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Accepted Version

Using deuterated PAH amendments to validate chemical extraction methods to predict PAH bioavailability in soils

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Abstract

Validating chemical methods to predict bioavailable fractions of polycyclic aromatic hydrocarbons (PAHs) by comparison with accumulation bioassays is problematic. Concentrations accumulated in soil organisms not only depend on the bioavailable fraction but also on contaminant properties. A historically contaminated soil was freshly spiked with deuterated PAHs (dPAHs). dPAHs have a similar fate to their respective undeuterated analogues, so chemical methods that give good indications of bioavailability should extract the fresh more readily available dPAHs and historic more recalcitrant PAHs in similar proportions to those in which they are accumulated in the tissues of test organisms. Cyclodextrin and butanol extractions predicted the bioavailable fraction for earthworms (*Eisenia fetida*) and plants (*Lolium multiflorum*) better than the exhaustive extraction. The PAHs accumulated by earthworms had a larger dPAH:PAH ratio than that predicted by chemical methods. The isotope ratio method described here provides an effective way of evaluating other chemical methods to predict bioavailability.

Keywords

Bioavailability; polycyclic aromatic hydrocarbons; earthworms; plants; deuterated

Capsule

A novel method using isotope ratios to assess the ability of chemical methods to predict PAH bioavailability to soil biota.

1. Introduction

Prolonged contact times between organic contaminants and soil decrease the bioavailability of these compounds for uptake by organisms or for degradation by microorganisms in a process often referred to as 'ageing' (Belfroid et al., 1995; Alexander, 2000; Northcott and Jones, 2001). Thus measuring the total concentration of organic contaminants present at contaminated sites may lead to over conservative risk assessments as only the bioavailable fractions can cause toxic effects. Recently, approaches for ecological risk assessment have been developed where bioavailability data, obtained from the results of bioassays are used (Harmsen, 2007). These bioassays only respond to the bioavailable fraction of contaminants (Jensen and Mesman, 2007), but their application can be time consuming and laborious. As a result a number of 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).

These chemical methods are normally validated in the literature 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). However, recent studies have shown distinct differences between the PAHs extracted using some of these techniques and those accumulated in earthworms and plants (Hickman and Reid, 2005; Bergknut et al., 2007; Gomez-Eyles et al., 2010). It is important to realise however, that these methods are meant to provide a measure of bioavailability not

bioaccumulation. Apart from being influenced by the bioavailability of the contaminant, the final concentration of an organic contaminant accumulated within a soil organism will also depend on the metabolic fate of the contaminant within the organism and the partitioning properties of the contaminant. Assessing chemical methods by comparing the concentration of a PAH they extract, with that accumulated in a soil organism is therefore not a fair test of their ability to predict PAH bioavailability (Gomez-Eyles et al., 2010).

An alternative way of assessing the ability of chemical methods involves predicting accumulation concentrations from concentrations measured by chemical methods and accounting for contaminant partitioning properties (Jonker et al., 2007; van der Heijden and Jonker, 2009). However these calculations do not account for differences in the metabolic fate of different contaminants and carry significant assumptions. When using passive sampling methods, like solid phase micro-extraction (SPME) fibres, these assumptions include using contaminant K_{ow} values as approximations for bioconcentration factors. When using mild solvent extractions (e.g. butanol) or depletive sampling extractions (e.g. cyclodestrin or tenax extractions) even further assumptions have to be made by using generically derived K_{oc} values (van der Heijden and Jonker, 2009). The latter is a very substantial assumption considering field contaminated soils have been shown to have K_{oc} values several orders of magnitude above generically derived ones (Hawthorne et al., 2002; Jonker, et al., 2007).

We propose a novel method to evaluate the ability of chemical extractions to predict PAH bioavailability to earthworms and plants that can account for differences in bioaccumulation concentrations caused by different contaminant properties. This

method follows the same principle used in a previous study on the effect of ageing in sediments on PAH accumulation at the top levels of aquatic food chains (Moermond et al., 2007). Here we spike a soil historically contaminated with PAHs, with deuterated PAHs (dPAHs) enabling a comparison of the extraction and uptake of freshly spiked PAHs and aged historic PAHs by chemical methods and accumulation bioassays. dPAHs have been used as internal standards in many studies involving PAHs as they have very similar properties to their respective undeuterated analogue PAHs (Bucheli et al., 2004; Bergknut, et al., 2007). They should therefore also have the same metabolic fate and partitioning properties as their respective undeuterated analogue PAHs. Consequently, a method that correctly predicts the fraction of PAHs available to earthworm and plants should extract the freshly spiked dPAHs and the aged historic PAHs in a similar ratio to that in which they are accumulated within earthworm and plant tissues. Comparing the ratio in which the chemical method extract the PAHs with that in which it accumulates in the soil organism, enables a fair assessment of these chemical methods to measure bioavailability. This cannot be achieved by simply comparing the concentration of a compound accumulated in a soil organism with that extracted by the chemical method.

This investigation aims to use this novel method to evaluate the ability of butanol and cyclodextrin extractions, two of the most widely reported methods, to predict PAH bioavailability to earthworm and plants in soils.

2. Experimental Section

2.1 Soil spiking and ageing

PAH-contaminated soil from a former gasworks site in the UK (Table 1) was passed through a 2 mm sieve. The <2mm fraction was spiked using a single-step spiking/re-hydration procedure (Reid et al., 1998) with a stock solution of deuterated PAHs (Sigma Chemicals, Poole, UK) in acetone, to final concentrations of 30 mg kg^{-1} of [$^2H_8$] naphthalene, [$^2H_{10}$] phenanthrene, [$^2H_{10}$] pyrene and 10 mg kg^{-1} of [$^2H_{12}$] benzo(a)pyrene. After addition of the stock solution, the soil was left uncovered in a fume cupboard for 24 h to ensure all the solvent had evaporated. After confirming 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. Samples of the soil were taken immediately after re-wetting to determine initial PAH concentrations. The remainder of the soil was used either in bioassays of 20 days duration (see below) or transferred to loosely sealed amber glass jars and aged for 20 days at 20ºC.

The same procedure was followed using a control soil (Broughton Loam, Kettering, UK) (Table 1), but this soil was spiked with fresh undeuterated PAHs as well as dPAHs to the same final concentrations as above. Exposing plants and earthworms to a soil freshly spiked with equal amounts of PAHs and dPAHs served as a control for any potential preferential accumulation of one kind of PAH over the other. When comparing ratios of dPAHs:PAHs between organisms and the chemical extractions we assume there is no difference between the uptake processes or the metabolic fate of dPAHs and PAHs within the organisms. Determining whether this assumption is true is therefore important when using these ratios to evaluate the potential of the chemical methods to predict the bioavailable fraction.

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152 **2.2 Soil extractions**

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154 To determine the total amount of PAHs in the soils five replicate 4 g portions of soil
155 were agitated in 10 ml of 1:1 by volume acetone/hexane mixture for 2 hours on an
156 orbital shaker (Orbital Shaker SO1, Bibby Sterilin Ltd, Stone, Staffordshire, UK) at
157 250 rpm. After extraction the samples were left to settle for 30 min, and then 2 ml of
158 solution were placed in a test tube containing 0.1 g of dry sodium sulphate before
159 transferring to gas chromatography vials for analysis (LOD=0.05 mg kg^{-1}). This
160 method was adapted from a mechanical shaking method previously reported to give
161 better recoveries than a Soxhlet extraction (Song et al., 2002).
162 Two different kinds of butanol extraction were carried out; a vortex extraction where
163 10 g of soil were mixed in 15 ml of butanol solvent and agitated for 120 s (Liste and
164 Alexander, 2002), and a shake (Reid et al., 2004) where 10 g of soil were mixed with
165 15 ml of butanol and placed on a rock and roll shaker for 12 hours. All butanol
166 extractions were passed through 0.45 µm polytetraflouroethylene (PTFE) filters
167 obtained from Chromacoal Ltd (Welwyn Garden City, UK) and were replicated 5
168 times before analysis by GC/MS. The method detection limits were 0.01 mg kg^{-1} and
169 0.015 mg kg^{-1} for the butanol mix and shake respectively.

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171 Cyclodextrin extractions (Stokes et al., 2005) were carried out in replicates of 5 by
172 mixing 1.5 g of soil with a 25 ml solution of 60-mM HPCD (Sigma Aldrich, Poole,
173 UK) in deionised water and agitating the mixture for 20 hours using an orbital shaker
174 at 250 rpm. The mixture was then centrifuged at 2500 rpm using a Mistral 3000i
175 centrifuge (MSE Sanyo-Gallenkamp, Leicester, UK) for 15 minutes and the

supernatant discarded. The resulting soil pellet was shaken with 25 ml of deionised water for 10 s, centrifuged again and the supernatant was again discarded to remove any remaining HPCD solution. The soil pellet was then exhaustively extracted using the acetone/hexane mechanical shaking extraction described above. GC/MS analysis of this exhaustive extraction measured the PAHs remaining in the soil after HPCD extraction (LOD=0.07 mg kg^{-1}).

All soil extractions were carried out after 20 days, once the earthworm and plant exposures had concluded. The extractions were carried out on both the soil that had been left in loosely sealed amber glass jars and also on the soil that had been used in the bioassays. An exhaustive acetone hexane extraction was also carried out on day 0 to determine the initial concentration of PAHs in the soils.

2.3 Earthworm bioassays

Earthworms (*Eisenia fetida*) were obtained from Blades Biological (Cowden, UK). Only adult earthworms with a clitellum were used in the bioassays. Five earthworms were exposed to 250 g of the spiked soils at 20ºC for 20 days in loosely sealed amber glass jars; 20 days was selected for consistency with the plant bioassays. After exposure, the earthworms were rinsed with water and kept on wet filter paper for 24 h to allow them to clear their guts. They were then cleaned, weighed and frozen at -20 ºC before being ground with 7 times their weight of dry sodium sulphate using a pestle and mortar. Earthworms were then extracted following a saponiphication 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 ultrasonicating the mixture at 45 ºC for 1 hour. The solvent layer was then cleaned on a deactivated silica column, pre-eluted with 5ml of hexane. The sample was then eluted with a further 5 ml of hexane before being concentrated down to 1 ml under a stream of nitrogen prior to analysis by GC/MS. Extraction efficiencies for all PAHs ranged between 80.2-103.5%.

2.4 Plant bioassays

Rye grass (*Lolium multiflorum*) was grown for 20 days in the soils in a temperature controlled greenhouse. The plants were harvested and the roots separated from the soil. Root samples were rinsed and ultrasonicated with deionised water to ensure complete removal of soil particles from the roots. The cleaned roots were freeze-dried (Super Modulyo 12K Freeze Dryer, Edwards, Crawley, West Sussex, UK) overnight. Once dried, the roots were ground, homogenized and weighed prior to ultrasonication for 2 hours in 10 ml of dichloromethane. The extracts were then concentrated down to 1 ml under a stream of nitrogen and passed through 0.45 µm filters before being transferred to GC vials. Solutions were analysed by GC/MS. Extraction efficiencies for all PAHs ranged between 84.7-100.3%.

2.5 GC-MS analysis

All samples were analysed using a Thermo Trace GC Ultra system equipped with a Thermo TR-5MS capillary column (dimensions: 30 m x 250 µm x 0.25 µm; Thermo Scientific, Runcorn, UK) operating with helium as a carrier gas, coupled to a Thermo ITQ 1100 mass spectrometer (MS) through a heated transfer line (300 ºC). The GC

injector (220 ºC) was operated in a pulsed splitless mode, 1µl aliquots were injected using an autosampler, and the GC oven was programmed to hold 60 °C for 3 min then ramped at 15 ºC/min to 290 ºC, and held for 10 minutes. The MS was operated with the ion source at 220 ºC and a damping flow of 0.3 ml min^{-1}.

2.6 Statistical Analysis

Statistical analysis was perfomed using R 2.9.2 (R Development Core Team). Differences between the ratios of dPAH: PAH accumulated in the organisms and those extracted by the different chemical methods were tested by performing an ANOVA after general linear modelling of the data. The general linear model was given a gamma distribution to account for the data being expressed as ratios.

3. Results and Discussion

3.1 PAH loss from the spiked soils

The loss of the freshly spiked 2 and 3-ringed PAHs and dPAHs (naphthalene and phenanthrene) during the 20 days of exposure was more rapid than that of the freshly spiked 4 and 5-ringed PAHs and dPAHs (pyrene and benzo(a)pyrene), as measured by the mechanical acetone hexane extraction, in both the gasworks and Kettering loam soils. This is consistent with previous reports that have shown a broad inverse relationship between the rate of biodegration and the number of rings in the PAH (Bossert and Bartha, 1986; Wild and Jones, 1993). Low-molecular weight PAHs are also more susceptible to abiotic processes like volatilisation (Park et al., 1990). The

loss of the freshly spiked 2 and 3-ringed PAHs during the 20 day exposures were significantly lower in the gasworks soil than in the Kettering loam ($p < 0.01$). The two soils were not characterised in sufficient detail to provide conclusive reasons for this, but it was probably occurred due to differences in physicochemical properties and microbial activities between the soils.

There was no significant difference in the loss of the dPAHs relative to their undeuterated analogues in all Kettering loam treatments ($p < 0.01$). This is to be expected as deuterated organic compounds are known to have very similar chemical and physical properties to their undeuterated analogues. However, there was a significantly smaller loss of naphthalene and phenanthrene from the soil used in the plant bioassays compared to loss from the soil kept in amber glass jars and the soil used for the earthworm bioassays ($p < 0.01$). This was despite the plant bioassay soil being left uncovered and in the light. These conditions are theoretically more conducive to abiotic loss processes such as volatilization or photodegration. This could indicate that most losses in this soil were due to biodegradation, and that the relatively higher soil moisture in the loosely sealed amber glass jars may have provided better conditions for microbial activity. There was a significantly larger decrease in the pyrene and benzo(a)pyrene concentrations in the Kettering loam used in the earthworm and plant bioassays relative to the soil that had not been exposed to any organisms ($p < 0.01$). Earthworms have been previously found to promote the degradation of PAHs (Ma et al., 1995) and a number of plant species have been shown to increase hydrocarbon degradation, although rye grass in particular had a smaller effect than others and has been shown to even decrease rhizosphere PAH degradation (Phillips et al., 2006; Phillips et al., 2008).

The loss of historic PAHs from the gasworks soils was higher than previously anticipated for a soil with contamination that had been ageing for decades. We hypothesise that introducing some freshly available dPAHs may have stimulated the microbial activity in the soil and induced the catabolism of some historic PAHs (Bauer and Capone, 1988; Reid et al., 2002). There was a greater loss of the freshly spiked deuterated naphthalene than that of its historic counterpart in both the soil that was not exposed to any organisms and the soil that was exposed to plants ($p<0.01$). However, this was generally not the case for the other dPAHs and their non-deuterated PAH counterparts. Faster degradation of the fresh and theoretically more available PAHs might have been expected, but the reduced losses relative to those in the Kettering loam coupled with the hypothesised induced catabolism of the historic PAHs may have prevented this from happening.

3.2 Comparing ratios of dPAH:PAH between chemical methods and earthworm bioassays

The ratios of dPAH to PAHs in the spiked gasworks soil are highly variable compared to those in the spiked Kettering loam (Figure 1). Note naphthalene is not included in these figures due to the low concentrations left in the soil after 20 days. However, it should be noted that the gasworks soil was not spiked with exactly the same concentration of dPAHs as the concentration of historic PAHs in the soil. The acetone

hexane extraction therefore gives an indication of the actual ratio of dPAH:PAH in the soil.

Low concentrations of phenanthrene and deuterated phenanthrene accumulated in the earthworms exposed to the gasworks soil, resulting in highly variable accumulation ratios. Differences between the dPAH:PAH ratios accumulated in the earthworms and those extracted by the chemical methods are therefore not statistically significant. However, there are highly significant differences in the ratios of dPAH:PAH accumulated in the earthworms exposed to the gasworks soil compared to those extracted by the chemical methods for the heavier 4-5 ring PAHs (pyrene and benzo(a)pyrene) ($p<0.001$). The ratios can be up to 6 times bigger in earthworm tissues relative to some chemical methods when considering benzo(a)pyrene. This implies that the benzo(a)pyrene fraction bioavailable to earthworms differs significantly to that predicted by the chemical methods. Earthworms accumulate an increasingly higher proportion of the fresh dPAHs with increasing PAH size. Although the mode of toxicity of benzo(a)pyrene to earthworms is non-polar narcosis it is a proven human carcinogen and as such is the main risk driver for many contaminated sites in the UK. Heavier PAHs have been shown to have relatively higher potencies as aryl hydrocarbon receptor agonists (Barron et al., 2004), and benzo(a)pyrene has a relative carcinogenic potency several order of magnitude higher than other PAHs like phenanthrene (Pufulete et al., 2004). Therefore it is important for chemical methods to correctly assess the bioavailablity of benzo(a)pyrene. A large number of investigations that attempt to validate the use of chemical methods to predict bioavailability often only use smaller 3-4 ringed PAHs like phenanthrene as models (Kelsey, et al., 1997; Tang and Alexander, 1999; Reid, et al., 2000; Liste and

Alexander, 2002), so care must be taken when extrapolating these results to the heavier more recalcitrant and toxic PAHs in soil.

It was expected that the dPAH:PAH ratios for the Kettering loam bioassays and chemical extractions would be at or close to unity as the 2 different kinds of PAHs were added on the same day and in equal concentrations to the soil. The results corroborate this, indicating that dPAHs have a similar behaviour to that of their analogue undeutrated counterparts. It is therefore safe to assume that any differences between the ratio of dPAH:PAH accumulated by the earthworms or plants and the ratios in the chemical extractions from the gasworks soil are because they are accessing different pools of PAHs and not because of any inherent difference in the uptake rate or metabolism of dPAHs and PAHs. This confirms that dPAH amendments can provide a good indication of the ability of a chemical method to predict the bioavailable fraction.

The fact that earthworms did not show signs of preferential accumulation of the dPAHs relative to the PAHs in the Kettering loam therefore confirms that the increased relative accumulation of the dPAHs from the gasworks soil is due to the higher availability of these freshly spiked dPAHs to earthworms relative to the historic PAHs. The chemical methods to predict bioavailability should have reflected this by extracting dPAHs and PAHs in a similar ratio to that accumulated in the earthworms. The concentrations of the different PAHs and dPAHs extracted by the different chemical methods were examined to determine whether the reason for their smaller dPAH:PAH ratios in the extractions relative to those in the earthworm were due to chemical methods extracting less dPAHs than those accumulated in the

earthworms, more of the historic PAHs than those accumulated in the earthworms, or a combination of the two. The concentrations in the acetone hexane extractions, the butanol mix and the cyclodextrin extractions indicated that the lower ratios were caused by a combination of both factors, whereas the butanol shake extractions had extracted higher concentrations of the historic PAHs. The concentrations of the dPAHs in both butanol extractions were similar but the 12 hour shake extracted even more of the historic PAHs, suggesting the increased contact time enabled the extraction of the more recalcitrant historic PAHs. Earthworms were therefore found to accumulate smaller amounts of historic PAHs than was predicted by any of the chemical methods. This is probably due to the lower chemical activity of historic PAHs relative to the freshly spiked dPAHs. Extraction methods like the ones used in this study involve shaking which maximises chemical potential gradients and minimises the kinetic constraints. This is not the case in the earthworm bioassays, where there will be a kinetic limitation of PAH uptake into the earthworms. Methods that provide a measure of the chemical activity of a substance, which is related to its energetic state (Reichenberg and Mayer, 2006), could therefore give a better indication of accumulation in soil organisms. Cyclodextrin and butanol extractions give a measure of the bioaccessible concentration, which is the portion of the total concentration that is or can become bioavailable (Alexander, 2000). This could explain why some studies have found poor correlations between the amounts of PAHs accumulated in earthworms and those extracted by butanol or cyclodextrin extractions (Hickman and Reid, 2005; Bergknut, et al., 2007; Gomez-Eyles, et al., 2010). There are a number of studies however in which butanol and cyclodextrin extractions provide a better indication of the bioavailable fraction of an organic contaminant than exhaustive extraction methods (Kelsey, et al., 1997; Liste and Alexander, 2002;

Hartnik et al., 2008). This is also true in this investigation as despite being significantly smaller than the ratio of dPAH:PAH accumulated in the earthworms, the ratios of dPAH:PAH extracted by the cylcodextrin and 120s butanol extractions are still closer to the bioassay values than the dPAH:PAH ratio of the exhaustive acetone hexane extraction.

3.3 Comparing ratios of dPAH:PAH between chemical methods and plant bioassays

The ratios of dPAH:PAH accumulated in the rye grass roots exposed to the gasworks soil are closer to those extracted by the chemical methods relative to the ratios accumulated in the earthworm tissues for pyrene and benzo(a)pyrene (Figure 2). Again most of the significant differences occur with the heavier 4-5 ringed PAHs. For pyrene all chemical extractions remove a significantly higher proportion of the historic PAHs except for the 120s butanol extraction ($p<0.05$). The acetone hexane and 12 hour butanol extraction also extracted a significantly higher proportion of the historic benzo(a)pyrene than that which accumulates in the plant roots ($p<0.01$). This is not the case for the cylodextrin and the 120s butanol extraction. The 120s butanol extraction and in some cases the cyclodextrin extraction therefore generally provide a better indication of the fraction of PAHs available to plants than the more exhaustive acetone hexane extraction. It is hard to validate these results in the literature as few investigations have been carried out attempting to relate chemical methods to predict bioavailability to plant accumulation, although in a previous investigation we found that a number of chemical methods did not improve the description of the variation in plant accumulation provided by an acetone hexane extraction (Gomez-Eyles, et al.,

2010). Tang and Alexander (1999) however found that a number of mild solvent extractions including butanol correlated strongly with anthracene accumulation in wheat and barley roots. No direct indication of how an exhaustive extraction compared with this was given.

Plants accumulated a much lower proportion of the freshly spiked dPAHs than the earthworms did. This could have occurred as plant roots are relatively static compared to earthworms. When exposed to the spiked gasworks soil they are likely to deplete the more readily available dPAHs surrounding them. The earthworms on the other hand are more mobile and are therefore likely to come across areas of soil they have not explored before. When exposed to these areas of soil, they will preferentially accumulate a higher proportion of the more bioavailable dPAHs before they move on to another area of soil where they will do the same. Differences in dPAH:PAH ratios between plants and earthworms could also be due to the earthworm tissues being more lipophilic than the root tissues causing more of the readily available dPAHs to partition into their tissues. Other reasons could include differences in the PAH uptake mechanisms between the two organisms.

4.0 Conclusions

In this investigation there are large differences between the ratios of dPAH:PAH accumulated in plants relative to those accumulated in earthworms suggesting there cannot be one sole chemical method to predict bioavailability. Factors like the behaviour of different soil biota within the soil or their different lipid contents have an important role in determining what fraction of a contaminant may or may not be

426 available to them. It is extremely challenging if not impossible to develop a chemical
427 method that is able to mimic soil organisms at a level in which differences between
428 species can be accounted for. Although in some cases the ratios extracted by the
429 chemical methods differ substantially from those accumulated in the earthworm
430 tissues, results from this investigation do suggest that cyclodextrin and short butanol
431 extractions extract a fraction of the PAHs which is closer to that bioavailable to
432 earthworms and plants than that extracted by an exhaustive extraction. Deuterated
433 PAH amendments could be used to evaluate the ability of other methods, like Tenax
434 extractions (Ten Hulscher, et al., 2003), solid-phase microextraction (SPME) fibres
435 (Van der Wal et al., 2004), poly-oxymethylene solid-phase extractions (POM-SPE)
436 (Jonker and Koelmans, 2001), persulphate oxidations (Cuypers et al., 2000) or super
437 critical carbon dioxide extractions (Kreitinger et al., 2007), to predict PAH
438 bioavailablity to different soil biota. We believe that using this isotope ratio method
439 can enable the comparison of methods that give an indication of the chemical activity
440 of a contaminant (e.g.SPME or POM) with those that give an indication of
441 contaminant accessibility (e.g. Tenax or cyclodextrin). This is of particular interest as
442 previously comparisons between methods have been made by comparing correlations
443 between chemical methods and bioaccumulation assays, or by using equilibrium
444 partitioning calculations to make predictions. In the former approach the correlations
445 are largely affected by the partitioning and metabolism of the contaminant within the
446 organism whilst the latter approach involves substantial assumptions, particularly
447 when using measurements from mild solvent and depletive sampling extractions. We
448 also suggest using a representative 5-ringed PAH like benzo(a)pyrene in tests of
449 chemical extractions due to the importance of this class of PAH in risk assessment. It
450 is therefore of particular importance that the fraction of the benzo(a)pyrene extracted

by the chemical methods examined in this investigation was the one that differed most substantially from that accumulated in the earthworms.

Acknowledgements

This study was funded by the Biotechnology and Biological Sciences Research Council (BBSRC).

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617 Table 1. Chemical and physical properties of the soils.

	pH	Total Organic Carbon (%)	Sand (%)	Silt (%)	Clay (%)
Kettering loam	7.1	1.99	66.9	21.7	11.8
Gasworks soil	7.4	10.6	81.1	16.7	2.24

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