

1 **A comparison of batch mode and dynamic physiologically based**
2 **bioaccessibility tests for PAHs in soil samples**

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14 **Abstract**

15 A fed state *in vitro* methodology capable of use in commercial testing laboratories has been
16 developed for measuring the human ingestion bioaccessibility of polyaromatic hydrocarbons
17 (PAHs) in soil (Fed ORganic Estimation human Simulation Test- FOREhST). The protocol
18 for measuring PAHs in the simulated gastro-intestinal fluids used methanolic KOH
19 saponification followed by a combination of polymeric sorbent solid phase extraction and
20 silica sorbent cartridges for sample clean-up and preconcentration. The analysis was carried
21 out using high pressure liquid chromatography with fluorescence detection. The repeatability

1 of the method, assessed by the measurement of the bioaccessibility of 6 PAHs
2 (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene,
3 dibenz[*ah*]anthracene and indeno[*1,2,3-c,d*]pyrene) in eleven gas works soils, was c.10%
4 RSD. The method compared well with the results from an independent dynamic human
5 simulation reactor comprising of the stomach, duodenal and colon compartments tested on
6 the same soils. The measured bioaccessible fraction of the soils varied from 10-60% for soils
7 containing 10-300 mg kg⁻¹ PAH (the sum of the six studied) with total organic carbon
8 concentrations in the soils ranging from 1-13%. A multiple regression model showed that the
9 PAH bioaccessible fraction could be explained using the PAH compound, the soil type and
10 the total PAH to soil organic carbon content. The method described here has potential for
11 site specific detailed quantitative risk assessment either to modify the risk estimation or to
12 contribute to the risk evaluation.

13 **Key words:** PAH, soil, gas works, bioaccessibility, TOC, *in vitro*, FOREhST.

14 **Introduction**

15 Soil has been identified as the primary reservoir for PAH in the UK [1] and ingestion of soils
16 is considered to be an important exposure pathway for humans [2]. For risk assessments
17 considering the ingestion pathway, it is not the total PAH concentration that is important but
18 the bioavailable fraction that enters the body. In this study, bioavailability is being discussed
19 in terms of people ingesting contaminated soil. In this situation oral *bioavailability* of a given
20 substance may be formally defined as the fraction of an administered dose that reaches the
21 central (blood) compartment from the gastrointestinal tract [2]. This term should not be
22 confused with the oral *bioaccessibility*, which is defined as the contaminant fraction of intake
23 that is soluble in the human gastrointestinal system and is therefore available for absorption

1 [2]. The methodologies used in this study estimate the bioaccessibility of PAHs using *in*
2 *vitro* physiologically based extraction methods.

3

4 The most commonly determined PAHs are the United States Environmental Protection
5 Agency list of sixteen "Consent Decree" priority pollutants [3, 4]. Of the sixteen,
6 benzo[*a*]pyrene (BaP) has been identified by the Environment Agency of England and Wales
7 (EA) as a non-threshold carcinogenic marker substance [5]. Soil Guideline Values (SGV) are
8 viewed as "trigger values", which are scientifically based generic assessment criteria (GAC),
9 for soil contamination, that are used to help evaluate the long to risks to human health.
10 Where soil concentrations exceed SGV, there may be a cause for concern to human health.
11 Such levels may pose a significant risk to human health, although further investigation and
12 evaluation of risks are required for the detailed risk assessment. No SGV for BaP has, to
13 date, been issued by the EA for use in the UK, although a recent publication [3] has
14 calculated GAC which are broadly equivalent to SGV values, of 0.83-2.1 mg kg⁻¹ for BaP in
15 residential and allotment soils varying in organic matter from 1% to 6%; for other parts of
16 Europe both the Danish and Flemish regulators have an intervention concentration/ cleanup
17 directive value of 1 mg kg⁻¹ [6, 7].

18

19 For inorganic contaminants a number of *in vitro* bioaccessibility methods have been
20 developed [8]. Importantly, since the bioaccessibility measurement from *in vitro* tests are
21 methodologically defined, it is only useful in a risk assessment if it can be shown that the
22 result is relevant to humans [9]. Soil arsenic bioaccessibility is now routinely considered in
23 UK site specific detailed quantitative risk assessments (DQRA) (e.g.,[10-14]). The results
24 from bioaccessibility tests are expressed as the bioaccessible fraction (BAF) as a percentage
25 using the expression:

$$BAF(\%) = \left(\frac{Element_{bioaccessible}}{Element_{total}} \right) \times 100$$

1

2 For inorganic bioaccessibility testing, evidence shows that the fasted state will give the most
3 conservative estimate of the bioaccessible fraction as these give rise to lower pH conditions
4 compared to the fed state [12-14]. A number of studies have shown that the presence of food
5 increases the bioaccessibility of organic contaminants [15-17]. This effect is probably due to
6 two separate influences. Food contains fat which can help mobilise hydrophobic organic
7 contaminants into the aqueous solution and, when food is present in the human gastro-
8 intestinal (GI) tract, the amount of bile salts increases and these act as a surfactant, greatly
9 reducing the surface tension of the digestive juice forming bile micelles with the organic
10 contaminant [16, 17].

11 A number of studies have used *in vitro* bioaccessibility tests to estimate the human ingestion
12 bioaccessibility of PAHs in soils. The German DIN standard bioaccessibility test [18], three
13 Chinese studies used fasted conditions [17, 19, 20], the Dutch National Institute for Public
14 Health and the Environment (RIVM) [21, 22], and Gron et al [6] have used the RIVM fed
15 state model [22].

16 In a different approach, PAH release from a contaminated soil, containing 49 mg PAH kg⁻¹,
17 using a SHIME (Simulator of the Human Intestinal Microbial Ecosystem) reactor comprising
18 the stomach, duodenal, and colon compartments [7] was investigated. The SHIME
19 effectively models the human GI [23, 24]. The SHIME reactor differentiates itself from other
20 *in vitro* intestinal models, because it is dynamic and comprises of the entire GI tract taking
21 into account the enzymatic processes in the stomach and duodenum and the different
22 characteristics of the microbiota along the colon reactors.

1 Vasiluk et al [25] studied the role of the human stomach membrane as a sink for desorbed
2 BaP from a soil/sediment matrix. The authors made a case that a membrane sink should be
3 considered in *in vitro* bioaccessibility testing suggesting that the driving force for uptake
4 from the soil is the fugacity gradient that exists between the gastrointestinal fluid and the
5 membrane.

6 Clearly, there are a number of experimental approaches to estimating the human
7 bioaccessibility of PAHs in soil samples, however, in order to produce comparative
8 reproducible and accurate data for risk assessments there needs to be a more standardised
9 approach to produce both comparable and validated data. To do this it is necessary to
10 develop a methodology which should be simple enough to be carried out by commercial
11 testing laboratories using standard laboratory equipment and analytical methodologies but
12 retain enough complexity to still be a reasonable representation of the human GI tract. In the
13 long term, this would involve the validation of the test against *in vivo* soil feeding trials and
14 evaluation of the method in an inter-laboratory trial. The main aim of this study was to
15 develop a standardised bioaccessibility test for PAHs in soils through the following
16 objectives:

- 17 • Develop and /or adapt a suitable *in vitro* batch test for PAHs in soil with reference to
18 the most up to date literature;
- 19 • Develop a robust analytical protocol for measuring PAHs in the simulated GI fluids
20 arising from the *in vitro* batch test over a suitable concentration range for PAH
21 contaminated soils;
- 22 • Assess the repeatability of the test for a range of soil types containing varying
23 concentrations of PAHs;

- 1 • Compare the absolute bioaccessible fractions against a well established independent
2 *in vitro* dynamic human GI simulator as guidance towards the potential of the batch
3 method for simulating human bioavailability.

4

5 **Materials and methods**

6 Six PAHs were chosen based on their toxicity [26]. These were, in alphabetical order,
7 benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF),
8 benzo[*a*]pyrene BaP, Dibenz[*a,h*]anthracene, DBA and indeno[*1,2,3-c,d*]pyrene (IP) .
9 Selected properties are shown in Table 1.

10 *Table 1 Selected PAH properties*

PAH & Abbreviation	Relative Molecular Mass	Number of Rings	TEF ^a	PEF ^c	Boiling Point °C ^d	Log K_{ow} ^d	EC (True) ^e
Benz[<i>a</i>]anthracene, BaA	228	4	0.1	0.145	435	5.91	25 (18)
Benzo[<i>b</i>]fluoranthene, BbF	252	5	0.1	0.141	481	5.80	29 (20)
Benzo[<i>k</i>]fluoranthene, BkF	252	5	0.1	0.1	481	6.00	29 (20)
Benzo[<i>a</i>]pyrene, BaP	252	5	1	1	496	6.04	30 (20)
Dibenz[<i>a,h</i>]anthracene, DBA	278	5	1 ^b	1.11	535	6.75	32 (22)
Indeno[<i>1,2,3-c,d</i>]pyrene, IP	276	6	0.1	0.232	534	6.55	34 (21)

11

12 ^a Toxic Equivalent Factor (TEF), the toxicity of the compound expressed relative to benzo[*a*]pyrene [26, 27];

13 ^b DBA TEF adopted from [27]

14 ^c Potency Equivalent Factor (PEF) values used to derive the Land Quality Management (LQM)/Chartered
15 Institute of Environmental Health GAC, where PEF reflects the relative carcinogenic potency of the PAH
16 congeners rather than the differences in their general toxicity [3]

17 ^d Boiling Point (°C) and Octanol water partition coefficients (Kow) [28]

1 ° Equivalent Carbon (EC) and true number of carbon atoms [3], where the EC is based on the boiling point of
2 the compound normalised to that of un-branched n-alkanes.

3

4 Full experimental details of the analysis of the total organic carbon (TOC) content, the total
5 PAH content of the soil, the bioaccessibility extraction and subsequent analysis of PAH in the
6 extract using methanolic KOH saponification followed by a combination of polymeric
7 sorbent solid phase extraction and silica sorbent cartridges for sample clean-up and
8 preconcentration followed by analysis by high pressure liquid chromatography with
9 fluorescence detection is supplied in the Supplemental Information.

10 **Soil Samples**

11 Eleven soil samples were investigated. The soils were collected from disused gas works sites
12 within the UK, and contained BaP concentrations ranging from 2 to 68 mg kg⁻¹. The soils
13 were prepared by first removing any large pieces of debris (stones, brick, pottery, plaster
14 etc.), freeze dried, followed by retention of the fraction that passed through a <250 µm sieve.
15 The <250 µm size fraction of soil was chosen as it is the fraction considered to be the upper
16 limit of particle size that is likely to adhere to children's hands, who are often the at risk
17 receptor for assessing contaminated sites [29] In addition, this fraction has been the
18 dominant soil fraction in bioavailability and bioaccessibility studies over the last decade [19,
19 20, 30-33].

20 ***In vitro* Bioaccessibility Test**

21 The RIVM have carried out extensive literature reviews of the pH, chemical environment and
22 transit times for the human stomach and upper intestine and have developed fed and fasted
23 bioaccessibility methods based on these findings [21, 22]. These methods have also been

1 used successfully for organic contaminants in soil [6, 16, 34, 35]. It was therefore decided to
2 adopt the fed state RIVM method, with minor modifications, for this study. In order to
3 distinguish this modified *in vitro* system from the RIVM method and other inorganic
4 methodologies the method was named as the Fed ORganic Estimation human Simulation Test
5 (FOREhST). The fed state was chosen as a number of studies have shown that for
6 hydrophobic organics the fed state gives the most conservative estimate of bioaccessibility
7 [15-17]. The RIVM study used an infant formula supplemented with vegetable oil as the
8 food component; This food component was chosen to represent an average diet for men and
9 women aged 19-65 in the Netherlands, with respect to their mean intake of energy and
10 nutrients [22]. Initial work in this study used an infant formula which, for routine use, was
11 found to be difficult to weigh out into the extraction tubes and, once opened, did not last long
12 before it degraded and was not suitable for use. The wet food product was replaced with a
13 freeze dried oatmeal and rice porridge infant food supplemented with sunflower oil to match
14 the macronutrient composition of the average diet of 4-6 year old children in Britain [36].
15 The FOREhST method is essentially a three stage static *in vitro* bioaccessibility test, which is
16 intended to simulate the physico-chemical conditions in the fed state. The method is carried
17 out at human body temperature (37°C) and utilises end-over-end rotation. The stages
18 involved in the methodology are suggestive of the saliva, gastric and intestinal (duodenal and
19 bile) phases of the human gastro-intestinal system, with sample collection (by centrifugation)
20 at the end of the extraction phase representative of small intestinal digestion. Gastro-
21 intestinal fluid pH, ratios and transit times are all adjusted, compared to a fasted static model,
22 to account for the physiological differences caused by the ingestion of food: saliva pH ($6.8 \pm$
23 0.5); gastric pH (1.3 ± 0.5); small intestinal pH (duodenal pH 8.1 ± 0.2 , bile pH 8.2 ± 0.2); GI
24 fluid ratio for saliva: gastric: duodenal: bile(1:2:2:1), GI transit time (gastric 2 hr, small
25 intestine 2 hr). For each contaminated soil under investigation, 0.3g of contaminated material

1 was extracted in triplicate, with extraction of each replicate on consecutive days in order to
 2 estimate the repeatability of the method. Sample blanks were extracted within each batch of
 3 samples under investigation. A detailed description of the FOREhST bioaccessibility test can
 4 be found in the Supporting Information.

5 **The SHIME method comparison**

6 The results of the FOREhST method were compared to the SHIME *in vitro* GI model [7, 23,
 7 24]. Each of the eleven soils were run in triplicate using the SHIME system with the same
 8 food component used in the FOREhST test. Full details of the conditions used for SHIME
 9 model are given in the supplementary information. The stomach and intestine extracts from
 10 the SHIME reactor were analysed for their PAH content using the same method used for the
 11 FOREhST extracts.

12 **Results and Discussion**

13 **Performance of the analytical method**

14 The performance of the test on spiked portions of the blank extraction solutions from both the
 15 FOREhST and SHIME methods was shown to be good: recoveries of c.90% or better with
 16 repeatability relative standard deviations of c. 5% or better (Table 2).

17 *Table 2 Solid phase extraction recoveries of 6 PAHs from various spiked saponified*
 18 *G.I fluids*

G.I PAH	No G.I Fluid		FOREhST Fed		SHIME Fed		SHIME Fasted	
	Av. %	RSD	Av. %	RSD	Av. %	RSD	Av. %	RSD
BaA	102.6	4.6	90.8	5.7	92.0	1.3	89.6	2.5
BbF	97.0	2.7	91.1	2.7	95.5	0.6	93.0	2.2
BkF	95.5	2.0	90.8	1.9	93.8	0.5	93.0	2.2
BaP	96.6	3.5	88.0	3.7	92.3	1.2	89.7	2.8
DBA	89.3	1.5	86.6	3.2	92.6	1.1	88.6	3.5
IP	89.1	1.4	89.2	2.2	90.5	1.1	89.4	3.5

19

1 **Comparison of the SHIME and FOREhST bioaccessibility results**

2 For the FOREhST method the majority of the samples have RSD values of 10% or better and
3 the SHIME method 15% or better.

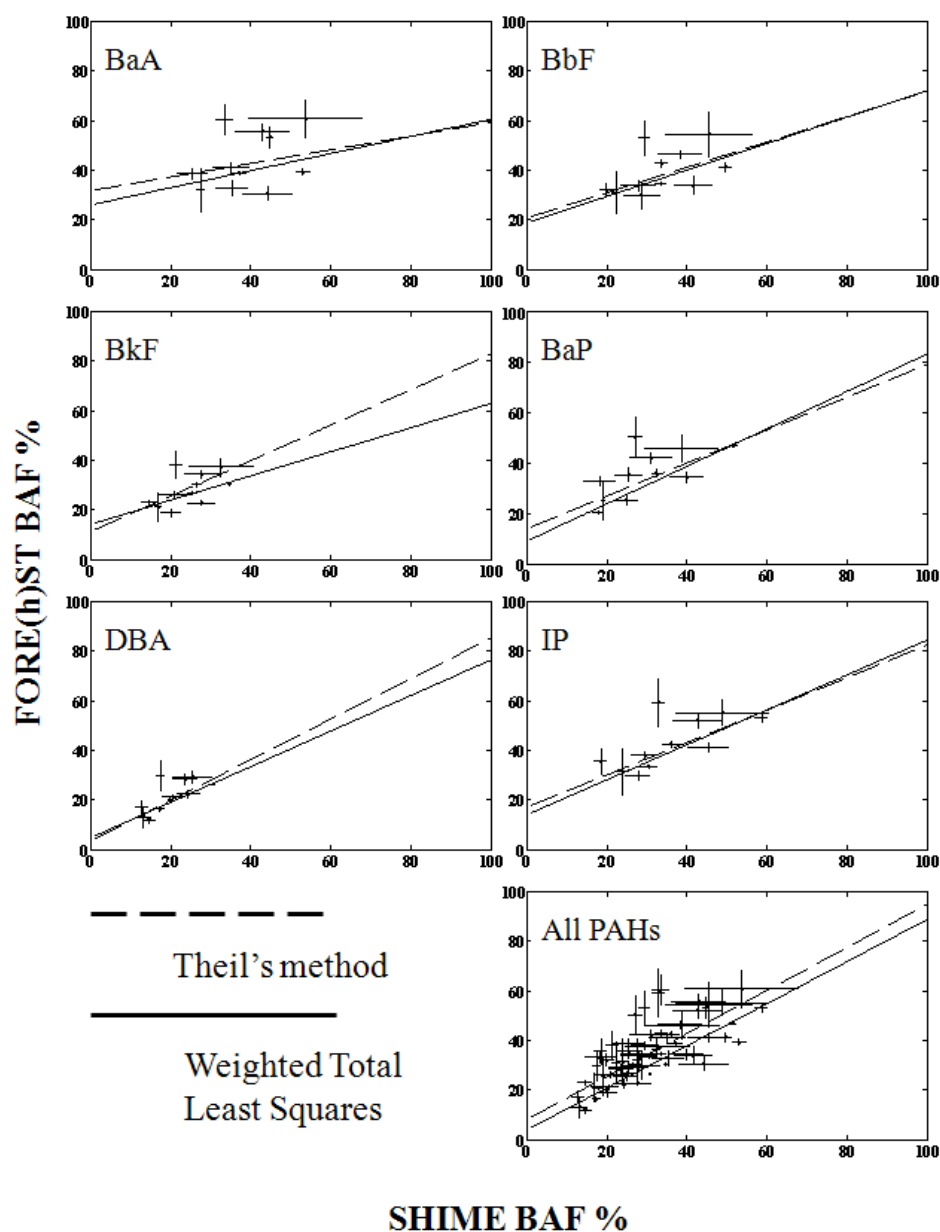
4 Since there is uncertainty associated with both measurements it is not appropriate to use
5 ordinary least squares regression. The weighted total least squares (WTLS) method which
6 takes account of the error on both sets of data [37] and Theil's non-parametric method that
7 makes no assumptions about distributions of errors and is robust to outliers have been used to
8 assess the linear relationship between the two measurements [38, 39].

9 *Table 3 Summary of the linear regression parameters for the SHIME FOREhST data*
10 *comparison*

PAH	WTLS Slope	WTLS Intercept	Theil Slope	Theil Intercept	r	p value
BaA	0.35	26.0	0.28	31.7	0.36	0.275
BbF	0.53	18.8	0.51	20.7	0.51	0.110
BkF	0.49	14.1	0.72	11.2	0.53	0.093
BaP	0.74	9.3	0.65	14.6	0.65	0.029
DBA	0.72	4.7	0.82	3.5	0.71	0.015
IP	0.71	14.0	0.65	17.1	0.68	0.021
All PAHs	0.85	3.9	0.87	8.1	0.74	<0.001

11

12



1

2 *Figure 1 Comparison of the BAF for the SHIME and the FORE(h)ST methods*

3 The slope and intercepts for both methods and Pearson correlation coefficients (r) and their
 4 associated significance are given in Table 3 and the scatter plots in Figure 1. There is
 5 reasonable agreement between the two regression methods. Although the original samples
 6 were chosen to have a range of total PAH (BaP ranges from c.2 to c. 70 mg kg⁻¹) the range of
 7 BAF for each individual PAH is relatively low, with BAF of the individual PAHs covering
 8 30 % or less of the total range of concentrations in the soil. This may account for the

1 relatively low r values and variability in slope for individual PAHs compared to the combined
2 data set. When all PAHs are considered together, there is highly a significant correlation
3 with a strong r value of 0.74 (Table 3). There is however a trend towards increasing r values
4 with increasing hydrophobicity (cf Table 1 K_{ow} and Table 3 r). BaA has a weak non-
5 significant correlation (0.36); Bbf and Bkf have moderate correlations (0.51 and 0.53) which
6 are significant at the 90% confidence level; BaP, IP and DBA have strong correlations (0.65,
7 0.68 and 0.71) which are significant the 95% confidence level. There is not enough data (the
8 variety of soils and PAH sources in this study are limited and the agreement when all PAHs
9 are considered is good) to conclude that the two methods do not agree for the least
10 hydrophobic PAHs. The trend, however, suggests that there is more variability in the BAF
11 for low Kow PAHs. A possible explanation for this is that PAH extraction from the soil is
12 through extraction into micelles which are formed in the GI extraction medium [22].
13 Research into the solubility of PAH in micelles [40] suggests that the more hydrophobic
14 compounds are held in the centre of the micelle whereas the less hydrophobic compounds are
15 solubilised at the interfacial medium. Therefore, during the *in vitro* extraction test used here,
16 once the hydrophobic PAHs are released from the soil they are held within the centre of the
17 micelle away from the soil surface but the less hydrophobic compounds at the surface of the
18 micelle can interact with the soil and be redistributed back onto the soil leading to a greater
19 variability the measured BAF.

20 Whilst Figure 1 shows the individual FOREhST BAF values for each PAH in each soil are
21 not always higher than the SHIME values, the slopes for the regression lines (Table 3) for all
22 individual PAHs and all PAHs combined together are all below unity showing that on
23 average the FOREhST method gives higher BAF than the SHIME method.

1 Taking all PAHs together slope of the line suggests that the SHIME method BAF values are
2 c. 80% of the FOREhST values showing that the FOREhST method over predicts compared
3 to the dynamic SHIME model.

4 The variability in BAF results between the soils in this study and PAHs is likely to be derived
5 from three sources:

- 6 i) The physico-chemical properties of the soils;
- 7 ii) The physico-chemical properties of the PAHs; and
- 8 iii) The original total concentration of PAH in the soil.

9 With respect to point iii) the mechanism for PAH sorption to soils is complex [41], with
10 multistage sequestration on to humic and fulvic acid polymer layers as well as adsorption on
11 to the soil mineral surfaces [42]. The different sorption sites on the soil will have different
12 PAH affinity and absolute capacity [41] depending on the make-up of the soil. The overall
13 PAH availability will therefore be a function of how much PAH is sorbed to the soil and how
14 tightly it binds to the different sorption sites on the soil surface.

15 The organic matter content of soils is often reported to be positively correlated with total
16 PAH content [43] which is related to the sorption capacity of the organic carbon in the soil.
17 Since there are varying concentrations of both TOC and PAHs in the soils under test the ratio
18 of the individual PAH concentration to the soil TOC (mg g^{-1}) was used to standardise the
19 amount of PAH relative to the TOC.

20 To understand how these parameters affect the final results, a simple linear regression model
21 was set up using the BAF value of the FOREhST method as the dependant variable (the Y
22 variable) and the soil sample, PAH molecule and the ratio of the original PAH concentration
23 to the TOC concentration (mg g^{-1}) as the independent variables (the X variables). Since soil
24 and PAH molecule are factors rather than continuous variables the regression was set up so

1 that the regression equation was relative to the soil with the lowest TOC (Soil 1) and the
 2 lowest molecular weight PAH (BaA, Table 1). The regression coefficients are given in Table
 3 4. The equation of the model is:

$$4 \quad BAF = \beta_0 + \beta_{PAH\ molecule} + \beta_{Soil} + \beta_1 \times \left(\frac{total\ PAH\ conc.}{TOC} \right)$$

5 For example, referring to Table 4, the predicted BAF for the PAH BbF in Soil 2 would be:

$$6 \quad BAF_{BbF,soil2} = 35.8 - 5.3 - 1.77 + 12.2 \times \frac{total\ BbF\ in\ Soil\ 2}{TOC\ in\ Soil2}$$

7 The model accounts for c. 90% of the variance in the data (Multiple R-squared: 0.94,
 8 Adjusted R-squared: 0.92). All of the parameters, apart from the effect of the PAH IP and
 9 soils 2, 4 and 8, are significant. In words, the model shows that on average c. 36% (intercept)
 10 of the PAH is bioaccessible. The coefficients associated with the factors show how much the
 11 average value is adjusted for specific PAHs and soils. The higher molecular weight PAHs
 12 have reduced bioaccessibility relative to BaA (negative coefficients ranging from -5.3 to -
 13 16.9%, Table 4) apart from IP which is not significantly different from BaA (p value of
 14 0.745, Table 4). Apart from IP the PAH coefficients indicate reduction of the BAF with log
 15 K_{ow} (see Table 1) i.e the higher the hydrophobicity the lower the amount of PAH extracted
 16 by the *in vitro* GI simulation test which is in general agreement with other bioaccessibility
 17 studies [17, 19]. IP, which has the second highest log Kow has a coefficient which is
 18 indistinguishable from BaA which has the lowest value. The reason for this is not clear since
 19 the literature does not show that IP sorption to soils shows any anomalous effects. The
 20 simulated stomach and intestine fluids for the SHIME and FOREhST methods contain a
 21 complex mixture of organic reagents and in the case of IP it appears that there is another
 22 property of IP besides log Kow which is governing its extraction in this matrix. The
 23 biological activity of PAHs in specific situations can be shown to be related to the shape of
 24 the molecule as defined by topographical properties e.g. [44] using a Quantitative Structure

1 Activity Relationship (QSAR) approach. The Pearson correlation coefficient between the
 2 coefficients for the PAHs (Table 4) and the Kow of the PAHs (Table 1) is weak (-0.29) but in
 3 a list of molecular descriptors for PAHs [45], there is a strong correlation (0.70) between the
 4 PAH coefficients and a topological descriptor called DECC which is an eccentricity index
 5 measuring the size and shape of the molecule. As only 6 PAHs have been investigated in this
 6 work there is not enough data to reach any conclusions regarding the anomalous IP
 7 behaviour, and a further study using a larger suite of PAHs would be required to test whether
 8 DECC or other properties are involved in the extraction mechanism.

9 *Table 4 Linear regression coefficients and associated statistics for the BAF MLR*
 10 *model*

Coefficient name	Independent Parameter	Coefficient value	Std. Error	p value
β_0	Intercept	35.8	1.9	<0.001
β_{BaA}	BaA	0	-	-
β_{BbF}	BbF	-5.3	1.5	<0.001
β_{BkF}	BkF	-13.5	1.6	<0.001
β_{BaP}	BaP	-9.0	1.6	<0.001
β_{DBA}	DBA	-16.9	2.2	<0.001
β_{IP}	IP	0.50	1.5	0.745
β_{Soil1}	Soil 1	0	-	-
β_{Soil2}	Soil 2	-1.77	2.0	0.371
β_{Soil3}	Soil 3	12.6	2.0	<0.001
β_{Soil4}	Soil 4	-4.1	2.8	0.138
β_{Soil5}	Soil 5	1.1	2.6	0.684
β_{Soil6}	Soil 6	-8.1	2.2	<0.001
β_{Soil7}	Soil 7	-8.0	2.1	<0.001
β_{Soil8}	Soil 8	-2.5	2.2	0.254
β_{Soil9}	Soil 9	6.4	2.1	0.004
β_{Soil10}	Soil 10	9.6	2.9	0.002
β_{Soil11}	Soil 11	10.1	3.1	0.002
β_1	PAH/TOC ratio	12.2	0.35	0.001

11

12 The soils show varying increases and decreases in bioaccessibility relative to soil 1
 13 (coefficients ranging from -8.1 to 12.6%). The coefficient for the PAH to TOC ratio also has
 14 a highly significant effect (p value = 0.001, Table 4). To give an idea of scale for this
 15 parameter, using BaP in soil 5 as an example, an increase in the BaP concentration of 14 mg

1 kg^{-1} increases the modelled BAF by 5% (from 39% to 44%) and increasing the TOC
2 concentration by 3% organic carbon decreases the BAF by 5% (from 39% to 34%)..

3 Similar regression results were obtained when the SHIME BAF replaced the FOREhST as
4 the independent variable in the linear model (data not shown). Despite the inclusion of the
5 PAH to TOC ratio in the model the effect of the different soils was also significant suggesting
6 that there are other soil properties which also control the BAF.

7 Although there have been a number of important findings arising from this study they cannot
8 be considered as generic until the methodology has been applied to a wider variety of soil
9 types and contamination sources as well as extending the PAHs investigated.

10 From the method development stand point the study has met the four objectives outlined in
11 the introduction as follows:

- 12 • The FOREhST method can be set up and used in any competent testing laboratory;
- 13 • The analysis of the PAHs in the simulated GI fluids is robust and accurate (Table 2);
- 14 • The bioaccessibility results for the soils studied were repeatable (mostly <10% RSD);
- 15 • The FOREhST method gives comparable results to the dynamic SHIME human GI model
16 giving some assurance that the method is likely to have some relevance to human
17 bioavailability; and
- 18 • The FOREhST method gives conservative results (c.20% higher) compared to the SHIME
19 method.

20 From a risk estimation standpoint the bioaccessibility data produced from this test could not
21 be used on their own as an input to a DQRA to derive site specific assessment criteria as
22 further validation tests are required which include:

- 23 i) Testing the applicability of the method on a wider variety of soil types and PAHs;

- 1 ii) Assessing the reproducibility of the method through an inter-laboratory trial;
- 2 iii) Validation of the results against *in-vivo* trials;
- 3 iv) The production of PAH reference soils to allow performance of the method to be
- 4 checked and monitored using standard QA/QC protocols;

5 Despite this, even at this early stage, bioaccessibility data from this test could be used to
6 provide information in a “lines of evidence approach” to aid the risk evaluation stage of
7 assessment.

8

9 **Interpretation of the bioaccessibility results**

10 From the point of view of risk assessment, taking into account that the study was carried out
11 on a limited range of soils, the fact that a quantitative model predicting BAF using soil and
12 PAH properties was achieved has the following consequences:

- 13 i) For well characterised sites it may be feasible to calculate on a site specific basis the
14 PAH BAF for a number of PAHs based on the measurements of a few “marker”
15 compounds;
- 16 ii) Quantitative data on the effect of different PAH to TOC ratios on the BAF factor can
17 provide invaluable information when regulators or developers are deriving site
18 specific assessment criteria for PAHs in soil; and
- 19 iii) The MLR model gives valuable insights into how the physico-chemical parameters
20 governing the soil/PAH system control the BAF and hence help to optimise both risk
21 evaluation and soil remediation strategies.

22 Acknowledgements

1 This paper is published with permission of the Executive Director of the British Geological
2 Survey. The project was funded by National Grid and project managed by Parsons
3 Brinkerhoff.

5 **Supporting Information Available**

6 Methods used for total organic carbon analysis and total PAH content of the soils, the
7 operating conditions for the SHIME method, the extraction and subsequent analysis of the
8 PAHs from the FOREhST method, tables containing the total PAH content of the soils and
9 the comparative bioaccessibility factors for all PAHs in all soils. This information is
10 available free of charge via the Internet at <http://pubs.acs.org>.

11 **References**

- 12 1. Menzie, C. A.; Potocki, B. B.; Santodonato, J., Exposure to Carcinogenic PAHs in the
13 Environment. *Environmental Science & Technology* **1992**, *26*, (7), 1278-1284.
- 14 2. Paustenbach, D. J., The practice of exposure assessment: A state-of-the-art review (Reprinted
15 from Principles and Methods of Toxicology, 4th edition, 2001). *J. Toxicol. Env. Health-Pt b-Crit.*
16 *Rev.* **2000**, *3*, (3), 179-291.
- 17 3. Nathanail, P.; McCaffrey, C.; Ashmore, M.; Cheng, Y.; Gillet, A.; Ogden, R.; Scott, D., *The*
18 *LQM/CIEH Generic Assessment Criteria for Human Health Risk Assessment* 2nd ed.; Land Quality
19 Press: Nottingham, 2009.
- 20 4. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY *Quality criteria for*
21 *water.*; EPA 440/5-86-001.; United States Environmental Protection Agency, Washington DC, USA.:
22 1987.
- 23 5. Department for the Environment Food and Rural Affairs and the Environment Agency
24 *Assessment of Risks to Human Health from Land Contamination: An Overview of the Development of*
25 *Guideline Values and related Research*; CLR7; 2002.
- 26 6. Gron, C.; Oomen, A.; Weyand, E.; Wittsiepe, J., Bioaccessibility of PAH from Danish soils.
27 *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental*
28 *Engineering* **2007**, *42*, (9), 1233-1239.
- 29 7. Van de Wiele, T. R.; Verstraete, W.; Siciliano, S. D., Polycyclic aromatic hydrocarbon
30 release from a soil matrix in the in vitro gastrointestinal tract. *Journal of Environmental Quality* **2004**,
31 *33*, (4), 1343-1353.
- 32 8. Wragg, J.; Cave, M. R. *In-vitro Methods for the Measurement of the Oral Bioaccessibility of*
33 *Selected Metals and Metalloids in Soils: A Critical Review*; R&D Project Record P5-062/TR/01;
34 Environment Agency: 2003.
- 35 9. Nathanail, C. P. Professional Practice Note: Reviewing Human Health Risk Assessment
36 Reports Invoking Contaminant Oral Bioavailability Measurements Or Estimates. www.cieh.org.uk

- 1 10. Nathanail, C. P.; McCaffrey, C.; Haynes, D., Assessing exposure to pedogenic arsenic
2 contamination at a dwelling in Northamptonshire, UK: a case study. *Soil Use Manage.* **2005**, *21*, (0),
3 508-517.
- 4 11. Nathanail, C. P.; Smith, R., Incorporating bioaccessibility in detailed quantitative human
5 health risk assessments. *J. Environ. Sci. Health, Part A* **2007**, *42*, (9), 1193-1202.
- 6 12. Maddaloni, M.; Lolacono, N.; Manton, W.; Blum, C.; Drexler, J.; Graziano, J., Bioavailability
7 of soilborne lead in adults, by stable isotope dilution. *Environmental Health Perspectives* **1998**, *106*,
8 1589-1594.
- 9 13. Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete,
10 W.; Van de Wiele, T.; Wragg, J.; Rempelberg, C. J. M.; Sips, A.; Van Wijnen, J. H., Comparison of
11 five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental*
12 *Science & Technology* **2002**, *36*, (15), 3326-3334.
- 13 14. Van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W., Inulin-type fructans
14 of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of*
15 *Applied Microbiology* **2007**, *102*, (2), 452-460.
- 16 15. Hack, A.; Selenka, F., Mobilization of PAH and PCB from contaminated soil using a
17 digestive tract model. *Toxicol. Lett.* **1996**, *88*, (1-3), 199-210.
- 18 16. Oomen, A. G.; Sips, A.; Groten, J. P.; Sijm, D.; Tolls, J., Mobilization of PCBs and lindane
19 from soil during in vitro digestion and their distribution among bile salt micelles and proteins of
20 human digestive fluid and the soil. *Environmental Science & Technology* **2000**, *34*, (2), 297-303.
- 21 17. Tang, X. Y.; Tang, L.; Zhu, Y. G.; Xing, B. S.; Duan, J.; Zheng, M. H., Assessment of the
22 bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an in vitro test.
23 *Environmental Pollution* **2006**, *140*, (2), 279-285.
- 24 18. DIN Soil Quality - Absorption availability of organic and inorganic pollutants from
25 contaminated soil material; DIN E 19738; Deutsches Institut fur Normung e. V.: Berlin, 2000.
- 26 19. Khan, S.; Cao, Q.; Lin, A. J.; Zhu, Y. G., Concentrations and bioaccessibility of polycyclic
27 aromatic hydrocarbons in wastewater-irrigated soil using in vitro gastrointestinal test. *Environmental*
28 *Science and Pollution Research* **2008**, *15*, (4), 344-353.
- 29 20. Lu, M.; Yuan, D.; Lin, Q.; Ouyang, T. Assessment of the bioaccessibility of polycyclic
30 aromatic hydrocarbons in topsoils from different urban functional areas using an in vitro
31 gastrointestinal test. <http://dx.doi.org/10.1007/s10661-009-0982-x>
- 32 21. Sips, A. J. A. M.; Bruil, M. A.; Dobbe, C. J. G.; Van de Kamp, E.; Oomen, A. G.; Pereboom,
33 D. P. K. H.; Rempelberg, C. J. M.; Zeilmakker, M. J. *Bioaccessibility of Contaminants form Ingested*
34 *Soil in Humans*; 711701012 RIVM: 2001.
- 35 22. Versantvoort, C. H. M.; van de Kamp, E.; Rempelberg, C. J. M. *Development and*
36 *applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from*
37 *food*; 3201020022004; RIVM: 2004.
- 38 23. Molly, K.; Vandewoestyne, M.; Desmet, I.; Verstraete, W., Validation of the Simulator of the
39 Human Intestinal Microbial Ecosystem (Shime) Reactor Using Microorganism-Associated Activities.
40 *Microb. Ecol. Health Dis.* **1994**, *7*, (4), 191-200.
- 41 24. Molly, K.; Woestyne, M. V.; Verstraete, W., Development of a 5-Step Multichamber Reactor
42 as a Simulation of the Human Intestinal Microbial Ecosystem. *Applied Microbiology and*
43 *Biotechnology* **1993**, *39*, (2), 254-258.
- 44 25. Vasiluk, L.; Pinto, L. J.; Walji, Z. A.; Tsang, W. S.; Gobas, F.; Eickhoff, C.; Moore, M. M.,
45 Benzo[a]pyrene bioavailability from pristine soil and contaminated sediment assessed using two in
46 vitro models. *Environmental Toxicology and Chemistry* **2007**, *26*, (3), 387-393.
- 47 26. Nisbet, I. C. T.; Lagoy, P. K., Toxic Equivalency Factors (TEFs) for Polycyclic Aromatic-
48 Hydrocarbons (PAHs) *Regulatory Toxicology and Pharmacology* **1992**, *16*, (3), 290-300.
- 49 27. Malcolm, H. M.; Dobson, S. *The calculation of an environmental assessment level (EAL) for*
50 *atmospheric PAHs using relative potencies*; Report No. DoE/HMIP/RR/94/041 Department of the
51 Environment: London, UK., 1994; pp pp. 34-46.
- 52 28. Alves de Lima Ribeiro, F.; Ferreira, M. M. C., QSPR models of boiling point, octanol-water
53 partition coefficient and retention time index of polycyclic aromatic hydrocarbons. *Journal of*
54 *Molecular Structure: THEOCHEM* **2003**, *663*, (1-3), 109-126.

- 1 29. Duggan, M. J.; Inskip, M. J.; Rundle, S. A.; Moorcroft, J. S., Lead in Playground Dust and on
2 the Hands of Schoolchildren. *Sci. Total Environ.* **1985**, *44*, (1), 65-79.
- 3 30. Casteel, S. W.; Weis, C. P.; Henningsen, G. M.; Brattin, W. J., Estimation of Relative
4 Bioavailability of Lead in Soil and Soil-Like Materials Using Young Swine. *Environmental Health*
5 *Perspectives* **2006**, *114*, (8), 1162–1171.
- 6 31. Kelley, M. E.; Brauning, S. E.; Schoof, R.; Ruby, M. V., *Assessing Oral Bioavailability of*
7 *Metals in Soil*. Battelle Press: Columbus Richland, 2002.
- 8 32. Schroder, J. L.; Basta, N. T.; Casteel, S. W.; Evans, T. J.; Payton, M. E.; Si, J., Validation of
9 the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils.
10 *Journal Of Environmental Quality* **2004**, *33*, (2), 513-521.
- 11 33. Tang, J. X.; Petersen, E. J.; Huang, Q. G.; Weber, W. J., Development of engineered natural
12 organic sorbents for environmental applications: 3. Reducing PAH mobility and bioavailability in
13 contaminated soil and sediment systems. *Environmental Science & Technology* **2007**, *41*, (8), 2901-
14 2907.
- 15 34. Hansen, J. B.; Oomen, A. G.; Edelgaard, I.; Gron, C., Oral bioaccessibility and leaching:
16 Tests for soil risk assessment. *Engineering in Life Sciences* **2007**, *7*, (2), 170-176.
- 17 35. Hurdzan, C. M.; Basta, N. T.; Hatcher, P. G.; Tuovinen, O. H., Phenanthrene release from
18 natural organic matter surrogates under simulated human gastrointestinal conditions. *Ecotoxicology*
19 *and Environmental Safety* **2008**, *69*, (3), 525-530.
- 20 36. Gregory, J.; Lowe, S.; Bates, C. J.; Prentice, A.; Jackson, L. V.; Smithers, G.; Wenlock, R.;
21 Farron, N., National Diet and Nutrition Survey :young people aged 4 to 18 years. In Agency, F. S.,
22 Ed. The Stationery Office: London, 2000; Vol. Volume 1: Report of the diet and nutrition survey.
- 23 37. Krystek, M.; Anton, M., A weighted total least-squares algorithm for fitting a straight line.
24 *Measurement Science and Technology* **2007**, *18*, 3438-3442.
- 25 38. Glaister, P., Robust linear regression using Theil's method. *Journal of Chemical Education*
26 **2005**, *82*, (10), 1472-1473.
- 27 39. Theil, H., A rank-invariant method of linear and polynomial regression analysis. *Indagationes*
28 *Mathematicae* **1950**, *12*, 85-91.
- 29 40. Lan Chun, C.; Lee, J.-J.; Park, J.-W., Solubilization of PAH mixtures by three different
30 anionic surfactants. *Environmental Pollution* **2002**, *118*, (3), 307-313.
- 31 41. Haritash, A. K.; Kaushik, C. P., Biodegradation aspects of Polycyclic Aromatic Hydrocarbons
32 (PAHs): A review. *Journal of Hazardous Materials* **2009**, *169*, (1-3), 1-15.
- 33 42. Luo, L.; Zhang, S. Z.; Ma, Y. B., Evaluation of impacts of soil fractions on phenanthrene
34 sorption. *Chemosphere* **2008**, *72*, (6), 891-896.
- 35 43. Nam, J. J.; Sweetman, A. J.; Jones, K. C., Polynuclear aromatic hydrocarbons (PAHs) in
36 global background soils. *Journal of Environmental Monitoring* **2009**, *11*, (1), 45-48.
- 37 44. Benísek, M.; Bláha, L.; Hilscherová, K., Interference of PAHs and their N-heterocyclic
38 analogs with signaling of retinoids in vitro. *Toxicology in Vitro* **2008**, *22*, (8), 1909-1917.
- 39 45. Todeschini, R.; Gramatica, P.; Marengo, E.; Provenzani, R., Weighted Holistic Invariant
40 Molecular Descriptors. Part 2. Theory Development and Applications on Modeling Physico-Chemical
41 Properties of PolyAromatic Hydrocarbons (PAH). . *Chemometrics and Intelligent Laboratory Systems*
42 **1995**, *27*, 221-229.
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1

Brief

- 2 The performance of a batch mode and a dynamic *in vitro* test for estimating the human
- 3 ingestion bioaccessibility of PAHs in soils are compared.