# *Sorptive physiologically based extraction of contaminated solid matrices: incorporating silicone rod as absorption sink for hydrophobic organic contaminants*

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# Sorptive physiologically based extraction of contaminated solid matrices: incorporating silicone rod as absorption sink for hydrophobic organic contaminants.

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## Abstract

The oral bioaccessibility of soil contaminants is increasingly assessed with Physiologically Based Extraction Tests (PBETs) to fine tune human health risk

assessment of contaminated land. In these tests the contaminant fraction that is desorbed into simulated digestive fluids is measured and classified as bioaccessible. However, this approach can lead to underestimations if the capacity of the fluids is insufficient to provide infinite bath conditions. To circumvent this artefact, we incorporated a silicone rod as an absorption sink into the PBET to continuously absorb mobilized contaminants and maintain the desorption gradient. Polycyclic aromatic hydrocarbons served as model contaminants and the colon extended PBET (CEPBET) as the extraction model. The inclusion of the silicone rod sink (1) increased the extraction capacity of the test by orders of magnitude, (2) ensured (near) infinite bath conditions and (3) allowed for simple back-extraction of PAHs for their quantification by GC-MS. The silicone rod provided fast enrichment when applied to the stomach and small intestine compartment, but was somewhat slower in the carbohydrate colon compartment. Finally, the sorptive-CEPBET was applied to wood soot and a kindergarten soil. The present article provides the basis for how an absorption sink can be integrated into PBET models.

## Introduction

Human exposure to hydrophobic organic contaminants (HOCs) through unintentional soil ingestion has received increasing attention during recent years.[1-3] Although the daily average soil intake for humans is estimated to be only 50-100 mg/day,[4] the high levels of HOCs in some soils make this exposure pathway important. Intestinal

secretions and enzymes can only mobilize a fraction of soil bound HOCs[5] while pinocytosis of soil particles is considered negligible.[6, 7] Therefore, risk assessment of the oral exposure route for hydrophobic organic soil contaminants has to take into account the HOC fraction that can become desorbed from soil under digestive conditions. This desorbable fraction is defined as bioaccessible, and is considered to be the maximum available fraction for absorption by the intestinal membranes.[8] Intestinal absorption will continuously removeHOCs from the digestive fluids and in this way maintain a gradient for further desorption.

Several *in-vitro* digestion models have been developed for measuring bioaccessibility of HOCs in contaminated soils.[3, 9-14] Such models offer a cheaper and more practical alternative compared to animal experiments. The common practice is to incubate the soil with a simulated digestive fluid, then separate the fluid from the digested soil and deduce bioaccessibility based on the amount of HOCs mobilized into the fluid.[1, 3, 15, 16] Such methods thus focus at the initial HOC partitioning into the solution, they do not take into account subsequent intestinal absorption which "consumes" HOCs and thereby maintains the desorption gradient for continuous HOC release from the soil.[17] Omitting this process will lead to an underestimation of the bioaccessibility,[18-20] unless the capacity of the applied fluid is sufficient to ensure infinite bath conditions.

The quantification on the mobilized HOC fraction requires phase separation, extraction, clean up and instrumental analysis, which for HOCs is laborious and possibly also associated with significant potential for error and experimental artefacts. The phase separation by filtration or centrifugation can be problematic for HOCs due to their hydrophobic nature and their tendency to sorb not only to soil but also to dissolved organic matter and labware surfaces.

In the present study, a "sorptive physiologically based extraction" is introduced by showing how a silicone rod can be incorporated as a large capacity absorption sink into an existing *in vitro* digestion model. The first purpose of this silicone rod is to continuously absorb the mobilized HOCs from the simulated gut fluid. This ensures that freely dissolved HOC concentrations remain low and that the chemical activity gradient that drives desorption is maintained during the incubation. The second purpose of the silicone rod is to serve as an analytical absorption phase that selectively samples the mobilized HOCs. At the completion of the incubation, the HOCs absorbed by the silicone rod are solvent extracted and measured by conventional instrumental analysis.

The application of silicone rods for the absorptive extraction and passive sampling of HOCs is well established, fully operational.[21, 22] Also the application of a sorbent material in an *in vitro* digestion model as a kind of passive sampler has been reported earlier and improved the correlation of *in vitro* with *in vivo* data.[23] The novelty in the current paper is the combination and careful alignment of a bioaccessibility extraction method with a modern absorptive sample preparation method. The simulated digestive fluids act as the mobilization medium that mimics the solubilization properties of the human digestive fluids. The silicone rod is dimensioned to serve as a high capacity absorption sink and analytical enrichment phase within the bioaccessibility extraction method. The silicone rod therefore yields measurements of accessible rather than total concentrations. We have recently reported on the integration of the silicone rod in cyclodextrin extractions, where it resulted in markedly higher estimates of microbial bioaccessibility.[24] The present study introduces this new concept for extractions with simulated digestive fluids within the area of human bioaccessibility research. For that purpose, polycyclic aromatic hydrocarbons (PAHs) were used as model compounds and the colon extended physiologically based extraction test (CEPBET)[15] as the

extraction model. The silicone rod was dimensioned based on silicone to fluid partition ratios, while the distribution of PAHs between the colloidal and aqueous phase in simulated fluids (speciation) was used to interpret absorption kinetics into the rod. Finally, the absorption sink was tested and this new configuration (sorptive CEPBET) was applied to soot and a kindergarten soil.

## Experimental section

Details of chemicals and materials used for passive dosing experiment, composition of recovery and internal standards and solvents used for rod and sample extraction are given in the Supporting Information (SI). The CEPBET[15]comprises three compartments: the stomach, the small intestine and the colon. Simulated digestive fluids of each compartment were prepared according to Tilson et al.[15] and their composition is given in the Supporting Information. The composition of these compartments has been validated against human subjects.

### *Contaminated solid matrices*

The method was applied to a wood soot and an urban surface soil sample, both containing elevated PAHs concentrations. The soot was selected as a relevant environmental matrix, since it is continuously produced from residential heating and it is one of the main PAH sources for urban soils.[25] Soot is also a worst case material for soil bioaccessibility extractions, due to its high $K_D$ values for PAHs.[26, 27] Soot material was sampled in 2009 from 8-10 family houses near Roskilde (Sealand, Denmark). This soot originated from wood fired stoves with a steel lined chimney and the microbial

PAH bioaccessibility of this material has been investigated in a previous study.[24] Total organic carbon content in soot sample was found to be 24.1%, a description of method analysis is given in supporting information. The surface soil originated from a Danish kindergarten yard, it was selected because children are more prone to accidentally ingest soil and it had PAH levels above Danish regulatory soil criteria. Atmospheric deposition of soot was estimated to be its main PAH source. Before use, soot was sieved through a 150 μm sieve and kindergarten soil through 250 μm sieve. Each collected fraction was well mixed before sub-sampling.

***Exhaustive extraction of PAHs***

PAHs were extracted from samples using a mixture of toluene and methanol (1:6), which provides higher extraction efficiency for soot-carbon materials compared to pure toluene.[28] 50 mg of soot (n=3) and 5 g of kindergarten soil (n=3), previously spiked with recovery standards, were Soxhlet extracted for 24 hours. Then solvent was evaporated to dryness and then switched to n-pentane, and then it was eluted through activated silica and activated alumina-B with n-pentane/dichloromethane. The obtained extract was evaporated under nitrogen, re-dissolved in 1 mL toluene and mixed with internal standards before GC-MS analysis.

***Absorption sink***

The silicone poly(dimethylsiloxane) (PDMS) was chosen as the absorption phase because of its well known partitioning properties,[29, 30] low internal diffusive resistance[31] and since its sorptive properties remain unaffected even in highly complex matrixes,[32] which is a crucial advantage due to the low pH and complex nature of simulated gut fluids. Several silicone formats were considered and the silicone rod was selected since

it allows the practical and efficient handling of a large silicone volume during bioaccessibility extraction and the subsequent back-extraction with a solvent. A silicone rod from Altec (Altec, Cornwall, United Kingdom) with a diameter of 3 mm (2.87-3.13 mm) was used as a sink. The rod mass was gravimetrically determined to 8.0 g/m, whereas the volume was calculated based on geometry to 7.3 $cm^3$/m, a detailed technical description of the rod is given in Gouliarmou et al. 2012.[24] Proper cleaning of the rod before its application is important to avoid analytical problems later. The reference cleaning method for silicone passive sampling materials at our laboratory is 100 hours Soxhlet extraction using ethylacetate. However, based on our previous experience we followed a less laborious procedure that was shown to be sufficient for the subsequent instrumental analysis by GC-MS.[24] Before use, the rod was cleaned by soaking once overnight in ethylacetate, followed by soaking three times overnight with methanol and then three times overnight with acetone. Finally, any adhering solvent was removed by at least four times overnight washes with Milli-Q water. Cleaned rods were stored until use in a sealed bottle with Milli-Q water.[24]

***Speciation of PAHs in simulated fluids***

Passive dosing was applied to determine 1) the distribution of PAHs between the colloidal and aqueous phase in simulated gut fluids (speciation) and 2) the silicone to fluid partition ratios ($K_{silicone,fluid}$). The passive dosing method enables one to study speciation of PAHs at a controlled freely dissolved concentration, the method has been described in detail by Gouliarmou et al.[33] Briefly, 500 ± 5 mg of medical grade silicone was cast into the bottom of 10 mL glass vials. The silicone in one group of vials was loaded with a mixture of naphthalene (NAPH), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLU) and pyrene (PYR), while the silicone in a second group

of vials was loaded with only benzo(a)pyrene (BaP). The loading level for the PAH mixture was set to 10% of saturation for each individual PAH, meaning that concentrations in silicone and water were at 10% of the respective water solubilities. BaP was loaded to 100% saturation, meaning that experiments were conducted at 100 % water solubility in both silicone and water.

The loaded vials were then equilibrated sequentially with water (Milli-Q), simulated fluid and again water. Equilibration was performed in triplicates by shaking overnight at 1000 rpm and 37 ± 1 ºC, which based on a 3 days pre-experiment was proven to be sufficient to reach equilibrium (SI Figure S1). PAH concentrations in the fluids ($C_{fluid}$) and water ($C_{water}$) were measured and free fractions (*ff*) were quantified according to equation: $ff = C_{water} / C_{fluid}$.[33] At the end of the experiment, PAHs were extracted from the silicone with two times 8 mL of methanol (overnight) and then measured by HPLC. Silicone to fluid partition ratios were determined as ratios of measured concentrations in silicone and the respective equilibrated solutions, which were then used for the subsequent sink dimensioning as well as the calculation of enhanced capacities.

***Sink dimensioning and enhanced extraction capacity of sorptive-CEPBET***

We define the absorption efficiency of the sink (*F*) as the fraction of the analyte that is absorbed by the sink from the simulated fluid. The predicted absorption efficiency at the thermodymamic equilibrium is given by:

$$F = \frac{m_{PAH\,(silicone)}}{m_{PAH\,(total)}} = \frac{1}{1 + \frac{V_{fluid}}{K_{silicone,\,fluid} \cdot V_{silicone}}} \qquad (1)$$

where $m_{PAH(silicone)}$ is the PAH mass absorbed by the rod and $m_{PAH(total)}$ is the initial PAH mass added in the simulated fluid. $V_{fluid}$ and $V_{silicone}$ are the volumes of simulated fluid and silicone rod, respectively.

The silicone rod volume required to obtain a given absorption efficiency ($F_{required}$) can then be calculated:

$$V_{silicone} = \frac{F_{required} \cdot V_{fluid}}{(1 - F_{required}) \cdot K_{silicone,\ fluid}} \quad (2)$$

In the present study we aimed for sufficient silicone volume to absorb 90% or more of mobilized analytes from each simulated fluid.

The inclusion of the sink increases the equivalent volume of the simulated fluid ($V_{equivalent}$) and thus the capacity of the system (simulated fluid + silicone) to receive PAHs. The enhanced capacity (EC) of the sorptive-PBET system relative to a PBET with the same simulated fluids is given by Eq. 3:

$$EC = \frac{V_{equivalent}}{V_{fluid}} = \frac{V_{fluid} + V_{silicone} \cdot K_{silicone,\ fluid}}{V_{fluid}} \quad (3)$$

The silicone rod should preferably also have a capacity for the analytes that exceeds the capacity of the sample. This will not always be strictly necessary within a dynamic system, as long the silicone rod operates in the kinetic regime and thus efficiently removes the analytes from the solution. The silicone mass was in the present study provided higher capacity since it was about 100 times higher than the organic carbon content of the sample. For sample types characterized by very high $K_D$ values, it can make good sense to further increase this ratio by simply decreasing the mass of the sample, which however will lead to lower analytical sensitivity of the method.

***Elimination kinetics and absorption efficiency of the sink***

The new sink had to be characterized and confirmed in terms of absorption kinetics and efficiency before its application into the CEPBET. Thus, an experiment was carried

out to assess 1) how fast PAHs were transferred from each fluid into the rod and 2) the experimental absorption efficiency of the rod at equilibrium. Experiments were performed separately for each simulated fluid (stomach, small intestine and colon), in parallel batch set ups with two replicates per fluid. To this end, 80 mL of fluid (37ºC) were spiked with 0.5 mL of a methanol solution containing the 6 PAHs yielding initial PAH concentrations between 150-300 μg/L. The fluid together with 2 m of silicone rod was placed into a 100 mL Pyrex bottle and closed with Teflon (PTFE)-lined screw caps. Bottles were shaken at 150 strokes/min inside the water bath (Grant OLS 200, Cambridge, England) at 37 ± 1 ºC. Sub-samples of 0.5 mL from each fluid were taken at predetermined time points, then extracted and analyzed by HPLC as described later. The PAH fraction that remained in the fluid at extraction time t ($F_{fluid(t)}$) was plotted against time and a one phase elimination model with plateau was fitted to the data:

$$F_{fluid}(t) = (1 - F_{fluid}(eq)) \cdot e^{-k_1 \cdot t} + F_{fluid}(eq) \qquad (4)$$

$F_{fluid(eq)}$ denotes the PAH fraction left in fluid at equilibrium and $k_1$ is the rate constant that characterizes the absorption kinetics into the silicone rod. Data were fitted by least squares using Graphpad Prizm 5 (San Diego, CA), the time to reach 95% of $F_{fluid(eq)}$ was calculated ($t_{95\%} = \ln(20)/k_1$ ), while the rod absorption efficiency was calculated based on a complete mass balance assumption ($F_{rod} = 1 - F_{fluid(eq)}$).

***Application to contaminated solid matrices and absorption efficiency***

*Application to contaminated solid matrices:* After sink dimensioning and confirmation, soot (n=3) and kindergarten soil (n=3) were incubated using the CEPBET with the silicone rod as an absorption sink. Sequential incubations were performed, where 100 mL glass bottles containing 1 g of sample, 2 meters of silicone rod and 80 mL of simulated stomach fluid ( pH 2.5 ) were placed in a shaking water bath (Grant

OLS 200, Cambridge, England) at 150 strokes/min and 37ºC. After 1 hour of incubation, stomach fluid was converted to small intestine fluid by pH adjustment to 7 and addition of 0.14 g bile salts and 0.04 g pancreatin, then bottles were resealed and incubation continued for further 4 hours. Finally, transition from small intestine to colon compartment was achieved by physical transfer: first the test substrate was recovered by centrifugation (3000g, 10 min), then substrate was added together with 80 mL colon fluid back to the bottle containing the rod and incubation continued for further 16 hours. Before addition to the test, all fluids were preheated in the water bath to 37ºC.

*Absorption efficiency:* To determine absorption efficiency of the rod for individual PAHs, the simulated fluids were spiked with a mixture of non-label PAHs and extracted with silicone rod but without the presence of the sample, while sample incubations were run in parallel and under identical experimental conditions.

Thus 80 mL of stomach and colon fluid (n=2, 37ºC) were spiked with 50 µL of a methanol PAH standard solution, initial PAH concentrations in spiked fluids ranged between 6-8 µg/L. Absorption efficiencies were determined separately for the stomach + small intestine compartments and for colon compartment. At the end of the incubations, rods were rinsed with Milli-Q-water and wiped with a lint free tissue. Then, recovery standards were added to the rods before they were extracted twice with 100 mL of acetone (>7 hours and then overnight). The two extracts were combined and evaporated under nitrogen, re-dissolved in 1 mL toluene and mixed with internal standards before GC-MS analysis. Acetone was chosen as the extraction solvent since it causes less swelling of silicone compared to other non polar solvents[34] and it is easier to evaporate relative to other polar solvents. The high extraction efficiency of this solvent extraction of the rods was previously verified.[24]

### *Instrumental analysis*

*HPLC analysis:* All samples from the passive dosing and sink confirmation experiments were mixed 1:1 (water, stomach fluid and small intestine fluid samples) or 1:2 (colon fluid) with methanol for preservation and extraction of analytes from matrix. Colon fluid samples were vortexed for 1 minute, then left overnight in the freezer and the supernatant was transferred to autosampler glass vials. The extraction efficiency of the method was tested and ranged between 98% and 120% (Table S2). These samples were then kept at -18ºC until analysis by HPLC with multiband fluorescence detection (Agilent 1100 HPLC equipped with a G1321A FLD operated at Ex: 260 nm and Em. 350, 420, 440 and 500 nm). Details are given in Gouliarmou et al 2012.[33]

*GC-MS analysis:* PAHs from rod, soot and kindergarten soil extracts were quantified using a Thermo Finnigan Trace 2000 GC with a 30 m x 0.25 mm x 0.25 μm 5% -Phenyl- methylpolysiloxane capillary column. Details are given in Gouliarmou and Mayer 2012. [24]

## Results and discussion

### *Speciation of PAHs in simulated fluids*

Free fractions (*ff*) of PAHs in the three simulated fluids were measured with passive dosing and then plotted against octanol – water partition ratios (Figure 1a). Free fractions ranged from 0.13 (BaP) to 1.00 (NAPH) in stomach fluid, from 0.08 (BaP) to 0.92 (NAPH) in the small intestine fluid and from 0.0016 (BaP) to 0.72 (NAPH) in the colon fluid. The somewhat lower free fractions in the small intestine and colon fluid were expected, since the addition of bile salts pancreatin and carbohydrates increases PAH binding.[1,15] The colon has the highest levels of these components (see Table X

Supporting Information). All measured free fractions were characterized by high precision with relative standard errors of typically less than 3.5 % (range of 0.9 – 8.7 %). Additionally, the earlier experienced analytical difficulties when applying non-equilibrium SPME to measure HOC speciation in simulated gut fluids[35] were efficiently circumvented, since the passive dosing technique was operated in the equilibrium regime and did not involve measurements on micrometer thin polymer coatings.[33]

The PAH - specific silicone to fluid distribution ratios ($K_{silicone,fluid}$) for the three fluids were measured and then plotted together with the $K_{silicone,water}$ against Log $K_{ow}$ (Figure 1b). Distribution ratios for stomach and small intestine fluid were rather similar and both characterized by a continuous increase from the least hydrophobic NAPH (558 L/L) to the most hydrophobic BaP (22 086 L/L). Silicone to colon distribution ratios were generally lower and did not show the same trend with PAH hydrophobicity. They were rather constant and ranged from 320 L/L (BaP) to 570 L/L (PHEN), which reflects the richer composition of the colon fluid.[15] These partition ratios indicate that silicone indeed can act as an efficient PAH absorption sink and simultaneously increase the capacity of the system (fluid + silicone) to receive PAHs.

***Sink dimensioning and enhanced extraction capacity of sorptive-PBET***

The required silicone volume to achieve a given equilibrium absorption efficiency was calculated (Eq. 2) using the measured $K_{silicone,fluid}$ values as input variables. Approximately, 30 cm of rod was estimated to be necessary to absorb at least 90% of all PAHs in the three fluids (80 mL) (Figure S3). However a 2 meter silicone rod (14.7 mL) was selected in the present study with the CEPBET method, which was found practical and was expected to provide faster absorption kinetics due to its higher surface

area (A= 377.4 $cm^2$ vs 57.1 $cm^2$ of 0.3 m rod). The required silicone volume for other bioaccessibility extraction methods can easily be adjusted and calculated by Eq. 2.

Based on the passive dosing results and Eq. 3, the inclusion of the silicone rod increased the capacity of the system to receive PAHs by 1-3 orders of magnitude (Figure 2). The enhanced capacity of the sorptive-CEPBET (fluid + silicone rod) relative to the simulated stomach fluid ranged from 105 (NAPH) to 4567 (BaP), and for simulated small intestine fluid from 103 (NAPH) to 4060 (BaP). The enhanced capacity was for both solutions analyte specific and increased with hydrophobicity of the PAHs (Figure 2). These results practically mean that the inclusion of the 14.7 mL of silicone rod resulted in an increase of extraction capacity for BaP, which is equivalent to a fluid volume of 365 L (= 80 mL × 4567) for stomach fluid and 325 L (= 80 mL × 4060) for small intestine fluid. The enhanced capacities for colon were generally lower ranging from 60 (BaP) to 110 (ANTH) (Figure 2), since the fed state of the colon compartment already provided a high capacity. It is important to note that the increase in capacity not only depends on the dimensions and characteristics of the absorption sink, but also on the volume and composition of the fluids as well as the analytes under consideration.

***Elimination kinetics and absorption efficiency of the sink***

In the present study, the silicone rod served two distinct purposes: (1) maintaining the diffusion gradient for the PAH desorption from the matrix and (2) enrichment of the analytes for subsequent back-extraction and instrumental analysis. For the first purpose, freely dissolved concentrations have to be kept at a very low level while for the second purpose it is the total concentrations in the medium that needs to be efficiently depleted. Elimination kinetics were determined on the basis of total

concentrations in the medium, and the translation to freely dissolved concentration can easily be done by multiplication with the free fractions from the passive dosing experiment.

The elimination kinetics of the 2 meter silicone rod for PHEN, PYR and BaP and all three fluids are shown in Figure 3, and are given for the other PAHs in SI (Figure S4). In stomach and small intestine compartment the elimination kinetics for all PAHs except BaP were fast and shorter than the incubation time. As Figure 3 shows, within 20 - 27 minutes the major PAH fraction was transferred from stomach and small intestine fluid to the rod, however BaP exhibited slower kinetics with $t_{95\%}$ being 4.1 h in stomach and 1.8 h in small intestine compartment (Figure S5). From a practical point of view the slower elimination of BaP in the stomach compartment (>incubation time) does not pose any problem, since stomach compartment is converted to small intestine by the addition of bile salts and pancreatin and the total incubation for stomach and intestine (1+4 hours) was sufficient. Also, the conversion of stomach fluid to small intestine accelerated the elimination kinetics for all PAHs and especially for the most hydrophobic BaP ($t_{95\%}$ = 1.8 h) (Figure S5). The reason is that the added bile salts lead to the formation of micelles, which act as diffusive carriers and facilitate the PAH transport through the aqueous unstirred boundary layer adjacent to the silicone rod. This is supported by earlier studies, which showed that 1) HOCs can be encapsulated into micellar hydrophobic cavities formed by bile salts[36, 37] and 2) bile micelles can act as a vehicle for PAHs and traverse the aqueous unstirred boundary layer adjacent to the intestinal wall of rats.[38] For both fluids, the elimination kinetics were found sufficient with regards to both maintaining the desorption gradient and transferring PAHs to the analytical sorbent.

Elimination kinetics of the colon compartment were slower compared to the other two compartments, which was compensated by the longer incubation time. $T_{95\%}$ in colon ranged between 0.84 h (NAPH) to 3.95 h (PYR) and BaP was again the slowest compound with a $t_{95\%}$ of 17.1 h. As Figure 4 shows, the elimination time generally increased with an increase in the colloidally bound fraction. This occurs becausecolloidal binding decreases the freely dissolved concentration of PAHs in the fluid and consequently the chemical activity gradient that drives the diffusive uptake into the rod. In the case of the colon compartment it is necessary to discuss both functions of the silicone rod: (1) Most importantly, in terms of maintaining the concentration gradient for the desorption, the method worked perfectly. The combination of rich colon medium and silicone rod effectively kept freely dissolved concentrations at an absolute minimum. (2) With regards to the efficient transfer of PAHs to the silicone rod, colon constituents and silicone rod apparently competed against each other, which slowed down the uptake into the rod. Fortunately, the elimination kinetics were generally still sufficient, but a faster elimination is desirable since it would enhance the performance and robustness of the method.

The absorption efficiency at equilibrium was high for all PAHs and all simulated fluids. This was confirmed by the level of the plateau reached with time in the elimination kinetics experiment (Figure 3, Figure S4). At equilibrium more than 97% of each PAH was absorbed from the simulated gastrointestinal fluids (Figure 3, Figure S4), which was in good agreement with the predicted absorption efficiencies (Figure S3).

In summary, the initial experiments confirmed that 2 meters of silicone rod provided sufficiently fast absorption kinetics and very high absorption efficiencies (>97%). The freely dissolved PAH concentrations were kept at a very low level and the gradient for

the desorption from the soil matrix was maintained. This supports the use of a silicone rod as an efficient analytical enrichment phase that rapidly absorbs PAHs from the solution. However, the combination of hydrophobic analyte and the very rich colon medium lead to somewhat slower absorption kinetics. These kinetics can be further optimized by reducing the medium volume or by reducing the binding within the colon medium. However, both of these optimization options were outside the scope of the present study to integrate the silicone rod into an existing PBET model.

***Application to environmental contaminated solid matrices and absorption efficiency***

*Absorption efficiency*. The measured absorption efficiencies quantified the analyte recovery between the spiking of the digestive solutions and the solvent extract of the silicone rods and thereby incorporated the efficiency of the rod extraction. Absorption efficiencies for the stomach and small intestine compartment was 55.5 – 86.3 %, and for the colon compartment 69.3 – 88.3 %. These values were generally lower than in the initial experiments (>97 %; Figure S4). The somewhat lower efficiencies in the final experiment can be explained by a combination of: (1) theyincluded the efficiencies for the rod extraction, (2) they were calculated as ratio between spiked and measured level rather than the concentration decrease in the solution and (3) they were measured at lower PAH concentrations (6-8 µg/L instead of 150 - 300 µg/L). The concentration level is relevant in this respect, since binding to proteins can increase with decreasing analyte concentration.[39]

The inclusion of an analytical enrichment phase brings new possibilities with regards to applying stable isotope standards into bioaccessibility extractions. They can be added (1) prior to the incubation of the matrix in the digestive fluids, (2) at the beginning of

the rod solvent extraction, (3) to the final rod extract or (4) to the rod before applying it in the test. Thus the efficiency of each step can be determined within sorptive bioaccessibility extractions, these data can be used for optimization, validation and dedicated process studies. The addition of standards to the digestive fluids at the beginning of the incubations seems presently the most practical approach, since it allows recovery determinations and corrections, while avoiding additional treatments required for the parallel incubations.

*Application to environmental contaminated solid matrices.* In the present study, all bioaccessibility measurements obtained with sorptive-PBET (Figure 4) were corrected for absorption efficiencies. The correction was chosen in order to account for both absorption and extraction efficiencies. This might led to a slight overestimation, which would bias the results in the conservative direction and was thus considered acceptable.

Mean values and standard errors (n=3) of the mobilized PAH fractions under digestive conditions were plotted for soot and kindergarten soil in Figure 4, together individual PAH fractions mobilized from soot when the sorptive bioaccessibility extraction (SBE) method, with cyclodextrin as a mobilization medium, was applied for 24 hours at room temperature. Results were expressed as the mass of PAHs absorbed by the sink relative to the initial PAH mass present in the matrix, while absolute accessibilities are given in Table S6. Measured fractions were precise with relative standard errors of typically less than 5.1% for soot and 13.3 % for kindergarten soil. For both matrices, mobilized fractions decreased with increasing PAH ring number (Figure 4) and ranged within 6 – 49.7 % and within 7.6-55% of total PAHs for kindergarten and soot respectively.

Measured mobilized fractions of the lighter PAHs were similar both for the SBE and sorptive-PBET method. However for the heavier PAHs, SBE yield 36% (PYR) – 73%

(D[a,h]A) higher bioaccessibility estimates than sorptive-CEPBET (Figure 4). These results emphasize the crucial role of the mobilization medium into sorptive bioaccessibility extraction methods. In SBE cyclodextrin was employed as a mobilization medium, which is an effective carrier for hydrophobic compounds and was previously shown to equalize desorption rates over a large hydrophobicity range, whereas in the sorptive-CEPBET desorption of PAHs depends on the composition of the simulated fluids.

***General features***

Silicone rod can serve as a high capacity absorption sink for HOCs in physiologically based extraction tests. The mobilization step of the pollutant from the soil matrix might appear to be most crucial, but will often be dependent on the efficient removal of desorbed pollutants by the second absorption step. In the present study, we showed that partitioning into a simple silicone sink can be used for the continuous absorption from simulated fluids to ensure (near) infinite sink conditions. This makes the method suited even for pollutant-sample combinations that are characterized by high $K_D$ values, such as historically contaminated soil, soot and charcoal.

The sorptive extraction into the silicone isolates target compounds from the solution matrix, which has two practically advantages: 1) No additional phase separation steps, such as filtration or centrifugation, were required prior to the extraction and instrumental analysis of the PAHs. This saves time and circumvents some of the uncertainties connected to the question of which PAH aggregate form or size actually is contributing to bioaccessibility.[16] 2) The final extract of the silicone rods will contain markedly reduced concentrations of interfering constituents originating from the soil

and the digestive fluids. In the present study, the obtained extracts could thus be analyzed without additional clean up steps.

Application of the analytical principle to other bioaccessibility extraction schemes and other solid matrixes such as sediment, sludge and biochar should be relatively simple. The applicability to other organic pollutants is also possible and probably mainly restricted by the ability of the rod to absorb them. The silicone rod is expected to be suited as high capacity sink for most hydrophobic organic compounds (Log $K_{ow}$ > 3-4),[34] which includes for instance brominated flame retardants, dioxins, chlorinated insecticides and pyrethroids. But, it should always be kept in mind that proper dimensioning and confirmation of the sink is important for valid measurements. The incorporation of an absorption sink will often be less necessary for more polar compounds including a wide range of degradation products, since aqueous fluids can offer infinite bath conditions and maintain the desorption gradient for such compounds.

Hopefully, the inclusion of the silicone sink will enhance bioaccessibility research by improving the performance and mechanistic basis of physiologically based extraction tests for hydrophobic organic chemicals. The new approach will generally lead to higher and more conservative bioaccessibility estimates, which should facilitate regulatory acceptance. Comparison and validation of sorptive physiologically based extractions with animal feeding studies are now needed.

ASSOCIATED CONTENT

**Supporting Information**

Additional graphs and tables as referenced in this article. This material is available free of charge via the Internet at https://pubs.acs.org

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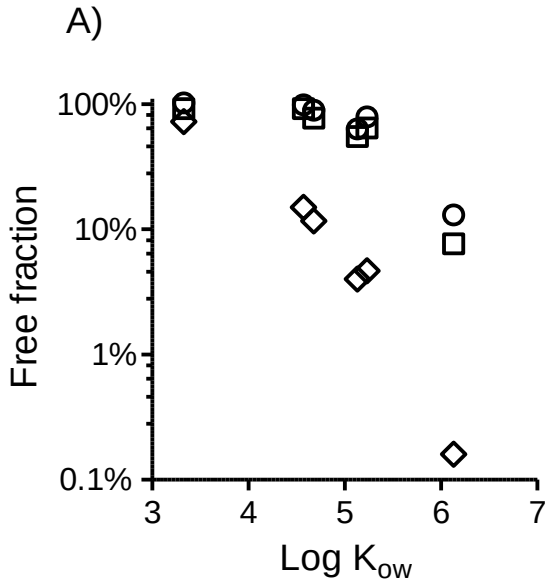


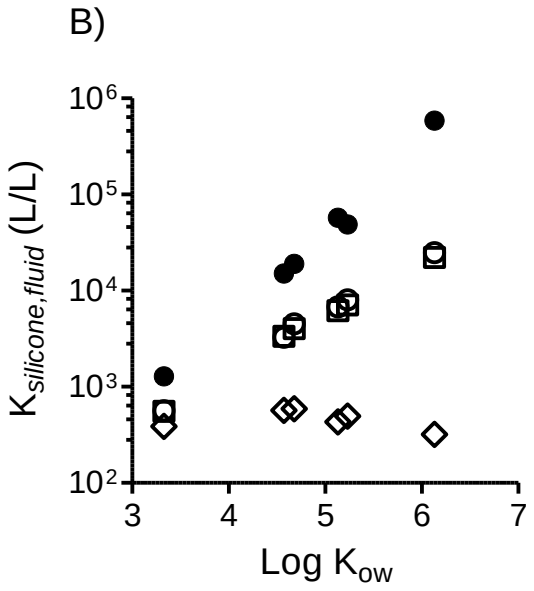



**Figure 1.** A) Free fractions of PAHs in simulated gastro-intestinal fluids obtained by passive dosing at 37°C, plotted against Log $K_{ow}$. Data show mean ± SEM, n = 3 and B) silicone to simulated fluid distribution ratios as measured in the present study and silicone to water distribution ratios obtained from Smedes et al. Data show mean ± SEM, n = 3 and were plotted against Log $K_{ow}$, stomach (○), small intestine (□)colon (◊)water (●).

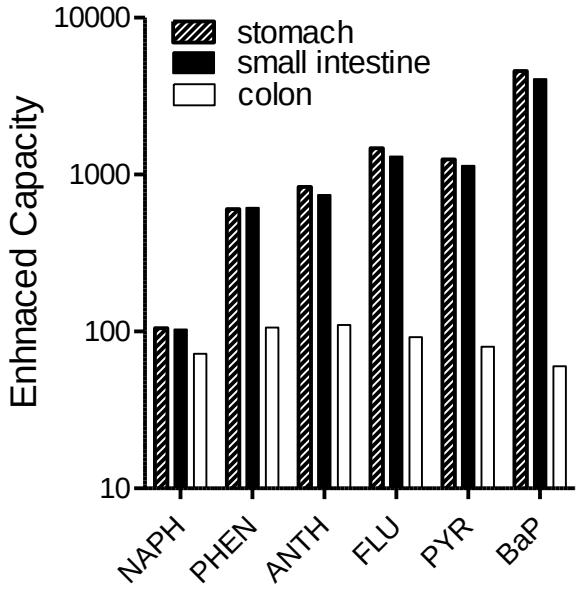
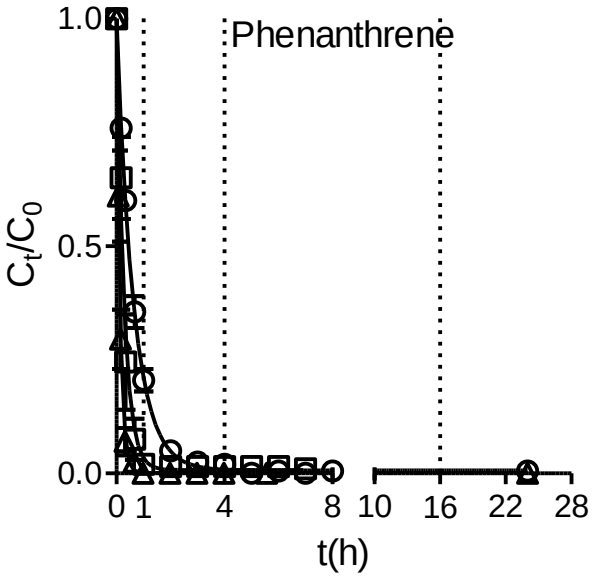



**Figure 2.** The enhanced capacity (EC) of the sorptive-PBET system (80 mL fluid + 14.7 mL silicone rod) relative to a PBET with the same simulated fluids (80mL fluid) is plotted for individual PAHs.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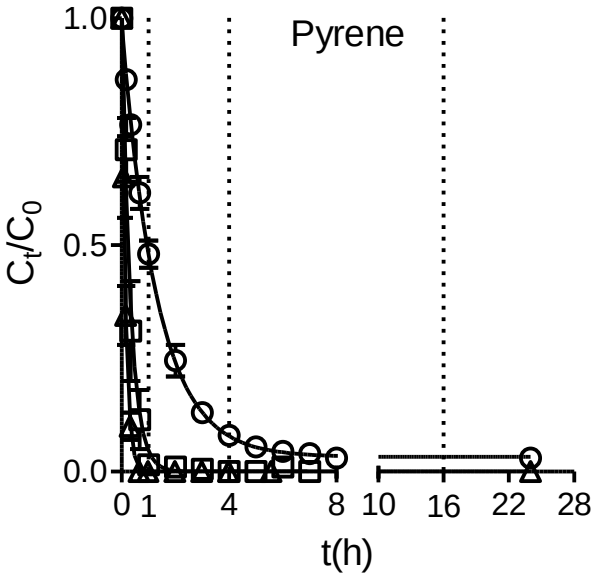
0.5
0.0
$C_t/C_0$
0 1 4 8 10 16 22 28
t(h)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0.0
$C_t/C_0$
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t(h)

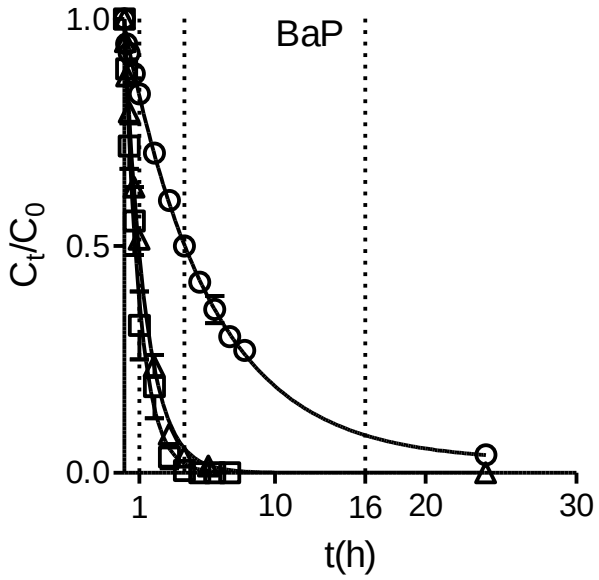


**Figure 3.** Elimination kinetics of PAHs from spiked simulated fluids into silicone rod. stomach (Δ)small intestine (□)fluid and circle colon (○). Data show mean PAH fraction of initial spike left in simulated fluid after time (t) of shaking at 37oC ± SEM (n= 2). Dashed lines indicate the incubation times in stomach (t=1), small intestine (t=4) and colon (t= 16) compartment of the extraction protocol.[15]

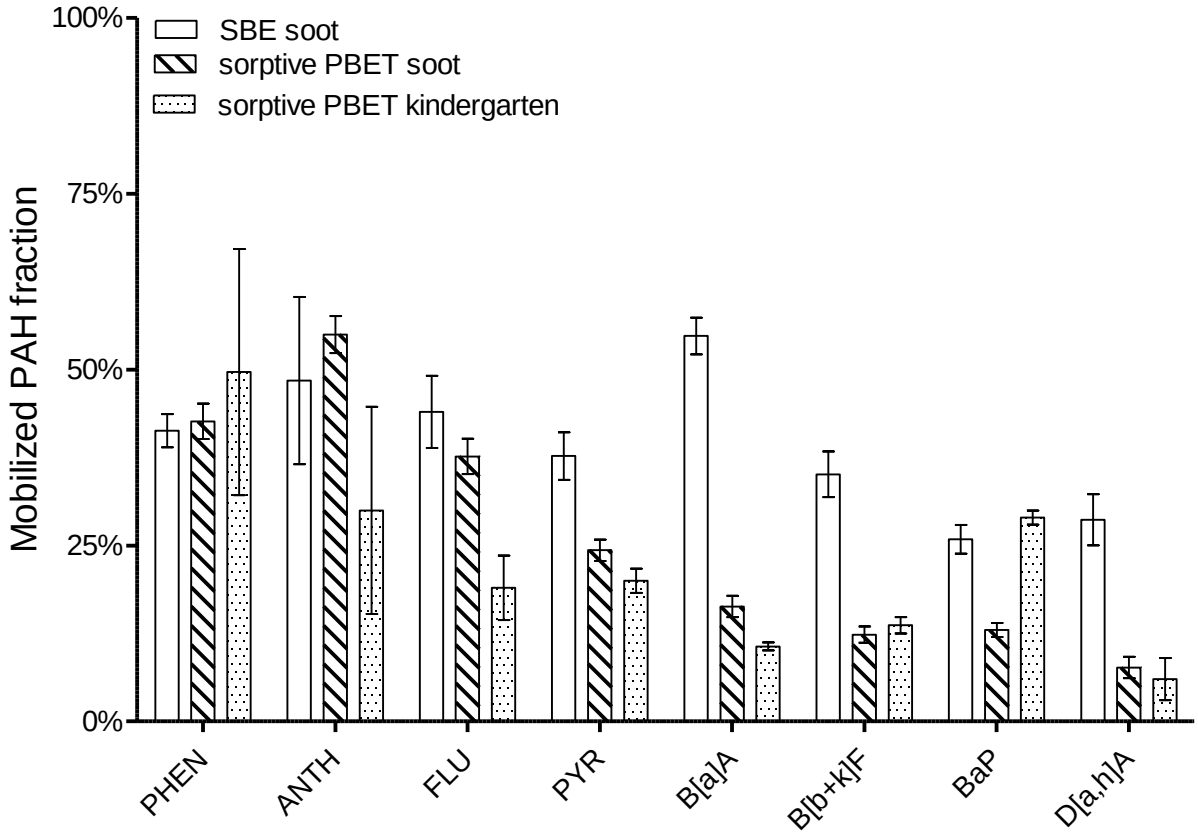


**Figure 4.** PAH fractions mobilized under *in vitro* digestive conditions and measured by sorptive-CEPBET were plotted for soot and kindergarten soil, together with PAH fractions mobilized from soot measured by SBE method and 24 hours incubation time at room temperature. Data show mean ± SEM (n=3). Abbreviations: phenanthrene (PHEN), anthracene (ANTH), fluoranthane (FLU), pyrene (PYR), benzo[*a*]anthracene (B[*a*]A), benzo[*b*+*k*]fluoranthene (B[*b*+*k*]FLU), benzo[*a*]pyrene (BaP), dibenzo[*a,h*]anthracene (D[*a,h*]A)

TOC/Abstract Art