University of Reading

DEVELOPING A SUSTAINABLE METHOD FOR THE DETERMINATION OF BIOACCESSIBILITY OF ORGANIC CONTAMINANTS FROM POLLUTED SITES

Thesis submitted for the Degree of Doctor of Engineering (EngD)

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.

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Abstract

The United Kingdom has an abundance of brownfield sites which can play a pivotal role in addressing the ever growing crisis of supply in the housing sector. However, many otherwise attractive sites are afflicted by the legacy pollutants of former industrial activities. Historically, contaminated land has been viewed as an issue which can only be addressed in the bluntest of ways. A contaminant is present, therefore risk is present, and developers either seek remediation or choose to build on a greenfield alternative. The result is an approach that sees brownfield land as a burden rather than opportunity. This project aims to explore other approaches. Bioaccessibility is increasingly being seen as a viable method for assessing contaminant risk in humans, as part of an approach that is more physiologically relevant and less conservative than traditional methods, with methods such as the Unified Bioaccessibility Method (UBM), human gastrointestinal based bioaccessibility extraction method, gaining increasing traction and acceptance as a viable testing method for the determination of bioaccessibility in inorganics such Arsenic, Lead, Zinc and Cadmium.

However, currently no standard method exists for the determination of bioaccessibility in organic contaminants, despite the development of several methodologies designed to assess bioaccessibility in these compounds. In the following thesis, oral bioaccessibility in PCBs is assessed using the FOREhST (Fed ORganic Estimation human Simulation Test) and CE-PBET (Colon Extended Physiologically Based Extraction Test) methods in 34 industrially contaminated soils. $\sum^{ICES\ 7}$ (the International Council for the Exploration of the Sea designated indicator congeners) bioaccessibility was recorded as 39.63% using the FOREhST method, though it was found that PCB 180 was consistently underestimated due to a saponification step included in the protocol. CE-PBET resulted in a significantly lower $\sum^{ICES\ 7}$ bioaccessibility of 15.21%. Although results varied, both methods demonstrated that the total contaminant approach is overly-conservative in the assessment of PCB contaminated soils.

As part of the thesis, a survey of PCB concentrations in the urban soils Central London was completed. The survey demonstrated low background levels of PCBs (15.1 μg/kg) dominated by isolated hotspots of elevated concentration (148.7 μg/kg), which may be attributed to re-emission events.

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CONFERENCE ATTENDANCE AND OUTREACH

- 7th International Workshop on Chemical Bioavailability, British Geological Survey, Nottingham, UK. 3-6th November, 2013. Poster presentation.
- Society of Brownfield Risk Assessment (SoBRA) Christmas Conference 2013, Royal Society of Chemistry, London, UK. 17th December, 2013. Poster presentation.
- Society of Environmental Geochemistry and Health (SEGH) 2014 Conference, Northumbria University, Newcastle upon Tyne, UK. 30th June – 4th July. Poster presentation.
- TSBE Annual Conference 2014, Henley Business School, Reading, UK. 8th July, 2014. Oral presentation and short paper.
- NERC Environment YES workshop, Old Trafford, Manchester, UK. 16th-17th October, 2014. Elevator pitch, group 'Dragon's Den' presentation and business pitch.
- Society of Brownfield Risk Assessment (SoBRA) Christmas Conference 2014, Royal Society of Chemistry, London, UK. 17th December, 2014. Poster presentation.
- 3rd UK and Ireland Exposure Science Meeting, Imperial College London, London, UK. 24th April, 2015. Poster presentation.
- TSBE Annual Conference, 2015, Henley Business School, Reading, UK. 8th July, 2015. Poster presentation and short paper.
- Society of Brownfield Risk Assessment (SoBRA) Summer Workshop: Uncertainty in Human Health Risk Assessment, North East Institute of Mining and Mechanical Engineering, Newcastle upon Tyne, UK. 15th July, 2015. Workshop facilitator/ leader.
- Scottish Contaminated Land Forum: 6th Annual Conference on the Advances in Land Contamination Assessment and Remediation, University of Strathclyde, Glasgow, UK. 16th September 2015. Poster presentation.
- 8th International Workshop on Chemical Bioavailability, University of Nanjing, Nanjing, China. 18th – 21st October, 2015. Oral presentation.
- 4th UK and Ireland Exposure Science Conference, Health and Safety Laboratories, Buxton, UK. 20th April 2016. Oral presentation.
- Ecobuild 2017, ExCeL, London. 9th March 2017. Oral Presentation.
- 5th UK Ireland Exposure Science Conference, University of Birmingham, Birmingham, UK. 29th March 2017. Poster presentation.

CHAPTER 1: INTRODUCTION

1.1 A refocusing on sustainability in remediation

It is estimated that some 15,000 ha of UK land is affected by contamination. Remediation allows for the rehabilitation of neglected, former industrial sites into renewed, safe environments. Pressure to preserve greenfield land, and to improve the sustainability and functionality of city regions, alongside a growing drive to provide more suitable land for business and housing, has led to a growing industry and government focus onto brownfield sites. Remediation techniques in the contaminated land sector are well established. Frequently these methods are energy intensive, costly and lengthy. However, in order to address the potential harm presented by the presence of contaminants in former industrial sites, such measures are essential. Remediation often requires the removal of contaminated material off site for disposal in landfill. Such activities contribute to elevated transport emissions, congestion, disturbance to local residents and the movement of problem material instead of eradication. In addition to the monetary costs of remediation, these measures act to dissuade the development and rehabilitation of contaminated land, with the consequence of additional pressure on greenfield spaces, and the prolonged blight of extensive, unsightly, potentially dangerous and extensive tracts of underused land in urban environments. This is exacerbated by the local impacts of longer commutes and additional traffic caused by the siting of residential and commercial sites on the edges of towns, when brownfields are frequently located in urban regions with convenient access to existing infrastructure.

A growing focus on sustainable remediation, including the development of novel *in situ* techniques, including the re-use of material on site, allowing the reduction of offsite movements and waste disposal, have improved the sustainability credentials of remediation activities. However, the complexity and cost of remediation is still seen as a barrier to brownfield development.

Bioaccessibility testing represents an opportunity to redefine afflicted sites through a physiologically relevant assessment, based on the proportion of a substance which is mobilised from soils, rather

than a total contaminant concentration assessment. Although robust methods have been developed, acceptance of bioaccessibility in contaminated land assessment faces barriers. This is particularly the case in terms of methods designed specifically for the assessment of persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), where acceptance of a standard methodology is absent. The need for reliable bioaccessibility methods for POPs is prescient due to their ubiquity in industrial and urban soils, environmental persistence and significant human health impact. There is a need for greater research into bioaccessibility of such organic compound groups, included the lengthening list of emerging organic contaminants (EOCs).

The methods investigated in this work aim to simulate the conditions within the human gastro-intestinal tract (GIT) using lab based tests, allowing for the calculation of oral bioaccessibility, a concept key to the understanding of the soil ingestion exposure pathway, which is of particular significance in residential land management due to the risk of accidental exposure to contaminated soils or intentional consumption through pica behaviour. The methods applied, CE-PBET (Colon Extended Physiologically Based Extraction Test) and FOREhST (Fed ORganic Estimation human Simulation Test), have been successfully applied to the measurement of bioaccessibility in PAH afflicted soils. This work investigates their application to PCBs, in order to widen the application of the tests.

The problems of contaminated land management and remediation are associated with the legacy of former industrial operations, regulation of known contaminants in use, and future emerging substances of concern. PCBs are typically considered a legacy pollutant, though re-emission and environmental persistence requires ongoing research and regulation. Bioaccessibility and bioavailability are fundamental concepts that are key to our understanding of soil health and sustainability, particularly in the built environment, and help us to better understand the linkages between human health and soil.

Researchers (Vane *et al.*., 2011; Ludwig and Steffen, 2017; Mineau, 2017; Zalasiewicz *et al.*., 2017) have suggested that Earth has entered the Anthropocene, an epoch dominated by mankind's influence on the natural world, typified by impacts on biodiversity, climate and geochemistry. Better understanding of the human health impacts of growing geochemical challenges is essential for long term management of soils and the societies which depend on them, and has never been more prescient.

1.2 Industrial context

The fields of bioaccessibility and bioavailability are defined by academic research, which has led to a growing understanding of the interactions between contaminated land and human health. However, the application of methods is dependent on interactions between academia, industry, business and regulatory bodies. The Engineering Doctorate (EngD) allows additional scope for greater understanding of these interactions. Study at the Technologies for Sustainable Built Environments (TSBE) Centre frames these academic issues as challenges for sustainability in the built environment.

EngD projects represent a linkage between academic institutions and industry sponsors, who work together to identify potential areas for applied and impactful research. Alongside the development of a PhD equivalent thesis, the EngD programme is designed to place emphasis on researchers as engineering professionals, and candidates focus on the application of research within the commercial and industrial sectors. The TSBE Centre achieves this through taught modules themed around sustainability, industry, construction standards and business.

1.2.1 Sponsorship organisation

The work presented in the following thesis was conducted in partnership between the University of Reading and the British Geological Survey (BGS). Both the University and BGS have a long and established record of groundbreaking and influential work in the field of bioaccessibility. The BGS maintain close relationships with the Bioaccessibility Research Group of Europe (BARGE), an organisation dedicated to the promotion of bioaccessibility research and practice, and aims to bring together institutions and researchers with an interest in bioaccessibility research (BARGE, 2016). Dr

Mark Cave, one of the industrial supervisors of this project, is the Chairman of BARGE, and Chris Collins, the lead academic supervisor, is a prominent member of the BARGE group, and has worked on the validation of the Environment Agency CLEA model, whilst maintaining directorship of the University of Reading's Chemical Analysis Facility.

1.3 Statement of project aims

Bioaccessibility testing is gaining increasing attention as a specialised method to measure the impact of contaminants, in soil and other media, on human health. In recent years, many such methods have been developed to assess contaminant bioaccessibility in soils affected by inorganic contaminants, such as mercury, lead, and chromium. The impacts of such methods have been felt not only in academia, but are beginning to play a role in risk assessment and policy, and have been proposed as a more physiologically relevant approach than total contaminant concentration derived assessment. Recent developments have led the field to expand into the area of organic geochemistry, with methodological developments such as the CE-PBET and FOREhST models showing promising results in PAH bioaccessibility. Despite this direction in the field, literature reveals an opportunity to gain a greater understanding of organic compound bioaccessibility, particularly in compound groups other than PAHs, such as brominated flame retardants, PCBDs and PCBs. Specifically; opportunities for greater research into the application of such methods, and their impact on sustainable city development have been identified. The results of this project will explore the possibilities of bioaccessibility testing as a weapon in the toolkit of assessors, and how the application of these methods can lead to a greater re-use of brownfield sites through a greater understanding of human interaction with soil-borne contaminants, leading to healthier, more sustainably designed and managed cities.

The aims of this project remain wide, but to summarise in a single sentence, the question this project aims to address is:

"*To what extent can bioaccessibility testing address contamination by organic pollutants in urban soils, and can it play a role in the development of a more sustainability-led redevelopment programme?*"

1.3.1 Research objectives

The aims described above will be fulfilled through the following research objectives:

1. Establish the current research level in the field of bioaccessibility in order to identify the issues to address, and any potential gaps in knowledge.
2. Compare the performance of the CE-PBET and FOREhST methods in the assessment of bioaccessibility in soil-bound PCBs.
3. Establish a background survey of PCBs within a large urban area, and establish a typical PCB profile, identifying any sources.
4. Apply a bioaccessibility testing approach to soil samples selected in objective 3, thus demonstrating the application of such methods in a real-world environment.
5. Explore the potential impacts, both financially and environmentally, of bioaccessibility testing of organic contaminants in soil, and how bioaccessibility assessment methods can lead to a more sustainable land-use regime.

These aims and objectives will be explored throughout the following thesis. Through the establishment of these objectives, it is hoped that a holistic approach can be followed, one that explores the problem of PCB contamination in soil, oversees the development of methodologies to assess the problem, and explores potential impacts.

1.4 Structure of thesis

This thesis is presented in 7 chapters. Following the introductory chapter, 5 chapters are presented in order to address the identified project aims and research objectives, followed by the overall conclusions.

The initial chapter following this introduction is Chapter 2, the preliminary literature review, which seeks to review the current state of knowledge in the field and explore the nature of the problem. This is presented in accordance with Objectives 1 and 5. Literature and further themes are additionally explored through further chapters.

Chapter 3 presents a survey of PCB concentrations in Central London, and is presented in accordance with Objective 3. In this chapter, the distribution and typical PCB profile of a large urban area are explored. This includes the investigation of potential sources of PCBs, and spatial dependence.

Chapter 4 presents PCB bioaccessibility data obtained through the application of the FOREhST methodology to a suite a 34 industrially contaminated soils. This chapter explores differences in bioaccessibility observed between congeners observed using the FOREhST method in preparation of comparison with the CE-PBET derived bioaccessibility data. This chapter is presented in accordance with Objective 2

Chapter 5 presents the results of bioaccessibility testing performed on a subset of 7 of the soils analysed in Chapter 4. These soils were selected on the basis of ∑ICES 7 PCB concentration, and the availability of limited bioavailability data collected as part of a parallel *in vivo* bioavailability study. CE-PBET data is explored alongside FOREhST data, including the investigation of heptachlorinated PCB loss through saponification, which was identified as part of Chapter 4. This chapter contributes to Objective 3.

Chapter 6 presents a discussion of the potential financial, environmental and sustainability associated impacts of bioaccessibility testing, and is presented in accordance with Objective 5.

The thesis is concluded in Chapter 7, with a summary of impacts, contributions and recommendations are presented. This chapter is presented within the context of the reseach aims and objectives identified in Chapter 1.

CHAPTER 2: APPROACHES TOWARDS CONTAMINATED LAND

This chapter aims to provide an overview of current research and policy. A survey of existing bioaccessibility methods is provided, alongside a thorough review of PCB-specific methods, presented as part of a wider discussion of the nature and challenges posed by PCB contamination.

2.1 Brownfield land, contaminated land and the remediation process

An increased pressure for available urban land, along with a movement towards a more sustainable approach to the re-use of land, has led to a focusing on former industrial sites as an opportunity for development (Cheng *et al.*., 2016; Morillo and Villaverde, 2017). As cities in the UK have become more extensive, the make-up of our urban landscape has changed to reflect the de-industrialisation of our economy. Formerly remote industrial sites are becoming surrounded by modern development (Foucault *et al.*., 2013), and have become prime candidates for redevelopment. The re-use of so-called 'brownfield' sites is paramount to the sustainable development goals of UK planning policy (Dixon *et al.*., 2011), and is actively encouraged through planning policy development and financial incentives for developers (Environment Agency, 2002), in preference to greenfield alternatives (Thornton *et al.*., 2007), often located with ready access to existing urban infrastructure and destinations (Bardos *et al.*., 2016a).

The redevelopment process for a brownfield site has been compared to that of the life-cycle of an industrially produced product, with the ecological and environmental costs of redevelopment, including the disposal of waste rubble and off-site clean-up of contaminated land factored and accounted for (Schrenk, 2002). This view is particularly applicable to the redevelopment of contaminated sites, as it allows for the calculation of the complete environmental and economic cost of contaminated land remediation and subsequent disposal. Disposal of contaminated material is largely conducted through off site removal and landfill (Rivett *et al.*., 2002), a process with

significant environmental and financial costs (Barrieu *et al.*., 2017), although recent developments have seen a growth in *in situ* methods including re-use of material on site and bioremediatory techniques (Hartley *et al.*., 2012; Chen *et al.*., 2015; Song *et al.*., 2015; Lefevre *et al.*., 2016; Song *et al.*., 2017), which are designed with sustainable remediation in mind (Favara and Gamlin, 2017; Huysegoms and Cappuyns, 2017).

Ultimately, the costs of remediation are passed onto the party deemed responsible for the source of contamination, or if unavailable, the current property owner (Environment Agency, 2009a). A holistic view such as this is useful, particularly when dealing with a site deemed to contain contaminated land and subject to potentially costly remediation. As assessments such as these could have potentially significant effects on the value, and development potential of brownfield sites, it is essential that the assessment of remediation cost is as accurate as possible. Contaminated land takes up a significant proportion of commercially available brownfield land, with an estimated 15,740 hectares affected, out of 63,750 hectares of brownfield land in 2008, with estimated clean-up costs of between £100,000 to £325,000 per ha (NERC and DTZ, 2009). However, land may be deemed contaminated without fitting the definition of 'brownfield' or 'derelict', though there is a greater likelihood of soil contamination where there has been a legacy of contaminant use or production.

2.2 Persistent organic pollutants

This project will focus on the assessment of bioaccessibility in persistent organic pollutants (POPs), a group of compounds principally associated with industrial activities described as a priority for elimination by the 2001 Stockholm Convention. The list of affected compounds and compound groups is not static, and is frequently supplemented with emerging contaminants. However, key, prominent compound groups are found, including DDT, PAHs and PCBs.

Many POPs are associated with heavy industrial practices, as a consequence of their on-site use, storage or manufacture. PAHs are closely associated with sites with a history of heavy industrial activity, such as former gasworks (Brown and Peake, 2006), coking plants (Smith *et al.*., 2006), petrochemical exploitation (Boitsov *et al.*., 2009), coal powered electricity generation (Lewtas, 2007) and the aluminium, iron and steel industries (Boffetta *et al.*., 1997). Railway facilities are associated with contamination from PAHs and PCBs (Department of Environment and British Railways Board, 1995) through on site use of oils, lubricants and the legacy of coal and diesel fuel use. Petrol stations and street side pollution have been identified as key sources of petrogenic and pyrogenic PAHs with a potentially wide-ranging impact across urban environments (Aichner *et al.*., 2007). Similarly, contamination by pyrogenic compounds, including PAHs, can be identified in garden and allotment soils. This can pose a unique risk if vegetables are grown in contaminated soil and later consumed (Fismes *et al.*., 2002; Samsøe-Petersen *et al.*., 2002).

2.3 Spatial distribution of contaminants

Sources of pollution are varied, and pollutant compounds are subject to distribution through dispersion, and may be identified remotely from their point of origin (Aichner *et al.*., 2007; Lehndorff and Schwark, 2009), depending on emission source, prevailing weather conditions, regional morphology (Lehndorff and Schwark, 2009) and physiochemical makeup of the pollutant compounds (Meharg *et al.*., 1998; Yamada *et al.*., 2003).

Spatial dependence is present in many types of geochemical measurements, it is typical for adjacently collected samples to show a greater degree of dependence than those collected a greater distance apart (Myers *et al.*., 1982). Methods, such as variogram analysis, have been developed in the field of geostatistics that can enable the researcher to interrogate data in terms of spatial dependence and potential spatial or temporal correlation (Rossi *et al.*., 1992; Gringarten and Deutsch, 1999). Such analysis can aid in the interpretation of environmental characteristics and interactions between environmental components including geochemical surveys (Yost *et al.*., 1982).

The variogram technique represents a key step in the assessment of the spatial structure of environmental data (Bogaert and Russo, 1999).

A key indicator of spatial dependence provided by the variogram technique is the range value. Sample locations separated by distances less than the calculated range spatially autocorrelated, those separated by distances greater than the range value are not (Esri, 2017).

Also of note is the role of intentional transport of pollutants following remediation of contaminated sites. Since off-site removal of contaminated material and disposal in landfill is the most prevalent method of remediation in UK contaminated sites (Rivett *et al.*., 2002; Environment Agency, 2007a), there is a possibility of transfer of pollutants, including PAHs and PCBs, into the surrounding matrix of the landfill following disposal, and possible leaching into the surrounding environment (Han *et al.*., 2013). There is also a higher risk of contaminant mobilisation through disturbance and subsequent volatilisation from the surrounding soil matrix, particularly in the case of low molecular weight compounds.

2.4 Pollutant linkages

In order to pose a risk to human health, compounds require a method, or methods, to transfer to the point at which they are considered harmful. As discussed, this may initially take the form of distribution through airborne particles, or it may be from direct contact with a contaminated soil or water matrix. Ingestion may be through inhalation, dermally through the skin or absorption in the gut. Removal of these linkages, or pathways, neutralises the immediate threat from the contaminant and can be considered an effective form of contaminant remediation. By definition, persistent organic contaminants are long lived. The lipophilic character of potentially harmful compounds such as PAHs mean they have a tendency to bioaccumulate in plant and animal tissues, travelling along the food chain. As such, they have a clear pathway to absorption in the gut, where they are able to accumulate within adipose body tissue (Geyer *et al.*., 1987). Crops grown in PAH contaminated soil show no reduction in plant growth, but show elevated levels of heavy weight PAH absorbed from

the soil matrix (Fismes *et al.*., 2002). This presents a risk of transfer of contaminants to the gut, despite there being no evidence of contamination from examination of the plants. In addition to this, there is a risk that contaminants can be ingested through the direct consumption of soil. This is a particularly high risk in young children who are more likely to ingest soil through playing on contaminated land, with ingestion arising from direct consumption of the soil, adhesion of soil to toys or other hand-to-mouth activities (Jacobsen, 1996; Nielsen and Kristiansen, 2005; Ko *et al.*., 2007).

2.5 Current UK policy on contaminated sites

Under Part 2A of the Environmental Protection Act (1990), it is the responsibility of the Local Authority (LA) to make the judgement on whether a site is deemed to be contaminated, and the degree of contamination. In most circumstances, the LA will seek to identify the party, the 'appropriate person', who is responsible for the contamination or allowed it to occur. Failing this, the LA will operate with the current landowner. A remediation notice is served by the LA and the appropriate person is notified of necessary remediation measures. In some circumstances, the LA may deem a site to be a 'special site', a term used to describe land:

- with a high potential to affect nearby watercourses or groundwater supplies;
- which has been formally, or is currently, used to manufacture explosives or refine oil;
- which has been used to dispose of acid tars;
- owned by the Ministry of Defence;
- which is a nuclear site;
- which is affected by radioactive contamination (DEFRA, 2012).

Designated special sites are administered by the Environment Agency[1] (EA) rather than the LA. PAH contaminated soil could be found at a number of 'special sites', particularly those associated with a legacy of heavy industry or oil refining. This was the case with the Bawtry gasworks site in Yorkshire, deemed a special site following the discovery of coal tar pits in close proximity to a groundwater aquifer, following redevelopment for housing (R. (National Grid Gas Plc (Formerly Transco Plc) v Environment Agency [2007] UKHL 30, 2007).

Soil Guidance Values (SGVs) were developed as a method to assess the risk posed to human health from land contamination. The values were developed by the Environment Agency to provide technical guidance to regulators, particularly to aid in the designation and remediation of contaminated land under Part 2A of the Environmental Protection Act 1990. SGVs represent 'trigger values', beyond which soil concentrations may pose a possibility of significant harm to human health, and are usually followed by further site examination and assessment of risk (Environment Agency, 2009d). Assessment of soil contaminant concentrations by SGV is therefore a logical 'first step' in the assessment of potential risk, to be followed by detailed quantitative risk assessment. On a precautionary basis, SGVs assume a 100% bioavailability scenario, representing a conservative assessment of risk, under which there is effectively no possibility of harm to human health (Nathanail and Smith, 2007).

SGVs were derived using the Contaminated Land Exposure Assessment (CLEA) model, a software suite which models the fate, transport and exposure risks posed by soil contaminants under defined exposure scenarios. The CLEA methodology resulted in the development of SGVs for three distinct exposure scenarios, representing 'residential', 'allotment' and 'commercial' land uses. Typically the exposure risk posed to a small child (6 year old) is modelled in the 'residential' and 'allotment' scenarios, due to the increased exposure to key pathways, such as the soil ingestion route, lower

[1] In England and Wales only. In Scotland the applicable organisation is the Scottish Environmental Protection Agency (SEPA), in Northern Ireland the Northern Ireland Environment Agency (NIEA) is the equivalent body.

body weight and an increased susceptibility to toxicity in some contaminants (Environment Agency, 2009c).

Environment Agency guidance published in 2009 provided SGVs for dioxins (PCDDs), furans (PCDFs) and dioxin-like PCBs. These compound groups were considered for assessment under common SGVs due to their common toxicity, tendency for environmental persistence and structural similarity (Environment Agency, 2009b). SGVs of 8 µg/kg (∑PCDD, PCDF, dioxin-like PCB) were derived for the 'residential' and 'allotment' land use scenarios; the 'commercial' value was calculated to 280 µg/kg (∑PCDD, PCDF, dioxin-like PCB).

Although the SGV approach in PCB assessment is limited, as dioxin-like PCBs are included within a suite of similar compounds, the guidance is useful as a measure of potential harm, and as a trigger value for further site specific assessment, including the potential application of bioaccessibility and bioavailability assessment.

Recent work has seen the development of a new range of assessment criteria in the form of Category 4 Screening Levels (C4SLs), which aim to replace SGVs as the standard guidance in generic site risk assessment. C4SLs were derived within the context of guidance produced by DEFRA in 2012, which introduced a categorisation method into the contaminated land assessment protocol under Part 2A of the Environmental Protection Act 1990 (DEFRA, 2012). TheC4SLs have been developed under the existing CLEA framework, and represent a movement towards a tiered approach to contaminated land assessment in the UK (CL:AIRE, 2014). Under the guidance, land is categorised in terms of risk to human health. Category 1 designation describes land which is clearly posing risk. Categories 2 sites are assessed to pose significant possibility of significant harm under the guidance of expert opinion and scientific guidance that analogous conditions on similar sites have, or are likely to pose, significant harm. Category 3 represents sites which do not meet the conditions for category 2 classification. Category 4 sites are those which clearly pose no potential significant possibility of

significant harm (SPOSH) risk, and should therefore not be considered as statutory contaminated land under Part 2A. (Ander *et al.*., 2013). Categorisations are summarised in Table 1.

Table 1: Summary of the new DEFRA contaminated land categories After (McCaffrey, 2013)**.**

Category 1	High probability of significant harm if no action taken.	Contaminated land
Category 2	Strong case for considering risks of sufficient concern.	
Category 3	Strong case does not exist, but not necessarily absent of risk	Not contaminated land
Category 4	Low or no risk.	

Currently, bioaccessibility testing is not utilised in the assessment of contaminated soils in the UK (Figure 1). The Environment Agency have stated that there is currently too much uncertainty in the relationship between bioaccessibility and the toxicity of contaminants, and are unable to recommend a standard testing method (Environment Agency, 2013), although providing a robust method is adopted, did not rule out bioaccessibility as a useful tool in site specific assessment (Lorenzi *et al.*., 2012).

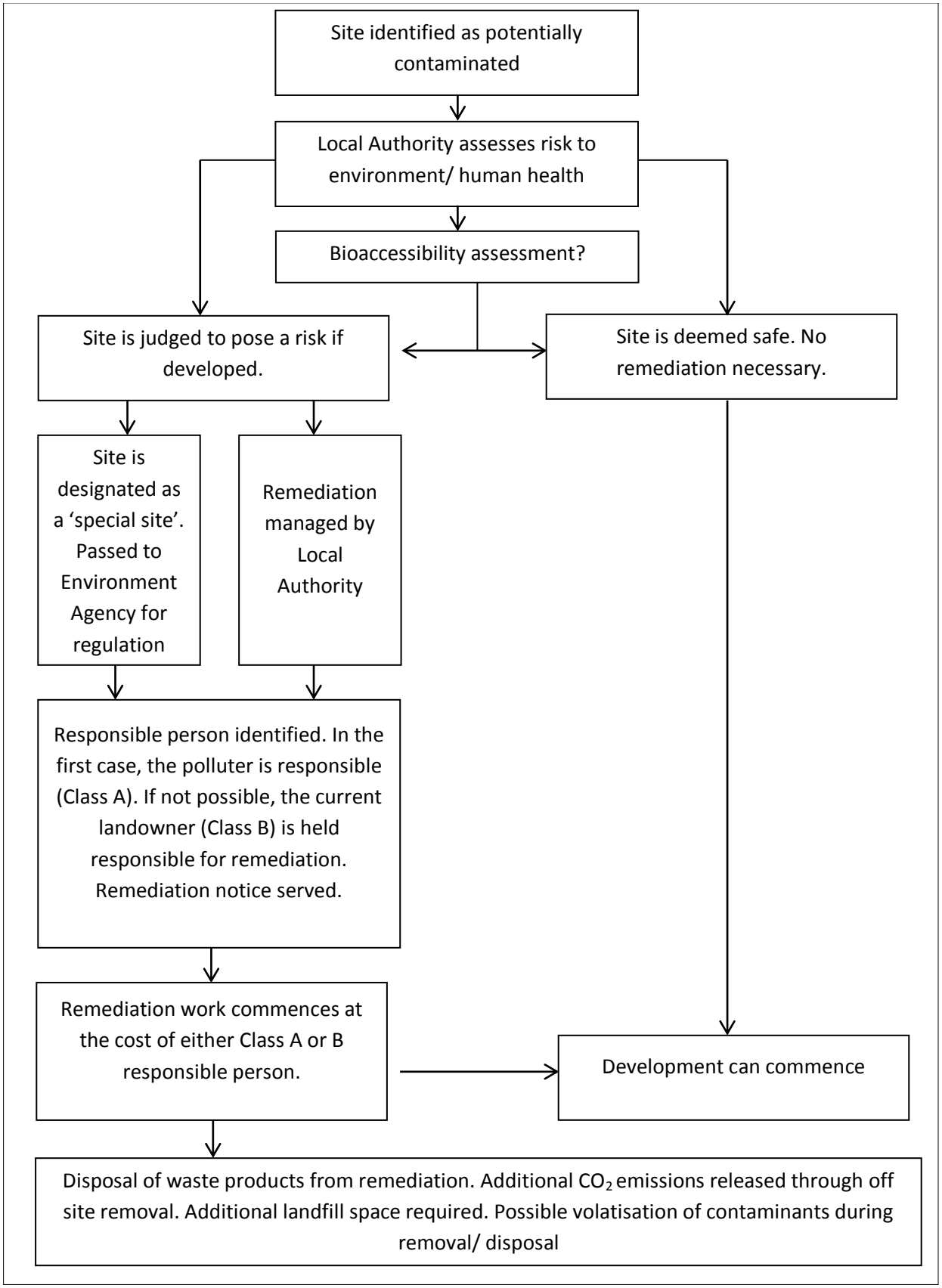

Figure 1: The development process for a contaminated site, with a proposed bioaccessibility assessment stage inserted. The remediation process is expensive, complicated and slows development

2.6 The bioavailability/ bioaccessibility question

The final pollutant linkage to be considered is that of bioaccessibility and bioavailability. Ingested, inhaled or dermally exposed compounds can be said to be in a state of *bioaccessibility*. If capable of interacting with the central blood cavity of the body, compounds can be referred to as being in a state of *bioavailability* (Tilston *et al.*., 2011; Ruby *et al.*., 2016). In the context of soil-borne pollutants, this is possible once desorption of contaminant compounds from contaminated soil particles occurs, thus becoming bioaccessible. In the gut, compounds are released from the soil matrix into solution, at which point they have the potential to enter the bloodstream, or become bioavailable. Bioavailability is a measure of the ability of a compound to enter the stage at which it may interact with the central blood cavity, risking accumulation within fatty tissues and toxicological effects (the extent of which, in both cases, is governed by the physiochemical properties of the specific compounds). Bioaccumulation of contaminants within tissues not subject to elimination by excretion or metabolism is of particular concern, as such sinks of bioaccumulated compounds can artificially extend the exposure of the organism to the harmful effects of the compound, long after physical contact has ceased (National Research Council, 2003).

However, there is frequently debate, discussion and confusion about these similar terms. There would appear to be several differing viewpoints on the definitions of 'bioavailability' and 'bioaccessibility', how the terms differ, at which point they crossover and how they interact as distinct processes within the systems of chemical ingestion.

Bioaccessibility is described in Semple *et al.*. (2004) as the point at which a compound interacts with, and begins a crossover of, a biological membrane. Following this crossover, the compound may be considered absorbed within the body and is free to transferral to the site of biological response (Fig. 2). Semple *et al.*. (2004) described this as the point at which a compound can be considered *bioavailable*. Similarly, Fernández-García *et al.*., (2009) define the bioaccessible and bioavailable processes as being distinct, successive stages of the digestive process;

"Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the digestive tract and thus becomes available for intestinal absorption. Bioaccessibility includes the entire sequence of events that take place during the digestive transformation of food material into that which can be absorbed by the body"

Under this definition, the bioaccessible fraction is the fraction of the released compound which has the potential to undergo bioactivity, to be absorbed by the body, stored in tissues or bioaccumulate. Thus, this becomes the bioavailable fraction. Therefore, the bioaccessible fraction is a necessary component of the bioavailable fraction, and enables bioactivity. The Fernandes-Garcia *et al.*., (2009) interpretation would seem to be in agreement with Semple at al. (2004), the crossover point at which a compound is no longer considered bioaccessible, but bioavailable, is when the compound is absorbed by a biological membrane in the central (blood) cavity, upon which the compound is able to bioaccumulate or become bioactive (Figure 2). Cave *et al.*., (2010) agrees with this view, drawing a clear dividing line between the bioaccessible fraction, which is present throughout the digestive tract and becomes mobilised through digestive processes, and the bioavailable fraction, which is considered present only when there is interaction with the central blood compartment.

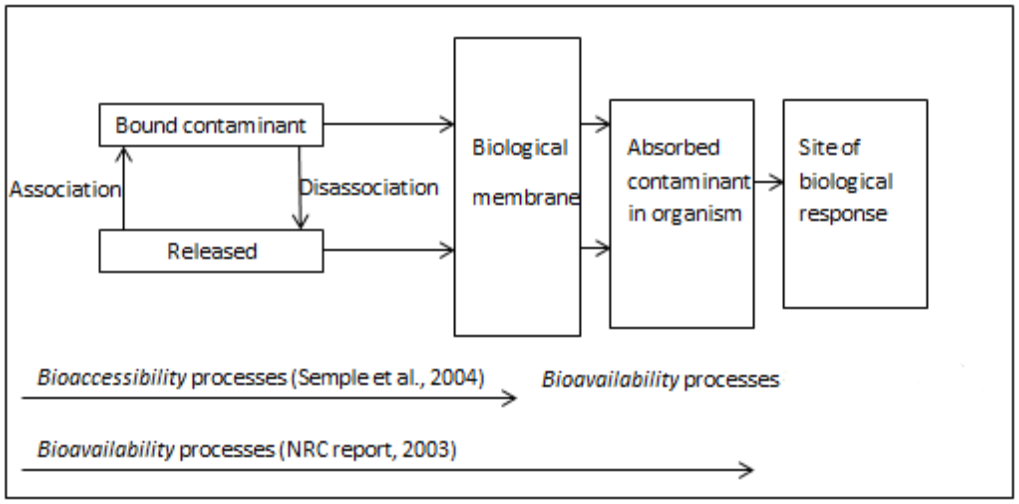

Figure 2: The differing definitions of bioaccessibility and bioavailability as described by Semple *et al.*. (2004), an adaptation of a figure from the National Research Council report 'Bioavailability of compounds in soils and sediments' (2003).

The National Research Council report 'Bioavailability of compounds in soils and sediments' defines the term as;

> *"…the individual physical, chemical, and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments."*

This interpretation notably omits the *bioaccessibility* stage, terming *bioavailability* as the sole process active during interactions between organism and pollutant. Under this interpretation, it can be assumed that all compounds released through the digestive process are bioavailable, and are therefore prone to bioaccumulation and bioactivity. This may be problematic in studies of gastrointestinal bioavailability, as it may lead to an over-exaggeration of the bioactive fraction of a compound is detected in the solutions resulting from digestion.

Opinions on terminology may differ, but these definitions are broadly in agreement. The NRC view on bioavailability does not consider a fraction which may be bioaccessible, but not immediately in a bioavailable state. This view is likely to cause over estimations of risk in samples from *in vivo* and *in vitro* gut models, and, whilst acknowledging the view that bioavailability plays a role in digestion, mirrors the current contaminated land policy view. The acceptance of distinct bioavailable and bioaccessible fractions provide a more accurate model of the absorptive properties of compounds and should be considered in current and future studies.

2.7 *In vitro* techniques for oral bioaccessibility assessment

In vitro techniques allow cost effective, relatively simple, repeatable assessment of oral bioaccessibility, without the ethical burden of *in vivo* experimentation. Lab based testing of sampled contaminated soils give a unique bioaccessibility and contaminant profile for each site – essential when considering the wide range of factors which can affect contaminant transfer from soils.

Several unique methods have been established for the assessment of bioaccessibility, and have been applied to the measurement of a wide range of pollutants (Table 2). A common feature of the techniques is the modelling of, at least a section of, the human gastro-intestinal (GI) tract. Such 'gut models' can provide an accurate simulation of the movement through, and assimilation of compounds into, the digestive system. The methods tend to differ in their complexity, and may not model the entire GI tract or the food content of the digestive system.

Table 2: Summary of recent studies of POP bioaccessibily in soils

Established method	Soil introduced (g)	Fed state?	Oral section present?	Stomach phase		Intestinal phase		Colon section present?	Compounds assessed	Typical values	Analytical technique	Reference
				pH	Time	pH	Time					
PBET	5	No	No	1.8-2.5	3 hours	7	3 hours	No	Pesticides, phenols, base neutral compounds	0.8 – 8.3% (gastric phase), 5.5% - 13.5% (small intestine phase) bioaccessibility. Estimated that the majority of POPs present in sample were excreted (>75%).	GC-MS	Scott and Dean, (2005)
N/A	3	No	No	1.5	2 hours	7.5	12 hours	No	Organochloride pesticides (OCPs), hexachloroethane isomers (HCHs)	4 – 97% bioaccessibility	GC-ECD	Tao *et al.*, (2009)
PBET	6	No	No	1.5	1 hour	7	4 hours	No	PAHs	3.9% to 54.9% for the gastric phase, 9.2% to 60.5% for the small intestine phase. Noted prominence of heavier molecular weight PAHs due to micellar formation associated presence of bile salts.	GC-MS	Tang *et al.*, (2006a)

PBET with Tenax sink	0.2	Yes, as a component of gut fluid.	No	2.5	1 hour	7	4 hours	No	PAHs	55.7 – 65.9% (spiked soils), 16.3 – 31% (field soils). Increases reported on samples extracted without the sink.	HPLC	Li *et al.*., (2015)
CE-PBET with silicone rod sink (Soprtive PBET)	1	Yes, as a component of gut fluid	No	2.5	1 hour	7	4 hours	Yes	PAHs	6 - 49.7% dependent on PAH. HMW compounds reflected lower bioaccessibility.	GC-MS	Gouliarmou *et al.*., (2013)
CE-PBET	1	Yes, as a component of gut fluid	No	2.5	1 hour	7	4 hours	Yes, 8 hours, pH 6.5.	PAHs	Small intestine 7 – 32.5%, colon phase 12.4– 34.4% bioaccessibiilty, varying between PAH compound. Significant increases on unfed PBET recorded.	GC-MS	Tilston *et al.*., (2011)
FOREhST	0.3	Yes, sunflower oil and infant porridge mixture.	Yes, 5 minutes, pH 6.8	1.6	2 hours	6	2 hours	No	PAHs	10 – 60% bioaccessibility	GC-MS	Cave *et al.*., (2010)

Bioaccessibility testing for metals is gaining increasing acceptance, with methods such as the UBM gaining particular prominence as a valuable method with proven *in vivo* validation. However, there is currently no universally accepted method for the assessment of bioaccessibility in organics, which require operating parameters distinct from the inorganic methods. This study will utilise the CE-PBET (Colon Extended Physiologically Based Extraction Test) model, designed at the University of Reading as an extension of the previous PBET model (Tilston *et al.*., 2011), and the FOREhST (Fed ORganic Estimation human Simulation Test) model designed by the British Geological Survey (BGS), adapted from the SHIME method devised by the Netherlands National Institute for Public Health and the Environment (RIVM) (Cave *et al.*., 2010). Both models operate in a fed state; food content is simulated within the system. The FOREhST method specifically simulates the stomach content of a British 4-6 year old, an age group particularly vulnerable to the mouthing of soil through pica or accidental ingestion (Lorenzi *et al.*., 2012).

CE-PBET extends the PBET model with the addition of an 8 hour colon section. The addition of the colon section produced a 50% increase in PAH bioaccessibility from lab prepared soils from the previous PBET model, attributed to a significantly longer incubation time within the colon section and the presence of carbohydrates, which assist PAH desorption from soils (Tilston *et al.*., 2011). The increased desorption of PAHs from the contaminant media in the colon section was monitored over 8 and 16 hour timescales, with no significant increase in desorption in the 16 hour extended incubation. Previous studies have overlooked the significance of the colon section in favour of an emphasis on the small intestine as a primary route to bioavailability (Oomen *et al.*., 2003).

2.7.1 The requirement for validation

Key to the adoption of a bioaccessible test as a trusted method of risk assessment is validation, typically performed through the comparison of derived bioaccessibility values with equivalent values obtained from an *in vivo* bioaccessibility model. *in vivoIn vivo* validation can be performed using multiple approaches, though methods such as the juvenile swine model have been found to represent the most accurate approaches in terms of simulating the human GI tract. The juvenile swine and mini pig models have been credited with accurate simulation of the digestive conditions of young children, which is desirable when assessing bioaccessibility and bioavailability of ingested soil due to the prominence of accidental soil ingestion and pica behaviour in this group, although models using species including mice, rats and goats have been utilised in bioavailability trials for organics (Ruby *et al.*., 2016).

Soils obtained for use in bioaccessibility tests in this work have been subject to limited *in vivo* bioavailability tests using the juvenile swine model, and the data is presented in Chapter 6. However, full *in vivo* validation of both methods is required for the protocols to accurately simulate physiological interactions with target compounds. Differences in physiochemical properties and the potential effects of metabolisation following gastrointestinal absorption in different compound groups require distinct *in vivo* study in order to maintain physiological and chemical validity.

2.8 Recent trends in bioaccessibility research

In order to measure recent trends in bioaccessibility testing, searches were performed for relevant terms using the ScienceDirect database. The rate of publications (since 2000) are shown in Figures 3 – 7. In all cases, the trend shows growth.

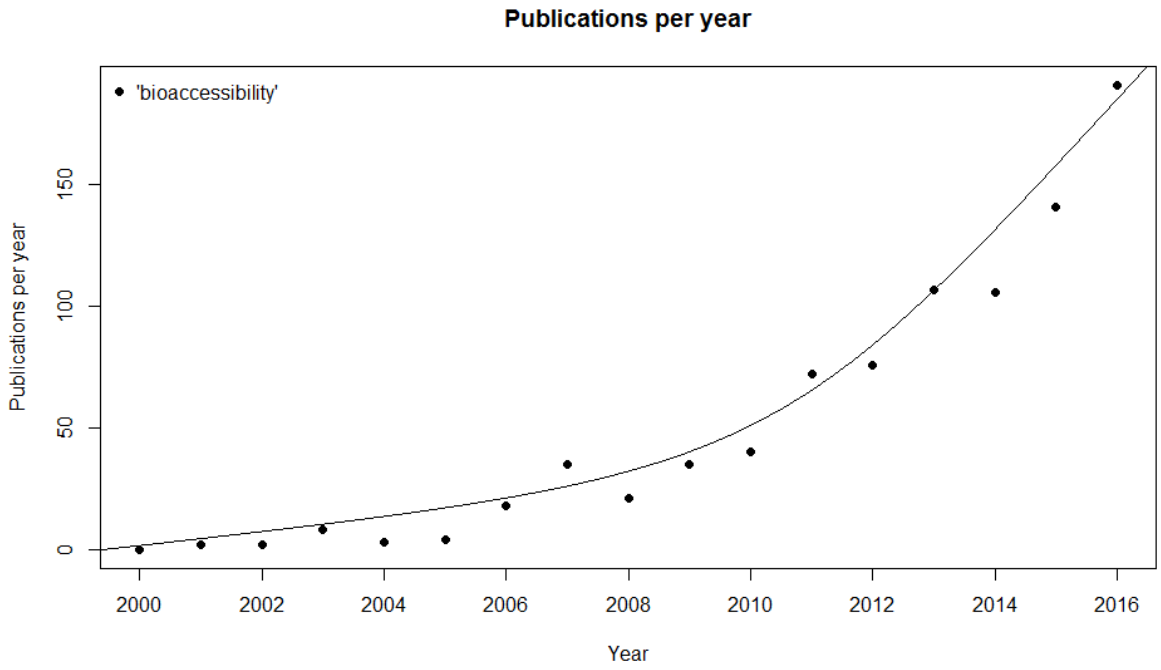

Figure 3: Publications per year recorded on the ScienceDirect database using the search term 'bioaccessibility'

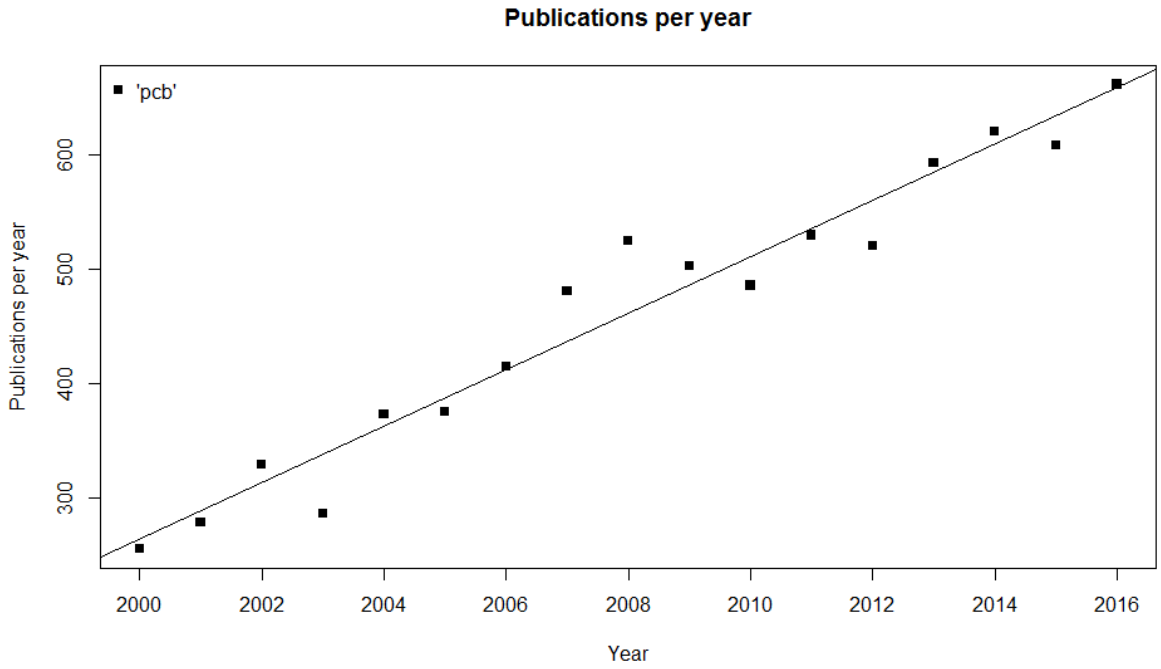

Figure 4: Publications per year recorded on the ScienceDirect database using the search term 'pcb'

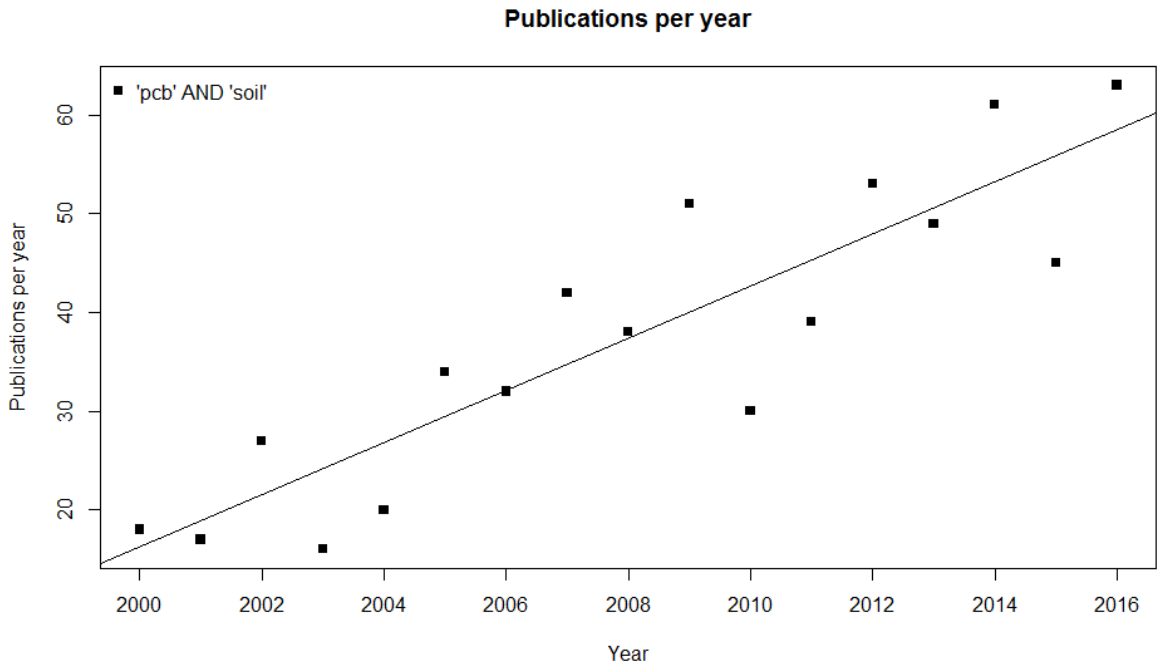

Figure 5: Publications per year recorded on the ScienceDirect database using the search terms 'pcb' and 'soil'

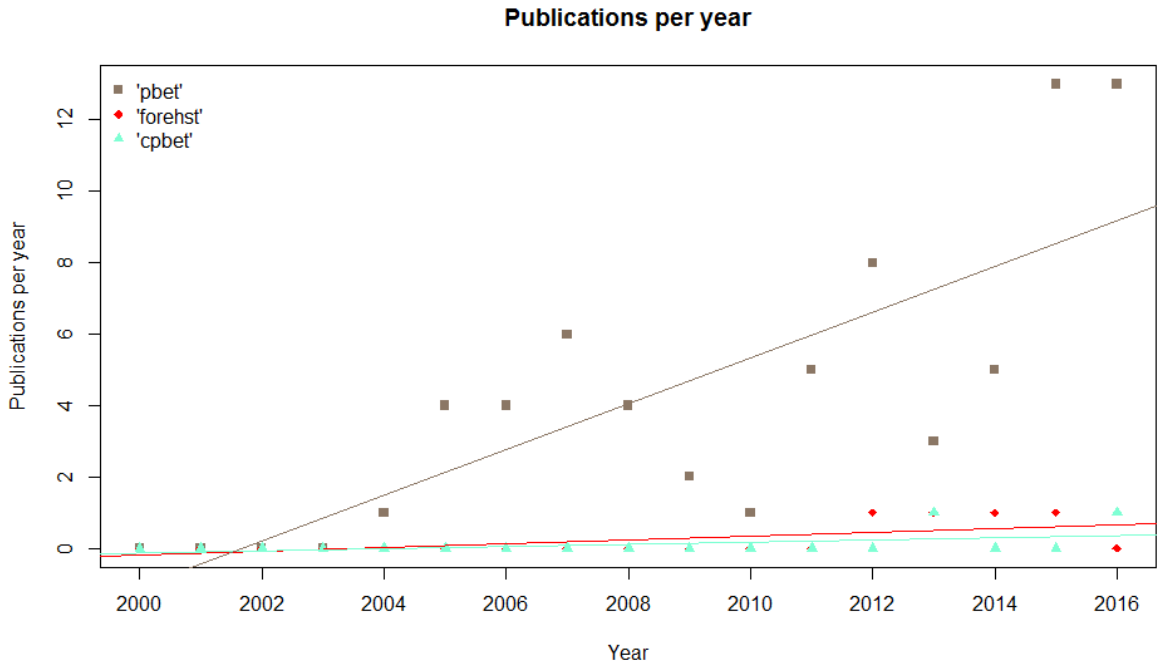

Figure 6: Publications per year recorded on the ScienceDirect database using the search terms 'pbet', 'forehst' and 'cpbet'.

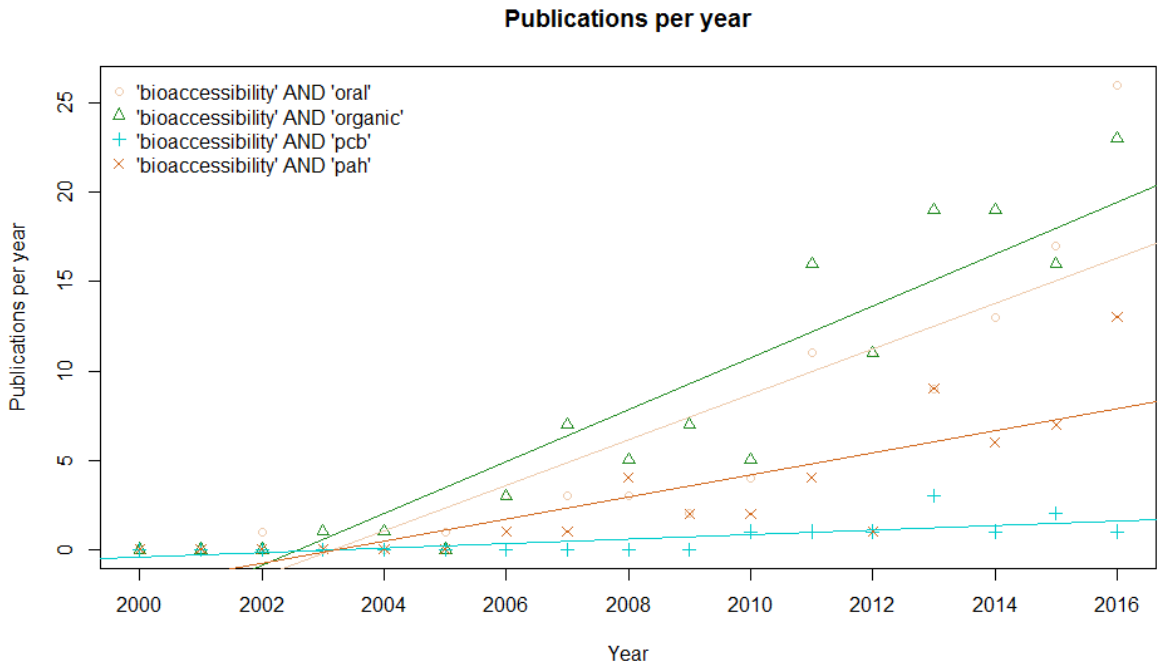

Figure 7: Publications per year recorded on the ScienceDirect database using various search terms associated with bioaccessibility.

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CHAPTER 3: THE UNIQUE CHALLENGE OF PCBS

The principal focus of this thesis is bioaccessibility in polychlorinated biphenyls (PCBs). This brief chapter aims to outline the specific challenges posed by this compound group. Particular reference will be made to physicochemical properties, environmental impact and accumulative properties, and the characteristic risks posed to human health by this group of compounds.

Entirely anthropogenic in origin, PCBs entered manufacture in 1929, with the introduction of Monsanto's Aroclor synthetic oil mixes. Rising fears over potential health risks, identification of carcinogenesis, particularly amongst 'dioxin like' congeners led to their widespread ban globally in the late 1970s, and ultimately through classification as a persistent organic pollutant (POP) under the 2001 Stockholm Convention (Fiedler *et al.*., 2013).

3.1 Distribution

As an anthropogenic pollutant associated with use in primarily industrial applications, the presence of PCBs is typically associated with sites of current or legacy industrial land uses. Indeed, soil PCB concentrations have been found to be typically elevated in urban, rather than rural environments, though environmental persistence and accumulation in biota, solis and sediments has led to global ubiquity (Cachada *et al.*., 2009). Although typically associated with point source emission (Vane *et al.*., 2007), PCBs have shown significant potential for re-emission and long-range atmospheric transport (Fu *et al.*., 2008; Jartun *et al.*., 2009).The widespread distribution, alongside anthropogenic

origin of PCBs has led to their usage as a key indicator of human impact on the global environment (alongside PAHs, total petroleum hydrocarbons and lead), and as a chemical signature of the Anthropocene (Vane *et al.*., 2011).

3.2 Environmental fate and food chain transfer

Despite the ban on their manufacture, PCBs continue to be of concern in the environment through their inherent resistance to degradation and environmental fate. PCBs represent a unique challenge in contaminated land management and remediation as they have an inherent longevity and resistance to degradation, along with significant associated health risks and high tendency to bioaccumulation due to lipophilicity (Henry and DeVito, 2003). Atmospheric half-life times of PCBs range from 10 – 20 years, as they are gradually affected by photodegradation and biodegradation processes. However, this is significantly longer when compounds are contained within soil to a depth greater than 1mm or in submerged sediment, with half-life times extended to the period of 80 – 100 years in Baltic sediment, and biodegradation effects are minimal in sediment and soil bound compounds (Sinkkonen and Paasivirta, 2000).

Although the accumulation of PCB material within soils and sediments remain a significant concern, and represent a significant global store of PCB compounds, the physicochemical properties and chemical stability of this compound group has led to food web accumulation and transfer between organisms (Boese *et al.*., 1997; Zimmerman *et al.*., 1997; Muir *et al.*., 2003). PCBs have been found to readily enter the food web through bioaccumulation principally by aquatic and terrestrial organisms. Subsequent consumption by humans and animals can lead to further accumulation of PCB compounds within their tissues (Beyer and Biziuk, 2009).

Trophic transfer and biomagnification of PCBs is well documented in marine environments, including within zooplankton, as evidenced by recent surveys of species in the Barents Sea, Arctic Ocean and Lake Ontario (Borgå *et al.*., 2005). Early studies identified elevated levels of PCBs in seabirds (Bogan and Bourne, 1972), a phenomenon which has been linked to the trophic transfer of xenobiotics

through the consumption of contaminated fish (Borlakoglu *et al.*., 1990), though additional sources such as marine plastics have also been identified as potential sources for PCBs in seabirds (Ryan *et al.*., 1988). The bioaccumulation of PCBs and other POPs in marine mammals and fish have led to concern about the potential impact on the health of humans with a diet rich in foods derived from these sources, including the indigenous population of the Aleutian Islands. Additional stresses are placed on such communities by the potential economic and societal impacts caused by developmental and reproductive disruption in fish species, resulting in diminished yields (Hardell *et al.*., 2010). The impact on remote communities highlights the global ubiquity of PCBs, furthered by trophic transfer and bioaccumulation of compounds in marine species.

3.2.1 The 1999 Belgian PCB incident

The tendency of PCBs to accumulate within fats, and to readily transfer to animal and human receptors was starkly illustrated by the contamination episode experience in 1999, when 100 Litres of PCB oil was accidentally introduced to a stock of recycled fat intended for use in animal feed production. The incident resulted in the distribution of PCB materials within animal feeds to more than 2500 farms. A widespread monitoring exercise was required to monitor the concentration of PCBs in milk and meat products, with the highest levels found in poultry, including egg laying hens and chicks, with levels of approximately 100 times the maximum recommended levels recorded in some samples of chicken meat (Bernard *et al.*., 2002), and in exceedance of local regulatory limits of 200 ng/g lipid weight ∑ICES 7 PCB in pork (Covaci *et al.*., 2002). The Belgian incident illustrates clearly the necessity for close monitoring of PCBs and other xenobiotics in the food chain, particularly in the case of farmed animals, due to the potential transfer of toxic materials to human receptors (Bernard *et al.*., 2002). The incident let to, in addition to greater public and scientific awareness of the hazards posed by PCB contamination, the introduction of systematic monitoring through a newly introduced national food safety agency in Belgium and the 2002 introduction of European standards for PCB and PCDD/Fs (polychlorinated dibenzodioxins and furans) in feedstocks (Covaci *et al.*., 2008; Hoogenboom *et al.*., 2015).

The long term human health impact of the Belgian incident is debated. Elevated levels of cancers in humans have been attributed to the episode, though studies suggest that the increased PCB burden in humans as a result of the incident was less than the PCB and dioxin burden of a typical human in the 1980s, or those regularly consuming contaminated seafood (Bernard, 2000; Bernard and Fierens, 2002), with a 2000 study identifying traces attributable to the release in blood plasma, but at concentrations too low to cause adverse public health effects (Debacker *et al.*., 2007). However, a recent study of the transfer of PCBs and PCDDs on the breast milk of mothers following a 2008 feedstock contamination event which affected Irish pork products found the environmental influence of the predominantly urban and industrial sample locations to play a more significant role in PCB burden than food chain contamination and the transfer of PCBs and PCDDs from contaminated livestock (Pratt *et al.*., 2012).

The incident illustrates not only the requirement for close monitoring of foodstock standards and potential contamination by PCB materials and the propensity for food chain transfer, but the requirement for close monitoring of the interaction between PCBs and human receptors and potential harm.

3.3 Common uses and Applications

It is the physicochemical properties which ultimately led to the ubiquity and environmental persistence of PCBs, which made them particularly attractive to widespread industrial application. PCBs were widely used in commercial and industrial applications prior to the ban on their production. The physicochemical properties of the compound group, including their resistance to degradation, electrical insulating properties, high boiling point and no-flammability, led to widespread adoption, usually in the form of a recognised technical mixture, such as an Aroclor. The US EPA lists common applications such as electrical heat transfer and hydraulic equipment, plasticising agents in paints, plastics and rubber products, pigments, dyes and carbonless copy paper, alongside other industrial applications within its PCB guidance documentation. The EPA

further highlights the range of products that, if produced before 1979, may contain PCB material, and should therefore be considered as potential sources of contamination:

- Motor and hydraulic oils;
- Transformers and capacitors;
- Electrical equipment, including voltage regulators, switches, re-closers, bushings, electromagnets;
- Electrical devices and appliances;
- Fluorescent light ballasts;
- Cable insulation;
- Thermal insulation (including fibreglass, felt, foam, cork);
- Adhesives and tapes;
- Oil based paints;
- Caulking;
- Plastics;
- Carbonless copy paper;
- Floor polish.

(US EPA, 2017)

Though not as significant, inadvertent formation has been identified as a secondary source of PCBs, including the production of PCB congeners during the manufacturing process used to develop pigments for paints, dyes and fabrics, which have subsequently been observed in waste water outlets from production facilities (Hu and Hornbuckle, 2010; Vorkamp, 2016). Such sources are significant as they represent a modern source of PCB emissions, and do not follow the Aroclor profile typical of contamination due to release from products where the inclusion of PCB material was intended, including non-Aroclor compounds such as PCB 11 (Rodenburg *et al.*., 2010).

PCB congener profile typically reflects patterns of use in terms of recognised congener formulations, however, this effect can be over-ridden by reworking of contaminant bearing sediments, and can be influenced by the length of time compounds are exposed to the open air, reflecting the depositional environment, as evidenced in the Mersey study by Vane *et al.*. (2007). Formulations vary globally, but frequently follow the congener pattern dictated by the Monsanto Aroclor formulations, or equivalent products produced under different tradenames (Table 3), with Aroclor mixtures 1242, 1248 and 1254 (and their technical equivalent mixes) most well studied to date. Distinct Aroclors and equivalent substances contain known standard mixtures of congeners which can be identified by GC/MS (Figure 8), though this profile can be altered through aging.

In some situations, dialectric fluids in capacitors or transformers may lack accompanying information indicating the presence or absence of PCBs, a known PCB formulation, or product name. In such cases, the likelihood of PCB-containing substances being present is based on the provenance of affected equipment. 'Open-use' of PCB materials was voluntarily restricted by Monsanto in 1972 due to increasing concerns about the environmental impacts of PCB use and disposal. As such, all Aroclors produced after this period were used in 'sealed' applications such as capacitor and transformer manufacture. 'Open-use' of PCB containing products in substances such as plasticisers and sealing pastes, cannot be discounted in other products and regions. Furthermore, bans were not global, and the potential for continued use of PCB based products should be considered. Sovol remained in large-scale production and use in the USSR until 1990, 11 years later than a 1979 US Congress/EPA imposed ban . Production (outside of the research environment) was banned internationally under the Stockholm Convention in 2001.

Table 3: Common PCB commercial PCB mixtures and their equivalent Aroclor product where data are available

Principal market	Manufacturer	Product	Aroclor equivalent	Chlorine by mass (%)[a]	Av. No. Cl / mol.[a]
USA/ UK	Monsanto	Aroclor 1221	N/A	21	1.15
USA/ UK	Monsanto	Aroclor 1232	N/A	32	2
USA/ UK	Monsanto	Aroclor 1242/ 1016**	N/A	42/ 41.5	3

USA/ UK	Monsanto	Aroclor 1248	N/A	48	4
USA/ UK	Monsanto	Aroclor 1254	N/A	54	5
USA/ UK	Monsanto	Aroclor 1260	N/A	60	6-6.3
USA/ UK	Monsanto	Aroclor 1262	N/A	62	6.8
USA/ UK	Monsanto	Aroclor 1268	N/A	68	8.7
USA/ UK	Monsanto	Aroclor 1270	N/A	70	10
Belgium/ France		Aceclor			
Italy		Apirollo	n.d.		
USA	American Corp.[c]	Asbetol	n.d.		
UK/ USA	General Electric/ Westinghouse[a]	Askarel	N/A	Variable ***	Variable ***
USA		Bakola 131			
USA	Allis-Chalmers[c]	Chlorextol			
Germany	Bayer[d]	Clophen A30 Clophen A40 Clophen A50 Clophen A60	1242 1248 1254 1260	42 48 54 60	3 4 5 6-6.3
USA		Diactor			
UK		Ducanol			
USA	Cornell Dubilier[c]	Dykanol			
USA	McGraw Edison[c]	Elemex			
Italy	Caffaro[c]	Fenclor 42 Fenclor 54 Fenclor 64 Fenclor 70 Fenclor DK	1242 1254 1260 1268 1270	40-42 52-54 60 65 71	
USA		Hydol			
USA		Inerteen			
Japan	Mitsubishi[d]	Kaneclor 200 Kaneclor 300 Kaneclor 400 Kaneclor 500 Kaneclor 600	1232 1242 1248 1254 1260	32-33 40-42 48 52-54 60	
USA		No-Flamol			
France	Prodolec[d]	Phenoclor DP30 Phenoclor DP40 Phenoclor DP50 Phenoclor DP60			
UK		Plastivar			
USA		Pydraul			
France	Prodolec[d]	Pyralene 2000 Pyralene 1500 Pyralene 3000	1232 1232 1242	32 32 42	2 2.5 3
USA	Eriez Magnets[c]	Pyranol			
UK	Monsanto[c]	Pyroclor			

USA	Kuhlman Electric[c]	Saf-T-Kuhl			
France	Monsanto[c]	Santotherm			
USSR*		Sovol	1254[b]		3
France		Therminol			
USSR*		Trichlorodiphenyl	1242[b]		5

a – (Erickson, 1997)

b –(Ivanov and Sandell, 1992)– Soviet mixtures

c - (US EPA, 1980)

d –(Fiedler, 2001)

* USSR during period of manufacture. Now former USSR states.

** Aroclor 1016 was introduced as a replacement product for Aroclor 1242, and contains an equivalent chlorination by mass (41.5%) .

*** Varies on application. A mixture of Aroclors 1242, 1254, 1016.

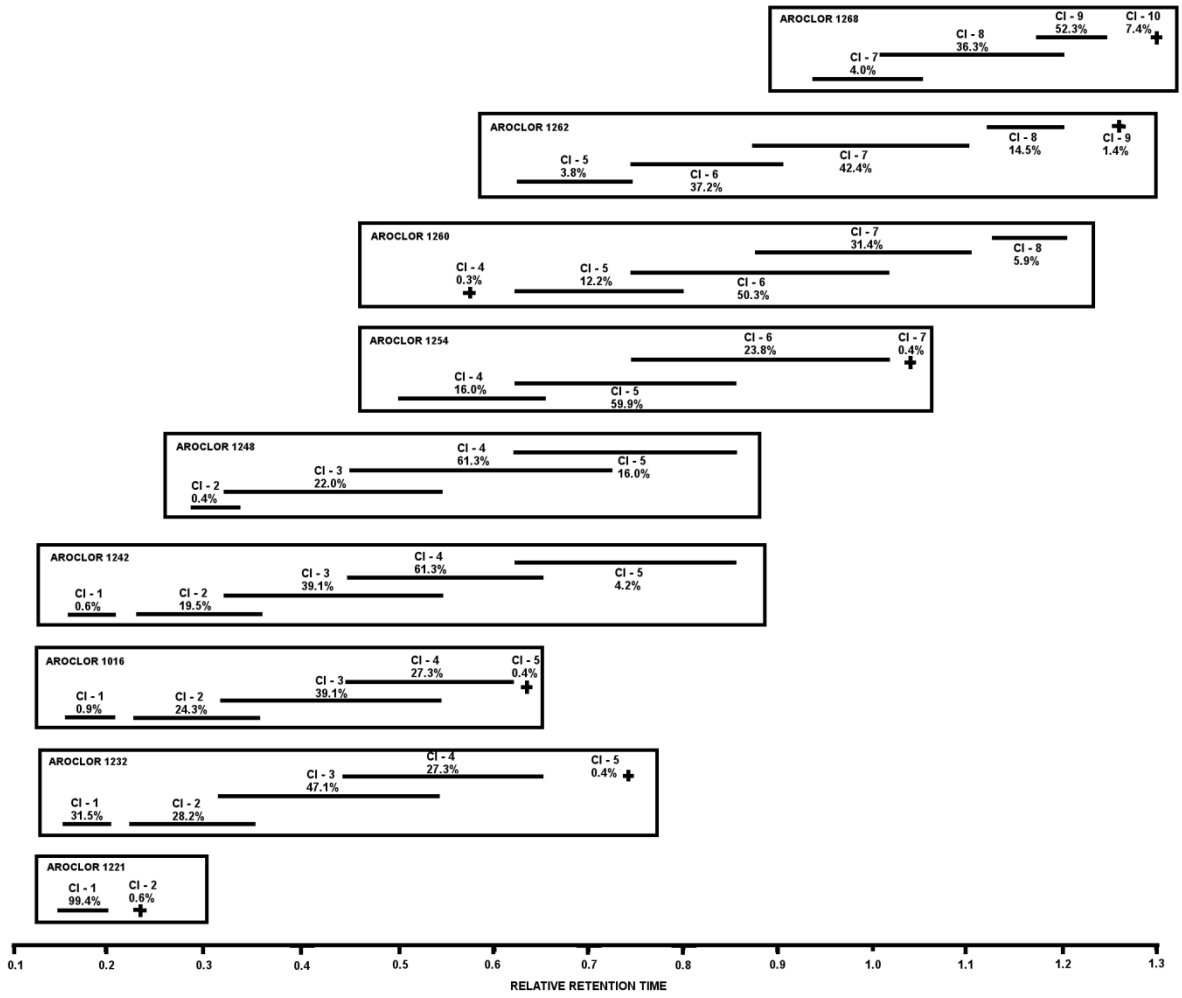

Figure 8: Relative GC-MS retention times and chlorination percentage distribution of homologs in Aroclor technical mixes. Retention times are relative to Chrysene-d 12 After Erickson (1997).

3.4 Physicochemical properties

There are a total of 209 PCB congeners, with varying molecular geometry, degree of chlorination and specific toxicities, representing distinct compound structures reflecting varying molecular geometry in terms of the extent of chlorination (maximum 10 –decachlorination) and chlorine substitution position around the biphenyl structure (Maervoet *et al.*., 2004), delimitating discrete compound homologue groups, based on chlorine substitution. Chlorine substitution represents a distinct controller on the physicochemical properties of the congener, dictating toxicity, potential for bioaccumulation, and tendency to biodegradation, with the higher-chlorinated compounds representing a greater resilience to degradation (Furukawa *et al.*., 1978). These properties can lead to preferential accumulation of higher chlorinated compounds during sequestration in soils and sediments, influencing the congener profile (Ehlers and Loibner, 2006; Environment Agency, 2009b), in addition to the influence of specific Aroclor congener mixes.

Cachada *et al.*., (2009) emphasises the importance of limiting analysis to select groups of congeners and understanding the history of the study site, to enable the focussed analysis of congeners of most concern. This may be adequate in areas affected significantly by high concentrations of Aroclors, such as sites in the USA and UK, but may not be transferrable for study in sites affected by a wider range of congeners, or other congener mixes, or in areas where compounds are suspected to have become transported following re-emission.

Emphasis should be on evaluation of toxic risk, persistence (preference for increased concentrations of non-volatile, heavier congeners in the environment/ prevented dispersal), and origin of pollution. As a response to these conditions, and the variability in physicochemical properties between congeners in the group, the International Council for the Exploration of the Sea (ICES) developed a 7 congener sub-group of compounds (PCB congeners 28, 52, 101, 118, 138, 153 and 180). The ICES-7 were selected on the basis of their environmental distribution, varied persistence between

congeners, and their representation of five major homologous series (tri-, tetra-, penta-, hexa- and hepta- chlorinated compounds) (Webster *et al.*., 2013; Glüge *et al.*., 2016a)

This group has been widely adopted by the scientific community as a convenient method to provide comparison between studies. Vane *et al.*. (2007) provides an extensive survey of PCB distribution in an estuary environment affected by a legacy of industry, focussing on the assessment of ICES-7 member congeners, and providing a clear PCB congener profile which reflects the industrial legacy of the study area. While the ICES-7 provide a useful range of congeners, particularly in comparison between studies, they have not been allocated on the basis of toxicity or dioxin like chemistry, and are therefore limited in the analysis of PCBs in the context of human health risk.

3.5 Nomenclature

PCBs represent a group of aromatic organic compounds subject to chlorination through the substitution of hydrogen atoms for chlorine within a biphenyl structure. As a compound group, PCBs may be interchangeably termed as chlorinated biphenyls, chlorinated diphenyls or polychlorobiphenyls (IARC, 2013). There are 209 distinct theoretical congeners within the group, dependent on the position and number of chlorine atoms present on the biphenyl ring structure.

A brief description of the standardised nomenclature, which has been developed to adequately distinguish between congeners and to apply short and long-form descriptive terms for individual members of the group, follows.

Using the standard Hill notation, a PCB compound can be characterised using the simple formula:

$C_{12}H_{(10-n\mathrm{Cl})}Cl_{(n\mathrm{Cl})}$

Where *n*Cl represents the number of chlorine atoms substituted to the biphenyl structure. This method is useful to determine the degree of chlorination within the compound. However, this notation fails to convey substitution position, and cannot determine individuals within the broad

homologue groupings of mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and deca chlorinated biphenyls (Erickson, 1997).

A descriptive and straight-forward nomenclature has been developed, which allows the chemist to determine the structure of the compound in terms of chlorination and the position of chlorine atom substitution. Each substation position is numbered 2–6 and 2'-6' (corresponding to the two rings of the biphenyl structure) (Figure 9). Under International Union of Pure and Applied Chemistry (IUPAC) guidance the substitution corresponding numbers are listed in ascending order (Mills *et al.*., 2007).

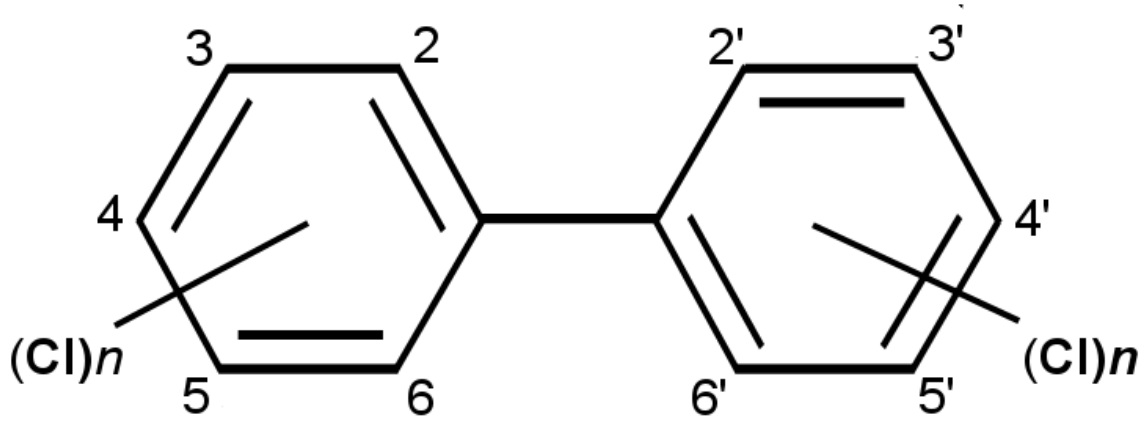

Figure 9: PCB structure, showing Cl substitution positions. Adapted from IARC (2013)

A convenient short-form systematic method of naming congeners was developed from this method, with each congener designated a number (1-209) (Ballschmiter and Zell, 1980). This numbering system has since been widely adopted by researchers and regulators, and is commonly found in PCB literature.

Aroclors follow a standard numbering system. The first two digits represent the number of carbon atoms in the compound structure (12 in the case of PCB based formulations, with the exception of Aroclor 1016, which was introduced as a replacement product for Aroclor 1242 - The chlorine content remains at approximately 42%), the second two digits represent the percentage of chlorine by mass in the Aroclor. The use of PCB material in such distinct mixtures allows the elimination of

the requirement to monitor all 209 PCB congeners, allowing focus on relevant compounds, as Aroclors can typically be associated with distinct congener profiles, though the range of potential mixtures complicates this process. Potential variability between batches of Aroclors is possible, and the chlorination process used in Aroclor production has been associated with the production of non-target congeners, including those in different homologue groups .

Table 4: Physicochemical properties of the ICES 7 PCBs, and a selection of commonly studied PAHs for comparison. Log Kow (n-octanol/ water partition coefficient) is presented as a measure of the tendency of the compound to absorb readily to organic matter, with a high value typically indicating a low affinity for water.

Compound	IUPAC congener no.	CAS no.	Cl no.	Log Kow	Water solubility (mg/L)
2,4,4' -trichlorobiphenyl	28	7012-37-5	3	5.62	0.27
2,2',5,5' – tetrachlorobiphenyl	52	35693-99-3	4	6.09	0.015
2,2',4,5,5' – pentachlorobiphenyl	101	37680-73-2	5	6.8	0.015
2,3',4,4',5 – pentachlorobiphenyl	118	31508-00-6	5	7.12	0.013
2,2',3,4,4',5' – hexachlorobiphenyl	138	35065-28-2	6	7.44	0.0015
2,2',4,4',5,5' – hexachlorobiphenyl	153	35065-27-1	6	7.75	0.00095
2,2',3,4,4',5,5' - heptachlorobiphenyl	180	35065-29-3	7	8.27	0.0038

Selected PAH data					
Compound	Empirical formula	CAS no.	No. benzene rings	Log Kow	Water solubility (µg/L)
Napthalene	C10H8	91-20-3	2	3.7	31
Fluorene	C13H10	86-73-7	3	4.18	1.9
Phenanthrene	C14H10	85-01-8	3	4.57	1.1
Fluoranthene	C16H10	206-44-0	4	5.22	0.26
Chrysene	C18H12	218-01-9	4	5.75	0.002
Benzo[a]pyrene	C20H12	50-32-8	5	6.04	0.004
Indeno[1,2,3-cd]pyrene	C22H12	193-39-5	6	7.66	0.06

3.6 Human health impacts

PCBs have been associated with a broad spectrum of human health impacts (Herrick *et al.*., 2007). Of most significant concern to human health are the 12 'dioxin-like' PCBs, which through coplanar structure (Figure 10), degree of chlorination and chlorine substitution position exhibit similar structures, and biological effects to dioxins such as 2,3,7,8-TCDD. These compounds have been found to bind to and activate the aryl hydrocarbon receptor (AhR), with neurological, reproductive, immunological and developmental effects in mammals (Van den Berg *et al.*., 2006; Environment Agency, 2009b; Zhang *et al.*., 2012). Dioxin like PCBs represent an important component of the total load of dioxins and dioxin-like compounds within many environmental media, such as animal produce and fish (Alcock *et al.*., 1998).

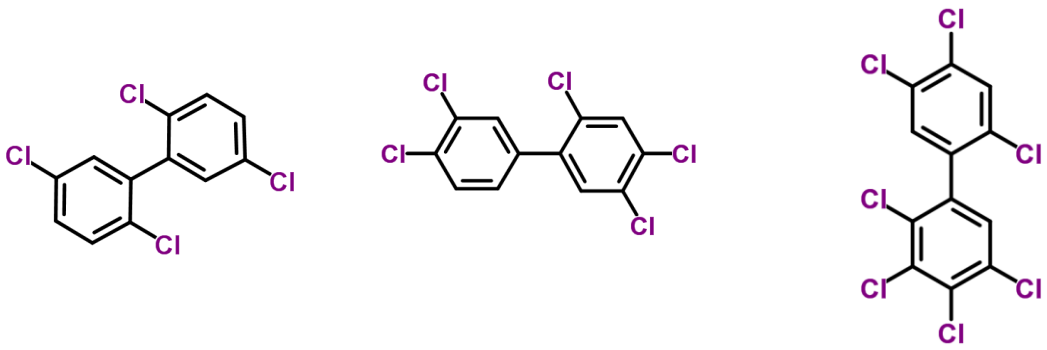

Figure 10: Structures of (L-R) PCB 52, 118 and 180. PCB 118 displays dioxin-like properties.

In order to evaluate the potential toxicity of dioxin like PCB congeners the World Health Organisation (WHO) have derived a method to determine Toxic Equivalency Factor (TEF) values. These values are determined on the basis of the estimated toxicity in relation to the toxicity value determined for2,3,7,8,- Tetrachlorodibenzo-p-dioxin (TCDD), a persistent organic compound associated with carcinogenic and tetratogenic effects in humans. Calculated values vary between congener, with PCB 126 representing the highest value (Table 5).

Although the TEF is a useful method to aid in the assessment of toxicity in DL PCB congeners, the specific human health risk is determined by the profile of compounds present. As shown in Table 5, TEF is not consistent between congeners, and the relative concentration of individual PCBs can vary

due to differences is PCB profile in Aroclors and the deterioration and preferential accumulation of compounds within the matrix (Birnbaum and DeVito, 1995; Giesy and Kannan, 1998).

Table 5: WHO derived TEF values for dioxin like PCB congeners (Van den Berg *et al.*., 2006)**.**

Compound	WHO TEF
2,3,7,8-TCDD	1
PCB 77	0.0001
PCB 81	0.0003
PCB 126	0.1
PCB 169	0.03
PCB 105	0.00003
PCB 114	0.00003
PCB 118	0.00003
PCB 123	0.00003
PCB 156	0.00003
PCB 157	0.00003
PCB 167	0.00003
PCB 189	0.00003

However, typically non-dioxin-like (NDL) PCBs are significantly more common in environmental matrices than DL compounds, and have a less characterised general toxicity profile (Hamers *et al.*., 2011), and are associated more closely with exposure from dietary sources in humans (Fattore *et al.*., 2008). *In vivo* studies of PCB toxicity profiles have identified NDL congeners as important contributors to detrimental neurodevelopmental in newborns, neurotoxicity, the promotion of tumour development and antiandrogenic impacts (Hamers *et al.*., 2011; Elnar *et al.*., 2012; Delannoy *et al.*., 2015).

It has been argued that a revised PCB nomenclature be developed, with emphasis placed on the distinct classes of metabolite compounds associated with the biotransformation of PCB within biotic tissues (Maervoet *et al.*., 2004), encouraging a greater emphasis on the role of PCBs as xenobiotic contaminants, and their interaction with organic tissues.

It has been recognised that contaminants such as PCBs represent a barrier to the re-use of contaminated land due to the high costs of necessary remediation, directed under regulations such as the Part 2A of the UK Environmental Protection Act, US federal and state regulations, and similar such regulations and requirements globally. Sound management of contaminated land is of utmost importance, and a degree of conservatism is prudent in order to eliminate potential risk. However, recent years have seen a growth in the development and application of bioaccessibility testing, particularly in the case of soil-borne inorganic substances such as Arsenic and Lead.

A growing field of evidence suggests that similar strides are possible in soils contaminated by PCBs (Table 6), and methods have been developed to study the bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) in ingested soil, such as the FOREhST and CE-PBET . Substances such as DDT and other organochloride pesticides. have been the subject of bioaccessibility studies . Polychlorinated dibenzonfurans (PCDFs) and PCBs continue to represent a challenge, and the bioaccessibility of emerging contaminants such as polybrominated diphenyl ethers (PBDEs) require further study. These compounds represent distinct hazards, and interact with soils and biota in unique ways. A key challenge for the bioaccessibility community is to identify and develop effective methodologies which show potential in application to multiple compound groups, with differing physicochemical properties, distribution and fate. The list of emerging organic pollutants continues to evolve, as reflected by the addition of emerging organic pollutants to international directives and regulations such as the First EU Watchlist .

Table 6: Summary of recent PCB bioaccessibility studies.

Study	Medium	Method	Summary of findings
Wang *et al.* (2013)	Dust (indoor and outdoor)	Combined intestinal/ gastric extraction.	Mean (all congeners) bioaccessibility of 27.9%. Bioaccessibility higher in LMW compounds. Attributed to lower Kow, therefore less soil/ sediment adsorption and greater affinity for the water column (gut fluid). This discounts the micellar effects of surfactants in the gut fluid or role of oily/ fatty or carbohydrate rich media.
Ertl and Butte (2012)	Indoor dust	Modified German guideline DIN 19738 (mouth/ stomach/ small intestine)	Mean of congeners (PCB 28, 101, 138, 153, 180) 63%. Increased bioaccessibility due to the presence of food and is observed, and the positive effect of gut fluid surfactants on bioaccessibility noted.
Xing *et al.* (2008)	Food	Goni *et al.* (2006)	Varied between media. 3% in fish, 25% in leafy vegetables. Attributed to retention of PCBs in lipid rich material in fish. Anticipates equilibrium of HOCs in bile/ GIT fluid micelles.
Kang *et al.*. (2013)	Workplace dust	PBET as used in Ruby *et al.* (1996)	Significant relationship observed between bioaccessibility and Kow Higher molecular weight compounds less likely to partition to GIT fluids. Equilibrium observed at 13.8 - 21.8% bioaccessibility. Final values varied between 33.5% (PCB 28) and 16.2% (PCB 194).
Shen (2016)	Food	GIT model incorporating mouth/ stomach/ small intestine sections.	Variability between foods. Vegetable based foods were lower than meat and eggs (Rice: 16.5%, cabbage: 4.2%; beef: 49%)
Hack & Selenka (1996)	Soil	GIT model incorporating stomach and small intestine. Fed/ unfed states.	3 – 22% in unfed state. Addition of lyophilised milk increases bioaccessibility to 40 -85%.
Oomen *et al.*. (2000)	Soil	Unfed GIT model incorporating mouth/ stomach/ small intestine sections.	Mean of congeners (PCB 28, 101, 138, 153, 180) 36%. Emphasised Importance of bile micelles to the increase of HOC bioaccessibility (PCBs and lindane were tested, and saw significant increases in bioaccessibility when bile salts were included in the simulated gut fluid).

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CHAPTER 4: POLYCHLORINATED BIPHENYLS (PCBS) IN THE URBAN SOILS OF CENTRAL LONDON, A EUROPEAN MEGACITY

4.1 Introduction

Polychlorinated biphenyls (PCBs) saw widespread use in a wide range of materials such as coolants, lubricants, transformer oils, sealants and calking agents (Herrick *et al.*., 2007) during the early twentieth century. PCBs were prized for their low reactivity and high chemical and thermal stability, though it is these qualities which contribute to their environmental persistence (Beyer & Biziuk 2009). Peak production was reached in the 1960s before phase-out in the late 1970s, following growing concern about their potential for harm and accumulation in the environment (US EPA, 1979) and a global ban through the Stockholm Convention on Persistent Organic Pollutants in 2001 (Zhang *et al.*., 2012).

In addition to their environmental persistence and accumulative potential PCBs are also toxic. PCBs have been found to possess endocrine disruptive qualities associated with developmental disorders in humans and other animals, and some congeners exhibit carcinogenicity (Colborn *et al.*., 1993; Abramowicz, 1995; Zhang *et al.*., 2012). Health risk from PCBs is significant through multiple exposure pathways (Kang *et al.*., 2013), but soil represents the most significant environmental store of PCBs (Harrad *et al.*., 1994).

PCBs represent a group of 209 congeners of halogenated aromatic hydrocarbons, described according to the position(s) of the chlorine atom substitution (Kodavanti *et al.*., 2001), which possess a highly lipophilic, hydrophobic character, associated with elevated rates of accumulation in soils and biota (Collins *et al.*., 2015), and in the adipose and organ tissues of humans (Dewailly *et al.*., 1999). The congeners range from mono- to deca-chlorinated compounds, but many are not commonly found in environmental samples or produced (Breivik *et al.*., 2002; Webster *et al.*., 2013). As such, surveys of the full range of 209 congeners are not typical, and congeners can be selected for quantification on the basis of background surveys of site history and potential PCB use, storage or manufacture (Hopf *et al.*., 2014), potential for harm, or to provide a representative sample of all

homologous series. In this study, PCB congeners 28, 52, 101, 118, 138, 153 and 180 were quantified. These are the congeners designated as the ICES (International Council for the Exploration of the Sea) 7, a group of congeners selected on the basis of their persistence and distribution in the environment, alongside their representation of five major homologous series (tri-, tetra-, penta-, hexa- and hepta- chlorinated biphenyls) and abundance in technical mixtures (Webster *et al.*., 2013; Glüge *et al.*., 2016b). Though typically associated with point sources, persistent semi-volatile compounds such as PCBs can be subject to diffuse dispersal and transport in air (Bennett *et al.*., 1998; Breivik *et al.*., 2002; Zhang *et al.*., 2007; Scheringer, 2009), including from secondary sources (Becker *et al.*., 2009) such as demolition or disturbance of in-situ equipment or PCB-containing materials with subsequent deposition to soil. PCBs therefore have the potential for diffuse contaminant in addition to point source pollutant through re-emission (Vane *et al.*., 2014).

Elevated soil PCB concentrations are associated with areas of heavy industry and centres of high population (Motelay-Massei *et al.*., 2004). A 2014 study of East London soils, including areas formally associated with heavy industry, found a mean soil ∑ICES-7 PCB concentration of 22 µg/kg, with a maximum value of 750 µg/kg (Vane *et al.*. 2014). A survey of Central London has not been conducted to date. This region is not associated with heavy industry presently, but as the centre of a major global megacity with a strong industrial history, industrial pollution of soils is expected be significant.

This study presents soil PCB concentrations at two depths (0-5 cm and 15 – 20 cm), at 69 sites across Central London. Potential sources of PCBs are investigated, including historical review of land use. Recorded concentrations are compared to similar studies of urban soil PCB concentration and congener profile, and normal background concentration (NBC) values determined.

4.2 Materials and methods

4.2.1 Soil sampling

Soils were sampled at a depth of 0-5 and 5-20 cm at each site (n=69) using a method described in (Vane *et al.*. 2014). Locations were selected across the study area every kilometre square and recorded using a Garmin handheld GPS device (accuracy ± 5m) (Figure 11). A Dutch auger was used to sample each location at both depths from the four corners and centre of a 20 by 20 m square grid, combined, and stored in a Rilsan bag, a high performance polymer sample bag used commonly in the collection environmental and forensic materials. The samples were stored at 4°C before freeze drying at -18°C. Soils were passed through a brass sieve with an aperture of 2 mm before agate ball milling, a method previously shown to provide high precision and accuracy in the detection of organic compounds in soils (Beriro *et al.*., 2014). Sampling was restricted to locations where the auger could be successfully inserted. A total of 138 samples were obtained which were suitable for use in this study.

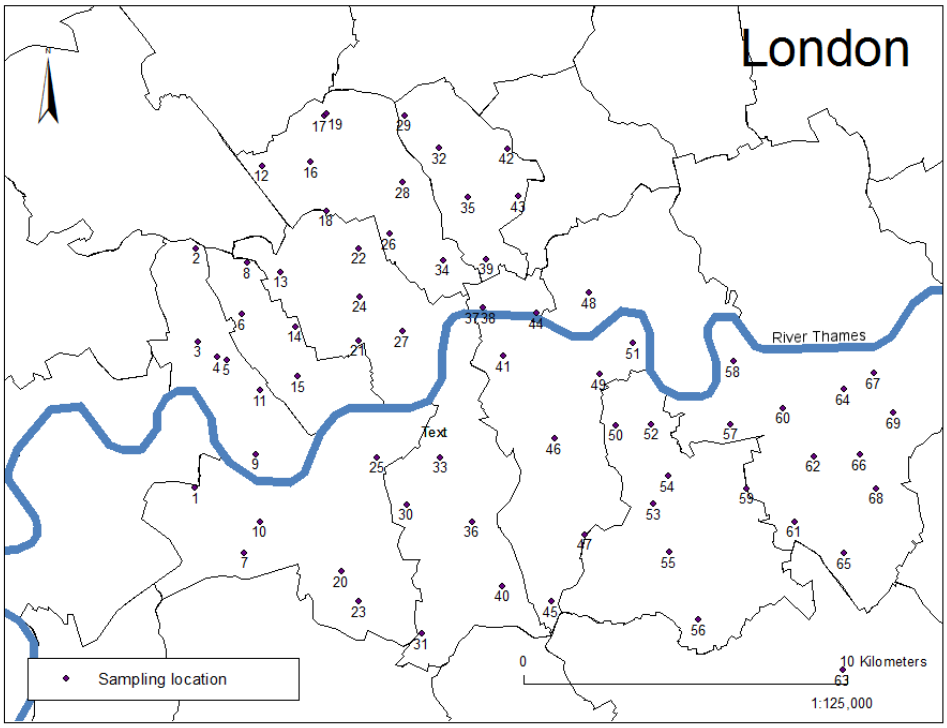

Figure 2: Sample locations in Central London

4.2.2 Investigation of spatial relationships

Sample sites were assessed in terms of their historical context through the examination of historic Ordnance Survey maps dating from 1920 to the present, in order to identify the land use, or domain i.e. developed or undeveloped, of the location, and any potential sources during the period in which PCB usage was common in industry, commerce and construction (1930 – 1979), and subsequent use of *in situ* equipment (1980 – present).

Variogram analysis has been applied to concentrations of the 7 ICES PCBs recorded in this study. Plots were performed in R 3.3.1 (R Core Team, 2016) using the software package 'automap' (Hiemstra *et al.*., 2009), which automatically fits an appropriate variogram model to the data, and are shown in Figures 14 and 15. Data from the two sample depths are shown independently.

4.2.3 Laboratory analysis of PCBs

Soils (10 g) were spiked with 50 µL of an internal standard solution of PCB 34 (18.92 ng/µL), PCB 62 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL) and PCB 173 (18.81 ng/µL), and 50 µL of

a recovery standard solution of PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL), and allowed to equilibrate for one hour, before being combined with a cleaned sand filler matrix of a ratio of soil/sand of 1:2 (w/w). Copper powder added to remove elemental sulphur, before ASE extraction using acetone/hexane (1:1 v/v). The ASE (Dionex 200, Dionex, Sunnyvale, CA) was operated at a pressure of 1000 psi, 100 °C oven temperature, 5 minute oven heat time, 5 minute static extraction time, acetone: hexane (1:1) solvent (60% of the cell volume flush volume), followed by a 1 MPa 60 second nitrogen purge. Extracts were reduced in volume under a stream of nitrogen to 3-4 mL and mixed with H_2SO_4 to release humic bound PCBs using method described by (Vane *et al.*. 2007). The supernatant was retained and reduced under a stream of nitrogen to 0.25 mL before transfer to a pre-filled Na_2SO_4 SPE cartridge (Agilent, Bond Elut TPH, 500 mg). The first fraction was eluted with 1.5 mL of pentane and discarded. The second fraction was eluted with 6 mL of hexane propanol (97:3 v/v) and retained, spiked with 100 µL of the analytical standard solution (PCB 29 (9.47 ng/µL) and PCB 157 (9.53 ng/µL)) and reduced to 200 µL under a stream of nitrogen.

Analysis was performed by combined gas chromatography-mass spectrometry (GC-MS) using a Fisons GC8000 gas chromatograph coupled to a Fisons MD800 single quadrupole mass spectrometer in full scan mode (ionisation energy 70eV, mass range 39-600 amu), using a method established in (Vane *et al.*., 2007). Sample injection volume was 1 µL. The GC was equipped with a Varian Factor Four VF-5s fused silica capillary column (60 m length, 0.32 mm inner diameter, 0.25 µm film thickness). The inlet was at a temperature of 280°C, and the detector at 250°C. The GC oven was temperature programmed from 100 °C (1 min isothermal) to 200 °C (at 5 °C/min) to 280 °C (at 2.4 °C/min) and held isothermally for 20 mins, to 300 °C (10 °C/min). Helium was used as the carrier gas at 16 psi. Quantification of PCBs was by selected ions (256.0, 292.0, 326.0, 360.0, 394.0).

4.2.4 Total organic carbon (TOC) analysis

TOC analysis was conducted using an Elementar Variomax CN analyser following acidification with HCl (50% v/v) to remove carbonate, as described in Vane *et al.*. (2007). The limit of quantification for a typical 300 mg sample was 0.18%.

4.2.5 Quality assurance and quality control

All glassware was pre-cleaned using a H_2CrO_4 method consisting of a 24 hour acid soak followed by rinsing with deionised water. HPLC grade solvents were used. Each ASE run consisted of 20 samples, and a method blank containing the internal standard solution introduced to the soil samples. All blanks produced results that were below the limits of detection. In addition, 7 CRM (BCR 481, an EU approved industrial soil with certified PCB concentrations) samples interspersed the soils, 1 per analytical run.

4.2.6 Limit of detection

LOD (limit of detection) values were determined for authentic standards using equation 1 (PCB 28: 0.0155 ng/µL, PCB 52: 0.0159 ng/µL, PCB 101: 0.0137 ng/µL, PCB 118: 0.0173 ng/µL, PCB 138: 0.0185 ng/µL, PCB 153 0.0199 ng/µL, PCB 180: 0.0272 ng/µL), using a method described in ICH (International Council for Harmonisation) guidance documents (ICH, 1994)

Equation 1:

$$LOD = \frac{3.3\,\sigma}{S}$$

Where:

σ = the standard deviation of the slope

S = the slope of the calibration curve, determined from the regression line of the calibration curve.

A method described by Wendelberger and Campbell (1994), which has been previously applied to environmental PAH studies, was applied. A zero value is given if the sample consists of entirely non-detects. If at least one congener is detected, non-detects are assigned the LOD value.

4.3 Results and discussion

PCB totals are given in terms of individual analysed PCB congener, as total ICES-7 selected congeners, and classified by homologous series (∑tri - ∑hepta –chlorinated compounds), thus allowing a full exploration of the role of PCB chemistry on accumulation and distribution within soils. These totals are summarised in Table 7, and shown in Figure 13.

4.3.1 PCB concentrations and congener profiles

The mean values, standard deviations, maximum and minimum values of individual PCB congeners, ∑ICES-7, 5 homologous series (tri, tetra, penta, hexa, hepta, a sum value of congeners in each homologous series) and $\sum^{\text{tri-hepta}}$ are given in Table 7. ∑ICES-7 values for both soil datasets are presented spatially in Figure 12. Maximum ∑ICES-7 concentration, across both soil datasets, was 148.7 µg/kg, with a mean value across all samples of 15.1 µg/kg (n=138).

This maximum value was identified in a soil from the X (0 – 5 cm depth) dataset (map ID 41, Newington/ Elephant & Castle). The mean ∑ICES-7 value of the X dataset was 10.9 µg/kg (n=69).

Within the A (15 – 20 cm depth) dataset, a maximum value of 135.8 µg/kg was identified (map ID 33, Stockwell). The mean value of the A dataset was 18.8 µg/kg (n=69).

A two-tailed Student's *t*-test conducted on the A and X datasets revealed no significant statistical difference between the two groups ($p \geq 0.01$).

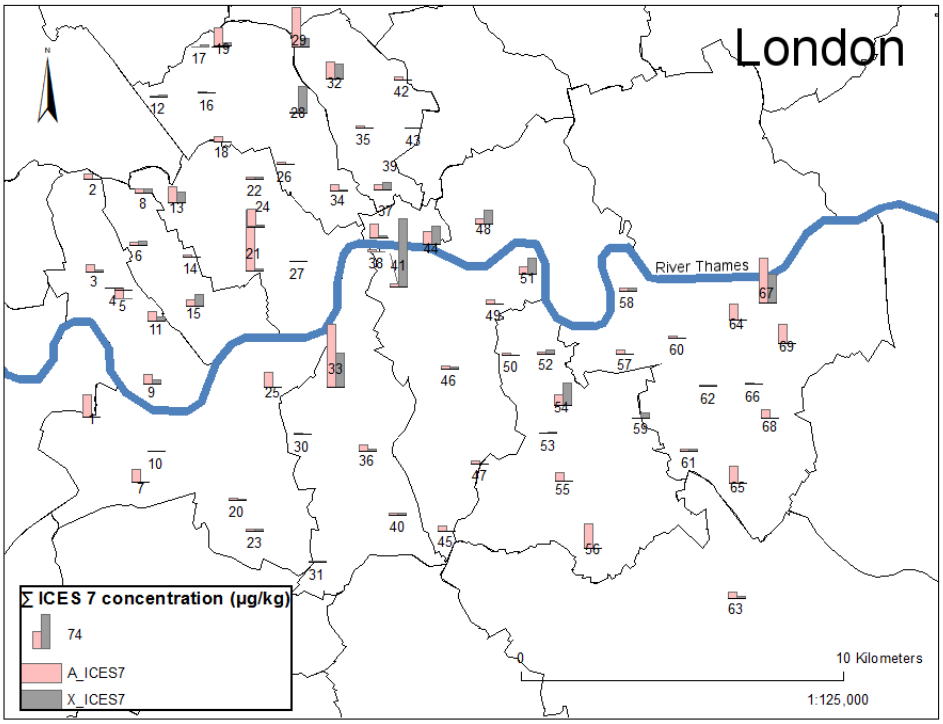

Figure 3: ∑ICES-7 concentrations recorded at each sampling sit

Table 7: Summary of PCB data recorded in Central London (µg/kg) Standard deviation is given in parenthesis.

All samples (n = 138)

	28	52	101	118	153	138	180	$\sum_{tri}$	$\sum_{tetra}$	$\sum_{penta}$	$\sum_{hexa}$	$\sum_{hepta}$	$\sum_{ICES-7}$
Minimum	0	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	35.15	10.33	109.57	45.41	43.67	62.72	18.44	35.15	10.33	127.06	106.39	18.44	148.72
Mean	3.14 (6.5)	0.66 (1.44)	4.28 (12.45)	2.65 (6.71)	1.32 (4.82)	2.40 (7.56)	0.67 (2.07)	3.14 (6.5)	0.66 (1.44)	6.93 (19.16)	3.71 (12.38)	0.67 (2.07)	15.14 (25.64)
Median	0.31	0.32	0.27	0.35	0.40	0.37	0.54	0.31	0.32	1.04	0.77	0.54	4.97
% of ∑ICES-7	20.71	4.43	27.16	18.38	8.69	16.73	4.38	20.71	4.43	45.78	24.53	4.38	100

X dataset – 0-5 cm depth (n = 69)

	28	52	101	118	153	138	180	$\sum_{tri}$	$\sum_{tetra}$	$\sum_{penta}$	$\sum_{hexa}$	$\sum_{hepta}$	$\sum_{ICES-7}$
Minimum	0	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	32.67	8.89	24.20	29.46	43.67	62.72	18.44	32.67	8.89	53.66	106.39	18.44	148.72
Mean	1.77 (4.94)	0.53 (1.20)	1.44 (4.18)	2.1 (5.03)	1.75 (6.23)	2.49 (8.38)	0.85 (2.92)	1.77 (4.94)	0.55 (1.20)	3.54 (8.78)	4.24 (14.19)	0.85 (2.92)	10.94 (23.22)
Median	0.31	0.32	0.39	0.35	0.40	0.37	0.54	0.31	0.32	0.62	0.77	0.54	3.23
% of ∑ICES-7	16.22	4.82	13.17	19.23	15.99	22.81	7.76	16.45	5.03	32.40	38.80	7.76	100

A dataset 5-20 cm depth (n = 69)

	28	52	101	118	153	138	180	$\sum_{tri}$	$\sum_{tetra}$	$\sum_{penta}$	$\sum_{hexa}$	$\sum_{hepta}$	$\sum_{ICES-7}$
Minimum	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Maximum	**35.15**	**10.33**	**109.57**	**45.41**	**22.57**	**42.71**	**0.54**	**35.15**	**10.33**	**127.06**	**49.45**	**0.54**	**135.81**
Mean	**4.56 (7.55)**	**0.80 (1.65)**	**7.12 (16.51)**	**3.20 (8.06)**	**0.88 (2.76)**	**2.30 (6.69)**	**0.49 (0.17)**	**4.56 (7.55)**	**0.80 (1.65)**	**10.32 (20.66)**	**3.19 (8.37)**	**0.48 (0.17)**	**18.83 (27.40)**
Median	**1.05**	**0.32**	**0.27**	**0.35**	**0.40**	**0.37**	**0.54**	**1.05**	**0.32**	**1.82**	**0.77**	**0.54**	**9.24**
% of ∑ICES-7	**23.76**	**4.21**	**35.68**	**16.95**	**4.67**	**12.20**	**2.54**	**23.76**	**4.21**	**54.82**	**16.93**	**2.54**	**100**

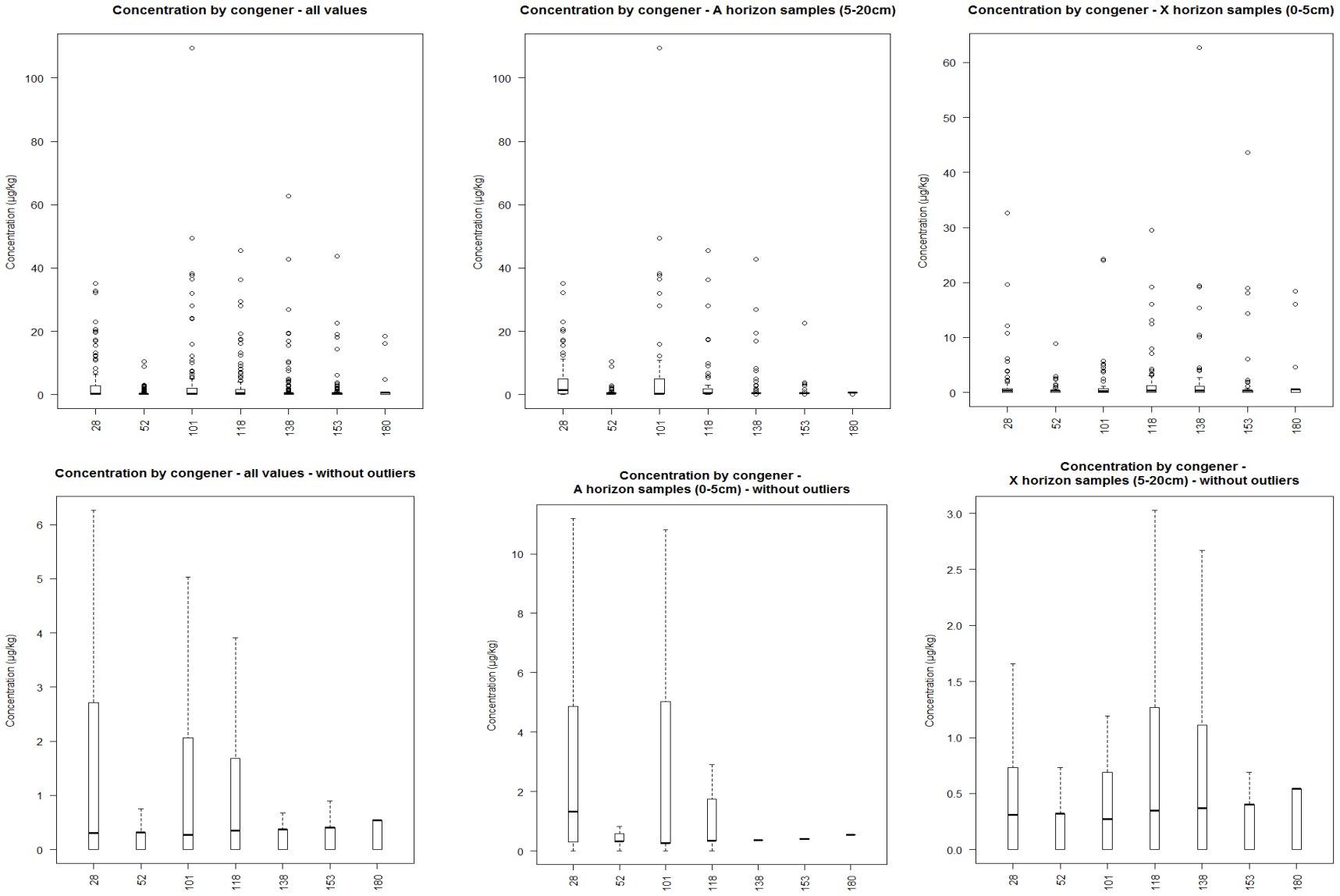

Figure 13: Concentration by congener. (L-R) All samples, A dataset values (5-20cm depth), X dataset values (0-5cm). With outliers (above), and with outliers removed (below).

4.3.2 Distribution of congeners and homologous series

Across all samples, ∑penta congeners formed the most dominant component of the ∑ICES-7 (45.8%), followed by ∑hexa (24.5%). ∑penta-hexa congeners made up 70.3% of the total ∑ICES-7 concentration. X dataset samples (0-5 cm depth) showed a more significant proportion of hexachlorinated congeners (∑penta: 32.4%; ∑hexa: 38.8% of ∑ICES-7), with ∑penta-hexa chlorinated compounds making up 71.2% of the ∑ICES-7 concentration. The A dataset samples (5-20cm depth) showed a more pronounced dominance of pentachlorinated congeners (∑penta: 54.8%).

Both soil datasets and the total dataset showed high concentrations of PCB 28 / ∑trichlorinated congeners compared to comparative studies (Table 11). Across all samples, the mean concentration was 3.1 µg/kg, representing 20.7% of the ∑ICES-7 profile, with a maximum value of 35.2 µg/kg. The X dataset mean value was 1.77 µg/kg, representing 16.22% of the ∑ICES-7, with a maximum value of 32.67 µg/kg. Within the A dataset, the mean value was 4.6 µg/kg, representing 23.8% of the ∑ICES-7, with a maximum recorded concentration of 35.2 µg/kg.

As this study, in common with many PCB surveys, focussed on the 7 congeners recommended for monitoring by the ICES, it is prudent to apply a risk assessment method which considers a sum ICES7 approach. One such approach is that of the Dutch VROM (Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieu/ Ministry of Housing, Spatial Planning and the Environment which specifies a target value of 20 µg/kg ∑ICES 7, to protect soil health over the long-term and to eliminate risks to humans and biota. An intervention value 1000 µg/kg is given as an indicator of perceived risk. None of the recorded ∑ICES 7 values were in exceedance of the intervention value. 27.54% *(n= 19)* of samples in the A dataset were in exceedance of the target value, a number which reduces to 15.94% *(n=11)* of samples in the X dataset. 8.7% *(n= 6)* of sample locations were in exceedance of the 20 µg/kg value in both A and X datasets.

4.3.3 Spatial dependence

Spatial correlation is suggested by the variogram output in samples of PCBs 28, 52, 101, 118, 138 and 153 in the ‘A’ dataset (Figure 14), and in 28, 52, 101, 118, 118, 138, 153 and 180 in the ‘X’ dataset

(Figure 15). Range values (an indicator of constant spatial dependence) vary between congeners. For the 'A' dataset range varied between 234 m to 1443 m, with a mean value of 721 m (with the PCB 180 variogram data omitted due to inadequate model fitting). This indicates dependence between values at data points located within the range value. The X dataset showed higher range values (837 m – 2565 m), with a mean value of 1396 m (Table 8).

These results, alongside similar results for ∑ICES 7 calculations, suggest a degree of spatial dependence which varies by congener.

The method was applied to a combined dataset, though this resulted in less clear variogram plots with larger nugget values, which typically indicates measurement error or variation at distances less than the sampling interval (Esri, 2017), though these potential errors are not identifiable in the individual datasets.

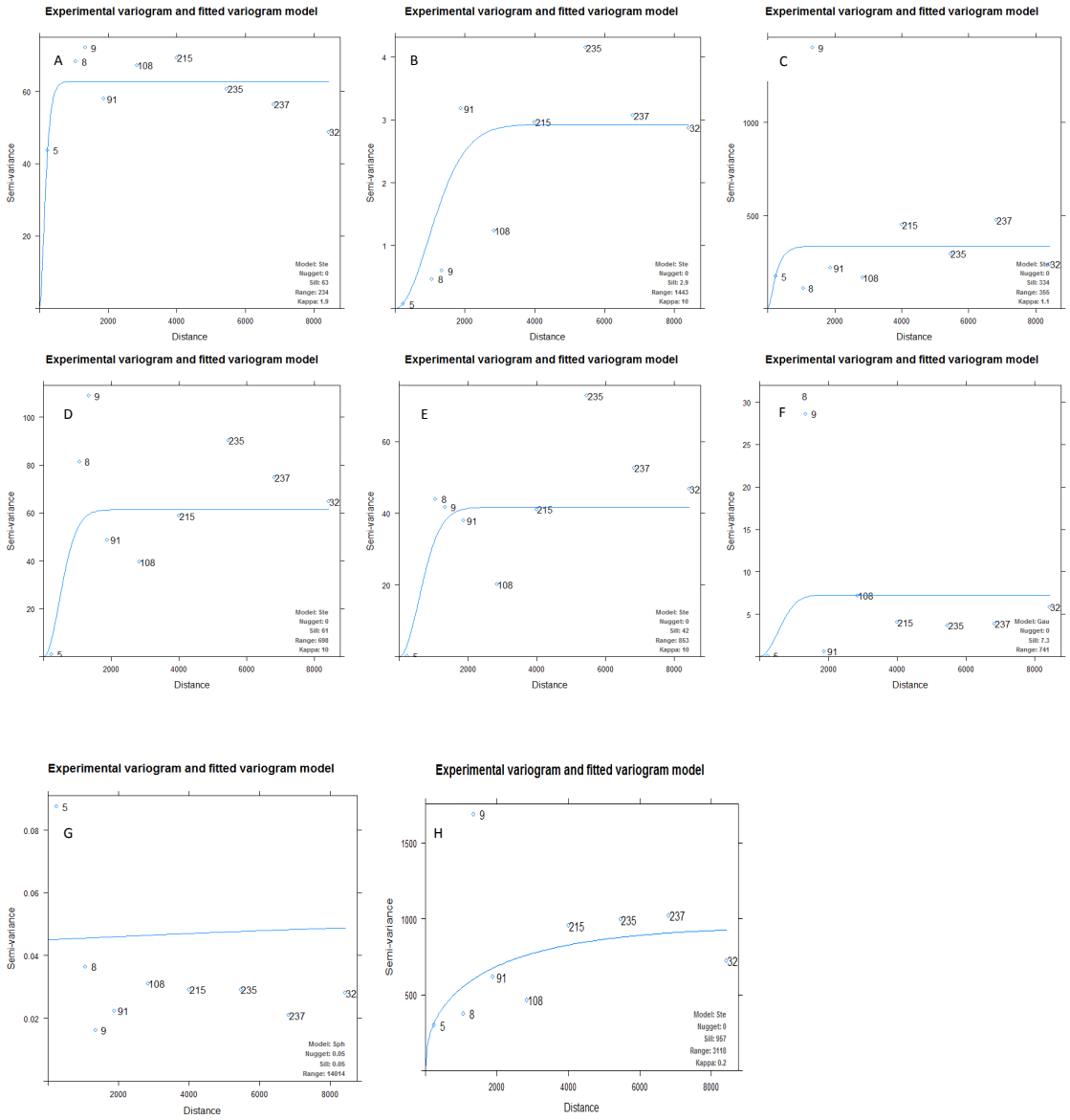

Figure 14: Variograms showing PCB 28 (A), 52 (B), 101 (C), 118 (D), 138 (E), 153 (F), 180 (G) and ∑ICES 7 (H) concentration (µg/kg) correlation with distance between data collection points (m) for Dataset 'A' (5 – 20 cm sample depth) samples.

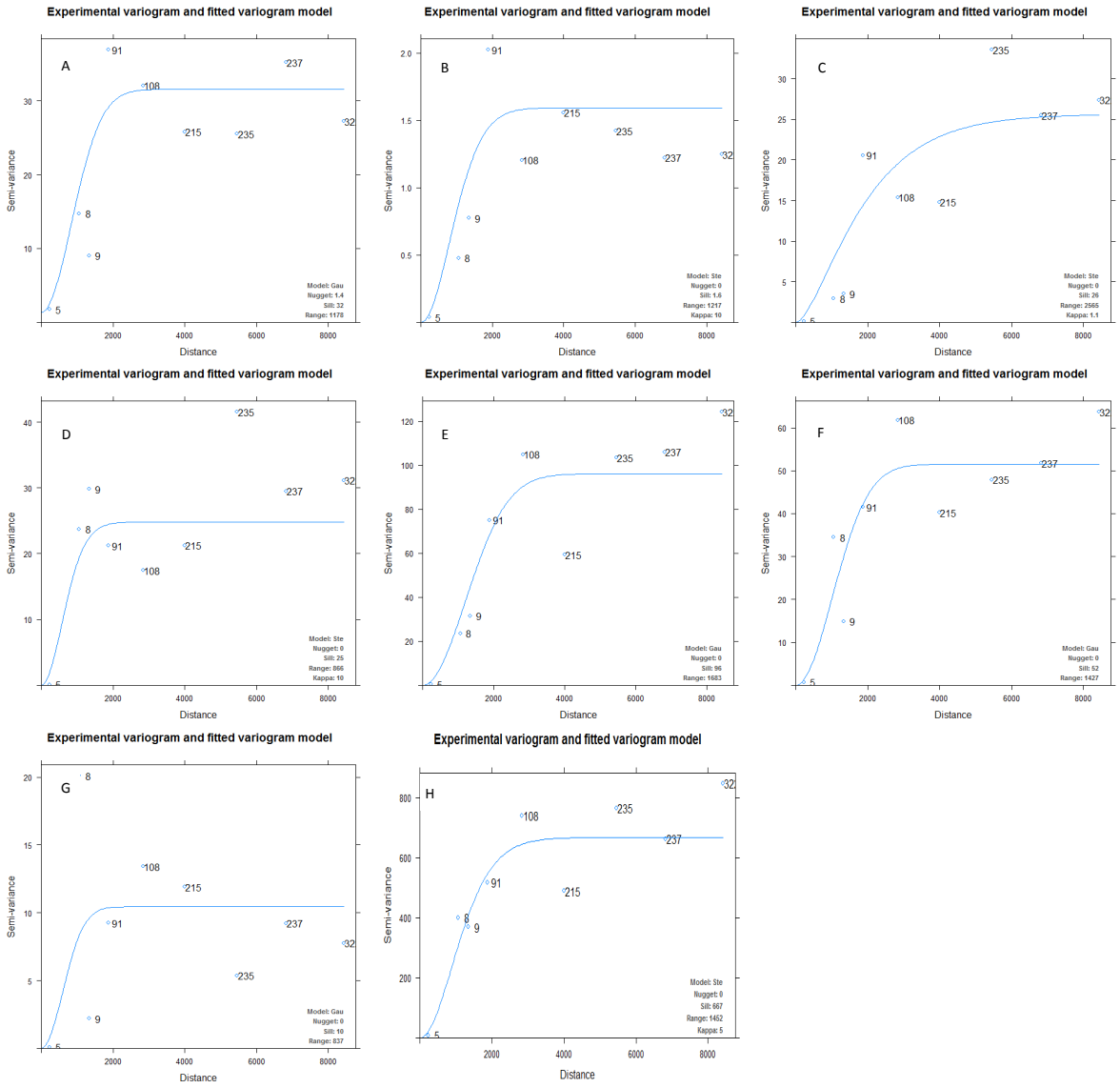

Figure 15: Variograms showing PCB 28 (A), 52 (B), 101 (C), 118 (D), 138 (E), 153 (F), 180 (G) and ∑ICES 7 (H) concentration (µg/kg) correlation with distance (m) for Dataset 'X' (0-5 cm sample depth) samples.

Table 8: Variogram parameters and model fitting methods (A and X datasets, combined datasets)

	Model	Nugget	Sill	Range (m)
A 28	Stein	0	63	234
A 52	Stein	0	2.9	1443
A 101	Stein	0	334	355
A 118	Stein	0	61	698
A 138	Gaussian	0	42	853
A 153	Stein	0	7.3	741
A 180	Spherical	0.05	0.05	14014
A ∑ ICES 7	Stein	0	957	3118
X 28	Gaussian	1.4	32	1178
X 52	Stein	0	1.6	1217

X 101	Stein	0	26	2565
X 118	Stein	0	25	866
X 138	Gaussian	0	96	1683
X 153	Gaussian	0	52	1427
X 180	Gaussian	0	10	837
X ∑ ICES 7	Stein	0	667	1452
Combined 28	Stein	42	47	1116
Combined 52	Gaussian	0.61	2.3	1386
Combined 101	Spherical	137	182	1001
Combined 118	Stein	0	43	214
Combined 153	Stein	12	29	2027
Combined 138	Stein	31	112	10254
Combined 180	Stein	0	5.2	120
Combined ∑ ICES 7	Stein	388	989	8413

4.3.4 Determination of site history and land use changes

Historical maps were examined to determine the historical context of the sample location points. Maps (Ordnance Survey County Series, 1:10,000 scale) dating from the 1920s to the present were examined to record any changes in land use, and any significant industrial activity in the vicinity of the data points. Location points were classified as 'Developed', 'Undeveloped' or 'Park land' for each of the historic periods, along with current land use. As anticipated, most sites have remained in a developed state throughout the examined period. For data points showing elevated levels of ∑ICES-7, or those with PCB 118 concentration above the residential SGV (8 µg/kg), evidence of land use changes or redevelopment within a radius of 500 m was examined to monitor the potential for re-emission from demolition or construction (Figure 16) using aerial photography in conjunction with Google Earth images and recorded in Table 10.

Table 9: Sample locations showing elevated PCB7 or PCB 118 concentrations in exceedance of the residential SGV (8µg/kg).

			PCB concentration (µg/kg)							
Sample ID	Map ID	Horizon	28	52	101	118	153	138	180	$\sum PCB_7$
654808	41	A	4.53	0.32	0.27	0.35	0.4	0.37	0.54	6.78
		X	0.31	1.48	23.98	16.01[a]	43.67	62.72	0.54	148.72[b]
650783	11	A	0.31	0.32	0.27	9.74[a]	2.85	4.97	0.54	19.01
		X	0.31	2.57	4.99	0.35	0.4	0.37	0.54	9.52
650174	44	A	2.72	0.32	5.68	9.1a	0.4	7.57	0.54	26.33
		X	32.67	0.32	0.27	3.18	0.4	0.37	0.54	37.76
655956	1	A	5.44	1.52	3.53	17.32[a]	3.79	16.91	0.54	49.06
		X	0	0	0	0	0	0	0	0.00
650376	29	A	32.17	1.74	4.59	28.09[a]	0.4	19.41	0.54	86.93[b]
		X	0.31	0.32	0.27	13.17[a]	0.4	4.56	0.54	19.57
653576	67	A	2.71	2.81	7.5	36.28[a]	22.57	26.88	0.54	99.28[b]
		X	2.06	2.43	5.72	19.15[a]	14.34	19.22	0.54	63.49[b]
652531	21	A	3.91	0.32	109.57	17.49[a]	0.4	0.37	0.54	132.60[b]
		X	0.31	0.32	0.27	1.27	0.4	0.37	0.54	3.48
654552	33	A	0.31	8.89	37.55	45.41[a]	0.4	42.71	0.54	135.81[b]
		X	0.31	0.32	24.2	29.46[a]	0.4	19.44	0.54	74.67[b]

Elevated values: [a] in exceedance of 8µg/kg residential SGV, [b] elevated $\sum PCB_7$ values.

Evidence of recent redevelopment and significant construction work was found at sample sites 41, 11, 44, 29, 67, 21, 33. Sample site 1 represented the only exception, with no legacy of industrial operations or significant demolition. The presence of construction and demolition raises the possibility of PCB contamination from re-emission.

Figure 16: Location of identified points of elevated concentration.

Table 10: Survey of construction and demolition activity in the vicinity of points of elevated concentration

Point	Region	Historical land use	Recent building work/ Redevelopment
21	Hyde Park/ Knightsbridge	Developed/ park land	Along South Carriage Drive. Land use has been largely static during all time scales. Perimeter of Hyde Park.
11	Earl's Court/ Hammersmith & Fulham	Developed	Demolition of nearby Earl's Court Exhibition Centre. Located in the vicinity of a Lillie Bridge London Underground depot, also due to be demolished.
1	Putney	Developed	None. Established residential suburb.
33	Stockwell	Developed	Significant redevelopment nearby, including Grantham Road and Union Road. Significantly urban in character. Approximately 2 km to the South East of the industrial Nine Elms area and Battersea Power Station.

41	Newington/ Elephant & Castle	Developed	Very significant in recent years, including large scale ongoing redevelopment at Elephant & Castle. Landmark developments such as the Strata SE1 building.
44	City of London	Developed	Very significant within the wider City of London. Construction evident within 500m of sample point, along Lower Thames Street. In the vicinity of many large developments including 20 Fenchurch Street and 30 St Mary Axe.
67	Woolwich	Developed	Significant in the local area. < 500 m from the site of Woolwich Power Station, demolished in 1978.
29	Upper Holloway	Developed	Some redevelopment around Archway station.

4.3.5 Relationship between TOC and PCB concentration

Percentage TOC values ranged from 1.75 - 11.85, with a mean value of 5.71. The relationship between ∑ICES-7 concentration and TOC was examined using a Pearson's correlation coefficient. Results showed no relationship (p=0.86) (Figure 17).

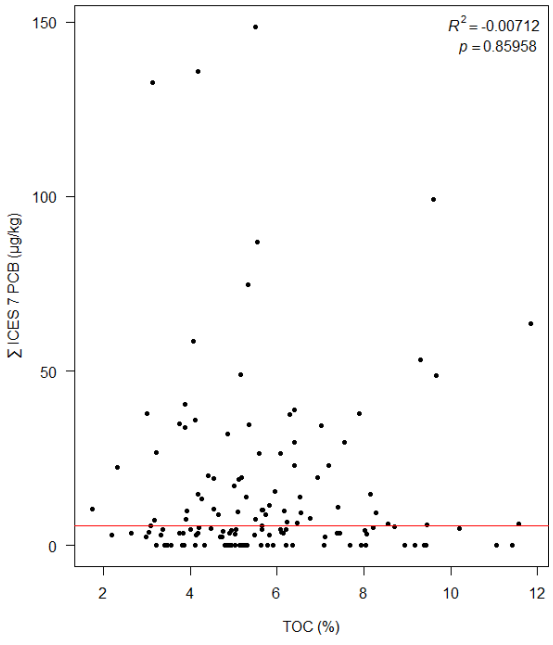

Figure 17: Plot of %TOC and ∑ICES-7 concentrations (all datapoints) (n=138)

4.3.6 Comparison with other studies

Although comparison between studies of PCB concentrations can be problematic due to differences in congeners analysed, mean ∑ICES-7 concentrations from this study are comparable with those from similar studies. Comparison with data obtained from similarly urban/ suburban environments show slight elevation in the London data (15.1 µg/kg) (Table 11). Mean concentrations from this study show lower levels than those recoded by Vane *et al.*. (2014) for East London soils. This may reflect the historically more industrially focussed character of the East London study area.

Median values show concentrations of ∑ICES-7 PCB (4.97 µg/kg). This value is in line with comparable mean values obtained by the Cachada *et al.*. (2009) survey of five major European cities, with higher median values were obtained for Glasgow (∑19: 22 µg/kg; ∑5: 9.4 µg/kg) and Torino (∑19: 14 µg/kg; ∑5: 6.6 µg/kg), and in the values recorded by Krauss & Wilcke in Bayreuth (∑12: 13 µg/kg) (Krauss and Wilke . Significantly, these sites represent regions of high levels of heavy industrial activity.

Median ∑ICES-7 values recorded in East London soils by Vane *et al.*. (2014) are similar to those recorded in this study (4.97 µg/kg). The median value is lower than the mean value in both studies, suggesting a large proportion of low concentration values in addition to the presence of elevated hotspots.

Table 11: PCB concentration from selected studies. Where required, data has been extracted for urban and suburban domains. The Rural domain has been disregarded where presented in the original study.

City	Study	Mean concentration (µg/kg)	Median concentration (µg/kg)		Land use
Central London	This study	15.1	5.0		Urban
East London	(Vane *et al.*., 2014)	22.0	4.9		Urban
England - rural	(Environment Agency, 2007b)	0.6	-		Rural
England - urban	(Environment Agency, 2007b)	1.8	-		Urban
Tarragona, Spain	(Nadal *et al.*., 2007)	4.4	-		Urban
Hong Kong	(Zhang *et al.*., 2007)	4.8	-		Urban
Glasgow	(Cachada *et al.*., 2009)	-	22.0[a]	9.4[b]	Urban
Aveiro, Portugal	Cachada *et al.*. 2009)	-	7.9[a]	2.6[b]	Urban
Ljubljana, Slovenia	Cachada *et al.*. 2009)	-	6.8[a]	2.1[b]	Urban
Uppsala, Sweden	Cachada *et al.*. 2009)	-	5.7[a]	2.3[b]	Urban
Torino, Italy	Cachada *et al.*. 2009)	-	14.0[a]	6.6[b]	Urban
Seine River basin	(Motelay-Massei *et al.*., 2004)	7.7	-		Urban/ suburban
Beijing	(Wu *et al.*., 2011)	3.15[c]	-		
Bayreuth, Germany	(Krauss and Wilcke, 2003)	-	13[d]		Urban

[a]∑19 PCB (congeners 1, 5, 18, 31, 44, 52, 66, 87, 101, 110, 138, 141, 151, 153, 170, 180, 183, 187, 206)
[b]∑5 PCB (congeners 52, 101, 138, 153, 180)
[c]∑6 PCB (congeners 28, 52, 101, 138, 153, 180)
[d]∑12 PCB (congeners 8, 20, 28, 52, 101, 118, 138, 153, 180, 199, 206, 209)

Comparisons between congener profiles can be drawn through normalisation of concentration values to PCB 153. A survey of five studies is presented in Table 12. The comparison shows elevated contributions of PCB 28, 101 and 118 in the normalised mean values recorded in this study, though application of the method to median values removed this effect. A relatively low mean concentration recorded for PCB 153 additionally contributes to the proportion of PCB 28, 101 and 118 shown in this comparison. Elevated concentrations of PCB 28 have been previously attributed to the leakage of PCB-bearing fluids from electrical equipment (Syed *et al.*. 2013), though there is no definitive evidence of this being the case in this data set. Additionally, lower-weight PCBs have been shown to accumulate in soils dominated by black carbon (Ali *et al.*. 2015), though no relationships were found in this study between TOC and ∑ICES-7 PCB or PCB 28 concentration.

Review of congener profiles across multiple studies shows considerable variance between values for similar land uses (Table 11), reflecting the environmental legacy of land use on soil chemistry.

Table 12: Congener concentrations normalised to PCB 153 concentration.

Location	Paper	28	52	101	118	153	138	180
Central London	This study (mean values)	2.38	0.51	3.11	2.11	1	1.92	0.50
Central London	This study (median values)	0.78	0.80	0.68	0.88	1	0.93	1.35
East London	Vane *et al.*. (2014)	0.16	0.59	0.65	0.41	1	1.16	0.27
Seine River Basin (Urban)	Motelay-Massei *et al.*. (2004)	1.06	0.59	n.d.	1.95	1	1.57	0.25
Seine River Basin (Suburban)		n.d.	0.69	0.37	0.65	1	0.29	1.17
Aveiro, Portugal	Cachada (2009)	n.d.	0.48	0.3	n.d.	1	n.d.	0.6
Glasgow, UK		n.d.	0.9	0.76	n.d.	1	n.d.	0.77
Ljubljana, Slovenia		n.d.	0.38	0.27	n.d.	1	n.d.	0.64
Torino, Italy		n.d.	0.21	0.48	n.d.	1	n.d.	0.6
Uppsala, Sweden		n.d.	0.28	0.28	n.d.	1	n.d.	0.69
Tarragona, Spain	Nadal *et al.*. (2007)	0.04	0.03	0.19	0.17	1	0.74	1.70

4.3.7 Calculation of normal background concentration (NBC) values

Recent methods have been developed to calculate NBC values from environmental datasets (Ander *et al.*., 2013). These methods have been applied successfully to inorganic contaminants in soil, such as Pb, Hg, Ni and Cd, with the purpose of defining the upper limit of 'normal' concentration. The method defines the upper limit to be the upper 95% confidence limit of the 95th percentile of the

dataset. The methodology was developed in order to measure background concentrations in regions affected by elevated natural levels of a contaminant, such those affected by geogenic As. The method has been used to generate NBC values for BaP and ∑16PAH in previous studies (Vane *et al.*., 2014), and here is applied to a PCB dataset. The NBC values given in this study assume a 'normal' background value for PCBs, though this may not be a realistic measure in soil contaminants of a purely anthropogenic nature, so NBCs are issued with caution.

The NBC given for PCB 118 is compared with the Environment Agency residential SGV for dioxins, furans and dioxin like PCBs of 8 µg/kg (Environment Agency, 2009b). The NBC of 6.9 µg/kg does not exceed the value for allotments and residential sites, and falls far below the value of 240 µg/kg for commercial sites.

Table 13: Calculated NBC values. Values calculated in Vane *et al.*. (2014) are given for comparison.

PCB congener/ summed set of congeners	NBC (µg/kg) (this study)	NBC (µg/kg) (Vane *et al.*., 2014)
PCB 28	8.1	1.8
PCB 52	0.61	2.9
PCB 101	5.6	7.8
PCB 118	6.9	4.4
PCB 138	0.58	10.5
PCB 153	0.65	9
PCB 180	0.78	4.7
$\sum^{ICES-7}$	52	180
$\sum^{3-Cl}$	8.1	22
$\sum^{4-Cl}$	0.61	20
$\sum^{5-Cl}$	20	48
$\sum^{6-Cl}$	1.4	66
$\sum^{7-Cl}$	0.78	73

Table 13 gives the calculated NBC values from this study, alongside those calculated using the same method and presented in Vane *et al.*. (2014), from East London sites. NBC levels are generally below the mean values detected (with the exception of PCB 28). This appears to be due to the necessity to remove outlier values prior the calculation of NBC, resulting in a drastically reduced mean value. This is supported by the lower median values shown in this study (Table 7), a phenomenon which has been identified in similar soil PCB surveys (Table 11). As PCB distribution frequently follows a pattern of point source emission, and there is no elevated natural, or geogenic background, this casts doubt on the legitimacy of the method in PCB analysis. Despite this, calculation of PCB 'background' values are useful in order to assess regional variations PCB levels, and enable a greater understanding of the legacy of industrial contamination in urban areas, and comparison between locales.

4.3.8 Standard recovery

Extraction efficiency was measured using the recovery standard methods described in 4.2.3. In summary, a recovery standard solution (50 µL) of PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL) were applied to each soil sample before the ASE extraction. A second set of analytical standards were introduced prior to GC/MS analysis via a solution (100 µL) of PCB 29 (9.47 ng/µL) and PCB 157 (9.53 ng/µL). Quantification of these standards was performed through analysis using a suite of internal standards compounds - PCB 34 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL) and PCB 173 (18.81 ng/µL). The concentrations of the standards were then compared and recoveries calculated.

Percentage recovery of the standards was as follows (Relative standard deviation in parenthesis):

Table 14: Standard recovery (%), London soil ASE samples.

PCB 19	PCB 147
55.24% (50.40%)	n.d.

The decision was taken to not correct the data on the basis of standard recovery. This is due to the limited range of standards available, which do not reflect the varied physicochemical properties of the wider PCB compound group. It would be possible to apply standards to each of the ICES 7 compounds, though this would significantly increase the cost of extraction and complexity of the analysis. Additionally, variations in standard recover reflect the complexity of working with very small samples of pure PCB material. PCB 147 recovery was hindered due to non-detect values in GC/MS. These results, combined with the high variability of PCB 19 recovery, suggest that standard concentrations were too low, and should be significantly increased in subsequent extractions.

This data is presented uncorrected due to the limitations posed by the standard protocol. Correction may be possible in future tests, but standards must be applied to all congener chlorination groups. Although this technique would add clarity, it would significantly increase the cost and complexity of the extractions.

4.4 Conclusions

Soil PCB concentrations were measured at 2 depths, across 69 sites in Central London. Findings include:

(i) $\sum^{ICES-7}$ concentrations ranged from 0 – 148.72 µg/kg across all values, with a mean of 15.14 µg/kg. Concentrations of $\sum^{ICES-7}$ at 0-5 cm depth ranged from 0- 148.72 µg/kg, with a mean of 10.94 µg/kg. Concentrations of $\sum^{ICES-7}$ at 5-10 cm depth ranged from 0- 135.81 µg/kg, with a mean of 18.83 µg/kg.

(ii) The congener profile is dominated by the penta- and hexa- chlorinated biphenyls (46% and 25% of $\sum^{ICES-7}$).

(iii) PCB 101 recorded the highest mean value, at 4.3 µg/kg. The maximum PCB 101 value was 109.6 µg/kg.

(iv) Median values show that the dataset is dominated by a small number of elevated values.

(v) Demolition and subsequent re-emission of PCBs is a potential source of peak values.

(vi) Mean concentrations are typically higher than in other surveyed urban areas. Mean values are lower than a previous study in East London, though reflect continuity.

(vii) NBC calculations appear were performed, but may not reflect true values. This is due to the nature of the dataset, which is dominated by a small number of elevated values. This may have implications for use of the NBC method in further PCB work.

(viii) TOC did not influence PCB concentration. It is likely that redistribution due to meteorological effects and diffuse sources have masked any changes in sorptive processes, though TOC may influence PCB behaviours at higher concentrations.

(ix) Mean and NBC values of the dioxin like compound PCB 118 fell below the residential SGV (mean value: 2.8 µg/kg, NBC: 6.9 µg/kg, residential SGV: 8 µg/kg), although this value was exceeded at 11 data points, across 8 locations. Additionally, PCB 118 represents only one of many dioxin-like PCBs, furans and dioxins which may be present in an urban soil, so should be considered relevant to further studies and risk assessments.

(x) Variogram analysis suggests potential spatial correlation, though correlations were clear only in isotropic variogram plots, suggesting a low degree of anisotropy between all congeners.

(xi) Data were compared to VROM Dutch assessment criteria in order to assess the impact of ∑ICES 7 congener levels on soil quality and sustainability indicators. In all cases, samples fell below the 'intervention value' of 1000 µg/kg, though 22% (30 out of 138 total samples) were in exceedance of the 'target value' of 20 µg/kg.

PCB values were typically low across the study area, though the data set is dominated by a small number of elevated values. A definitive explanation for the elevated values is open to discussion, though re-emission of PCBs from recent demolition and building activity is a potential source,

alongside the transport of wind-blown contaminants from historic sources such as incinerators, power stations and heavy industrial point sources.

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CHAPTER 5: THE APPLICATION OF AN *IN VITRO* GASTROINTESTINAL MODEL TO ASSESS BIOACCESSIBILITY IN SOIL-BOUND PCBS

5.1 Introduction

As an alternative to analysing the total concentration of a contaminant in a soil sample, bioaccessibility is gaining increasing prominence as a suitable method for assessing potential harm from contaminated soils and sediments to humans (Collins *et al.*., 2015). Methods have been developed that allow the determination of oral bioaccessibility to humans – the fraction that has entered solubility in the gastro-intestinal fluids prior to transfer of xenobiotics into the bloodstream, such as As, Sb, Cd, Cu, Zn, Ni, Cr and Pb (Denys *et al.*., 2012a; Cave *et al.*., 2016), brominated flame retardants (Abdallah *et al.*., 2012), pesticides (Tao *et al.*., 2011; Shi *et al.*., 2017) and PAHs (Tilston *et al.*., 2011; Lorenzi *et al.*., 2012). This study presents PCB (polychlorinated biphenyl) bioaccessibility data obtained using the FOREhST method, which has been specifically designed to simulate oral bioaccessibility in organic contaminants (Cave *et al.*., 2010; Lorenzi *et al.*., 2012).

Particularly amongst young children, ingestion of soil is frequently regarded as a key exposure pathway for potentially harmful xenobiotics (Chaney *et al.*., 1996), with studies assessing the typical consumption of soil to be 30 – 200 mg/day via hand to mouth behaviour alone (van Wijnen *et al.*., 1990), increasing with pica behaviour to an estimated 1000 – 5000 mg per day (Centre for Disease Control and Prevention, 2011). The field of bioaccessibility testing has developed as a response to the demand for better understanding of the ingestion pathways, and to provide a more physiologically relevant assessment of risk from soil contaminants (Juhasz *et al.*., 2007), and can contribute as part of a robust body of evidence in human health risk assessment and policy formation (BARGE, 2016). This study aims to apply bioaccessibility testing to assess the behaviour of a distinct group of organic contaminants associated with former industrial sites, which have been found to present a significant health risk.

PCBs are a group of organochloride compounds frequently identified as a key legacy industrial pollutant, one of twelve persistent organic pollutant groups (POPs) described by the Stockholm Convention (Xu *et al.*., 2013). The Convention recognises PCB contamination as an ongoing area of concern, identifying the target of 'environmentally sound management' of global PCB usage and waste by 2028 (Stockholm Convention, 2008), with the PCB Elimination Network (PEN) established in 2009 to assist in the delivery of this aim. PCBs represent a challenge to developers and land quality assessors as they are characterised by their widespread ubiquity, particularly in former industrial, urban sites (Krauss and Wilcke, 2003; Davis *et al.*., 2007; Vane *et al.*., 2007; Cachada *et al.*., 2009; Vane *et al.*., 2014). They are typified by their bioaccumulative potential, resistance to physical, chemical and enzymatic breakdown (Zhang *et al.*., 2012) and risk to human health (Tilson and Kodavanti, 1998; Kodavanti *et al.*., 2001; Aminov *et al.*., 2013), and classified as Group I carcinogens by the International Agency for Research on Cancer (IARC) (Lauby-Secretan *et al.*., 2016).

Bioaccessibility testing represents a unique opportunity to introduce physiologically based evaluation into risk assessment. In addition, the development of lab-based methods allow researchers to refocus away from animal-based testing, with the added costs and ethical questions raised, as encouraged in such measures as the EU REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) program, which aims to limit the use of animal testing in toxicity assessment (Schoeters, 2010). However, despite these efforts, validation with an animal model remains the 'gold standard' measure of *in vitro* model performance, and *in vivo* derived bioavailability data should be taken into consideration when assessing the relevance of *in vitro* derived bioaccessibility values (Collins *et al.*., 2015).

5.2 Methodology

5.2.1 Soils

34 soils, which had previously been analysed and studied as part of a companion *in vitro* bioavailability study, were extracted using the FOREhST method (Cave *et al.*., 2010) to determine bioaccessibility the full procedure is detailed below. PCB concentrations had been previously

determined using an ASE method as described in Delannoy *et al.*., (2015). This enables the potential comparison of bioaccessibility with initial concentration and bioavailability data. The soils were collected as part of a previous study investigating soil-PCB bioavailability using a swine model. Samples were taken during 2013 from five sites located in France, identified from the BASOL French public database of contaminated land. Samples were taken from a soil depth of 5 cm, freeze-dried and sieved to 500 µm (Delannoy *et al.*., 2015).

5.2.2 Extraction vessel

The extraction vessel used in the FOREhST procedure was replaced following initial tests showing reduced analyte recovery (analyte concentration was below the limit of detection), which was attributed to a failure to sufficiently combine solutions by mixing, combined with an accumulation of hydrophobic compounds in oil droplets, becoming retained in the glassware as a result of the narrow aperture of the vessel. A replacement was sourced, which has a wider bore (28 mm) than the previous vessel (10 mm). This replacement was the Pyrex 8422-100 100 mL round-bottom tube (Cole-Palmer, Vernon Hills, USA). A PTFE liner was cut to fit the screw-on cap. The replacement vessel allows more complete removal of supernatant. The former and current glassware designs are shown in Figure 18. The replacement vessel not only aids the transfer of supernatant, but allows for the use of reusable screw caps in place of crimp fittings.

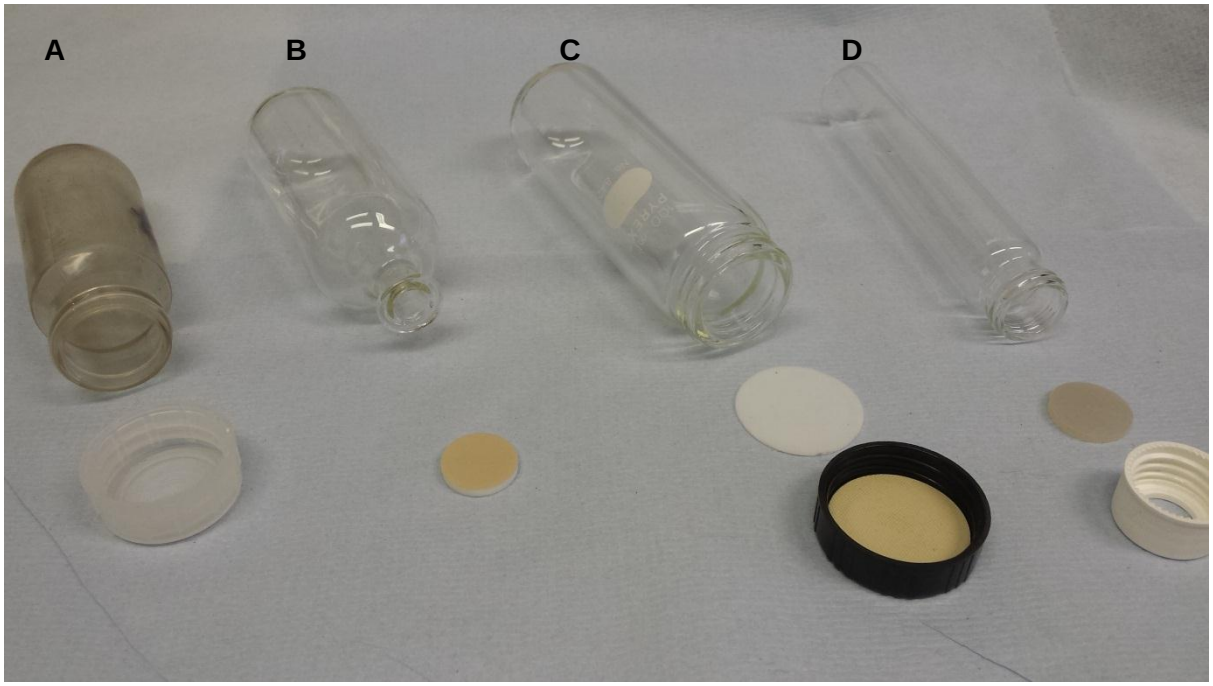

Figure 18: (L-R) Standard Nalgene UBM vessel (A); FOREhST vessel with narrow aperture, PTFE lined septum, crimp cap (not pictured) (B); updated FOREhST vessel with wider diameter aperture, PTFE liner, screw cap (C); 60 mL collection vial, PTFE lined setptum, screw cap (D).

5.2.3 Preparation of simulated digestive solutions

In preparation for the procedure, simulated digestive fluids are prepared (saliva, gastric, duodenal and bile). In the case of each of the fluids, reagents are mixed in separate 'organic' and 'inorganic' 500 mL flasks with Milli-Q (Merck, Darmstadt, Germany) de-ionised water, and combined into a 2 L Nalgene container in preparation for use. Reagents are listed in Table 15.

Table 15: Reagents used in preparation of simulated gastrointestinal fluids.

	Saliva	Gastric	Duodenal	Bile
	Reagent	Reagent	Reagent	Reagent
Inorganic component (concentration in 500 mL flask)	Potassium chloride (1792 mg/L) (VWR); Sodium phosphate (1776 mg/L) (VWR); Potassium thiocyanate (400 mg/L) (VWR); Sodium sulphate (1140 mg/L) (VWR); Sodium chloride	Sodium chloride (5504 mg/L) (VWR); Sodium phosphate (533 mg/L) (VWR); Potassium chloride (1649 mg/L) (VWR); Calcium chloride (799 mg/L) (VWR); Ammonium chloride (612 mg/L) (VWR);	Sodium chloride (14024 mg/L (VWR)); Sodium bicarbonate (11214 mg/L) (VWR); Monopotassium phosphate (160 mg/L) (Sigma Aldrich); Potassium chloride (1129 mg/L)	Sodium chloride (10518 mg/L) (VWR); Sodium bicarbonate (11570 mg/L) (VWR); Potassium chloride (753 mg/L) (VWR); Hydrochloric acid – 37% (180 µL/L) (VWR)

	(596 mg/L) (VWR); Sodium hydroxide (244 mg/ L) (VWR);	Hydrochloric acid - 37% (6.5 mL/L) (VWR)	(VWR); Magnesium chloride (100 mg/L) (VWR); Hydrochloric acid - 37% (180 µL/L) (VWR)	
Organic component (concentration in 500 mL flask)	Urea (400 mg/L) (Sigma Aldrich)	Glucose (1300 mg/L) (Sigma Aldrich); Glucoronic acid (40 mg/L) (Sigma Aldrich); Urea (170 mg/L) (Sigma Aldrich); Glucosamine hydrochloride 660 (mg/L) (Sigma Aldrich)	Urea (200 mg/L) (Sigma Aldrich)	Urea (500mg/L) (Sigma Aldrich)
Additional reagents (concentration in 1 L carboy)	Amylase (290 mg/L) (Sigma Aldrich); Mucin (25 mg/L) (Sigma Aldrich); Uric acid (15 mg/L) (Sigma Aldrich)	Bovine serum albumin (1000mg/L) (Sigma Aldrich); Mucin (9000 mg/L) (Sigma Aldrich); Pepsin (2500 mg/L) (Sigma Aldrich)	Calcium chloride (200 mg/L) (VWR); Bovine serum albumin (1000 mg/ L) (Sigma Aldrich); Pancreatin (9000 mg/L) (Sigma Aldrich); Lipase (1500 mg/L) (Sigma Aldrich)	Calcium chloride (222 mg/L) (VWR); Bovine serum albumin (1800 mg/L) (Sigma Aldrich); Bile (30000 mg/L) (Sigma Aldrich)
pH	6.8 (+/- 0.5)	1.4 (+/- 0.5)	8.1 (+/- 0.2)	8.2 (+/- 0.2)
pH (combined solutions)	Saliva: gastric (1:2) 1.6 (+/- 0.2)			
	Saliva: gastric: duodenal: bile (1:2:2:1) (v/v/v/v) 6.0 (+/- 0.75)			

5.2.4 FOREhST procedure

Containers of pre-prepared simulated salivary, gastric, duodenal and bile fluids were placed into the heated water bath to bring them to a temperature of 37° C in preparation for the procedure. They were then shaken, and pH recorded. If the target pH were not observed (Table 15), adjustments were made using HCl (60% v/v) or NaOH (10 M). Solution pH measurements were recorded from individual fluids and prepared mixes, representing different stages of the process (saliva + gastric; saliva + gastric + duodenum + bile). The salivary and gastric solutions were then returned to the water bath to maintain temperature, and the duodenal and bile fluids set aside.

The extraction vessel was loaded with a 0.3 g sample of the soil to be tested, 0.813 g HiPP Organic Creamy Porridge (Hipp UK Ltd, Reading, UK) and 50 µL of sunflower oil (Morrisons, Ltd, Bradford, UK). The addition of components was checked using a laboratory balance, and equipment cleaned with acetone between handling samples. A blank sample, prepared without a soil sample but with all other vessel components, was prepared alongside other samples. A 50 µL surrogate standard solution of PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL) was then added, before 2.45 mL Milli-Q de-ionised water, and 4.5 mL of the prepared saliva solution. The lids of the sample containers were then screwed tight with a PTFE liner, and turned end-over-end, fully submerged in the water bath for five minutes. The vessels were then removed, opened, and 9 mL of simulated gastric solution added. The vessels were once again sealed, submerged and turned end-over-end in the water bath for a further two hours, representing the 'stomach' phase of the procedure. Once two hours had elapsed, the duodenal and bile fluids were placed into the water bath to raise temperature in preparation for use. The FOREhST vessels were removed and re-opened. 9 mL simulated duodenal fluid and 4.5 mL simulated bile fluid were added, and the pH of the solution recorded. If the solution pH failed to meet the target of 6.0 (+/- 0.75), hydrochloric acid (HCl) or sodium hydroxide (NaOH – 10.0 M) was added, dropwise, to correspondingly lower or raise the value accordingly. The vessels were then sealed for a final time, submerged and turned end-over-end in the water bath for a further 2 hours, representing the 'intestinal' phase. At the end of the process, the vessels were removed and placed directly into the centrifuge, and subjected to centrifugation at 3500 g for five minutes, separating the suspended solids from a distinct supernatant solution of approximately 30 mL. The supernatant was retained in a pre-cleaned 60 mL collection vial and sealed with a PTFE lined cap for further analysis. The pellet was discarded.

In preparation for the clean-up procedure, the supernatant was vortex mixed for 30 seconds to ensure homogenisation, and 6 mL sub-sampled into a further pre-cleaned 60 mL collection vial. A 100 µL recovery standard spike of PCB 29 (9.47 ng/µL) and PCB 157 (9.53 ng/µL) were added to monitor the performance of the clean-up process.

To ensure quality control, each soil was extracted in triplicate, and extracted as intra-run replicates. Each run of the FOREhST process contained 18 soil samples, interspersed with an EU approved recognised CRM with known PCB concentrations (industrial soil BCR 481), and a method blank containing the full FOREhST solution, food element and standards. CRM consistency was measured, with bioaccessibility data presented in the Supporting Information. A total of 7 runs of the method were performed, with three inter-run replicates of each sample included. All glassware was pre-cleaned using a H_2CrO_4 method consisting of a 24 hour acid soak followed by a deionised water rinse and air drying. and HPLC grade solvents were used throughout.

Additional blanks were introduced immediately prior to GC/MS. All blanks produced results that were below the limit of detection.

5.2.5 Supernatant clean-up

5.2.5.1 Saponification

The alkaline saponification method applied has been previously used to successfully isolate PAHs from the FOREhST supernatant (Cave *et al.*., 2010) through the breakdown of interference materials such as humic acids and methyl esters. A solution of methanolic potassium hydroxide (KOHMe) (6 M) was prepared by adding 84 g of KOH to 250 mL of methanol, mixing gently to encourage dissolution of the pelleted KOH, and 30 mL of the KOHMe solution was added to the 6 mL subsample of each FOREhST supernatant. Samples were then heated at 100°C for one hour, removed from the oven and gently turned, shaken and returned to the oven for a further 30 minutes. The samples were then removed from the oven and allowed to cool. The resultant solution was bronze coloured, semi opaque, and contained a crude soap, produced as a result of the saponification technique. Once cooled, the samples were prepared for liquid/liquid extraction.

5.2.5.2 Liquid/liquid extraction

A liquid/liquid extraction technique was employed In order to transfer the target PCB compounds from the saponified FOREhST solution into hexane (C_6H_{14}), in preparation for analysis. The saponified samples were transferred to 100 mL flasks and combined with 60 mL of de-ionised Milli-Q water.

Four mL of hexane were added, the flasks vigorously shaken for 30 seconds and allowed to settle. The hexane phase was then removed via a Pasteur pipette and retained. A further 3 mL of solvent was added, the shaking and settling procedure repeated, hexane retained, and repeated. A total of 10 mL hexane was retained. The remaining FOREhST solution was removed and disposed of safely. The hexane phase was evaporated under a gentle stream of nitrogen to 0.5 mL.

5.2.5.3 Chemical Drying

The hexane sample was chemically dried using a Na_2SO_4 method. A clean glass Pasteur pipette, fitted with glass wool wadding and filled with Na_2SO_4 sorbent to form a column, was prepared (Figure 19). The column was conditioned with 1 mL hexane, which was allowed to drip through the column under gravity, without the use of a vacuum manifold. Once the hexane had fully dripped through the column, the 0.5 mL hexane containing the target PCBs was introduced to the column. Once again, the hexane was permitted to drip freely through the column without the use of a vacuum manifold, and collected. 1 mL of hexane was added to the empty vial which contained the sample. This was then added to the column and the elute collected. This procedure was repeated for a further 2 washes. The resulting hexane sample is then concentrated by evaporation under a gentle stream of nitrogen to 0.5 mL, in preparation for solid phase extraction.

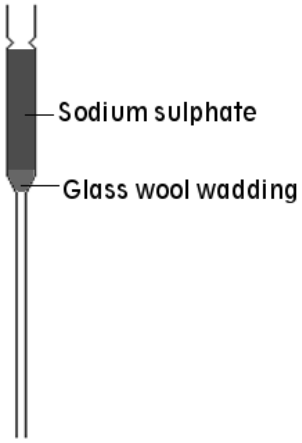

Figure 19: Sodium sulphate (Na_2SO_4) column used for sample drying.

5.2.5.4 Solid Phase Extraction

A glass column was prepared consisting of a clean glass pipette fitted with a wadding of glass wool, and filled with equal quantities of Silica (SiO_2) and Aluminium Oxide (Al_2O_3) (Figure 20). The column was conditioned with 2 mL hexane, which was allowed to fully drip through the column, and discarded. The 0.5 mL PCB-laden hexane was then introduced to the column and allowed to drip through under gravity. The eluted hexane was discarded as the PCB analytes were retained within the column matrix. Nine dichloromethane aliquots of 0.9 mL were then introduced to the column and gradually allowed to elute through the column under gravity, and collected in a clean vial. The sample was then concentrated under a gentle stream of nitrogen to 1.0 mL and a known volume and concentration of analytic standards added, allowing comparative analysis of recovery standards and analytes. If low concentrations are expected, further concentration of the sample to 200 µL is advised at this point, prior to GC/MS analysis.

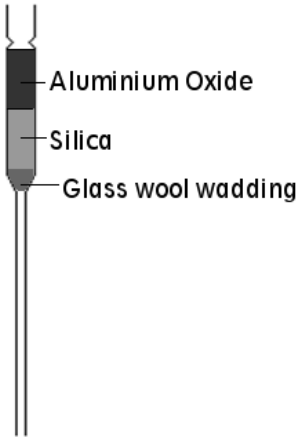

Figure 20: Prepared solid phase extraction column. Use of a standard Pasteur pipette is more cost effective than disposable SPE cartridges.

5.2.6 Analysis

Gas chromatography/ mass spectrometry (GC/MS) analysis was performed using a Fisons GC8000 gas chromatograph coupled to a Fisons MD800 operating in full scan mode (ionisation energy 70 eV, mass range 39-600 amu), as described in Vane *et al.*., (2007). The GC was equipped with a Varian FactorFour VF-5s fused silica capillary column (60 m length, 0.32 mm inner diameter, 0.25 µm film thickness). The inlet was at a temperature of 280°C, and the detector at 250°C. The GC oven was

temperature programmed from 100 °C (1 min isothermal) to 200 °C (at 5 °C/min) and then to 280 °C (at 2.4 °C/min) and held isothermally for 20 mins before ramping again to 300 °C (10 °C/min). Helium was used as the carrier gas at 16 psi. Quantification of PCBs was by selected ions (256.0, 292.0, 326.0, 360.0, 394.0). Data was collected and chromatograms integrated using the Xcalibur 2.0.7 software package (Thermo Fisher Scientific Inc., 2007).

5.3 Results

5.3.1 Soil PCB concentrations

Total soil ∑ICES-7 PCB concentrations ranged from 0.64 µg/g to 1882.62 µg/g. Individual congener concentrations varied, but followed a consistent congener profile dominated by hexachlorinated PCBs (congeners 138 and 153) (Table 16). In 26 of the soils PCB 28 concentrations were found to be below the level of quantification. ∑ICES 7 concentration in excess of the Dutch VROM 'intervention value' of 1000 µg/kg, was evident in all but one of the soils. All samples were in exceedance of the VROM soil 'target value' of 20 µg/kg, and are in excess of typical concentrations of PCBs ubiquitous in the environment (∑7 (England), urban: 1.77 µg/kg, rural: 0.63 µg/kg) (Environment Agency, 2007b). In all samples, the concentration of PCB 118 is in exceedance of the Environment Agency residential and allotment SGV (8 µg/kg) for dioxins, furans and dioxin like PCBs (8 µg/kg), 87.5% of the samples were in exceedance of the Commercial SGV (240 µg/kg).

Table 16: PCB concentrations of the tested soils

Congener	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑ICES-7
Mean concentration (µg/g) (RSD - %)	0.65 (322.75)	1.33 (158.76)	21.55 (45.63)	4.25 (148.75)	95.22 (215.69)	55.04 (204.39)	67.56 (213.28)	245.58 (210.30)
Proportion of total (%)	0.33 (355.17)	4.75 (110.84)	14.11 (58.22)	7.14 (91.68)	32.05 (21.81)	22.62 (14.59)	19.00 (54.84)	100 (0)

(RSD)

5.3.2 TOC

The potential influence of TOC on POP sorption dynamics has been well documented, with sorption of HOC strongly dependent on organic content within the soil matrix, and showing clear influence (Tang *et al.*., 2006a). However, this effect has been shown to be diminished by meteorological and geochemical processes (Vane *et al.*., 2007; Environment Agency, 2007b). TOC showed wide variability between samples, ranging from 0.05% to 25.73 % (RSD = 98.15%). No clear relationship was identified between TOC and ∑ICES 7 PCB concentration (Pearson's product correlation coefficient p = 0.86) (Figure 21). Similarly, bioaccessibility data showed TOC to have no influence on oral bioaccessibility in this dataset (Pearson's product correlation coefficient p = 0.86) (Figure 22). It can therefore be concluded that, in this group of samples, PCB input has a greater role in soil PCB concentration than TOC, over-riding the effect of TOC on sorptive behaviours.

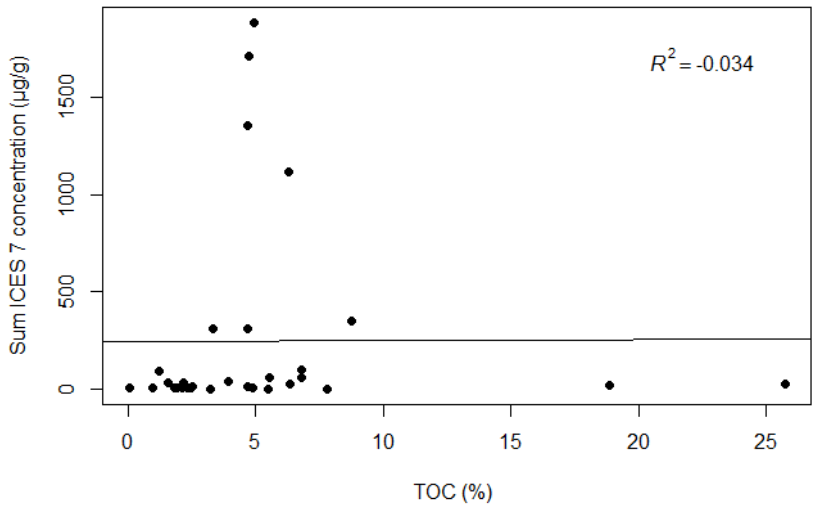

Figure 21: TOC (%) plotted against ∑ICES7 PCB concentration (µg/g). No relationship between initial PCB concentration and TOC was identified.

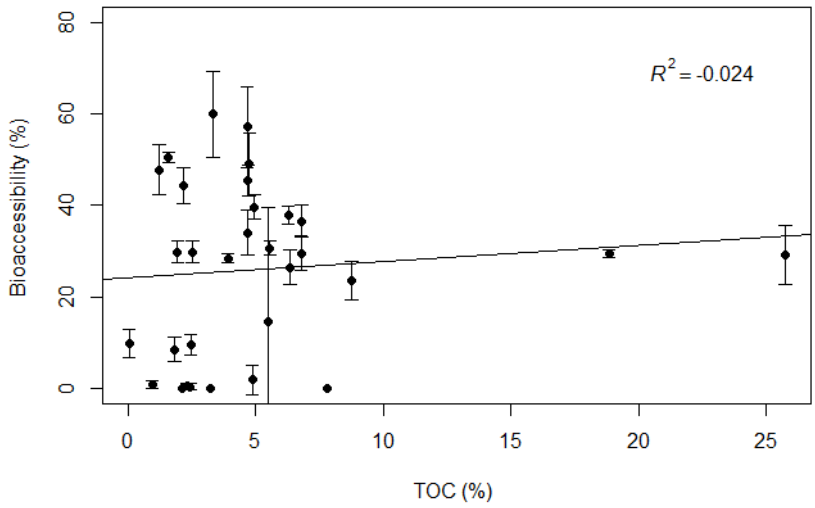

Figure 22: TOC (%) plotted against bioaccessibility (%). TOC appears to have little bearing on the bioaccessibility of bound compounds.

5.3.3 Bioaccessibility data – ∑ICES 7 concentrations and individual congeners

Bioaccessibility of ∑ICES 7 compounds in all soils varied from 0% – 57.12% (RSD = 70.92%), with a mean value of 25.03%. Bioaccessibility appears to vary more significantly in soils with a ∑ICES 7 PCB concentration lower than 500 µg/kg (Figure 23). As discussed, TOC appeared not to be an influencing factor on bioaccessibility.

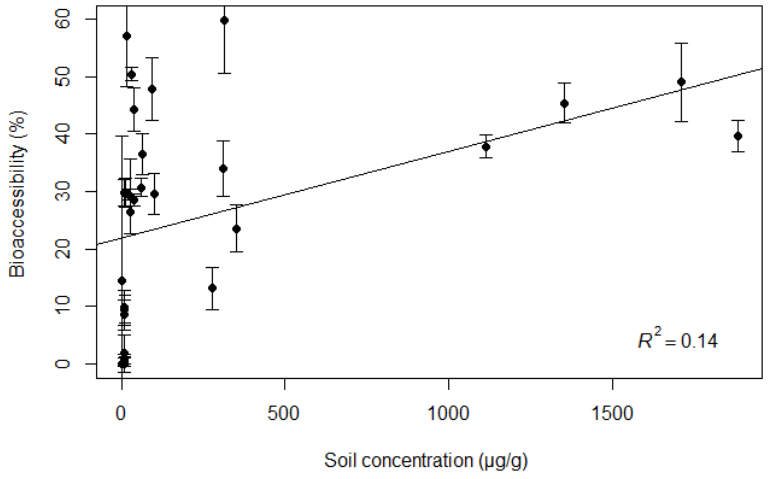

Figure 23: ∑ICES 7 Soil bioaccessibility plotted against initial soil concentration (µg/g).

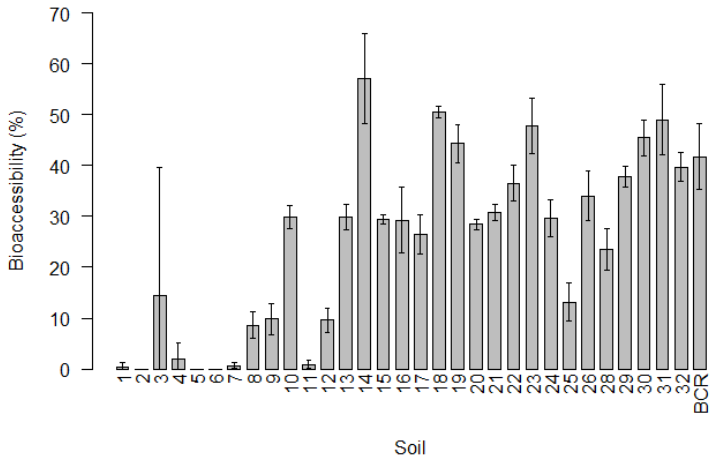

Figure 24: ∑ICES 7 bioaccessibility (%) per soil. Some variability exists between bioaccessibility values. Soils are arranged by ∑ICES 7 PCB concentration, with soil 1 representing the lowest concentration. Soil 32 was the most heavily contaminated sample.

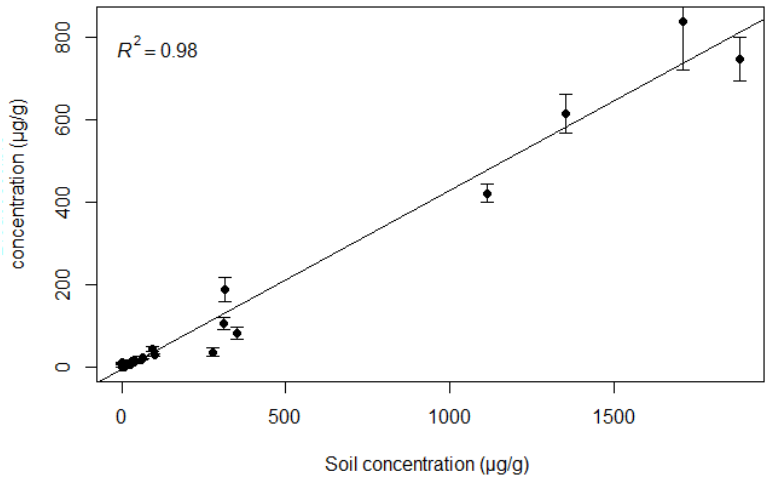

Figure 25: Initial soil concentration (∑ICES 7) plotted against ∑ICES 7 PCB concentration in the bioaccessible fraction (the bioaccessible concentration) (µg/g). A linear relationship is evident between total soil concentration and the bioaccessible concentration, suggesting a linear relationship.

Individual congener bioaccessibility was consistent between PCB congeners 28, 52, 101, 118, 153 and 138 (mean values varying between 30.18% - 37.54%) (Table 17, Figure 26). However, recorded values for PCB 180 were significantly lower, with a mean value of 1.61%. The trend is visible when bioaccessibility is recorded by homolog, with bioaccessibility in heptachlorinated compounds, which is otherwise consistent in tri-, tetra-, penta- and hexa- chlorinated congeners (Figure 27). Although low bioaccessibility has been recorded for HMW compounds in PAH studies (Tang *et al.*., 2006b), the reduction in PCB 180 recovery is so markedly reduced compared to other homologs is potentially evidence of a systematic error in the extraction or clean-up procedure, which requires investigation.

Previous studies have indicated the potential for reduction in the recovery of high molecular weight PCBs during the saponification process (Erickson, 1997). In light of these findings, it was decided that an investigation into the effect of saponification on PCB 180 was required.

Table 17: Bioaccessibility (%) values obtained for all soils (n = 34)

Congener	28	52	101	118	153	138	180	∑ICES 7
Mean	34.57	30.38	37.54	30.18	32.49	30.18	1.61	25.03
Maximum	60.04	80.26	81.48	96.25	70.92	64.72	4.39	57.12
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Standard deviation	22.84	27.47	28.51	28.74	23.56	21.03	1.63	17.75
Relative standard deviation (%)	66.06	90.44	75.94	95.23	72.52	69.68	101.14	70.92

Additional bioaccessibility calculations were conducted using the BCR 481 soil, and are shown in Table 18

Table 18: Bioaccessibility values obtained using the FOREhST method with the BCR 481 CRM material. These values have been derived from ASE in order to obtain the full ICES 7 congener data.

Congener	28	52	101	118	153	138	180	Sum 7
Run 1	0.00	34.71	30.40	48.08	47.24	39.15	1.29	31.34
Run 2	31.76	70.59	56.35	112.64	72.66	56.85	4.71	49.76
Run 3	47.01	90.45	58.51	116.38	66.25	51.76	5.19	46.71
Run 4	39.39	70.00	55.47	113.55	72.71	56.82	5.18	49.81
Run 5	81.31	72.57	50.53	89.48	52.75	40.59	4.33	37.46
Run 6a	99.10	75.85	51.04	102.55	54.13	42.26	4.51	38.73
Run 6b	69.88	70.94	50.82	79.71	55.50	44.32	4.31	39.26
Run 7	78.77	75.73	52.47	107.64	57.57	44.81	4.67	40.87
Mean	55.90	70.10	50.70	96.25	59.85	47.07	4.27	41.74
SD	32.27	15.76	8.71	23.25	9.54	7.10	1.25	6.51
RSD (%)	57.73	22.48	17.18	24.15	15.94	15.09	29.27	15.59

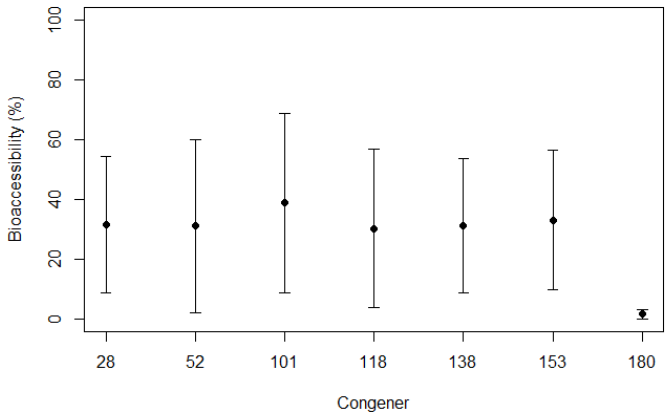

Figure 26: Congener specific bioaccessibility (%) across all tested soils and CRM. Reduced recovery in PCB 180 is clear. Recovery is consistent between the other ICES congeners.

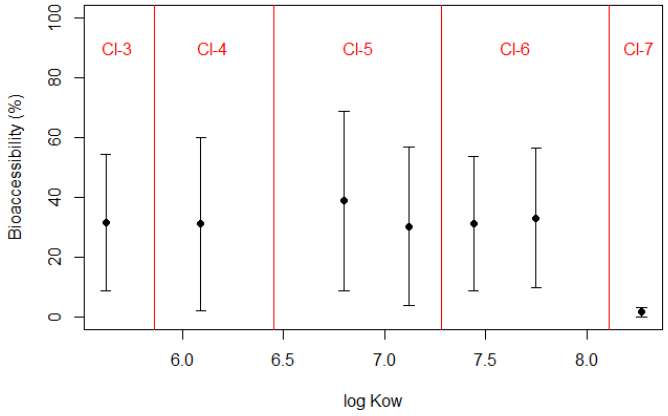

Figure 27: Bioaccessibility expressed by LogKow/ PCB homolog (%). The trend demonstrated in Figure 26. Is evident. CL-3 to CL-6 congeners show consistent bioaccessibility values.

5.3.4 PCB 180 recovery

In all cases, PCB 180 recovery was found to be significantly reduced (0-4.38%) bioaccessibility in all soils, with a mean recovery of value of 2.94% (sd = 1.58%). This observation was similarly apparent in CRM samples, reporting a PCB 180 bioaccessibility of 4.27% (sd = 1.25%). Additionally, most samples were affected by integration issues in chromatograms. GC/MS integration errors have been encountered in previous studies in high molecular weight compounds, including PCB 180 (Delannoy *et al.*., 2014) which have impaired or prevented analysis.

Effects of saponification on high molecular weight PCBs have been documented in previous work, and appear to present an explanation for greatly reduced PCB 180 recovery in this study. PCB 173 analytical standard was added post-saponification in this study, and was therefore unaffected.

In order to explore the likelihood of saponification related heptachlorinated compound loss, a range of samples were prepared and subject to two distinct clean-up processes with the saponification step implemented and removed (Table 19). Samples were prepared through the spiking of an internal standard solution to a 6 mL sample of FOREhST supernatant previously obtained from CRM extractions. This procedure was repeated to test internal standard integrity under the same conditions using blank samples prepared using a 6 mL sample of pre-prepared FOREhST simulated gut solution, as described in Table 19.

Table 19: Procedures undertaken to test for saponification related analyte loss.

Sample	Standard spike	Clean-up procedure	Saponification?
C1 –CRM C1 – BLANK	50 µL solution of PCB 34 (18.92 ng/µL, PCB 62 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL), PCB 173 (18.81 ng/µL)	LLE: Addition of 20 mL of Acetone: Hexane solution (1:1) followed by shake at 120 RPM for one hour. SPE: Al_2O_3/ SiO_2 following Na_2SO_4 chemical drying.	Yes
C2 – CRM C2 – BLANK		As above.	No
F1 – CRM F1 – BLANK		LLE: Typical FOREhST method. As detailed in 2.4. SPE: Al_2O_3/ SiO_2 following Na_2SO_4 chemical drying.	Yes

F2 – CRM F2 - BLANK		As above.	No

In samples where the saponification step has been omitted, chromatogram interpretation is significantly improved in 394.00 ion, representing the heptachlorinated compounds. Other molecular groups are unaffected by any impact caused by the saponification process (Figure 28).

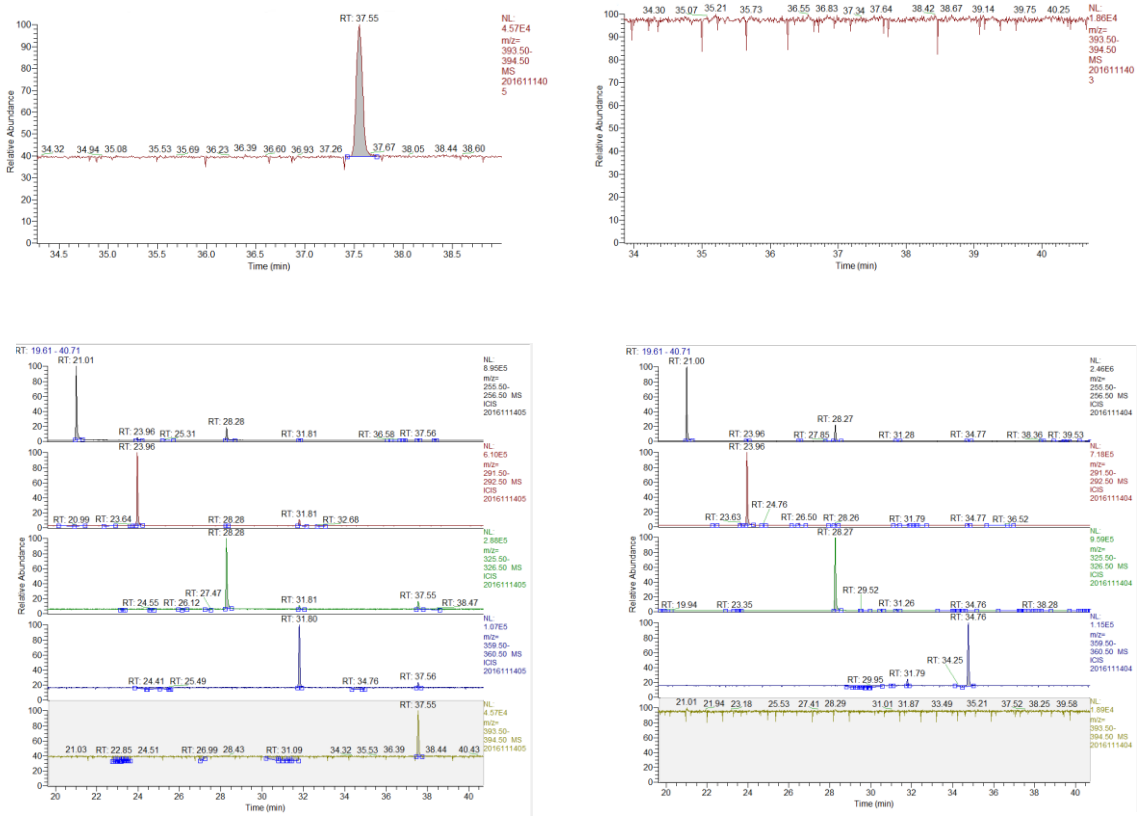

Figure28: Chromatograms showing PCB 173 recovery in unsaponified (L) and saponified (R) samples.

5.3.5 Standard recovery

In order to monitor potential losses of analyte compounds due to sample handling (potential losses associated with the liquid:liquid extraction process, SPE process and sample concentration), standards were introduced at key points during the extraction protocols. A 50 µL solution of PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL) was introduced as a surrogate standard via a soil spike.

These standards were introduced to monitor losses throughout the extraction procedure. Prior to the liquid:liquid extraction, a 100µL recovery standard solution of PCB 29 (9.47 ng/µL) and PCB 157 (9.53 ng/µL) were introduced to monitor the performance of the clean-up procedure. Internal standards of PCB 34 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL) and PCB 173 (18.81 ng/µL) were introduced prior to GC/MS in order to quantify the analyte, surrogate, and recovery compounds.

Percentage recovery of the standards was as follows (Relative standard deviation in parenthesis):

Table 20: Surrogate and recovery standard recovery, FOREhST extractions (% recovery).

Surrogate standards		Recovery standards	
PCB 19	PCB 147	PCB 29	PCB 157
70.70% (24.02%)	80.89% (30.13%)	82.38% (14.23%)	63.25% (26.07%)

The decision was taken to not correct the data on the basis of standard recovery. This is due to the limited range of standards available, which do not reflect the varied physicochemical properties of the wider PCB compound group. It would be possible to apply standards to each of the ICES 7 compounds, though this would significantly increase the cost of extraction and complexity of the analysis. Additionally, variations in standard recovery reflect the complexity of working with very small samples of pure PCB material.

5.4 Conclusions

Congener bioaccessibility ranged from 0 – 96.25% across all monitored compounds, with the highest value identified in PCB 118. However, mean bioaccessibility values ranged from 30.18 – 37.54% across PCBs 28, 52, 101, 118, 153 and 138, with a much lower value of 1.61% identified in PCB 180. ∑ICES 7 bioaccessibility ranged from 0 – 57.12%, with a mean value of 25.03%. This mean value has been affected by the low recovery of PCB 180 in this study, which can be addressed using an adapted clean up procedure.

These values are in contrast to soil PCB bioaccessibility of 36% recorded in Oomen *et al.* (2000) and 3 – 85 % in Hack & Selenka (1996). In terms of PCB bioaccessibility from dust samples, Wang *et al.* (2013) recorded similar values (27.9%), while Ertl and Butte (2012) recorded a mean value of 63%.

No clear relationship was identified between TOC and bioaccessibility in this study, despite very clear variation in TOC levels between tested soils. Although TOC has been identified as a significant determinant on bioaccessibility in previous PAH and PCB studies, this phenomenon appears to have been overridden by the processes of the extraction test, such as the desorption of analytes into micellar rich simulated gut fluids, and is not found to be significant enough to impact extraction.

Recovery of tri-, tetra-, penta- and hexa- chlorinated compounds remained consistent, though recovery of the heptachlorinated PCB 180 was reduced. With the exception of PCB 180, this suggests consistency in bioaccessibility despite variation in LogKow. The relationship between bioaccessibility and LogKow will be observed in further extractions using the CE-PBET method, and clean-up methods amended to address the problem of low PCB 180 recovery. Differential bioaccessibility in PCBs in accordance with chlorination has been observed in other studies, with HMW compounds showing reduced recovery (Wang *et al.*., 2013). However this was shown as a progressive reduction in bioaccessibility as chlorination increased, to a heptachlorinated value of 15.4%. This study shows consistent bioaccessibility values accompanied by an abrupt reduction to 4.39% in heptachlorinated compounds (PCB 180).

A linear relationship between initial PCB concentration and the bioaccessible concentration was identified in all congeners and ∑ICES 7 data. This suggests that bioaccessibility may be, to an extent, predictable in PCBs, and further highlights the consistency of the method, though, once again, this was not as well observed in PCB 180, which can be attributed to quantification difficulties caused by low concentrations.

PCB 180 recovery was consistently low. Although quantification of HMW PCBs has been identified as problematic in previous work, it was attributed in the case of this study to the saponification stage of the clean-up procedure. Tests on heptachlorinated compounds (PCB 173 and 180) have revealed significantly greater recoveries of these compounds using clean-up methodologies that omit the saponification stage, with other homologs unaffected, It is therefore recommended that saponification is omitted in further work, or, if deemed necessary due to high lipid content extraction fluids, is closely monitored and adjusted where required.

5.4.1 Implications and Interpretation

(i) A mean ∑ICES 7 bioaccessibility of 25% suggests the total contaminant approach to be overly conservative, though this value is reduced by artificially low recoveries detected in PCB 180. With the exception of PCB 180 individual congener bioaccessibility ranged from 30 – 38%. This is consistent with other *in vitro* PCB assessments, but lower than the values obtained using the same method for PAH assessment, though PAHs showed more variability between specific compounds.

(ii) A systematic error resulted in artificially lowered PCB 180 concentrations. This has been attributed to dechlorination processes during the MeKOH saponification stage of the clean-up procedure.

(iii) Relationships between soil total and bioaccessible fraction PCB concentrations appear to be linear in all congeners and ∑ICES 7 values. This suggests the potential for predictive model development.

(iv) Mean bioaccessibility remained consistent between congeners, suggesting that the micellar, carbohydrate rich media of the simulated gut fluids were sufficient to overcome preferential desorption of lower molecular weight compounds, as has been seen in other PCB and PAH bioaccessibility studies.

(v) Bioaccessibility appeared to be maintained across soil samples. TOC did not appear to be a controlling factor on PCB bioaccessibility.

(vi) The bioaccessible fraction was difficult to determine in samples with a ∑ICES 7 PCB concentration of <10 µg/g.

(vii) Potential for further work with emerging organic contaminants is clear – oral bioaccessibility remains a dominant contaminant pathway, though other exposure routes may be explored through dermal and respiratory bioaccessibility methods.

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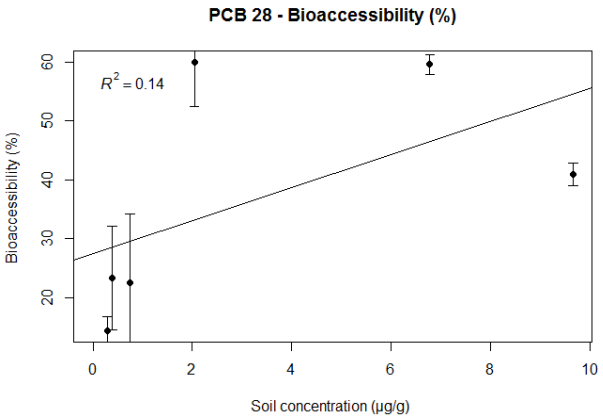
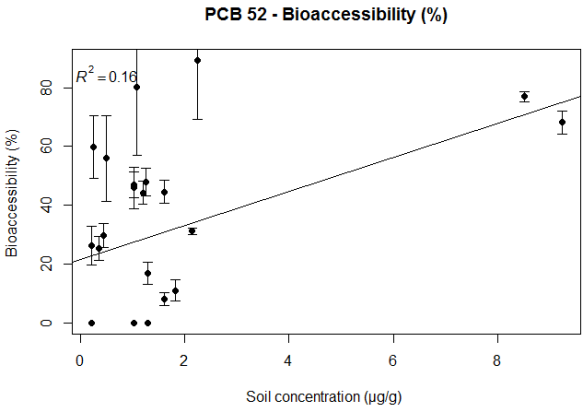
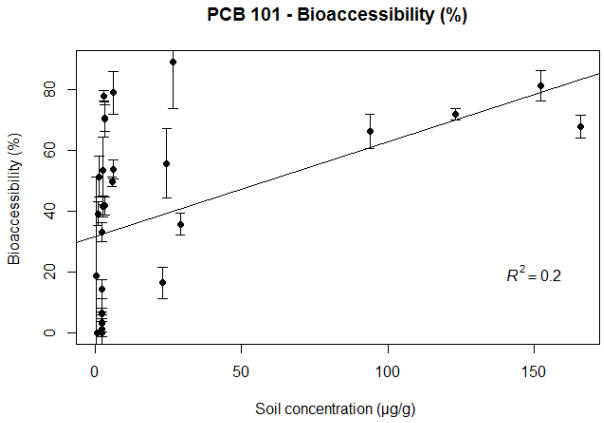
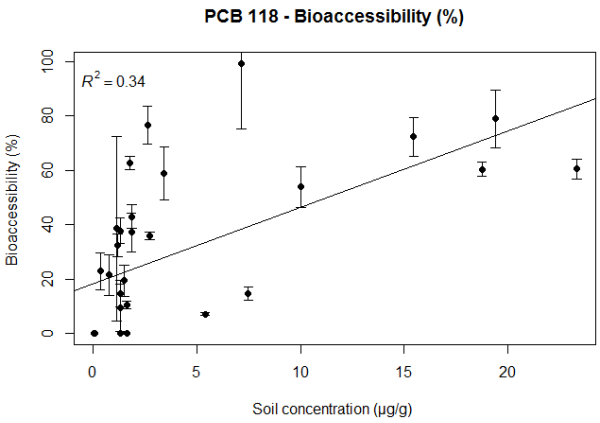
4.6 Supplementary information

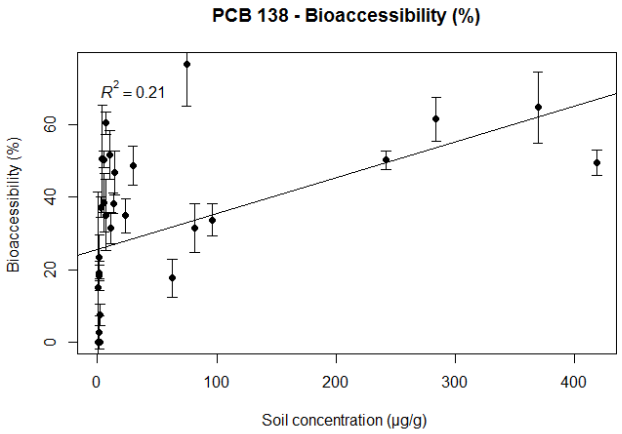
Plots of initial vs. bioaccessible concentrations (congener specific)

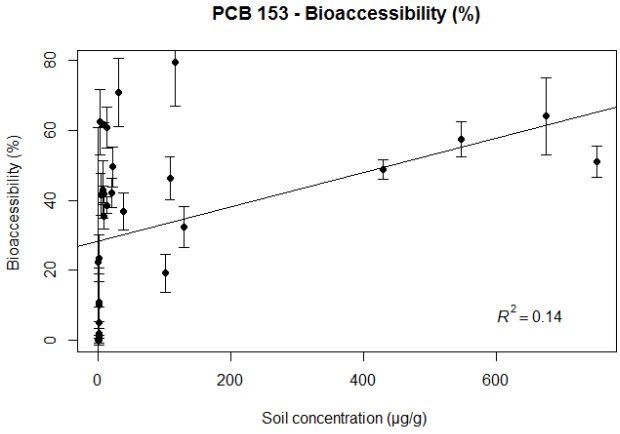


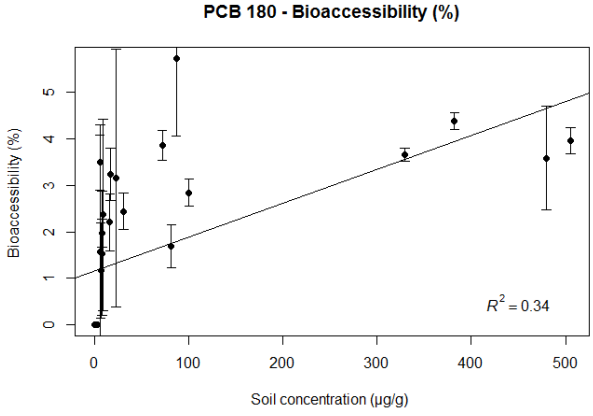




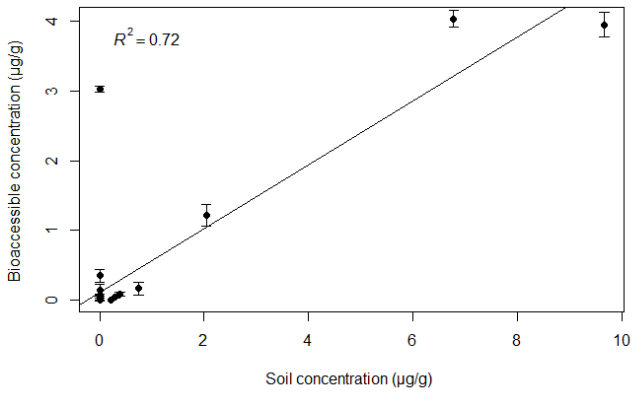




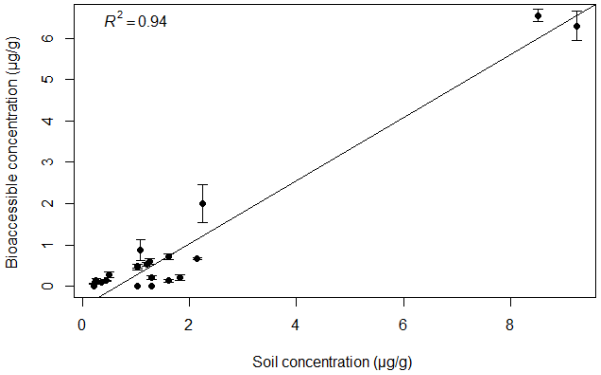




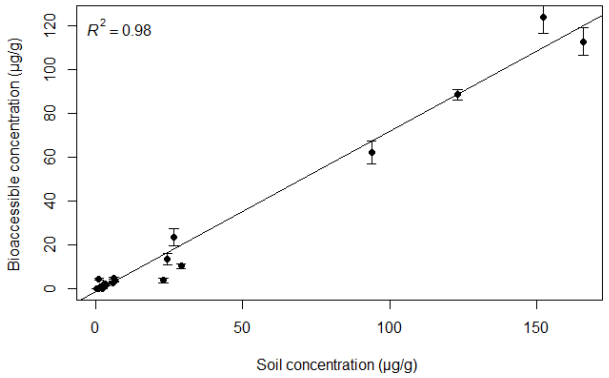
PCB 28: Total vs.bioaccessible concentration



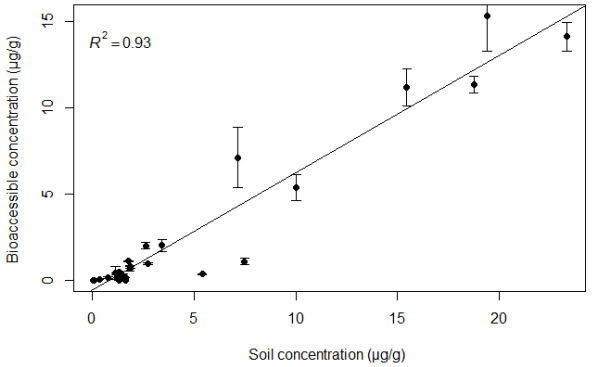
PCB 52: Total vs. bioaccessible concentration



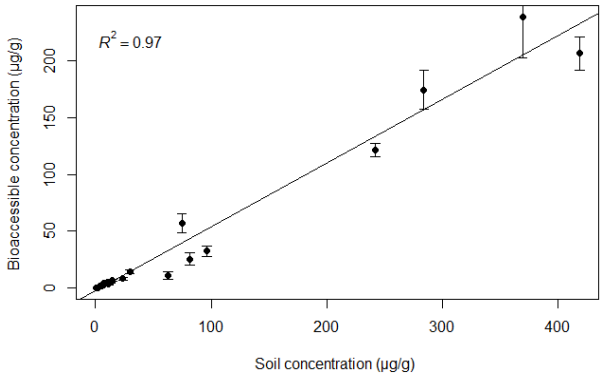
PCB 101: Total vs. bioaccessible concentration



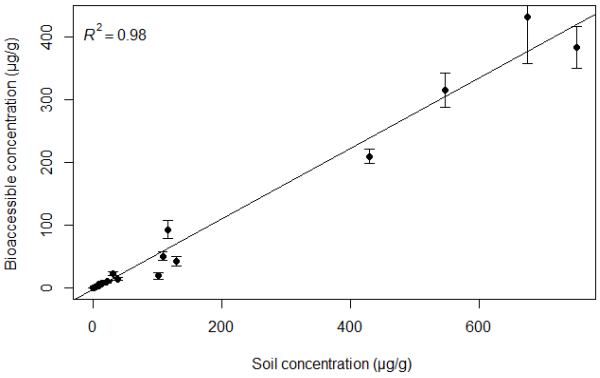
PCB 118: Total vs. bioaccessible concentration



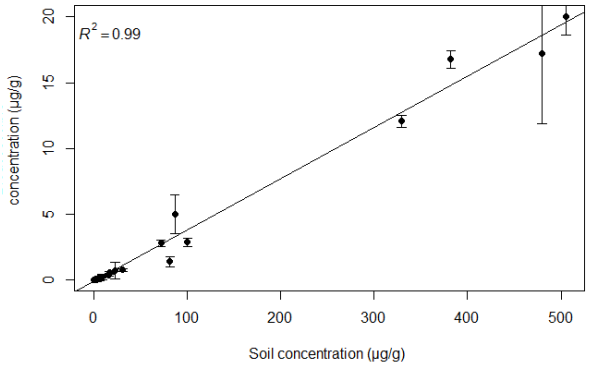
PCB 138: Total vs. bioaccessible concentration



PCB 153: Total vs. bioaccessible concentration



PCB 180: Total vs. bioaccessible concentration



CHAPTER 6: ASSESSMENT OF BIOACCESSIBILITY IN SOIL-BOUND PCBS – A COMPARISON AND EXPLORATION OF RESULTS FROM THE CE-PBET AND FOREHST METHODS.

6.1 Introduction

Despite an ever-growing demand for urban land suitable for development, and an increasing emphasis on the prioritisation of brownfield sites in preference to greenfield projects, and a growing focus on soil health as an indicator of sustainability (Cheng *et al.*., 2016; Morillo and Villaverde, 2017), many sites are overlooked due to the costs and difficulties associated with contaminated land remediation (NERC and DTZ, 2009; Bartke *et al.*., 2016). The outcome is often loss of greenfield sites (Thornton *et al.*., 2007) in preference to peri-urban developments, unsuitable in terms of amenity provision and transport impact (Bardos *et al.*., 2016b), and an over-abundance of under-utilised derelict land within the urban environment (Lai and Zhang, 2016; Loures and Vaz, 2016). The result is an unhealthy, an deprived cityscape and a rural environment degraded through urban sprawl. Where remediation is conducted, costs to the developer can be high (Barrieu *et al.*., 2017), as can be the burden of waste disposal, energy expenditure, and carbon emissions where *in situ* applications take place (Huysegoms and Cappuyns, 2017). Recently, more holistically oriented approaches focussed on the wider impacts of remediation in terms of social impacts and the sustainability agenda have been developed (Cappuyns, 2016). These include new methods which approach the remediation problem from less of a 'rigid engineering' approach than traditional techniques, with a sustainability focus in mind, including novel *in situ* approaches (Hartley *et al.*., 2012; Chen *et al.*., 2015; Lefevre *et al.*., 2016; Song *et al.*., 2017).

Attention is turning to the development of methods and incentives to establish a more sustainability-driven approach (Favara and Gamlin, 2017; Huysegoms and Cappuyns, 2017), which removes the barriers to brownfield redevelopment. In the case of contaminated land, these barriers may be tackled in terms of more representative risk assessment and analysis of potential harm through the fine tuning of risk assessments. This approach may help to institute a less-conservative

approach, and bring more sites back into suitability. One such method is bioaccessibility assessment, a range of *in vitro* methodologies arising out of a necessity to model and predict the bioavailability of xenobiotic substances, a key component of risk assessment (Ollson *et al.*., 2017).

Bioaccessibility is increasingly being considered as a suitable method for the assessment of ingested contaminants in favour of a 'total contaminant' approach, allowing the calculation of a more physiological relevant value (Collins *et al.*., 2015; Shi *et al.*., 2017). Ingestion of soil is a major source of exposure for xenobiotics (Chaney *et al.*., 1996; Oomen *et al.*., 2002). Defined as the proportion of a contaminant which has become mobilised from the ingested media into a digestive chyme before absorption across the intestinal wall into the blood stream (Oomen *et al.*., 2002; Collins *et al.*., 2015), the bioaccessible fraction therefore represents a 'worst case' scenario, whilst avoiding the over-conservatism inherent to the total concentration approach, and reduces uncertainty (Juhasz *et al.*., 2007).

Methods have been developed to assess the bioaccessibility of contaminants, initially in soil-bound and aqueous phase inorganic contaminants such as heavy metals, and increasingly in the study of persistent organic pollutants (POPs) (Cave *et al.*., 2010; Lorenzi *et al.*., 2012; Collins *et al.*., 2015; Rodríguez-Navas *et al.*., 2017a). Of these, the CE-PBET and FOREhST methods have met with success in PAH studies (Cave *et al.*., 2010; Tilston *et al.*., 2011; Lorenzi *et al.*., 2012; Collins *et al.*., 2013; Nathanail and Ogden, 2013; Collins *et al.*., 2015) and to assess bioaccessibility in DDT (Smith *et al.*., 2012) and brominated flame retardants (Abdallah *et al.*., 2012; Garcia-Alcega *et al.*., 2016; Kademoglou *et al.*., 2017) . In this study, the results of the CE-PBET and FOREhST methods will be compared across a range of soils which have been subject to an *in vitro* (juvenile swine) bioavailability model in a parallel study (Delannoy *et al.*., 2015) in order to present initial validation work and comparison of bioaccessibility and bioavailability data.

This study addresses the bioaccessibility of soil-borne PCBs (polychlorinated biphenyls), a group of organic compounds identified as a POP group under the Stockholm Convention (Xu *et al.*., 2013).

Although impacted by successive manufacture and sale bans during the 1970s – 1990s, PCBs remain a significant legacy pollutant PCBs are characterised by their environmental persistence, inertness and resistance to degradation in the environment and within biota (Zhang *et al.*., 2012), physicochemical properties which cause accumulation in soils, sediment, organic matter and biota. Due to their widespread use, environmental persistence and bioaccumulative tendency, PCBs remain ubiquitous, particularly in urban soils (Tilson and Kodavanti, 1998; Kodavanti *et al.*., 2001; Aminov *et al.*., 2013). PCBs are characterised by elevation in former industrial, urban areas (Krauss and Wilcke, 2003; Davis *et al.*., 2007; Vane *et al.*., 2007; Cachada *et al.*., 2009; Vane *et al.*., 2014), though they can be distributed through processes such as wind and water transport, or intentional redistribution due to disposal of PCB products or PCB impacted rubble and other waste, and re-emission (Fu *et al.*., 2008; Jartun *et al.*., 2009).

This study aims to assess the applicability of two established bioaccessibility methods by addressing the following questions:

1. Are the results between methods significantly different? If so, why?
2. How do the bioaccessibility results compare with bioavailability data?
3. How do results compare with previous (PAH) studies using the same protocols?

Fundamentally, this study aims to investigate the appropriateness of bioaccessibility testing, using two proven methods CE-PBET and FOREhST. Additionally the study extends the application of these methods to PCBs, and opens up the possibility of their application to similar groups of organic compounds.

6.2 Methodology

Extractions were performed using two established protocols: The FOREhST and CE-PBET methods. In both tests, 5 replicates of 7 soils (Table 21). Each run included a sample of a recognised CRM (PCB industrial soil BCR 481) and method blank alongside the tested soil samples. All glassware was

cleaned using a chromic acid (H_2CrO_4) method, which included a 24 hour acid soak, followed by rinsing with deionised water and air drying.

Table 21: Samples used in the comparison study. Soils represent 7 field soils, with an additional CRM.

Process	Samples per soil	Soils
CE-PBET	5	8
FOREhST (without saponification)	5	8
FOREhST (with saponification)	3	8

6.2.1 Preparation of simulated gut fluids

6.2.1.1 CE-PBET fluids

Simulated digestive fluids were prepared prior to the extraction. In the CE-PBET method, fluids were divided into a stomach/ small intestine fluid, and a colon section. Reagents used and quantities are listed in Table 22. Each fluid was prepared separately in quantities as required. Fluids were prepared on the day before the planned extraction. Milli-Q de-ionised water was used throughout to create solutions and to rinse glassware prior to use. All equipment was cleaned prior to use with acetone rinse process

Table 22: Reagents used in the preparation of CE-PBET simulated gut fluids.

Stomach/ small intestine section fluid	Colon section fluid
Sodium malate (500 mg/L) (VWR); Sodium citrate (500 mg/L) (Sigma Aldrich); Lactic acid (420 µL/L) (Sigma Aldrich); Acetic acid (500 µL/L) (Sigma Aldrich); Pepsin (1250 mg/L) (VWR); Starch (5000mg/L) (VWR); Peptone from casein (3400 mg/L) (VWR); Tryptone (6100 mg/L) (VWR); Yeast extract (4500 mg/L) (VWR); Casein (3000 mg/L) (Sigma Aldrich); Pectin (2000 mg/L) (Sigma Aldrich); Xylan (2000 mg/L) (Sigma Aldrich); Arabinogalactan (2000 mg/L) (Sigma Aldrich); Guar Gum (1000 mg/L) (Sigma Aldrich); Inulin (1000 mg/L) Sigma Aldrich);	Starch (5000 mg/L) (VWR); Calcium chloride (150 mg/L) (VWR); Monopotassium phosphate (500 mg/L) (Sigma Aldrich); Dipotassium phosphate (500 mg/L) (Sigma Aldrich); Sodium chloride (6100 mg/L) (VWR); Magnesium sulphate (1250 mg/L) (VWR); Potassium chloride (4500 mg/L) (VWR); Iron sulphate (5 mg/L) (VWR); Sodium bicarbonate (1500 mg/L) (VWR); Bile salts (400 mg/L) (Sigma Aldrich); Mucin (5000 mg/L) (Sigma Aldrich); Cysteine hydrochloride (800 mg/L) (Sigma Aldrich); Pectin (2000 mg/L) (Sigma Aldrich); Yeast extract (4460 mg/L) (VWR); Peptone (3300 mg/L) (Sigma Aldrich); Tryptone (5000 mg/L) (Sigma Aldrich) Inulin (1000 mg/L) (Sigma Aldrich); Xylan (2000) (Sigma Aldrich); Casein (3000 mg/L) (Sigma Aldrich); Arabinogalactan (2000 mg/L) (Sigma Aldrich); Guar gum (1000 mg/L) (Sigma Aldrich)
Target pH	
2.5 (+/- 0.5)	6.5 (+/-0.5)

6.2.1.2 FOREhST digestive fluids

The FOREhST protocol requires the preparation of digestive fluids as described in Cave *et al.*. (2010). Distinct saliva, gastric, duodenal and bile fluids were prepared in 500 mL batches of 'organic' and 'inorganic' constituent parts, before thorough mixing in 2 L Nalgene containers and storage until use (Table 23). As with CE-PBET solutions, Milli-Q de-ionised water was used throughout to create the solutions and to rinse glassware, and equipment was cleaned using acetone. Solutions were prepared a maximum of two days before use.

Table 23: Reagents used in the preparation of FOREhST simulated gut fluids.

	Saliva	Gastric	Duodenal	Bile
	Reagent	Reagent	Reagent	Reagent
Inorganic component (concentration in 500 mL flask)	Potassium chloride (1792 mg/L) (VWR); Sodium phosphate (1776 mg/L) (VWR); Potassium thiocyanate (400 mg/L) (VWR); Sodium sulphate (1140 mg/L) (VWR); Sodium chloride (596 mg/L) (VWR); Sodium hydroxide (244 mg/ L) (VWR);	Sodium chloride (5504 mg/L) (VWR); Sodium phosphate (533 mg/L) (VWR); Potassium chloride (1649 mg/L) (VWR); Calcium chloride (799 mg/L) (VWR); Ammonium chloride (612 mg/L) (VWR); Hydrochloric acid - 37% (6.5 mL/L) (VWR)	Sodium chloride (14024 mg/L (VWR)); Sodium bicarbonate (11214 mg/L) (VWR); Monopotassium phosphate (160 mg/L) (Sigma Aldrich); Potassium chloride (1129 mg/L) (VWR); Magnesium chloride (100 mg/L) (VWR); Hydrochloric acid - 37% (180 µL/L) (VWR)	Sodium chloride (10518 mg/L) (VWR); Sodium bicarbonate (11570 mg/L) (VWR); Potassium chloride (753 mg/L) (VWR); Hydrochloric acid – 37% (180 µL/L) (VWR)
Organic component (concentration in 500 mL flask)	Urea (400 mg/L) (Sigma Aldrich)	Glucose (1300 mg/L) (Sigma Aldrich); Glucoronic acid (40 mg/L) (Sigma Aldrich); Urea (170 mg/L) (Sigma Aldrich); Glucosamine hydrochloride 660 (mg/L) (Sigma Aldrich)	Urea (200 mg/L) (Sigma Aldrich)	Urea (500mg/L) (Sigma Aldrich)
Additional reagents (concentration in 1 L carboy)	Amylase (290 mg/L) (Sigma Aldrich); Mucin (25 mg/L) (Sigma Aldrich); Uric acid (15 mg/L) (Sigma Aldrich)	Bovine serum albumin (1000mg/L) (Sigma Aldrich); Mucin (9000 mg/L) (Sigma Aldrich); Pepsin (2500 mg/L) (Sigma Aldrich)	Calcium chloride (200 mg/L) (VWR); Bovine serum albumin (1000 mg/ L) (Sigma Aldrich); Pancreatin (9000 mg/L) (Sigma Aldrich); Lipase (1500 mg/L) (Sigma Aldrich)	Calcium chloride (222 mg/L) (VWR); Bovine serum albumin (1800 mg/L) (Sigma Aldrich); Bile (30000 mg/L) (Sigma Aldrich)
pH	6.8 (+/- 0.5)	1.4 (+/- 0.5)	8.1 (+/- 0.2)	8.2 (+/- 0.2)
pH (combined solutions)	Saliva: gastric (1:2) 1.6 (+/- 0.2)			
	Saliva: gastric: duodenal: bile (1:2:2:1) 6.0 (+/- 0.75)			

6.2.2 Bioaccessibility assessment procedures

Extractions were performed using the CE-PBET and FOREhST methods. Though common in their role as a simulated gastro-intestinal simulation procedure, the methods present unique methodologies.

6.2.2.1 CE-PBET procedure

Pre-prepared stomach/ small intestine solution was warmed in a heated water bath set to 37® C. The solution pH was then recorded. Alterations were made using HCl (60% v/v) or NaOH (10 M) if required, until the target pH of 2.5 (+/- 0.5) was reached. The colon solution was set aside until required. The tested soil (0.5 g) was then added to a 100 mL glass centrifuge vessel, using equipment rinsed with acetone between samples. The warmed stomach/ small intestine solution (40 mL) was then added alongside a 50 µL aliquot of surrogate standard solution PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL). The vessel was then sealed using a PTFE liner pre cleaned using acetone. The samples were then rotated in the bath for 1 hour to simulate the transit of the contaminant through the stomach section of the GIT. The samples were then removed and 0.07 g of bile salts and 0.02 g of pancreatin were added to convert the stomach solution into a simulated small intestine fluid. pH was adjusted to 7.0 (+/- 0.75) using a saturated sodium bicarbonate solution. The vessels are then resealed, and turned in the water bath for a further 4 hours, simulating transit through the small intestine. Vessels are then removed from the water bath, and centrifuged for 15 minutes at 3500 RPM, causing a separation of supernatant and a compacted soil pellet. The supernatant was decanted into a 60 mL collection vial and retained for clean-up and analysis.

The colon solution was pre-warmed to 37°C in the hot water bath, shaken by hand and pH tested. Adjustments, if required, were performed using dropwise addition of HCl (60% v/v) and NaOH (10.0 M) as necessary, with a target pH of 6.5 (+/-0.5). The colon solution (40 mL) was added to each of the vessels, re-sealed using a PTFE liner, and shaken briefly by hand to break up the pellet. The vessels are then returned to the water bath and rotated for a further 16 hours, simulating transit through the colon. The vessels are then removed, and centrifuged for 15 minutes at 3500 RPM,

separating a supernatant from the pellet. The supernatant was decanted into a 60 mL collection vial and retained for clean-up. The pellet sample was retained within the vessel for further analysis.

A 50 µL aliquot of internal standard solution was added to the supernatant samples, consisting of PCB 34 (18.92 ng/µL), PCB 62 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL) and PCB 173 (18.81 ng/µL). Acetone:hexane (1:1) (20 mL v/v) was added and the sample briefly shaken by hand. The sample was then placed on an orbital shaker, and shaken for 1 hour at 120 RPM. The hexane layer was then removed via Pasteur pipette and retained for solid phase extraction and analysis. This process was repeated with the colon supernatant samples.

The soil pellet was subjected to a similar process. Acetone:hexane (1:1) (20 mL v/v) was added to the vessel containing the pellet. The vessel was then shaken by hand to break up the pellet. The vessel was then placed on an orbital shaker for 1 hour at 120 RPM before removal of the hexane layer via Pasteur pipette.

A glass wool wadded anhydrous Na_2SO_4 filled glass column was used to chemically dry the hexane samples. The samples were passed through the column following conditioning with a 1 mL hexane elution, and collected in a clean glass vial. A further 1 mL of hexane was added to each of the vials which previously contained the hexane samples. This was performed a further 2 times. The elute was retained and combined with the sample hexane for solid phase extraction and analysis.

6.2.2.2 FOREhST procedure

Until the saponification stage, the FOREhST protocol was followed as detailed in 4.2.3.

The saponification process associated with the FOREhST methodology, as tested successfully in PAH studies (Cave *et al.*. 2010), was found to cause a systemic reduction in the recovery of PCB 180 and other hepta-chlorinated compounds. As such, saponification has been omitted in this study. However, data are provided from previous FOREhST extractions with the saponification step included to aid in comparison and assess method performance. The saponification process entailed

the addition of 30 mL of methanolic potassium hydroxide (KOHMe) to a 6 mL subsample of the produced supernatant, which was then heated in an oven at 100 C for 1 hour, carefully turned, then returned to the oven for a further 30 minutes. Liquid / liquid extraction was then performed using hexane. The full process is detailed in 4.2.5.2

Following liquid/ liquid extraction and chemical drying, hexane samples from both extraction protocols were concentrated to 0.5 mL under a gentle stream of nitrogen and subject to a common Al_2O_3 and SiO_2 SPE method, which has previously been used to clean-up supernatant samples produced under the FOREhST protocol (Cave *et al.*. 2010). The process entailed the elution of samples through a column filled with equal quantities of Al_2O_3 and SiO_2 following preconditioning with a 2 mL sample of hexane. The samples were allowed to drip through, without the use of a vacuum manifold or other source of outside influence. The process results in a PCB sample retained within the column matrix, which was washed out through the elution of 9 x 0.9 mL aliquots of dichloromethane, which was collected for analysis. The PCB-laden DCM sample was then concentrated to 200 µL before GC/MS analysis.

6.2.3 GC/MS analysis

Gas chromatography/ mass spectrometry was performed using a Fisons GC8000 GC coupled to a Fisons MD800 mass spectrometer. The GC oven was fitted with a Varian FactorFour VF-5s fused silica capillary column (60 m length, 0.32 mm inner diameter, 0.25 µm film thickness). The instrument was operated in a full scan mode (ionisation energy 70 eV, mass range 39-600 amu), with helium as a carrier gas at 16 psi, as described in Vane *et al.*. (2007). The oven was temperature programmed from 100 °C (1 minute isothermal) to 200 °C (5 °C/minute) to 280 °C (2.4 °C/ minute), held isothermally for 20 minutes, then ramped to 300 °C at 10 °C/ minute. Quantifcation of independent PCB congeners was performed by selected ions (256.0, 292.0, 326.0, 394.0), and data collected using the Xcalibur software package (Thermo Fisher Scientific Inc., 2007). Integration was performed using a process of manual identification to reduce the risk of errors caused by an automated peak integration process.

6.3 Results

6.3.1 Soil PCB concentrations

Seven soils were selected for further analysis from an original dataset of 34 soils to provide sufficient range of ∑ICES 7 concentration, which have been previously extracted using the FOREhST method (Chapter 5) on the basis of availability of limited *in vivo* data and the widespread range of soil-PCB concentrations. In the tested soils, ∑ICES 7 PCB ranged from 8.22 µg/g to 1113.70 µg/g (Table 24). Congener profile was dominated by hexa- and hepta- chlorinated compounds (PCB 138, 153 and 180) (Table 25). All soils were in exceedance of the Dutch VROM 'intervention value' of 1000 µg/kg and 'target value' of 20 µg/kg. The Environment Agency residential and allotment SGV for dioxin like PCBs, dioxins and furans was exceeded by PCB 118 concentrations in all soils. The commercial PCB 118 SGV of 240 µg/kg was exceeded in all soils with the exception of soil 15, with a PCB 118 concentration of 40 µg/kg. Initial PCB concentrations as found in this selection of soils would be in exceedance of human health and sustainable use indicators in a 'total concentration' assessment, and would present a significant level of risk to the health of humans and other biota.

Table 24: ∑ ICES 7 concentrations for tested soils. CRM value is a concentration calculated using ASE. This was performed in order to generate a ∑ICES 7 figure for this soil.

Soil	∑ICES 7 concentration (µg/g)
10	8.22
14	16.42
15	23.46
18	31.58
22	63.90
26	311.69
29	1113.70
CRM (BCR 481)	451.14

Table 25: Mean composition of soils used in the bioaccessibility extractions.

Congener	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑ICES 7
Mean concentration (µg/g) (RSD %)	1.21 (97.01)	1.18 (23.85)	19.02 (178.65)	5.31 (127.32)	83.74 (187.55)	52.98 (169.81)	62.07 (194.18)	224.14 (181.39)
Proportion of total (%)	0.54	0.53	8.48	2.37	37.36	23.64	27.69	100

The BCR 481 CRM was limited by the limited congener range of certification. Of the ICES 7 compounds, certification is available for congeners PCB 101, PCB 118, PCB 153 and PCB 180 (Table 26). The CRM material was therefore assessed using the ASE method described in chapter 4, in order to provide a full range of ICES 7 data to use in bioaccessibility calculations.

Table 26: BCR 481 certified concentration values (European Commission, 1994)**.**

Congener	Certified value (mg/kg)	Uncertainty (mg/kg)
PCB 101*	37.0	3.0
PCB 118*	9.4	0.7
PCB 128	9.1	0.8
PCB 149	97.0	7.0
PCB 153*	137.0	7.0
PCB 156	7.0	0.5
PCB 170	52.0	4.0
PCB 180*	124.0	6.0

The ASE process was performed with five repeat samples of the CRM soil, and the extracted data is shown in Table 27.

Table 27: PCB concentration determined for the BCR soil from ASE extraction.

Congener	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180	∑ICES 7
Mean concentration (mg/kg)	0.3	2.9	46.5	7.4	155.8	121.6	116.7	451.1
Relative standard deviation (%)	5.8	3.6	19.0	8.7	27.5	28.0	39.9	23.0

6.3.2 TOC

Across CE-PBET and FOREhST extracted samples TOC was not shown to be a confounding or determining factor on oral bioaccessibility (Figure 29). This is consistent with previous work performed using the FOREhST method (Chapter 4). These results appear in contradiction of previous studies, where HOC sorption rates have been shown to dependence on TOC (Tang *et al.*., 2006a).. Similarly, TOC showed little relationship with initial PCB concentration in the soils.

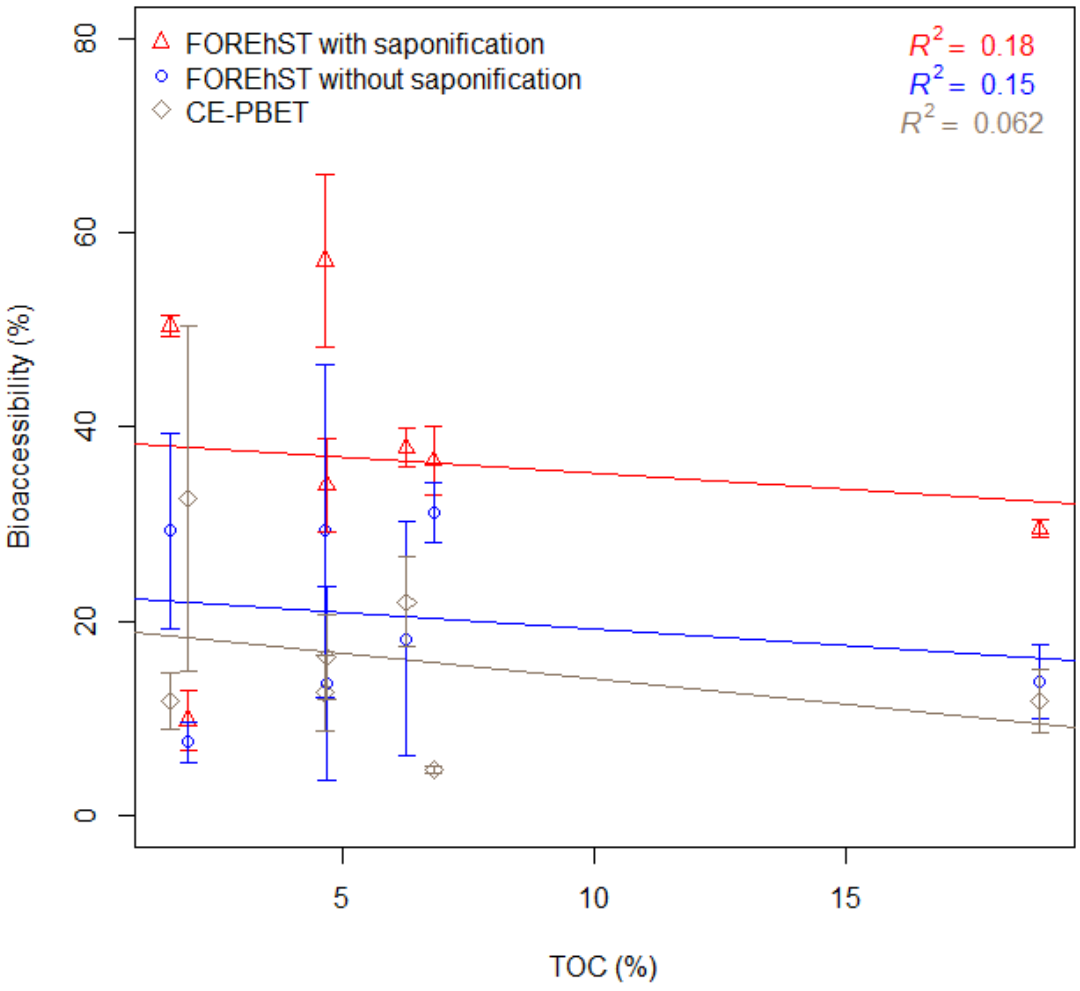


Figure 29: TOC (%) plotted against oral bioaccessibility for CE-PBET, FOREhST, and FOREhST (without saponification) extractions. TOC fails to predict bioaccessibility in all three methods.

6.3.3 Bioaccessibility data - ∑ICES 7 concentrations and individual congeners

Table 28: Congener by congener bioaccessibility values (%), omitting CRM bioaccessibility.

	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180
CE-PBET (%) (RSD %)	9.38 (94.39)	22.60 (61.71)	18.00 (52.18)	16.08 (64.64)	16.15 (59.97)	17.82 (66.20)	17.45 (44.72)
FOREhST without saponification (%) (RSD %)	20.90 (19.60)	19.58 (39.26)	21.58 (40.80)	15.38 (44.35)	22.59 (50.55)	20.89 (46.52)	24.81 (57.39)
FOREhST (%) (RSD %)	41.67 (62.37)	54.45 (31.65)	55.68 (26.79)	45.19 (48.62)	47.74 (27.63)	45.05 (27.43)	2.26 (76.25)

Table 29: Bioaccessibility values recorded using the BCR 481 CRM These values were derived from ASE treatment of the CRM to ascertain ICES 7 concentrations, due to limited certified congener concentrations.

	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180
CE-PBET (%) (% RSD)	9.15 (21.79)	12.02 (24.87)	9.81 (24.19)	17.19 (112.03)	9.62 (29.73)	10.70 (20.77)	5.00 (49.01)
FOREhST without saponification (%) (RSD %)	49.15 (26.61)	45.92 (17.56)	35.93 (20.87)	48.06 (25.70)	46.38 (23.55)	36.60 (21.81)	42.31 (27.83)
FOREhST (%) (RSD %)	55.90 (57.73)	70.10 (22.48)	50.70 (17.18)	96.25 (24.15)	59.85 (15.94)	47.07 (15.09)	4.27 (29.27)

6.3.3.1 CE-PBET

Under the CE-PET protocol, ∑ICES 7 PCB bioaccessibility varied between 4.62% - 32.54%, with a mean value of 15.21% (RSD = 59.80%) (Tables 28 & 29). As discussed, TOC appeared to have no influence on bioaccessibility in this dataset (Figure 29). In previous analysis of the full range of soils using the FOREhST method (Chapter 5), bioaccessibility appears to show greater variation in lower concentration samples. Figure 30 may reflect this phenomenon in terms of one sample with a comparatively high variability though variability was seen in samples of a ∑ICES 7 concentration lower than 100 µg/kg, which is not seen in the CE-PBET data.

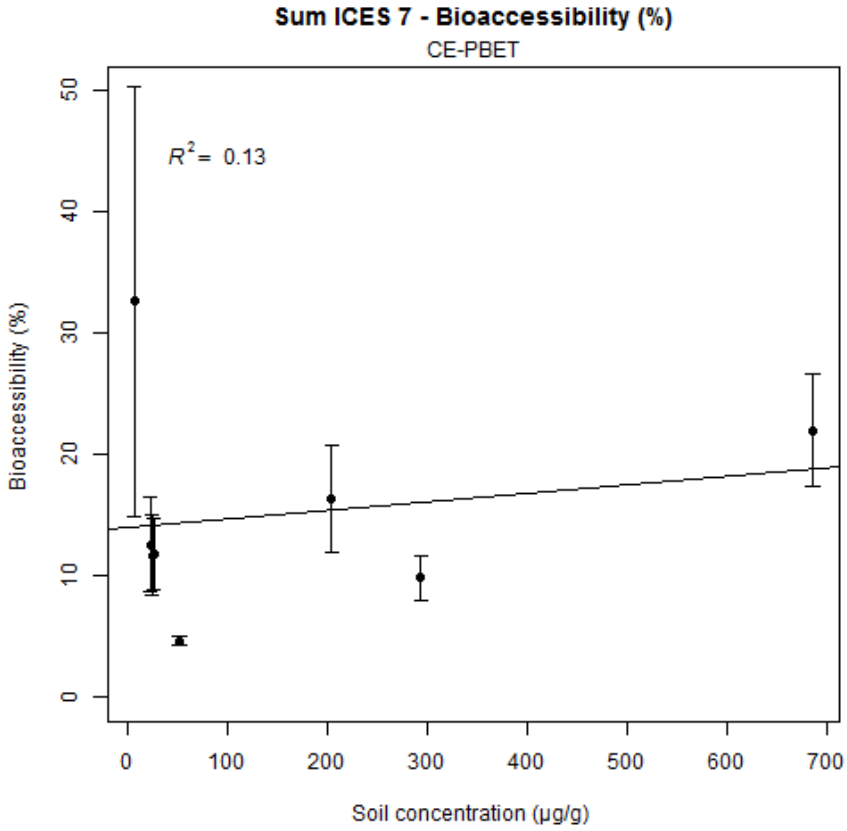


Figure 30: Oral bioaccessibility calculated using the CE-PBET method. ∑ICES 7 values.

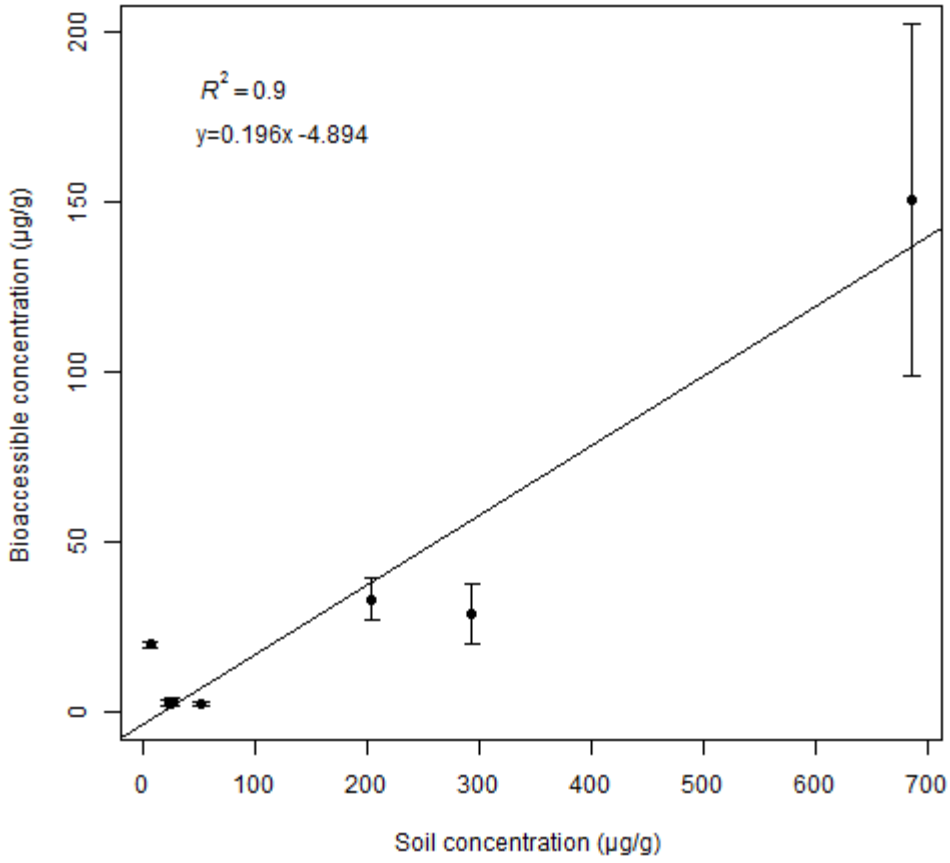


Figure 31: Linear response plot showing the concentration detected in the bioaccessible fraction (combined stomach/ small intestine and colon phases) using the CE-PBET method. Linearity is evident, though the relationshipis less clear than seen in FOREhST data.

A linear relationship between total concentration identified through the CE-PBET process, and the total bioaccessible concentration (identified as the cumulative stomach/ small intestine and colon PCB concentration) (R^2 = 0.9) (Figure 31). This reflects similar relationships identified in previous FOREhST extractions (Figure 32), though this relationship is less prominent in CE-PBET data.

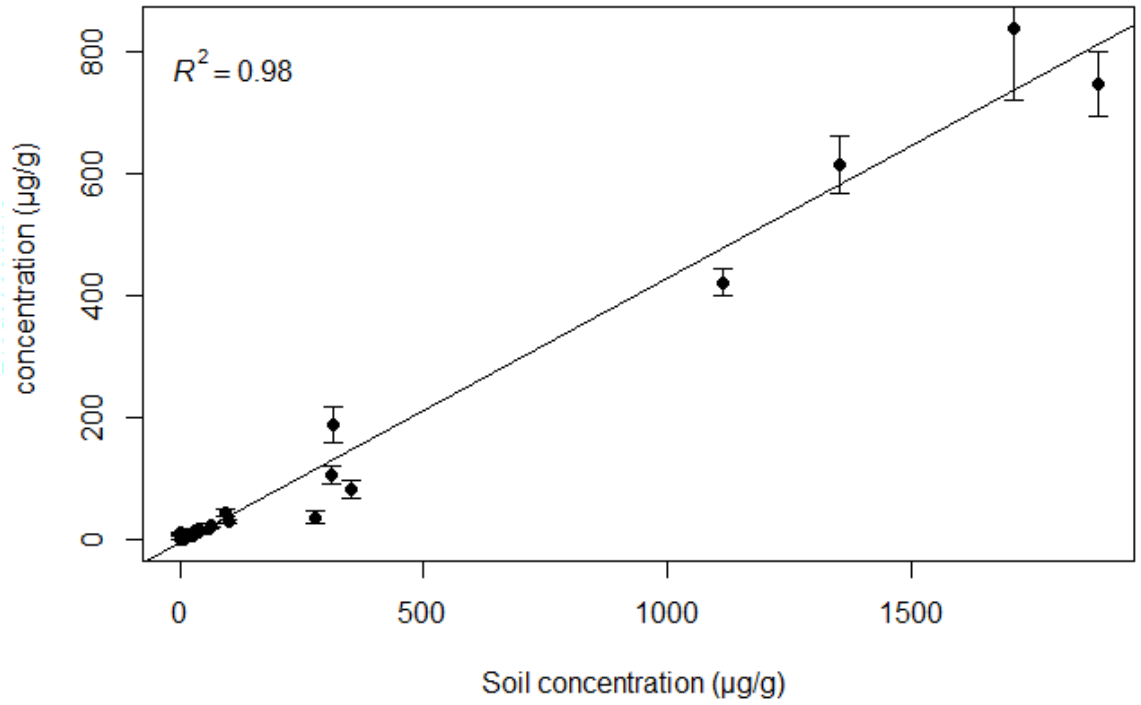


Figure 32: Linear response curve obtained from FOREhST extraction of the 34 soils, plotting initial soil concentration against the bioaccessible fraction. Linearity is clear, and error is than in CE-PBET data and FOREhST unsaponified data.

6.3.3.1.1 Impact of the colon section, and PCB distribution between phases

Bioaccessibility studies of soil bound PAHs have found the extended colon section of the extraction to be a significant driver in soil-PAH desorption and elevated bioaccessibility in comparison to PBET extractions. Tilston *et al.*. (2011) recorded field soil PAH concentrations of up to 4.6 times greater in the carbohydrate rich colon media than in the combined stomach and small intestine phases of the PBET extraction, the colon extended method an expansion of a PBET method previously adjusted to contain a fed element to aid in the desorption of hydrophobic organic compounds from soils. These PAH studies established an upper desorptive limit, or equilibrium, after 8 hours of exposure to the colon medium. In the case of this study, the full colon section period of 16 hours has been applied to maximise exposure of PCB bearing soils in case of an absence of equilibrium.

The results of the CE-PBET extraction in this study showed that bioaccessibility was elevated by the presence of the colon section, but this failed to produce increases comparable with PAH extractions, despite the full 16 hour exposure period (Table 30, Figures 34 & 35). This presents the possibility of either a much lower equilibrium of PCB concentration in colon fluid than equivalent PAH extractions,

or an upper limit in the desorption rate of PCBs from the soil sample which is less prominent in PAH extractions. The inclusion of a 'sink', comprised of a section of hydrophobic material with properties consistent with HOC sorption, such as sections of silicone rod has been found to overcome the effects of compound desorption equilibria in gut model fluids, and may be applicable to the CE-PBET method. However, the linearity of the relationship between dose and response (Figures 33 and 34), indicating the absence of an upper desorptive limit, suggest that this approach may be limited in this case. In the case of all tested soils, the majority (69.25% - 95.23%, with a mean of 85.01%) of ∑ICES 7 PCB was retained in the resultant soil 'pellet', the sample of soil combined with a small coating of solid mass from the gut fluid, retained following centrifugation and colonic supernatant removal.

Table 30: Calculated proportions of ∑ICES 7 PCB in CE-PBET sections and retained soil pellet

Soil	Stomach/ small intestine phase (%)	Colon phase (%)	Soil pellet (%)
10	29.56	1.19	69.25
14	9.38	3.24	87.38
15	10.80	0.76	88.44
18	9.33	3.04	87.62
22	3.59	1.19	95.21
26	12.68	3.52	83.80
29	15.24	6.47	78.30
BCR	7.02	2.93	90.06
Mean	12.20	2.79	85.01
Standard deviation	7.84	1.83	8.00
Relative Standard deviation (%)	64.23	65.68	9.42

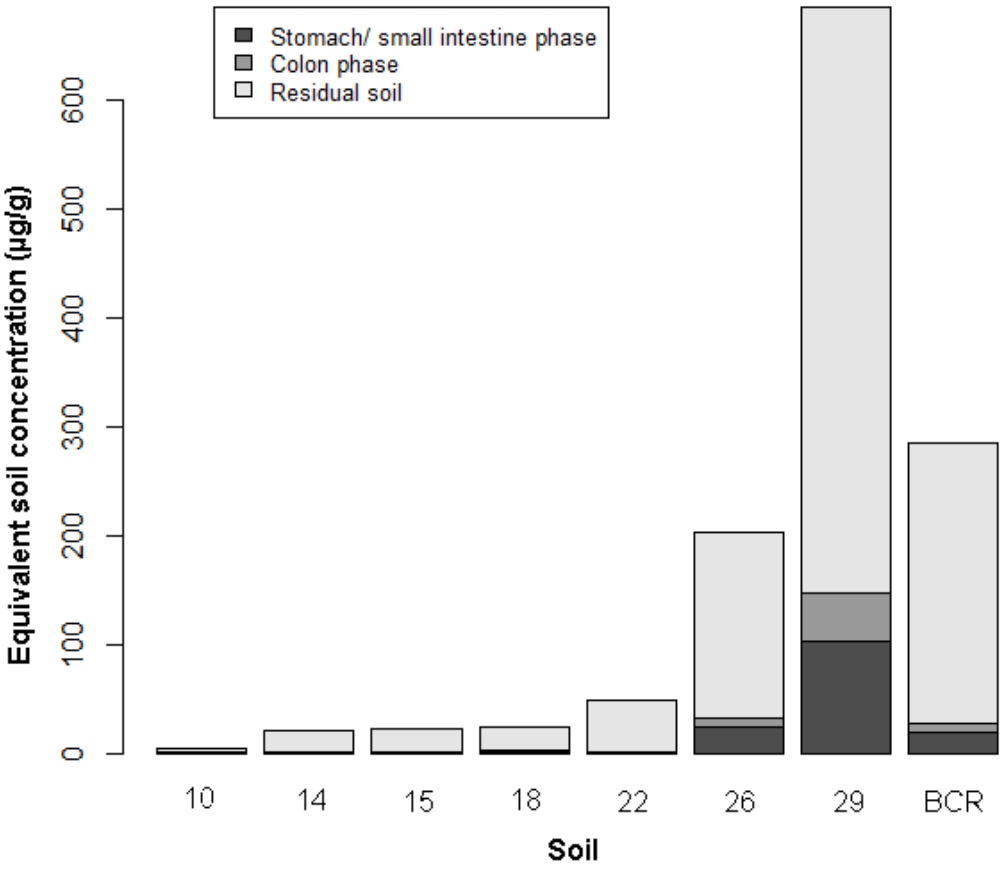


Figure 33: Concentration of ∑ICES 7 PCB in residual soil, stomach/ small intestine and colon phases after CE-PBET for 7 industrially contaminated soils, and BCR 481 (CRM). The BCR data represents a calculated figure derived using an ASE method in order to obtain a ∑ICES 7 total.

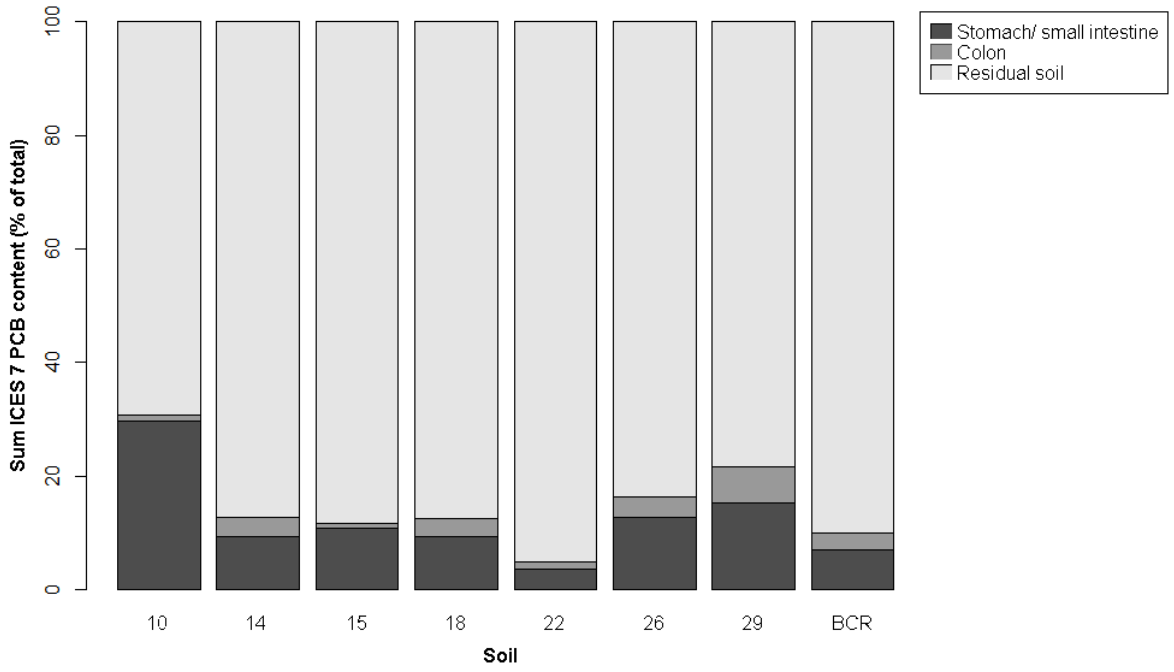


Figure 34: Proportion of ∑ICES 7 PCB in the stomach/ small intestine, colon, and residual soil of 7 industrially contaminated soils, and the BCR 481 recognised CRM, after the CE-PBET process. The BCR data represents a calculated figure derived using an ASE method in order to obtain a ∑ICES 7 total.

6.3.3.2 FOREhST

Following an initial 34 soil survey of bioaccessibility using the FOREhST method in Chapter 4, comparative extractions were performed on the eight soils processed with the CE-PBET method using the FOREhST method. During the initial 34 soil survey, it was determined that PCB 180 recovery was significantly reduced, with mean PCB 180 bioaccessibility calculated as 1.61%, compared with a ∑ICES 7 value of 57.12%, reflected in CRM samples. Due to the low recovery of PCB 180 it should be omitted for further bioaccessibility calculations. This was traced to a MeKOH saponification step included in the FOREhST protocol, which was affecting the detection of heptachlorinated biphenyls in supernatant samples through dechlorination (Erickson, 1997). MeKOH saponification is a technique found to be effective as a method of extraction of HOCs from matrices such as foods, soils and biota. In the case of the FOREhST process, this was applied to aid in the extraction of PAHs, and later PCBs, from a lipid rich matrix, containing remnant food constituents

humic acids and potentially colloidal soil particles (Cave *et al.*., 2010). In order to eliminate this effect, samples have been extracted using FOREhST without the saponification step, and presented alongside those with the saponification step included for comparison with CE-PBET results.

Linearity is evident in the relationship between soil ∑ICES 7 PCB concentration and bioaccessible fraction, though the relationship shows a stronger correlation in the saponified samples, despite reduced PCB 180 recovery (R^2 = 1, R^2 = 0.78) (Figure 35).

In all of the eight comparison soils and the CRM, bioaccessibility values for ∑ICES 7 were greater in the saponified samples, with a mean value of 39.63% for saponified samples (minimum value 29.49, maximum value 57.12), compared with a mean value of 23.07% in the samples extracted using the FOREhST protocol with the saponification step removed (Figure 36). ANOVA analysis reveals a significant difference between the bioaccessibility values obtained with and without the saponification step (p= 0.009). With the notable exception of PCB 180, this pattern was reflected in individual PCB congener bioaccessibility (Figure 37). However, in all soils except soil 10, FOREhST bioaccessibility (with the saponification step in included or omitted) was greater than the CE-PBET calculated equivalent. With the exception of PCB 180 in the case of saponified samples, this is equally true of individual PCB congener and ∑ICES 7 bioaccessibility calculated from all soils (Figure 38).

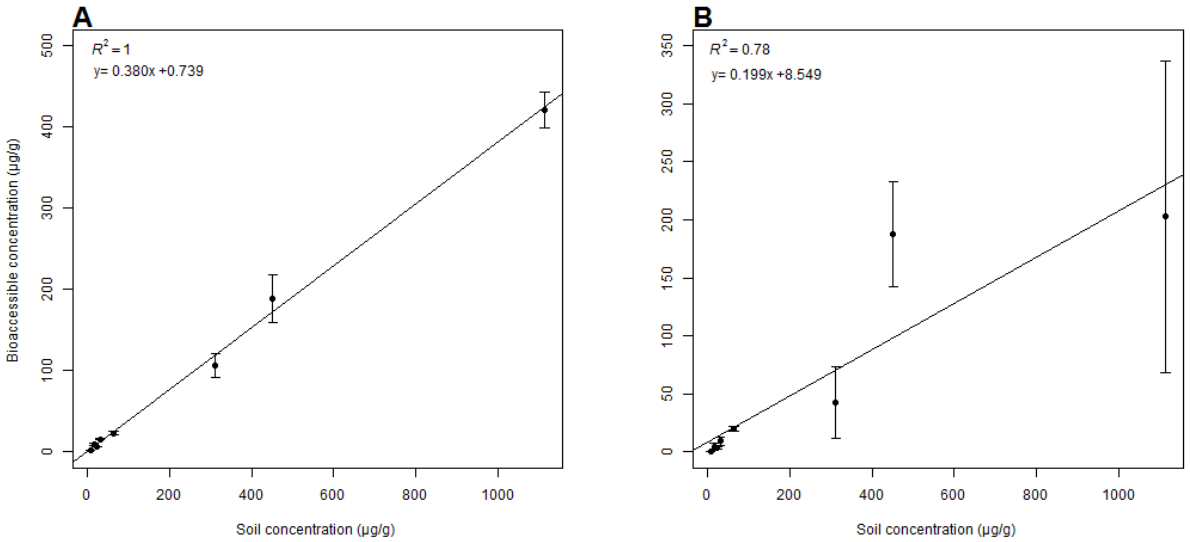


Figure 35: Linear response curves of bioaccessible concentration obtained from FOREhST extractions of the group of 7 soils and CRM tested with the CE-PBET methodology. (A) with the saponification step included in the cleanup (n=3); and (B) with the saponification step omitted (n=5). In both cases the relationship appears linear, though this is more prominent in those samples treated with saponification. This is in spite of greatly reduced recovery of PCB 180 in this method.

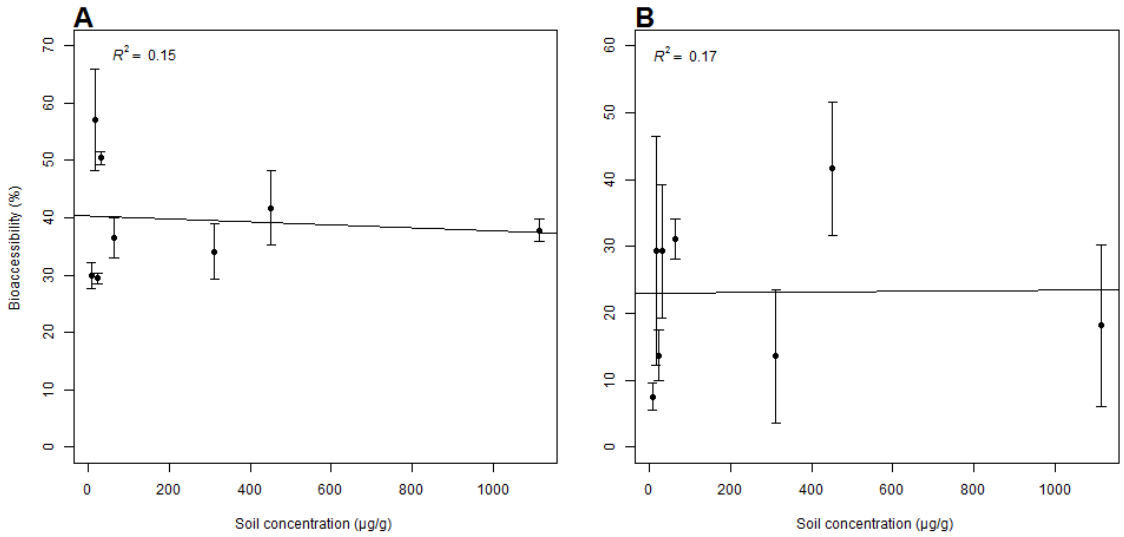


Figure 36: Bioaccessibility values obtained from the group of 7 soils and CRM. (A) using the saponification cleanup method and (B) with the saponification step omitted.

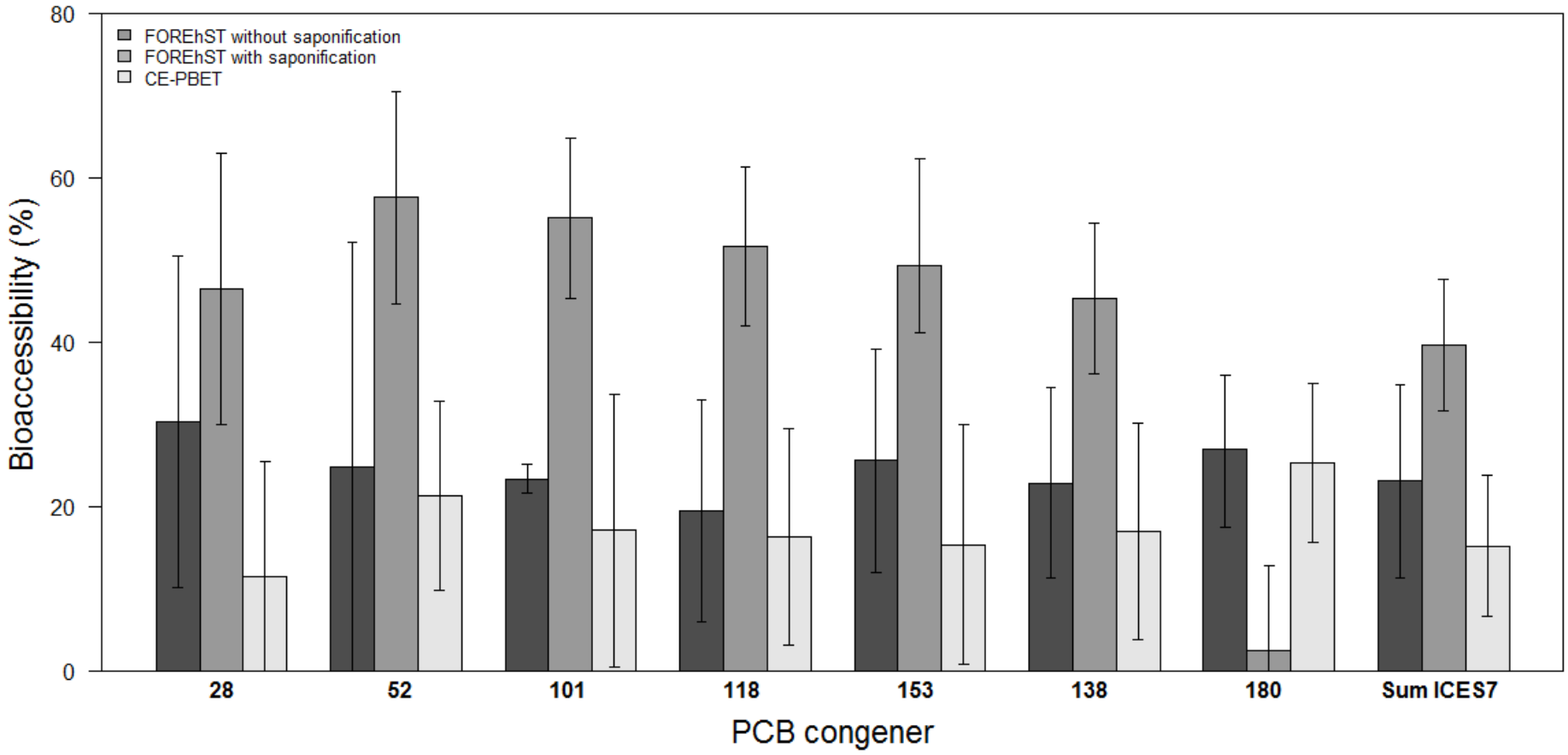


Figure 37: PCB congener bioaccessibility calculated using CE-PBET, FOREhST and FOREhST (without saponification) for 7 industrially contaminated soils and a recognised CRM (BCR 481). THE BCR data represents a calculated figure derived using an ASE method in order to obtain a ∑ICES 7 total.

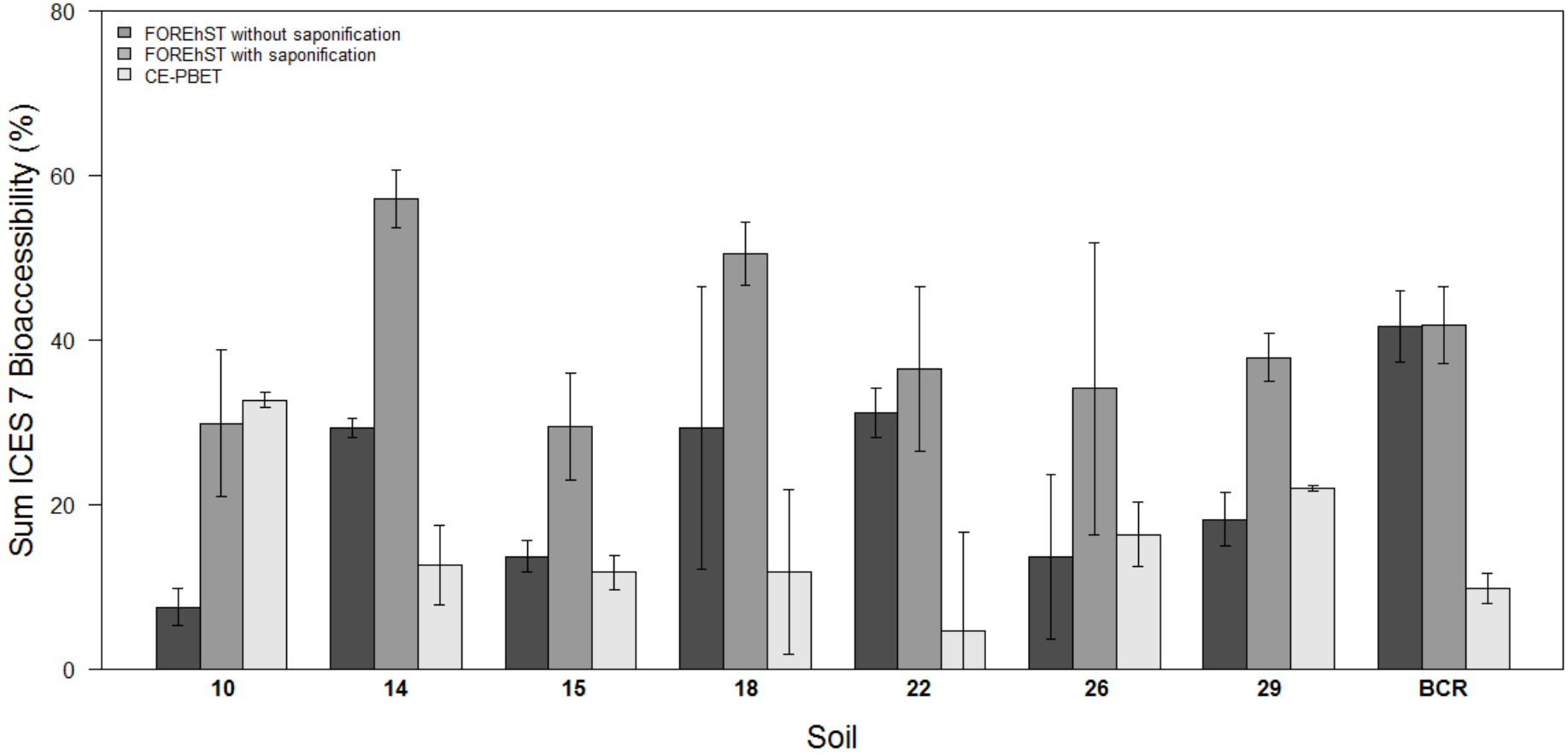


Figure 38: ICES 7 PCB bioaccessibility (per soil) calculated using the CE-PBET and FOREhST methodologies (with and without saponification during the cleanup procedure). THE BCR data represents a calculated figure derived using an ASE method in order to obtain a ∑ICES 7 total.

6.3.4 Correlation between initial soil concentration and bioaccessible concentration

Plotting the initial concentration of tested soils against the bioaccessible concentration (in the case of CE-PBET extractions, a total bioaccessibility value, combining the small intestine/ stomach section with the colon section), enables the construction of a 'dose-response' curve. These curves have been constructed from data obtained from extractions of soils using the CE-PBET, and FOREhST methodology with a saponification step included, and removed. The resultant curves appeared to show linearity. These relationships have been further investigated using the resultant regression statistics. Denys *et al.* (2012), in their *in vivo* validation work with the calculated bioaccessibility of heavy metals in soils using the UBM method, demonstrated a process of benchmarking in order to test the robustness of linear relationships between contaminant bioaccessibility and bioavailability. Benchmarks developed covered the R^2, slope and y intercept values:

- The intercept is not significantly different from 0;
- The slope should be between 0.8 and 1.2;
- The R^2 value should be greater than 0.6 (Denys *et al.*., 2012b).
- This method has been applied to investigate ∑ICES 7 soil concentrations and bioaccessible fraction response.

R^2 values in all cases were above the 0.6 benchmark defined by Denys *et al.*, though the increased standard deviation reflected by the FOREhST (without saponification) data extended beneath the limit. Otherwise, FOREhST (0.929) and CE-PBET (0.985) values were robust (Figure 39).

Intercept values showed greater variability between the methods, revealing varying degrees of bias between methodologies. The CE-PBET value, in particular, suggests a negative bias in bioaccessible concentration in compared to initial soil values. This is not reflected in FOREhST data, which tends towards a slight positive bias.

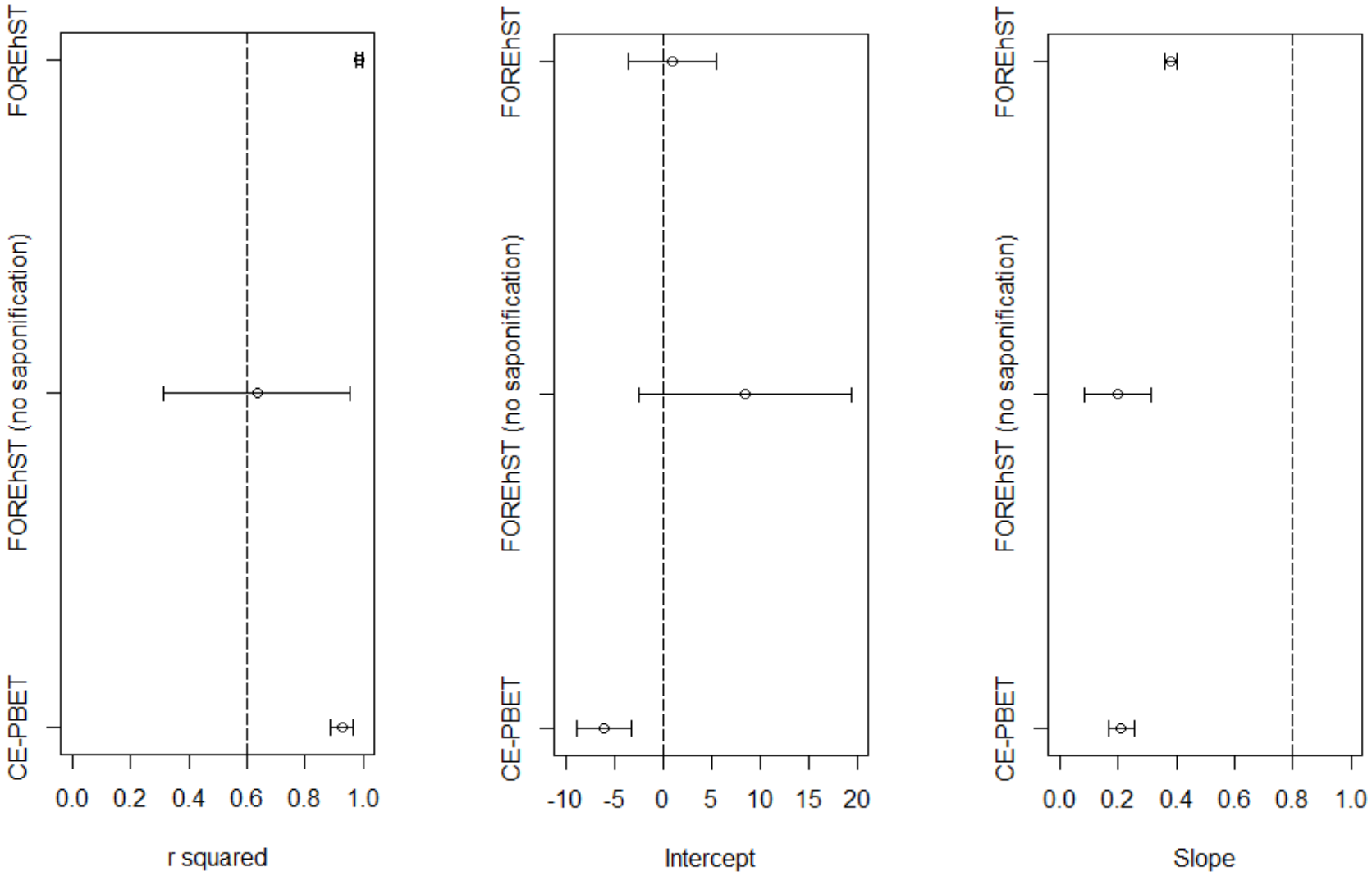


Figure 39: Summary of regression statistics from the ∑ICES 7 initial soil dose response curves for FOREhST, FOREhST (without saponification) and CE-PBET. Error bars represent satandard deviation. Dotted lines show benchmark values described in Denys *et al.* (2012)

Slope values describe the nature of the relationship between the initial and bioaccessible values, expressed as a gradient or 'slope' value. A plot of bioaccessible concentration against initial soil concentration would not be expected to show a 1:1 relationship, as predicted in Denys (2012), as the bioaccessible fraction necessarily reflects a lower concentration than the official total value. As such, all three methodologies exhibit slope values beneath the lower benchmark value of 0.8. Lower values recorded for FOREhST (without saponification) (0.199) (and CE-PBET (0.210) than the value recorded for FOREhST (0.381) reflect typically lower bioaccessibility rates recorded in these methods.

6.3.5 Comparison with in vivo studies

Bioavailability, a concept common in pharmaceutical and animal sciences, was described in Littell *et al.*. (1997) as a measure of 'the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilised in metabolism by the animal', a measure of the 'effectiveness' of a drug or potentially harmful substance. The concept of bioaccessibility helps to describe the absorption of a substance into the central body cavity (Oomen *et al.*., 2002), and can be effectively described in terms of a consequence of bioaccessibility (or, the absorption of a given substance across a membrane, and movement within the body to an effective 'point of harm', following release into a bioavailable matrix from a source, or carrier matrix, such as a soil, dust or foodstuff. One can imagine the bioaccessible fraction as a source for a *potentially* bioavailable substance, which has become liberated from a source material. As such, bioaccessibility and bioavailability are inextricably linked, and bioaccessibility, given a reliable relationship with bioavailability data, can be used as an effective predictor of potential ham.

Bioavailability was calculated using a juvenile swine model for a group of four PCBs (congeners 101, 138, 153, 180), with data published in (Delannoy *et al.*., 2015). Although the availability of this data is limited to a subset of soils, and limited to four PCBs due to difficulties in peak integration of the analytical chromatography, it is provided in this study for the purpose of guidance.

The adopted methodology was presented in Denys *et al.*. (2012), and represents a repeated means approach, developed as a method of alleviating the effect of significant error typically associated with bioaccessibility and bioavailability data. This approach results in the development of high, low and median R2 values (Table 31), which are presented as a measure of linearity in the bioaccessibility/ bioavailability plots.

Bioaccessibility was found to be consistently lower than bioavailability in all cases (Figure 40). Linear relationships between bioaccessibility and bioavailability were absent in most cases, though FOREhST derived bioaccessibility appears to show a degree of correlation in PCB 153 that is not replicated in other method/ compound combinations (R^2= 0.35). The range of bioavailability data, limited to a selection of four PCBs, eliminated the possibility of a ∑ICES 7 comparison.

Table 31: R^2 values calculated for bioaccessibility/ relative bioavailability comparison in selected PCB congeners.

		R^2		
Method	Congener	Low	Median	High
FOREhST	101	0.00	0.28	0.84
	138	0.00	0.17	0.83
	153	0.00	0.35	0.93
	180	0.00	0.15	0.83
FOREhST (no saponification)	101	0.00	0.11	0.75
	138	0.00	0.18	0.87
	153	0.00	0.14	0.84
	180	0.00	0.16	0.90
CE-PBET	101	0.00	0.15	0.83
	138	0.00	0.15	0.85
	153	0.00	0.12	0.79
	180	0.00	0.12	0.75

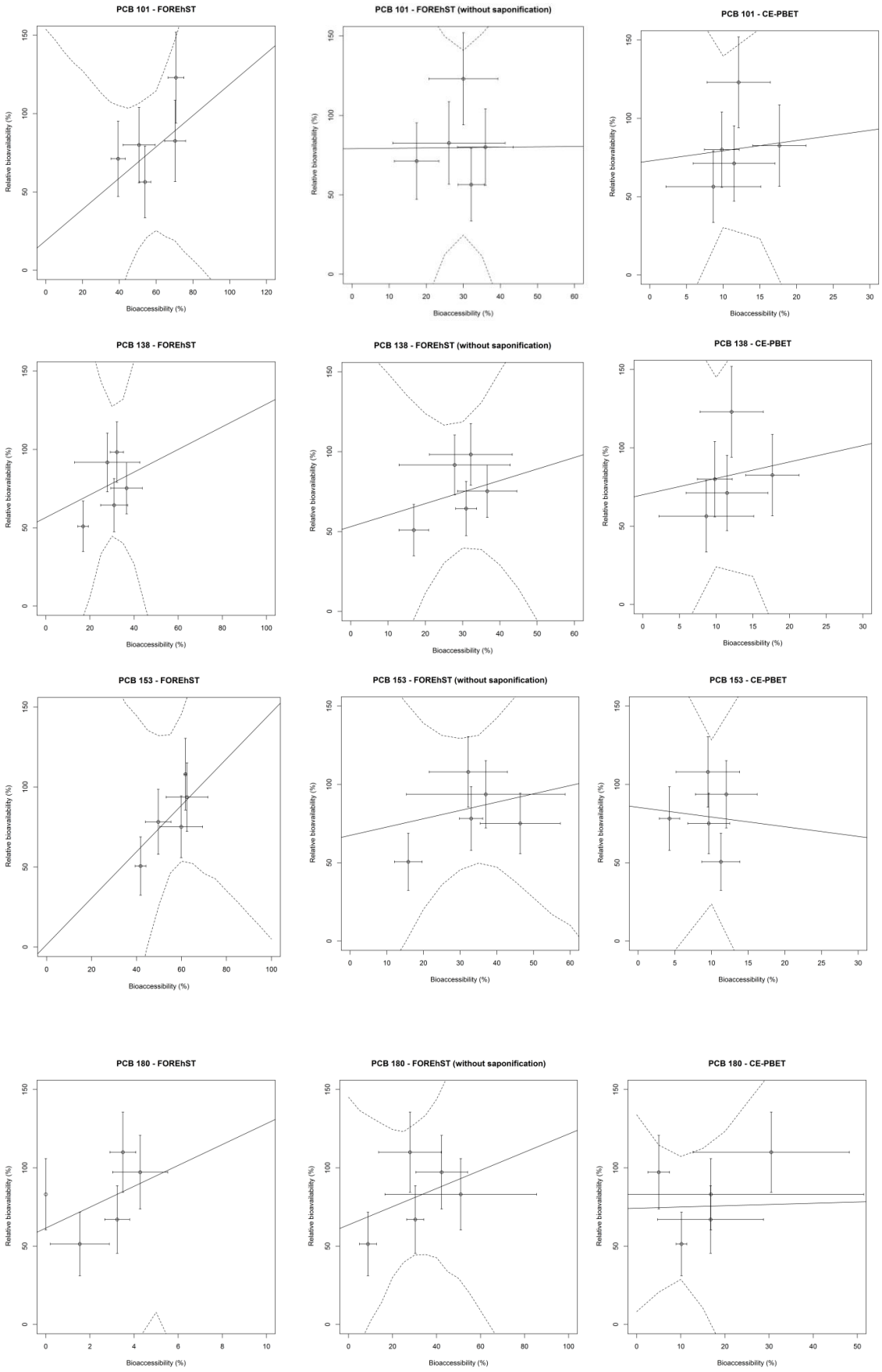


Figure 40: Bioaccessibility/ relative bioavailability correlation plots for selected PCB congeners. The solid line is the line of best fit, dashed lines mark the 95% confidence intervals.

6.3.6 Comparison of bioaccessibility values between methods

Observation of plotted data (Figures 37 and 38) suggest differences between the mean values obtained through the use of the three methodologies. The FOREhST method, with the saponification step in place, appears to provide consistently higher percentage bioaccessibility values in all soils (Figure 38) and across all congeners, with the exception of PCB 180. These patterns are confirmed through statistical testing. Differences between the mean ICES 7 bioaccessibility values were investigated via an ANOVA test, revealing significant difference between the three methods (p = 0.00029) (<0.01). Further investigation of variables using the Tukey's HSD test revealed statistically significant differences between the values obtained from FOREhST and CE-PBET (p = 0.00024), FOREhST and FOREhST without saponification (p = 0.00923) (<0.01), but not between CE-PBET and FOREhST (without saponification) (p = 0.28303) (>0.01).

6.3.7 Consistency between congeners

Statistical testing was also applied to investigate the potential differences, or potential consistency between congener bioaccessibility obtained with each method. Initial observation of the plotted data suggest a degree of consistency between congeners and chlorination homologs (Figure 41). An ANOVA test confirmed this, with no significant difference between congener means detected in the CE-PBET (p = 0.875) and FOREhST (without saponification) (p = 0.161) datasets (> 0.01). Significant difference between congener bioaccessibility was identified in the FOREhST data (p = 0.0000004) (<0.05), with a Tukey's HSD test revealing the significant difference to be present only in the comparison of PCB 180 data to other congeners. There is no statistical difference between the mean bioaccessibility values of non-PCB 180 congeners. This echoes the conclusions of Chapter 4.

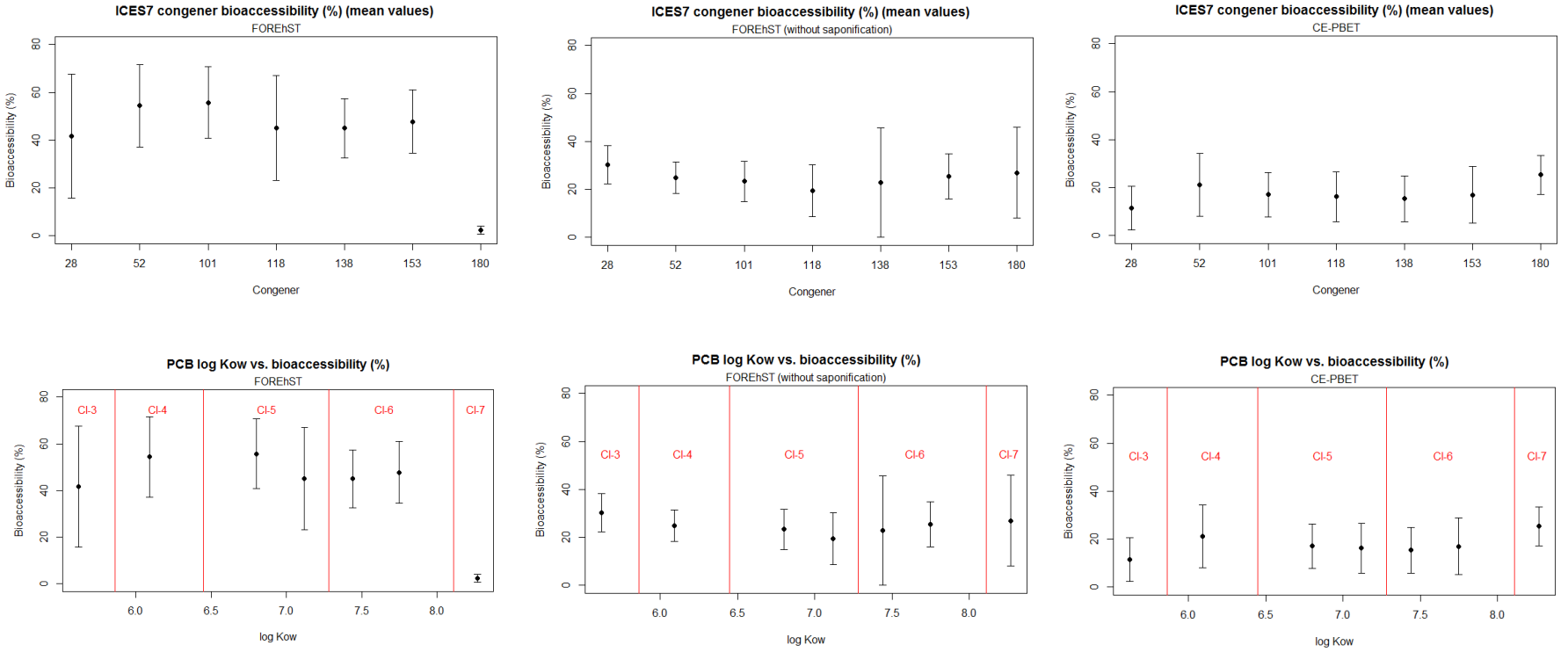


Figure 41: Congener specific % bioaccessibility (top) and the % bioaccessibility of PCB homologs expressed in terms of LogKow (lower) of 7 industrially contaminated soils, measured using the FOREhST methodology, FOREhST with the saponification step omitted, and CE-PBET. Error bars represent standard deviation around the mean value.

In order to monitor potential losses of analyte compounds due to sample handling (potential losses associated with the liquid:liquid extraction process, SPE process and sample concentration), standards were introduced at key points during the extraction protocols. A 50 µL solution of PCB 19 (20.01 ng/µL) and PCB 147 (19.31 ng/µL) was introduced as a surrogate standard in the form of a soil spike. Internal standards of PCB 34 (19.77 ng/µL), PCB 119 (18.92 ng/µL), PCB 131 (19.24 ng/µL) and PCB 173 (18.81 ng/µL) were introduced prior to the clean-up procedure in order to quantify the analyte and surrogate standard compounds.

Percentage recovery of the standards was as follows (Relative standard deviation in parenthesis):

Table 32: Surrogate standard recovery obtained using the FOREhST method without the saponification stage and CE-PBET (% recovery rates).

FOREhST method without saponification		CE-PBET method	
PCB 19	PCB 147	PCB 19	PCB 147
46.67% (17.70%)	21.8% (59.50%)	41.91% (143.66%)	n.d.

As with previous extractions, the data was not corrected for standard recovery to the variation in physicochemical properties of the analytes. Once again, it would be possible to apply a wider range of standards to reflect these variations, but with significant cost and complexity increases. The methods tested showed greater variation and lower recovery than the standard FOREhST methodology. PCB 147 was beneath the limit of detection in CE-PBET standards, which may be attributed to reduced bioaccessibility data recorded for this method.

6.4 Conclusions

This chapter set out to investigate and identify any differences in bioaccessibility recorded for 7 industrially contaminated soils (alongside a suitable CRM) using three different extraction methodologies. This followed a previous survey of a wider group of 34 industrially contaminated soils using the FOREhST method alone.

It was identified in previous work that a saponification stage in the standard FOREhST procedure caused a discrepancy in the recovery of heptachlorinated compounds, which was reflected in extremely low recorded values for PCB 180. As such, a modified FOREhST methodology, with the saponification step removed, was introduced to investigate the potential for a PCB focused FOREhST methodology which better reflected anticipated PCB 180 recovery.

Analysis of bioaccessibility recorded reveals the original FOREhST methodology to provide consistently higher values than both the CE-PBET method and the FOREhST method with the saponification step removed, with a mean ∑ICES 7 bioaccessibility of 39.63% in comparison 23.07% recorded using the amended method, and 15.21% in CE-PBET. This value includes the reduced recovery of PCB 180, which was found to be significantly lower than the recovery of other congeners using the same method. ICES 7 values obtained using the FOREhST method without saponification and CE-PBET were found to be not statistically different. The impact of the colon section was found to increase bioaccessibility in all congeners, though the increase was limited, and was less pronounced tahan in previous PAH studies, where the colon section was found to make up a far larger proportion of the bioaccessible fraction. TOC appeared to have no effect on bioaccessibility. This supports the findings of Chapter 5, which explored the bioaccessibility of 34 industrially contaminated soils, and came to the same conclusion.

In terms of individual congener bioaccessibility, congeners were found to be consistent across each extraction methodology (with the exception of PCB 180 in the unmodified FOREhST protocol). This is in spite of variation in LogKow for individual PCB homologs (Figure 41). Consistent bioaccessibility between congeners in the unsaponified samples confirms the low recovery of PCB 180 in FOREhST extractions is associated with the cleanup methodology, as opposed to systematic reduced heptachlorinated compound recovery, as suggested in Chapter 5

All methods exhibited linear relationships between the initial soil PCB concentration and response. This expands upon the findings of Chapter 5, with linearity representing a potential opportunity to

predict, to a limited extent, bioaccessibility on the basis of initial soil concentration. The linearity of this relationship suggests a high degree of consistency within the method data.

Comparison with bioavailability failed to show meaningful relationships or significant correlation, though this was hampered by limited bioavailability data. The comparisons with bioavailability are presented in this work by way of an indicator, and should not be considered as a suitable validation experiment for any of the investigated extraction methods. More work in the validation of these methods would be useful.

This data is presented uncorrected due to the limitations posed by the standard protocol. Correction may be possible in future tests, but standards must be applied to all congener chlorination groups. Although this technique would add clarity, it would significantly increase the cost and complexity of the extractions.

6.4.1 Implications and findings

(i) Mean bioaccessibility was found to be significantly higher in the unmodified FOREhST methodology than in the adapted protocol, which omitted the saponification step of the cleanup procedure. This suggests that further work should continue with the original methodology, but omit PCB 180 as a target compound. As an alternative, further work may suggest an alternative to saponification that allows for the breakdown of fatty acids and other post-digestive products without the breakdown of PCB 180, or an adapted saponification process may be developed.

(ii) Bioaccessibility derived from the CE-PBET method was significantly lower than in the case of FOREhST, though it was not significantly different to those samples not treaded with saponification. Though initially this suggests that FOREhST represents a more conservative assessment protocol, further tests of CE-PBET with a saponification step may show increases in bioaccessibility in non-hepta- PCB congeners.

(iii) The colon section of the CE-PBET extraction increased bioaccessibility, but the increase was far less dramatic than in previous PAH studies. Again, a modified cleanup methodology may improve this recovery, and experiments into a suitable saponification technique are advised.

(iv) Mean bioaccessibility remained constant between congeners and PCB homologs. This may suggest that the carbohydrate rich micellar gut fluids using in FOREhST and CE-PBET overcome the preferential desorption of lower molecular weight compounds seen in previous HOC studies.

(v) TOC does not appear to be a controlling factor on PCB bioaccessibility.

(vi) Limited tests reveal poor association with relative bioavailability, though it is advised that further *in vivo* comparison, following through bioaccessibility methodological development, is essential for model validation.

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CHAPTER 7: THE ROLE OF BIOACCESSIBILITY IN SUSTAINABLE CONTAMINATED LAND MANAGEMENT

The contaminated land problem is one of potential damage, ongoing damage and legacy, and has been described as one of the most significant anthropogenic pressures on the ecological function of European soils (Cachada *et al.*., 2016). Over 300,000 ha of land in the UK are classed as 'potentially contaminated' (Hou and Al-Tabbaa, 2014). However, this figure represents an estimate – the true extent of the contaminated land issue, particularly in the case of legacy pollutants, is not known in detail, and is subject to detailed site investigation and risk assessment, including, crucially, the correct definition of 'contaminated' land (DEFRA, 2006). Contaminated land represents a sizable challenge, or opportunity, for sustainable development in the UK.

Recent years have seen an increase in the prominence of the sustainability concept in the fields of land management and remediation (Hou and Al-Tabbaa, 2014; Bardos *et al.*., 2016a; Cappuyns, 2016; Ridsdale and Noble, 2016; Huysegoms and Cappuyns, 2017), including the growing focus of government incentivisation of the redevelopment of brownfield sites, including those affected by contaminated land (Dixon, 2012; Burke *et al.*., 2015). It is important therefore to consider the potential for bioaccessibility to be integrated into the sustainability led land management approach. Sustainable remediation has been described as 'an integrated consideration of social economic and environmental factors' (Bardos *et al.*., 2016a), a definition formed through the work of the EU CLARINET group, which also promoted the concept of placing risk assessment at the core of the determination of remediation requirement, including impact on receptors and the severity of potential harm. Bardos *et al.*. (2016) goes on to describe a sustainability –led contaminated land regime that considers societal harm, and the need to consider the role of remediated land following the completion of works. It is within this concept bioaccessibility testing is framed.

The successful and relevant assessment of contaminated land, from a risk perspective (as advocated by the CLARINET group), requires an understanding of the contaminant as a potential cause of harm.

As such it is necessary to consider the movement of the contaminant following ingestion, and the readiness of a substance to leave the soil to enter a bioaccessible state, or to become bioavailable within the bloodstream. Total contaminant approaches do not allow this nuanced, detailed approach, but assume an effective 100% bioavailability, although assessment techniques typically consider estimates on typical human exposure rates as a sum of exposure routes, and are based on toxicity based tolerable daily intake values calculated from *in vivo* exposure tests (Environment Agency and DHI, 2005).

Bioaccessibility tests act as predictors of bioavailability, present the opportunity for the modelling of post consumption contaminant behaviours, and can provide a physiologically relevant, conservative assessment, without the absolutism of the total contaminant ingestion approach. Bioaccessibility assessment also allows the simulation of multiple contaminant pathways. Oral ingestion is the most prominent, though respiratory and dermal pathways are prominent exposure routes. In this study, focus is on the ingestion pathway. Bioaccessibility research has, so far, focused largely on the ingestion pathway due to the prominence of this route in the soil contamination environment, and the establishment of reliable *in vitro* testing.

Bioaccessibility testing allows the application of physiologically relevant assessment criteria at the heart of land management, and can contribute, amongst other factors towards a less conservative and more sustainability and health focused approach (Koch and Reimer, 2012). The influence of bioaccessibility derived data is to effectively raise the threshold by which land is classified as 'contaminated', or a designated Part IIA site under the guidance of the Environmental Protection Act 1990, in tandem with site specific assessment criteria derived from the Contaminated Land Exposure Assessment (CLEA) model (Nathanail, 2005; Gay and Korre, 2006). The result of reclassification has the potential to unlock some, if not all, of the 300,000 ha of land considered to be contaminated under current 'total contaminant' approaches. Avoidance of the contaminated land designation results in the avoidance of remediation, and the costs environmental and financial costs associated.

Although it is unclear how profitable the inclusion of a standard bioaccessibility element in a site specific contaminated land assessment may be, since this is dependent on the value of the site, the extent of remediation and building costs, it is clear that methods to reliably increase the threshold into the status of 'contaminated land' can have a fundamental impact on the need for remediation. Remediation avoidance allows financial savings to be made, and eliminates the need for energy intensive remediation methods, excess road traffic movements and landfill disposal of contaminated materials. This helps to incentivise the re-use of brownfields in preference to greenfield, uncontaminated sites, as the uncertainty, complexity and clean-up cost otherwise represents a barrier to the redevelopment of contaminated land (Schadler *et al.*., 2011).

A potential limit to the usefulness of bioaccessibility lies in the awareness of assessment techniques and rates of uptake amongst regulatory bodies. A 2009 survey of Local Authorities in England and Wales conducted by the Chartered Institute for Environmental Health, Newcastle City Council and the University of East Anglia found that 70% of respondents agreed that bioaccessibility is seen as a 'useful tool that facilitates contaminated land management'. The same survey found that 78% of respondents cited a lack of adequate guidance as a barrier to the implementation of bioaccessibility techniques, with 71% citing uncertainties about bioaccessibility as a limit to the usefulness of the application of bioaccessibility assessment (EUGRIS, 2010). Although academic research into bioaccessibility has increased in the intervening years since the survey was conducted, and standardised protocols have been introduced for the assessment of bioaccessibility from metals in environmental samples (International Organisation for Standardisation, 2007), through methods such as the Unified Bioaccessibility Method (UBM) (Cave *et al.*., 2016), there is currently no standardised methodology for the assessment of bioaccessibility in POPs or emerging organic contaminants, contaminant groups associated with elevated human health risk and environmental accumulation (Rodríguez-Navas *et al.*., 2017b). In addition, it is important that methods exhibit repeatable and reproducible results in inter-laboratory trials, and maintain consistency with data obtained through *in vivo* trials (Collins *et al.*., 2015).

With an ever growing demand for suitable construction land, and increased focus on the redevelopment of brownfield in preference to greenfield sites, and an increasingly visible focus on sustainability in land management and remediation, assessors require a wide range of tools to better address the problem of contaminated land. Bioaccessibility represents on one of the most powerful techniques available to address uncertainty and over-conservatism in contaminated land management, with significant potential environmental and financial benefits. An increased focus on brownfield development contributes to cleaner, more functional and sustainably managed urban areas. Despite this, technical barriers to the widespread adoption of bioaccessibility remain. The work presented in this thesis aims to contribute to the knowledge that can help to unlock the potential of these methods.

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CHAPTER 8: CONCLUSIONS

This chapter summarises the key conclusions and findings of the thesis sections, and assesses the potential impact of findings and contribution to knowledge. This will include potential industrial, policy and academic impacts, reflecting the unique nature of the EngD programme. The originally laid out aims and objectives will be revisited in order to assist in the assessment of impact and significance of the work. Limitations, potential improvements and strengths are also discussed, along with recommendations and any potential opportunities for further work.

8.1 Impacts, implications and contribution to knowledge

The nature of this project is such that the findings of this thesis contribute to academic knowledge, alongside potential industrial and procedural impact. These impacts will be measured against the initial project aim and research objectives.

The overarching aim of the thesis was to answer the research question:

"*To what extent can bioaccessibility testing address contamination by organic pollutants in urban soils, and can it play a role in the development of a more sustainability-led redevelopment programme?*"

This aim was developed as a refinement of a statement of aims made in Chapter 1. The statement of aims was developed as a synthesis of the initial project title:

"Developing a sustainable method for the determination of bioaccessibility of organic contaminants from polluted sites".

Research objectives were developed to aid in the development of a thesis and research plan to address the research question.

1. *"Establish the current research level in the field of bioaccessibility in order to identify the issues to address, and any potential gaps in knowledge."*

The thesis set out to review previous bioaccessibility research, including work done on different compound groups and organic and inorganic contaminants. This led to reviews of the major bioaccessibility methods, particularly those which have been applied to organic contaminants. It was identified that research into bioaccessibility, bioaccessibility in organics, oral bioaccessibility and bioaccessibility associated with both PAHs and PCBs has been steadily increasing since 2000. However, review of literature in the bioaccessibility field identified the need for greater application, testing and assessment of methods for the testing of bioaccessibility in organics.

Further review of literature has led to the identification of PAHs and PCBs as prime candidates for bioaccessibility study. These compounds are associated with ubiquity, particularly in urban areas, though they are equally associated with point sources of elevated levels. These compound groups have both been identified as POPs under the Stockholm Convention on Persistent Organic Pollutants, represent a risk to human and environmental health, and can act as a significant barrier to redevelopment in brownfield land if identified. In addition, PAHs and PCBs have a strong affinity for sorption to soil particles, and are associated with accumulation and long-term storage within soils and sediments.

The project found focus on PCBs. PAHs remain a significant source of risk and a barrier to development, and are relatively understudied compared to inorganic contaminants in the bioaccessibility field. However, both the FOREhST and CE-PBET protocols have been used to assess bioaccessibility in soil-bound PAHs. The application of such extraction methods to different compound groups, representing different physicochemical properties, background concentrations and risks, is key to the development of a unified method. In addition, access was granted due to the work and collaborative projects of BGS to PCB contaminated samples suitable for bioaccessibility testing, background concentration study, and suitable CRMs.

Analysis of research trends using ScienceDirect shows a gradual increase in PCB research, despite the clear status of PCBs as a legacy contaminant, since 2000. This reflects the continued focus on PCBs

within the scientific and regulatory community, a result of the ubiquity, potential for risk and environmental resilience of these compounds.

Research trends point towards increasing interest in bioaccessibility testing, including in POP assessments. The focus of this research will continue to be PAHs and PCBs, but greater emphasis is likely to be places on emerging contaminants, such as PBDEs and HBCDs. The modelling of bioaccessibility in different pathways, such as dermal and respiratory bioaccessibility, is a potential further direction for the field, alongside emerging *in silico* methods.

2. *"Compare the performance of the FOREhST and CE-PBET methods in the assessment of bioaccessibility in soil-bound PCBs."*

The CE-PBET and FOREhST methods were the focus of this project, representing two of the most prominent extraction methods specifically designed for use in the assessment of bioaccessibility in organic contaminants. Since both methods simulate a fed-state human GIT system, there is potential to compare bioaccessibility results, amend the methods and suggest refinements.

In order to develop a comparison study, it was necessary to acquire a set of soils affected by PCBs which could be used with both methods. As a consequence of recent collaboration between the Université de Lorraine (UdL) and BGS, 34 soils, industrially contaminated with PCBs, were made available. These soils ranged from 0.64 – 1882.62 µg/g in $\sum^{ICES\ 7}$ concentration, and were sampled by UoL staff for the purposes of bioavailability testing using a juvenile swine model. In addition, the BCR 481 CRM was used throughout ($\sum^{ICES\ 7}$ 314.5 µg/g certified concentration).

Initial assessment of the 34 soils was performed using FOREhST, which provided an initial indicator of the performance of the method with PCB contaminated material. This study is described in Chapter 5. The initial survey resulted in a mean $\sum^{ICES\ 7}$ bioaccessibility of 25%. This figure included consistently low % bioaccessibility values recorded for PCB 180, which has been attributed to a systematic loss of heptachlorinated compounds during the saponification process which is included

as part of the standard FOREhST supernatant cleanup procedure. The mean ∑ICES 6 (PCB 28, 52, 101, 118, 138 and 153) bioaccessibility ranged from 30 – 38%. This value is consistent with previous PCB studies, but is lower than PAH values obtained using the FOREhST method. With the exception of PCB 180, % bioaccessibility remained consistent between congeners despite varying LogKow. This suggests that the micellar, carbohydrate rich gut media was sufficiently efficient in the promotion of desorption from soil and containment within the gut media to overcome anticipated preferential desorption of lower molecular weight PCBs. This behaviour was replicated with soil TOC, which did not appear to be a controlling factor on bioaccessibility.

One of the headline findings from the initial survey of 34 soils using FOREhST was the linearity between initial soil and bioaccessible fraction PCB concentration, equivalent to a linear dose/ response curve. This suggests that that an upper limit of desorption was not reached, and raises the possibility of accurate prediction of bioaccessible concentrations on the basis of initial soil PCB concentrations.

The potential impact of this section of the work is associated with the impact on the wider field of bioaccessibility. The study helps to provide understanding in the currently understudied field of organic contaminant specific bioaccessibility. Additionally, the findings provide an essential insight into the risk to human health posed by PCBs, and their behaviour within a simulated gut system. PCB studies, in addition to previous PAH studies, promote a greater acceptance of FOREhST as unified bioaccessibility method for organics, and potentially opens the door to future studies with other organic xenobiotics, including a wide range of emerging contaminants.

Following the initial survey of bioaccessibility in the full set of 34 soils, 7 were selected on the basis of the provision of a wide range of $\sum^{ICES\ 7}$ PCB concentrations, and availability of *in vivo* data for bioavailability comparison. Once again, the BCR 4811 CRM was also tested. In order to address the reduced PCB 180 bioaccessibility in the original 34 soil FOREhST extractions, further FOREhST

extractions were performed with the saponification step removed, and the bioaccessibility data compared with both the original FOREhST and CE-PBET data.

Removal of the saponification step prevented the loss of PCB 180, though the overall $^{ICES\ 7}$ bioaccessibility was significantly lower (in comparisons of the 7 selected soils and the CRM, the original method produced a $\sum^{ICES\ 7}$ value of 39.63%, the amended version recorded 23.07%). This suggests that the inclusion of the saponification step results in a more conservative bioaccessibility assessment, while still reducing the threshold for harm. It is, therefore, recommended that the full method be followed as this allows a more cautious approach. There may be potential for an adapted saponification method which reduces the loss of heptachlorinated congeners, while retaining the conservative approach of the original method.

With CE-PBET, mean $\sum^{ICES\ 7}$ bioaccessibility values were significantly lower than FOREhST (15.21%), though they were not significantly different to values recorded with the FOREhST method without saponification. This may suggest that the CE-PBET protocol may benefit from a saponification step to enable more conservative results. The colon section did increase bioaccessibility, but earlier results in PAH studies, where a dramatic increase occurred, were not replicated. As in the original FOREhST 34 soil study, bioaccessibility remained consistent between congeners in all methodologies, and soil TOC did not influence the results.

Limited relative bioavailability data via a juvenile swine study were available for comparison, though this was limited to PCBs 101, 138, 153 and 180. Linearity between bioaccessibility and relative bioavailability was absent in all but the PCB 153 data in the case of FOREhST. However, bioavailability comparison is provided as guidance only, and further work is required in this area. A full validation of the bioaccessibility methods may reveal greater and more reliable relationships, and is a logical next step.

This work contributes to the further development of the FOREhST and CE-PBET methods, and contributes to the wider field of bioaccessibility, particularly in the relatively understudied category of organic contaminant bioaccessibility. With all extraction methods, it is clear that the bioaccessible fraction is less than the total figure, and assessment is currently overly conservative. If bioaccessibility bears out in future *in vivo* work to show a close correlation with bioavailability, this conclusion will be confirmed, and bioaccessibility testing may contribute towards more a more sustainable land-use regime, as lower, more physiologically relevant values, inform on the suitability of redevelopment and remediation necessity. There appears to be significant potential for bioaccessibility to be applied as part of a body of evidence approach. Indeed, linear relationships between soil concentration and bioaccessible concentration suggest that the bioaccessibility value may be calculable from initial soil concentration tests, as a guidance document for future work.

3. *"Establish a background survey of PCBs within a large urban area, and establish a typical PCB profile, identifying any sources."*

Working with the BGS allowed access to Central London soil samples, collected at two depths, which were collected as part of a wider survey of London soil and sedimentary geochemistry. Sixty-nine locations were available for analysis for soil PCB at the two depths. The study followed previous work in assessing PCB and PAH concentrations in East London.

$\sum^{ICES\ 7}$ concentrations ranged from 0 - 148.72 µg/kg across all values, with a mean value of 15.14 µg/kg. A median value of 4.97 µg/kg reflects the distribution of PCB concentrations across the study site, which is dominated by a small number of elevated levels. The Central London values were presented in comparison with values obtained in East London, and some of the key studies of PCB levels in urban areas globally. Mean values obtained for Central London were slightly lower than those recorded for East London (22 µg/kg), though the median value was consistent (4.9 µg/kg), suggesting that, once again, the distribution was dominated by a small number of isolated values. Values were consistently higher than comparable urban regions in terms of $\sum^{ICES\ 7}$ congeners.

In terms of London, the lower mean value is perhaps to be expected of a part of London less traditionally associated with heavy industry than the East, though the elevated values in comparison to other urban areas, and the Environment Agency background value for urban soils in England (1.77 μg/kg) reflect the dominance of PCB containing substances in construction, building maintenance and industrial process in the Greater London area.

The mean concentration of the dioxin-like compound PCB 118 (2.78 μg/kg) fell below the residential SGV of 8 μg/kg determined for dioxin-like compounds, but was found to be in exceedance in 8 locations. PCB 118 represents only one of a number of dioxins, dioxin like PCBs and furans which may be potentially present, so elevated levels of PCB 118 should be considered relevant to further urban soil surveys. Additionally, all samples were found to be below the VROM Dutch $\sum^{ICES-7}$ intervention value of 1000 μg/kg, though 22% of locations were found to be in exceedance of the 20 μg/kg target value.

The congener profile was dominated by penta- and hexa- chlorinated compounds, 45.78% and 24.53% of $\sum^{ICES-7}$ respectively, which is consistent with an Aroclor 1254/ 1260 profile. This profile is consistent with the conclusion that re-emission from demolition, or gradual release following deterioration of materials containing PCBs, may act as a significant source of PCBs in Central London, as these technical mixtures would be typical as ingredients in building materials used during the period of widespread PCB application. This finding suggests that buildings constructed before outright bans on the use of PCBs represent a potential source for PCBs as components of materials such as paints, sealants and caulking.

In order to assess a potential 'baseline' value for London, normal background concentration (NBC) calculations were performed. This calculation was based on a method which has been successfully applied to studies aiming to determine baselines of metals in soils, including Pb, Hg, Ni and Cd, and has more recently been applied to PAH and PCB studies. This method allowed the determination of NBC values for each of the ICES 7 compounds and $\sum^{ICES-7}$. However, NBC values were found to be

lower than mean values, in some cases values seemed artificially, or unrealistically low. This has been attributed to the nature of the distribution typical to soil PCB. The NBC method eliminated identified outliers from the dataset. While this does not affect background calculations in geogenic contaminants, as is frequently the case in metals, outliers are often key to the understanding of anthropogenic contaminants, such as PCBs, which are typically associated with elevated point source emissions.

4. *"Apply a bioaccessibility testing approach to soil samples selected in Objective 3, thus demonstrating the application of such methods in a real-world environment."*

As planned, samples were selected for consideration with the FOREhST bioaccessibility method on the basis of the results of the Central London survey. However, none of the individual PCB congeners were high enough in concentration to be identified following the procedure, resulting in an effective 0% bioaccessibility. Although these results were disappointing, the results of investigations associated with Objective 2 suggest that bioaccessibility may be used as a precautionary measure in contaminated soil. As stated, the PCB levels in London were typically below regulation values. It seems appropriate that bioaccessibility may not be appropriate for very low level contamination, but will play a decisive role in the assessment of high concentration samples. Soils with a $\sum^{ICES-7}$ concentration in excess of the VROM intervention value of 1000 µg/kg will benefit from bioaccessibility testing. If the results of the studies presented are accurate, a bioaccessibility factor of 30 - 40% may be appropriate for PCB-laden soils, allowing the safe development of land containing soils of up to 2500 – 3000 µg/kg $\sum^{ICES-7}$ PCB concentration, avoiding the requirement for costly and extensive remediation.

5. *"Explore the potential impacts, both financially and environmentally, of bioaccessibility testing of organic contaminants in soil, and how bioaccessibility assessment methods can lead to a more sustainable land-use regime."*

The potential impacts on sustainable contaminated land management were initially explored in Chapter 7. A clear movement towards sustainability is taking place in contaminated land management, and focus has shifted towards the redevelopment of brownfield land in favour of greenfield sites. Bioaccessibility represents a key tool in the redevelopment of brownfield sites affected by contaminated land. In reassessing the risks posed by soil contaminants through bioaccessibility, emphasis is placed on the fraction of a contaminant that has the potential to cause harm, allowing the safe use of land previously deemed unusable due to the presence of contaminants. The uncertainty, complexity and financial costs of remediation represent a significant barrier to the redevelopment of contaminated land, and bioaccessibility is one of the key tools in the arsenal of assessors to approach contaminated land in a more sustainability focussed and physiologically relevant way.

The widespread adoption of bioaccessibility testing into the land-use regime has been hindered by technical barriers, especially in the case of POPs, where standard assessment protocols are yet to be established. Surveys have found that Local Authority officers recognise the potential of bioaccessibility modelling, but feel that guidance is inadequate and bioaccessibility represents an unacceptable level of uncertainty. These issues can only be addressed through the continued research of methods, and the validation of bioaccessibility results through comparison with *in vivo* bioavailability data. The evolving nature of the contaminated land issue is such that unified methods should possess the flexibility to adapt to emerging contaminants, while retaining relevance to legacy pollutants. The application of methods to different classes of organic contaminants allows exploration of his versatility, and has been influential in the adoption of PCBs as target compounds during this work. The FOREhST and CE-PBET methods have been applied successfully to PAH bioaccessibility, and ongoing research continues into emerging contaminant groups.

8.2 Limitations and future research directions

The validation of bioaccessibility protocols is essential to assess the physiological relevance of bioaccessibility data. Although initial bioavailability correlations were investigated through the course of this work, this was limited by the availability of applicable *in vivo* data. A clear next step would be to supplement these initial findings with *in vivo* data, which can be correlated to the bioaccessibility data obtained through both methods. However, these initial, limited *in vivo* comparisons suggest potential links with bioavailability, and should be investigated in further studies.

Two surrogate standards were used in this study, which limited their ability to trace each PCB homolog group throughout the bioaccessibility processes and cleanup protocols. Although the process may be prohibitively costly, further research into differential bioaccessibility between individual congeners or compound homologs may be achieved through the use of mass-labelled surrogate standards. However, the spiking of these standards would require very high precision due to the very low concentrations typically required.

The spatial survey of Central London revealed the limitations of the use of the NBC calculation procedure which has been previously applied to, principally but not exclusively, geogenic soil contaminants, such as Pb. The technique appears robust in studies where the distribution and impact of geogenics, though it has since been applied to PAH and PCB surveys. Although in the case of PAHs non-anthropogenic sources are common, the anthropogenic nature and typical distribution of PCB contamination, in predominantly point source locations, limits the usefulness of the technique, since it relies on the elimination of outlier values and calculated means, which are skewed by the distribution of the data, as reflected by the far lower median concentrations.

In-depth study of the distribution of congeners was conducted using a multiple component analysis (MCA) approach, though this did not yield definitive results. The MCA process aims to identify

relationships between associated values, such as elevated concentrations of contaminants in environmental samples. However, this was inconclusive in the case of the London data. The initial findings of the MCA analysis are given in Appendix A for reference, though no definitive findings were extracted from the data.

Further research into the bioaccessibility of POPs should consider the application of methods to emerging contaminants. Indeed, research into emerging contaminant bioaccessibility is increasing, including recent publications by colleagues at the University of Reading. Further application of CE-PBET and FOREhST to emerging contaminants will advance the case for the establishment of a unified method for organics.

New methodologies are being explored to investigate bioaccessibility in exposure pathways other than the oral route, including dermal and respiratory bioaccessibility. The future of bioaccessibility testing may lie in *in silico* work. The *in vitro* modelling of bioaccessibility in multiple exposure pathways can contribute to this work. *In silico* modelling would represent further financial and practical advantages in the land quality management, though the same limitations that apply to *in vitro* bioaccessibility methods apply here – all methods must be fully validated against *in vivo* bioavailability.

8.3 Final remarks

A running theme throughout this thesis has been one of 'sustainable remediation', a concept which appears to be growing in prominence as a fundamental tenet of brownfield rehabilitation. Bioaccessibility testing, in reappraising risk from contaminated land in a manner which is less conservative, yet cautious, and physiologically relevant, is fundamental to this concept. The methods presented in this thesis, FOREhST and CE-PBET, have shown applicability to the measurement of PCB bioaccessibility. Although the findings vary between the methods, values obtained clearly indicate that the total contaminant approach is over-estimating the risk of soil borne PCBs, as has been shown previously in PAH tests. Continued research into these methods will inevitably include the

application of bioaccessibility to emerging organic contaminants, and they are likely to retain their place at the forefront of bioaccessibility science.

This project has sought to unite geochemistry and the study of bioaccessibility with sustainability, and has achieved this through using the unique resources available. These have included the expertise of world leading academics in bioaccessibility, the provision of test soils through collaboration, and the development of a survey of geochemistry in Central London, in addition to the expertise of academics and industry leaders in the TSBE Centre.

TSBE Centre projects are varied, but share a common goal, to introduce more sustainable ways of managing the built environment. Provision of safe soils on which to build is absolutely key to maintaining the health of the built environment, and bioaccessibility remains a powerful tool to achieve a more sustainable brownfield and contaminated land strategy.