

Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability

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1 **Relative proportions of polycyclic aromatic hydrocarbons differ**
2 **between accumulation bioassays and chemical methods to predict**
3 **bioavailability**

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Abstract

Chemical methods to predict the bioavailable fraction of organic contaminants are usually validated in the literature by comparison with established bioassays. A soil spiked with polycyclic aromatic hydrocarbons (PAHs) was aged over six months and subjected to butanol, cyclodextrin and tenax extractions as well as an exhaustive extraction to determine total PAH concentrations at several timepoints. Earthworm (*E. fetida*) and rye grass root (*L. multiflorum*) accumulation bioassays were conducted in parallel. Butanol extractions gave the best relationship with earthworm accumulation ($r^2 \leq 0.54$, $p \leq 0.01$); while cyclodextrin, butanol and acetone-hexane extractions all gave good predictions of accumulation in rye grass roots ($r^2 \leq 0.86$, $p \leq 0.01$). However, the profile of the PAHs extracted by the different chemical methods was significantly different ($p < 0.01$) to that accumulated in the organisms. Biota accumulated a higher proportion of the heavier 4-ringed PAHs. It is concluded that bioaccumulation is a complex process that cannot be predicted by measuring the bioavailable fraction alone.

Keywords

bioavailability, polycyclic aromatic hydrocarbons, earthworms, plants, accumulation

Capsule

The ability of chemical methods to predict PAH accumulation in *E. fetida* and *L. multiflorum* was hindered by the varied metabolic fate of the different PAHs within the organisms

1. Introduction

As organic compounds age in soil they become less available for uptake by organisms, and are thus less likely to have toxic effects or be degraded by soil microorganisms (Alexander, 2000). The biological effects of a contaminant are therefore not related to its total concentration, but to the bioavailable fraction. This is the fraction of the contaminant that is biologically available for uptake.

Regulators and the public are used to a system where total concentrations are considered as well founded and definitive values, although there are now new approaches for ecological risk assessment where bioavailability data, obtained from the results of bioassays, have a more important role (Harmsen, 2007). Bioassays only respond to the bioavailable fraction of contaminants and have the advantage of being able to consider site-specific effects of mixtures of contaminants and their metabolites (Jensen and Mesman, 2007). Although they are the most established method of quantifying bioavailability, their application may be time consuming and laborious, so a large number of theoretically more time and cost-efficient chemical methods for predicting bioavailability have been published in the scientific literature (Kelsey et al., 1997; Reid et al., 2000; Ten Hulscher et al., 2003).

The most frequent approach to evaluate chemical methods for the prediction of polycyclic aromatic hydrocarbon (PAH) bioavailability is by comparing how they approximate or correlate with the amount of organic compound accumulated by soil biota such as earthworms and to a lesser extent plants, or the amount degraded by microbes (Kelsey et al., 1997; Tang and Alexander, 1999; Reid et al., 2000; Liste and

Alexander, 2002; Tang et al., 2002; Ten Hulscher et al., 2003; Hickman and Reid, 2005; Bergknut et al., 2007). When correlating chemical predictors of bioavailability to bioassays it is important to consider that the bioavailability being measured is specific to the organism used in that particular bioassay, and also to be aware that the determination of earthworm or plant accumulation does not necessarily measure contaminant bioavailability, but rather measures an interaction end-point between the organism and the compound (Hickman and Reid, 2005).

Earthworms are appropriate model organisms for bioavailability as they live in intimate contact with the soil, have a thin and permeable cuticle and consume large volumes of soil (Jager et al., 2005). They have been used in many studies as reference systems for organic compound bioavailability due to their importance in the terrestrial food chain, their potential to accumulate contaminants and ease of handling in the laboratory (Kelsey et al., 1997; Tang and Alexander, 1999; Liste and Alexander, 2002; Tang et al., 2002; Ten Hulscher et al., 2003; Van der Wal et al., 2004a; Hickman and Reid, 2005; Bergknut et al., 2007). Considerably less work has been carried out using plant accumulation as a reference system (Tang and Alexander, 1999; Tao et al., 2006a).

This study aims to compare how a range of chemical methods (namely extractions using butanol, cyclodextrin or tenax), frequently tested in isolation, predict PAH bioavailability using two different accumulation bioassays (earthworms and plants) as reference systems. It is important to consider different reference systems as bioavailability has been shown to vary between different organisms (Kelsey et al., 1997; Stroo et al., 2000).

2. Methods

2.1 Soil spiking and ageing

A 2 mm sieved Kettering Loam soil (Broughton Loam, Kettering, UK) (Table 1) was spiked using a single-step spiking/re-hydration procedure (Reid et al., 1998) with a stock solution of naphthalene and acenaphthene (2-ringed PAHs), fluorene and phenanthrene (3-ringed PAHs) and fluoranthene and pyrene (4-ringed PAHs) (Sigma Chemicals, Poole, UK) in acetone, to final concentrations of approximately 90 and 450 mg kg⁻¹ total PAH with equal concentrations of each PAH. After addition of the stock solution, the soil was left uncovered in a fume hood for 24 h to ensure all the solvent had evaporated. After checking for removal of the solvent by olfactory detection and checking for residual wetting in the soil, the spiked soil was re-wetted to 60% of its water holding capacity, transferred to 10 loosely sealed amber glass jars (5 for each concentration) and aged for 6 months at 20°C. After 0, 1, 2, 3 and 6 months, 2 jars (1 of each concentration) were emptied for use in the different chemical extractions and bioassays. A non-spiked 2mm sieved Kettering Loam soil was used as a control in all soil extractions and bioassays.

2.2 Soil extractions

To determine the total amount of PAHs in the soil, five replicate 2 g portions of soil were agitated in 10 ml of 1:1 by volume acetone/hexane mixture for 1.5 hours on an end over end shaker. After extraction the samples were left to settle for 30 min, and then 2 ml of solution were placed in a test tube containing 0.1 g of dry sodium

126 sulphate before transferring to gas chromatography (GC) vials for analysis. This
127 method was adapted from a mechanical shaking method previously reported to give
128 better recoveries than a Soxhlet extraction (Song et al., 2002). This adapted method
129 was also found to give better recoveries than a soxhlet extraction in a preliminary
130 study. Native PAH concentrations in the control soils were below the method
131 detection limit (0.5 mg kg^{-1}).

132
133 Three different kinds of butanol extraction were carried out; a vortex extraction where
134 10 g of soil were mixed in 10 ml of butanol solvent and agitated for 50 s (Swindell
135 and Reid, 2006) or 120 s (Liste and Alexander, 2002) and then left to settle for 30
136 minutes, and a shake (Reid et al., 2004) where 10 g of soil were mixed with 15 ml of
137 butanol and placed on an end over end shaker for 12 hours and then left to settle for
138 30 minutes. All butanol extractions were replicated 5 times and analysed using GC-
139 FID. The method detection limits were 0.10 mg kg^{-1} and 0.15 mg kg^{-1} for the butanol
140 mix and shake respectively.

141
142 Cyclodextrin extractions (Stokes et al., 2005) were carried out in triplicate by mixing
143 1.5 g of soil with a 25 ml solution of 60-mM HPCD (Sigma Aldrich, Poole, UK) in
144 deionised water and agitating the mixture for 20 hours using an orbital shaker (Orbital
145 Shaker SO1, Bibby Sterilin Ltd, Stone, Staffordshire, UK) at 200 rpm. The mixture
146 was then centrifuged at 2500 rpm using a Mistral 3000i centrifuge (MSE Sanyo-
147 Gallenkamp, Leicester, UK) for 30 minutes and the supernatant discarded. The
148 resulting soil pellet was shaken with 25 ml of deionised water for 10 s, centrifuged
149 again and the supernatant was again discarded to remove any remaining HPCD
150 solution. The soil pellet was then exhaustively extracted using the acetone/hexane

mechanical shaking extraction described above. GC analysis of this exhaustive extraction measured the PAHs remaining in the soil after HPCD extraction. The method detection limit was 0.67 mg kg⁻¹.

Tenax extractions (Ten Hulscher et al., 2003) were also carried out in triplicate by mixing 1.4 g of soil and 1 g of Tenax TA[®] beads (60/80 mesh, 177-250 µm, Sigma Aldrich) in 70 ml of deionised water and placing them on an end over end shaker for 6 hours. The beads were separated from the soil, rinsed with distilled water to remove soil particulates and solvent extracted by ultrasonication in 10 ml of hexane for 1 hour. The solvent samples were then analysed by GC-FID. The method detection limit was 0.71 mg kg⁻¹.

Chemical extractions were carried out in months 0, 1, 2, 3 and 6 except the Tenax extractions that were only carried out at months 0, 2 and 6.

2.3 Earthworm bioassays

Earthworms (*Eisenia fetida*) were obtained from Blades Biological (Cowden, UK). Only adult worms with a clitellum were used in the bioassays. Five worms were exposed to 300 g of the spiked soil (after 0,1,2,3 and 6 months of ageing) at 20°C for 14 days. After exposure, the worms were rinsed with water and kept on wet filter paper for 24 h for depuration of their guts. They were then weighed and frozen at -20 °C before being ground with 7 times their weight of dry sodium sulphate using a pestle and mortar. Tissues were then extracted following a saponification method to remove fat from the earthworms (Contreras-Ramos et al., 2008). This consisted of

adding 10ml of 0.5M KOH and 10 ml of a 1:1 acetone/hexane solvent mixture to the ground earthworm and ultrasonicing the mixture at 45 °C for 1 hour. The solvent layer was then cleaned on a deactivated silica column, pre-eluted with 5ml of 1:1 acetone/hexane. The sample was then eluted with a further 5 ml of 1:1 acetone/hexane before being concentrated down to 1 ml by nitrogen blowdown prior to analysis by GC/MS.

2.4 Plant bioassays

Rye grass (*Lolium multiflorum*) was grown for 4 weeks in the soil (after 0,1,2 and 3 months of ageing) in a temperature controlled greenhouse. After 4 weeks the plants were harvested and the roots separated from the soil. Root samples were rinsed with deionised water, wiped with tissue paper and freeze-dried (Super Modulyo 12K Freeze Dryer, Edwards, Crawley, West Sussex, UK) overnight. The dried roots were then ground, homogenized and weighed prior to ultrasonication for 2 hours in 10 ml of dichloromethane. The extracts were then concentrated down to 1 ml by nitrogen blowdown and passed through 0.45 µm filters obtained from Chromacoal Ltd (Welwyn Garden City, UK) before being transferred to GC vials. Solutions were analysed by GC/MS.

2.5 GC-FID analysis

Soil extraction samples were all analysed using an Agilent 6890N Network GC system equipped with a HP5 capillary column (dimensions: 30 m x 320 µm x 0.25 µm; Agilent Technologies Inc, Santa Clara, USA), operating with helium as a carrier gas. The oven was configured to 50 °C, and held for 1 minute, then ramped to 280 °C

at a rate of 15 °C min⁻¹, and held for 8 minutes. The injector and the FID were held at 300 °C.

2.6 GC-MS analysis

Plant and earthworm samples were all analysed using an Agilent 7890A Network GC system equipped with an HP5 capillary column (dimensions: 30 m x 250 µm x 0.50 µm; Agilent Technologies Inc), operating with helium as a carrier gas and coupled to an Agilent 5975C mass spectrometer (MS) through a heated transfer line (250 °C). The GC injector (300 °C) was operated in a pulsed splitless mode, 1 µl aliquots were injected using an autosampler, and the GC oven was programmed to hold 45 °C for 2.25 min then raise the temperature by 40 °C/min to 300 °C, which was held for 6 minutes. The MS was operated in single ion monitoring (SIM) mode with electric impact ionization.

2.7 Statistical Analysis

Chemical extractions and bioassays were compared using General Linear Regressions in Genstat Release ver. 7 (Lawes Agricultural Trust, Rothamsted Experimental Station).

3.0 Results

3.1 PAH loss from spiked soil

The loss of 2 and 3-ringed PAHs during the ageing period was more rapid than that of the heavier 4-ringed PAHs as measured by the mechanical acetone hexane extraction (Figure 1). All the naphthalene was depleted after 2 months and only pyrene and fluoranthene remained in the soil in month 6 at both concentrations. The initial rate of PAH loss was significantly greater for the 450 mg kg⁻¹ concentration where 25% of the original spike remained after 1 month compared to 50% in the 90 mg kg⁻¹ treatment ($p<0.01$). All the naphthalene was lost from both soils over the following month and less than 5% of the original spike of the other 2-3 ring PAHs remained after month 3. Less than 20% of the original amount of pyrene and fluoranthene remained in the soil spiked with 90 mg kg⁻¹ and less than 10% in the 450 mg kg⁻¹ soil after 6 months.

3.2 Soil extractions – Total PAH

The acetone hexane extraction extracted significantly more PAHs than any of the chemical methods used to predict bioavailability at all five time points ($p<0.01$) (Figure 2). All the extractions were significantly different from each other over the 6 month period ($p<0.05$). Raising the contact time in the butanol extractions from 50s to 120s and 120s to 12h led to a significant increase in the total amount of PAHs extracted ($p<0.05$).

3.3 Bioassay data

There was no significant difference between the total amount of PAHs extracted from the earthworms exposed to either soil after 0 and 1 months ($p < 0.01$) (Figure 3). After month 1 there was a significant decline in PAH accumulation in the earthworms exposed to the soil spiked with a total of 450 mg kg^{-1} PAH at each successive time point ($p < 0.01$). There was no significant decline in earthworm accumulation in the 90 mg kg^{-1} soil between month 2 and 3 but there was between months 1 and 2 and months 3 and 6 ($p < 0.01$).

The only significant decline in the total amount of PAH accumulated in the rye grass roots was between months 0 and 1 in the soil spiked with 450 mg kg^{-1} PAH ($p < 0.01$) (Figure 4).

3.4 Comparing chemical extractions with bioassay data

General Linear Regression suggests that 12 h butanol extractions explain a larger proportion of the variation in total PAH accumulated in earthworm tissue than any other chemical extraction (Table 2). The r^2 values are generally higher for the heavier 4-ringed PAHs (fluoranthene and pyrene) than for the 3-ringed PAHs (fluorene and phenanthrene). r^2 values for acenaphthene are deceptively high as it virtually disappears from the soil after month 2. Regression analysis was not possible for naphthalene as it was not detected in earthworm tissue.

Only cyclodextrin extractions explain a larger proportion of the variation in total PAH accumulated in plant tissue than the acetone hexane extraction (Table 3). Napthalene values are not included as it was only detectable in the soil in months 0 and 1.

Comparisons between plants and earthworms should not be made using these values as plants were not sampled in the month 6 time point. Tenax extractions are not included in these tables as they were not performed throughout all time points either.

3.5 Composition of accumulated and extracted PAHs

For the month 0 soils there was a significantly larger percentage contribution of 4-ringed PAHs in both the earthworm and plant accumulation bioassays relative to the chemical extractions ($p < 0.01$), with the proportion of 4-ringed PAHs being less than 40% in all chemical extractions (Figure 5). The soils still contained a substantial amount of 2 and 3-ringed PAHs. There was also a significantly higher contribution of 2-ringed PAHs in the Tenax and cyclodextrin extractions than in any of the other extractions or bioassays ($p < 0.05$).

On month 2 however, when the soils contained a substantially smaller amount of the 2 and 3-ringed PAHs, there was no significant difference between the PAH profiles of earthworms and butanol extractions in the soil spiked with 90 mg kg^{-1} PAH ($p < 0.05$) (Figure 5). All other extractions had significantly different compositions than the earthworms ($p < 0.05$), but they were substantially closer than in month 0. The proportion of 4-ringed PAHs was higher than 60% for the acetone hexane and tenax extractions and higher than 90% in all others. There was no significant difference

between the acetone hexane and plant extractions in the soil spiked with 450 mg kg⁻¹ PAH ($p < 0.05$), there was a difference between all others ($p < 0.01$), but again they were substantially closer than in month 0.

4. Discussion

4.1 PAH loss from spiked soil

The low-molecular weight PAHs exhibited the highest loss rates. These PAHs are susceptible to abiotic processes like volatilization (Park et al., 1990). This together with biodegradation is most likely responsible for the rapid loss of the 2-ringed PAHs in the first month. For the remainder of the PAHs, biodegradation is likely to have been the main loss process. There is a broad inverse relationship between the rate of biodegradation and the number of rings in the PAH (Bossert and Bartha, 1986; Wild and Jones, 1993) which is consistent with only the 4-ringed PAHs being detectable in the 6 month old soils.

4.2 Soil extractions

The non exhaustive extractions only recovered a fraction of the PAHs extracted by the acetone hexane extraction at all time points. This has been reported in other papers where the fraction recovered by these non exhaustive extractions has been related to the bioavailable fraction (Kelsey et al., 1997; Reid et al., 2000). However, the different extraction techniques generally extracted different amounts of PAHs over

the different time points. Differences between different chemical methods to predict PAH bioavailability have also been found in a previous study, where a number of chemical extractions were compared using PCA (Bergknut et al., 2007).

The fact that increasing the contact time of the butanol extractions significantly increased the amounts of PAHs extracted has important implications when trying to measure the bioavailable fraction as will be discussed in the following section. Differences in extraction between the varying contact times were not as pronounced in previous studies. Swindell and Reid (2006) found a vortexing time of 50s to be appropriate as an approximation for the rapidly desorbing fraction and that increasing it to 120s as in Liste and Alexander (2002) made very little difference to the value obtained.

4.3 Comparing chemical methods with the earthworm accumulation bioassay

The regressions show that the exhaustive extraction using acetone hexane does not provide the best prediction of PAH accumulation in earthworms. Butanol extractions had the better regression results. This is in line with earlier studies where mild solvents were initially proposed as chemical methods to predict bioavailability (Kelsey et al., 1997; Liste and Alexander, 2002), although much higher r^2 values (>0.90) have been found in other studies with butanol (Tang and Alexander, 1999). The extraction with the longest contact time 12h, between the solvent and the soil, showed the best correlations. Different contact times and mild solvents of varying strength have been found to correlate differently with different bioassays and other chemical methods to predict bioavailability (Kelsey et al., 1997; Tang and Alexander,

1999; Liste and Alexander, 2002; Tang et al., 2002; Swindell and Reid, 2006; Bergknut et al., 2007). A more rigorous defence of the extraction time chosen is required.

Some studies have found butanol and other mild solvents to provide a poor indication of earthworm bioavailability (White et al., 1997; Johnson et al., 2002). Jonhson et al. (2002) suggest that butanol may be a good mimic of the passive uptake of chemicals by organisms through their outer epidermis, but that it is less effective at predicting the amount absorbed through the gut as here the soil structure and extraction conditions are altered. Gut uptake could be higher than passive uptake through the outer epidermis (Landrum, 1989), although this may not be the case with *E. fetida* as it is an epigeic earthworm species and therefore consumes less soil than endogeic earthworm species. This may be the reason for butanol having a relatively good correlation in this study and strong correlations in the previously mentioned studies where *E. fetida* was used as the test species, but not with the endogeic *Aporrectodea longa* used in Johnson *et al.* (2002). It is therefore important to be aware of these differences between species as results in investigations like this one are greatly influenced by the choice of species.

Cyclodextrin extractions only slightly improved the prediction of PAH accumulation relative to the acetone hexane extraction. There are some studies indicating that cyclodextrin extractions of organic pesticides are a good indicator of earthworm bioavailability (Hartnik et al., 2008), but most studies using PAHs indicate a poor correlation with earthworm accumulation (Hickman and Reid, 2005; Bergknut et al., 2007). Reasons for this include that earthworms have complex accumulation mechanisms, and that they can access compounds from both the aqueous and the solid

phase (Gevao et al., 2001), suggesting the simple aqueous to hydrophobic sink model provided by cyclodextrin or Tenax extractions may not account for the complexity of the system. However, it is also important to consider that the lower sensitivity of these methods due to the dilution stages and smaller masses of soil used in the extractions relative to the butanol extractions could be another reason for their poorer predictability.

The butanol and cyclodextrin extractions account for a larger percentage variance in the amount of PAHs accumulated in the earthworm tissue than the acetone hexane extractions, but there is still a large proportion of the variation in accumulated PAHs unaccounted for by these extraction methods.

4.4 Comparing chemical methods with the plant accumulation bioassay

The chemical methods to predict bioavailability did not improve the description of the variation in plant accumulation provided by the acetone hexane extraction. Other attempts to compare plant accumulation with extractions using this solvent mixture were not found, but a good correlation with hexane extractions was also observed by Tao *et al.* (2006a). Here the amount of PAHs extracted by the water and hexane fractions of a sequential extraction scheme using an accelerated solvent extraction system was found to correlate well with accumulation in wheat roots. Tang and Alexander (1999) found that a number of mild solvent extractions including butanol correlated strongly ($r^2 > 0.89$) with anthracene accumulation in wheat and barley roots, but no direct indication of how an exhaustive extraction compared with this was given. Tenax extractions have been shown to have potential to predict toxicity to

plants as measured by the emergence of lettuce seedlings (Cofield et al., 2008), but no studies have attempted to correlate either cyclodextrin or tenax extractions with plant accumulation to date. Further investigation is required in this field as non exhaustive methods to predict bioavailability should theoretically provide a better indication of bioavailability to plants than exhaustive ones. Predicting the amount of PAHs that will accumulate in plants is important from a human health perspective, as food ingestion is the main source of human exposure to PAHs, with the major dietary contributions being cereals and vegetables (Phillips, 1999).

It should be noted that in this study and in the one by Tao *et al.* (2006a), the plant roots were only rinsed with water prior to analysis. It is therefore possible that the root extractions included some PAHs sorbed to the root surface and therefore not strictly accumulated within the roots (Tao et al., 2006b).

4.5 Composition of accumulated and extracted PAHs

The PAH profile of the earthworms and plants was different from the profile obtained by the soil extractions. Bergknut *et al.* (2007) observed a higher proportion of 5- and 6-ringed PAHs accumulated in earthworms than those extracted by a series of chemical extractions using mild solvents and cyclodextrins amongst others. The higher octanol-partitioning coefficient (K_{ow}) of these heavier PAHs was given as the reason for their increased accumulation. A strong negative correlation ($r^2=0.93$) between $\log K_{ow}$ and PAH elimination rate from earthworm tissue (Matscheko et al., 2002) and the fact that earthworms have been found to promote the degradation of the more readily biodegradable PAHs (Ma et al., 1995) may have contributed to the

increased accumulation of the heavier 4-ringed PAHs in the earthworms of this study. Similar studies have also shown that PAHs with higher K_{ow} accumulate more in plant roots than those of lower K_{ow} (Gao and Ling, 2006), confirming earthworm and plant accumulation are not only controlled by the bioavailable fraction of the contaminant but also by contaminant characteristics. The greater proportion of heavier PAHs accumulated by the plants and worms is highly significant from a risk assessment point of view as these are generally the more toxic/carcinogenic/mutagenic components. If a soil were to be extracted with a surrogate chemical assay the wrong bioavailability/toxicity profile might be assumed. The same overall PAH concentration as that of a bioassay may be obtained but hidden in that is the greater proportion of the heavier and more toxic PAHs.

Tenax extractions have been found to provide good predictions of bioaccumulation of PCBs and some organic pesticides in oligochaetes (You et al., 2006; Landrum et al., 2007). This was not the case for a number of PAHs including phenanthrene, and the authors believed the most logical reason for this was that some PAHs are readily biotransformed by some oligochaetes unlike most chlorinated compounds. Similarly measuring the desorption of two pesticides into the aqueous phase using cyclodextrin extractions has been found to predict pesticide uptake into earthworms, but not pesticide bioaccumulation within the earthworm tissues (Hartnik et al., 2008). Differences in bioaccumulation rates between compounds cannot necessarily be explained by differences in the bioavailable fraction and are most likely due to different metabolic fate in the organisms (Hartnik and Styris have, 2008). Chemical methods to predict bioavailability therefore cannot account for biological factors, like elimination or biotransformation, which affect the accumulation of chemicals. This is

probably one of the main reasons for the bioavailable fraction predicted by methods such as cyclodextrin extractions to correlate strongly with microbial mineralisation (Reid et al., 2000; Hickman and Reid, 2005; Allan et al., 2006; Papadopoulos et al., 2007), but only correlate weakly with earthworm accumulation (Hickman and Reid, 2005). The fact that the composition of the PAHs accumulated in plants and earthworms also differed despite them being exposed to exactly the same soil reiterates this point. PAHs will have different metabolic fates in different organisms and it will be hard if not impossible to develop a chemical method that can mimic soil biota to this level.

Some authors have used the Equilibrium Partitioning (EP) theory to account for the different biota to sediment accumulation factors (BSAF) of different contaminants (Krauss and Wilcke, 2001; Van der Wal et al., 2004b; Kreitinger et al., 2007). Van der Wal *et al.* (2004b) for example used EP theory considering contaminant K_{ow} and pore water concentrations as measured by SPME fibres, to relate the bioavailable fraction as measured by the SPME fibres to accumulation in earthworms. Measuring the bioavailable fraction of a contaminant in this way and then combining it with EP theory to obtain a prediction of earthworm accumulation may be a better way of predicting earthworm and plant accumulation. However, Bergknut *et al.* (2007) found poor correlations between PAH accumulation in earthworms and PAHs extracted by SPME fibres using the method considering EP theory as proposed in Van der Wal *et al.* (2004b). Using contaminant K_{ow} on its own to predict accumulation may therefore not be sufficient and other factors like organism specific uptake and detoxification mechanisms may need to be included in the calculation. More research into this issue is vital as being able to predict the uptake of PAHs by plants and

earthworms has important implications both for human health and the environment due to their accumulation potential up the food chain and their carcinogenicity.

5.0 Conclusion

Using accumulation bioassays to assess the capability of chemical methods to predict the bioavailability of readily biotransformable or biodegradable PAHs is not a fair test of their potential as bioavailability indicators. Even if they do provide a good estimate of the bioavailable fraction other processes influence the accumulation of contaminants in soil biota, including the physicochemical properties of the contaminant and the characteristics of soil biota themselves. Modelling these contaminant properties and soil biota uptake, biotransformation and elimination mechanisms may be the best way of predicting the amount of contaminant bioaccumulated in soil biota using the bioavailable fraction measured by chemical methods.

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Table 1. Chemical and physical properties of the Kettering loam soil.

pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)
7.1	5.0	66.9	21.74	11.76

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687 Table 2. Results of General Linear Regressions between the total and individual
688 amount of PAHs extracted by the acetone hexane shake (AH), the cyclodextrin
689 extraction (CD), the 50s butanol mix (BM50s), the 120s butanol mix (BM120s) and
690 the 12h butanol shake (BS12h) relative to the amounts accumulated in the earthworm
691 *E. fetida*.

	AH	CD	BM50s	BM120s	BS12h
Acenaphthene r^2 p	0.81 <0.01	0.78 <0.01	0.80 <0.01	0.82 <0.01	0.86 <0.01
Fluorene r^2 p	0.03 0.30	0 ^a	0 ^a	0.02 0.31	0.06 0.24
Phenanthrene r^2 p	0 ^a	0 ^a	0 ^a	0.01 0.34	0.20 0.11
Fluoranthene r^2 p	0.55 0.01	0.40 0.03	0.63 <0.01	0.59 0.01	0.47 0.02
Pyrene r^2 p	0.55 0.01	0.48 0.02	0.76 <0.01	0.62 <0.01	0.47 0.02
Total PAH r^2 p	0.48 0.02	0.51 0.03	0.54 0.01	0.60 0.01	0.64 <0.01

^a Residual variance exceeds variance of response variate

Table 3. Results of General Linear Regressions between the total and individual amount of PAHs extracted by the acetone hexane shake (AH), the cyclodextrin extraction (CD), the 50s butanol mix (BM50s), the 120s butanol mix (BM120s) and the 12h butanol shake (BS12h) relative to the amounts accumulated in the rye grass (*L. multiflorum*) roots.

	AH	CD	BM50s	BM120s	BS12h
Acenaphthene r^2 p	0.84 0.01	0.85 0.01	0.76 0.01	0.75 0.02	0.94 <0.01
Fluorene r^2 p	0.64 0.04	0.68 0.03	0.68 0.03	0.66 0.03	0.62 0.04
Phenanthrene r^2 p	0.07 0.31	0.11 0.27	0.09 0.29	0.06 0.32	0 ^a
Fluoranthene r^2 p	0.80 0.01	0.84 0.01	0.70 0.03	0.36 0.12	0 ^a
Pyrene r^2 p	0.73 0.02	0.78 0.01	0.57 0.05	0.19 0.21	0 ^a
Total PAH r^2 p	0.95 <0.01	0.97 <0.01	0.93 <0.01	0.86 0.01	0.82 0.01

^a Residual variance exceeds variance of response variate