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Oral Bioavailability of Nonpolar Organic Chemicals in Soil for Use in Human Health Risk Assessment

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Preface

Methods to assess the potential effect of soil pollution on human beings has historically relied on total concentrations of pollutants in soil, but the link between pollution concentration in soil and the risk is not linear. Research has shown that the potential risk associated with pollution in soil is often less than if organisms were exposed directly to the pollutants, as pollutants dissolve in varying degrees in the human gastrointestinal system. In addition, pollutants can be very strongly absorbed to the soil and thus reduce the potential risk of uptake by humans (or other receptors), that are exposed to soil. The consequence is that if the bioavailability of substances in soil is not included in risk assessment, clean-up objectives may be too restrictive in relation to the objective of protecting f.ex. soil-eating children, and thus significantly increase expenditure of remedial actions.

In Denmark, there are no specific guidelines on the application of tests for in vitro bioavailability when doing risk assessment at contaminated sites. The Danish Environmental Protection Agency has therefore given a grant to this fact-finding project to review literature on bioavailability studies and methods in relation to human toxicology (soil-eating children) for selected PCBs and PAHs, including a summary of the regulatory practice in the United States, Australia, Canada and Europe.

The literature study could form the basis for preparation of practical EPA-guidelines for application of bioavailability tests.

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Summary and Conclusion

This report provides a review of bioavailability studies and methods in relation to HHRA for PAHs and PCBs. An extensive body of literature on assessing the oral bioavailability of PAHs in soil using *in vivo* and *in vitro* approaches is available, while studies on the oral bioavailability of PCBs in soil, especially using *in vitro* approaches, are very limited. Based on a critical review of the studies reported in the literature, the CE-PBET is recommended as the *in vitro* method to evaluate oral PAH bioavailability in soil due to its simplicity, cost effectiveness, and potential for standardization. Several aspects in study design have also been recommended to ensure the *in vitro* test consistent with a good practice established by the BARGE. A single standard *in vitro* method for PCB bioaccessibility measurement cannot be recommended due to data limitation, but approaches being used for PAHs may provide a useful template and similar aspects in study design to PAHs should be considered for an *in vitro* approach for PCBs.

This report summarizes various differences among countries (i.e., the US, UK, The Netherlands, France, Canada, and Australia) in the regulatory approaches to evaluating and using bioavailability in risk assessment, including definitions of terms, test methods that are deemed acceptable, reporting requirements, regulatory frameworks, and guidance on specific chemicals. The review reveals very limited application of relative bioavailability adjustments for organic chemicals in HHRAs. Adjustments reflecting reduced bioavailability of soil metals are much more common, with standardized methods and regulatory guidance available from several countries. Nevertheless, site-specific data from well designed and documented studies can support adjustments to exposure assessments and risk-based soil cleanup levels for contaminated land. Although substantial scientific justification is needed, such studies support more realistic assessments of cleanup levels needed to protect public health.

Methods for assessing the oral bioavailability of PAHs and PCBs in soil require additional research and development (especially for PCBs) to fill data gaps and support broader application of bioavailability adjustments in risk assessment. Key issues are summarized below:

- Bioavailability research should include a variety of field soils that reflect a wide range of source materials and soil properties, in order to obtain a comprehensive understanding of the soil-chemical interactions as well as the factors (i.e., different types of organic carbon) likely to control oral bioavailability and absorption of PAHs and PCBs in humans.
- Due to variations of bioavailability with chemical concentration, as well as concentration-dependent soil interactions, bioavailability research should include samples covering a range of environmentally relevant concentrations.
- More research is required to establish a standard protocol of *in vitro* test for assessment of bioaccessibility of PAHs and PCBs in soil. Large scale inter-laboratory comparative studies could help identify a common procedure that is applicable at different concentrations in different soil types. Quality control schemes should also be developed.
- Inclusion of food or a lipid sink is a critical component of in vitro studies for nonpolar organic chemicals.

Although validation of *in vitro* methods by comparison with *in vivo* data is desired, assessing PAHs and PCBs *in vivo* is not straightforward. Without reliable *in vivo* methods, their use to validate *in vitro* may not be feasible.

Sammenfatning og konklusion

Denne rapport giver et overblik over biotilgængelighedsstudier og metoder i forhold til human toksikologi (jordspisende børn) for udvalgte PAH'er og PCB'er. Litteraturgennemgangen viser, at der eksisterer en del viden om *in vivo* og *in vitro* tests til vurdering af oral biotilgængelighed af PAH'er i jord, hvorimod det er meget begrænset, hvad der findes af viden om oral biotilgængelighed af PCB'er i jord ved hjælp af især *in vitro* tests.

Ud fra en kritisk gennemgang af litteraturstudierne anbefales CE-PBET (Colon Extended Physiologically-Based Extraction Test) som *in vitro* metode til at vurdere den orale biotilgængelighed af PAH i jord. Metoden er enkel, kost effektiv og er egnet til standardisering. Der er givet en række anbefalinger til justering af *in vitro* testen for at sikre, at testen følger god praksis fastlagt af BARGE (Bioaccessibility Research Group in Europe).

Data mangler for at kunne udpege en enkelt standard metode til *in vitro* test af PCB biotilgængelighed i jord, men metoder, test design og vurderinger anvendt på PAH kan give et brugbart udgangspunkt for en kommende *in vitro* test overfor PCB.

Denne rapport giver desuden et overblik over forskelle i den regulatoriske praksis i forskellige lande (USA, UK, Holland, Frankrig, Canada og Australien) ved anvendelse og vurdering af biotilgængelighed i forbindelse med risikovurderinger, heriblandt definitioner af begreber, accepterede test metoder, rapporterings-/dokumentationskrav, lovgivningsmæssige rammer og vejledende retningslinjer for specifikke kemiske stoffer. Litteraturgennemgangen viser, at biotilgængelighedsaspekter og –tests anvendes i begrænset omfang ved sundhedsmæssige risikovurderinger af organiske stoffer i jord. Derimod bruges biotilgængelighedstests og –data ofte til at justere risikovurderinger for metaller i jord, og der findes standardiserede metoder og vejledninger hertil i flere lande.

Ikke desto mindre kan stedspecifikke data fra godt designet og dokumenterede undersøgelser af biotilgængelighed støtte tilpasninger i eksponeringsvurderinger og justeringer af jordkvalitets- og oprensningskriterier for forurenet jord. Selv om mere omfattende videnskabelige begrundelser kan være nødvendige, understøtter sådanne undersøgelser og tests mere realistiske vurderinger af oprensningskriterier, der tager hensyn til biotilgængelighed og beskytter menneskers sundhed.

Metoder til vurdering af den orale biotilgængelighed af PAH'er og PCB i jord kræver yderligere forskning og udvikling (især for PCB) for at kunne understøtte en bredere anvendelse af biotilgængelighedsdata i risikovurderinger med henblik på at justere eksisterende jordkvalitets- og oprensningskriterier. Centrale emner, der bør undersøges, er opsummeret nedenfor:

- Forskning indenfor biotilgængelighed bør omfatte en række jorde, der afspejler en bred vifte af kilder til PAH- og PCB-forurening og jord egenskaber, for at opnå en mere tilbundsgående forståelse af de kemiske processer i jord samt de faktorer (dvs. forskellige typer af organisk kulstof), der styrer oral biotilgængelighed og absorption af PAH'er og PCB i mennesker.

- Biotilgængelighed afhænger af kemisk koncentration og koncentrations-afhængige jord interaktioner - yderligere forskning bør derfor omfatte prøver, der dækker en række miljømæssigt relevante koncentrationer.

- Mere forskning er påkrævet for at fastlægge en standardprotokol for *in vitro*-test til vurdering af biotilgængeligheden af PAH'er og PCB i jord. Storskala sammenlignende undersøgelser med relevante laboratorier kunne evt. bidrage til udvikling af en fælles procedure, der kan bruges overfor forskellige koncentrationer i forskellige jordtyper. Procedure for kvalitetskontrol bør også udvikles.

- *In vitro*-undersøgelser for upolære organiske kemikalier bør omfatte fødevarer eller belysning af, hvilken effekt fedtstoffer i kroppen har på biotilgængeligheden.

Validering af *in vitro*-metoder ved sammenligning med *in vivo* data er ønskeligt, men vurdering af PAH'er og PCB *in vivo* er ikke ligetil. Uden pålidelige *in vivo* metoder, kan disse sandsynligvis ikke anvendes til at validere in vitro-tests.

Acronyms and abbreviations

ACS:	Assessment of Site Contamination
AF:	Absorption Factor
ATSDR:	Agency for Toxic Substances and Disease Registry
AUC:	Area under the Concentration versus Time Curve
BaP:	Benzo(a)pyrene
BARC:	BioAccessibility Research Canada
BARGE:	Bioaccessibility Research Group in Europe
CE-PBET:	Colon Extended PBET
CEC:	Cation Exchange Capacity
CSF:	Cancer Slope Factor
DDT:	Dichlorodiphenyltrichloroethane
DEPA:	Danish Environmental Protection Agency
DL-PCB:	Dioxin-Like PCB
DQRA:	Detailed Quantitative Risk Assessment
EFSA:	European Food Safety Authority
FOREhST:	Fed ORganic Estimation human Simulation Test
HHRA:	Human Health Risk Assessment
HIL:	Health Investigation Level
HQ:	Hazard Quotient
IARC:	International Agency for Research on Cancer
IPCS:	International Programme for Chemical Safety
INERIS:	French National Institute for Industrial Environment and Risks
IVBA:	In Vitro Bioaccessibility Assay
IVD:	In Vitro Digestion
IVG:	In Vitro Gastrointestinal
kg:	kilogram
Koc:	Soil-Organic Carbon Partition Coefficient
Kow:	Octanol-Water Partition Coefficient
Kp:	Soil-Water Partition Coefficient
mg:	milligram
NDL-PCB:	Non-Dioxin-Like PCB
NEPC:	National Environment Protection Council
NEPM:	National Environment Protection Measure
NOM:	Natural Organic Matter
NRC:	National Research Council
PAH:	Polycyclic Aromatic Hydrocarbon
PBET:	Physiologically-Based Extraction Test
PCB:	Polychlorinated Biphenyl
PDMS:	Poly(dimethylsiloxane)
PQRA:	Preliminary Quantitative Risk Assessment
RBA:	Relative Bioavailability Adjustment
RBALP:	Relative Bioavailability Leaching Procedure
RfD:	Reference Dose
RIVM:	Het Rijksinstituut voor Volksgezondheid en Milieu (National Institute of Public Health and the Environ-ment, Netherlands)
RSL:	Regional Screening Level
SBRC:	Solubility Bioavailability Research Consortium
SHIME:	Simulator of Human Intestinal Microbial Ecosystem
SOP:	Standard Operating Procedure

TCDD:	Tetrachlorodibenzo-p-dioxin
TDI:	Tolerable Daily Intake
TEQ:	Toxicity Equivalent
Tiny-TIM:	Tiny TNO in vitro model
TOC:	Total Organic Carbon
TRV:	Toxicity Reference Value
TRW:	Technical Review Workgroup for Metals and Asbestos
UBM:	Unified BARGE Method
µm:	micron or micrometer
UK:	United Kingdom
US:	United States
USEPA:	United States Environmental Protection Agency

1. Introduction

This report addresses scientific issues relevant to characterizing the oral bioavailability of nonpolar organic chemicals in contaminated soil, and its application in setting risk-based soil cleanup levels for contaminated land. Human health risk assessment (HHRA) forms the basis for deriving risk-based cleanup levels and assumptions about the bioavailability of chemicals in soil are inherent in the HHRAs. The report summarizes the findings from literature review on bioavailability studies and methods in relation to HHRA for selected nonpolar organic chemicals, including the regulatory practice in Europe, United States (US), Canada, and Australia.

Soil cleanup levels for contaminated land are not typically adjusted to account for reduced bioavailability of chemicals in soil. However, the relationship between the soil concentration and the health impact on humans who are exposed to the contaminated soil may be modified if the chemical is tightly bound or incorporated into the soil particles in such a way that it is poorly absorbed into the body. Considering only the concentration in soil tends to lead to overly restrictive remedial requirements, which in turn lead to higher remediation costs without commensurate increases in health protection. Assessing the bioavailable fraction of the given chemical when conducting risk assessments will provide more realistic estimates of remediation needs.

The term bioavailability refers generally to the degree to which chemicals are absorbed or transferred across membranes and enter the systemic circulation after contact with external or internal body surfaces. More specific definitions for the purposes of this document are provided in front of this chapter. A chemical that is completely absorbed would be considered 100 percent bioavailable; however, it is seldom the case that an oral dose of a chemical is completely absorbed. Bioavailability varies greatly from chemical to chemical, depending upon a number of factors that differ between inorganic and organic chemicals. In the case of nonpolar organic chemicals, bioavailability tends to increase as molecular weight decreases and lipid solubility increases. The physical form of a chemical within a particular environmental medium (e.g., sequestration of organic compounds in soil pore spaces) is key to controlling bioavailability from soil.

In risk assessment, both exposure assessment and toxicity assessment involve dose terms, and the combination of these two terms to generate risk estimates needs to be carefully defined in terms of bioavailability to ensure that comparable doses are being used. Variation in chemical bioavailability among exposure media should be quantified to ensure that intake estimates may be accurately compared to tolerable daily intakes (TDIs) or toxicity reference values (TRVs). The use of appropriately derived bioavailability adjustments could reduce uncertainties associated with intake estimates and contribute to more meaningful and realistic risk results for use in risk management decisions (e.g., establishing cleanup goals).

In 2004 and 2005, the Danish Environmental Protection Agency (DEPA) published two reports reviewing methods for measuring bioavailability of soil contaminants and making recommendations for the application of the results in setting soil cleanup levels (DEPA 2004, 2005). A condensed version of these reports was published in English by the UK Environment Agency (2005). These reports were focused on selected metals (cadmium, lead and nickel) and polycyclic aromatic hydrocarbons (PAHs). Results from some *in vitro* methods of measuring relative bioavailability of soil lead and cadmium were judged to provide a sufficient basis for adjusting soil clean up levels to reflect reduced bioavailability. In contrast, available methods for nickel and PAHs were only judged to provide qualitative information on the protectiveness of cleanup levels. Methods for assessing nonpolar organic chemicals have been improved considerably in the past decade, and an updated assessment for these chemicals is the focus of this report.

The nonpolar organic chemicals selected for this bioavailability evaluation include PAHs and polychlorinated biphenyls (PCBs). These chemicals are frequently associated with the greatest risk in contaminated soils in Demark, and are also commonly found to have reduced bioavailability in weathered soils as compared with more soluble forms used in toxicity studies. In addition, soil quality criteria were developed by DEPA for several PAHs, including benzo(a)pyrene (BaP), dibenzo(a,h)anthracene, and total PAHs (sum of BaP, benzo[b+j+k]fluoranthene, dibenzo[a,h]anthracene, fluoranthene, and indeno[1,2,3-c,d]pyrene).

This report will assess the feasibility of including nonpolar organic chemicals in national guidelines for *in vitro* tests (laboratory tests) of the bioavailability of hazardous substances as a part of risk assessments of contaminated soil. Information needed to develop guidelines for application of *in vitro* tests to evaluate bioavailability of PAHs and PCBs in contaminated soil will be identified. Oral bioavailability of chemicals in soil is currently an active area of research. Consequently, it is recognized that some of the underlying information and concerns identified in this review may need to be updated in the future. An overriding goal of the review is to promote use of the best available science in assessing contaminated soil in Denmark.

This report consists of the following sections:

- Summary and Conclusions — Summarizes the use of bioavailability data for PAHs and PCBs in risk assessment and setting risk-based cleanup levels, and discusses limitations and data needs
- Section 1, Introduction — Describes the purpose of the report and the document organization.
- Section 2, Bioavailability and Factors Influencing Bioavailability — Defines bioavailability terminology and describes site-specific factors influencing bioavailability.
- Section 3, Measuring Bioavailability – Provides a general review of *in vivo* and *in vitro* approaches to measure oral bioavailability.
- Section 4, Regulatory Uses of Bioavailability — Discusses the role of bioavailability in risk assessment and in adjustment of soil clean-up levels, and summarizes recent regulatory practice in the US, United Kingdom (UK), The Netherlands, France, Canada, and Australia.
- Section 5, Chemical-Specific Bioavailability Summaries — Discusses critical aspects of environmental chemistry and mammalian toxicokinetics that may influence the design and conduct of oral bioavailability studies, summarizes the *in vivo* and *in vitro* studies and methods to measure oral bioavailability in the literature, and provides *in vitro* study design guidelines.

References cited in this report are provided in Appendix 1.

2. Bioavailability and Factors Influencing Bioavailability

As noted earlier, variation in chemical bioavailability among exposure media should be quantified to ensure that intake estimates may be accurately compared to TRVs. When a difference is identified for a particular chemical, an adjustment may be applied to the estimated intake of the chemical from an environmental medium to make the estimates comparable to doses used in dose-response analyses of toxicity studies. There are multiple terms used in the literature that describe the absorption of chemicals by living organisms. This section provides definitions for the terms relating to bioavailability and the physical and chemical factors that influence the bioavailability of chemicals in humans.

2.1 Definitions: Absolute and Relative Bioavailability

When evaluating oral exposures to chemicals in humans, the most commonly applied definition of bioavailability is absorption and uptake into systemic circulation. Definitions of bioavailability used in ecological risk assessment differ (National Research Council ([NRC] 2003), and these differences lead to reliance on different methods of testing bioavailability (e.g., those reviewed by Cui et al. 2012).

Absolute Bioavailability: In the context of mammalian toxicology and HHRA, oral bioavailability is defined as the fraction of an ingested dose of a chemical (i.e., the administered dose) that is absorbed and reaches the blood stream. Absolute bioavailability is the fraction of an administered dose that is absorbed:

$$Absolute\ Bioavailability\ (fraction) = \frac{Absorbed\ Dose}{Administered\ Dose}$$

Due to differences in chemical characteristics and the mechanisms by which they are absorbed, chemicals vary greatly in their intrinsic bioavailability. Lipid solubility is a critical factor controlling bioavailability of nonpolar organic chemicals in soils because these chemicals are absorbed via the same mechanisms governing absorption of lipids.

Relative Bioavailability: The chemical and physical form in which a chemical exists in environmental media will cause its bioavailability to vary in different settings (NRC 2003). For nonpolar organic chemicals present in environmental media, the nature of their interactions with the matrix in which they are present over time will govern their bioavailability relative to chemicals freshly mixed into a matrix. The differences in bioavailability of chemicals in different settings are termed the relative bioavailability. Relative bioavailability is typically measured by comparing the bioavailability of a chemical in the environmental medium of interest relative to its bioavailability in the dosing medium used in the critical toxicity study. A relative bioavailability factor (RBA) may be calculated in several ways, and stated as either a fraction or a percent. RBA can be described as the absolute bioavailability of a chemical in soil divided by the absolute bioavailability from the dosing medium used in the toxicity study:

$$RBA\ (fraction) = \frac{Absorbed\ Fraction\ from\ Soil}{Absorbed\ Fraction\ from\ Dosing\ Medium\ Used\ in\ Toxicity\ Study}$$

When absolute bioavailability is not known, RBA can be calculated by comparing tissue levels after adjusting to comparable doses. For example, for chemicals excreted in the urine, the urinary excretion fractions may be compared. If a chemical accumulates in a tissue, tissue levels may be compared.

The RBA may be used to directly modify risk-based cleanup levels or applied in site-specific risk assessment to account for the difference in absorption between the chemical in the environmental medium and in the TRV studies. Typically, the most bioavailable form of a chemical is used in toxicity studies. When a less bioavailable form is present in soil the RBA will be less than 1.0. An RBA may be greater than 1.0 if a more bioavailable form of the chemical is present in an environmental medium than in the medium used in the toxicity studies.

Bioaccessibility: Practically, much research on relative bioavailability has been focused on *in vitro* benchtop studies that are designed to predict the dissolution of a chemical in the human gastrointestinal tract. The fraction of administered chemical that dissolves in these systems is termed bioaccessibility (Ruby et al. 1999). Variations in solubility within the gastrointestinal tract control the relative bioavailability of chemicals, so bioaccessibility provides a measure of RBA.

2.2 Factors Influencing Bioavailability

Characteristics of the soil or waste material at a specific site, such as organic carbon and clay content, affect the bioavailability of nonpolar organic chemicals. Particle size distribution and the period of time since the contamination occurred are other key factors affecting bioavailability.

This discussion primarily focuses on soil. Other waste materials may have different forms or reduced organic content and different pH when compared to soils, as well as highly variable particle size ranges. In all cases it is important to fully characterize the material being evaluated with regard to particle size distribution and other characteristics.

2.2.1 Particle Size

Many of the processes governing chemical interactions with soil particles will vary with particle size. Available surface area is increased as particle size decreases, and chemical reactivity of fine particles is expected to be greater than in coarse particles. When humans contact soil, fine particles are also more likely to adhere to hands and be available for ingestion after hand-to-mouth contact. Hand-to-mouth contact is highest in young children, leading to higher soil ingestion rates.

Site contamination is often reported based on analysis of bulk soil. In contrast, much research on soil chemical bioavailability has been performed using a fine fraction of soil. The fraction < 250 microns (µm) has frequently been used because it was shown to include particles most likely to adhere to skin (Kissel et al. 1996). After a comprehensive review of data from the last 30 years, Ruby and Lowney (2012) recommended a soil particle size of <150 µm for future studies on the oral bioavailability and bioaccessibility of PAHs in soil. DEPA has traditionally used bulk soils in bioavailability analyses so as to include cases where bulk soil is intentionally ingested (UK Environment 2005). The significance of the selected size fraction will depend on the particle size distribution in a specific soil and on whether or not the fine fraction is enriched in the contaminant.

2.2.2 Soil Characteristics

Soil characteristics such as organic matter content, cation exchange capacity, and pH may affect relative bioavailability of both organic and inorganic chemicals (Datta and Sarkar 2005, Hack and Selenka 1996, Kelley et al. 2002, NRC 2003, Ruby 2004, Yang et al. 2002). These factors affect chemical solubility and mobility, which in turn influences the dissolution of a chemical within the gastrointestinal tract.

Soil characteristics may vary over time by both physical and chemical processes (Kelley et al. 2002). These processes generate mineral matter of different particle sizes that combine with detritus and living organic matter in the formation of soil. Metabolism of nutrients by microorganisms, macroorganisms, fungi, and plants also contributes to chemical reactions in soil.

Fine soil fractions are dominated by clay minerals with large surface area-to-volume ratios and highly reactive surfaces (Kelley et al. 2002). In temperate climates, negatively-charged aluminosilicate minerals, organic matter, and metal hydrous oxides predominate in clay (Kelley et al. 2002). The negatively-charged minerals in clay, measured as the cation exchange capacity (CEC), provide reactive surfaces important for soil—contaminant interactions influencing mobility and bioaccessibility. CEC provides important information regarding a soil's potential to bind contaminants.

Both polar and nonpolar contaminants react with organic matter in soil. Organic matter, including geologic material, detritus, and living organisms, also contains small pore spaces that provide hydrophobic sites for contaminant absorption (Kelley et al. 2002).

2.2.3 Interaction between Soil and Organic Chemicals

As noted earlier, important differences exist in the interactions of nonpolar and polar organic chemicals with soil. Nonpolar organic compounds are mainly found in association with organic matter such as soot particles and humic material, while polar organic chemicals are found in association with mineral components of soil (NRC 2003). This report focuses on nonpolar organic chemicals, i.e., PAH and PCBs. Additional information regarding factors influencing interactions of polar organics (e.g., organic acids such as phthalates) with soil is provided by NRC (2003).

Within the soil environment, nonpolar organic chemicals are usually present in four fractions: water-dissolved fraction, rapidly desorbing fraction, slowly desorbing fraction, and nonextractable fraction (Ortega-Calva et al. 2015, Ruby et al. 2016). The water-dissolved fraction is determined by chemical water solubility. As solubility increases, the potential for uptake and absorption by organisms increases. The other three fractions of nonpolar organic chemicals are determined by the amount and form of organic matter content in soil. Organic matter in soil is derived primarily from microbial and fungal decomposition of plant matter. Humans also may contribute organic matter to soil through waste disposal, contamination, or deposition of airborne particulates. Organic matter present in soil provides a substrate for adsorption of organic compounds, and consists of two major forms: natural organic matter (NOM) and black carbon domains (Accardi-Dey and Gschwend 2002). The fraction of organic chemical weakly adsorbed within NOM or on mineral surface (rapidly desorbing fraction) can be considered as potentially available for uptake by organisms living in soil (Cornelissen et al. 1998). The fraction of organic chemical strongly adsorbed to the surface or residing within narrow nanopores of more carbonized materials (i.e. black carbon) has reduced tendency to partition out of the sorbed phase into the aqueous phase. This slowly desorbing fraction is considered as unavailable for degradation by soil organisms and is only extractable from the soil matrix using harsh solvents (Stokes et al. 2006). Some organic chemical molecules can be so tightly bound or entrapped within the black carbon domains that they cannot be removed even by vigorous solvent extractions (Jonker et al. 2005, Jonker and Koelmans 2002, Stokes et al. 2006). This fraction is considered as the nonextractable fraction. While the oral bioavailability of organic chemicals for humans is complex, it is likely that only the water-dissolved and rapidly desorbing fractions are bioavailable, while the slowly desorbing and nonextractable fractions, especially those strongly adsorbed to black carbon domains, may limit the release of organic chemicals in the gastrointestinal tract. Therefore, the source and form of organic matter in soil into which the organic chemicals have partitioned will act as controlling factors in determining the oral bioavailability (Ruby et al. 2016). The fraction of a chemical in the slowly desorbing and nonextractable fractions is expected to increase with the length of time that organic chemicals have been in soil due to the effects of

aging or weathering, resulting in lower bioavailability for chemicals at sites contaminated long ago. Over time, sequestration of organic chemicals in soil may occur through several processes, including diffusion into pore spaces or less accessible soil matrix, and adsorption into more strongly sorbing black carbon phases or into even more inaccessible nanopores within the black carbon particles (Alexander 2000, NRC 2003, Ruby et al. 2016).

The capacity for microbiological degradation is often used to assess the weathering of nonpolar organic chemicals, and decreased microbial degradation is associated with decreased mobility and solubility. Over time, microbes have a reduced ability to reach chemicals as more are sequestered in pore spaces in soil particles (Alexander 2000). The pore spaces in soil particles may be as small as 5 nanometers. Bacteria and fungi may have diameters closer to 1000 nanometers and cannot contact chemicals in the nanopores, thus protecting the chemicals from biodegradation. Mobilization of organic chemicals from nanopores into larger soil pore spaces is also limited by lack of advection of water into the nanopores and associated diffusion. Biodegradation may be a significant factor in reducing concentrations of organic chemicals in soil soon after contamination, but also tends to make chemicals more water-soluble (Alexander 1999). Biodegradation results in byproducts that are more water-soluble than the parent chemical, with increased mobility and an increased potential for absorption.

Quantifying the solubility of a chemical in soil assists in understanding the potential bioavailability because greater solubility generally results in an increased potential for absorption. Several different partition coefficients are used to quantify the solubility of a chemical or affinity for organic carbon in soil, including octanol-water partition coefficient (K_{ow}), soil-organic carbon partition coefficient (K_{oc}), and soil-water partition coefficient (K_p) (Chung and Alexander 2002, NRC 2003). K_{ow} is a chemical property that can be measured in a laboratory test of the partitioning of chemicals between octanol, representing soil organic matter, and water. K_{oc} and K_p are site-specific parameters that will vary based on site soil characteristics (Chiou 2002, Chiou and Kile 2000). The K_{oc}, which represents the partitioning of a chemical into soil organic carbon, is a more direct measure of a chemical's affinity for organic matter. K_p represents the affinity of an organic chemical for a particular soil type. K_p can be divided by the site-specific fraction of organic carbon in soil to calculate the K_{oc}. Higher K_{ow}, K_{oc}, and K_p values are expected to result in stronger chemical adsorption to soil particles.

3. Measuring bioavailability

Measurement of chemical absorption is complicated by the variety of factors controlling absorption of different chemicals, as well as by differences among chemicals in metabolism, disposition and excretion. Many chemicals are absorbed by systems supporting absorption of nutrients. In vivo methods using laboratory animals or human subjects to measure absorption have been developed to support studies of nutrients and pharmaceuticals, as well as toxicants. In vitro methods that do not rely on laboratory animals are also used, but are generally limited to assessing relative bioavailability.

Due to a desire to reduce the use of laboratory animals, much emphasis has been placed on in vitro tests over the past twenty years or more. Nevertheless, the reliability of these tests is often assessed based on how the results compare to those of in vivo studies. With some nonpolar organic chemicals such as PAHs, there is considerable question about the reliability of in vivo methods (Ruby et al. 2016). This presents a difficulty in assessing in vitro results, because it is not apparent how these tests may be validated. In the following sections, general approaches for in vivo and in vitro studies are briefly described, with chemical-specific issues described in greater detail in Section 5.

3.1 *In Vivo* methods

This section first provides a general review of approaches used to measure bioavailability in laboratory animals. Rodents, primates and swine have all been used. Selection of the animal model will depend on the toxicokinetic profile of the chemical of interest, chemical concentrations in soil and sensitivity of analytical methods, how bioavailability will be measured (excreta, blood, tissue), and resources available.

One or more doses of the chemical of interest are administered to the test animals, and then selected tissues or excreta are analyzed for chemical concentrations at various time periods following the dosing. A soluble form of the chemical of interest or the form used in the toxicity study typically serves as the reference material. The time period of dosing and time of tissue analysis is dependent on known toxicokinetic properties of the chemical. For example, PCBs and dioxins/furans accumulate in adipose tissue, whereas PAHs are readily metabolized and do not accumulate in tissues. Age of the animal may affect absorption, as well as whether the animal has recently been fed (i.e., fasting or non-fasting condition). The study design must account for these factors to accurately estimate relative bioavailability in humans.

Modifications to standard methods for assessing bioavailability may be needed to ensure that representative environmental concentrations of chemicals in weathered soils can be tested. Radiolabeled organic chemicals or radioisotopes of metals, are often for assessing chemical bioavailability because they are not confounded by metabolism of test chemicals and allow detection of small doses. Testing of weathered site soils must instead rely on chemical analysis, so larger doses may be needed to meet analytical requirements. Larger doses may be impractical for typical environmental contamination, e.g., if an upper limit is imposed on doses by size of a soil bolus that can be administered to an animal or by the palatability of feed containing too much soil. When test soils are not representative of the majority of soils at a site, it will be especially important to evaluate the relevance of the higher concentration samples to the soil and chemical characteristics over the concentration range of interest.

The most comprehensive approach to measuring absorption is a mass balance approach that tracks the total amount of the administered chemical, measuring the amount of chemical in tissues and excreta. In the simplest case, the absorbed fraction of the administered chemical will be the total amount of chemical found in tissues and urine, and the unabsorbed fraction will be the amount in feces. This approach only applies to chemicals that are not excreted back into the gastrointestinal tract via the bile. The primary in vivo approaches are described below.

3.1.1 Blood Concentrations

Blood concentrations may be tracked over time by collecting repeated samples of blood after a single dose administration. Area under the concentration (AUC) versus time curve is then calculated for the orally administered chemical and bioavailability is calculated by comparison with the AUC for an intravenously administered dose. This approach generally works best for chemicals that are readily absorbed and excreted quickly, such as arsenic (Freeman et al. 1995, Roberts et al. 2002). Relative bioavailability may be determined by dividing the AUC calculated from test animals dosed orally with site soil by the AUC calculated from test animals given an oral reference dose of a soluble form of the chemical. The AUC approach has also been adapted to predict bioavailability of slowly excreted chemicals (i.e., lead) by giving repeated daily doses until a steady-state is approximated and then measuring AUC for a 24-hour period (Casteel et al. 1997).

3.1.2 Fecal Excretion

For chemicals without biliary excretion, measurement of chemical concentrations in feces will indicate the fraction of the dose that is not absorbed. The duration of the study must accommodate the transit time of the chemical through the gastrointestinal tract to allow for complete collection. The fraction of dose absorbed is the administered dose minus the amount of chemical that is excreted in feces. If an intravenous dose of a chemical results in fecal excretion that suggests biliary excretion, and a different approach is needed to measure bioavailability for that chemical.

3.1.3 Urinary Excretion

Some chemicals are excreted primarily in urine. In such cases the fraction of a dose excreted in urine represents the fraction absorbed. Relative bioavailability may be determined by dividing the fraction of the dose in urine following an oral dose with site soil by the fraction of the dose in urine following an oral reference dose.

3.1.4 Tissue Concentrations

Tissue concentrations may be used to assess bioavailability for chemicals that accumulate in specific tissues. Following a specified dosing period, the tissues that preferentially accumulate the chemical of interest are analyzed. Relative bioavailability is calculated by dividing the tissue concentration following an oral dose with site soil by the tissue concentration following an oral reference dose.

3.2 *In Vitro* Methods

Laboratory extraction tests (i.e., *in vitro* tests) that simulate dissolution of chemicals in the gastrointestinal tract are now widely used to assess the relative bioavailability of soil arsenic and lead. These *in vitro* tests have been applied to other metals with varying success, and have been modified to adapt them for use with organic chemicals (Kelley et al. 2002, NRC 2003, Ruby et al. 1999, Sips et al. 2001, Collins et al. 2015, Ruby et a. 2016). The general approach involves incubation of site soil or reference chemical in a low pH solution for a time period that mimics the residence time of food in a child's stomach (i.e., the "stomach phase"). The solution is then sometimes incubated in a higher pH solution for a time period that represents the residence time of food in the small intestine (i.e., "intestinal phase"). During the incubating time periods, various enzymes and acids are added to the solution to mimic the digestion process. In these tests, the amount of chemical dissolved in the final solution over the amount of chemical

added represents the bioaccessible fraction, and is used as a measure of relative bioavailability.

In general, the in vitro methods originating in Europe tend to be more complex in order to mimic gastrointestinal tract chemistry as closely as possible and yield an extraction system that more accurately predicts uptake in humans. In contrast, the in vitro methods originating in Canada, US, and Australia tend to rely on correlation with in vivo data in order to accurately predict the bioavailable fraction as measured in animal models. As a result, these tests can be less complex because they are focused on capturing the critical test components that allow for a correlation with animal data (Ruby et al. 2016).

For nonpolar organic chemicals, two concerns have driven modifications to the methods used to assess metals: 1) the need to include food or a "lipid sink" to provide a phase into which organic chemicals may partition, and 2) consideration of the potential for chemical metabolism and absorption in the large intestine or colon. Without a lipid sink, nonpolar organic chemicals may be retained on soil particles with associated underestimation of bioaccessibility. The lack of an extended "colon" phase may also lead to underestimation of bioaccessibility if chemicals are absorbed in the lower gastrointestinal tract, or if they are metabolized by gut bacteria, and the metabolites later absorbed. The duration for food passage through the colon accounts for almost 80 percent of the transit time through the human digestive tract, and the extended incubation time afforded by the colon may be important for absorption of organic chemicals with higher log K_{OW} (Collins et al. 2013). The colon consists of an aqueous medium rich in carbohydrates that may facilitate desorption of organic chemicals from soil. Microbial transformation of organic chemicals occurs in the colon compartment (van de Wiele et al. 2005), and the bioaccessibility can be underestimated if only parent compounds are measured. Measuring both parent and gut metabolites deserves further attention in future studies (Tilston et al. 2011).

Five types of *in vitro* methods developed to assess the bioaccessibility of organic chemicals (i.e., PAHs) are described by Cui et al. (2016), including physiologically-based extraction test (PBET), simulator of the human intestinal microbial ecosystem (SHIME), The Netherlands National Institute of Public Health and the Environment (RIVM) method, Fed ORganic Estimation human Simulation Test (FOREhST), and *in vitro* gastrointestinal (IVG) method. The operational parameters and components in gastrointestinal solution for these *in vitro* methods are listed in Table S1 of Cui et al. (2016). These approaches are described below.

PBET

The PBET assay was developed in the UK and modified based on the assay for metal-contaminated soils (Ruby et al. 1996, 2002). The PBET involves a gastric phase with a solution pH at 1.5 representing the fasted state, and an intestinal phase under a neutral condition with the presence of bile, pancreatic enzymes, and proteins (such as mucin). The incubation time of gastric and intestinal phases is typical residence time in the digestive tract.

The colon extended PBET (CE-PBET) was developed in the UK by adding a colon extraction phase to the PBET (Tilston et al. 2011) to better represent potential absorption in the large intestine. The CE-PBET was operated in the fed state, which may further enhance bioaccessibility. However, the challenge of mimicking microbial effects in the colon phase is not addressed by the CE-PBET.

SHIME

The SHIME assay was initially developed in Belgium by Molly et al. (1993) as an automated multistage reactor consisting of five compartments (including various gastrointestinal and colon phases). The reactor was later developed into the SHIME assay to measure PAH bioaccessibility (van de Wiele et al. 2004). The SHIME reactor represents the entire gastrointestinal tract with enzymatic processes in the stomach and duodenum, and a colon simulator with a microbial community from the human colon in which potential microbial degradation is considered (Siciliano et al. 2010). Potential microbial degradation in the colon compartment could lead to the production of metabolites that are more toxic, and hence a greater risk to humans, than the parent chemicals. Measuring both parent and microbial metabolites may more accurately measure bioaccessibility if metabolites are also absorbed. However, large-scale application of SHIME assay can be a challenge due to its relatively complicated operation and maintenance, i.e., the odor due to the colon microbial community.

RIVM Method

The RIVM method was developed in The Netherlands for both fed and fasted states, including the saliva, gastric and intestinal phases (Sips et al. 2001, Hagens et al. 2008, Versantvoort et al. 2004). RIVM assay has not been widely applied probably due to the limited availability of references (Sips et al. 2001, Versantvoort et al. 2004). However, the FOREhST, which was developed based on the RIVM assay, is a popular method to assess the bioaccessibility of organic chemicals.

FOREhST

The FOREhST is an *in vitro* method developed in the UK, which was modified based on the RIVM and unified Bioaccessibility Research Group in Europe (BARGE) method (UBM) assays (Cave et al. 2010, Sips et al. 2001, Wragg et al. 2009). The FOREhST includes the saliva, gastric and intestinal phases and it incorporates food into the assay and adjusts the gastrointestinal components to simulate the fed state which have the potential to mobilize organic chemicals from sample matrices, and therefore bioaccessibility for the worst-case scenario could be evaluated. However, the addition of milk powder as the food makes it difficult for analysis. The saponification process to remove the lipid from milk is usually concurrent with low recovery of PAHs after intestinal phase extraction.

IVG Method

The IVG assay was initially developed in US to assess arsenic bioaccessibility in soils by Rodriguez et al. (1999) and Basta et al. (2007). Compared with other methods, the IVG assay has limited application for bioaccessibility of organic chemicals in soils, and poor correlation with RBA results using a juvenile swine model was observed. The poor correlation was attributed to the poor thermodynamic equilibrium during the two-hour IVG extraction, which may be overcome by extending the extraction time. However, the static nature of the IVG assay, i.e., with no dynamic sorption process by intestinal cells, is another limiting factor to accurately estimate bioavailability in soil.

Regulatory acceptance of *in vitro* methods as an approach to assess chemical bioavailability in soil requires scientific justification. These methods may be supported when there is a good understanding of the processes controlling chemical dissolution in the gut and assurances that relative bioavailability is not being underestimated. The strongest justification comes from formal validation efforts in which *in vitro* bioaccessibility results are compared to bioavailability data from *in vivo* studies. Processes for validation and regulatory acceptance of toxicological test methods have been developed by the US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 1997). Adaptation of this validation process to *in vitro* bioaccessibility methods is described by Ruby et al. (1999), Schoof (2004), and USEPA (2009). Validation of *in vitro* test results with *in vivo* test results has been mainly conducted for lead (Drexler and Brattin 2007, Ruby et al. 1999, USEPA 2009) and arsenic (Bradham et al., 2015, Brattin et al. 2013, Diamond et al. 2016), and the data on organic chemicals are very limited.

Compared to *in vivo* methods for bioavailability measurement, *in vitro* methods have several advantages, such as relative simplicity, sustainability, reduced costs, and the ability to develop a reproducible standard operating procedure (SOP). The *in vitro* tests that have been used to measure the bioaccessibility of PAHs and PCBs on an experimental basis are further discussed in Section 5.

Over ten years ago, DEPA commissioned a series of studies assessing various *in vitro* bioaccessibility testing methods for metals and PAHs (DEPA 2004, 2005). A great deal of variability was observed among the methods assessed and ultimately, the RIVM method was recommended for evaluating oral bioaccessibility under fasted conditions for metals and under fed conditions for PAHs (DEPA 2005).

4. Regulatory uses of bioavailability

Given an understanding of the differences between relative bioavailability, bioaccessibility, and the factors influencing the bioavailability of organic compounds in soil, these concepts can then be applied to risk assessment methodology. This section describes the applicability of bioavailability to risk assessment, including the role of bioavailability in risk assessment, and its application in risk assessment. Current regulatory approaches to evaluating and using bioavailability in risk assessment in the US, UK, The Netherlands, France, Canada, and Australia are also summarized.

4.1 Role of Bioavailability in Risk Assessment

Oral TRVs used to estimate risks or to derive risk-based cleanup levels are typically calculated as intakes or administered doses. A RBA as a unitless fraction may be applied to account for differences between exposure medium and toxicity study dosing medium as follows:

$$Intake_{unadjusted} * RBA = Intake_{adjusted}$$

The default assumption in the absence of a bioavailability adjustment is that the bioavailability of a chemical in soil is comparable to its bioavailability in the exposure medium from the toxicity studies used to derive the TRV, i.e., the RBA is assumed to be 1.0. For most legacy contaminated sites, RBA will be less than 1.0. Use of bioavailability adjustments may reduce risk estimates and provide support for higher cleanup levels while providing adequate protection of public health.

One key consideration in the application of bioavailability adjustments for mixtures of nonpolar organic chemicals is that the mixture in soil must be mimicked in the reference material tested in the bioavailability study unless the focus is on one indicator chemical. Mixtures of organic chemicals such as PCBs or PAHs are often assessed using toxic equivalency factors, so the relative concentrations of such mixtures should be matched in the reference material.

For *in vitro* studies, additional steps are required before applying a RBA in risk assessment, that is first to calculate bioaccessibility, and then to evaluate how representative the bioaccessibility data are of relative bioavailability. The *in vitro* test method should include a reference material that is the same as the chemical form used in the TRV study. If this cannot be achieved, a comparable soluble form of the chemical could be used. Bioaccessibility from the *in vitro* test can be estimated as follows:

$$Bioaccessibility = \frac{\%\ Recovery\ of\ Soil\ Sample}{\%\ Recovery\ of\ Reference\ Material}$$

Ideally, validation of *in vitro* test results with *in vivo* data would show how representative the bioaccessibility data are of relative bioavailability. Lead is a good example for which sufficient data are available to support a robust regression equation that is used to convert estimates of bioaccessibility, as measured in the USEPA lead *in vitro* bioaccessibility assay (IVBA) to relative bioavailability before application in risk assessment. Such conversion may be needed because, while the correlation between *in vivo* and *in vitro* data is strong, the relationship may not be 1:1 and thus, whenever sufficient data are available, IVBA values need adjusting before being used as a surrogate for *in vivo* RBA. The regression equation for lead is as follows:

$$Relative\ Bioavailability\ (fraction) = 0.878 * In\ Vitro\ Bioaccessibility\ (fraction) - 0.028$$

Arsenic is another example for which a robust regression equation is available to convert estimates of bioaccessibility to relative bioavailability before application in risk assessment. The regression equation for arsenic developed by Diamond et al. (2016) is as follows:

$$Relative\ Bioavialability\ (\%) = 0.79 * In\ Vitro\ Bioaccessibility\ (\%) + 3$$

While defensible correlations between *in vitro* and *in vivo* data for other chemicals may become available in future, up to now such correlation is very limited for organic chemicals. As a practical matter, the correlations are close to 1:1 over much of the range of reported bioaccessibilities. For that reason, it has typically been assumed that bioaccessibility determined as the ratio of dissolution from soil to dissolution from reference material, may be used directly as a RBA value to adjust intake estimates. A detailed, scientifically-based rationale should be provided to support bioavailability adjustments in HHRA if a robust correlation between *in vitro* and *in vivo* data is not available for the chemical evaluated.

4.2 Regulatory Approaches to Evaluating and Using Bioavailability in Risk Assessment

The bioavailability of organic chemicals in soil is an important and active area of research for environmental scientists, although this area remains only partially recognized by regulators. Over the past few years, there has been growing acknowledgment of the need to consider potential reduced bioavailability of organic chemicals in risk assessment frameworks, to. By providing site-specific relative bioavailability data, more realistic decision-making on organic chemical contamination can be achieved, rather than relying on the overly conservative, traditional approach of using total concentrations (Ortega-Calvo et al. 2015).

Scientific developments on bioavailability cannot always be easily translated into ready-to-use regulatory approaches, and such regulatory frameworks are in various stages of development in different countries. To facilitate the implementation of bioavailability in risk assessment and management, the approaches to evaluating bioavailability must be standardized, clearly articulated, and well-justified. The continuing growth of the knowledge base of bioavailability science will enhance the potential for its regulatory implementation (Ortega-Calvo et al. 2015).

There are various differences among countries in the regulatory approaches to evaluating and using bioavailability in risk assessment, including definitions of terms, test methods that are deemed acceptable, reporting requirements, regulatory frameworks, and guidance on specific chemicals. A brief summary of regulatory practice in the US, UK, The Netherlands, France, Canada, and Australia is provided in the following sections.

4.2.1 United States

The US has long acknowledged that differences in chemical absorption in the dosing medium of TRV studies and the site exposure medium should be considered in risk assessment (USEPA 1989); however, formal guidance on incorporation of relative bioavailability into risk assessments is much more recent. Guidance on how to assess site-specific oral bioavailability of metals in soils was issued in 2007 (USEPA 2007a), providing technical and policy guidance to USEPA staff on making risk management decisions for contaminated sites. A decision framework on how to evaluate and incorporate site-specific oral bioavailability information into the risk-based decision-making process is also provided to improve site-specific risk estimates and derivation of cleanup levels. The guidance document includes a description of how USEPA would evaluate whether a specific bioavailability method has been validated for regulatory risk assessment purposes.

As a companion to the general guidance for soil metals, USEPA issued detailed guidance for estimating the relative bioavailability of lead in soil and soil-like materials (USEPA 2007b) using either a swine model or an *in vitro* model. Juvenile swine is considered to be a good physiological model for gastrointestinal absorption in children. Estimates of lead relative bioavailability from a series of *in vivo* swine studies conducted with 19 soil and soil-like test materials (i.e., soils and mining tailings) ranged from 6 percent to 105 percent, forming the basis for USEPA's default assumption of 60 percent relative bioavailability. When a site-specific RBA estimate is available, it may replace USEPA's default assumption. The guidance also describes the IVBA method which correlates well with the *in vivo* model results, and provides a regression equation to use in converting the *in vitro* bioaccessibility results to an RBA (see Section 4.1). USEPA has published a SOP for IVBA to evaluate lead bioaccessibility in soil (USEPA 2012a), which is based on previous work conducted by Ruby et al. (1993, 1996) and Drexler and Brattin (2007). This was followed by a short report documenting the compliance of the USEPA lead IVBA method with validation and regulatory acceptance criteria (USEPA 2009).

The relative oral absorption of soil arsenic has been tested in a series of studies in juvenile swine (USEPA 2010a), cynomolgus monkeys (Roberts et al. 2007), and mice (Bradham et al. 2011, Makris et al. 2008). These studies included soils from mining and smelting sites, sites with historical arsenical pesticide use, and hazardous waste sites. Based on an upper percentile from a data set of 103 estimates of arsenic relative bioavailability reported in these studies, USEPA (2012b) recommends a default value of 60 percent for arsenic relative bioavailability in soil when compared to water, which is supported by the fact that less than 5 percent of the arsenic relative bioavailability estimates exceeded 60 percent. In general, USEPA recommends that efforts be made to collect data that support site-specific estimates, rather than relying on the default value. Several research groups have developed good correlations between *in vitro* and *in vivo data* for arsenic (Bradham et al. 2015, Brattin et al 2013, Diamond et al. 2016), but the USEPA's validation program for arsenic *in vitro* test currently is still underway.

Estimates of RBA for dioxins/furans in soils have been compiled and summarized by USEPA based on six *in vivo* studies conducted with rabbits, rats, and swine (USEPA 2010c). The average RBA estimates for dioxins/furans in swine and rats ranged from 28 percent to 41 percent, as compared to a lipid or organic solvent vehicle as the reference material (e.g., corn oil). In 2015, USEPA published a relative bioavailability assay evaluation framework for *in vivo* tests for dioxins/furans (USEPA 2015). In this framework, USEPA recommends that estimates of the RBA for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity equivalent (TEQ) in soil being used in risk assessment, and the soil fraction of <250 µm should be used in the *in vivo* bioassay. Currently, there is no general consensus on the preferred animal model for estimating RBA for dioxins/furans or which tissue would provide reliable predictions of the TEQ body burden. USEPA recommends the *in vivo* study should include a complete analysis of dioxin/furan congeners and soil characterization, e.g., total solids, pH, total organic carbon (TOC), and grain size distribution. A range of soil concentrations should also be tested to assess the depend-

ence of RBA on concentration. USEPA has not yet published an evaluation framework for *in vitro* tests for dioxins/furans.

USEPA does not currently have approved *in vivo* or *in vitro* protocols of evaluating bioavailability for other metals or organic compounds. Some regional USEPA and state regulatory offices provide additional guidance on use of bioavailability adjustments in risk assessment.

4.2.2 United Kingdom

The UK Environment Agency does not provide a recommendation for specific *in vivo* or *in vitro* test methods (UK Environment Agency 2007, 2011). Application of bioavailability adjustments is allowable under limited conditions. In 2005, the UK Environment Agency recognized the usefulness of bioavailability adjustments in risk assessment, but required "suitable justification" for the use of bioaccessibility data (UK Environment Agency 2005). Suitable justification included the following:

- A detailed description of the sample collection;
- Preparation, and analysis and quality assurance methods;
- An understanding of the uncertainties in the test method;
- Application of the bioaccessibility data only to the soil and dust ingestion pathway;
- No extrapolation of data from one chemical to another; and
- Potential for planned land use changes that could affect oral bioaccessibility by causing changes in chemical sequestration.

A bioaccessibility protocol, the UBM has been developed by the BARGE based on the modifications with the RIVM method in order to harmonize the use of bioaccessibility in HHRAs for contaminated soils in Europe (http://www.bgs.ac.uk/barge/ubm.html). The UBM has been applied to risk assessments of arsenic, chromium, and lead contamination in urban UK soils (Broadway et al. 2010, Farmer et al. 2011, Wragg and Cave 2012).

4.2.3 The Netherlands

RIVM developed an *in vitro* bioaccessibility test method for lead and BaP (Sips et al. 2001). Like many other *in vitro* methods, the RIVM method is based on the digestive system of a child in a fasted state. This method may also be modified to the *in vitro* digestion (IVD) model to allow for evaluating the bioaccessibility of lead under an "average physiological state" by conversion between only fasted and only fed physiological states (Hagens et al. 2008). More recently, the RIVM has examined the predictability of three *in vitro* models for lead currently used in the Netherlands: the IVD model, the Tiny TNO *in vitro* model (Tiny-TIM), and the UBM. By comparing the results of these three *in vitro* models with the results of an *in vivo* bioavailability study conducted on juvenile swine, RIVM found that the UBM is the best model for estimating the bioaccessibility of lead in soil (van Kesteren et al. 2014). Further, based on the results of the swine study, a lead RBA ranging from 0.58 (50th percentile) to 0.84 (80th percentile) is recommended to be used in risk assessment. The current Dutch soil quality standard for lead, which was derived based on a RBA of 0.4 from the IVD and Tiny-TIM model in the study of Hagens et al. (2009). van Kesteren et al. (2014) suggest this standard may need to be re-evaluated.

4.2.4 France

Researchers at the French National Institute for Industrial Environment and Risks (INERIS) contributed to the BARGE effort to harmonize the use of bioaccessibility in HHRAs for contaminated soils in Europe (http://www.bgs.ac.uk/barge/ubm.html), and development of the bioaccessibility protocol UBM based on the modifications with the RIVM method. *In vivo* validation of the UBM for the bioaccessibility of lead, cadmium, and arsenic in soils has been undertaken by INERIS and the Polytechnique de Lorraine, Nancy using a juvenile swine model (Denys et al. 2012). In addition, evaluation of the UBM has also been undertaken by an international interlaboratory comparison exercise (Wragg et al. 2009). The UBM has been applied to risk as-

sessments of urban and agricultural soils contaminated with cadmium, lead, and zinc from smelter emissions in France (Pelfrene et al. 2011, Roussel et al. 2010).

4.2.5 Canada

Health Canada guidance includes two tiers of risk assessment, preliminary quantitative risk assessment (PQRA, Health Canada 2012) and detailed quantitative risk assessment (DQRA, Health Canada 2010). PQRA is used to rank the potential human health risks posed by federal contaminated sites, and a default RBA value of 1.0 is usually assumed. If a RBA value other than 1.0 is used, it must be sufficiently documented for peer review. DQRA makes greater use of site-specific assumptions, with the objective of providing more accurate and realistic estimate of exposures and risk. The DQRA guidance includes a substantial discussion of various aspects of bioavailability assessment, including consideration for the use of oral bioavailability adjustments. A site-specific bioavailability study is usually necessary to obtain an appropriate adjustment value, and the potential use of oral bioavailability adjustments in DQRA needs to be thoroughly considered on a case-by-case basis.

In 2011, a report titled “Guidance on Consideration of Oral Bioavailability of Chemicals in Soil for Use in Human Health Risk Assessment” was issued by Health Canada (ENVIRON 2011). Now Health Canada has drafted formal guidance on oral bioavailability of substances in soil and soil-like media under the Federal contaminated site risk assessment program (soon to be released). Both of these documents address scientific issues relevant to characterizing the oral bioavailability of substances in contaminated soil relative to bioavailability in other exposure media, and how such information may be used in HHRA for federal contaminated sites in Canada. The formal guidance document only addresses metals in soil, but the 2011 guidance also provided summaries of relative bioavailability of nonpolar organic chemicals in soil.

BioAccessibility Research Canada (BARC) and Health Canada designed and sponsored a round robin study conducted by the Royal Military College of Canada to determine how results would vary among laboratories that used different *in vitro* methods to measure the bioaccessibility of inorganic contaminants (BARC 2011). It reported that the bioaccessibility results ranged widely, but the reproducibility for several elements (all laboratories/methods combined) was comparable to the uncertainty resulting from analysis at accredited laboratories. BARC (2011) also conducted validation of arsenic and lead bioaccessibility results from several simpler and more physiologically-based *in vitro* methods with *in vivo* swine bioavailability studies.

4.2.6 Australia

Australia has conducted pioneering work on the introduction of bioavailability in full-scale land management with consideration of lead and arsenic bioavailability in its National Environment Protection Measure (NEPM). On behalf of the Australia National Environment Protection Council (NEPC), Ng et al. (2009, 2010) conducted a review of bioaccessibility testing protocols and methods for application of bioavailability adjustments in risk assessment. Ng et al. (2009, 2010) provided recommendations on how to incorporate bioavailability data into risk assessments. Risk assessment practitioners were directed to USEPA (2007a) for further guidance on selection of *in vitro* and *in vivo* test methods. *In vitro* assays were judged appropriate for estimating relative bioavailability for lead and arsenic, and could be used for Tier 2 risk assessments.

In 2013, NEPC issued the *Guideline on Site-Specific Health Risk Assessment Methodology* (NEPC 2013a). The guideline considers a number of *in vitro* methods appropriate to measure arsenic and lead relative bioavailability, including the Relative Bioavailability Leaching Procedure (RBALP) (USEPA 2007a), the Solubility Bioavailability Research Consortium (SBRC) method (Kelley et al. 2002), and the IVG method (Basta et al. 2007, Rodriguez et al. 1999). The guideline also requires using site-specific bioavailability assessments in addition to the generic RBA values in risk assessment. Furthermore, NEPC develops health investigation levels (HILs) for arsenic and lead in soil based on generic RBA values (25-70 percent for arsenic and 50 percent for lead) (NEPC 2013b).

Currently, bioavailability of organic compounds and metals other than lead and arsenic is yet to be incorporated in the NEPM Assessment of Site Contamination (ACS) at the NEPM. Research toward the development of SOPs is the focus of Australian studies with a view to inclusion of bioavailability in the next revision of the NEPM.

5. Chemical Specific Bioavailability Summaries

A summary of bioavailability information for the selected nonpolar organic chemicals, PAHs and PCBs, is presented in this section. These chemicals are frequently associated with the greatest risk in contaminated soils in Demark, and are also commonly found to have reduced bioavailability in weathered soils as compared with more soluble forms used in toxicity studies.

The focus of this section is to identify critical aspects of environmental chemistry and mammalian toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) of the selected chemicals that may influence the design and conduct of relative oral bioavailability studies of the chemicals in soil. The *in vivo* and *in vitro* studies of oral bioavailability in the literature are also summarized to illustrate these points. Finally, study design guidelines are provided for each of the chemicals evaluated. Due to the large number of efforts underway by international scientific and regulatory communities to further develop the methods to measure oral bioavailability, it is encouraged that a search of the current guidance and literature databases be conducted prior to initiating new studies.

5.1 Polycyclic Aromatic Hydrocarbons

PAHs are a class of hydrocarbons composed of multiple aromatic rings. PAH contamination of urban soil is widespread due to a variety of anthropogenic activities. PAHs may be released to the environment during the processing of coal and petroleum products, through emissions from power plants and incinerators as byproducts of incomplete combustion, during chemical production processes, and from vehicles and wood preservation activities.

PAHs exhibit toxic, mutagenic, teratogenic and carcinogenic properties (International Agency for Research on Cancer [IARC] 2010). Oral TRVs for noncancer endpoints have been derived by USEPA, RIVM, and the Agency for Toxic Substances and Disease Registry (ATSDR) for a number of individual PAHs, and USEPA and RIVM have also developed TRVs for cancer endpoints (ITER 2016). Risk-based soil cleanup levels are often derived using an oral cancer slope factor derived by USEPA as the TRV. The Danish EPA soil quality criterion is 4 milligram per kilogram (mg/kg) for total PAHs, 0.3 mg/kg for BaP, and 0.3 mg/kg for dibenzo(a,h)anthracene. These criteria represent the soil concentrations corresponding to an excess lifetime cancer risk of one-in-a-million (10^{-6}) (DEPA 2004). In the critical studies that form the basis of the USEPA oral TRV, BaP was administered to mice in the laboratory chow diet. BaP is well absorbed when administered with food containing lipids. Thus, the bioavailability of PAHs in soil is expected to be reduced when compared to the bioavailability of PAHs in diet, resulting in an RBA less than 1.0.

5.1.1 Toxicokinetics

There have been relatively few studies of PAH oral bioavailability in humans or animals. The oral absorption of PAHs in animals and humans has been shown to be high from food or vegetable oil with absorption rates ranging from 87 to almost 100 percent when ingested in food or oil by rats and hamsters (Magee et al. 1996). In the studies reviewed by Magee et al. (1996), the absorption of PAHs did not significantly vary with the individual compounds, dose levels, or non-soil dosing vehicle. In some cases, lower rates have been reported when doses were extremely high (Ramesh et al. 2004). For example, absorption of 100 mg/kg of BaP in peanut oil was found to be 40 percent in rats (Ramesh et al. 2001).

PAHs are lipophilic, allowing them to dissolve into and be transported by diffusion across cell membranes including those lining the gastrointestinal tract (IARC 2010). Smaller PAHs, i.e., those with two or three rings, are absorbed more rapidly and completely than those with five or six rings. Absorbed PAHs are widely distributed throughout the body, but may achieve higher concentrations in fatty tissues. PAH metabolism is extensive, yielding more soluble metabolites, such as epoxides, phenols, and dihydrodiols. These metabolites form conjugates with sulfate, glutathione or glucuronic acid that are excreted in feces via bile and in the urine.

Determination of PAH oral bioavailability in animal studies is challenging due to the complexity of toxicokinetics within the human body. Bioavailability is typically calculated by measuring the amount of chemical (and its metabolites) in blood, urine, feces, or tissues as well as measurement of biomarkers (e.g., DNA adduct formation in lung and liver tissue, liver enzyme induction). However, all these approaches have certain limitations as described in Ruby et al. (2016) and Juhasz et al. (2014).

Blood

Assessment of PAH bioavailability using blood as an endpoint is complicated by the fact that PAHs may be absorbed from the gastrointestinal tract and metabolized in the hepatic portal system but may not reach the systemic circulation (due to biliary excretion). Thus, the fraction excreted in the bile is not accounted for if the amount in the systemic circulation is measured. On the other hand, metabolized PAHs may enter the systemic circulation, and not be quantified as bioavailable if only the parent compound is measured.

Feces

PAHs in feces have been used as an endpoint for the upper-bound estimation of bioavailability, subtracting PAH excreted in feces from the administered dose to estimate absorbed dose. However, fecal contents reflect both the fraction absorbed and excreted (through bile) in addition to unabsorbed PAHs, and distinguishing between the two for the purpose of estimating bioavailability is difficult. An additional confounding factor for feces-based estimates is the enterohepatic recirculation of PAHs, which not only delays the elimination of PAHs in feces but also affects the forms of PAHs in feces.

Urine

Use of urinary excretion to measure PAH bioavailability has been focused on metabolite excretion, but a principal limitation of using urinary metabolites as an indicator of PAH absorption arises from the fact that urinary excretion is a minor pathway of PAH elimination from the body. Thus, urine excretion has substantial uncertainty even for estimating relative bioavailability.

Tissue

After absorption, PAHs that enter the systemic circulation are readily distributed and stored in tissue in proportion to their lipid content. Use of tissue as an endpoint for evaluating PAH relative bioavailability is based on the assumption that with repeated doses, and once a steady state has been achieved between blood and tissues, the concentration of a PAH or metabolite in tissues will be proportional to the absorbed dose. However, self-induction of metabolism that occurs with repeated doses can produce differences in metabolic clearance among animals ingesting PAHs in soil versus diet, and the direct proportionality between tissue concentrations and absorbed dose needed for bioavailability determination may be lost.

Biomarkers

The use of biomarkers such as DNA adduct formation or liver enzyme induction as endpoints for bioavailability measurements can potentially provide relevant indicators of the internal doses of PAHs. However, it may not fit the classical definition of bioavailability, and using an RBA based on internal dose metrics (i.e., biomarkers) is incompatible with a TRV based on external doses.

5.1.2 Behavior in Soil

PAHs are characterized by low water solubility, low vapor pressure, lipophilic properties and long half lives in soils (Juhasz and Naidu 2000), and interact with soil components which could affect their oral bioavailability. As discussed in detail in Section 2.2.3, only the water-dissolved and rapidly desorbing fractions of PAHs are considered bioavailable, while the slowly desorbing and nonextractable fraction, especially the fraction strongly adsorbed to black carbon domains, may limit the release of PAHs in the gastrointestinal tract. Therefore, the PAH source material and organic carbon form into which the PAHs have predominantly partitioned will act as controlling factors in determining the oral bioavailability (Ruby et al. 2016).

Oral bioavailability may also be dependent on the PAH concentrations in soil. Studies indicated that adsorption to black carbon is competitive and nonlinear (Cornelissen et al. 2005, Ghosh et al. 2003), while adsorption to NOM is noncompetitive and linear (Schwarzenbach et al. 2003). Therefore, the lower the concentration of PAHs in soil, the more likely that black carbon will dominate sorption (Cornelissen et al. 2005). However, at higher concentrations, PAHs will compete against other organic contaminants and native organic compounds in soils and such effects can saturate or block the available surface adsorption sites (Cornelissen et al. 2005, Kwon and Pignatello 2005). NOM may therefore dominate sorption at high PAH concentrations. The adsorption of PAHs to black carbon can be up to two orders of magnitude higher than that predicted for NOM (Cornelissen et al. 2005, Hong et al. 2003). Therefore, studies of oral bioavailability conducted at elevated PAH concentrations (tens to thousands of mg/kg) may overestimate oral bioavailability compared to what would be seen at more environmentally relevant concentrations (Ruby et al. 2016).

The Danish EPA soil quality criteria for PAHs is in the range of 0.3 to 4 mg/kg, and bioavailability adjustment may greatly affect the results of risk assessment and risk management decision-making for sites with PAH concentrations near this range. However, as indicated in Section 5.1.3 and 5.1.4, bioavailability studies are usually conducted at concentrations much higher than this range (up to 5,000 mg/kg), and application of the RBA values derived from these studies to risk assessment may overestimate the risk.

As discussed in Section 2.2.3, it is also important to consider the effects of aging or weathering on PAH soil interactions, which are likely to reduce the oral bioavailability of PAHs in soil (Ruby et al. 2016). Thus, studies conducted with soils spiked with PAHs in the laboratory may produce higher oral bioavailability measurements than studies conducted with weathered soils in the natural environment.

5.1.3 *In Vivo* Studies and Methods

Ruby et al. (2016) provides a comprehensive review of 21 *in vivo* studies of PAH bioavailability in soil, and Ramboll Environ reviewed two additional *in vivo* studies (Juhasz et al. 2014, Peter et al. 2016). These studies provide a strong basis to evaluate the current state of science regarding *in vivo* approaches used to measure PAH bioavailability in soil. Some of the key experimental parameters used in these *in vivo* oral bioavailability studies were summarized in Tables 2 and S1 of Ruby et al. (2016), and are discussed below.

The 23 *in vivo* studies reviewed by Ruby et al. (2016) and Ramboll Environ used a variety of animal models (including mice, rats, mini pigs, juvenile swine, and goats) to evaluate the RBA of various PAHs (including anthracene, BaP, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, phenanthrene, pyrene, carcinogenic PAHs, and total PAHs).

The 23 studies were conducted with soils from various PAH sources, including contaminated field soil (21 studies) and uncontaminated soil spiked with PAHs in laboratory (four studies). In the four studies that evaluated the effect of aging on RBA, Goon et al. (1991) showed a slight reduction in RBA values (14% to 27% reduction after 6 to 12 months of aging), and Bordelon et al. (2000), Reeves et al. (2001), and Duan et al. (2014) showed no reduction in RBA values after aging for 3 to 12 months.

A wide range of soil concentrations for total PAHs have been studied, from <1 mg/kg to >4,000 mg/kg, with only a few studies in the range of more environmentally relevant concentrations (i.e., <10 mg/kg). As discussed in Section 5.1.2, it appears that studies conducted in the higher concentration range may tend to overestimate RBA for soils in the lower concentration range. Use of high concentrations may also minimize the difference between weathered and spiked soils. A majority of the studies (18 out of 23) reported the soil particle size tested, but more than half of them (11 studies) were much larger than the particle sizes (<150 µm to <250 µm) considered as the fraction most likely to adhere to human hands and be incidentally ingested through hand-to-mouth contact (Collins et al. 2015, Kissel et al. 1996, Ruby and Lowney 2012).

Only 10 of the 23 studies reported the TOC concentrations of the soils tested, and none of them characterized the types of organic carbon in the test soils, which is needed to fully understand the effects of PAH source materials and different forms of organic carbon on RBA values. There was some indication that soil TOC is inversely related to the RBA of BaP (Duan et al. 2014, Goon et al. 1991), but the data were very limited.

Among 10 of the 23 studies reviewed, RBA values were either not reported, or could not be calculated from the data presented in the publication. Among the other 13 studies, the reported or calculated RBA values for PAHs ranged from <0.6 percent to approximately 100 percent in contaminated field soil, and from 50 percent to 100 percent in uncontaminated spiked soil. As described above, in the critical studies that form the basis of the USEPA oral TRV, BaP was administered to mice in the laboratory chow diet. Therefore, absorption of PAHs from soil relative to absorption from diet is the appropriate metric for determining RBA for use in HHRA. However, as in shown in Tables 2 and S1 of Ruby et al. (2016), absorption from the diet is not always selected as the basis for calculating RBA values reported in the literature.

In summary, the 23 *in vivo* studies reviewed provide a basis for the conclusion that the bioavailability of PAHs from soil is reduced relative to absorption from diet, and that the default assumption of 100% RBA likely overestimates actual exposure to PAHs in soil. However, a wide range of RBA values were reported, and because of the limited scope of each individual study and the large variability in study designs, it is difficult to compare results directly across studies. These studies also not provide a strong basis for further understanding of the factors controlling PAH oral bioavailability in a broader context beyond the individual samples tested.

5.1.4 *In Vitro* Studies and Methods

Although an extensive body of literature used *in vivo* methods for determination of PAH bioavailability in soil, the time required for *in vivo* studies and the expense of animal trials preclude their use as routine bioavailability assessment tools. As a result, rapid and cost effective *in vitro* methods simulating human gastrointestinal conditions have been developed in order to estimate PAH bioaccessibility in soil. Over ten years ago, DEPA conducted a series of studies assessing various *in vitro* bioaccessibility testing methods for PAHs (DEPA 2004, 2005), and the RIVM method (Sips et al. 2001) was recommended for evaluating oral PAHs bioaccessibility under fed conditions (DEPA 2005). *In vitro* methods for assessing the bioavailability of nonpolar organic chemicals have been improved considerably in the past decade. This report focuses on the up-to-date knowledge of *in vitro* methods for PAH bioaccessibility in soil based on a comprehensive review of literature papers, especially those published in the last ten years.

Ruby et al. (2016) reviewed 34 *in* vitro studies of PAH bioaccessibility in soil, and Ramboll Environ reviewed two additional *in vitro* studies (Collins et al. 2013, Juhasz et al. 2014). These studies include a wide range of approaches, and provide a strong basis to evaluate the current state of science for measuring PAH bioaccessibility in soil. Some of the key experimental parameters used in these studies were summarized in Tables 3 and S2 of Ruby et al. (2016), and are discussed below.

The 34 *in vitro* studies reviewed by Ruby et al. (2016) and Ramboll Environ evaluated oral bioaccessibility of various PAHs, including anthracene, BaP, chrysene, dibenzo[a,h]anthracene, fluoranthene, naphthalene, phenanthrene, and total PAHs. The 34 studies were conducted with soils from various PAH sources, including contaminated field soil (29 studies) and uncontaminated soil spiked with PAHs in laboratory (six studies). In the two studies that evaluated the effect of aging on bioaccessibility, Duan et al. (2014) showed a reduction of BaP bioaccessibility from 75% to 55% after 90 days of aging, and Minhas et al. (2006) showed no reduction in chrysene bioaccessibility after aging for 6 or 12 months.

A wide range of soil concentrations for total PAHs have been studied, from <1 mg/kg to 5,000 mg/kg in contaminated field soil, with one third of the studies conducted in the range of more environmentally relevant concentrations (< 10 mg/kg). As discussed in Section 5.1.2, it appears that studies conducted in the higher concentration range may tend to overestimate RBA for soils in the lower concentration range. A majority of the studies (26 out of 34) reported the soil particle size tested, and but some of them (eight studies) were much larger than the fine particle sizes (<150 µm to <250 µm) most likely to adhere to children's hands.

Among the 34 studies reviewed, 24 studies reported the TOC concentrations of the soils tested, but only a few of them characterized the types of organic carbon in the test soils limiting evaluation of RBA variation with PAH source materials and different forms of organic carbon on RBA values. Seven studies indicated that soil TOC is inversely related to bioaccessibility.

As described in Section 3.2, the *in vitro* methods developed to date for PAH bioaccessibility measurements have been categorized into five types by Cui et al. (2016), including PBET, SHIME, RIVM method, FOREhST, and IVG method. The advantages and disadvantages of each *in vitro* method are discussed in Section 3.2. Below is a summary of the studies using one of the five *in vitro* methods to assess PAH bioaccessibility:

- PBET: PBET - Khan et al. (2008), Li et al. (2015), Lu et al. (2010), and Tang et al. (2006); CE-PBET - Collins et al. (2013), Gouliarmou et al. (2013), and Tilston et al. (2011).
- SHIME: Cave et al. (2010), Siciliano et al. (2010), and van de Wiele et al. (2004).
- RIVM Method: Grøn et al. (2007), Pu et al. (2004), and Sips et al. (2001).
- FOREhST: Cave et al. (2010), Juhasz et al. (2014), and Lorenzi et al. (2012).
- IVG Method: James et al. (2011).

In recent years, emphasis has been placed on some modifications and improvements to overcome the limitations of traditional *in vitro* methods, including the use of a sorption sink to overcome solubility constraints associated with hydrophobic organic chemicals, and the use of epithelial Caco-2 cell lines to simulate sorption in the human gastrointestinal tract (Cui et al. 2016).

As discussed above, PAHs are characterized by low water solubility and high hydrophobicity, and they tend to partition into a lipophilic phase. Therefore, PAH bioaccessibility may be underestimated via *in vitro* methods without lipophilic phase. The presence of food or a lipid source is a critical factor in the absorption of PAHs. Among the 34 studies reviewed, 13 studies incorporated food components into their assays, and the food sources used in these studies were highly variable.

Addition of a lipid/sorption sink provides a desorption gradient, enhancing the desorption of PAHs from soil matrix, and mimicking the large surface area and sorptive potential of the human gastrointestinal tract (Collins et al. 2013, Gouliarmou and Mayer 2012, Gouliarmou et al. 2013, Hurdzan et al. 2008, James et al. 2011, Juhasz et al. 2016, Li et al. 2015, Vasiluk et al. 2007, Wang et al. 2011, Zhang et al. 2015a, b). These sorption sinks include C18 membrane, poly(dimethylsiloxane) (PDMS) rods, and Tenax. However, it is difficult to conclude which sorption sink is the most appropriate until several issues have been fully investigated, including the possible difference in sorption rate between sorption sink and intestinal cells, the combined effect of sorption sink and components in gastrointestinal solution, and validation with *in vivo* data.

Bile salts in the small intestine, in the presence of lipids and cholesterol or dietary lipids, may form mixed micelles into which the PAHs can partition (Guyton and Hall 1996, van Schooten et al. 1997). These mixed micelles likely enhance the absorption of PAHs in the intestinal epithelium (Hack and Selenka 1996, Holman et al. 2002, Oomen et al. 2000, 2004, Tao et al. 2011). Most of the 34 *in vitro* studies reviewed by Ruby et al. 2016 and Ramboll Environ have bile salts as one of the components in intestinal solution.

Caco-2 cells have been added to several *in vitro* assays to mimic the uptake process of PAHs across intestinal epithelial cells (Minhas et al. 2006, Vasiluk et al. 2007). Caco-2 cells are an enterocyte cell line with transport properties from a human colon adenocarcinoma (Artursson et al. 1996). Caco-2 cells can differentiate spontaneously in culture and produce a monolayer of epithelial cells, which share many morphological and functional characteristics of mature enterocytes in small intestine (Hidalgo et al. 1989). Given the fact that isolation and incubation of intestinal cells are still impractical, Caco-2 cells could be used as a good model to evaluate the transport of organic chemicals in intestinal phase. However, debate still exists about how Caco-2 cells would affect PAH bioaccessibility (Buesen et al. 2003, Tao et al. 2009, 2011, Vasiluk et al. 2007, Wang et al. 2011). The other drawback is that studies on toxic effect of PAHs to Caco-2 cells are rather limited (Lampen et al. 2004, Niestroy et al. 2011). PAH-induced toxicity may influence the transport, metabolites, and uptake process of PAHs by Caco-2 cells, consequently changing the bioaccessibility results.

Among 11 of the 34 studies reviewed, PAH bioaccessibility values were either not reported, or could not be calculated from the data presented in the publication. Among the other 23 studies, the reported or calculated bioaccessibility values for PAHs ranged from 0.1 percent to 76 percent in contaminated field soil, and from 2 percent to 89 percent for uncontaminated spiked soil. Most of the reported bioaccessibility values were <50%. Validation of an *in vitro* test against RBA results from an *in vivo* animal model (i.e., *in vitro* to *in vivo* correlation) has been attempted in 5 out of the 34 studies on the bioaccessibility of PAHs in soil (Duan et al. 2014, Grøn et al. 2007, James et al. 2011, Pu et al. 2004, Stroo et al. 2005). However, neither of these five studies produced a strong and robust *in vitro* to *in vivo* correlation based on *in vitro* and *in vivo* data of high quality (Ruby et al. 2016). To date, *in vitro* test results appear to underestimate bioavailability predicted by *in vivo* studies. This is possibly a consequence of the *in vitro* tests not being operated in the most conservative condition, i.e., not in fed state and not including a colon compartment or a sorption sink (Collins et al. 2015).

In summary, the 34 *in vitro* studies reviewed provide a basis for the conclusion that the bioavailability of PAHs from soil is reduced relative to absorption from diet, and that the default assumption of 100% RBA likely overestimates actual exposure to PAHs in soil. Due to the variability in study designs and wide range of soil PAH concentrations tested, it is difficult to compare results across studies and identify factors controlling PAH bioaccessibility. The validation of *in vitro* methods with *in vivo* data has so far been limited, but lack of a widely accepted *in vivo* method limits the viability of validation efforts.

5.1.5 Study Design Guidelines

In vitro bioaccessibility testing is a useful and practical approach to evaluating oral PAH bioavailability in soil because of its relative simplicity, sustainability, reduced costs, and the ability to develop a reproducible SOP. As indicated in Collins et al. (2015), selection of a specific *in vitro* test method should consider the good practices established by BARGE:

- It should be physiologically based, mimicking the human GI physicochemical environment in the stomach and small intestine;
- It should represent a conservative case;
- There should be one set of conditions for all potentially harmful elements being studied;
- It must be demonstrated that the test is a good analogue of *in vivo* conditions; and
- The test must be able to produce repeatable and reproducible results within and between testing laboratories.

Based on a critical review of the five *in vitro* approaches in the literature, the CE-PBET is recommended as the *in vitro* method to evaluate oral PAH bioavailability in soil, with consideration of the following aspects in study design:

- Digestive compartment: CE-PBET consists of three digestive compartments, including stomach, small intestine, and colon. The addition of water-based digestive compartment (i.e., saliva) is not needed and of limited importance since soil remains in this compartment for a relatively short period of time (less than five minutes) and PAHs have low water solubility. The addition of the colon compartment is recommended since it increases the potential bioaccessibility and represents a conservative condition.
- Operational Parameters and Components in Gastrointestinal Solution: The operation parameters and components in the solution for each of the three digestive compartments for CE-PBET are listed in Table 1 below:

Table 1: Operational Parameters and Components in Gastrointestinal Solution for CE-PBET

Phase	Composition	pH	Time (hour)	Soil/Liquid Ratio
Gastric	0.5 g/L sodium malate, 0.5 g/L tri-sodium citrate, 420 µL lactic acid, 500 µL glacial acetic acid, 1.25 g/L pepsin	2.5	1	1:100
Intestinal	0.5 g/L pancreatin, 1.78 g/L bile	6.5	4	1:100
Colon	4 g/L mucin, 4.5 g/L NaCl, 4.5 g/L KCl, 1.5 g/L $NaCHO_3$, 1.25 g/L $6H_2O{\cdot}Na_2SO_4$, 800 mg cysteine hydrochloride, 500 mg K_3PO_4, 0.19 g/L $CaCl_2$, 0.5 g/L K_2HPO_4, 50 mg haemin, 5.0 mg $7H_2O{\cdot}FeSO_4$, 0.4 g/L bile	7.0	8	1:100
Diet	5.0 g/L starch, 3.4 g/L peptone, 6.1 g/L tryptone, 4.5 g/L yeast extract, 3.0 g/L casein, 2.0 g/L pectin, 2.0 g/L xylan, 2.0 g/L arabinogalactan, 1.0 g/L guar gum, 1 g/L inulin	--	--	--

Abbreviations:
g/L: gram per liter
mg: milligram
µL: microliter
Source: Cui et al. (2016).

- Food and Sorption Sink: The CE-PBET should be operated in fed state and a sorption sink is required, both of which maximize desorption of PAHs from soil and represent a conservative condition.
- Microbial community: A microbial community is preferred to be included in the colon compartment since it takes into account the potential microbial degradation of PAHs.

Both parent compound and gut metabolites need to be quantified in order to accurately measure bioaccessibility.

- Test Soil: Site-specific bioavailability studies must use aged/weathered site soils that are representative of the site conditions. A soil particle size of <150 µm to <250 µm is recommended for the soil tested as it is considered as the fraction most likely to adhere to human hands and be incidentally ingested through hand-to-mouth contact. Although people may intentionally ingest soil, this is a relatively rare occurrence and testing bulk soil is not recommended.

For studies evaluating oral RBA for PAH mixtures, rather than focusing on an indicator chemical such as BaP, it will be necessary to develop a reference PAH mixture with the same components present in each soil sample.

Use of CE-PBET to evaluate oral PAH bioavailability in soil should include a detailed, scientifically-based rationale to support bioavailability adjustments. Ideally, the robustness of the selected test would be confirmed through inter-laboratory comparison. While validation with *in vivo* studies is desirable (Collins et al. 2015), the lack of a reliable *in vivo* method for PAHs means that validation is not currently feasible.

5.2 Polychlorinated Biphenyls

PCBs are a class of chemicals synthesized by catalyzed chlorination of biphenyl (European Food Safety Authority [EFSA] 2010). Depending on the number of chlorine atoms and their position, there are 209 different PCB congeners. Due to their physico-chemical properties, such as chemical stability, low heat conductivity, and high dielectric constants, PCBs were widely used in a number of industrial and commercial applications. Although the manufacture, processing and distribution of PCBs have been prohibited in almost all industrial countries since late 1980s, they still can be released into the environment through poorly maintained hazardous waste sites, leakage from electrical transformers, deterioration of paint and sealants in older buildings, improper disposal of consumer products, and waste burning in municipal and industrial incinerators. There are no known natural sources of PCBs in the environment (EFSA 2010).

The ubiquitous presence of PCBs in the environment causes concerns due to their persistence, potential bioaccumulation in animal and human tissues, and toxicity. The IARC has classified PCBs as human carcinogens (IARC 2016). Other toxic effects such as endocrine disruption, neurotoxicity, reproductive/developmental abnormalities, and immune dysfunction are also well known (EFSA 2010). Based on structural characteristics and toxicological effects, PCBs can be divided into two groups. One group consists of 12 congeners that adopt a coplanar structure and have the capability to bind to the Ah receptor, thus showing toxicological properties similar to dioxins. This group of PCBs is therefore called dioxin-like PCBs (DL-PCBs). The other group, non-dioxin-like PCBs (NDL-PCBs), refers to PCB congeners that do not share the same mode of toxic action as dioxins (EFSA 2010).

Oral TRVs for noncancer endpoints have been derived by USEPA, RIVM, ATSDR, and the International Programme for Chemical Safety (IPCS) for PCBs, and USEPA, ATSDR, IARC, IPCS, and RIVM have also developed TRVs for cancer endpoints (ITER 2016). The USEPA oral carcinogenic TRV for PCBs is based on a study in which female Sprague-Dawley rats were fed diet to which PCBs were added. PCBs are well absorbed when administered with food containing lipids. Thus, the bioavailability of PCBs in soil is expected to be reduced when compared to the bioavailability of PCBs in diet, resulting in an RBA less than 1.0.

5.2.1 Toxicokinetics

The oral absorption of PCBs from food has been shown to be high in both animals and humans. As discussed in IARC (2016), the absorption of PCBs from breast milk and food generally ranged from 60 percent to 100 percent in two human studies, with the exception that the absorption of PCB 202 was less than 52 percent (Dahl et al. 1995, Schlummer et al. 1998). These results suggest that the less chlorinated congeners were generally more completely absorbed compared to those with a higher degree of chlorination. Less chlorinated PCBs also exhibited greater absorption in rats than more chlorinated congeners (e.g., 95 percent for dichlorobiphenyls, but only 75 percent for octachlorobiphenyls, Tanabe et al. 1981).
Evaluation of PCB relative bioavailability is complicated by its variation with dietary lipid levels and human body burden levels. Schlummer et al. (1998) observed that lower absorption of PCBs occurred in older individuals who had higher dietary lipid levels and body burdens compared to younger individuals, and as the PCB levels in human tissue increase, absorption decreases due to the lower concentration gradient across the intestinal lumen.

Once absorbed, PCBs distribute preferentially to adipose tissue and concentrate in human breast milk due to its high fat content (IARC 2016). Liver is the major organ of metabolism, and PCB metabolism depends on the degree and the position of the chlorine atoms of individual congeners. In general, congeners with more than four chlorine substituents are more slowly metabolized than those with four or fewer chlorines (IARC 2016). Therefore, highly chlorinated congeners persist in the human body with half-lives of approximately 8–15 years, while less chlorinated congeners have shorter half-lives and are known to be readily cleared from the body (Grandjean et al. 2008, Ritter et al. 2011, Thomas et al. 1999). PCBs are primarily excreted after they have been conjugated and transformed into more polar and water-soluble metabolites. The major routes of excretion of PCBs are fecal and urinary (IARC 2016). The metabolites may also be excreted in bile back into the intestines.

Determination of PCB oral bioavailability in animal studies is challenging due to the complexity of toxicokinetics within the human body. PCB bioavailability is typically calculated by measuring the amount of chemical (and its metabolites) in blood, adipose tissue, or feces. Since PCBs distribute preferentially to adipose tissue after absorption, adipose tissue is the primary measurement endpoint among the *in vivo* studies. However, all these approaches have certain limitations, similar to those encountered with testing PAH bioavailability.

Adipose Tissue

After absorption, PCBs that may enter the systemic circulation are readily distributed preferentially to adipose tissues. Use of adipose tissue as an endpoint for evaluating PCB relative bioavailability is based on the assumption that with repeated doses, and once a steady state has been approximated between blood and adipose tissues, the concentration of a PCB or metabolite in adipose tissue will be proportional to the absorbed dose.

Blood

Due to their lipophilicity, PCBs are absorbed from the gut primarily via the lymphatic system, and are less likely than PAHs to exhibit liver first pass metabolism. This makes blood a more reliable endpoint for assessing PCB oral absorption. Similar to PAHs, metabolized PCBs may enter the systemic circulation, and not be quantified as bioavailable if only the parent compound is measured.

Feces

PCBs in feces have been used as an endpoint for the upper-bound estimation of bioavailability, subtracting PCB excreted in feces from the administered dose to estimate absorbed dose. However, fecal contents reflect both the fraction absorbed and excreted in bile, as well as unabsorbed PCBs. Due to greater tissue retention, this is less of a problem for PCBs than for PAHs.

5.2.2 Behavior in Soil

PCBs are generally characterized by low water solubility, high lipophilicity, and high persistence. Different PCB congeners exhibit a wide range of lipophilic and stability properties, as well as affinity for organic carbon, which make PCBs a challenging group to study for bioavailability. The fate of different PCB congeners in the human digestive system is related to their chemical and physical properties (i.e., degree of chlorination) as well as interaction and partition with soil constituents.

PCBs are expected to behave similarly to PAHs, with only the water-dissolved and rapidly desorbing fractions of PCBs being bioavailable, while the slowly desorbing and nonextractable fraction, especially strong absorbed to black carbon domains, may limit the release of PCBs in the gastrointestinal tract. The characteristics of soil organic matter is thought to be one of the most important factors that determine the strength of interactions between soil and PCBs, and further the PCB bioavailability (Cornelissen et al. 2005, Pignatello 1998, Pignatello and Xing 1996). Delannoy et al. published a series of papers in 2014-2015 to evaluate the effects of soil organic matter on the relative bioavailability of NDL-PCBs (Delannoy et al. 2014a, b, 2015). They concluded that the more condensed organic matter (i.e., activated carbon added to the artificial soil or black carbon in the field soil) strongly reduces PCB bioavailability, while the less condensed organic matter (i.e., fulvic acid or humic acid) does not seem to have a significant effect. Soil clay content and pH is shown to have a rather limited impact on PCB bioavailability due to the apolarity of NDL-PCBs (Delannoy et al. 2015).

As discussed for PAHs, PCB concentrations in soil may also affect oral bioavailability due to the absorption to different organic carbon forms (i.e., NOM versus black carbon). Studies of oral bioavailability conducted at elevated PCB concentrations (hundreds of mg/kg) may overestimate oral bioavailability compared to the range of concentrations more likely to be considered in remediation decisions (i.e., less than 25 to 50 mg/kg). The Danish EPA does not publish a soil quality criterion for PCBs. The USEPA Regional Screening Level (RSL) for total PCBs is 0.23 mg/kg for residential soil and 0.94 mg/kg for industrial soil (USEPA 2016), but actual site cleanup levels are often higher. PCB bioavailability studies are sometimes conducted at concentrations much higher than this range (up to 300 mg/kg), and application of the RBA values derived from these studies to risk assessment may overestimate the risk.

As discussed in Section 2.2.3, it is also important to consider the effects of aging or weathering on PCB soil interactions, which are likely to reduce the oral bioavailability of PCBs in soil. Thus, studies conducted with soils spiked with PCBs in the laboratory may produce higher oral bioavailability measurements than studies conducted with weathered soils in the natural environment.

5.2.3 *In Vivo* Studies and Methods

A literature search for *in vivo* studies of PCB bioavailability in soil yielded only nine papers, using a variety of animal models (including rats, juvenile swine, hens, and goats). With a relative similarity of physiology, growth, and absorptive mechanisms to humans, juvenile swine model is frequently used to evaluate the bioavailability of PCBs through soil ingestion (Delannoy et al. 2014a, b, 2015). The goat or hen model could be used to evaluate the bioavailability of PCBs through indirect exposure, i.e. ingestion of animal products (e.g., milk or egg) (Feidt et al. 2013, Fournier et al. 2012). Some of the key experimental parameters used in these *in vivo* oral bioavailability studies are discussed below.

The six NDL-PCBs, including PCB 28, 52, 101, 138, 153, and 180, which constitute the most abundant PCB congeners found in environmental matrices like soil (EFSA 2010, Meijer et al. 2003) have been most frequently evaluated. The sum of the six NDL-PCBs is often referred to as indicator PCBs, because they are easily quantified compared to other NDL-PCBs and represent all relevant degrees of chlorination. The EFSA Scientific Panel on Contaminants in the

Food Chain considers the indicator PCBs as most suitable for a risk assessment of NDL-PCBs on the basis of available data (EFSA 2010). Some DL-PCBs, such as PCB 77, 105, 118, 126, and 169, were also evaluated in the *in vivo* studies.

As indicated in Section 5.2.2, studies conducted with soil spiked with PCBs in the laboratory may produce higher oral bioavailability measurements than studies conducted with weathered soil in the natural environment. Four out of the nine studies were conducted with uncontaminated soil spiked with PCBs in the laboratory and aged for 24 hours to six months (Delannoy et al. 2014a, b, Fries and Marrow 1992, Pu et al. 2006). One study was conducted with uncontaminated soil spiked with PCBs in the laboratory and aged for eight years through a freezing process (Fries et al. 1989); however, the freezing process (halting chemical reactions) is not considered comparable to aging/weathering which involves chemical transformations. Fries and Marrow (1992) showed no reduction in bioavailability after aging for five days or six months. Four out of the nine studies were conducted with contaminated field soil from industrial sources (Delannoy et al. 2015, Fouchecourt et al. 1998) or in the vicinity of a former fire (Feidt et al. 2013, Fournier et al. 2012).

A wide range of soil PCB concentrations have been studied, from < 0.1 mg/kg to 300 mg/kg, with only three studies (Feidt et al. 2013, Fournier et al. 2012, Fries and Marrow 1992) in the range of more environmentally relevant concentrations (i.e., <10 mg/kg). Five of the nine studies reported the soil particle size tested, but only two of them used the particle size of <125 µm to <250 µm (Fries and Marrow 1992, Fries et al. 1989). The particle sizes used in other studies ranged from <500 µm to <2,000 µm.

Eight of the nine studies reported the TOC concentrations of the soils tested, and four of them characterized the types of organic matter in the test soils (Delannoy et al. 2014a, b, Delannoy et al. 2015, Feidt et al. 2013). It was found that the more condensed organic matter (i.e., activated carbon added to the artificial soil or black carbon in the field soil) strongly reduces PCB bioavailability, while the less condensed organic matter (i.e., fulvic acid or humic acid) does not seem to have a significant effect.

Among the nine studies, the reported or calculated RBA values for PCBs ranged from 36 percent to approximately 100 percent in field contaminated soil, and from 3 percent to approximately 100 percent in uncontaminated spiked soil. As described above, in toxicity studies used to assess PCB dose response and derive oral TRVs, PCBs were administered through diet. Therefore, absorption of PCBs from soil relative to absorption from diet is the appropriate metric for determining RBA for use in HHRA. In eight out of the nine studies reviewed, absorption from the diet or oil was selected as the basis for calculating the RBA values.

In summary, of the nine *in vivo* studies reviewed, only the four studies conducted with field contaminated field soil provide a general but limited basis for the conclusion that the bioavailability of PCBs from soil is reduced relative to absorption from diet, and that the default assumption of 100% RBA likely overestimates actual exposure to PCBs in soil. However, variability is expected in the behavior of different PCB congeners, and the available data is not sufficient to make broad generalizations. The impact of varying concentrations and site conditions on soil PCB oral bioavailability requires more investigation.

5.2.4 *In Vitro* Studies and Methods

In vitro studies used to assess PCB bioaccessibility in soil are limited, with only three papers (Hack and Selenka 1996, Oomen et al. 2000, Pu et al. 2006) identified in a literature search. Although these studies provide a limited basis to evaluate *in vitro* approaches to measure PCB bioaccessibility in soil, many of the findings for PAHs are applicable to testing PCBs. Some of the key experimental parameters used in these *in vitro* oral bioaccessibility studies are discussed below.

The three *in vitro* studies reviewed evaluated oral bioaccessibility of some or all of the six NDL-PCBs most abundant in soil, including PCB 28, 52, 101, 138, 153, and 180. Studies by Oomen et al. (2000) and Pu et al. (2006) were conducted with uncontaminated soil spiked with PCBs in laboratory and aged for two weeks and 24 hours, respectively. Hack and Selenka (1996) was conducted with 18 contaminated field samples of soil, sewage sludge, asphalt, metal scrap, and blast sand residue.

The soil PCB concentrations tested in the three *in vitro* studies were < 1 mg/kg, 7 and 14 mg/kg, and 300 mg/kg, respectively. None of the three studies reported the soil particle size tested. Among the three studies, only Pu et al. (2006) reported the TOC concentrations of the soils tested, and results indicated that soil TOC is inversely related to bioaccessibility. However, none of the three studies characterized the types of organic carbon in the test soils.

Hack and Selenka (1996) used a modified PBET to evaluate PCB bioaccessibility in soil. The modified PBET involved two phases, i.e., gastric and intestinal phases. The pH of gastric solution was 2, which was followed by neutral intestinal solution (pH = 7) with the presence of bile, pancreatin, and trypsin. The incubation time of gastric and intestinal phase was two and six hours. It was found that bioaccessibility of PCBs in soil was enhanced when whole milk powder was added to the system. The authors concluded that this is probably due to the formation of mixed micelles which are known to readily solubilize organic chemicals and facilitate their absorption.

Oomen et al. (2000) used a modified RIVM method to evaluate PCB bioaccessibility in soil. The modified RIVM method involved three phases, i.e., saliva, gastric, intestinal phases. The pH of gastric solution was 1, which was followed by neutral intestinal solution (pH = 8) with the presence of bile, pancreatin, and other proteins. No food or sorption sink was added to the system. The incubation time of gastric and intestinal phase was both two hours. It was found that bioaccessibility of PCBs in soil increased as more bile or protein was added to the system. A follow-up study by Oomen et al. (2001) examined transport of the mobilized PCBs across the intestinal wall with addition of intestinal epithelial Caco-2 cells to simulate the human intestinal environment. High absorption efficiencies and accumulation into the epithelial cells was observed.

Pu et al. (2006) also used a modified RIVM method to evaluate PCB bioaccessibility in soil. The modified RIVM method involved three phases, i.e., saliva, gastric, intestinal phases. The pH of gastric solution was 3, which was followed by neutral intestinal solution (pH = 7) with the presence of bile, pancreatin, and other proteins. No food or sorption sink was added to the system. The incubation time of gastric and intestinal phase was both two hours. Pu et al. (2006) has attempted to validate their *in vitro* test against RBA results from an *in vivo* rat model, but no significant correlation was observed. The *in vitro* test underestimated PCBs released from a soil in the *in vivo* assay, which is possibly a consequence of the *in vitro* test not including food or a sorption sink (Collins et al. 2015).

Among the three studies reviewed, the reported bioaccessibility values for PCBs ranged from 6 percent to 40 percent in field contaminated soil without the addition of milk powder, from 43 percent to 85 percent in field contaminated soil with the addition of milk powder, and from 30 percent to 79 percent in uncontaminated spiked soil.

In summary, the three *in vitro* studies provide an inadequate basis to determine if the bioavailability of PCBs from soil is reduced relative to absorption from diet. A wide range of bioaccessibility values were reported, and none of the studies incorporated a lipid sink into their analyses which may underestimate the PCB bioaccessibility.

5.2.5 Study Design Guidelines

Methods for assessing the relative bioavailability of PCBs in soil are not yet well established. Both in vivo and in vitro studies conducted to date have significant design limitations. Only three *in vitro* studies were conducted in the literature to evaluate oral PCB bioavailability in soil. Based on such limited data, a single standard *in vitro* method for PCB bioaccessibility measurement cannot be recommended. More research is needed to fill in the data gaps, and approaches being used for PAHs may provide a useful template. The following aspects in study design should be considered:

- Digestive compartment: The *in vitro* test should consist of at least two digestive compartments, i.e., stomach and small intestine. The addition of water-based digestive compartment (i.e., saliva) is not needed and of limited importance since soil remains in this compartment for a relatively short period of time (less than five minutes) and PCBs have low water solubility. More research is needed to understand if the addition of a colon compartment (i.e. CE-PBET) is relevant for PCBs. Since PCBs are absorbed via uptake into the lymphatic system, absorption in the colon may not be as important as it may be for PAHs.
- Operational Parameters and Components in Gastrointestinal Solution: The operational parameters and components in the solution for each digestive compartment should be standardized. The parameters and components listed in Table 1 for measurement of PAH bioaccessibility using CE-PBET could be considered.
- Food and Sorption Sink: The *in vitro* test should be operated in fed state and a sorption sink may be required, both of which maximize desorption of PCBs from soil and represent a conservative condition.
- Test Soil: Site-specific bioavailability studies should include soil samples that are representative of the range of concentrations being considered for remediation. A soil particle size of <150 µm to <250 µm is preferred for the soil tested as it is considered as the fraction most likely to adhere to human hands and be incidentally ingested through hand-to-mouth contact.

For PCB mixtures, due to variations in behavior among different congeners, the use of a representative reference mixture is important when studying the relative bioavailability of PCBs. Use of an *in vitro* test to evaluate oral PCB bioavailability in soil should include a detailed, scientifically-based rationale to support bioavailability adjustments.

Appendix 1. References

Accardi-Dey A, Gschwend PM. 2002. Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. Environ. Sci. Technol. 36 (1):21-29.

Alexander M. 1999. Biodegradation and bioremediation. Second edition. Academic Press, San Diego, CA.

Alexander M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ. Sci. Tech. 34(20):4259-4265.

Artursson P, Karlsson J, Ocklind G, Schipper N. 1996. Studying transport processes in absorptive epithelia. In: Shaw, A.J. (Ed.), Epithelial Cell Culture: a Practical Approach. IRL Press at Oxford University, Oxford, UK, pp. 111-133.

Basta N, Foster J, Dayton E, Rodriguez R, and Casteel S. 2007. The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils. J of Environ. Sci. Health Part 42:1275-1281.

Bioaccessibility Research Canada (BARC). 2011. Round Robin Experiment: Variability of Bioaccessibility Results Using Seventeen Different Methods on a Standard Reference Material (NIST 2710). Prepared by Environmental Science Group, Royal Military College of Canada.
Bordelon NR, Donnelly KC, King LC, Wolf DC, Reeves WR, George SE. 2000. Bioavailability of the genotoxic components in coal tar contaminated soils in Fischer 344 rats. Toxicol. Sci. 56:37-48.

Bradham KD, Nelson C, Juhasz AL, Smith E, Scheckel K, Obenour DR, Miller BW, Thomas DJ. 2015. Independent Data Validation of an in Vitro Method for the Prediction of the Relative Bioavailability of Arsenic in Contaminated Soils. Environ. Sci. Technol. 49:6312−6318.

Bradham KD, Scheckel KG, Nelson CM, Seales PE, Lee GE, Hughes MF, Miller BW, Yeow A, Gilmore T, Harper S, Thomas DJ. 2011. Relative bioavailability and bioaccessibility and speciation of arsenic in contaminated soils. Environ. Health Perspect. 119(11):1629-1634.

Brattin W, Drexler J, Lowney Y, Griffin S, Diamond G, Woodburry L. 2013. An in vitro method for estimation of arsenic relative bioavailability in soil. J. Toxicol. Environ. Health, Part A. 76:458–478.

Broadway A, Cave MR, Wragg J, Fordyce FM, Bewley RF, Graham MC, Ngwenya BT, Farmer JG. 2010. Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment. Sci. Tot. Environ. 409:267–277.

Buesen R, Mock M, Nau H, Seidel A, Jacob J, Lampen A. 2003. Human intestinal Caco-2 cells display active transport of benzo[a]pyrene metabolites. Chem. Biol. Interact. 142:201-221.

Casteel SW, Cowart RP, Weis CP, et al. 1997. Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL site of Aspen, Colorado. Fund. Appl. Toxicol. 36:177–187.

Casteel SW, Weis CP, Henningsen GM, and Brattin WJ. 2006. Estimation of relative bioavailability of lead in soil and soil-like materials using young swine. Environ Health Perspect

114:1162-1171.

Cave MR, Wragg J, Harrison I, Vane CH, Van de Wiele T, De Groeve E, Nathanail CP, Ashmore M, Thomas R, Robinson J, Daly P. 2010. Comparison of batch mode and dynamic physiologically based bioaccessibility tests for PAHs in soil samples. Environ. Sci. Technol. 44:2654-2660.

Chiou CT. 2002. Partition and Adsorption of Organic Contaminants in Environmental Systems: Hoboken, NJ, John Wiley & Sons.

Chiou CT, and Kile DE. 2000. Contaminant sorption by soil and bed sediment--Is there a difference?: U.S. Geological Survey Fact Sheet 087-00. http://toxics.usgs.gov/pubs/FS-087-00/.

Chung N, and Alexander M. 2002. Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil. Chemosphere 48(1):109-115.

Collins CD, Craggs M, Garcia-Alcega S, Kademoglou K, Lowe S. 2015. Towards a unified approach for the determination of the bioaccessibility of organic pollutants. Environment International 78:24-31.

Collins CD, Mosquera-Vazquez M, Gomez-Eyles JL, Mayer P, Gouliarmou V, Blum F. 2013. Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils? Environ. Pollut. 181:128-132.

Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Koelmans AA, Van Noort PCM. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ. Sci. Technol. 39:6881-6895.

Cornelissen G, Rigterink H, Ferdinandy MMA, Van Noort PCM. 1998. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environ. Sci. Technol. 32:966- 970.

Cui X, Mayer P, and Gan J. 2013. Methods to assess bioavailability of hydrophobic organic contaminants: Principles, operations, and limitations. Environ. Pollut. 172:223-234.

Cui X, Xiang P, He R, Juhasz A, Ma L. 2016. Advances in in vitro methods to evaluate oral bioaccessibility of PAHs and PBDEs in environmental matrices. Chemosphere 150:378-389.

Dahl P, Lindström G, Wiberg K, Rappe C. 1995. Absorption of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans by breast-fed infants. Chemosphere 30(12):2297–306.

Datta R, and D Sarkar. 2005. Consideration of soil properties in assessment of human health risk from exposure to arsenic-enriched soils. Integr. Environ. Assess. Manage. 1(1):55-59.

Delannoy M, Rychen G, Fournier A, Jondreville C, Feidt C. 2014a. Effects of condensed organic matter on PCBs bioavailability in juvenile swine, an animal model for young children. Chemosphere 104: 105–112.

Delannoy M, Schwarz J, Fournier A, Rychen G, Feidt C, 2014b. Effects of standard humic materials on relative bioavailability of NDL-PCBs in Juvenile Swine. PLoS ONE 9, e115759.

Delannoy M, Fournier A, Dan-Badjo AT, Schwarz J, Lerch S, Rychen G, Feidt C. 2015. Impact of soil characteristics on relative bioavailability of NDL-PCBs in piglets. Chemosphere 139: 393–401.

Denys S, Caboche J, Tack K, Rychen G, Wragg J, Cave M, Jondreville C, and Feidt C. 2012. In Vivo Validation of the Unified BARGE Method to Assess the Bioaccessibility of Arsenic, Antimony, Cadmium, and Lead in Soils. Environ. Sci. Technol., 46 (11), pp 6252–6260.

Danish Environmental Protection Agency (DEPA). 2004. Human biopløselighed af jordforureninger. 1-6-2004. Miljøstyrelsen. (by Grøn, C., Asmussen, O.W. and Samsø-Petersen).

Danish Environmental Protection Agency (DEPA). 2005. Test for bioaccessibility of metals and PAH from soil. Test selection, validation, and application. Prepared for Danish Environmental Protection Agency. July. (by Grøn, C. and Asmussen, O.W).

Diamond GL, Bradham KD, Brattin WJ, Burgess M, Griffin S, Hawkins CA, Juhasz AL, Klotzbach JM, Nelson C, Lowney YW Scheckel KG, Thomas DJ. 2016. Predicting oral relative bioavailability of arsenic in soil from in vitro bioaccessibility, J. Toxicol. Environ. Health, Part A, 79:4, 165-173.

Drexler JW, Brattin WJ. 2007. An in vitro procedure for estimation of lead relative bioavailability: With validation. Human Ecol. Risk Assess. 13:383-401.

Duan LC, Palanisami T, Liu YJ, Dong ZM, Mallavarapu M, Kuchel T, Semple KT, Naidu R 2014. Effects of ageing and soil properties on the oral bioavailability of benzo[a]pyrene using a swine model. Environ. Int. 70, 192-202.

ENVIRON. 2011. Guidance on Consideration of Oral Bioavailability of Chemicals in Soil for Use in Human Health Risk Assessment. July.

European Food Safety Authority (EFSA). 2010. Results of the monitoring of non-dioxin-like PCBs in food and feed. EFSA J 8: 1701–1736.doi:10.2903/j.efsa.2010.1701.

Farmer JG, Broadway A, Cave MR, Wragg J, Fordyce FM, Graham MC, et al. 2011. A lead isotopic study of the human bioaccessibility of lead in urban soils from Glasgow, Scotland. Sci. Tot. Environ. 409: 4958-4965.

Feidt C, Ounnas F, Julien-David D, Jurjanz S, Toussaint H, Jondreville C, Rychen G, 2013. Relative bioavailability of soil-bound polychlorinated biphenyls in lactating goats. J. Dairy Sci. 96, 3916–3923.

Fouchecourt MO, Berny P, and Riviere JL. 1998. Bioavailability of PCBs to male laboratory rats maintained on litters of contaminated soils: PCB burden and induction of alkoxyresorufin O-dealkylase activities in liver and lung. Arch. Environ. Contam. Toxicol. 35(4):680-687.

Fournier A, Feidt C, Travel A, Bizec BL, Venisseau A, et al. 2012. Relative bioavailability to laying hens of indicator polychlorobiphenyls present in soil. Chemosphere 88: 300–306.

Freeman GB, Schoof RA, Ruby MV, Davis AO, Dill JA, Liao SC, Lapin CA, Bergstrom PD. 1995. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in Cynomolgus monkeys. Fund. Appl. Toxicol. 28:215–222.

Fries GF, Marrow GS. 1992. Influence of soil properties on the uptake of hexachlorobiphenyls by rats. Chemosphere 24, 109–113.

Fries GF, Marrow GS, Somich CJ. 1989. Oral bioavailability of aged polychlorinated biphenyl residues contained in soil. Bull. Environ. Contam. Toxicol. 43, 683–690.

Ghosh U, Zimmerman JR, Luthy RG. 2003. PCB, PAH speciation among particle types in contaminated harbor sediments and effects on PAH bioavailability. Environ. Sci. Technol. 37 (10), 2209- 2217.

Goon D, Hatown NS, Klan MJ, Jerniganon JD, Farmer RG. 1991. Oral bioavailability of aged soil-absorbed benzo[a]pyrene (BaP) in rats. Toxicology 11:1356.

Gouliarmou V, Collins C, Christiansen E, Mayer P. 2013. Sorptive physiologically based extraction of contaminated solid matrices: incorporating silicone rod as absorption sink for hydrophobic organic contaminants. Environ. Sci. Technol. 47, 941-948.

Gouliarmou V, Mayer P. 2012. Sorptive bioaccessibility extraction (SBE) of soils: combining a mobilization medium with an absorption sink. Environ. Sci. Technol. 46, 10682-10689.

Grandjean P, Budtz-Jørgensen E, Barr DB, Needham LL, Weihe P, Heinzow B. 2008. Elimination half-lives of polychlorinated biphenyl congeners in children. Environ. Sci Technol. 42(18):6991–6.

Grøn C, Oomen A, Weyand E, Wittsiepe J. 2007. Bioaccessibility of PAH from Danish soils. Journal of Environmental Science and Health Part A 42:1233-1239.

Guyton AC, Hall JE. 1996. Textbook of Medical Physiology, 9th ed.; W.B. Saunders Company: Philadelphia, PA.

Hack A, Selenka F. 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicol Lett. 88(1-3):199-210.

Hagens W, Lijzen J, Sips A, Oomen A. 2008. The bioaccessibility and relative bioavailability of lead from soils for fasted and fed conditions. Derivation of the “average physiological state” correction factor. RIVM Letter Report 711701080/2008, SIR Advisory Report No. 11190. The Netherlands National Institute of Public Health and the Environment (RIVM), Bilthoven.

Hagens WI, Walraven N, Minekus M, Havenaar R, Lijzen JPA, Oomen AG. 2009. Relative oral bioavailability of lead from Dutch made grounds. National Institute for Public Health and the Environment, Report number 711701086.

Health Canada. 2010. Federal Contaminated Site Risk Assessment in Canada, Part V: Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRA).

Health Canada. 2012. Federal Contaminated Site Risk Assessment in Canada, Part I: Guidance on Human Health Preliminary Quantitative Risk Assessment (PQRA), Version 2.0.

Hecht SS, Grabowski W, Groth K. 1979. Analysis of feces for benzo(a)pyrene after consumption of charcoal-broiled beef by rats and humans. Cosmet. Toxicol. 17:223-227.

Hidalgo IJ, Raub TJ, Borchardt RT. 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96, 736-749.

Holman H, Goth-Goldstein R, Aston D, Yun M, Kengsoontra J. 2002. Evaluation of gastrointestinal solubilization of petroleum hydrocarbon residues in soil using an in vitro physiological

based model. Environ. Sci. Technol. 36:1281-1286.

Hong L, Ghosh U, Mahajan T, Zare RN, Luthy RG. 2003. PAH sorption mechanism and partitioning behavior in lampblack-impacted soils from former oil-gas plant sites. Environ. Sci. Technol. 2003, 37 (16), 3625-3634.

International Agency for Research on Cancer. (IARC). 2010. Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 92, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures.

IARC. 2016. Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 107, Polychlorinated Biphenyls and Polybrominated Biphenyls. February.

International Toxicity Estimates for Risk (ITER). 2016. Database Accessed August 2016. https://iter.ctc.com/publicURL/pub_search_list.cfm.

James K, Peters RE, Laird BD, Ma WK, Wickstrom M, Stephenson GL, Siciliano SD. 2011. Human exposure assessment: a case study of 8 PAH contaminated soils using in vitro digestors and the juvenile swine model. Environ. Sci. Technol. 45, 4586-4593.

Jonker MTO, Hawthorne SB, Koelmans AA. 2005. Extremely slowly desorbing polycyclic aromatic hydrocarbons from soot and soot-like materials: Evidence by supercritical fluid extraction. Environ. Sci. Technol., 39 (20), 7889- 7895.

Jonker, MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: mechanistic considerations. Environ. Sci. Technol. 36, 3725-3734.

Juhasz AL, Naidu R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo(a)pyrene. Int. Biodeterior. Biodegr. 45:57-88.

Juhasz A, Smith E, Weber J, Rees M, Rofe A, Kuchel T, Sansom L, Naidu R. 2007a. Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. .Chemosphere 69:961-966.

Juhasz AL, Tang W, Smith E. 2016. Using in vitro bioaccessibility to refine estimates of human exposure to PAHs via incidental soil ingestion. Environ. Res. 145:145-153.

Juhasz AL, Weber J, Stevenson G, Slee D, Gancarz D, Rofe A, Smith E. 2014. In vivo measurement, in vitro estimation and fugacity prediction of PAH bioavailability in post-remediated creosote-contaminated soil. Sci. Total. Environ. 473-474:147-154.

Kelley ME, Brauning SE, Schoof RA, et al. 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, OH. www.battelle.org/bookstore.

Khan S, Cao, Q, Lin AJ, Zhu YG. 2008. Concentrations and bioaccessibility of polycyclic aromatic hydrocarbons in wastewater irrigated soil using in vitro gastrointestinal test. Environ. Sci. Pollut. Res. 15:344-353.

Kissel JC, Richter KY, Fenske RA. 1996. Factors affecting soil adherence to skin in hand-press trials. Bull Environ Contam Toxicol 56:722-728.

Kwon S. Pignatello JJ. 2005. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): Pseudo pore blockage by model lipid components and its implications for N2-probed surface properties of natural sorbents. Environ. Sci. Technol. 39 (20):7932-7939.

Lampen A, Ebert B, Stumkat L, Jacob J, Seidel A. 2004. Induction of gene expression of xenobiotic metabolism enzymes and ABC-transport proteins by PAH and a reconstituted PAH mixture in human Caco-2 cells. Biochim. Biophys. Acta 1681:38-46.

Li C, Cui XY, Fan YY, Teng Y, Nan ZR, Ma LQ. 2015b. Tenax as sorption sink for in vitro bioaccessibility measurement of polycyclic aromatic hydrocarbons in soils. Environ. Pollut. 196:47-52.

Lorenzi D, Entwistle J, Cave M, Wragg J, Dean JR. 2012. The application of an in vitro gastrointestinal extraction to assess the oral bioaccessibility of polycyclic aromatic hydrocarbons in soils from a former industrial site. Anal. Chim. Acta 735:54-61.

Lu M, Yuan DX, Lin QM, Ouyang T. 2010. Assessment of the bioaccessibility of polycyclic aromatic hydrocarbons in top soils from different urban functional areas using an in vitro gastrointestinal test. Environ. Monit. Assess. 166:29-39.

Magee B, Anderson P, Burmaster D. 1996. Absorption adjustment factor (AAF) distributions for polycyclic aromatic hydrocarbons (PAHs). Hum. Ecol. Risk Assess. 2(4):841-873.

Makris KC, Quazi S, Nagar R, Sarkar D, Datta R, Sylvia VL. 2008. In vitro model improves the prediction of soil arsenic bioavailability: Worst-case scenario. Environ. Sci. Technol. 42(16):6278-6284.

Meijer SN, Ockenden WA, Sweetman A, Breivik K, Grimalt JO, et al. 2003. Global distribution and budget of PCBs and HCB in background surface soils: Implications for sources and environmental processes. Environ Sci Technol 37: 667–672.

Minhas JK, Vasiluk L, Pinto LJ, Gobas FAPC, Moore MM. 2006. Mobilization of chrysene from soil in a model digestive system. Environ. Toxicol. Chem. 25 (7):1729- 1737.

Molly K, Woestyne MV, Verstraete W. 1993. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl. Microb. Biotechnol. 39:254-258.

National Environment Protection Council (NEPC). 2013a. Schedule B4, Guideline on Site-Specific Health Risk Assessment Methodology. National Environment Protection Measure, Assessment of Site Contamination.

NEPC. 2013b. Schedule B7, Appendix A1, The Derivation of HILs for Metals and Inorganics. National Environment Protection Measure, Assessment of Site Contamination.

National Research Council (NRC). 2003. Bioavailability of contaminants in soils and sediments, processes, tools, and applications. The National Academies Press, Washington, DC. 420 pp.

Ng J, Juhasz A, Smith E, Naidu R. 2009. Contaminant bioavailability and bioaccessibility. Part 2: Guidance for industry. CRC CARE Technical Report No. 14, CRC for Contamination Assessment and Remediation of the Environment, Adelaide, Australia.

Ng J, Juhasz A, Smith E, Naidu R. 2010. Contaminant bioavailability and bioaccessibility. Part 1: A scientific and technical review. CRC CARE Technical Report No. 14, CRC for Contamina-

tion Assessment and Remediation of the Environment, Adelaide, Australia.

Niestroy J, Barbara A, Herbst K, Rode S, van Liempt M, Roos PH. 2011. Single and concerted effects of benzo[a]pyrene and flavonoids on the AhR and Nrf2-pathway in the human colon carcinoma cell line Caco-2. Toxicol. Vitro 25:671-683.

Oomen AG, Rompelberg CJM, Van de Kamp E, Pereboom DPKH, De Zwart LL, Sips AJAM. 2004. Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. Arch. Environ. Contam. Toxicol. 46:183–188.

Oomen AG, Sips AJAM, Groten JP, Sijm DTHM, and Tolls J. 2000. Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. Environ. Sci. Technol. 34:297-303.

Oomen AG, Tolls J, Kruidenier M, Bosgra SSD, Sips AJAM, Groten JP. 2001. Availability of polychlorinated biphenyls (PCBs) and lindane for uptake by intestinal Caco-2 Cells. Environ. Health Perspect. 109:731-737.

Ortega-Calvo J, Harmsen J, Parsons JR, Semple KT, Aitken MD. 2015. From Bioavailability Science to Regulation of Organic Chemicals. Environ. Sci. Technol. 49:10255−10264.

Pelfrêne A, Christophe Waterlot, Muriel Mazzuca, Catherine Nisse, Géraldine Bidar, Francis Douay. 2011. Assessing Cd, Pb, Zn human bioaccessibility in smelter-contaminated agricultural topsoils (northern France). Environ. Geochem. Health 33:477-493.

Peter RE, James K, Cave M, Wickstrom M, Siciliano SD. 2016. Is received dose from ingested soil independent of soil PAH concentrations: animal model results. Environ Toxicol Chem. Jan 27. doi: 10.1002/etc.3384. [Epub ahead of print]

Pignatello JJ. 1998. Soil organic matter as a nanoporous sorbent of organic pollutants. Adv Colloid Interface Sci 76–77:445–467.

Pignatello JJ, Xing BS. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environ Sci Technol. 30:1–11.

Pu X, Lee LS, Galinsky RE, Carlson GP. 2004. Evaluation of a rat model versus a physiologically based extraction test for assessing phenanthrene bioavailability from soils. Toxicol Sci. 79(1):10-17.

Pu X, Lee LS, Galinsky RE, Carlson GP, 2006. Bioavailability of 2,3',4,4',5-pentachlorobiphenyl (PCB118) and 2,20,5,50-tetrachlorobiphenyl (PCB52) from soils using a rat model and a physiologically based extraction test. Toxicology 217:14–21.

Ramesh A, Inyang F, Hood DB, Archibong AE, Knuckles ME, Nyanda AM. 2001. Metabolism, bioavailability, and toxicokinetics of benzo[a]pyrene [B(a)P] in F-344 rats following oral administration. Exp. Toxic. Pathol. 53:253-270.

Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH. 2004. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Intl. J. of Tox. 23:301-333.

Reeves WR, Mcdonald TJ, Bordelon NR, George SE, Donnelly KC. 2001. Impacts of aging on in vivo and in vitro measurements of soil bound polycyclic aromatic hydrocarbon availability. Environ. Sci. Technol. 35:1637-1643.

Ritter R, Scheringer M, MacLeod M, Moeckel C, Jones KC, Hungerbühler K. 2011. Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. Environ. Health Perspect. 119(2):225–31.

Roberts SM, Weimar WR, Vinson JRT, Munson JW, Bergeron RJ. 2002. Measurement of arsenic bioavailability in soil using a primate model. Toxicol. Sci. 67:303–310.

Roberts SM, Munson JW, Lowney YW, Ruby MV. 2007. Relative oral bioavailability of arsenic from contaminated soils measured in the Cynomolgus monkey. Toxicol. Sci. 95(1):281-288.

Rodriguez RR, Basta NT, Casteel SW, Pace LW. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33(4):642–649.

Roussel H, Waterlot C, Pelfrêne A, Pruvot C, Mazzuca M, Douay F. 2010. Cd, Pb and Zn Oral Bioaccessibility of Urban Soils Contaminated in the Past by Atmospheric Emissions from Two Lead and Zinc Smelters. Archiv. Environ. Contam. Toxicol. 58:945-954.

Ruby MV. 2004. Bioavailability of soil-borne chemicals: Abiotic assessment tools. Human Ecol. Risk Assess. 10: 647-656.

Ruby MV, Davis A, Link TE, Schoof RA, Chaney R, Freeman G, Bergstrom P. 1993. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27(13):2870-2877.

Ruby MV, Davis A, Schoof R, Eberle S, Sellstone C. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30(2):422-430.

Ruby MV, Fehling KA, Paustenbach DJ, Landenberger B, Holsapple M. 2002. Oral bioaccessibility of dioxins/furans at low concentrations (50 – 350 ppt TEQ) in soil. Environ. Sci. Technol. 36(22):4905-4911.

Ruby MV, Lowney YW. 2012. Selective soil particle adherence to hands: implications for understanding oral exposure to soil contaminants. Environ Sci Technol. 46(23):12759-71.

Ruby MV, Lowney YW, Bunge AL, Roberts SM, Gomez-Eyles JL, Ghosh U, Kissel JC, Tomlinson P, Menzie C. 2016. Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil-State of the Science. Environ. Sci. Technol., 50:2151-2164.

Ruby MV, Schoof R, Brattin W, Goldade M, Post G, Harnois M, Mosby DE, Casteel SW, Berti W, Carpenter M, Edwards D, Cragin D, Chappell W. 1999. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. Environ. Sci. Technol. 33:3697–3705.

Schlummer M, Moser GA, McLachlan MS. 1998. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. Tox. Appl. Pharm. 152:128-137.

Schwarzenbach RP, Gschwend PM, Imboden DM. 2003. Environmental Organic Chemistry, 2nd ed.; John Wiley & Sons, Inc.: Hoboken, NJ.

Siciliano SD, Laird BD, Lemieux CL. 2010. Polycyclic aromatic hydrocarbons are enriched but bioaccessibility reduced in brown field soils adhered to human hands. Chemosphere 80: 1101-1108.r

Sips A, Bruil M, Dobbe C, van de Kamp E, Oomen A, Pereboom D, Rompelberg C, Zeilmaker M. 2001. Bioaccessibility of contaminants from ingested soil in humans, Method development and research on the bioaccessibility of lead and benzo(a)pyrene. RIVM Report 711701012/2001. The Netherlands National Institute of Public Health and the Environment (RIVM), Bilthoven.

Stokes JD, Paton GI, Semple KT. 2006. Behavior and assessment of bioavailability of organic contaminants in soil: Relevance for risk assessment and remediation. Soil Use Manage. 21:475-486.

Stroo H, Nakles D, Kreitinger J, Loehr R, Hawthorne S, Luthy R, Holman H, La Pierre A. 2005. Improving risk assessments for manufactured gas plant soils by measuring PAH availability. Integ. Environ. Assess. Manage. 1(3):259-266.

Tanabe S, Nakagawa Y, Tatsukawa R. 1981. Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. Agric. Biol. Chem. 45:717-726.

Tang XY, Tang L, Zh, YG, Xing BS, Duan J, Zheng MH. 2006. Assessment of the bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an in vitro test. Environ. Pollut. 140:279-285.

Tao S, Li L, Ding JN, Zhong JJ, Zhang DY, Lu Y, Yang YF, Wang XL, Li X, Cao J, Lu XX, Liu WX. 2011. Mobilization of soil bound residue of organochlorine pesticides and polycyclic aromatic hydrocarbons in an in vitro gastrointestinal model. Environ. Sci. Technol. 45: 1127-1132.

Tao S, Lu Y, Zhang DY, Yang YF, Yang Y, Lu XX, Sai DJ 2009. Assessment of oral bioaccessibility of organochlorine pesticides in soil using an in vitro gastrointestinal model. Environ. Sci. Technol. 43:4524-4529.

Thomas GO, Sweetman AJ, Jones KC. 1999. Metabolism and body-burden of PCBs in lactating dairy cows. Chemosphere 39:1533–1544.

Tilston EL, Gibson GR, Collins CD. 2011. Colon extended physiologically based extraction test (CE-PBET) increases bioaccessibility of soil bound PAH. Environ. Sci. Technol. 45:5301-5308.

United Kingdom Environment Agency. 2005. International Workshop on the Potential Use of Bioaccessibility Testing in Risk Assessment of Land Contamination. Science Report SC040054. October.

UK Environment Agency. 2007. In-vitro Bioaccessibility Testing: Current Science and Way Forward (Environment Agency Science Update 2).

UK Environment Agency. 2011. Oral bioaccessibility testing.

United States Environmental Protection Agency (USEPA). 1989. Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A). Interim Final. EPA/540/1-89/002. Office of Emergency and Remedial Response. Washington, D.C. December.

USEPA. 2007a. Guidance for evaluating the oral bioavailability of metals in soils for use in human health risk assessment. OSWER Directive 9285.7-80. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. May.

USEPA. 2007b. Estimation of relative bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. May.

USEPA. 2009. Validation assessment of in vitro lead bioaccessibility assay for predicting relative bioavailability of lead in soils and soil-like materials at Superfund sites. OSWER 9200.3-51. Office of Solid Waste and Emergency Response. Washington D.C. June.

USEPA. 2010a. Relative Bioavailability of Arsenic in Soils at 11 Hazardous Waste Sites Using an In Vivo Juvenile Swine Method. OSWER 9200.0-76. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. June.

USEPA. 2010b. Final Report Bioavailability of Dioxins and Dioxin-Like Compounds in Soil. December.

USEPA. 2012a. Standard Operating Procedure for an In vitro Bioaccessibility Assay for Lead in Soil. EPA document 9200.2-86. April.

USEPA. 2012b. Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil. OSWER 9200.1-113. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. December.

USEPA. 2015. Soil Dioxin Relative Bioavailability Assay Evaluation Framework. OSWER 9200.2-136. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. February.

USEPA. 2016. Regional Screening Levels. May.
van de Wiel JAG, Fijneman PHS, Duijf CMP, Anzion RBM, Theuws JLG, Bos RP. 1993. Excretion ofbenzo[a] pyrene and metabolites in urine and feces of rats: Influence of route of administration, sex and long-term ethanol treatment. Toxicology 80:103- 115.

van de Wiele TR, Vanhaecke L, Boeckaert C, Peru K, Headley J, Verstraete W, Siciliano S. 2005. Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites. Environ. Health Perspect. 113:6-10.

van de Wiele TR, Verstraete W, Siciliano S. 2004. Polyaromatic hydrocarbon release from a soil matrix in the in vitro gastrointestinal tract J. Environ. Qual. 33:1343–1353.

van Kesteren PCE, Walraven N, Schuurman T, Dekke RA, et al. 2014. Bioavailability of lead from Dutch made grounds: A validation study. National Institute for Public Health and the Environment, Report Number 607711015.

van Schooten FJ, Moonen EJC, vander Wal L, Levels P, Kleinjans JCS. 1997. Determination of polycyclic aromatic hydrocarbons (PAH) and their metabolites in blood, feces, and urine of rats orally exposed to PAH contaminated soils. Arch. Environ. Con Tox 33:317-322.

Vasiluk L, Pinto LJ, Walji ZA, Tsang WS, Gobas F, Eickhoff C, Moore MM. 2007. Benzo[a]pyrene bioavailability from pristine soil and contaminated sediment assessed using two in vitro models. Environ. Toxicol. Chem. 26:387-393.

Versantvoort CHM, van de Kamp E, Rompelberg CJM. 2004. Development and applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from food, 3201020022004. RIVM.

Wang B, Xue M, Yan Lv, Yu Y, Zhong JJ, Su YH, Wang R, Shen GF, Wang XL, Tao S. 2011. Cell absorption induced desorption of hydrophobic organic contaminants from digested soil residue. Chemosphere 83:1461-1466.

Weis CP, LaVelle JM. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. Chem. Speciation Bioavailab. 3(3/4):113-119.

Wragg J, Cave M. 2012. Assessment of a geochemical extraction procedure to determine the solid phase fractionation and bioaccessibility of potentially harmful elements in soils: A case study using the NIST 2710 reference soil. Analytica chimica acta 722:43-54.

Wragg J, Cave MR, Taylor H, Basta N, Brandon E, Casteel S, Grøn C, Oomen A, van de Wiele T. 2009. Inter-Laboratory Trial of a Unified Bioaccessibility Procedure; OR/07/027. British Geological Survey.

Yang JK, Barnett MO, Jardine PM, Basta NT, Casteel SW. 2002. Adsorption, sequestration, and bioaccessibility of As(V) in soils. Environ. Sci. Technol. 36(21):4562-4569.

Zhang YY, Pignatello JJ, Tao S, Xing BS. 2015a. Bioaccessibility of PAHs in fuel soot assessed by an in vitro digestive model with: effect of including an absorptive sink. Environ. Sci. Technol. 49:3905-3912.

Zhang YY, Pignatello JJ, Tao S, Xing BS. 2015b. Bioaccessibility of PAHs in fuel soot assessed by an in vitro digestive model with absorptive sink: effect of food ingestion. Environ. Sci. Technol. 49:14641-14648.

Oral Bioavailability of Nonpolar Organic Chemicals in Soil for Use in Human Health Risk Assessment

This report provides a review of bioavailability studies and methods in relation to human health risk assessment for PAHs and PCBs. Moreover standard in vitro methods to evaluate oral PAH and PCB bioavailability in soil are assessed with the aim of giving support to adjustments to risk-based soil cleanup levels for contaminated land. Furthermore various differences among countries (i.e., the US, UK, The Netherlands, France, Canada, and Australia) in the regulatory approaches to evaluating and using bioavailability in risk assessment are summarized, including definitions of terms, test methods that are deemed acceptable, reporting requirements, regulatory frameworks, and guidance on specific chemicals.

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