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BIOACCUMULATION OF TETRACHLOROBIPHENYL AND HEXACHLOROBIPHENYL CONGENERS BY *YOLDIA LIMATULA* AND *NEPHTYS INCISA* FROM BEDDED SEDIMENTS: EFFECTS OF SEDIMENT- AND ANIMAL-RELATED PARAMETERS

JAY C. MEANS*† and ANNE E. MCELROY‡
†Aquatic Toxicology Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA
‡Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, New York 11794, USA

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Abstract—Sediments from Narragansett Bay (RI, USA) were fortified with two chlorobiphenyl congeners, 2,4,2′,4′-tetrachlorobiphenyl (IUPAC 47) and 2,4,5,2′,4′,5′-hexachlorobiphenyl (IUPAC 153), and equilibrated for various times up to 30 d to assess the bioaccumulation potential of sediment-sorbed polychlorinated biphenyls by the benthic invertebrates *Yoldia limatula* and *Nephtys incisa*. Bioaccumulation was investigated at steady state and using a single-compartment kinetic model over exposure periods of up to 60 d for *Nephtys* and 30 d for *Yoldia*. Normalization of exposure and tissue accumulation data to hydrophobic reservoirs yielded accumulation factors (AFs) that fell within model prediction ranges. However, persistent, statistically different values of AFs were obtained from sediments with varying organic carbon contents. Growth of the organisms, feeding strategies, and lipid content were all significant variables in interpreting wet weight steady-state accumulation. Kinetically determined AF values were not statistically different from those measured at steady state. A role of interstitial water colloidal organic matter in mediating bioaccumulation was strongly suggested by the results.

Keywords—Bioaccumulation Polychlorinated biphenyl congeners Sediments Accumulation factors

INTRODUCTION

Bedded sediments represent major reservoirs for a wide variety of hydrophobic refractory organic contaminants entering aquatic environments. As a result of the persistence of many of these chemical compounds, sediments represent a potential long-term ecological threat both to organisms living and feeding in these contaminated environments through direct exposure, as well as to other biological components of the ecosystem, including humans, through food-chain transfer of these contaminants. Numerous monitoring studies have documented the widespread contamination of coastal and estuarine sediments with polychlorinated biphenyls (PCBs) both nationally [1–4] and internationally [5]. Polychlorinated biphenyls are a complex class of toxic substances containing 209 individual congeners. These congeners have been implicated in several studies as reproductive and immune system toxicants and as potential carcinogens or tumor promoters [6,7]. Polychlorinated biphenyls also have been shown to induce mixed-function oxidase isoenzymes, which are involved in xenobiotic metabolism in most species of mammals, including humans [8] and aquatic organisms [9,10].

The study of bioaccumulation of PCBs in aquatic organisms continues to be an area of intensive research, particularly in urban harbors and in evaluating dredge material disposal strategies [11–13]. The United States Environmental Protection Agency (USEPA) is developing sediment quality criteria that take into consideration the bioaccumulation potential of sediment-bound contaminants. Bioconcentration of PCBs from water into several species of fish and epibenthic bivalves has been reported in numerous papers [1–5, 14]. However, studies of the chemical and physical factors that control the bioaccumulation of PCBs into benthic organisms, those organisms living in the most intimate contact with contaminated sediments, have been relatively limited. Swindoll and Applehans [15] reported that bioaccumulation was dependent upon a number of biotic and abiotic factors including sediment texture and organic carbon content. Rubinstein et al. [16] reported bioaccumulation of PCBs and selected metals from sediments in three benthic invertebrate species. Subsequently, Rubinstein et al. [17] reported that PCB mixtures (Aroclor 1254) were accumulated rapidly into the benthic worm *Nereis virens* from a contaminated sediment and further demonstrated that the PCB residues could be transferred through *Nereis* up the food chain to benthic-feeding fish such as juvenile spot (*Leiostomus xanthurus*).

The bioaccumulation of PCBs or other neutral hydrophobic organic compounds from sediment has been hypothesized to be controlled in part by sediment/water partitioning [11,18–20]. Hydrophobic compounds are strongly sorbed to sediments, exhibiting high sediment/water partition coefficients, which have been shown to be highly correlated to sediment organic carbon contents [21]. More research is required to definitively establish the relative contributions of various potential routes of exposure, such as pore water via respiration or sediment organic matter via ingestion, or a mixture of these two processes. The basic assumptions made in this conceptual model are that, during bioaccumulation, the sediment phase is at equilibrium with the pore-water phase, that the fugacity or activity of the chemical is the same in each phase at equilibrium, that the organism receives an exposure equivalent to the aqueous phase exposure concentration only of the chemical and that the sediment-bound fraction of the contaminant is available to the organism through desorption into the aqueous phase either in pore water or in the digestive tract [22]. Because

* To whom correspondence may be addressed.

the USEPA is developing sediment quality criteria [23] based upon these and other assumptions, elucidation of the factors that may control accumulation processes in the benthos is important. The review of the issues and assumptions made in these sediment quality criteria estimations has been published by Di Toro et al. [22].

Significant advances in the understanding of the physical and chemical factors that control the sorption process, as well as reliable predictive equations for estimating sorption from compound properties such as water solubility and octanol/water partition coefficients (log K_{ow}) and sediment organic carbon content, have been developed [21,24], and recently reviewed by Schwarzenbach et al. [25]. The phenomenon of bioaccumulation from sediments generally may be conceived of as a two-stage equilibrium process involving desorption from sediment (S) into interstitial pore water (W) followed by bioaccumulation from the interstitial pore water into organismic tissues (T), predominantly the lipid pools. The equilibrium constant (K_p) and the bioconcentration factor (BCF) control these processes.

$$S \underset{Kp}{\longleftrightarrow} W \underset{BCF}{\longleftrightarrow} T$$

Research in a number of laboratories has suggested that predictive bioaccumulation models can be used to estimate exposure of benthic organisms from sediments [11,18,19]. However, results from further research in our laboratory (J.C. Means and A.E. McElroy, unpublished data) and the work of Lee and his colleagues [26,27] have suggested that a toxicokinetic model of bioaccumulation from sediments may be more appropriate to estimate bioaccumulation in benthic organisms that utilize sediment carbon pools as a source of energy and nutrients. Davis and Means [28] observed that both the number and type of benthic infaunal organisms could profoundly influence contaminant mobilization from bedded sediments in both the dissolved and particulate flux rates. These preliminary studies, however, raised many more questions concerning organism–sediment interactions as they relate to contaminant mobilization and bioavailability to other components in aquatic ecosystems. Among the issues raised was the degree to which bioturbation by infaunal communities influences bioavailability to other infaunal organisms, benthic organisms, epibenthic organisms, and pelagic organisms. The important issue of food-chain transfers of contaminants between trophic levels also has not been studied in controlled systems.

In the present investigation, we report the results of studies designed to examine the influence of several sediment-related and animal-related experimental variables upon the rates and extent of steady-state bioaccumulation of tetrachlorobiphenyl and hexachlorobiphenyl in two species of benthic deposit-feeding invertebrates, *Nephtys incisa* and *Yoldia limatula.*

METHODS AND MATERIALS

The Appendix contains a summary of the experimental materials used in these investigations.

Sediments

Two natural sediments with varying organic carbon content and known levels of PCB contamination were collected from sites in Narragansett Bay, Rhode Island, USA, and wet sieved (2-mm pore sieve) to remove organisms and coarse materials such as shell hash, rocks, and detritus. The sediments were homogenized on a roller mill for 1 week prior to analyses for sediment characteristics and spiking with [^{14}C]chlorobiphenyls. Sediment slurries were equilibrated with the isotope for 7 or 30 d as described in McElroy and Means [19].

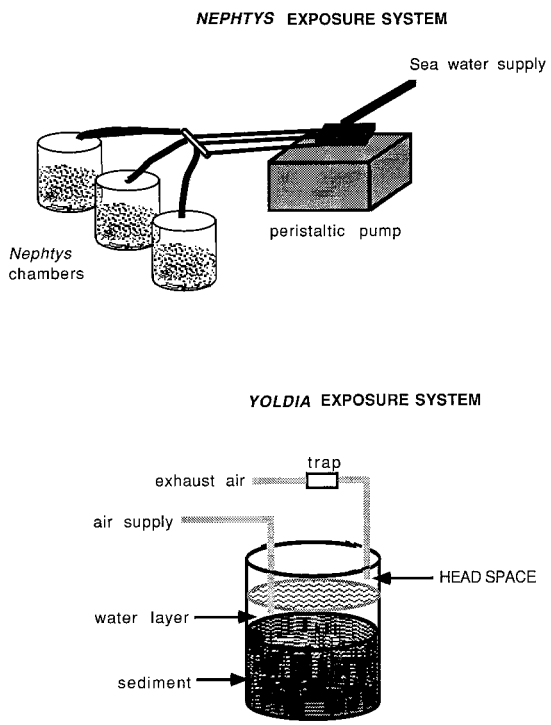

Fig. 1. Design of exposure chambers for bioaccumulation experiments.

Compounds

Two chlorobiphenyl congeners, 2,4,2′,4′-tetrachloro[UL, ^{14}C]biphenyl (IUPAC 47)(TCBP) and 2,4,5,2′,4′,5′-hexachloro[UL, ^{14}C]biphenyl (IUPAC 153)(HCBP), were selected to represent two common chlorination groups of PCBs, with different aqueous solubilities, sediment/water partition coefficients, and bioconcentration factors as shown in the Appendix. Each of the compounds was >98% radiochemically pure as verified by reverse-phase liquid chromatography followed by liquid scintillation counting (LSC) and >99% chemically pure as verified by capillary-column gas chromatography using electron capture detection.

Organisms

Two species, the polychaete *N. incisa* and the protobranch bivalve *Y. limatula,* were selected to assess the differences in the uptake kinetics from sediments as well as potential differences in the equilibrium bioaccumulation factors in two deposit-feeding species with different feeding modes. The species selected represent two major classes of benthic infaunal organisms found in estuaries in the northeastern region of the United States [29]. These or related species are important food organisms for commercially harvested demersal fish, which represent the next trophic level [30].

Figure 1 shows schematic diagrams of the exposure chambers used for *Nephtys* and *Yoldia.* For *Nephtys,* each chamber consisted of a 150-ml glass beaker filled with approximately 70 g (wet weight) of bedded, wet, radiolabeled sediment. Each chamber was covered with a Nitex (Aquacenter, Inc., Leland, MS, USA) screen that prevented the organisms from leaving the chamber and allowed the overlaying water in each chamber

to receive a flow of 5 ml/min of oxygenated seawater (ambient salinity = 20 × 10^{-3}), which was coarse sand filtered (~1 mm) to remove large particulate matter. This flow of water insured that the organisms had an adequate oxygen supply and that soluble wastes were flushed from the chamber but was not sufficient to cause sediment resuspension within the chambers. *Nephtys* itself was not considered an aggressive sediment-disrupting organism [31]. All chambers were maintained at 20 ± 1°C in a water bath throughout the course of the experiments.

Yoldia is an aggressive sediment ''conveyor belt'' feeder [32] and as a result, a more complex exposure chamber was required to prevent the fine fraction of the sediments in the chambers from being depleted during the course of the experiments. Figure 1 shows the chambers used for *Yoldia*. Each chamber consisted of a 75-mm-diameter × 150-mm-high glass container filled with approximately 80 g (wet weight) of bedded, wet, labeled sediments and 100 ml of seawater. The chamber was fitted with a cap that had an air inlet and outlet tube. Air was supplied to each chamber at a rate of 50 ml/min to provide for an oxygenated water column in each chamber and to purge volatile metabolic wastes such as ammonia. The outlet tube was fitted with a polyurethane foam plug trap to recover any PCBs volatilized from the chamber during the course of the experiment. Previous studies verified that these traps were >99% efficient in removing PCBs from humidified air samples. Water loss in the chambers was monitored daily and chambers were refilled approximately every 3 d or as needed. The foam plugs were collected and analyzed at the times organisms were sacrificed for analysis.

Experimental design

For *Nepthys*, one chamber containing each equilibration time/sediment combination (LTJT, STJT, LTSP, STSP, where LT = long term, ST = short term, and JT and SP are sediment was sacrificed at time intervals of 0, 2, 5, 10, 20, 30, and 60 d. For *Yoldia*, one chamber containing each sediment (JT, SP) was sacrificed at time intervals of 0, 2, 5, 10, and 30 d in the HCBP experiment and 0, 2, 4, 7, and 10 d in the TCBP experiment. At the time of collection, the organisms in each chamber were divided into two groups, weighed, and frozen for subsequent analysis. Tissue samples were ground in anhydrous sodium sulfate and extracted four times in acetonitrile (ACN). The extracts were combined, reduced in volume on a rotary evaporator, and subsampled for radioactivity and lipid content.

The overlying water in each chamber was decanted into 250-ml stainless steel centrifuge tubes and centrifuged at 10,400 *g* at 4°C for 20 min. The overlying water (*Yoldia* chambers only) was then extracted three times with methylene chloride. The extracts were combined, dried over anhydrous sodium sulfate, concentrated in a rotary evaporator, transferred to a scintillation vial, and evaporated to near dryness under nitrogen. The residue was then taken up in a known volume of hexane and sampled in triplicate (1 ml) for LSC analysis.

The sediment in each chamber was transferred to centrifuge tubes as described above and the interstitial water was separated by centrifugation (10,400 *g*, 4°C, 20 min). After centrifugation, the interstitial water was extracted three times with methylene chloride. The extracts were reduced in volume and sampled in triplicate (2 ml) for LSC counting. The centrifuged sediments were resuspended, homogenized, and sonicated with pesticide grade ACN. The suspension was subsampled (approximately 2.5–3.0 g dry weight) and extracted as described in McElroy and Means [19].

Table 1. Sediment concentrations of radiolabeled polychlorinated biphenyl congeners

Congener[a]	Species	Sediment[b]	Concentration (μg/g dry wt.) ± SD	*n*
TCBP	*Yoldia limatula*	SP	3.23 ± 0.03	5
		JT	2.21 ± 0.04	4
HCBP	*Yoldia limatula*	SP	1.34 ± 0.05	7
		JT	1.12 ± 0.03	7
	Nephtys incisa	SP	1.36 ± 0.16	14
		JT	1.03 ± 0.09	14

[a] TCBP = 2,4,2′,4′-tetrachlorobiphenyl; HCBP = 2,4,5,2′,4′,5′-hexachlorobiphenyl.
[b] SP = Sabin Point, JT = Jamestown (see Appendix).

The polyurethane foam plugs from the chambers, which were contained in the barrels of 50-ml glass syringes, were extracted by drawing up and extruding 6 ml of acetone repeatedly through the foam plug. This extraction was performed three times with fresh portions of acetone and each extract was counted for ^{14}C separately. The total disintegrations per minute (dpms) in each extract were totaled to yield a measure of PCB volatilization during the course of the exposure experiment.

All quantification of PCBs was performed by LSC using Dimilume (Packard Instruments, Meriden, CT, USA) as the scintillant on a Packard Tricarb-460-C counter (Parkard Instruments, Meriden, CT, USA), with corrections made for quench, chemiluminescence, and background. Each set of samples was counted twice to insure that chemiluminescence was absent or minimized. All measurements were made in duplicate or triplicate samples. Mean dpms were converted to masses of PCB using the specific activities of the individual radiolabeled compounds. No corrections of the specific activities for dilution by the unlabeled congeners were necessary because the levels of the unlabeled congeners 47 and 153 were very low (<3%) relative to the labeled congener added.

RESULTS AND DISCUSSION

Sediment and pore-water concentrations

Analyses of radiolabeled PCB congener concentrations in sediments over the course of each experiment showed that there were no significant changes in any of the experimental chambers, confirming that the organisms were exposed to a constant amount of contaminant throughout the experiments. Mean concentrations for all sediment samples analyzed are shown in Table 1. Concentrations of added PCBs were in the ppm range, similar to naturally incurred PCB residues at our high organic carbon site. No degradation of these two congeners was observed either in the sediments or in the tissues of the two species of organisms used. Also, the total amount accumulated by the biomass in each chamber was small (<2%) relative to the total mass of available contaminant in the sediments in each chamber. Thus, accumulation in the biomass would not be expected to deplete the sediment pool of PCB. Mean pore-water concentrations of PCBs were also relatively constant throughout the course of the experiments (Table 2). It should be noted that pore-water concentrations within the *Nephtys* chambers were stable and lower (0.19 ng/ml and 0.37 ng/ml for SP and JT, respectively) than those in the *Yoldia*

Table 2. Pore-water concentrations of radiolabeled polychlorinated biphenyl congeners

Congener[a]	Species	Sediment[b]	Concentration (μg/L) ± SD Expected[c]	Measured[d]	*n*
TCBP	*Yoldia limatula*	SP	0.087	0.83	3
		JT	0.123	1.22	3
HCBP	*Yoldia limatula*	SP	0.00125	0.27 ± 0.12	6
		JT	0.00216	0.53 ± 0.12	6
	Nephtys incisa	SP	0.00127	0.19 ± 0.04	12
		JT	0.00198	0.37 ± 0.16	12

[a] TCBP = 2,4,2′,4′-tetrachlorobiphenyl; HCBP = 2,4,5,2′,4′,5′-hexachlorobiphenyl.
[b] SP = Sabin Point, JT = Jamestown (see Appendix).
[c] Calculated from organic carbon/water partition coefficient (K_{oc}) (see Appendix) for TCBP or HCBP for each sediment using means of concentrations above.
[d] Excludes data from day 0 and 2; ST and LT equilibration times are pooled for *Nephtys* data.

chambers (0.27 ng/ml and 0.53 ng/ml for SP and JT, respectively), especially if the data for day 0 and day 2 time points are excluded from the *Nephtys* data set to eliminate the effects of the initial assembly of the chambers and the initial burrowing of the organisms. The higher degree of variability in the *Yoldia* chambers was attributed to the aggressive sediment processing of these organisms, which continually created and destroyed burrow chambers within the sediment bed and created new sediment interfaces for desorption into the interstitial water. Also, the respiratory and feeding behavior of *Yoldia* resulted in dilution of interstitial water with overlying water, which contributed to the variability observed in the interstitial water concentrations. The overlying water was continuously purged with air, which provided a simulated advective removal process to these chambers, making them more similar to the chambers used in the *Nephtys* experiments where overlying water was continuously flushed from the chamber with fresh seawater.

Comparison of predicted pore-water concentrations (Table 2) based upon values of the organic carbon/water partition coefficient (K_{oc}) for TCBP and HCBP with measured values showed that pore-water concentrations were well above those predicted from the K_{oc}, suggesting that interstitial dissolved organic carbon (DOC) may have altered the partition coefficients. The influence of interstitial water colloidal organic matter upon K_p values [33] was predicted by Means and Wijayaratne [34] and Wijayaratne and Means [35] for other classes of hydrophobic contaminants. Interstitial water measurements by Brownawell and Farrington [3] for a broad spectrum of PCB congeners were consistent with this prediction and recently Burgess et al. [36] isolated PCB-bearing interstitial water colloids from contaminated sediments. The increase in interstitial water concentrations (reduction of sorption K_p) was greater for HCBP (>100 times) than for TCBP (>10 times), as predicted by Brownawell and Farrington [3]. Investigations of the influence of DOC upon bioaccumulation of hydrophobic contaminants such as polycyclic aromatic hydrocarbons (PAHs) [37] and PCBs [14] have shown that DOC exerts a protective effect by reducing bioavailability. When considered in the context of the current experiment, we suggest that even though the interstitial water concentrations are increased by the presence of DOC, the protective effect of the DOC may partially or totally counterbalance these higher concentrations by making the compound unavailable to the organism.

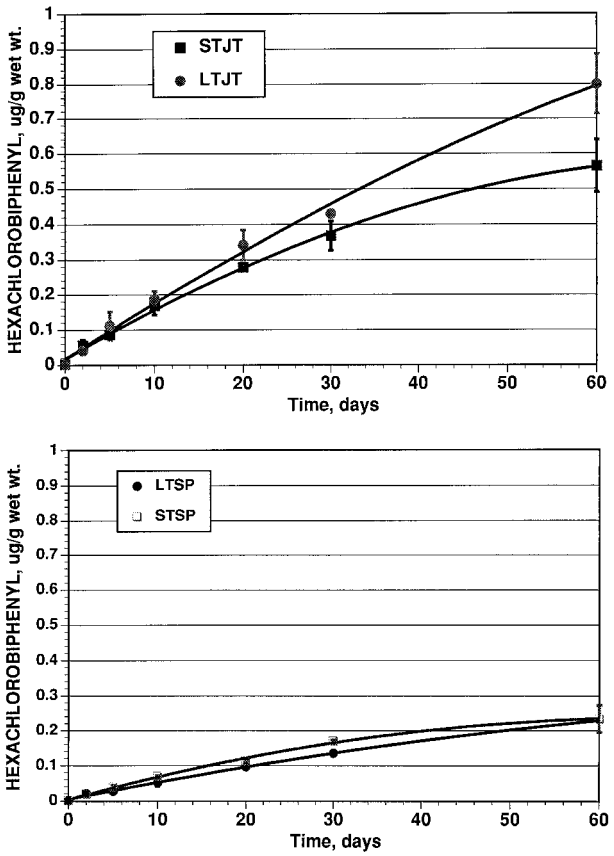

Fig. 2. Bioaccumulation of hexachlorobiphenyl from two contaminated sediments in *Nephtys incisa*.

Bioaccumulation kinetics and equilibrium constants

Figure 2 shows the accumulation of the HCBP congener in *Nephtys* from each equilibration time/sediment type source over a period of 60 d. All data are expressed in terms of μg/g wet weight of HCBP accumulated. Although initial accumulation in tissues is rapid (measurable at 2 d), tissue concentrations of HCBP continue to increase throughout the 60-d period. A Levenberg–Marquardt iterative, nonlinear chi square analysis [38] was used to model the accumulation data and indicated that more than 100 d would have been required for these organisms to reach steady-state HCBP concentrations (on a wet weight basis) relative to the constant sediment concentrations. Higher concentrations of HCBP were reached in *Nephtys* exposed to sediment JT than in those exposed to sediment SP even though the absolute concentration of HCBP was slightly higher in sediment SP. If the mean absolute amounts of HCBP accumulated at the end of 60 d (0.681 μg/g wet weight and 0.153 μg/g wet weight, for JT and SP, respectively) are adjusted for the organic carbon (OC)-normalized sediment HCBP concentrations, then values of 0.0128 μg/g wet weight/(μg/g OC) and 0.00445 μg/g wet weight/(μg/g OC) are obtained. Thus, *Nephtys* accumulated approximately 2.87 times as much HCBP from sediment JT as from sediment SP on an equal-exposure basis (1 μg HCBP/g OC in the sediment). It should be noted that based upon partitioning theory, the steady-state level of accumulation observed in individual organisms from a constant exposure source will

Table 3. Analysis of variance for bioaccumulation data (wet weight) in *Nephtys incisa*

Source of variation[a]	d.f.	F[b]
Sediment type/location (JT/SP)	1	423.2***
Exposure time (d)	1	1526***
Equilibration time (LT/ST)	5	5.4*
Sediment/location × exposure time	1	181.6***
Location × equilibration time	5	1.5 NS
Exposure time × equilibration time	5	18.5**

[a] JT = Jamestown, SP = Sabin Point; LT = long term, ST = short term.
[b] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS = not significant.

also be a function of the tissue lipid mass in the organism, so the differences observed here must further be adjusted for any differences in lipids. This is discussed in the next section of the paper.

The effect of PCB-spike contact time with sediments during the labeling procedure on the relative bioavailability of HCBP in sediments was addressed in the LT versus ST (30-d versus 7-d equilibration period) comparison in this experiment. The data for all times over the 60-d exposure period for LT and ST were examined in a three-way analysis of variance (ANOVA) for significant differences using equilibration time as one test variable. The three-way ANOVA addressing the influence of sediment type/location, equilibration time with the spiked isotope, and time of exposure on body burdens of HCBP in *Nephtys* revealed that all three factors contribute significantly to the variation observed (Table 3). Length of exposure (F = 1,562) and sediment type/location (F = 423) were highly significant at a greater than $p < 0.001$ level (F[1,5] = 47.2). Equilibration time with the isotope (F = 5.4) was barely significant at the $p < 0.05$ level (F[5,5] = 5.05). In sediment SP, no significant differences were found between the amounts accumulated by *Nephtys* from LT versus ST exposures. In sediment JT, a significant difference was observed. In this case, the amount accumulated by the *Nephtys* from the LT treatment was greater than that from the ST treatment. This finding is counterintuitive to the trend expected if equilibration time were important for comparisons with the field, because the ST equilibration would be expected to yield an exposure substrate in which HCBP would be more bioavailable to the organism [39]. The fact that this expected difference in bioavailability did not occur in either sediment suggests that, for the PCBs investigated, realistic bioaccumulation data can be obtained in laboratory experiments with spiked compounds using reasonable equilibration periods (1–4 weeks). As there were no significant differences in sediment SP and only small numerical differences (factor of ~1.1) in sediment JT, the LT and ST data sets for *Nephtys* were combined for all subsequent comparisons with other data in these studies.

Figure 3 shows the HCBP bioaccumulation data for *Yoldia* in the SP and JT sediments over a 30-d time course. A number of similarities and differences between the *Nephtys* and *Yoldia* data sets are apparent. First, a similar trend with respect to the relative availability of HCBP from the two sediment types was consistent. At 30 d, *Yoldia* had accumulated 0.719 μg/g wet weight and 0.231 μg/g wet weight from the JT and SP sediments, respectively. After normalization for the differences in HCBP concentrations for the two sediments, values of 0.0124 μg/g wet weight/(μg/g OC) and 0.00684 μg/g wet weight/(μg/g OC) are obtained, which yield a JT/SP ratio of 1.81,

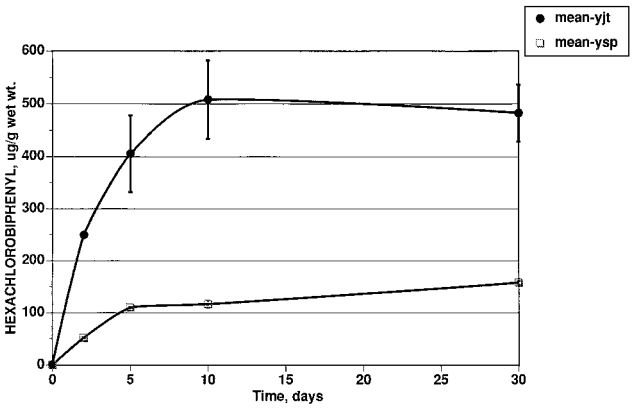

Fig. 3. Bioaccumulation of hexachlorobiphenyl from two contaminated sediments in *Yoldia limatula*.

which is similar to the ratio of 2.86 found in *Nephtys*. Differences may also be due in part to the types and amounts of lipids present.

The rate of HCBP accumulation in *Yoldia* was much more rapid than in *Nephtys*, with *Yoldia* reaching steady-state HCBP concentrations with respect to the constant sediment exposure concentration by 10 d. No significant changes in tissue wet weight concentrations of HCBP were observed between day 10 and 30 in this experiment. This is in distinct contrast to the pattern observed in wet weight concentrations in *Nephtys*, which continued to increase throughout a 60-d exposure period. A one-way ANOVA showed that the differences in the amount accumulated as a function of sediment type/location (F = 23.0) were significant for *Yoldia* ($p < 0.003$).

A number of biological differences exist between these two deposit-feeding organisms that are likely to influence bioaccumulation of organic contaminants. *Yoldia* is a very aggressive deposit feeder, processing up to three times its wet weight in sediment per day, depending upon sediment texture and temperature [32], whereas *Nephtys*, a deposit-feeding worm, feeds much less actively [31].

The accumulation of TCBP in *Yoldia* from sediments JT and SP over a 10-d time period are shown in Figure 4. *Yoldia* accumulated TCBP to final levels of 4.47 μg/g wet weight and 1.21 μg/g wet weight from the JT and SP substrates, respectively, over the 10-d period. After normalization of the exposure concentrations to a 1.0 μg/g OC basis, these levels correspond to 0.0390 μg/g wet weight/(μg/g OC) and 0.0149 μg/g wet weight/(μg/g OC) for the JT and SP samples, respectively. Thus, 2.62 times more TCBP was accumulated in *Yoldia* from sediment JT versus from sediment SP. The rate

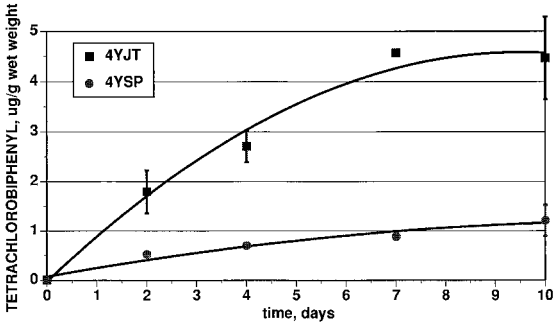

Fig. 4. Bioaccumulation of tetrachlorobiphenyl from two contaminated sediments in *Yoldia limatula*.

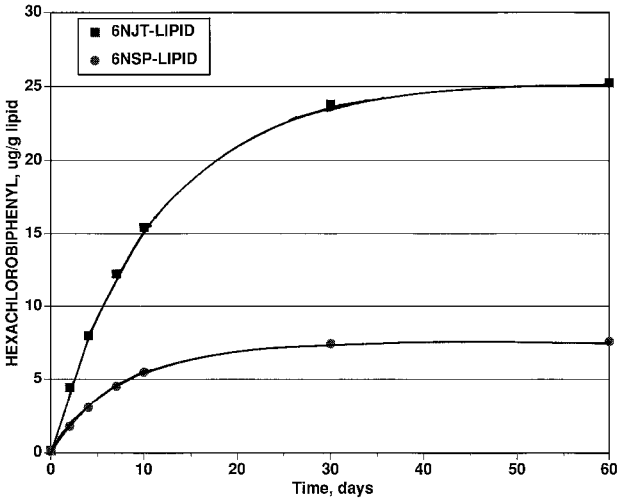

Fig. 5. Accumulation kinetics of tetrachlorobiphenyl and hexachlorobiphenyl from contaminated sediments in *Nephtys incisa* tissue lipid.

of accumulation of TCBP in *Yoldia* appeared to be similar to rates observed for HCBP. The nonlinear regression of these data yielded time-to-95% of steady state for TCBP concentrations (wet weight basis) of 15.6 and 10.7 d for the JT and SP sediments, respectively. These values suggest that the 10-d exposure period was shorter than the period needed for the organisms to equilibrate with the low OC JT sediment but was sufficient for the SP sediment. A one-way ANOVA showed that the differences in the amount accumulated as a function of sediment type/location ($F = 13.4$) were significant ($p < 0.011$) with a greater accumulation observed for the JT sediment (low OC).

As mentioned, lipid pools of a variety of organisms have been correlated with bioaccumulation potential, so we examined both the equilibrium and kinetics of accumulation of HCBP and TCBP residues from JT and SP sediments into lipids in *Nephtys* and *Yoldia.* Figure 5 shows the uptake of HCBP on a lipid weight basis in *Nephtys.* Examination of these data shows that the lipid pools within *Nephtys* are equilibrating with the sediments more rapidly than is shown in the wet weight basis data. Nonlinear regression of these data yielded values for time-to-95% steady state of 32 d and 23 d for the JT and SP sediments, respectively, when the data for ST and LT spike equilibration times are pooled. An analysis of the lipid content of *Nephtys* as a function of time (SP and JT data pooled for each day) shows a clear trend of increasing lipid content over the 60-d course of the experiment. These data suggest that the increasing wet weight concentrations of HCBP in *Nephtys* were the result of growth (addition of lipid relative to total wet weight) rather than slow equilibration of the tissues with the external source of HCBP.

A similar examination of the data for *Yoldia* (Fig. 6) shows that the lipid pools of these organisms also reached apparent steady state with the PCBs in sediments more rapidly than was indicated in the whole tissue data, although the differences were less dramatic. Nonlinear regression of HCBP data yielded equilibration times of 3.8 and 14.4 d for the JT and SP sediments, respectively, whereas, as stated earlier, steady state on a wet weight basis was achieved in 8.9 and 14.6 d, respectively. For the accumulation of TCBP in *Yoldia,* the lipid pools of the organism reached apparent steady state with the JT sediment at a rate slightly faster than was indicated on a wet weight basis (12.2 d versus 15.6 d). The lipid normalization for the SP-exposed organisms could not be obtained directly by nonlinear regression because of the ''sawtooth'' trend in the wet weight data (Fig. 4). However, normalization of these data to the average lipid content of 1.01% followed by nonlinear regression (Fig. 6) suggest that the lipid pools were at apparent steady state in less than 10 d and that lipid-normalized TCBP concentrations would be approximately 108 μg/g lipid for *Yoldia* exposed to SP sediment (estimated by linear regression of the data from days 2, 4, 7, and 10 of the experiment: slope = 0.686, intercept = 40.9, $R^2 = 0.916$).

Table 4 presents a comparison of the measured final concentrations of HCBP and TCBP achieved in *Nephtys* within 60 d and in *Yoldia* within 30 d with calculated steady-state tissue concentrations estimated by nonlinear regression of the

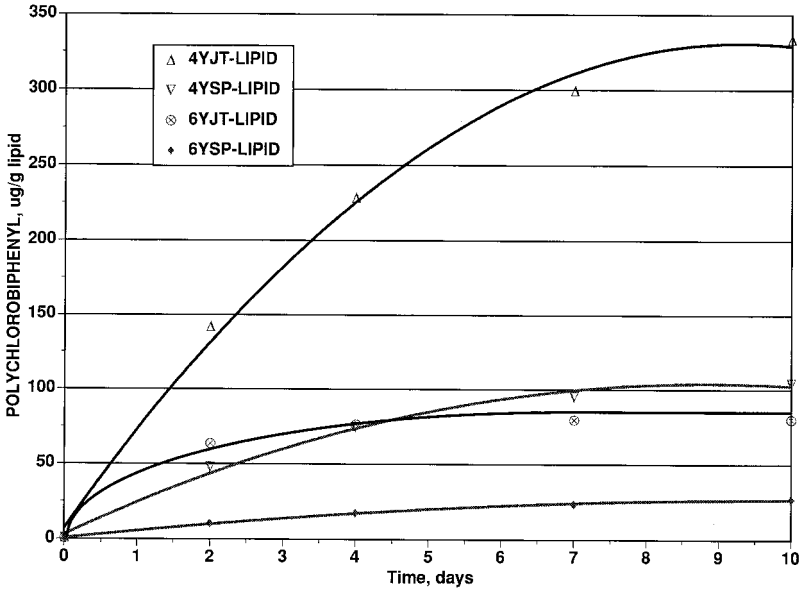

Fig. 6. Accumulation kinetics of tetrachlorobiphenyl and hexachlorobiphenyl from contaminated sediments in *Yoldia limatula* tissue lipid.

Table 4. Comparison of measured (final) and predicted[a] (steady-state) HCBP and TCBP concentrations (wet wt. versus lipid normalized) for *Nephtys incisa* and *Yoldia limatula* exposed to JT and SP sediments[b]

Sediment[c]	Species[d]	Measured concentration[e] (μg/g) Wet	Measured concentration[e] (μg/g) Lipid	Predicted concentration[e] (μg/g) Wet	Predicted concentration[e] (μg/g) Lipid	T_c[f]	K_d[f]
HCBP							
JT	Y	0.719 (99)	63.2 (79)	0.721	79.9	0.770	0.786
	N	0.681 (89)	27.3 (108)	0.762	25.3	0.043	0.093
SP	Y	0.231 (108)	29.4 (96)	0.214	30.6	0.185	0.208
	N	0.234 (84)	8.88 (117)	0.278	7.6	0.027	0.126
TCBP							
JT	Y	4.47 (86)	437 (120)	5.21	364	0.784	0.246
SP	Y	1.21 (108)	76.2 (≈101)	1.12	(≈75.8)[g]	NA	NA

[a] Predicted from nonlinear regression of data combining all data for long-term and short-term equilibration times where applicable.
[b] HCBP = 2,4,5,2′,4′,5′-hexachlorobiphenyl, TCBP = 2,4,2′,4′-tetrachlorobiphenyl.
[c] JT = Jamestown, SP = Sabin Point.
[d] Y = *Yoldia limatula*, N = *Nephtys incisa*. NA = not analyzed.
[e] Values in parentheses represent the percent of measured values relative to predicted values reported in the table.
[f] T_c and K_d are uptake and depuration rate constants, respectively, calculated by nonlinear regression from lipid-normalized data as in Foster et al. [18]; units are d^{-1}.
[g] Estimated by linear regression of data from days 2, 4, 7, and 10. Intercept, $y = 75.8$.

time course data on both a wet weight and lipid weight basis. Foster et al. [18] demonstrated that nonlinear regression could be used to predict final tissue concentrations of some hydrophobic compounds in benthic organisms exposed to contaminated sediment in short-term experiments. They further suggested that the kinetic parameters for the uptake (T_c) and depuration (K_d) rate constants (calculated on a lipid weight basis and derived from the same kinetic analysis) could be used to predict the bioaccumulation factor (BAF) at steady state. The data in Table 4 demonstrate that measured values were between 84 and 108% of predicted wet weight concentrations and between 79 and 120% of predicted lipid weight concentrations of HCBP and TCBP in both organisms. It is clear from these data that in most cases, the measured values on a wet weight basis tend to underestimate steady-state wet weight concentrations regardless of the compound, organism, or sediment used. These underestimates could be as much as 20% even after 60 d of exposure in *Nephtys* (e.g., for *Nephtys*, the predicted times to apparent steady state were greater than 110 d). In contrast, if the accumulation data were calculated on a lipid weight basis, much shorter predicted and observed times to steady state were found for both species. Effective experimental exposure times could be reduced to approximately 30 d for *Nephtys* and between 4 and 15 days for *Yoldia*. As noted earlier, our data suggest that organism growth is in part responsible for the differences observed. As a practical consideration, minimization of exposure times reduces the probability of organism mortality, while effecting several economies in experimental costs and time.

Table 5 presents a comparison of measured and predicted hydrophobic reservoir normalized accumulation factors (AFs) or preference factors (PFs) for the current set of experiments. Both the values of AFs estimated from the kinetic analysis of the experimental data and the 1/PF calculated directly from the data at the end of each time course have been normalized for both tissue lipid content and sediment total OC [18,19]. Examination of these data indicates that the kinetic approach to determining the AF for both compounds yields values very similar to those determined at steady state. The AF approach is intended to predict the maximum tissue concentrations of a contaminant based on its concentration on an OC basis in the sediment and on a lipid concentration basis in tissues. The model predicts values of AF or 1/PF of 0.5 to 2.0. Clearly, the majority of our data fall within the model. The exceptions are that the actual amount of HCBP accumulated in *Nephtys* was less than predicted in both JT and SP sediments (Table 4), with the ''sequestering effect'' of SP OC being greater than the JT organic matter. Conversely, in the case of TCBP accumulation in *Yoldia* from sediment JT, the organism accumulated almost twice the amount predicted by the model (Table 5). Similar differences in the behavior of hydrophobic chlorinated compounds such as HCBP have been observed by other

Table 5. Comparison of measured and predicted HCBP and TCBP accumulation factors for *Nephtys incisa* and *Yoldia limatula* in JT and SP sediments

Compound[a]	Sediment[b]	AF[c]	1/PF[d]
Nephtys incisa			
HCBP	JT	0.464	0.423 ± 0.063
	SP	0.212	0.212 ± 0.044
Yoldia limatula			
HCBP	JT	0.980	1.68 ± 0.358
	SP	0.891	0.862 ± 0.028
TCBP	JT	3.18	3.46
	SP	0.932[e]	0.924

[a] HCBP = 2,4,5,2′,4′,5′-hexachlorobiphenyl, TCBP = 2,4,2′,4′-tetrachlorobiphenyl.
[b] JT = Jamestown, SP = Sabin Point.
[c] AF = accumulation factor. Calculated from uptake rate constant to depuration rate constant (T_c/K_d) ratio using method of Foster et al. [18].
[d] PF = preference factor. Calculated as presented in McElroy and Means [19].
[e] Calculated by linear regression of all data for days 2, 4, 7, and 10.

Table 6. Comparison of observed accumulation factors with accumulation factors derived from measured and calculated interstital water concentrations[a] as the exposure medium and using a lipid-based bioconcentration factor (BCF)[b] for HCBP and TCBP

Compound[c]	Species	Sediment[d]	Accumulation factor		
			Measured	Calculated	Observed
TCBP	*Yoldia limatula*	JT	11.0	1.24	3.18
		SP	12.4	1.24	0.932
HCBP		JT	387	1.58	0.980
		SP	340	1.57	0.891
HCBP	*Nephtys incisa*	JT	152	0.813	0.464
		SP	122	0.813	0.212

[a] Measured and calculated interstitial water concentrations (IW) taken from Table 2.
[b] Calculated from BCF for molluscs [40] and then normalized for average lipid content. Tissue concentrations were calculated as follows: (IW × BCF × kg/1,000 g)/(g lipid/g total wet wt.) = μg chlorinated biphenyl/g lipid.
[c] TCBP = 2,4,2′,4′-tetrachlorobiphenyl, HCBP = 2,4,5,2′,4′,5′-hexachlorobiphenyl.
[d] JT = Jamestown, SP = Sabin Point.

investigators (H. Lee II, personal communication; J.C. Means, unpublished data). The fact that differences persist between AFs calculated for different sediments ($p < 0.05$), even after normalization for sediment OC, suggests that other factors relating to organic carbon type may influence the bioavailability of sorbed contaminants.

The role of colloidal organic matter present in interstitial waters may be a critical factor. Table 6 shows the observed AF data using mean experimental values (measured) of interstitial water TCBP or HCBP concentrations versus AF values derived from predicted (calculated) interstitial water TCBP or HCBP concentrations (see Table 2). Both sets of data are estimated using the same lipid-based BCF estimated from the K_{ow} for the compounds (Appendix) [40]. It is interesting to note that the AFs calculated using the equilibrium partitioning model (calculated) yielded values that are generally higher than the experimentally observed AFs, except for the case of TCBP in *Yoldia* from sediment JT (Table 6) [19], but that are orders of magnitude lower than those calculated from measured rather than predicted interstitial water concentrations of TCBP or HCBP. This suggests that only a fraction of the total interstitial water chlorinated biphenyls were bioavailable.

Those AFs calculated from equilibrium partitioning theory for TCBP and HCBP fall within the model (AF ≈ 0.5–2) yet the values calculated from the actual experimental interstitial water concentrations of TCBP are far above (9–10 times) those calculated from the equilibrium partitioning model. This suggests that although the interstitial concentrations of PCBs may be elevated due to the presence of colloidal organic matter, all of these PCB residues are not readily available for accumulation. It is important to note that the AF predicted for TCBP in sediment JT is considerably below the actual experimental AF (1.24 versus 3.18).

For HCBP, the AFs calculated from equilibrium partitioning theory also fall within the model. A closer correspondence between calculated values of AF and experimentally observed values of AF were found for sediment JT (0.813 versus 0.464 in *Nephtys* experiments and 1.58 versus 0.980 in *Yoldia* experiments). This was less the case for the high OC SP sediment (0.813 versus 0.212 in *Nephtys* experiments and 1.57 versus 0.891 in *Yoldia* experiments). The values for AFs calculated from the actual experimental interstitial water concentrations of HCBP are a factor of more than 100 times above those calculated from the equilibrium partitioning model. This suggests that although the interstitial concentrations of HCBPs may be greatly elevated due to the presence of colloidal organic matter, the vast majority of these PCB residues are not readily available for accumulation by benthic organisms, similar to the data for TCBPs.

These results suggest that further study of the role of colloidal organic matter in mediating sediment partitioning and bioconcentration in deposit-feeding organisms needs to be undertaken. Also, an investigation of the influence of the quality of the organic matter in sediments as it relates to sorptive properties of contaminants and the role, if any, that benthic organisms play in modifying quality of sediment organic matter through their feeding, mixing, and secretion activities needs to be conducted. Further, a better understanding of bioavailability in relation to the different types of lipid pools within the organism is also needed.

CONCLUSIONS

The bioavailability of sediment-bound TCBP and HCBP was assessed using two deposit-feeding benthic invertebrates, *Nephtys incisa* and *Yoldia limatula*. The amount of HCBP accumulated in both species and the amount of TCBP accumulated in *Yoldia* were inversely related to the OC content of the exposure sediments. On an equal-exposure basis, organisms accumulated more (2.86 in *Nephtys* and 1.81 in *Yoldia*) HCBP from the low OC (JT) sediment than from the high OC (SP) sediment, on a tissue wet weight basis. Similar but larger differences (2.62 times) were also observed for TCBP accumulation in *Yoldia*. Normalization of tissue data for lipid content reduced these differences to a factor of approximately two for both species and both compounds. Examination of the lipid-normalized tissue concentration data revealed that the lipid pools of the organism equilibrated with the external exposure concentration of PCB congeners fairly rapidly (5–20 d), whereas wet weight basis concentrations appeared to increase for as much as 60 d or longer for *Nephtys*. This suggests that growth of the organism during the course of the exposure must be factored into the analysis of bioaccumulation data.

The fact that lipid and organic carbon normalization of the bioaccumulation data yields accumulation factors that differ consistently by a factor of two to four suggests that some other sediment-related or organism-related factor(s) are active in limiting the availability of PCBs in high-organic, fine-grained sediment relative to the low-organic carbon, fine-grained sediment. One such factor that was addressed experimentally in this study was the effect of equilibration time of the contaminant upon the availability of the contaminant to *Nephtys*. Examination of these data failed to reveal any consistent differences in the relative availability of HCBP attributed to equilibration times of 1 week and 1 month. A second sediment-related factor that may contribute to differences in bioavailability is the presence of colloidal solids in the interstitial water of the exposure microcosms. Comparison of predicted versus observed interstitial water concentrations suggested that colloidal solids may have increased interstitial concentrations of TCBP and HCBP. This solubility enhancement, however, yielded no significant increases in bioaccumulation, as observed in two deposit-feeding species of benthic organisms. This suggests that although elevated levels (10–100 times expected)

of colloidally bound contaminants may be detected in interstitial waters and may be transported from the sediment bed, these bound contaminants are not necessarily available to deposit-feeding organisms for bioaccumulation.

The accumulation data collected in these experiments were examined using the kinetic approach proposed by Foster et al. [18]. Steady-state, lipid-normalized AFs determined directly were compared to AFs determined from nonlinear regression of the accumulation kinetics. No significant differences were observed in the AFs obtained from these two approaches. This suggests that shorter accumulation experiments may be sufficient to generate reliable estimates of steady-state tissue concentrations of sediment-sorbed contaminants.

Comparison of both the kinetic and steady-state data for the two species of deposit-feeding organisms yields two major conclusions concerning the possible biological differences. First, it is clear from both the wet weight data and the lipid-normalized data that *Yoldia* accumulated HCBP more rapidly than did *Nephtys* exposed in the same sediments ($p < 0.001$). Because both organisms ingest carbon and nutrients from the organic matrix of the sediments, it is likely that aggressive sediment processing exhibited by *Yoldia* is largely responsible for the observations, although other factors related to digestion and respiration or the composition of tissue lipids in *Yoldia* may also play a role. Second, although differences were observed in the kinetics of HCBP accumulation, only small differences (approx. a factor of two) were observed in the steady-state concentrations of HCBP in the two species.

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APPENDIX

List of experimental parameters

Sediments

Jamestown (JT), Narragansett Bay, Rhode Island, USA
- 1.93 ± 0.06% Organic carbon (n = 4)
- Sandy silt
- 50 ppb Total polychlorinated biphenyls (PCBs) as Aroclor 1254
- Long-term equilibration = 30 d (LT)
- Short-term equilibration = 7 d (ST)

Sabin Point (SP), Narragansett Bay, Rhode Island, USA
- 3.97 ± 0.10% Organic carbon (n = 4)
- Silty clay
- 904 ppb Total PCBs as Aroclor 1254
- Long-term equilibration = 30 d (LT)
- Short-term equilibration = 7 d (ST)

Compounds

TCBP
- 2,4,2′,4′-Tetrachlorobiphenyl
- (UL) ^{14}C Specific activity = 10.5 mCi/mmol, ≈7.97×10^4 disintegration per minute (dpm)/μg
- Log octanol/water partition coefficient (K_{ow}) = 6.29
- Log organic carbon (sediment)/water partition coefficient (K_{oc}) = 5.97[a]
- Log bioaccumulation factor (wet basis) (BAF) = 4.07[b]

HCBP
- 2,4,5,2′,4′,5′-Hexachlorobiphenyl
- (UL) ^{14}C Specific activity = 12.5 mCi/mmol, ≈7.72×10^4 dpm/μg
- Log octanol/water partition coefficient (K_{ow}) = 7.75
- Log organic carbon (sediment)/water partition coefficient (K_{oc}) = 7.43[a]
- Log bioaccumulation factor (wet basis) (BAF) = 5.30[b]

Organisms

Nephtys incisa
- A nonselective, subsurface deposit-feeding polychaete worm
- Four per chamber
- Average initial wet weight (ww) = 0.50 g
- Average initial lipid content = 0.91% (weight [w]/ww)

Yoldia limatula
- A subsurface ''conveyor-belt'' deposit-feeding protobranch bivalve
- Ten per chamber
- Average initial wet weight (ww) (HCBP experiment) = 0.30 g
- Average length (HCBP experiment) = 17 mm
- Average initial wet weight (TCBP experiment) = 0.66 g
- Average length (TCBP experiment) = 31 mm
- Average initial lipid content (HCBP experiment) = 0.47% (w/ww)
- Average initial lipid content (TCBP experiment) = 1.01% (w/ww)

[a] Calculated from K_{ow} according to Means et al. [21].
[b] Calculated from K_{ow} according to Hawker and Connell [40].