volume of poorly-perfused tissues (pp) = BW-V_{wp}-V_{bone}
P_{liver}	100	a, b	Partition coefficient, liver/plasma
P_{kidney}	100	a, b	Partition coefficient, kidney/plasma
P_{wp}	100	a, b	Partition coefficient, well-perfused tissues/plasma
P_{pp}	20	a, b	Partition coefficient, poorly-perfused tissues/plasma
P_{bone}	1000	a, b	Partition coefficient, bone/plasma
Transfer rates used in this study			
Rate	*Units*	*Calculation / value*	
K12	day^{-1}	$Q_{liver}/(V_{liver}*P_{liver})$	
K21	day^{-1}	Q_{liver}/V_{plasma}	
K23	day^{-1}	Q_{kidney}/V_{plasma}	
K32	day^{-1}	$Q_{kidney}/(V_{kidney}*P_{kidney})$	
K24	day^{-1}	Q_{bone}/V_{plasma}	
K42	day^{-1}	$Q_{bone}/(V_{bone}*P_{bone})$	
K25	day^{-1}	Q_{wp}/V_{plasma}	
K52	day^{-1}	$Q_{wp}/(V_{wp}*P_{wp})$	
K26	day^{-1}	Q_{pp}/V_{plasma}	
K62	day^{-1}	$Q_{pp}/(V_{pp}*P_{pp})$	
A_{gi}	unitless	0.06-0.12[c]	
eU	day^{-1}	0.47[d]	
eB	day^{-1}	0.2[d]	

[a]O'Flaherty (1991), [b]O'Flaherty (1993), [c]Rabinowitz et al. (1976), [d]Morisawa et al. (2001)

Part I5: Nickel PBTK model equations and parameters

Gut: $\frac{dAMT_{gut}}{dt} = -Kf - \left(K1 \times AMT_{gut}\right) - \left(\rho K1 \times AMT_{gut}\right)$

Serum: $\frac{dAMT_{serum}}{dt} = Kf + (K1 \times AMT_{gut}) + (K21 \times AMT_{tissues}) - (K12 \times AMT_{serum}) - (eU \times AMT_{serum})$

Urine: $\frac{dAMT_{urine}}{dt} = (eU \times AMT_{serum})$

Tissues: $\frac{dAMT_{tissues}}{dt} = (K12 \times AMT_{serum}) - (K21 \times AMT_{tissues})$

Faeces: $\frac{dAMT_{faeces}}{dt} = \left(\rho K1 \times AMT_{gut}\right)$

Where,
AMT is the mass of Ni in compartments.
The absorbed fraction of Ni dose in the gut (A_{gut}) was 0.011, as determined by Sunderman et al. (1989). The daily rate constant for faecal excretion of unabsorbed Ni in dose was expressed as ρK1, and ρ was calculated as (1-A_{gut})/A_{gut}.

Table I5: Physiologically based parameters for the Ni PBTK model

Nickel			
Parameter Symbol	**Parameter Value**	**Reference**	**Description**
A_{gut}	0.7 ± 0.4 %	a	Mass fraction of Ni dose absorbed from the gut
	2.95 ± 1.32 %	b	
K1	0.33 ± 0.24 /hr	a	Alimentary absorption of Ni from ingested dose
Kf	$1.05*10^{-4}$ ± $3.6*10^{-5}$ mg/hr	a	Alimentary absorption of Ni from dietary (baseline) Ni intake (*pseudo-zero order*)
eU	0.15 ± 0.11 /hr	a	Urinary elimination rate of nickel
K12	0.37 ± 0.34 /hr	a	Nickel transfer rate from serum to tissues
K21	0.1/hr	a	Nickel transfer rate from tissues to serum

[a]Sunderman et al. (1989), [b]Nielsen et al. (1999)

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Appendix J: Outputs of Selected Simulations

Part J1: Selected simulations showing predicted blood Pb concentrations

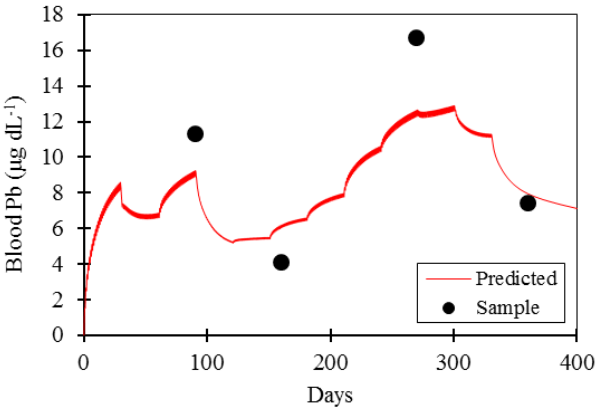

Fig. J1-A: Predicted blood Pb concentration for participant P04. Daily intake rate (IR, in µg day^{-1}) of Pb was calculated using the participant's produce consumption rates. IR was determined for each month, ranging from 20 to 154 µg day^{-1}. Measured blood Pb concentrations are also indicated.

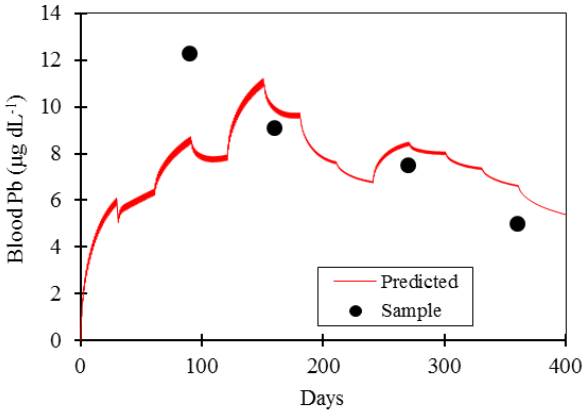

Fig. J1-B: Predicted blood Pb concentration for participant P09. IR for each month ranged from 17 to 150 µg day^{-1}. Concentrations of Pb in blood samples are indicated.

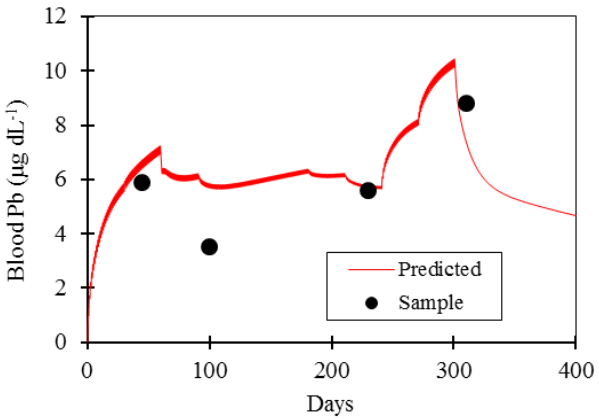

Fig. J1-C: Predicted blood Pb concentration for participant P10. IR for each month ranged from 30 to 120 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

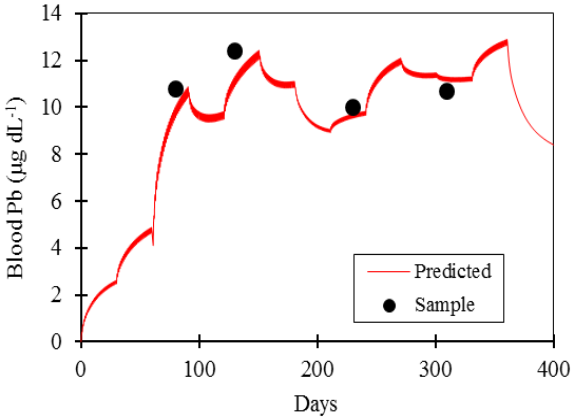

Fig. J1-D: Predicted blood Pb concentration for participant P11. IR for each month ranged from 40 to 186 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

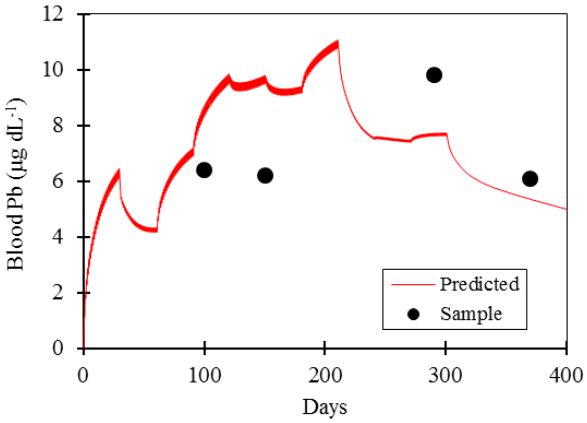

Fig. J1-E: Predicted blood Pb concentration for participant P15. IR for each month ranged from 22 to 137 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

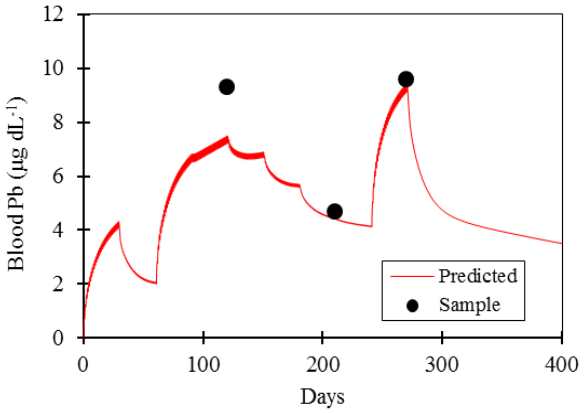

Fig. J1-F: Predicted blood Pb concentration for participant P17. IR for each month ranged from 10 to 120 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

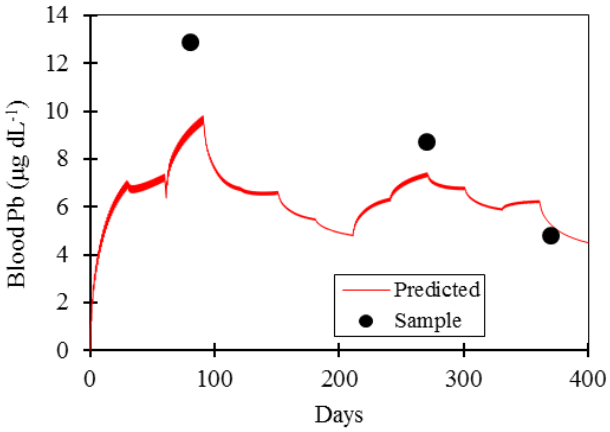

Fig. J1-G: Predicted blood Pb concentration for participant P22. IR for each month ranged from 10 to 139 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

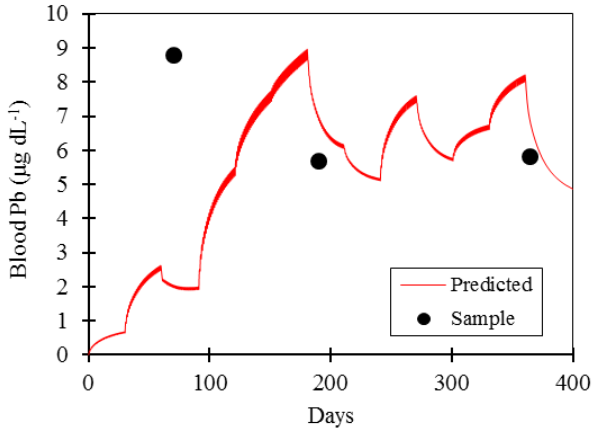

Fig. J1-H: Predicted blood Pb concentration for participant P23. IR for each month ranged from 10 to 115 µg day^{-1}. Measured Pb concentrations in blood samples are indicated.

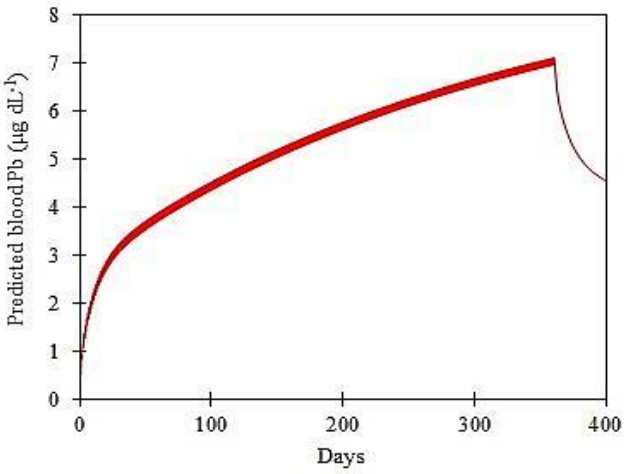

Fig. J1-I: Predicted blood Pb concentration for participant P01, simulated using the average daily Pb intake rate (IR) of 50 µg day^{-1}. Continuous daily exposure was simulated for a period 1 year.

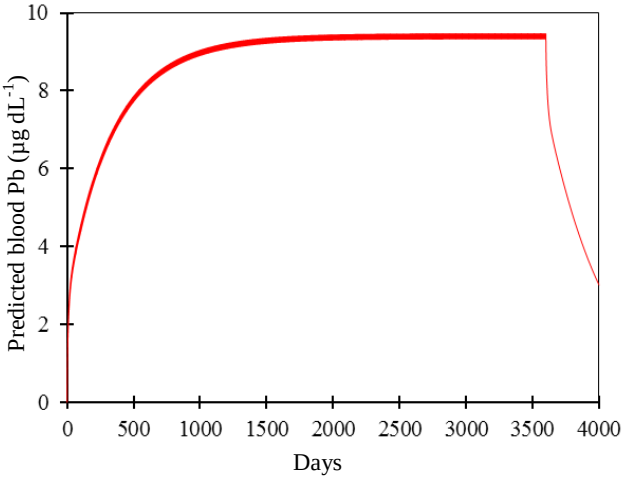

Fig. J1-J: Predicted blood Pb concentration for participant P01, simulated using the average daily Pb intake rate (IR) of 50 µg day^{-1}. Continuous daily exposure was simulated for a period 10 years. Predicted blood Pb becomes steady at 9.4 µg dL^{-1} after about 1500 days (~ 4 years) of continuous daily exposure.

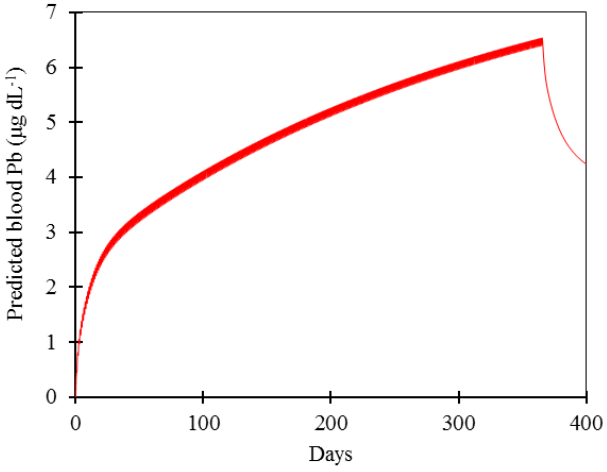

Fig. J1-K: Predicted blood Pb concentration for participant P15, simulated using the median daily Pb intake rate (IR) of 45 µg day^{-1}. Continuous daily exposure was simulated for a period 1 year.

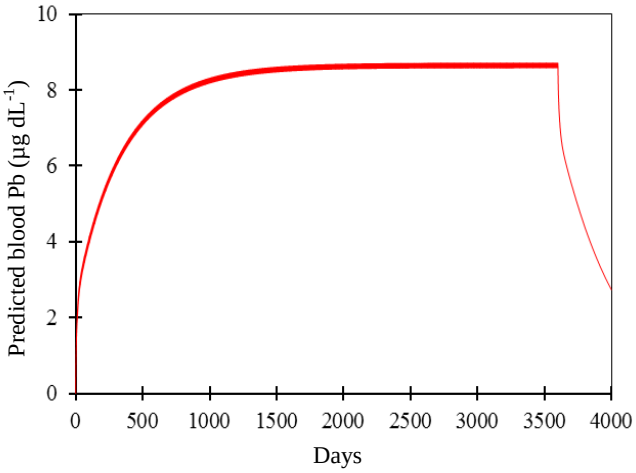

Fig. J1-L: Predicted blood Pb concentration for participant P15, simulated using the median daily Pb intake rate (IR) of 45 µg day^{-1}. Continuous daily exposure was simulated for a period 10 years. Predicted blood Pb becomes steady at 8.6 µg dL^{-1} after about 1500 days (~ 4 years) of continuous daily exposure.

Part J2: Selected simulations of inorganic As in urine

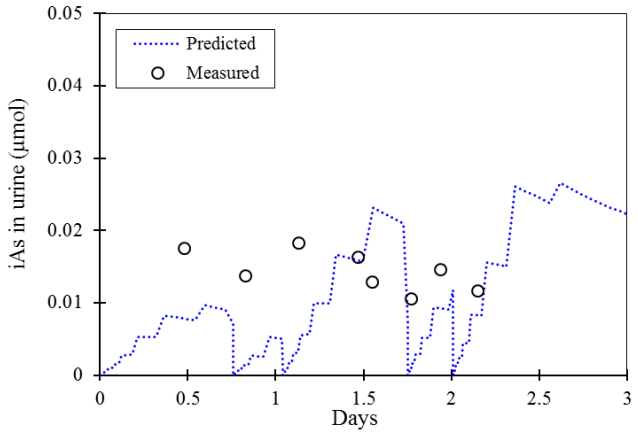

Fig. J2-A: Predicted and measured iAs in urine for participant P12. Simulated doses of iAs varied between 0.1 and 1.5 µmol.

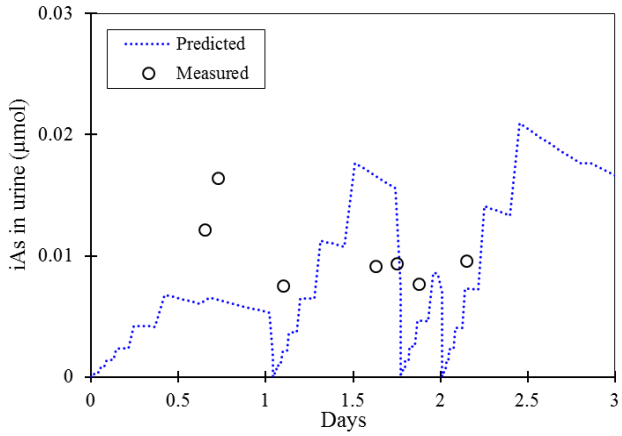

Fig. J2-B: Predicted and measured iAs in urine for participant P13. Simulated doses of iAs varied between 0.6 and 1.2 µmol.

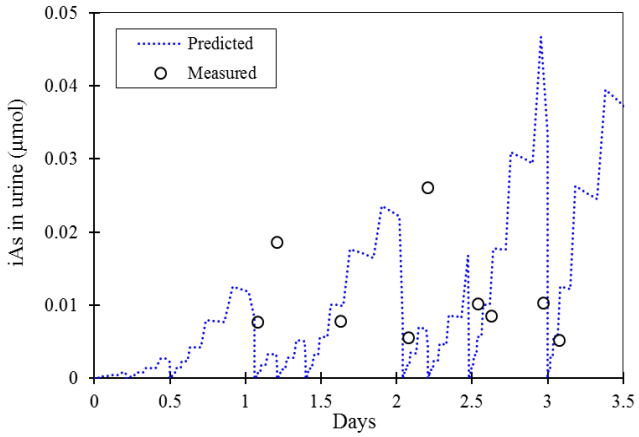

Fig. J2-C: Predicted and measured iAs in urine for participant P15. Simulated doses of iAs varied between 0.2 and 2.5 µmol.

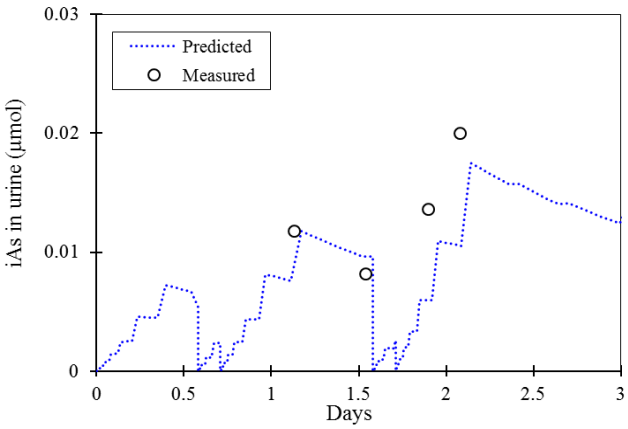

Fig. J2-D: Predicted and measured iAs in urine for participant P22. Simulated doses of iAs varied between 0.1 and 0.9 µmol.

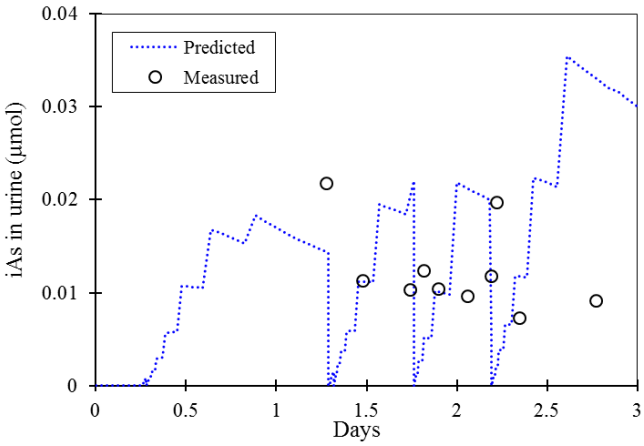

Fig. J2-E: Predicted and measured iAs in urine for participant P25. Simulated doses of iAs varied between 0.01 and 1.6 µmol.

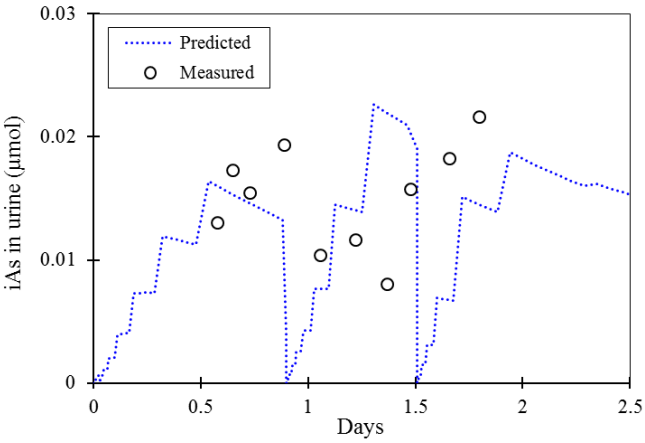

Fig. J2-F: Predicted and measured iAs in urine for participant P26. Simulated doses of iAs varied between 0.1 and 1.5 µmol.

Part J3: Summary of output of Cd simulations and measured Cd in urine

Participant ID	Calculated Cd doses (µg day^{-1})[a]	Simulated Exposure Frequencies (years)[b]	Predicted Cd in Urine (µg L^{-1})	Median of measured Cd in Urine (µg L^{-1})[c]
P01	8.3	20	0.12	0.117
P02	7	20	0.08	0.101
P03	2.2	1	0.003	0.015
P04	1.4	2.5	0.003	0.020
P05	7.8	20	0.1	0.121
P07	1.8	2	0.004	0.020
P08	5.5	3	0.015	0.037
P09	7.1	3.5	0.021	0.053
P10	9.7	17	0.1	0.123
P11	10.4	17	0.11	0.132
P12	9.8	20	0.12	0.154
P13	6	20	0.07	0.068
P15	3.8	8	0.022	0.042
P16	5.8	4	0.02	0.037
P17	7	4	0.023	0.020
P18	6.4	2	0.013	0.042
P19	3.5	20	0.041	0.037
P20	2.4	1.5	0.004	0.042
P21	3.8	20	0.044	0.073
P22	3.9	20	0.045	0.033
P23	4.5	30	0.07	0.051
P24	6.5	30	0.1	0.088
P25	4.8	19	0.054	0.044
P26	6.4	19	0.1	0.077
P28	6.1	0.2	0.005	0.018
P29	9.3	5	0.04	0.055
P31	4.3	1	0.002	0.018
P32	8	6	0.036	0.057
P33	7.3	7	0.04	0.046
P34	1.4	17	0.015	0.051
P35	2.2	17	0.023	0.040
P36	4.5	0.2	0.004	0.033
P38	1.5	1.5	0.003	0.018
P39	2.6	6	0.012	0.022

[a]A participant's daily intake rates (IR) were averaged over 12 months to obtain a representative annual IR value used as the dose. Excludes extra data collected over the 2 to 3 consecutive days.

[b]Based on the reported number of years a participant has been using their allotment.

[c]For urinary concentrations ≥LOD.

Part J4: Selected simulations of Cr(III) in urine

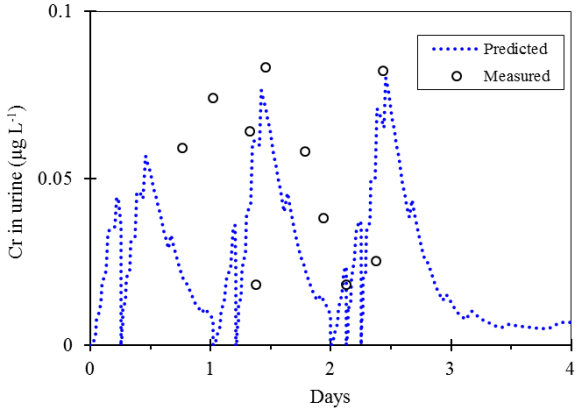

Fig. J4-A: Predicted and measured Cr(III) in urine for participant P09. Simulated doses varied between 42 and 72 µg Cr(III).

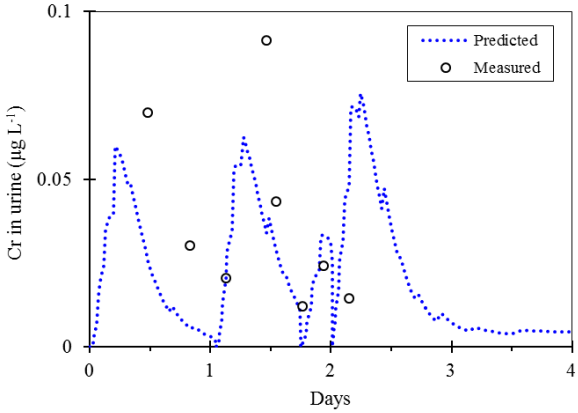

Fig. J4-B: Predicted and measured Cr(III) in urine for participant P12. Simulated doses varied between 45 and 86 µg Cr(III).

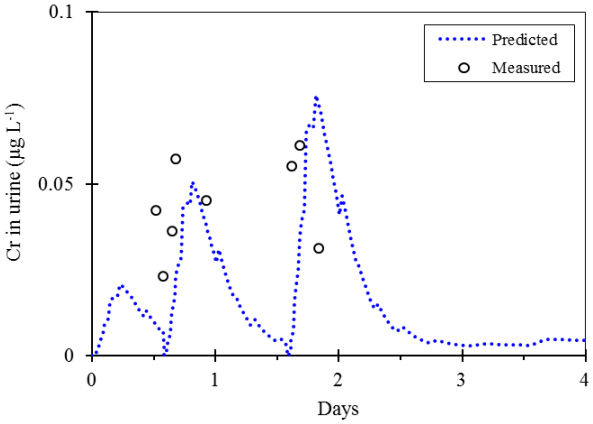

Fig. J4-C: Predicted and measured Cr(III) in urine for participant P21. Simulated doses varied between 25 and 92 µg Cr(III).

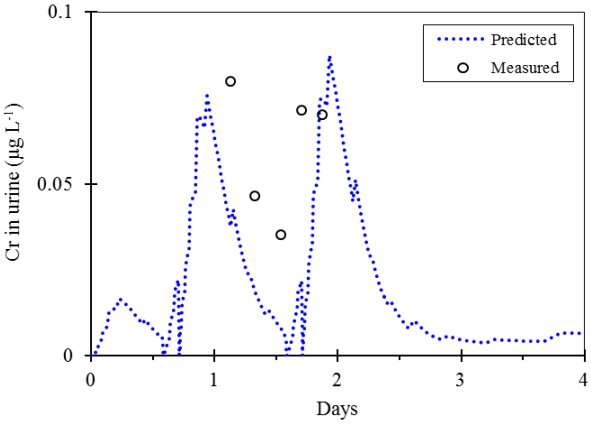

Fig. J4-D: Predicted and measured Cr(III) in urine for participant P22. Simulated doses varied between 25 and 86 µg Cr(III).

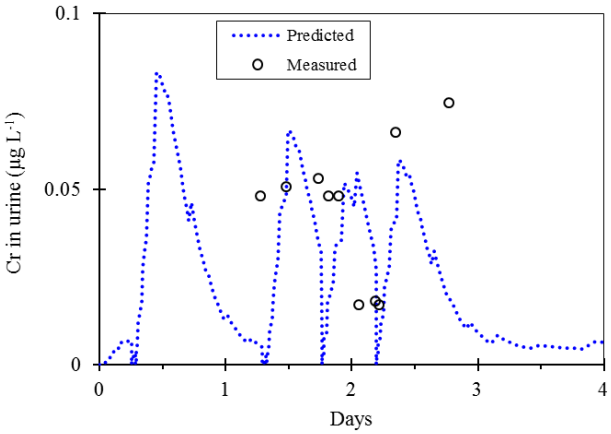

Fig. J4-E: Predicted and measured Cr(III) in urine for participant P25. Applied doses varied between 11 and 83 µg Cr(III).

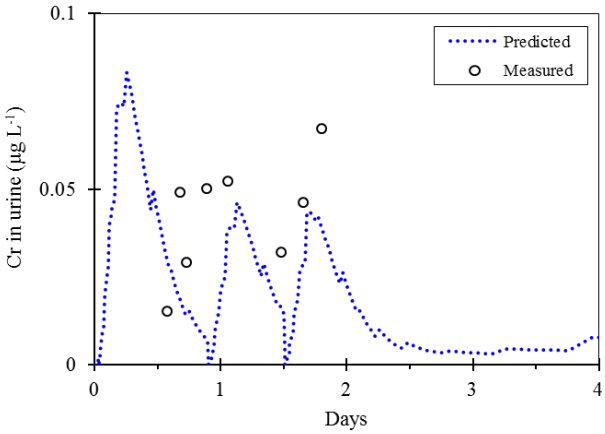

Fig. J4-F: Predicted and measured Cr(III) in urine for participant P26. Applied doses varied between 48 and 68 µg Cr(III).

Part J5: Selected simulations of Ni in urine

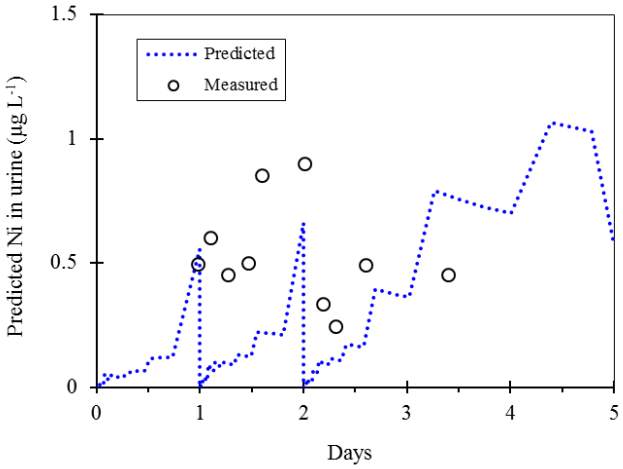

Fig. J5-A: Predicted and measured Ni in urine for participant P01. Simulated doses varied between 50 and 90 µg Ni.

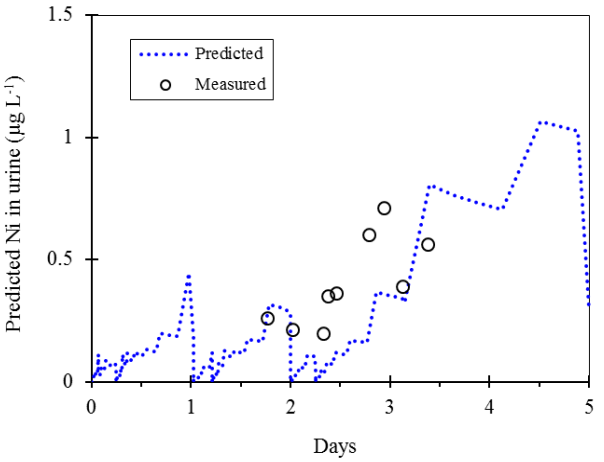

Fig. J5-B: Predicted and measured Ni in urine for participant P09. Simulated doses varied between 12 and 102 µg Ni.

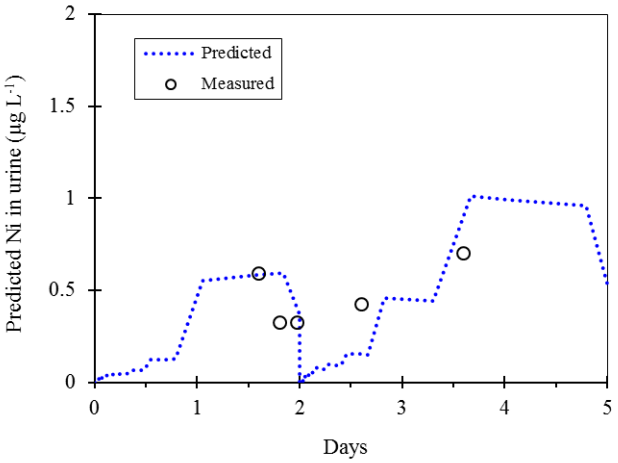

Fig. J5-C: Predicted and measured Ni in urine for participant P10. Simulated doses varied between 83 and 100 µg Ni.

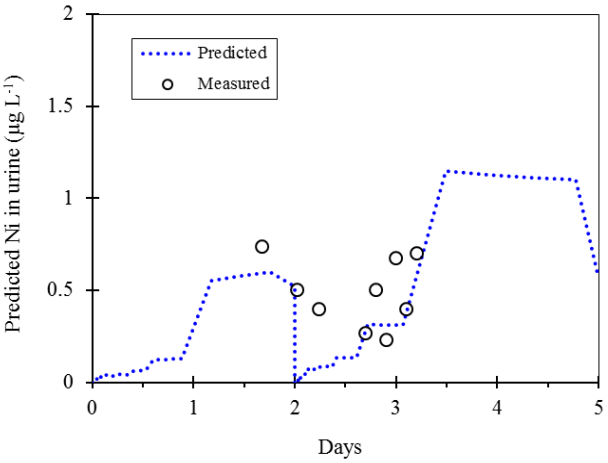

Fig. J5-D: Predicted and measured Ni in urine for participant P11. Simulated doses varied between 80 and 100 µg Ni.

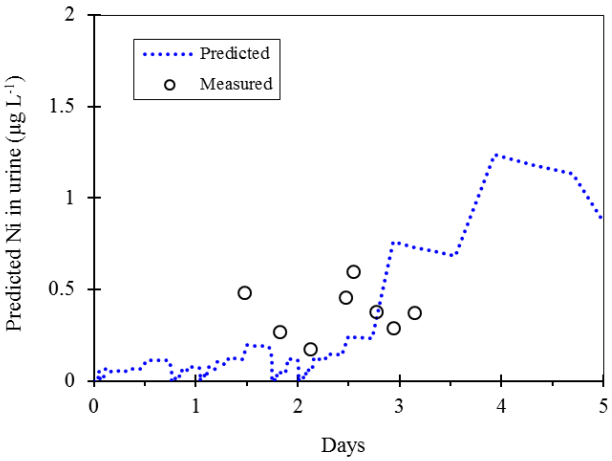

Fig. J5-E: Predicted and measured Ni in urine for participant P12. Simulated doses varied between 32 and 155 µg Ni.

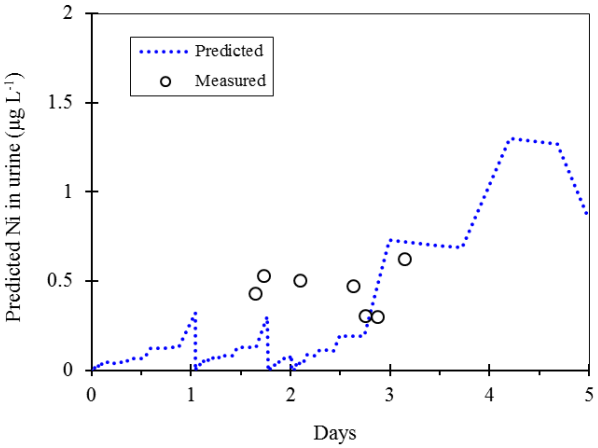

Fig. J5-F: Predicted and measured Ni in urine for participant P13. Simulated doses varied between 17 and 105 µg Ni.