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Relative Importance of Ingested Sediment and Pore Water as Bioaccumulation Routes for Pyrene to Oligochaete (*Lumbriculus variegatus*, Müller)

MATTI T. LEPPÄNEN* AND JUSSI V. K. KUKKONEN

University of Joensuu, Department of Biology, P.O. Box 111, FIN-80101 Joensuu, Finland

It is generally accepted that sediment ingestion is an important route in accumulation of highly hydrophobic sediment-bound contaminants. The significance of this route is, however, difficult to quantify reliably. For this purpose, the relative importance of pore water and ingested sediment as sources was studied by exposing individual oligochaetes of different size to radiolabeled pyrene spiked lake sediment for 28 days. Simultaneously, their ingestion behavior (egestion rate) was followed. The design allowed comparison of the bioaccumulation process between individuals ingesting and noningesting sediment. Pyrene accumulated mainly through ingested material. After 8 days of exposure, approximately 61% of the body burden had accumulated via ingested material. Uptake clearance rates differed between worm groups, which started sediment ingestion at different points of time. This was probably due to decreasing bioavailability. The data signify the importance of ingested material in bioaccumulation of hydrophobic chemicals in deposit feeders. The method offers a biologically sound and reliable tool for assessing the bioavailability of chemicals from pore water and ingested sediment for *Lumbriculus variegatus*.

Introduction

Standard bioaccumulation experiments (*1*) provide information on the potential of organisms to accumulate sediment associated contaminants. The resulting body burdens are known to be influenced by sediment and chemical characteristics, and also the organisms themselves [see review by Landrum and Robbins (*2*)]. For compounds with log K_{ow} < 5, the major route for the accumulation is pore water (*3, 4*). For more hydrophobic compounds, the contribution of ingested material in accumulation increases (*5*−*8*). For example, a recently presented model for predicting bioaccumulation of organic chemicals by benthic organisms signifies the relevance of feeding mode and diet in this process (*9*). Better knowledge of the relative importance of different uptake routes in accumulation would help researchers to understand more precisely the circumstances in bioavailability and to formulate more precise accumulation models. However, the significance of different routes in accumulation process is difficult to study with satisfaction, and new approaches are needed.

* Corresponding author fax: +358 13 251 3590; e-mail: mtl@cc.joensuu.fi.

Lumbriculus variegatus has been commonly used in bioaccumulation assays during the recent years (*8, 10*−*12*). It fulfills the criteria set for a test organism (*13*), and it is exposed to all relevant accumulation routes. Its surface egestion behavior can also be used to estimate sediment ingestion rate. Our previous results (unpublished data) have shown that this endpoint was sensitive to sediment characteristics and that sediment ingestion was completely ceased during reproduction by architomy, a feature which was employed in the present work.

The primary objective of this study was to compare the significance of pore water and ingested sediment as accumulation routes of sediment-bound pyrene in *Lumbriculus*. A 28-day bioaccumulation experiment with a radioactive chemical was established to follow the relationship between sediment toxicant concentration and organism accumulation. Together with the tissue concentration analyses, the egestion rate of individuals with different sediment ingestion behavior was followed to determine the duration of the noningesting period and the onset of sediment ingestion as well as to compare possible relationship between gut passage time and accumulation of contaminants.

Materials and Methods

Test Rationale. Architomic reproduction mode of *L. variegatus* induces worms of sufficient size to fragment into two parts approximately from the middle of the body. After fragmentation, new individuals regenerate fresh segments for tail (former anterior end) and head/prostomium (former posterior end). During this process, worms do not ingest sediment, and this pause has been observed to last approximately 6−7 days with posterior end in Lake Höytiäinen sediment. It is also quite common that the posterior part divides further, resulting in the pause in feeding to prolong 10−12 days with the new posterior part (unpublished data). *Lumbriculus* feeds in subsurface sediment and egests all ingested material on the sediment surface, which allows simultaneously both the quantification of egestion rate and the detection of onset of ingestion.

These features in reproduction and feeding behavior were used as a basis for experimental design. Three worm groups were established to study the significance of sediment ingestion on accumulation of pyrene (Figure 1). Group 1 consisted of the smallest worms [5−9 mg wet weight (ww)] found in the culture aquarium. Previous experiments have shown that worms of this size seldom divide during a 28-day test and that they start to feed almost immediately in the sediment. To establish the second group, 85 large worms [12−21 mg wet weight (ww)] were selected from the same culture and placed individually into beakers with clean, unspiked Lake Höytiäinen sediment. After 2 days, most of the worms had divided and the posterior ends were selected for the bioaccumulation test. The group 3 worms were born during the accumulation test from group 2 posterior worms by architomy 5−7 days after the beginning of the exposure. Only 17 out of 80 worms in group 2 reproduced twice. This second fragmentation took place before they started ingestion, judging from the empty guts and lack of faecal pellet production. The second fragmentation was noticed by two protruding tails above the surface. The new posterior parts (group 3 worms) were sieved and placed to new beakers on day 8 and anterior parts were discarded. As a result of this procedure, we had worms which started ingestion at the beginning of the test (group 1 = G1), after the test day 6 (group 2 = G2f), or after the test day 9 (group 3 = G3) but they all had an equal opportunity to accumulate pyrene from

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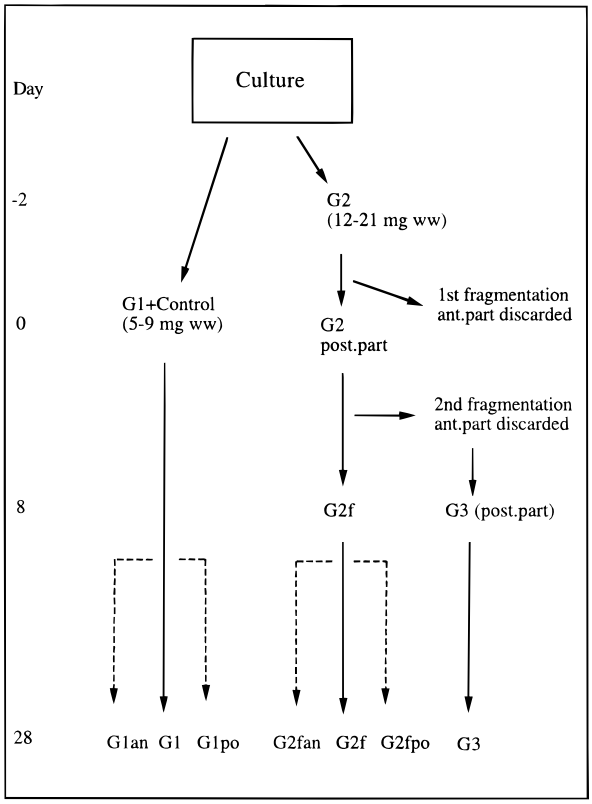

FIGURE 1. Test rationale and representation of selection process for the posterior parts of worms after architomic reproduction. Day zero denotes the start of the exposure. Dashed lines represent fragmentation of G1 and G2f worms during the second week of the test. Resulting anterior and posterior fragments were not used in any of the analyses.

the very beginning of the test.

Sediment Spiking. Pump-collected, fine-grained sediment from Lake Höytiäinen (an unpolluted, oligotrophic lake in Eastern Finland) was used as a test medium [86% of dry weight (dw) belonging to size class < 63 μm, TOC 3.6%]. The sediment has low concentrations of PCBs (6.5 pg g^{-1} dw as TEQs) and PAHs (total 250 ng g^{-1} dw) which are delivered by atmospheric transport (*14*). Also, metal concentrations are typical for an unpolluted lake (*14*).

Wet sediment (3010 g) was spiked with pyrene-4,5,9,10-^{14}C (Sigma Chemical Co., St. Louis, specific activity 32.3 mCi $mmol^{-1}$) in two aliquots. The chemical dissolved in ethanol (activity 0.03 Ci μL^{-1}) was added dropwise to sediment while mixing. The aliquots were mixed for 4 h at room temperature with a specially designed rotating metal blade. After mixing, aliquots were combined and stored at +5 °C in dark for two weeks before use. As a result of spiking, concentration of radiolabeled pyrene was 901 ± 27 pmol g^{-1} dw (65 000 ± 2000 dpm g^{-1} dw, n = 3) based on liquid scintillation counter (LSC) measurements. A control sediment was prepared with the same manner adding same amount of ethanol (0.26 μL g^{-1} ww) during the mixing. The purity of pyrene stock solution was 96.5 ± 0.2% (n = 2) as determined with thin-layer chromatography and LSC. After run in hexane:benzene (4:1, v:v), the silica gel plate was divided to segments of uniform size, and the radioactivity of the scraped segments was counted by LSC. The segment of the highest counts also fluoresced at 254 nm.

Test Procedure. A 50 mL beaker with 20 mL of spiked or control sediment containing one worm was used as an experimental unit. Group 1 had 40 experimental units which were sampled randomly in triplicates during the 28-day accumulation experiment. Group 2 had 80 experimental units, 17 of which fragmented during the test days 5 and 7 and formed group 3. Also, these groups were sampled in triplicates. No additional nourishment was used during the experiment.

Each beaker had a 2−3 mm layer of combusted quartz sand on sediment surface. Egestion rate of each individual was followed during the whole test by collecting faecal pellets from the sand surface. The egestion rate was used as a surrogate of their sediment ingestion behavior. We assume that weighing faecal pellets represents ingestion rate of worms with reasonable accuracy, because assimilation efficiencies of deposit feeders are fairly low (*15*). This is probably emphasized with nonselective *Lumbriculus* feeding on sediment with low OC content. The pellet production of five worms in separate beakers with clean sediment served as controls for egestion rate.

The same artificial freshwater as in culture conditions [(*16*) pH 7.0, hardness 1.0 mmol L^{-1} as Ca + Mg] was used in the tests, except that the pH was adjusted to 6.5 (pH in sediment 6.2). Water renewal was done at the time of faecal pellet collection or at least every fourth day. Previous experiments with the same sediment had shown that oxygen saturation stays above 75% with this renewal scheme (unpublished data). Also, in the present experiment, saturation was above 85% when measured from control beakers. The measured low total ammonia concentration (0.1 mg L^{-1} in pore water) in this sediment should not affect worms (*17*). Samples from overlying water (6 mL) were taken at the time of faecal pellet collection from three replicate beakers and analyzed for pyrene.

Analyses. After sieving from the sediment, worms were purged in clean artificial freshwater for 10 h. Brooke et al. (*18*) suggest that 12−24 h is a sufficient period for gut purging. However, as *L. variegatus* is able to eliminate pyrene in a water-only system (*10*), although very slowly, a 10 h purge period was used in our test. After purging, the guts were mainly empty and the few sediment pellets left inside the worms were assumed not to have a significant effect on tissue concentrations.

Individual worms with empty guts were carefully blotted dry, weighed, and placed in LSC vials with 1 mL of tissue solubilizer (Lumasolve) and dissolved at 50 °C for overnight. Next day, 12 mL of LSC cocktail (Lumagel Safe) was added with 500 μL 1 M HCl. Vials were shaken cautiously and ^{14}C-activity was counted using LKB Wallac 1217 liquid scintillation counter after settling periods of 2 weeks and 2 months. Measurements from the 2 month settling period were used, since variability in counts between replicates was decreased. Triplicate sediment samples were taken on days 0, 14, and 28 and treated as worm samples before activity counts. Water samples (6 mL) were mixed with 6 mL of LSC cocktail and shaken to form a gel. Counts were obtained after a few days settling period. The data were corrected for quench using the external standards ratio method after correcting for background.

Lipids were determined using a microgravimetric method (*19*) from five worm groups: large (>12 mg ww) and small (5−9 mg ww) worms directly from culture (day −2), anterior parts of group 2 worms after second fragmentation (Figure 1) at test day 8, group 2f anterior fragments (G2fan in Figure 1) at test day 28, and group 1 posterior fragments (G1po) at test day 28. Single individuals in five replicates were used for each lipid group.

Model. The data from an accumulation experiment were fit for a two-compartment, first-order rate coefficient model with a special lambda (λ) value included to represent the decline in bioavailable fraction during the experiment (*7*).

$$C_a(t) = [k_s C_s(0)(e^{-\lambda t} - e^{k_e t})]/[k_e - \lambda]$$

where $C_a(t)$ is the concentration of the chemical in the organism (pmol g^{-1} wet animal) at time t, $C_s(0)$ is the initial chemical concentration in the sediment (pmol g^{-1} dry weight), λ is the rate constant which represent decline in bioavailable fraction (h^{-1}), k_s is the uptake clearance of the chemical from sediment (g dry sed g^{-1} wet animal h^{-1}), k_e is the elimination rate constant of the chemical (h^{-1}) and t is time (h). The λ was used because accumulation data suggested that bioavailable fraction of pyrene had decreased during the experiment. λ is the slope of the regression between natural logarithm of pyrene sediment concentration and time ($\lambda = 0.000\ 109\ 4$). Therefore, it actually represents decline in chemically extractable pyrene not in bioavailable fraction. However, it is assumed that there is a relationship between chemical extractability and bioavailability, although the fraction or rate of process is not necessarily the same (*7*). The elimination rate coefficient in G3 worms was close to zero, and an estimation of uptake clearance coefficient was also obtained by fitting a model without elimination rate constant.

The accumulation model assumes that sediment concentrations represent bioavailable fraction. However, in this study, no attempt was made to estimate true concentrations in source compartments. Therefore, uptake clearance and elimination coefficients do not represent correct estimates, but their mutual proportions are valid and comparable with each other. This statement holds only with worm groups of the same feeding behavior. Noningesting and ingesting worms probably have different source compartment concentrations and coefficients are therefore not comparable with each other.

The model also assumes that organisms do not biotransform pyrene, which is confirmed by Harkey (*20*). We also assume that the worms did not significantly gain weight during the test. The data were fit for the least-squares nonlinear regression method using SYSTAT (*21*).

Statistics. Uptake clearance and elimination constants were compared following the procedure described by Ratkowsky (*22*). Basic ANOVA and *t*-test were used for comparing means of lipid groups and worm tissue concentrations at the end of the test. Levene's test was used to check homogeneity of error variances. The least-squares linear regression was used to estimate the relationship between egestion rate and time in each sediment ingesting worm group. The regressions were calculated for the periods, when egestion increased with time (21−165 h for G1, 165−452 h for G2f and 281−504 h for G3 as recorded from the start of the exposure). The slopes of equations were compared by analysis of covariance (homogeneity of regression coefficients) and Tukey test (*23*). At each time point, data was obtained from the same individuals used for tissue concentration analysis. Significance level of 0.05 was employed to detect statistical difference.

Results

During the experiment, a small decline in pyrene concentration in sediment was observed. However, this was not significant at 5% probability level (the fit of linear regression: $F = 4.57$, df = 1,7, $p = 0.07$). The average chemical concentrations in overlying water were 0.16 ± 0.08 pmol mL^{-1}. On the basis of measurements from the overlying water and the water renewal schedule, it was estimated that approximately 1% of total pyrene was removed via water phase from beakers during the experiment. The removal of faeces was not assumed to affect the sediment chemical concentration, because *L. variegatus* ingests mainly bulk sediment (*24*). No mortality or sediment avoidance was observed during the test; on the contrary, reproduction by architomy was frequent. An unplanned reproduction in groups G1 and G2f was noted mainly during the second week

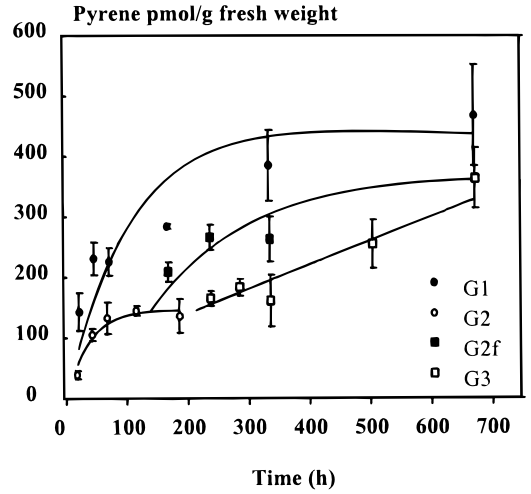

FIGURE 2. Accumulation of pyrene in sediment ingesting worms (G1, G2f, and G3) and noningesting worms (G2). Lines represent fit of the accumulation model and dots represent observed tissue concentrations (±SD, $n = 3$). The fifth observation in group 2 (186 h) consists of worms after second fragmentation (posterior parts = G3) which have not yet started sediment ingestion.

of exposure (Figure 1). Those individuals were not used in the accumulation calculations. Lipid content of worms (range 6.0−7.2%) did not change over the course of the test (ANOVA: $F = 1.05$, df = 4,19, $p = 0.41$).

The delay in the start of egestion occurred as expected with recently fragmented worms allowing comparison in accumulation between sediment ingesting (G1) and noningesting worms (G2) (Figure 2). Pyrene tissue concentrations in the noningesting worms (G2) increased slowly as compared to the ingesting worms (G1). Regeneration of the new head segments (prostomium) was completed after day 6 (144 h, G2f) or after day 9 (216 h, G3), and thereafter, both clearance and elimination constants differed between ingesting worms (k_s, df = 2,44; $F = 36.51$; $p < 0.001$ and k_e, df = 2,44; $F = 24.47$; $p < 0.001$) (Table 1). Group 1 and 2f reached apparent steady state at the end of the 28-day test but the pyrene body burdens in group 1 were significantly higher than in group 2f. λ correction was not included for these analyses and the mean pyrene sediment concentration was used. The change in constants, however, was minor (for example, the clearance constants without λ were $0.005\ 40 \pm 0.000\ 86$, $0.001\ 65 \pm 0.000\ 34$ and $0.000\ 29 \pm 0.000\ 08$ g dry sediment g^{-1} fresh worm h^{-1} for G1, G2f, and G3, respectively), and the effect on the interpretation of the results was assumed to be insignificant. If we assume that noningesting (G2) and ingesting (G1) worms differ from each other only in relation to their feeding behavior and that uptake from both sources is independent and additive, we can estimate the proportion of the chemical accumulated via pore water (plus via direct contact of integument with sediment particles). At day 8, 39% of pyrene had accumulated via pore water. At the steady state, estimation was not calculated since the concentration in source compartment could not be reliably estimated due to the decrease in the bioavailable fraction.

Egestion rates of worms were followed throughout the test; it was observed that the progress in egestion rates differed between the worm groups (Figure 3). The control worms reproduced unexpectedly during the second week, and their egestion rate was not followed thereafter. The total amount of faecal pellets produced by G1 worms was significantly lower during the first 9 days compared to control worms (*t*-test, $T = -2.313$; df = 12, $p = 0.04$). The decline in egestion rates in each worm group at a certain stage of exposure may be related to pyrene tissue concentrations. However, the

TABLE 1. Pyrene Uptake Clearance (k_s) and Elimination (k_e) Constants (±ASE) for Sediment Ingesting (G1, G2f, and G3) and Noningesting Worms (G2)[a]

vhd	k_s (g sed g^{-1} h^{-1})	k_e (h^{-1})	C_{D28}	T	df	p
G1	0.00476 ± 0.00071	0.00920 ± 0.00154	468 ± 84			
G2f	0.00159 ± 0.00033	0.00586 ± 0.00153	364 ± 50	2.65	11	0.02
G3	0.00027 ± 0.00009	−0.00262 ± 0.00132	362[c]			
G3[b]	0.00047 ± 0.00005					
G2	0.00437 ± 0.00061	0.02649 ± 0.00468	136 ± 28[d]			

[a] Final worm concentrations (C_{D28}, pmol g^{-1} ww ± SD) and t-test results between worm groups G1 and G2f are also tabulated. [b] k_s constant based on model without elimination constant, k_e. [c] A single observation. [d] Value at day 8.

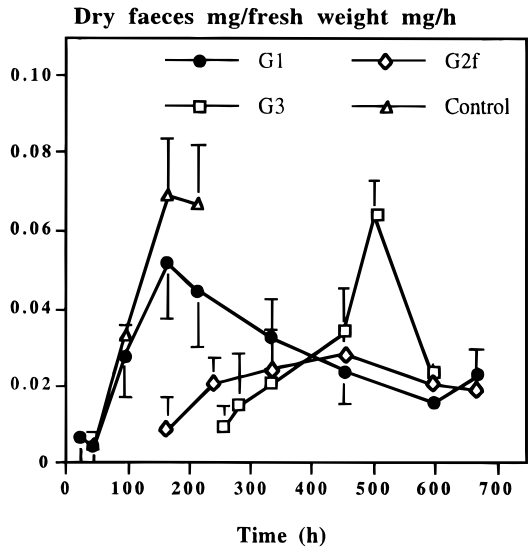

FIGURE 3. Onset and progress of sediment ingestion as average egestion rates (±SD, n = 1−16) of worm groups during the exposure.

relationship between body residues and egestion rates is beyond the scope of this study. The average egestion rates in each worm group are presented because it permits comparison between gut passage time and accumulation of contaminants. The slopes of regression lines characterizing correlation between egestion rate and time were different between worm groups (ANCOVA, $F = 15.01$, df = 2,28, $p < 0.0001$). For example, the slope in group 3 was significantly larger than in group 2f (Tukey, $q = 14.33$, df = 3,28, $p < 0.0001$), and the slope in group 1 was significantly larger than in group 3 (Tukey, $q = 4.41$, df = 3,28, $p < 0.025$). As the clearance rates decreased in the order G1 > G2f > G3, we can conclude that the uptake of chemicals was independent of gut passage time within the measured range of egestion.

Discussion

The data of this study corroborates previous findings (*5*−*8*) of the importance of ingested sediment in bioaccumulation of hydrophobic contaminants. Ingested material was the major route for pyrene accumulation in *L. variegatus*. According to Harkey et al. (*25*), benzo[*a*]pyrene (BaP) tissue concentrations in nonfeeding amphipods were approximately half of those in feeding individuals after 7- and 14-day exposures in Lake Michigan sediment. The exposure of contaminated sediment to digestive fluids of marine deposit feeders has been shown to increase bioavailability (*26*), and hence, higher tissue concentrations in feeders are expected to result from solubilization of contaminants. Digestion and absorption of food can even cause contaminant fugacity in the gut to exceed that in the organism, which leads to net uptake of chemicals across gut and maybe to biomagnification (*27*).

In the present study, the distinction between the relative differences in accumulation routes was based on normal reproductive behavior of oligochaetes residing inside the sediment. Although the architomic reproduction mode is very simple, its effects on worm physiology are unknown. For example, metabolic activity may be different during the regeneration period. However, if we assume that transfer of nonionic hydrophobic contaminants from pore water to organisms is mainly governed by the laws of thermodynamics, the possible differences in physiology probably have only minor effect on accumulation process as far as lipid content remains the same. On the other hand, recently fragmented individuals may have been less active burrowers than actively feeding worms, hence experiencing lower cumulative pore water concentrations. The worms protruded the tails above the surface intermittently for variable periods to egest during the exposure. Chemical concentration in the overlying water, however, was so low that accumulation from this source is assumed to be insignificant. Degradation of pyrene in sediment is possible, although the rate is probably low (*28*). The significant difference in tissue concentrations between ingesting and noningesting worms was obtained during the first 186 h. Therefore, we assume that mainly parent compound was taken up during the first week of exposure. If the microbial degradation products and worm metabolites had been produced during the test, their effect on results would probably have shown later. We feel that the method can be reliably used to study the significance of these routes in accumulation of sedimentary contaminants. The only vital requirement for the use of the method is the knowledge of the size at which *L. variegatus* reproduces in test sediment. This appeared to be related to culture conditions and sediment characteristics (unpublished data).

Pore water concentrations were not measured during the test. As the pore water data would have helped to estimate the relative contribution of sediment particles and pore water as pyrene source, a separate experiment was conducted. Sediment from the same sampling location was spiked with radiolabeled pyrene and handled exactly in the same manner as during the present test producing concentrations of 1044 ± 33 pmol g^{-1} dw and 0.43 ± 0.02 pmol mL^{-1} ($n = 4$, $\log K_{oc} = 4.7$) for sediment and pore water, respectively, after 2 weeks storage time. Total concentration in 1 g of wet sediment was 144.9 ± 4.5 pmol of which pore water constituted only 0.36 ± 0.02 pmol (freely dissolved and DOC associated). If we assume that the sediment used in the test had approximately the same pyrene distribution between solids and water, we can speculate the source of contaminant keeping in mind that, in this fine fractioned sediment, nonselectively feeding *Lumbriculus* ingests also pore water. The large difference in pyrene distribution between solids and pore water would have allowed much larger difference in uptake and tissue concentrations between ingesting and noningesting worms than measured. This indicates that pyrene sorbed to particles is much less bioavailable than pyrene in pore water. The larger tissue concentrations in sediment ingesting worms can be due to digestive enzymes which solubilize

pyrene and/or the gut architecture which allows more efficient penetration of contaminant through membranes than outer integument. If the digestive agents had been responsible for the difference, we would have seen a positive relationship between increasing gut residence time (decreasing egestion rate) and uptake. Unfortunately, the decrease in bioavailable pyrene (sorption of pyrene to unavailable pool, see below) during the test masked the possible effect of gut residence time.

What is also noteworthy is the difference in tissue concentrations at apparent steady state (day 28) between the feeding worm groups (G1 vs G2f). Three possible reasons can be presented. First, physiology of worms may have differed although lipid content was equal between the worm groups. The size of the worms offers no explanation either as the average weights of G1 and G2f individuals at day 28 were 7.0 ± 1.0 and 7.6 ± 1.3 mg ww, respectively, and the fresh weight of a single individual in group 3 was 4.4 mg. Individuals from the groups 2f and 3 had gone through reproduction by architomy just prior to the exposure (G2f) or during the exposure (G3), which may have affected their physiology by some unquantified way.

Second, ingestion rate of deposit feeders have been shown to affect contaminant tissue concentration (*6*, *24*, *25*). However, as we have already noted, the accumulation of pyrene cannot be related to gut passage time in this study.

Third, aging of the contaminated sediment changes bioavailability of PAHs (*7*, *29*, *30*). The bioavailability of chemicals in sediment is based on thermodynamic balance between storage (particles, DOC) and freely dissolved pool (*7*). Time to reach equilibrium between adsorption and desorption processes of hydrophobic organic chemicals may even take months (*31*) and this process is expected to be related to changes in bioavailability. Aging of spiked sediment for 1 week resulted in 38% lower uptake clearance rate (k_s) of [^{14}C]BaP compared to freshly added [^{3}H]BaP in a *Diporeia* spp. experiment (*30*). Most of this sorption to unavailable fraction was observed to take place during the first week of storage in Lake Michigan sediment. Decrease in k_s, due to aging, was also reported for pyrene (*29*). The decrease in the bioavailable fraction can explain why the uptake clearance rates and apparent steady-state tissue concentrations differed between the three worm groups in the present study. The sorption of pyrene to a slowly reversible particle pool and/or dissolved organic matter pool during the exposure may have resulted in the lower uptake clearance rates and body burdens of the G2f and G3 worm groups.

The presented data does not confront solids with pore water as pyrene source but compares the relative contribution of pore water and ingested sediment (solids and pore water) in accumulation process. The method can be applied to test accumulation routes of chemicals with different K_{ow} properties which could improve our understanding of bioaccumulation process beyond chemical partitioning between particulate and aqueous phase. Similarly, including the effect of sediment-contaminant contact time in designs could offer a possibility to estimate whether changes in bioavailability are taking place mainly in particle or in aqueous phase (see Figure 6 in ref 7). These designs, however, are only able to refer to the source location and route of uptake. The sediment characteristics which modify bioavailability within compartments require additional measurements and are not yet fully understood (*30*, *32*).

The study supports strongly the view that feeding behavior of aquatic invertebrates is a significant factor in accumulation of hydrophobic contaminants. Deposit-feeding animals possess a variety of feeding strategies. For example, polychaetes can be divided to different groups according to the morphology of intestine which reflects variations in diet quality and digestive kinematics (*33*). As the present results indicate, the uptake of hydrophobic contaminants takes place mainly through the ingestion; hence, the diversity in digestive environment among benthic organisms may partly explain why the equilibrium partitioning theory has failed to predict bioavailability in some earlier studies [see review by Landrum et al. (*31*)]. The feeding behavior of organisms cannot be neglected when modeling the fate of sediment associated contaminants.

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